Fluorescent and Photochromic Fluorescent Compounds for Applications in Optical Nanoscopy

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Hiermit möchte ich für die Überlassung des interessanten Themas und große Unterstützung während meiner Promotionszeit Herrn Prof. Dr. Armin de Meijere, Herrn Prof. Dr. Stefan W. Hell und Herrn Dr. Vladimir N. Belov ganz herzlich danken.

to my family

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Abbreviations

ACN	acetonitrile;
9-BBN	9-borabicyclo[3,3,1]nonane;
Boc	*tert*-butoxycarbonyl;
BODIPY	4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene;
DCC	dicyclohexylcarbodiimide;
DHP	3,4-dihydro-*2H*-pyran;
DIAD	diisopropyl azodicarboxylate;
DIEA	*N,N*-diisopropylethylamine;
DMAA	*N*,*N*-dimethylacetamide;
DMAP	4-(*N*,*N*-dimethylamino)pyridine;
DMF	*N*,*N*-dimethylformamide;
DMSO	dimethyl sulfoxide;
DPPA	*O*,*O*-diphenylphosphorylazide;
DTT	dithiothreitol;
EDC	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide;
EDTA	ethylenediaminetetraacetic acid;
Fmoc	9-fluorenylmethyloxycarbonyl;
FmocCl	9-fluorenylmethylchloroformiate;
HATU	*O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate;
HPLC	high-pressure liquid chromatography;
HPTLC	high performance thin layer chromatography;
Im	imidazole;
LDA	lithium diisopropylamide;
NBD	4-amino-7-nitrobenz-2-oxa-1,3-diazol;
NBS	*N*-bromosuccinimide;
NCS	*N*-chlorosuccinimide;
NHS	*N*-hydroxysuccinimide;
NMR	nuclear magnetic resonance;
PBS	phosphate-buffered saline;
PMB	4-methoxybenzyl;
PPTS	pyridinium *p*-toluenesulfonate;
Py	pyridine;

TBDPS	*tert*-butyldiphenylsilyl;
TFA	trifluoroacetic acid;
THF	tetrahydrofuran;
THP	tetrahydro-*2H*-pyranyl;
TMEDA	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine;
TMP	2,2,6,6-tetramethylpiperidine;
TMS	trimethylsilyl;
TsOH	*p*-toluenesulfonic acid;
TSTU	*N*,*N*,*N*′,*N*′-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate;
Z	benzyloxycarbonyl.

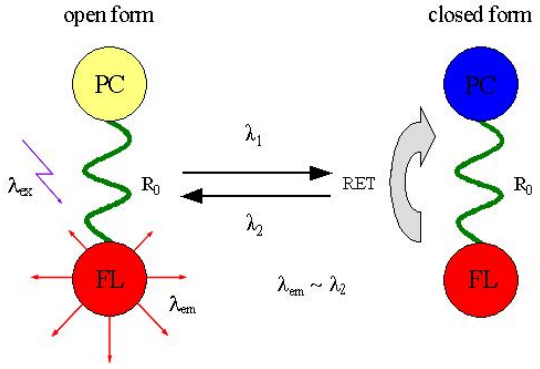
Preface

According to the Abbe principle, the optical resolution of a light microscope cannot be better than one half of the wavelength of the used light. The Abbe law is no more valid nowadays, and the diffraction barrier in optical microscopy has been overcome.[1] A diffraction-unlimited resolution has been achieved using various physical concepts.

Reversible optical fluorescence modulations form a basis of a new physical concept in far-field optical microscopy that allows one to achieve a resolution better than 100 nm.[1a]

Photochromic compounds may be reversibly interconverted by light between two isomers with different absorption spectra, and therefore they are known to be good switchable units. Differences in the absorption spectra may be used for modulation of a secondary function on a molecular or supramolecular level. The switchable fluorescence signal is often used as a parameter, which can easily be detected with high sensitivity.

Photochromic fluorescence resonance energy transfer (pcFRET) could be an attractive tool for improving the optical resolution by imaging of objects bearing switchable fluorescent markers.[2] Let us consider a label consisting of a photochromic compound and a fluorescent dye connected with a linker (Scheme 1). If the fluorescent signal of the labelled object can be modulated by reversible switching of the photochromic unit from a colorless to a colored form, the optical resolution may be improved, provided that many (hundreds or thousands) cycles between the fluorescent and non-fluorescent states are possible without a considerable loss of contrast (caused by photobleaching).[2b]



Scheme 1. Resonance energy transfer (RET) between the closed form of the photochromic unit (PC) and the fluorophore (FL) connected by a bridge with length R_o. λ_1: UV light which transforms the PC into the colored state and switches off the fluorescence; λ_2: visible light which restores the initial state of the PC unit and switches on the fluorescence; λ_{ex}: excitation light

which probes fluorescence; λ_{em}: emitted light. Fluorescence is efficiently quenched, if λ_{max} of the closed form (λ_2) is near to λ_{em} and $R_o < R_F$, where R_F is the Förster radius (distance between PC and FL, where the efficiency of the energy transfer (E_{RET}) is 50%). $E_{RET} \approx R_F^6/(R_F^6 + R_o^6)$.[3]

An advantage of this approach compared with other superresolution techniques[1c] is that the switching event is triggered by light with relatively low intensity. These regularities were used for the design of the photochromic fluorescent compounds described in this work.

The present work is organized in three chapters. Some principles of optical microscopy with superresolution imaging are described in Chapter 1, and published data on photochromic 1,2-bis(3-thienyl)perfluorocyclopentenes and switchable fluorescent compounds are reviewed in Chapter 1.

In Chapter 2, our own results concerning the synthesis and properties of new photochromic and photochromic fluorescent compounds are presented. Novel, practically useful 1,2-bis(3-thienyl)perfluorocyclopentenes have been developed and prepared in the present work, and their use in superresolution imaging have been evaluated. It has been shown that good performance in aqueous solutions is very difficult to achieve. Nevertheless, new water-soluble photochromic and photochromic fluorescent compounds with an improved switching behavior in water have been prepared.

Chapter 3 deals with the synthesis of fluorescent GM1 ganglioside derivatives – model compounds – for a new superresolution technique, a combination of stimulated emission depletion (STED) and fluorescence correlation spectroscopy (FCS). This combination drastically reduces the focal volume of the FCS method and enables directly to observe nanoscale dynamics of lipid molecules in membranes of living cells.[4]

Chapter 1. Fluorescence modulation by the use of photochromic compounds

1. Main principles of optical nanoscopy

Optical resolution in far-field microscopy was assumed to be limited by the Abbe principle to about half of the wavelength of the used light.[5] Several inventions in the last 10–15 years demonstrated that the diffraction barrier may be overcome by using a reversible or irreversible photoswitching of the fluorescence between a dark and an emitting state.[1,6] Breaking the diffraction barrier enables detailed visualization of cellular objects in biology and writing of nanostructured patterns with focused visible light.[2b]

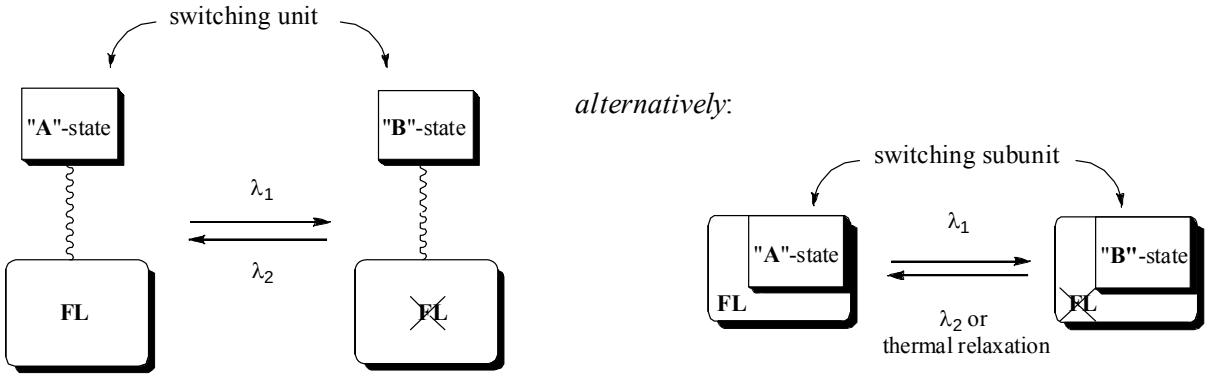
Reversible saturable (switchable) optically linear fluorescence transitions (RESOLFT), the first family of the superresolution techniques, is based on a localized squeezing of the effective observation volume by means of illumination patterns presenting one or more intensity zeros in the focal plane. This can be achieved using a spot-like (focal) illumination, scanned through the sample, or by structure illumination. For example, a very important novel method of the stimulated emission depletion (STED) microscopy uses the ground (singlet) state of the fluorophore (S_0) as a dark state, and the first excited state (S_1) as a bright one. In practical applications of the STED method, a focused pulse excites fluorescence in a small spot (with dimensions limited by diffraction), and immediately after that a red-shifted doughnut-shaped STED beam switches off the fluorescence of excited molecules by stimulated emission ($S_1 \rightarrow S_0$) everywhere, except in the very center of the doughnut, where the quenching intensity is zero. For squeezing the fluorescence to a very small central spot, the depletion rate should exceed the rate of the spontaneous transition to the ground state S_0. Fluorescent lifetimes of organic fluorophores ($\tau_{fl} \sim 10^{-9}$ s) and their optical cross-sections of the $S_1 \rightarrow S_0$ transitions ($\sigma \sim 10^{-16}$ cm^2) imply that the STED pulse should have a very high power $I_{STED} \gg I_S \equiv (\sigma\tau_{fl})^{-1} \sim 10^{25}$ photons/(cm^2×s) ≅ 10 MW/cm^2. I_S is a threshold intensity depending on the dye employed and the depletion wavelength used. The resolution enhancement scales roughly with $(1 + I_{STED}/I_S)^{0.5}$. These huge light intensities inevitably cause photobleaching of fluorophores, and therefore STED microscopy ultimately requires the most photostable fluorescent dyes. High fluorescence quantum yields (Φ_{fl}) are also required to increase the sensitivity of the method and reduce the imaging time. Moreover, if a resolution in the molecular scale is desired, the fluorophores should be suitable for single molecule detection.

Oppositely to RESOLFT, were the position of the subdiffraction-sized emitting spot is known and controlled, another family of techniques, based on the imaging of stochastically switched-on single emitters has emerged. In them the position of the observed volume is a priori

unknown, and its size is reduced to a single molecule. The main advantage of this single-molecule localization microscopy (SMLM, also known as photo activation localization microscopy [PALM] and stochastical optical reconstruction microscopy [STORM]) over the RESOLFT techniques is that it is not necessary to force the marker molecules through several photoswitching cycles. However, its application requires samples with very low background levels, and thus a high contrast between the molecular states of the markers used.

Besides the effective switching of fluorescence, several requirements are important for the markers to perform well in SMLM. First, highly photostable fluorophores with high fluorescence quantum yields are necessary to yield a large number of photons (in the "on" state). Second, a high contrast between the two states (fluorescent and dark) is required for measuring densely stained samples and localization of a large number of markers. High contrast means that all the markers in the dark state produce a negligible contribution to the background during the imaging of the switched-on ones. Finally, the switching reactions should be easily controlled, to keep the desired fraction of markers all of the time in the "on" state.

Photoswitching of the fluorescence between a dark and an emitting state can be implemented by several ways. First, the fluorescent dye can be covalently attached to a switching unit, thus the fluorescence in one form is quenched due to the intramolecular interactions (energy transfer). Second, the fluorescent compound can already have a switching subunit in the molecule (Scheme 1.1).[7]



Scheme 1.1. Photoinduced transitions of switchable fluorescent dyes between fluorescent and non-fluorescent states.

Finally, the non-switchable fluorescent dyes can be reversibly transformed to their metastable dark state, such as the triplet, without chemical transformations.[8] However, this method requires huge light intensities, and sometimes special buffers with an oxygen scavenger are needed. Moreover, this superresolution imaging method of the ground state depletion and single molecule return (GSDIM) requires new photostable fluorescent dyes with recovery times

from several tens to several hundreds of milliseconds, minimal content of the dye in the ground state after the pump pulse and the possibility to enhance the recovery by irradiation with the UV laser (375 nm).

For applications in optical microscopy, the switchable fluorescent labels should meet the following general requirements:

- high photostability
  - fatigue- and photoresistance of the photochromic unit
  - resistance of the fluorophore against bleaching
- high fluorescent modulation (>90%)
- high fluorescent quantum yields (Φ_{fl})
- thermal stability of the fluorescent and non-fluorescent forms
- fast switching times
- switching in aqueous medium or on a biological object
- large separation between the absorption bands of the photochromic unit (350 nm < λ_{OF} < λ_{ex} < λ_{em} < λ_{CF})
- linker between the fluorophore and the photochromic unit with a reactive group

The photochromic diarylethenes are widely used as switching units for fluorescence modulation due to their outstanding properties.

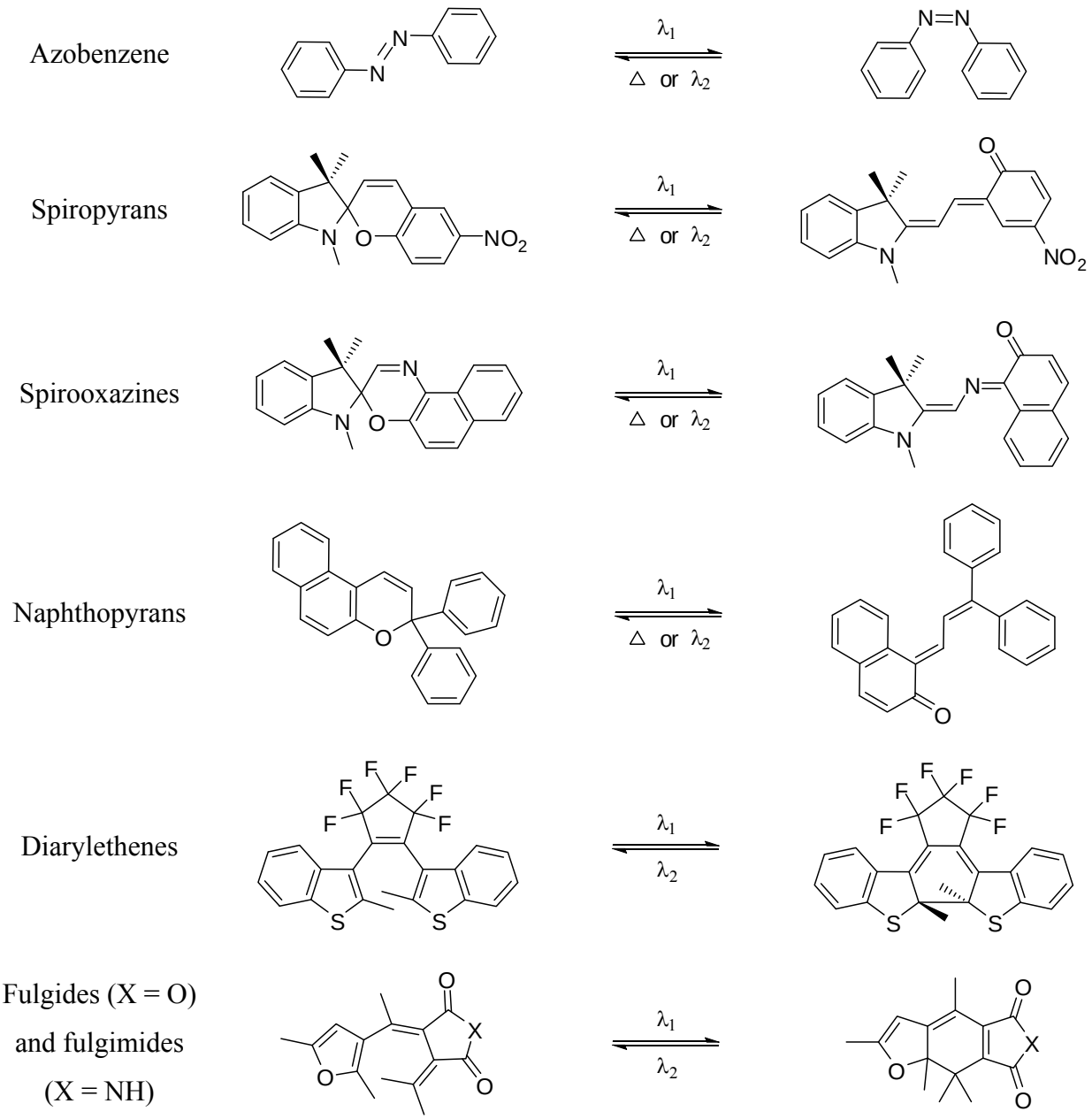
2. Photochromism and types of photochromic compounds

Photochromism may be defined as a reversible transformation of a chemical species by photoirradiation between two forms having different absorption spectra[9]. Discovered in the late 1890s, this term was suggested by Y. Hirshberg in 1950. It combines the Greek words „phot“ and „chroma“, which mean light and color, respectively.

The two isomers of photochromic compounds differ from one another not only in the absorption spectra but also in various physical and chemical properties, such as refractive indices, dielectric constants, oxidation-reduction potentials, and structures. Due to the instant property changes caused by photoirradiation, these materials may be used in various optoelectronic devices, such as optical memory, photo-optical switching, display construction, and nonlinear optics.[10]

Typical photochromic compounds, such as azobenzenes, spiropyrans, spirooxazines, and naphthopyrans (Scheme 2.1), undergo thermally reversible photochromic reactions. Photogenerated isomers are thermally unstable and return to the initial forms in the dark. On the other hand, diarylethenes and furylfulgides exhibit thermally irreversible photochromic

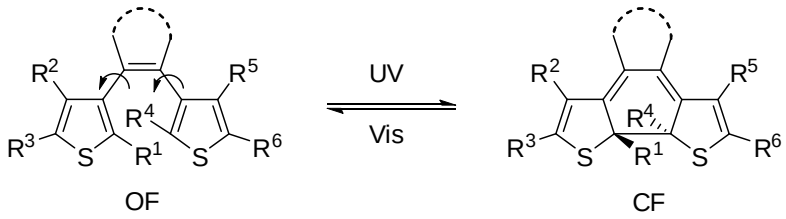
reactions. Photogenerated species are thermally stable and do not return to the initial isomers at room temperature.



Scheme 2.1. Types of the photochromic compounds.

Photochromic diarylethenes with heterocyclic aryl groups, especially with thienyl groups, are the most promising candidates for photonic applications because of their fatigue resistance and thermal stability of the closed-ring isomers.[11]

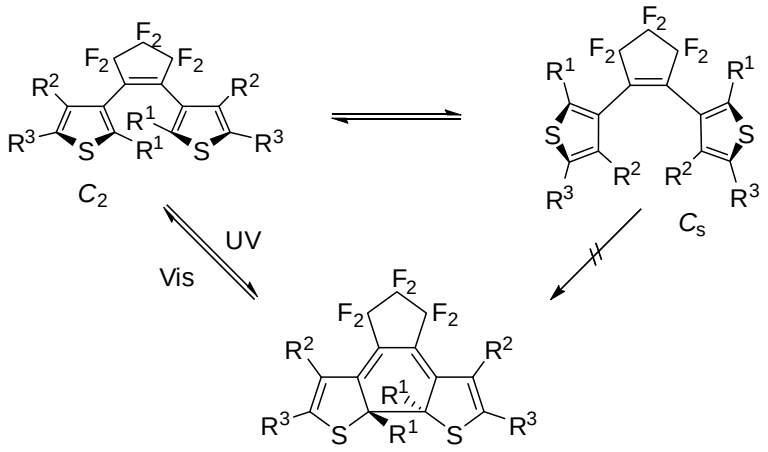
Photochromic reaction of diarylethenes is based on reversible transformations between the open-ring isomer with a 1,3,5-hexatriene fragment and the closed-ring isomer with a cyclohexadiene structure. According to the Woodward–Hoffmann rules[12] the cyclization reaction is allowed in the conrotatory mode in the photoexcited state, as shown in Scheme 2.2.



Scheme 2.2. Photochromism of 1,2-bis(3-thienyl)ethenes.

The colorless open-ring isomer converts to the colored closed form upon irradiation with UV light, and the colored isomer returns to the initial colorless form upon irradiation with visible light. However, each of these forms may be colorless, if the absorption bands are observed below 400 nm. In this case an "invisible photochromism" takes place.[13] The photochromic compounds can repeat photoinduced coloration/decoloration cycles more than 10^4 times,[14] and both forms were estimated to be stable for more than thousand years at room temperature.[15]

In solution, the open form of dithienylethenes may exist in two conformations: an antiparallel (aryl rings with C_2 symmetry) and a parallel (aryl rings with mirror C_s symmetry), as shown in Scheme 2.3. Normally, in the equilibrium they are present in almost equal amounts.



Scheme 2.3. Parallel and antiparallel conformations of dithienylperfluorocyclopentenes.

Upon irradiation with UV light, the conrotatory photocyclization reaction can proceed only from antiparallel conformers.[16] The cyclization quantum yield depends on the ratio between antiparallel and parallel conformers, and was found not to exeed 50%.[17] Bulky substituents at the 2 and 2' positions of the thienyl rings increase the population of the antiparallel conformation and increase the cyclization quantum yield,[18] but decrease the stability of the closed-ring isomer.[19]

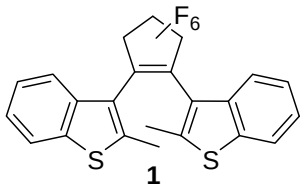
The presence of the two forms (parallel and antiparallel) in solution also affects the NMR spectra.[20] The activation barrier of the conformational changes was estimated to be 67–71

kJ/mol, and the coalescence temperature was found to be higher than room temperature (293 K).[21]

The 1,2-bis(3-thienyl)ethene system may contain maleic anhydride, maleimide, perfluorocyclopentene, and cyclopentene units as a central binding part.[22] Diarylethenes with a perfluorocyclopentene bridging unit exhibit excellent photochromic properties.

3. General strategies for the synthesis of photochromic diarylperfluorocyclopentenes

For the first time, diarylperfluorocyclopentenes were mentioned in 1992, when Hanazawa *et al.* reported that the perfluorocyclopentene moiety shifted the absorption band of the closed form to longer wavelengths and increased the stability of these photochromic compounds in comparison with a non-cyclic analogue.[14b]



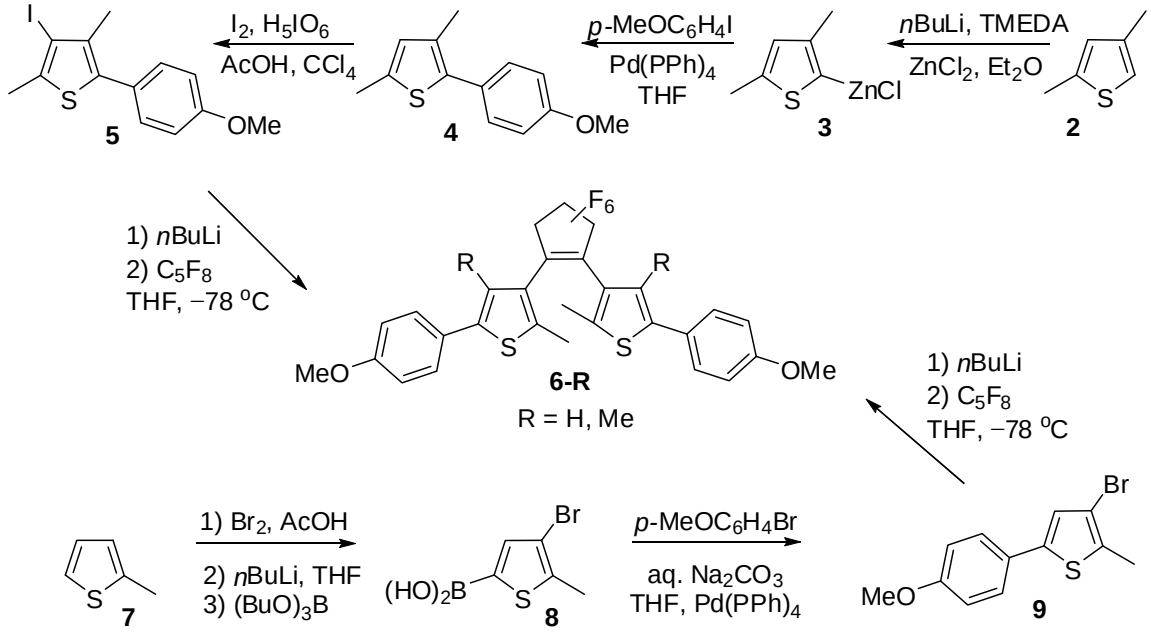
1

Scheme 3.1. The first reported diarylperfluorocyclopentene.

The photogenerated closed-ring isomer of 1,2-bis(2-methylbenzo[*b*]thiophen-3-yl)perfluorocyclopentene (**1**, Scheme 3.1) with an absorption maximum at 526 nm was thermally stable, and the absorption intensity remained unchanged after heating for more than 6 months in toluene at 80 °C. The authors reported that even after 14000 coloration/decoloration cycles in methylcyclohexane in the presence of air the perfluorocyclopentene derivative **1** retained about 90% of its performance.

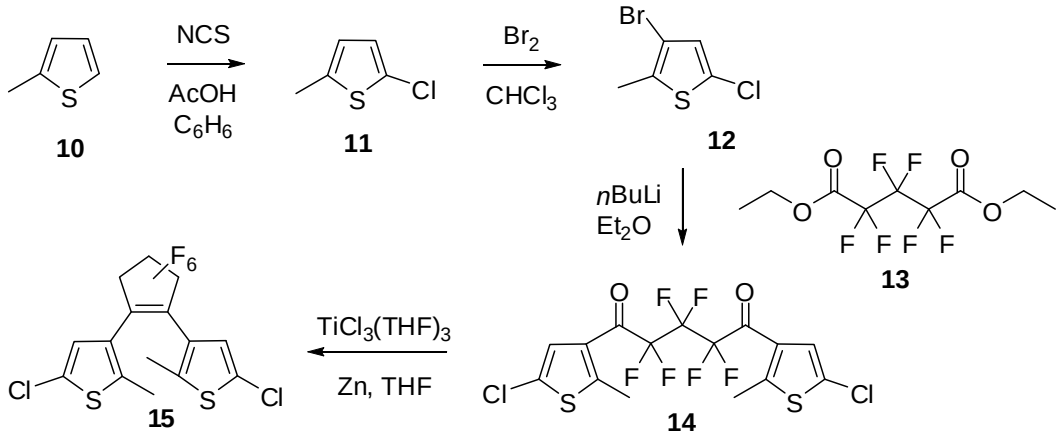
Although many functionalized diarylethenes with highly attractive photochromic properties have been synthesized, their preparations are far from being trivial.

The perfluorocyclopentene fragment can be introduced to the final molecule in two different ways. The first procedure involves the halogen-lithium exchange in 3-bromo- or 3-iodothiophenes with butyllithium followed by the addition of perfluorocyclopentene.[14b] The substituted halogenated intermediates can by obtained either via a zink derivative[20] or, alternatively, via Suzuki coupling with areneboronic acid[23] (Scheme 3.2). After lithiation, the corresponding 3-lithio-5-phenylthiophenes were treated with octafluorocyclopentene at low temperature and symmetric perfluorocyclopentenes **6**-Me and **6**-H were obtained in 71 and 90% yields, respectively.



Scheme 3.2. Two synthetic routes towards symmetric 1,2-bis(3-thienyl)ethenes.

Another method was reported in 1999 and based on the reaction of lithiated 3-bromo-2-methyl-5-chlorothiophene (**12**) with ethyl hexafluoroglutarate (**13**) followed by a McMurry coupling (Scheme 3.3).[24] At –78 °C, only the bromine atom was substituted with lithium, while the lithium-chlorine exchange at ambient temperature presented an additional possibility to functionalize the photochromic compound **15**.

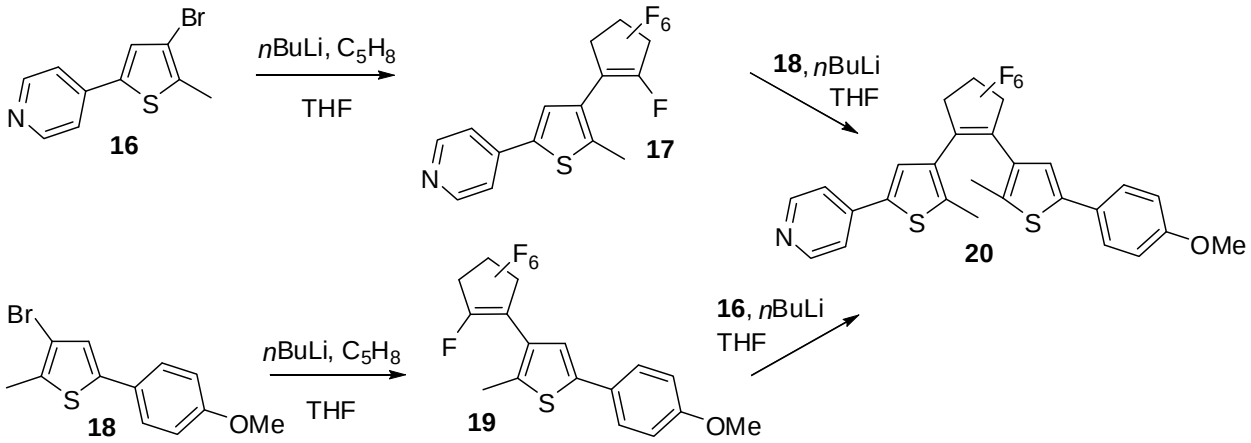


Scheme 3.3. Synthesis of symmetric 1,2-bis(3-thienyl)ethene **15**.

In spite of the high cost of octafluorocyclopentene and difficulties to handle it (b. p. 28 °C), the first method is widely used.

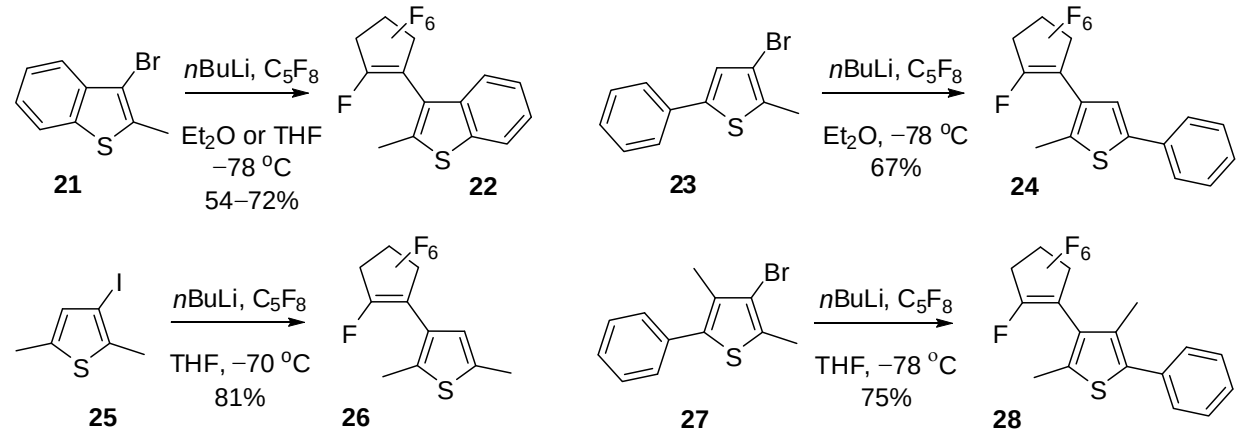
Monosubstituted perfluorocyclopentenes may be prepared by controlling the ratio between octafluorocyclopentene and aryllithium. These compounds can be used for the synthesis of the non-symmetric diarylethenes with various (heterocyclic) aryl groups. The first reported non-

symmetric photochromic compound **20** was obtained along two routes from the bromo derivatives **16** and **18** and monosubstituted perfluorocyclopentenes **17** and **19** in total yields of 26 and 23%, respectively (Scheme 3.4).[25] The open-ring isomer of **20** has an absorption band at 296 nm in benzene. After irradiation with 365 nm light, a new absorption band of the closed-ring isomer appears at 602 nm.



Scheme 3.4. Synthesis of non-symmetric 1,2-bis(3-thienyl)ethene **20**.

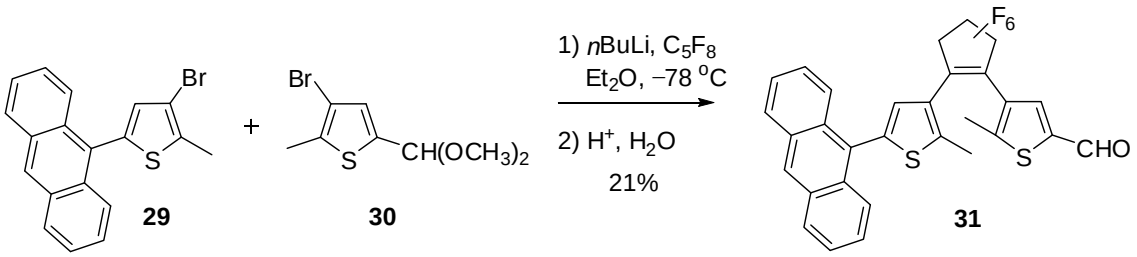
Useful heptafluorocyclopentenes are presented in Scheme 3.5: 3-(heptafluorocyclopent-1-enyl)-2-methylbenzo[*b*]thiophene (**22**),[26] 2,5-dimethyl-3-(heptafluorocyclopent-1-enyl)thiophene (**26**),[27] 3-(heptafluorocyclopent-1-enyl)-2-methyl-5-phenylthiophene (**24**),[28] 2,4-dimethyl-3-(heptafluorocyclopent-1-enyl)-5-phenylthiophene (**28**).[29]



Scheme 3.5. Examples of useful arylheptafluorocyclopentenes.

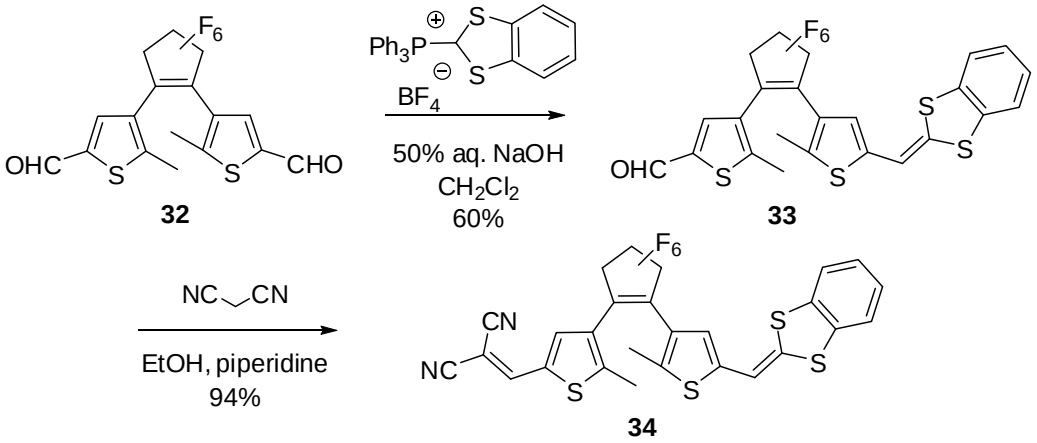
In particular, 3-(heptafluorocyclopent-1-enyl)-2-methyl-5-phenylthiophene (**24**) has been widely used for the synthesis of different non-symmetric photochromic compounds.[30]

Alternatively, non-symmetric diarylethene **31** can be prepared by the addition of perfluorocyclopentene to a mixture of the two lithiated aryl derivatives (Scheme 3.6).[31] However, in this case the yield was low (21%), and two symmetric photochromic by-products were isolated.



Scheme 3.6. Synthsis of non-symmetric perfluorocyclopentene **31**.

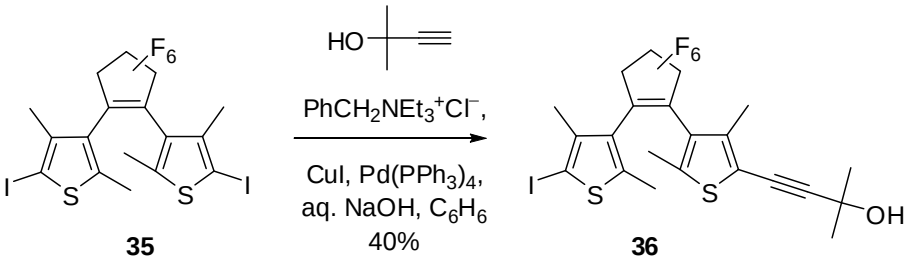
Another option to generate non-symmetric diarylethenes is chemical modification of symmetric compounds. For example, the Wittig condensation of **32** with one equivalent of 2-triphenylphosphonio-l,3-benzodithiole tetrafluoroborate afforded the mono-adduct **33** which could be easily isolated in 60% yield (Scheme 3.7).[32] Monoaldehyde **33** was then condensed with malonodinitrile to give 94% of the non-symmetric push-pull compound **34**. The closed form of **34** was found to absorb at 828 nm in benzene (the ultimate value for all photochromic diarylethenes), but was thermally unstable.



Scheme 3.7. Modifications of symmetric 1,2-bis(3-thienyl)ethenes.

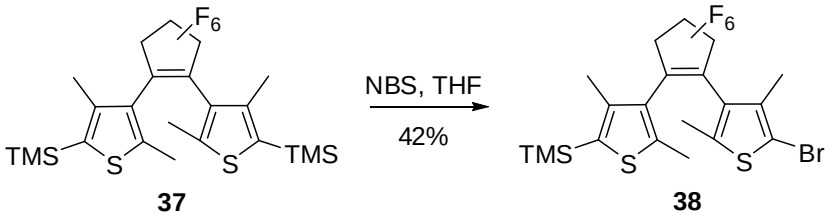
In another investigation, 1,2-bis-(2,4-dimethyl-5-iodothiophen-3-yl)perfluorocyclopentene (**35**) was coupled with 1 equivalent of 2-methyl-3-butyn-2-ol in the presence of benzyltriethylammonium chloride, CuI and $Pd(PPh_3)_4$ in benzene with 5.5 M NaOH at room temperature to give the derivative **36** in 40% yield (Scheme 3.8). It was used for the synthesis of

multi dithienylethene arrays with two, three or four ethynyl-bridged 1,2-bis(2,4-dimethylthiophen-3-yl)perfluorocyclopentenes.[33]



Scheme 3.8. Modifications of symmetric 1,2-bis(3-thienyl)ethenes.

Treatment of compound **37** with 1 equivalent of NBS resulted in 42% of the monobromide **38** (obtained by replasement of one TMS group with bromine) (Scheme 3.9) which was further used in the synthesis of a fused dithienylethyl trimer.[34]



Scheme 3.9. Modifications of symmetric 1,2-bis(3-thienyl)ethenes.

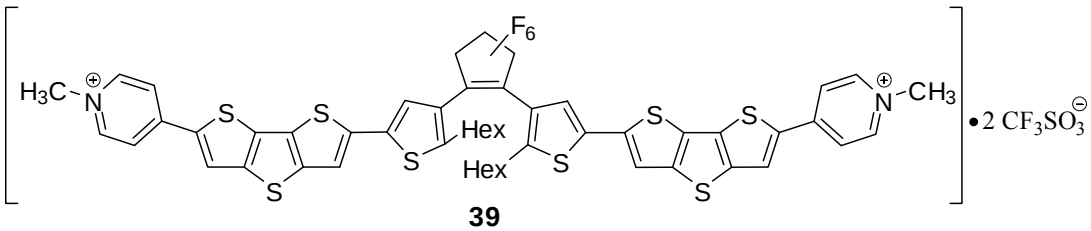
4. Photochromic fluorescent 1,2-bis(3-thienyl)perfluorocyclopentenes

Fluorescent photochromic compounds described in the literature may be used as optical memory materials and fluorescent probes. Non-destructive readout capability is indespensible for applications in the field of recording media. The fluorescence readout method is very sensitive, and the light with low power energy destroys the recorded information to a minimal extent. Three separated wavelength for writing, reading, and erasing cycles allow to change the fluorescence intensity reversibely.

Several photochromic fluorescent diarylethenes were reported in the last 14 years.[35]

4.1. Fluorescence modulation achieved by changes in conjugation

The fluorescence may be quenched by the photoinduced electron transfer (PET), and several fluorescent photoswitchers based on this principle have been synthesized. The compounds **39** and **40** designed by Tsvigoulis and Lehn at the begining of the 1990s have good fluorescence modulation.[36]



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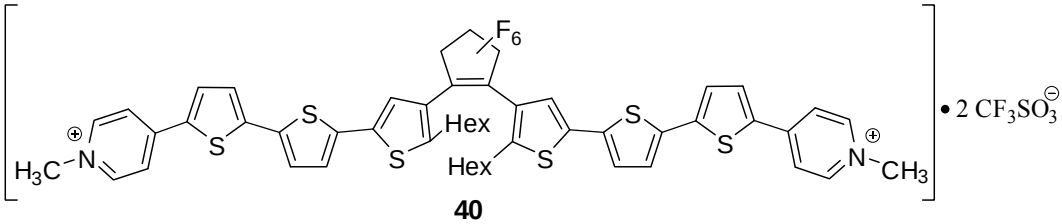
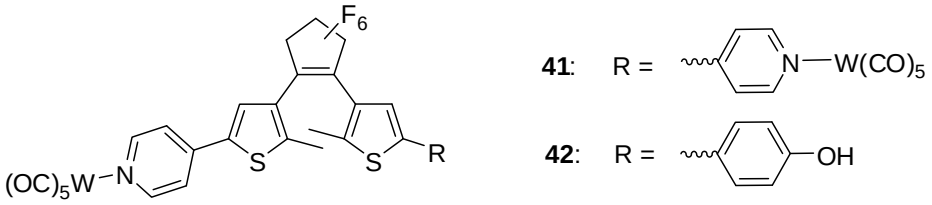


40

The open-ring isomers **39** and **40** underwent photoinduced cyclization upon irradiation with 312 nm light, and the closed-ring isomers were formed with a maximal conversion of 92%. The irradiation with visible light (λ >600 nm) restored the initial absorption spectra. Excitation with 400–500 nm light of the open-ring isomers **39** and **40** produced a fluorescence signal with a maximum at about 589 nm and 611 nm, respectively, and only slightly influenced the cycloreversion of the closed-ring isomers. The closed forms displayed only a weak fluorescence (3–4%), and the achieved modulation of the fluorescence was 88–89%. Although the properties of this system are not perfect, these compounds are interesting for potential applications as a non-destructive readout and storage media.

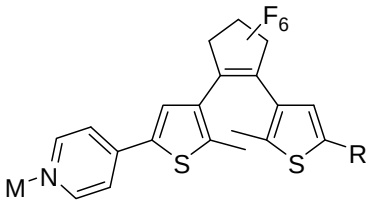
At the end of the 1990s the group of Lehn reported the properties of the tungsten and rhenium complexes of 1,2-bis(3-thienyl)perfluorocyclopentene with a pyridine ring.[37]



41: R = N—W(CO)$_5$

42: R = OH

It is interesting, that the fluorescence signal of the closed-ring isomers of **41** and **42** was stronger than that of the open-ring isomers. For compounds **41** and **42**, the conversion at the photostationary state (PSS) was reported to be 90%, and the fluorescence modulation was found to be 62 and 79%, respectively. Among the complexes **41**–**44**, the compound **42** was a promising candidate for the non-destructive readout system, because its fluorescence intensity (excited at 200–400 nm) was high. The fluorescence quantum yield of the closed form of **42** was found to be 0.15, and for the open form – only 0.03 (upon excitation at 240 nm).

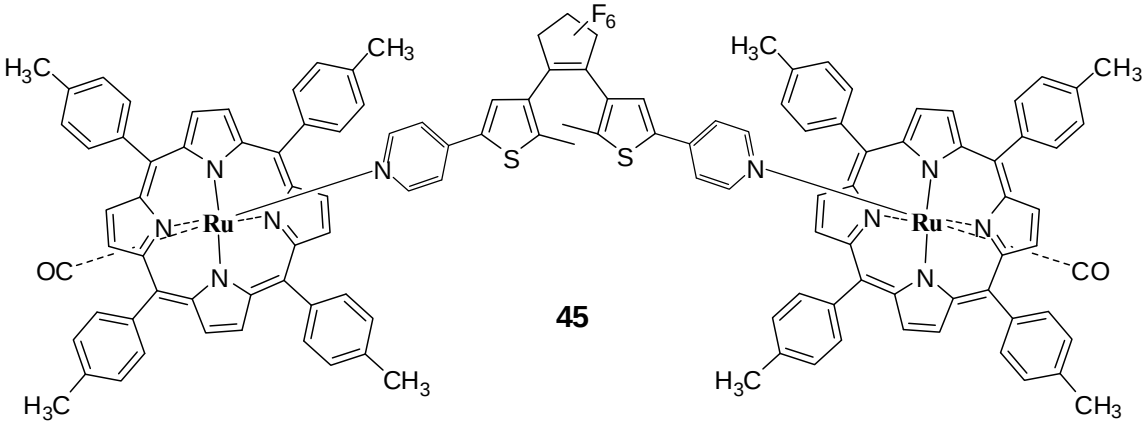


43: M = $[Re(bpy)(CO)_3](CF_3SO_3)$,
R = N-$[Re(bpy)(CO)_3(CF_3SO_3)]$

44: M = $[Re(bpy)(CO)_3](CF_3SO_3)$,
R = *p*MeOPh

The open-ring isomers of the complexes **43** and **44** emit at 372 nm and 368 nm (upon excitation at 320 nm), and the fluorescence quantum yields were reported to be 0.04 and 0.18, respectively.

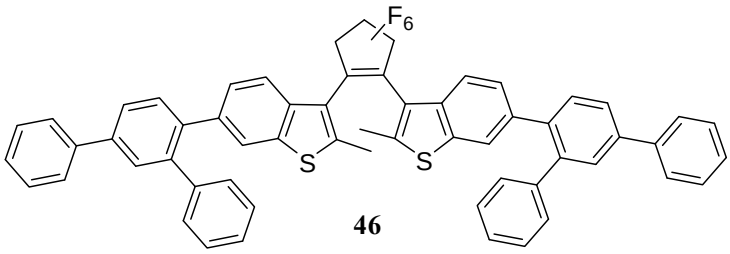
In 2001 the group of Branda described complexes of diarylethenes with transition metals.[38] In compound **45** each of the two pyridine groups was axially coordinated with one porphyrinato(ruthenium) complex.



45

The open form of **45** showed phosphorescence at 730 nm upon excitation with 400–480 nm light. Irradiation of **45** with 365 nm light produced the non-emitting closed-ring isomer with a 95% conversion, and irradiation with 580 nm light regenerated the open form. The modulation of the emission signal in deoxygenated benzene was found to be around 87%.

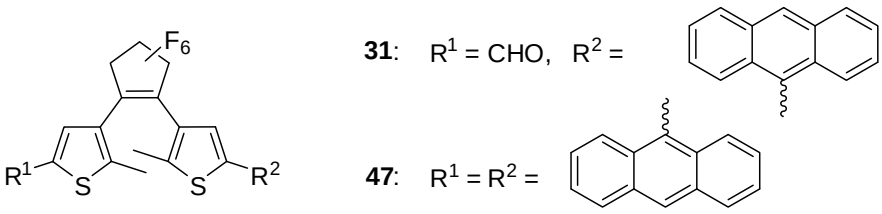
Both forms of the diarylethene **46** synthesized in the Irie's group are fluorescent, but the fluorescence maxima were observed at different wavelengths.[39]



46

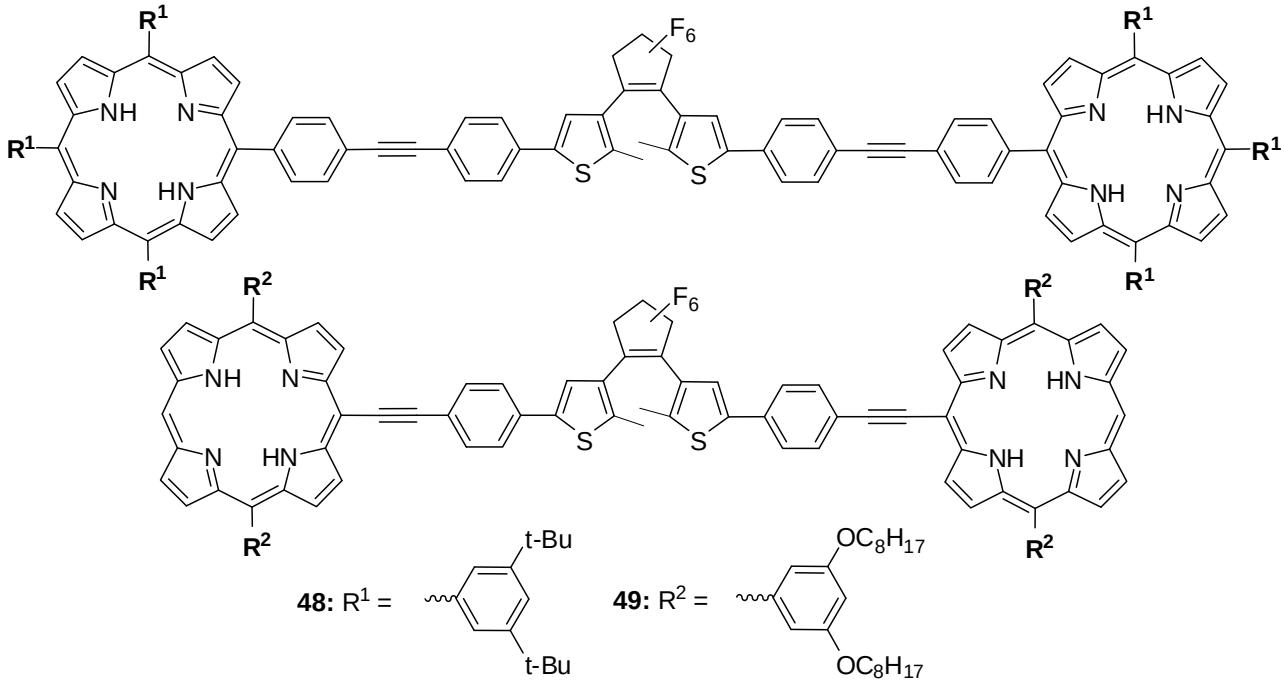
The authors investigated bulk amorphous films prepared from the monomeric compound **46** with high glass–liquid transition temperature (T_g = 127 °C). The conversion of **46** to a closed-ring isomer in the PSS was found to be 29% (upon irradiation with 313 nm light), and two

emission bands were observed at 450 nm and 650 nm, corresponding to the open and closed form, respectively.



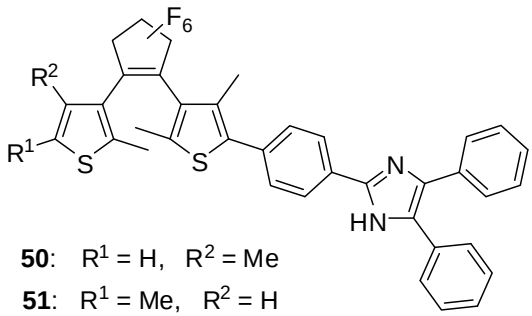
In 2001 the group of Kryschi described in detail the fluorescence dynamics of the anthracene-substituted diarylethenes **47** and **31**.[31] The fluorescence of the open-ring isomers **47** and **31** (at ca. 450 nm) was quenched in the closed-ring isomers obtained upon irradiation with UV light. However, the fluorescence quantum yields were found to be low and depend on the polarity of the solvent.

At the same time, the group of Irie reported the synthesis and photochemical properties of diporphyrin derivatives **48** and **49**.[40]



The open-ring isomer **48** displayed fluorescence maxima at 650 nm and 717 nm (upon excitation at 420 nm), and the intensity of the fluorescence in the closed-ring isomer was low. Conversions of the open to closed form upon irradiation at 330 nm were found to be 75 and 20% for the compounds **48** and **49**, respectively. The open-to-close photoisomerisation of **49** was rather slow compared with **48**, and the cyclization and cycloreversion quantum yields of both compounds were also low.

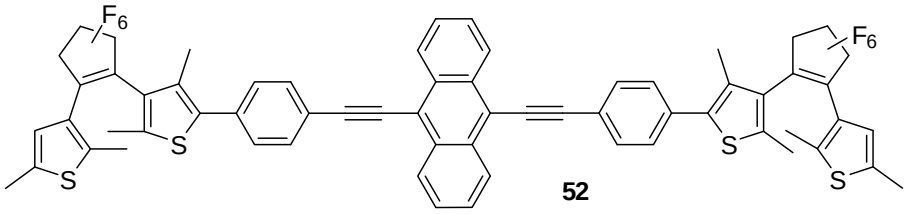
Irie and co-workers prepared the fluorescent diarylethenes **50** and **51** with a 2,4,5-triphenylimidazole chromophore.[41]



50: R^1 = H, R^2 = Me
51: R^1 = Me, R^2 = H

Compounds **50** and **51** reversibly underwent photocyclization and ring-opening reactions by alternate irradiation with UV (366 nm) and visible (>480 nm) light. The conversions between open and closed isomers in the photostationary state were 67 and 82% for **50** and **51**, respectively. The fluorescence intensity changed also reversibly in the course of photochromic reactions. The open-ring isomers of compounds **50** and **51** emit at 410 nm (when excited at 313 nm), but the fluorescence quantum yields were quite low (4.5 and 7.7%, respectively). However, the fluorescence modulation for compound **50** was high and estimated to be about 96%.

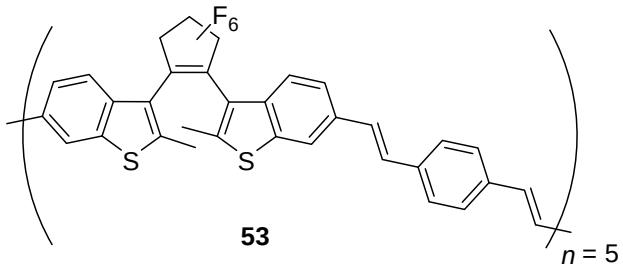
The fluorescent photochromic compound **52** prepared by Irie's group in 2001 had two diaryethene residues connected through a fluorescent bis(phenylethynyl)anthracene.[42]



52

The fluorescence quantum yield for compound **52** was found to be 0.83 for the open form and less than 0.1% for the closed-ring isomer (only one diarylethene residue participated in the photocyclization). This compound also exhibited a laser emission, and the emission intensity was reversibly switched by alternative irradiation with 313 nm and λ >500 nm light.

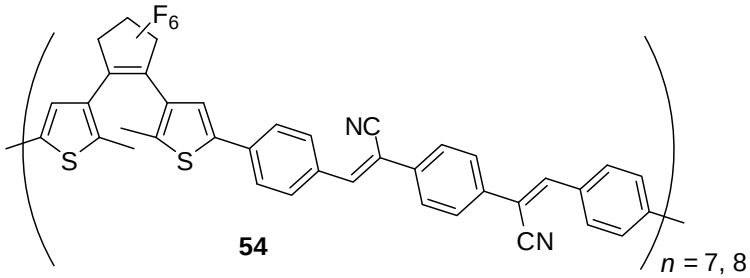
In 2002, Korean researchers synthesized the highly fluorescent photochromic diarylethene oligomer briged by a 4-phenylenevinylene fragment.[43]



53 *n* = 5

After excitation with 381 nm light, a strong fluorescence of the open-ring isomer was observed at 450 nm, and the fluorescence quantum yield of the oligomer **53** was found to be 0.53. Upon irradiation with 325 nm light, the colorless solution turned red (with a cyclization quantum yield 0.38). The closed form of **53** showed only a weak fluorescence, and the fluorescence modulation was found to be ca. 76%.

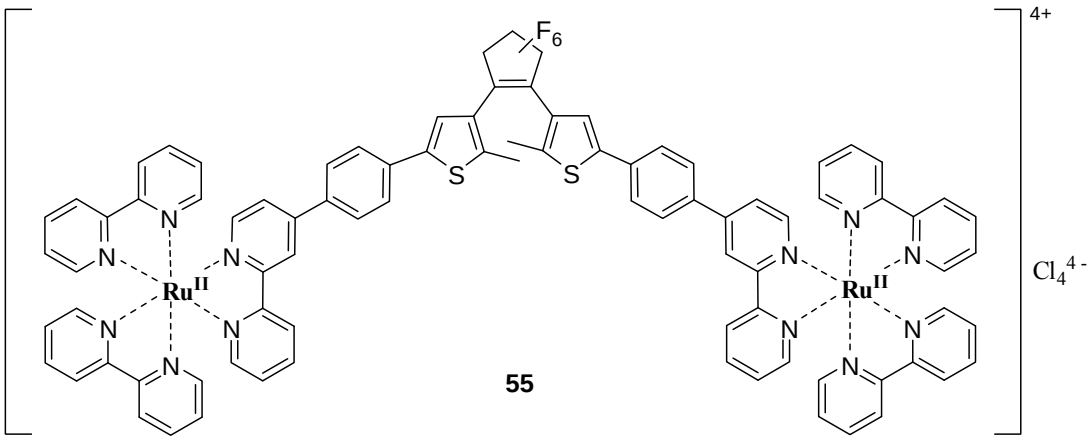
In Park's group, the fluorescent photochromic polymer films were developed.[44]



54 *n* = 7, 8

Oligomer **54** showed the photochromic behavior in solution and in polymer films; the cyclization and cycloreversion quantum yields were 0.36 and 0.0075, respectively. Upon excitation with 400 nm light, the fluorescence modulation (at about 510 nm) between the open form and the PSS in solution or film reached 83 and 90%, respectively.

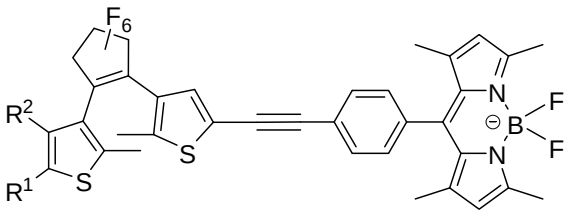
In 2003 another group of researchers developed and characterized dithienylethene-based molecular switch **55** with two ruthenium complexes.[45]

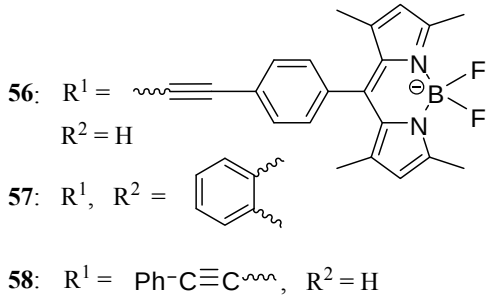


55

Excitation with 334 nm light resulted in light emission with a maximum at 630 nm. A high emission modulation (97%) of **55** was observed; however, this excitation wavelength also produced the cyclization to the non-emitting closed-ring isomer. The emission quantum yield was low, but in deaerated solutions a two-fold increase was observed.

In 2005 Neckers's group reported properties of diarylethenes connected with a BODIPY fluorophore.[46]



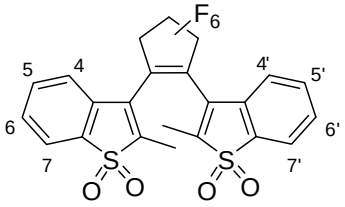


56: R^1 = $R^2 = H$

57: R^1, R^2 =

58: R^1 = Ph–C≡C, $R^2 = H$

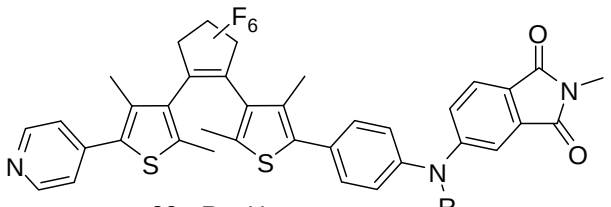
The open forms of compounds **56**, **57** and **58** strongly emit at about 520 nm (when excited with 480 nm light) with quantum yields 0.27, 0.34 and 0.32, respectively. The conversions of compounds **56**, **57** and **58** to almost non-fluorescent closed-ring isomers (upon irradiation at 254 nm) were estimated to be 96, 81 and 95%, respectively. The fluorescence can be recovered by irradiation with visible light; thus the modulation of the fluorescence for compound **56** reached 90%.



59

In 2005 Jeong *et al.* reported a new fluorescent photochromic diarylethene system **59**, which was obtained by oxidation of 1,2-bis(2-methylbenzo[*b*]thiophene-3-yl)perfluorocyclopentene (**1**). In contrast to previous examples, the fluorescence quantum yield of the derivative **59** increased after the photocyclization.[47] The closed-ring isomer of compound **59** was ca. 10 times more fluorescent than the open-ring isomer (excitation at 400 nm), and the switching could be repeated for at least 10 times upon alternating irradiation with visible and UV light. The introduction of acetyl groups in the positions 6 and 6' of the oxidized benzothiophene residues[48] along with the heptyl groups to the reactive carbon atoms[49] drastically increased the fluorescence intensity of the closed-ring isomers, and the fatigue resistance of the modified compounds improved.

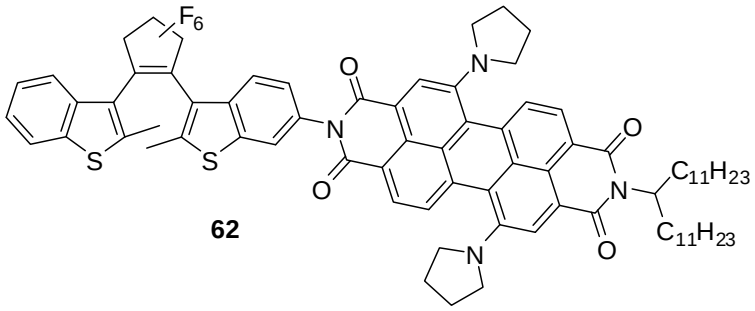
In 2008 our group reported the synthesis of photochromic diarylethenes and their fluorescent and solvatochromic properties.[50]



60: R = H
61: R = Boc

The fluorescence emission was modulated by irradiation with UV and visible light, which caused the photochromic reactions. The emission wavelength and efficiency strongly depended on the polarity of the solvent. The fluorescence maxima for the open form of **61** (excitation at 370 nm) shifted to longer wavelength on changing the solvent from cyclohexane to dioxane. For compound **60** the fluorescence quantum yield was reduced from 0.23 in cyclohexane to 0.016 in dioxane. The high cyclization quantum yield of **60** (0.5 in cyclohexane) drastically decreased with increasing solvent polarity.

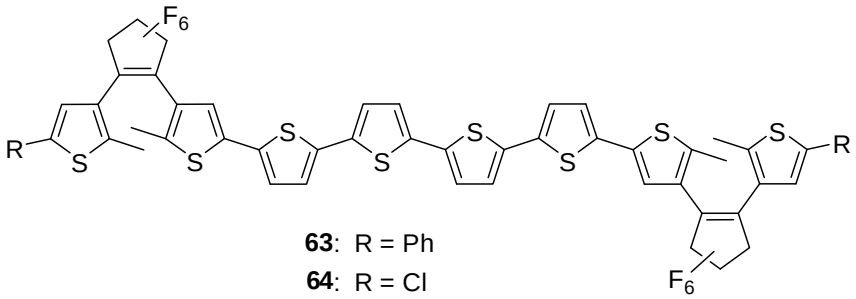
Recently, the group of Würthner designed a fluorescent diarylethene-perylene bisimide photochromic system.[51]



62

Both the open and closed forms of compound **62** exhibited the characteristic UV/Vis spectra of 1,7-dipyrrolidinylperylene bisimides, with an absorption maximum at around 700 nm. The fluorescent quantum yield decreased with increasing of the dielectric constant of the solvent (ε). In low-polar solvents (chloroform), no fluorescence quenching was observed. In acetone (high ε-value), the quantum yields of the fluorescence were 0.11 and 0.03 for open- and closed-ring isomers of **62**, respectively.

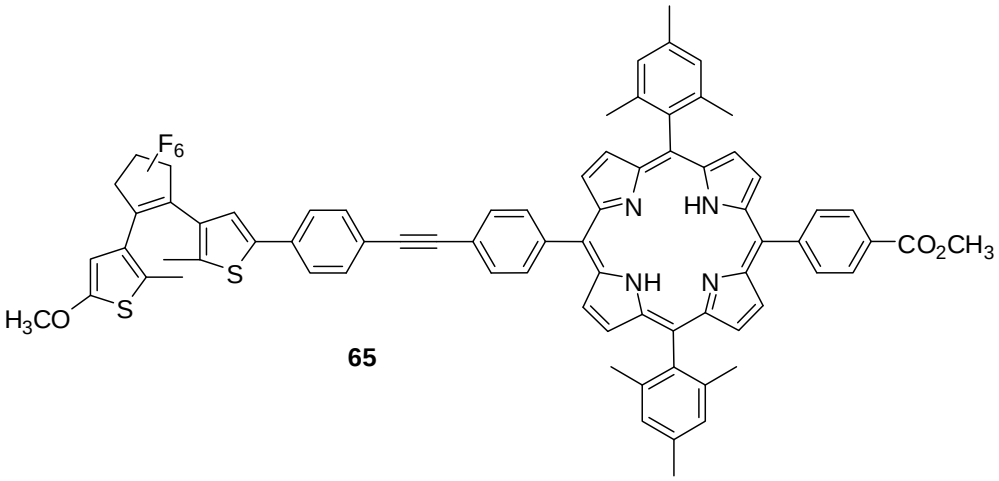
Quite recently, the group of Feringa reported the synthesis and spectroscopic properties of the photoswitchable sexithiophene.[52]



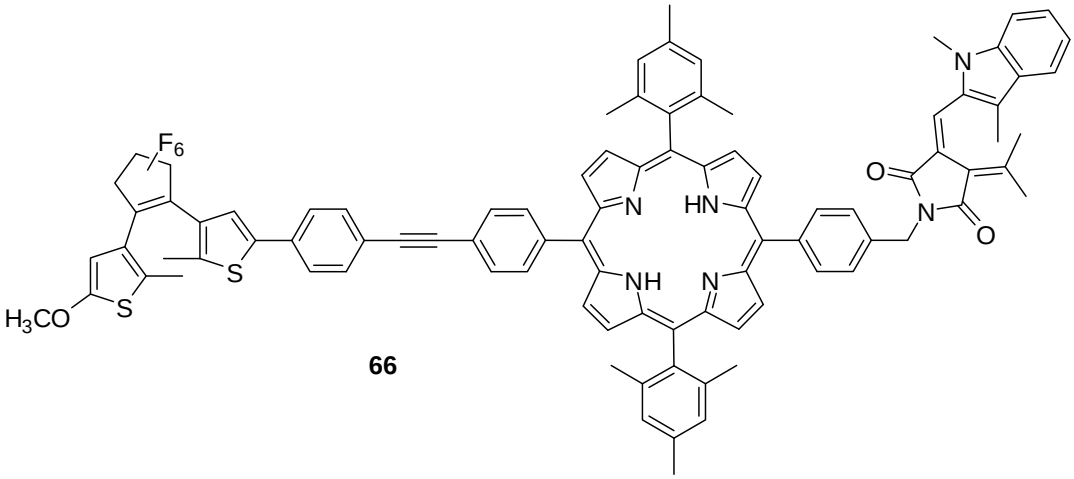
63: R = Ph
64: R = Cl

Irradiation of the adducts **63** and **64** with UV light resulted in the appearance of a new absorption band at 634 nm and 567 nm, respectively, and the photochromic response was reversed upon irradiation with visible (>500 nm) light. For compound **63**, the modulation of the fluorescence was about 98%, indicating that at the photostationary state (at 365 nm) an essentially complete conversion of the open to closed form was achieved. The fluorescence quantum yields for **63** and **64** were found to be 0.28 and 0.09, respectively.

In the group of Gust the fluorescent dithienylethene–porphyrin dyad **65** and the fulgimide–porphyrin–dithienylethene triad **66** were synthesized and studied.[53]



65



66

The absorption spectrum of a model dyad **65** showed porphyrin Q-bands in the region of 500–600 nm and a Soret band at 417 nm. Irradiation with UV light provided the almost non-fluorescent closed-ring isomer with broad absorption at 600 nm. Visible irradiation of the closed form returned the compound **65** to its open form, which exhibited typical porphyrin fluorescence at 650 nm and 717 nm (excitation at 470 nm). Modulation of the fluorescence was found to be around 80%. In the case of triad **66**, transformation between the different forms of both photochromic fragments proceeded at different wavelengths. It was used for design of logic gates and, in particular, for an all-photonic molecular keypad lock. The compound **66** had the strongest and the weakest fluorescence signal at 650 nm (excited at 470 nm), when only fulgimide and diarylethene fragments were in the closed form, respectively.

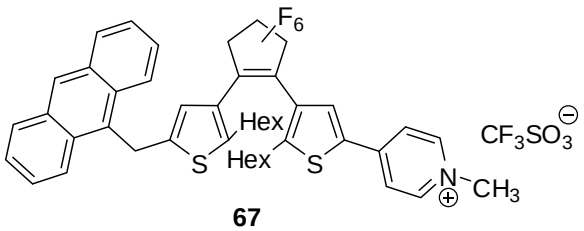
4.2. Fluorescence modulation achieved by pcFRET

Fluorescence modulation can also be achieved, if a fluorescent group is attached to a diarylethene derivative through a saturated spacer. In this case, quenching of the fluorescence is observed, when the absorption band in one of the two states of the photochromic fragment is significantly overlapping with an emission band of the fluorophore. The absence of the fluorescence is explained by the intramolecular photochromic fluorescence resonance transfer of energy (pcFRET).

The efficiency of the energy transfer depends on the distance between the photochromic and fluorescent parts and the Förster radius (critical transfer distance R_o). The latter is proportional to the degree of the overlapping of the absorption band of the photochromic unit and the emission band of the fluorescent dye.[3]

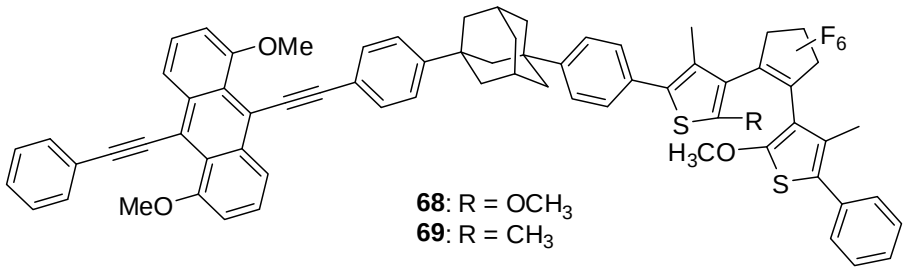
Not very many compounds of this type are known. This can be explained by synthetic difficulties, because in most cases preparations of these non-symmetric compounds are multistep and tedious.

In 2000 Endtner *et al.* designed and synthesized the first fluorescent photochromic compound, in which diarylethene and antracene moieties were connected through a methylene group.[54]



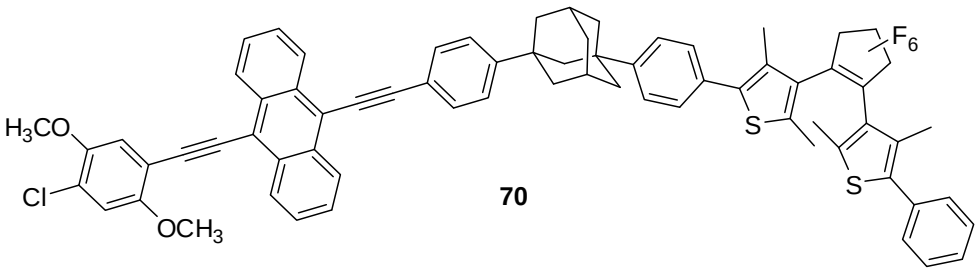
67

The open-ring isomer of **67** showed a fluorescence band with a vibrational fine structure characteristic for anthracene. The fluorescence signal of the closed-ring isomer was fully quenched. In the photostationary state upon irradiation at 355 nm, the conversion of the compound **67** was found to be 87%. No fluorescence quantum yield has been reported for this substance.



68: R = OCH_3
69: R = CH_3

In 2002 and 2004 Irie *et al.* designed and synthesized fluorescent photoswitching molecules in which two different photochromic diarylethenes and highly fluorescent 2,5-dimethoxybis(9,10-phenylethynyl)anthracene were linked through an 1,3-adamantanediyl bridge. Their photochromic performance in solution as well as in polymer films under single-molecule conditions have been studied in detail.[55] The compound **68** with two methoxy groups in the diarylethene fragment had one of the lowest photocycloreversion quantum yields ever reported ($<10^{-4}$ in toluene at 630 nm), while the non-symmetric assembly **69** had a higher value (1.5×10^{-3} at 600 nm). The fluorescence quantum yield in the open-ring isomer of the adduct **68** was shown to be 0.73, while the closed form was found to be almost non-fluorescent (Φ_{fl} <0.001), and the calculated energy-transfer efficiency was close to 99.9%. However, no quantitative data for the conversion and the residual fluorescence in solution have been published.

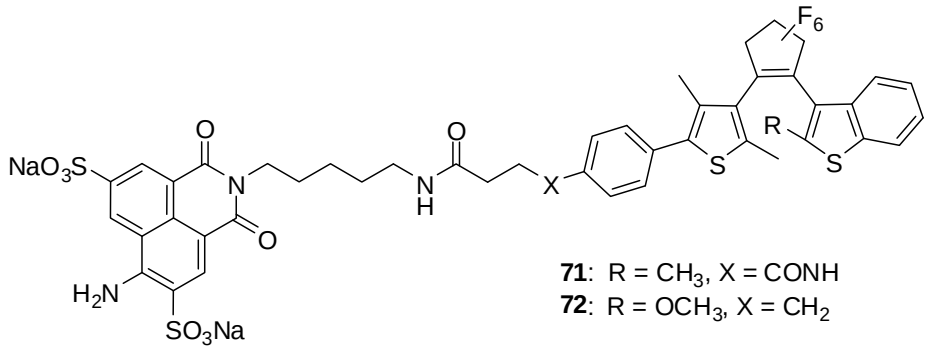


70

In 2002 Kawai *et al.* reported the synthesis of the diarylethene linked with a 9,10-bis(phenylethynyl)antracene unit connected through a rigid adamantyl spacer (compound **70**).[39b] Characteristic fluorescent bands of bis(phenylethynyl)antracene unit in this compound were found to be at 484 nm and 515 nm, and the fluorescence quantum yield was as high as 84%. The closed-ring isomer showed almost no fluorescence, which indicated efficient fluorescence

quenching by the photochromic unit. However, low conversion (about 58%) between the open and closed forms of **70** in photostationary state resulted in the high residual fluorescence.

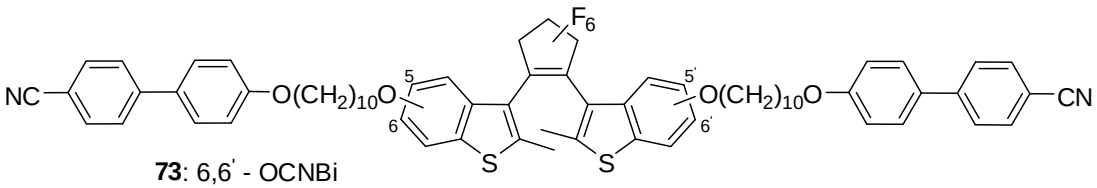
Giordano *et al.* in 2002 reported an assembly in which a relatively long and flexible cadaverine linker acylated with succinic or butanoic acid residues connected the Lucifer Yellow fluorophore with different diarylperfluorocyclopentene acceptors.[2a]



71: R = CH_3, X = CONH
72: R = OCH_3, X = CH_2

When the diarylethene was in the open form, the fluorescence was not quenched by the diarylethene unit. In the photostationary state, the decrease in fluorescence for compounds **71** and **72** was found to be 65 and 84% in methanol upon irradiation at 313 and 320 nm, respectively. Interestingly, the photochromic units alone, without the fluorophore, were converted to the closed forms nearly quantitatively by irradiation even at the band edge (340 nm; 92 and 97% conversion in PSS for 2-methyl- and 2-methoxybenzo[*b*]thiophene derivatives, respectively). The researchers did not report the fluorescence quantum yields, but we can assume, that for the connected fluorophore, the fluorescence quantum yield was not better than for Lucifer Yellow (Φ_{fl} = 0.21 in water).

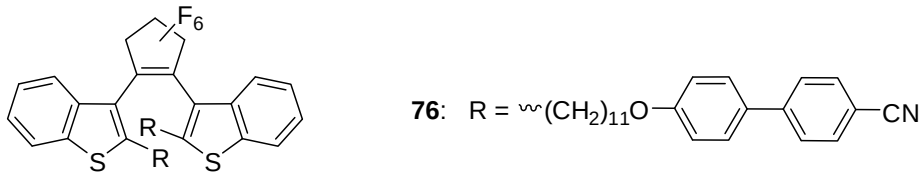
In 2004 Frigoli and Mehl reported a fluorescent photochromic system based on a 1,2-bis(2-methylbenzo[*b*]thiophen-3-yl)perfluorocyclopentene group, connected by decyl spacers with two cyanobiphenyl (CNBi) groups.[56]



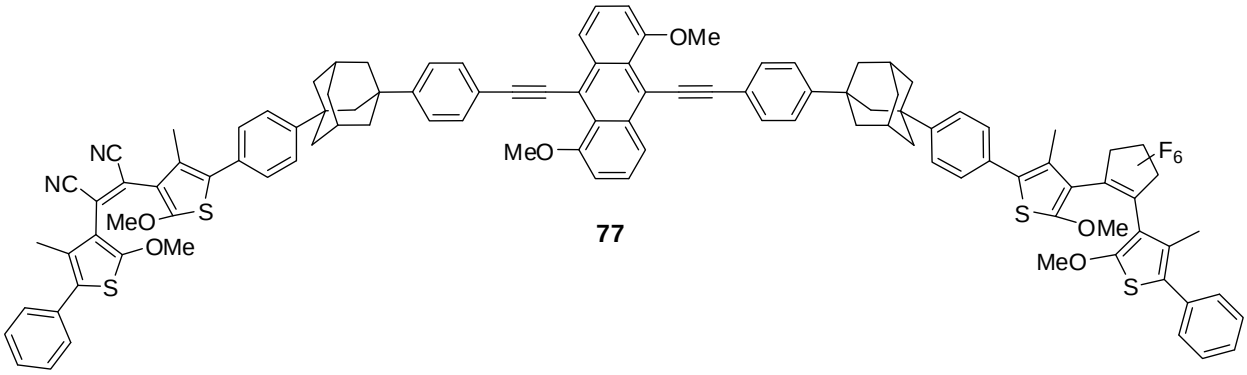
73: 6,6' - OCNBi
74: 5,6' - OCNBi
75: 5,5' - OCNBi

After irradiation with UV light, the compounds became red (**73**), red-purple (**74**) and purple (**75**). The cycloreversion quantum yields were reported to be high (0.16–0.41 at 546 nm light). The quenching of the fluorescence in PSS in cyclohexane was found to be 36, 26 and 23%

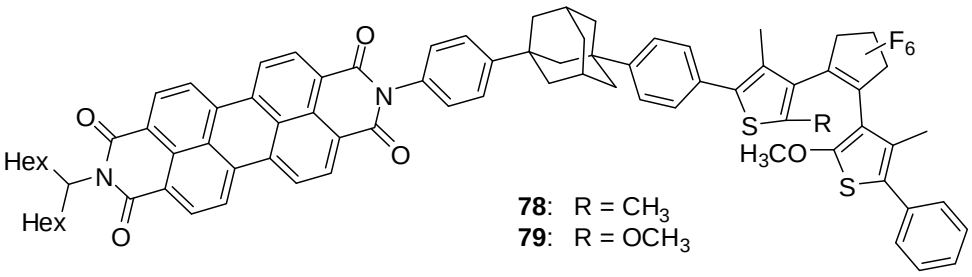
for compounds **73**, **74** and **75**, respectively. Even though compounds **73**–**75** have two strongly fluorescent cyanobiphenyl groups (Φ_{fl} = 0.74), after coupling with the photochromic unit, the observed fluorescence quantum yields were generally low (~1–2%).



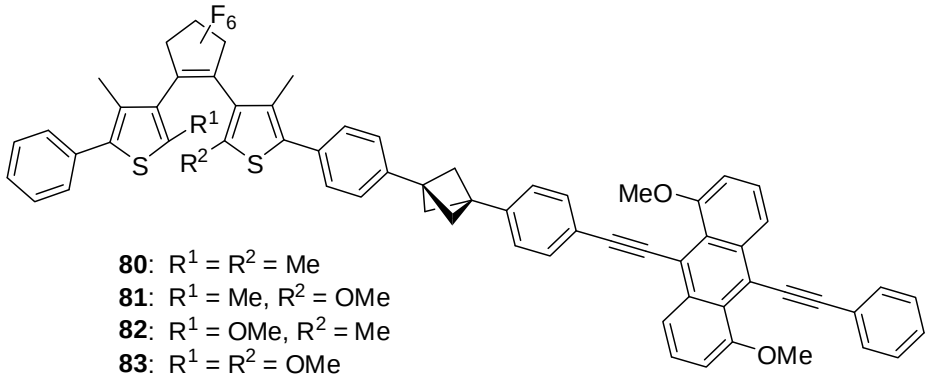
The same authors also obtained diarylethene derivative **76** with cyanobiphenyl groups in 2,2'-positions attached to the photochromic part via a long alkyl spacer.[57] Upon excitation at 313 nm, the fluorescence quantum yield for compound **76** was found to be 0.13 for the open-ring isomer. A spacer with eleven CH_2-groups considerably lowered the FRET efficiency, and the fluorescence modulation was found to be only 5%, though the conversion in the PSS was found to be high (62%).



A short report of Irie's group published in 2005 dealt with an adduct of the dicyano-type diarylethene unit, perfluorocyclopentene-type diarylethene unit and fluorescent bis(phenylethynyl)anthracene unit, which were covalently linked through two rigid adamantyl spacers (compound **77**).[58] Two photochromic residues underwent the photocyclization at different wavelengths, because the absorption band of dicyano-type diarylethene expanded over 400 nm, and for the perfluorocyclopentene-type diarylethene was below 400 nm. The researchers concluded that the fluorescence properties of this molecule may be used for the construction of the molecular logic gate (at the single molecule level). Unfortunately, no quantative data for the fluorescence modulation and quantum yield of **77** have been reported.

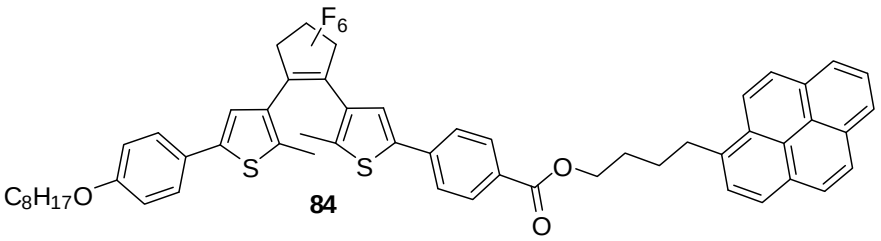


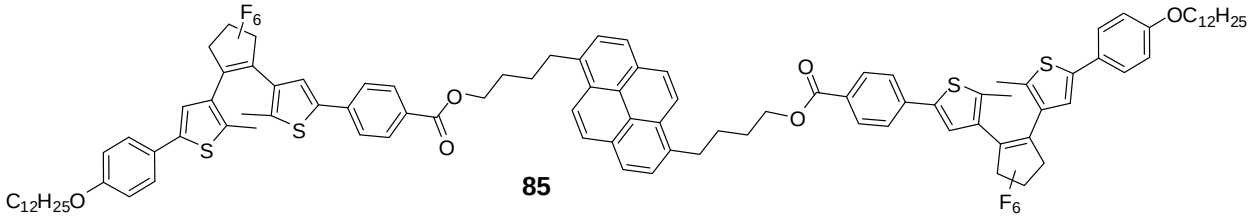
Further development of these systems has been reported later:[59] the more stable perylenebisimide unit was used instead of bis(9,10-phenylethynyl)antracene. For each of the compounds **78** and **79** in the open forms, two main fluorescence peaks were found at 489 nm and 525 nm (excitation at 313 nm), respectively, and fluorescence quantum yields were close to 0.98 in dichloromethane. The fluorescence modulation for compound **79** was as high as 96%. The absorption bands of the open-ring isomers of **78** and **79** were found at 495 nm and 525 nm, and, after irradiation with UV light, new bands of the closed forms at 640 nm appeared. The authors showed that compounds **78** and **79** had similar cyclization quantum yields, but the cycloreversion quantum yields were markedly different. The cycloreversion quantum yield of closed-ring isomer of **79** was 1% of that of **78**. This decrease was attributed to the presence of methoxy substituents at the reactive carbons.[60] The photochromic reactions of **78** and **79** were also studied at the single-molecule level in various polymer matrices.



In 2007 de Meijere *et al.* reported various dithienylethene derivatives connected with 1,5-dimethoxy-9,10-di(phenylethynyl)antracene through the short and rigid 1,3-bicyclo[1.1.1]pentanediyl linker.[61] The bicyclo[1.1.1]pentane fragment provided the shortest possible distance between the energy donor and acceptor groups and an angle of 180° favorable for the high RET efficiency. The photoconversion of compounds **81**–**83** with one or two methoxy groups at 2,2'-positions was found to be close to 100% (upon irradiation with 313 nm light), which resulted in an extremely high fluorescence modulation in acetonitrile solution.

Unfortunately, the fatigue resistance of the photochromic unit was shown to decrease from compound **80** to **83**.



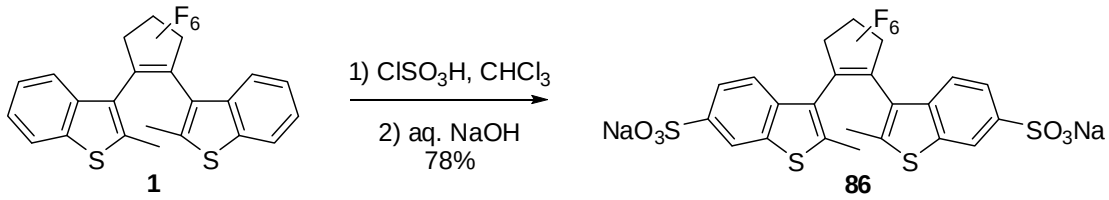


Recently, the group of Irie designed and synthesized the diarylethene-pyrene diad **84** and diarylethene-pyrene-diarylethene triad **85** with a long linker between the photochromic part and the fluorescent pyrene. The goal was to investigate the photoinduced two-dimensional ordering change at the interface between a solution and a highly ordered phase on pyrolytic graphite.[62] Upon irradiation with UV light (λ >313 nm), the colorless ethyl acetate solution turned blue, and irradiation with visible light (λ >480 nm) restored the initial state. The conversion from the open-ring isomer with an absorption maximum at 276 nm to the closed-ring isomer with an absorption maximum at 606 nm was 97% for diarylethene **84** (at 313 nm). For the dimer **85**, the contents of the open-open, the open-closed, and the closed-closed isomers in the photostationary state under irradiation with 313 nm light were 0.1, 8.2 and 91.7, respectively. Unfortunately, no data on fluorescent properties were reported in this publication.

5. Photochromic diarylperfluorocyclopentenes with improved solubility in aqueous media

Water solubility of the molecular probes is very important for their applications in life sciences. Even a low content of other solvents in water or aqueous buffers may cause protein denaturation or cell death. Probably, the problems associated with bad performance in water or aqueous buffers may be solved by the synthesis of hydrophilic photochromic diarylethenes.

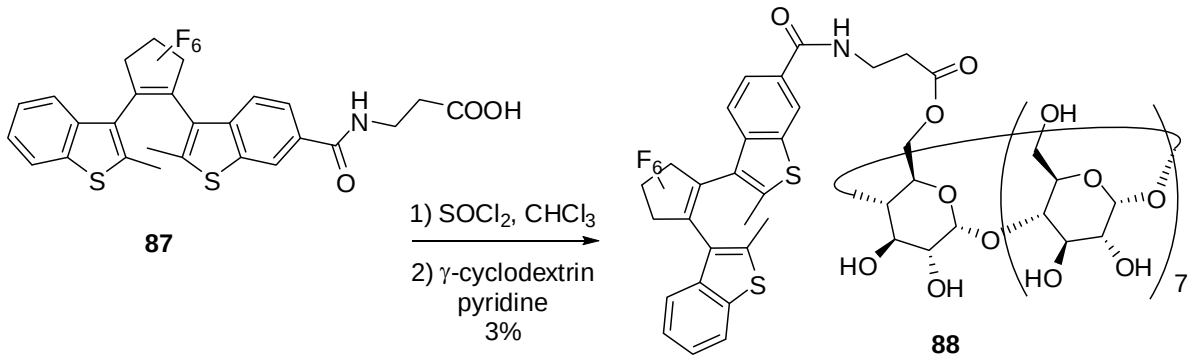
Already in 1997, Irie's group reported the preparation of the water-soluble 1,2-bis(2-methyl-1-benzo[*b*]thiophene-3-yl)perfluorocyclopentenes with two sulfonic acid groups attached to the benzene rings.[63] Compound **86** was synthesized by treatment of the diarylethene **1** with chlorosulfonic acid and subsequent hydrolysis with 1% aqueous NaOH (Scheme 5.1).



Scheme 5.1. Synthesis of water-soluble perfluorodiarylethene **86**.

In aqueous solution compound **86** showed photochromic activity: after irradiation at 313 nm the initial colorless solution turned red, and new absorption bands appeared at 358 and 529 nm; the reverse reaction was driven with visible light (>480 nm). The goal of this work was to analyze the changes in properties by placing the molecule into cyclodextrins. Cyclodextrins have relatively non-polar cavities of different sizes (depending on the number of glucopyranose units) and polar outer shells, and therefore they can enclose various types of organic molecules and provide them with hydrophilic properties. The two conformations of the open form of the photochromic diarylethenes (parallel and antiparallel) differ in their structures and the ability to be incorporated in such cavities. After addition of an excess of γ- or *β*-cyclodextrins, the cyclization quantum yield of compound **86** in aqueous solution increased from 0.32 to 0.45 and 0.49, respectively. NMR measurements indicated that in the case of *β*-cyclodextrin 97% of **86** was in the antiparallel conformation, and without *β*-cyclodextrin only 64%. These results demonstrated that the increase in the quantum yield was due to the increase in the population of the antiparallel conformer (conformational restriction) in cyclodextrin cavities.

Later it was reported that the formation of a self-inclusion complex for the 1,2-bis(1-benzo[*b*]thiophen-3-yl)perfluorocyclopentene **88** modified with γ-cyclodextrin depends on the solvent nature.[64]

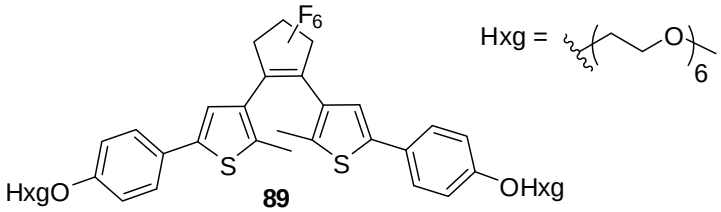


Scheme 5.2. Synthesis of the water-soluble complex **88**.

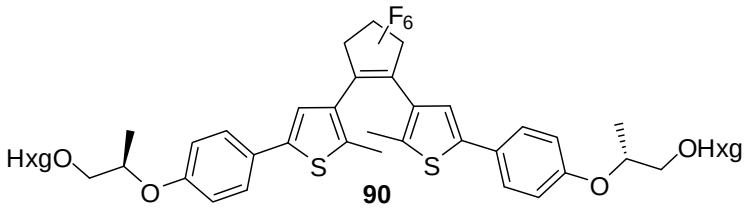
NMR measurements confirmed that the self-inclusion takes place in an aqueous solution, while in methanol the diarylethene residue of **88** is outside the cavity. Compound **88** was

obtained from the acid **87** with γ-cyclodextrin in anhydrous pyridine in low yield (Scheme 5.2). In aqueous solution its photochromic properties were similar to those of compound **86** mentioned above.

A series of reports described photochromic diarylethenes with amphiphilic side chains. In the first study,[65] diarylethenes with hexa(ethyleneglycol) units have been prepared and their photochromic properties and self-assembling behavior were studied.



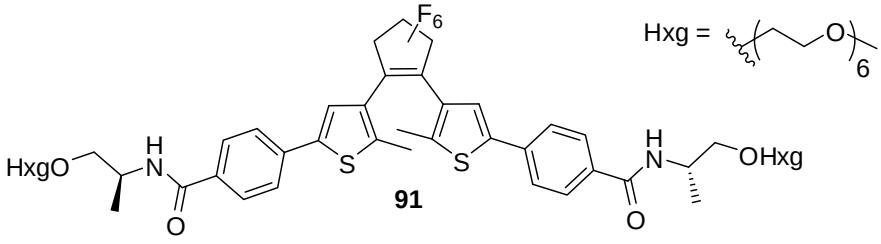
89



90

Compounds **89** and **90** showed excellent photochromic performance in organic solvents and even in aqueous media, in which the photoconversions under irradiation with 313 nm light reached 91 and 86%, respectively. The absorption maxima of the colorless open-ring isomers in aqueous solution were observed at 289 and 294 nm for compounds **89** and **90**, and the absorption maxima of the closed-ring isomers were found to be at 573 and 583 nm, respectively. However, due to hydrophobic interactions, these compounds were found to be self-associated (nanodomains of around 100 nm in size in water at room temperature), and their solution became cloudy upon heating. In general, self-association is undesirable for fluorescent probes, because the fluorescence of the aggregates may be quenched.

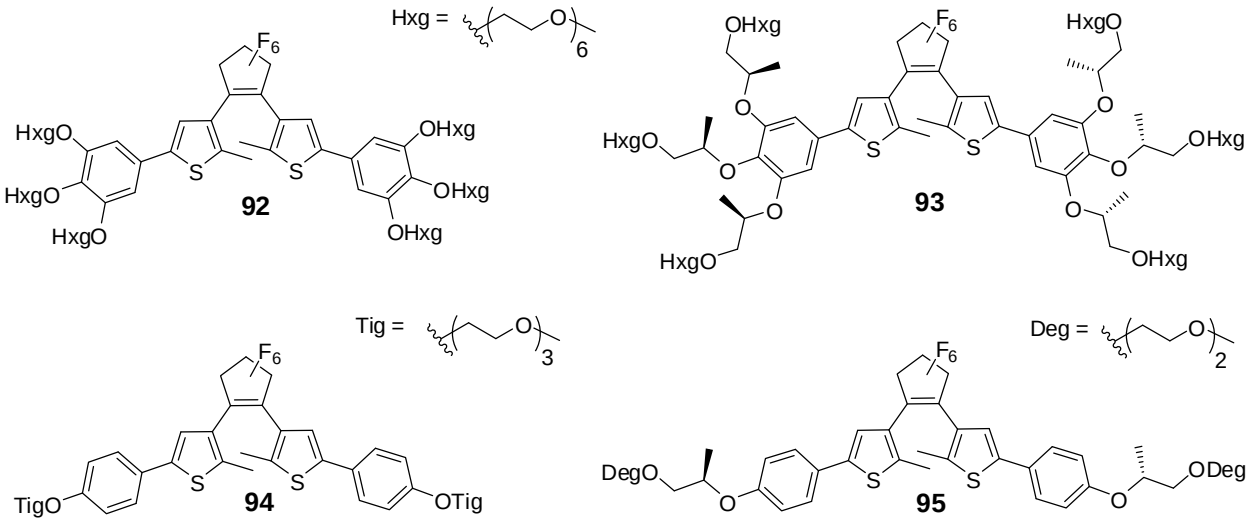
In 2008 the same group reported a similar hexa(ethyleneglycol)diarylethene hybrid structure with an amide group nearby the aromatic core.[66] The colorless open form of **91** absorbed around 310 nm, and the absorption maximum of the closed-ring isomer was located at 598 nm in water. Similar self-assembled chiral structures have been observed.



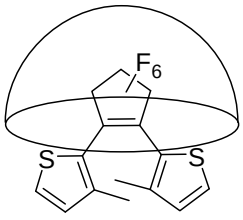
91

Owing to the intermolecular hydrogen bonds of amide groups, the amphiphilic molecules are densely packed in the case of the closed-ring isomer in water. As a result, the cloud-point temperature for compound **91** in the closed form decreased to 22 °C, while for the open form it was found to be 45 °C.

Recently, a number of diarylethene derivatives **92**–**95**, which differed in the lengths and number of oligo(ethyleneglycol) side chains have been prepared.[67] The size of the aggregates and the self-assembling behavior depended on the proportions of the amphiphilic side chain and the hydrophobic core moiety in the whole structure. Compounds **92** and **93** with six hexa(ethyleneglycol) side chains and enhanced solubility were shown to give molecularly dispersed solutions in water, while the solubility of compounds **94** and **95** (with shorter side chains) in water was very poor.



Quite recently, researchers reported the diarylethene derivative encapsulated into water-soluble nano-cavitand and its photochromic behavior in aqueous solution.[68]



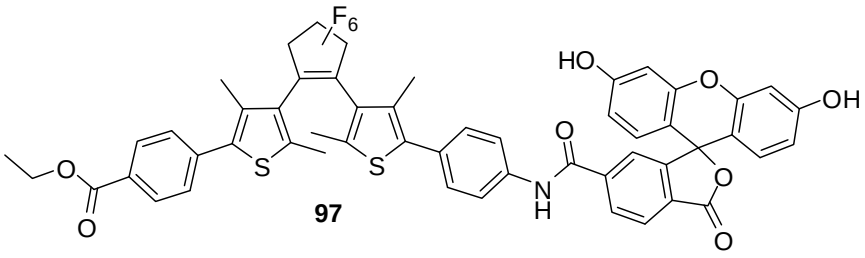
96

They used the smallest diarylethene, 1,2-bis(3-methyl-2-thienyl)perfluorocyclopenthene **96**, and the nano-cavitand with an external coat of carboxylic acid groups and an internal hydrophobic pocket with a volume, large enough to encapsulate this diarylethene derivative. The NMR analysis revealed that 1:1 complex was formed, and perfluorocyclopentene moiety was

included into the hydrophobic empty pore of the cavitand. Upon irradiation with UV and visible light, reversible photochromic reactions of **96** were observed in a sodium borate buffer solution. Unfortunately, the authors did not discuss the possibility of using the larger diarylethene derivatives.

However, all these symmetric photochromic compounds lack an additional functional group, which is necessary for attaching them to fluorescent parts. Moreover, their absorbance at 375 nm (optimized for UV lasers and high aperture optics) is too low, and they cannot be used for effective switching.

In 2007 the group of Irie attempted to use the new perfluorocyclopentene derivative as a fluorescence switching agent for labelling of proteins. They synthesized a fluorescent diarylethene **97** with fluoresceine as a fluorescent dye.[69]



97

Reversible fluorescence switching was observed along with the photochromic reaction. Irradiation at 365 nm produced the closed-ring isomer with absorption at 580 nm, and the fluorescence intensity decreased. After irradiation with visible light (λ >550 nm), both absorption and fluorescence spectra returned to their original state. Modulation of the fluorescence was found to be 60% due to the moderate conversion between the open and closed forms in PSS. The fluorescence quantum yields in ethanol before and after UV light irradiation were 0.71 and 0.14 (PSS), respectively. After deprotection of the carboxy group of **97**, the fluorescent photochromic compound was decorated with a succinimidyl ester group and attached to a protein. However, the absorption and fluorescence spectral changes of the labelled protein were reported for the solution in PBS buffer with 30% of EtOH; such conditions are inappropriate for life science applications.

Chapter 2. Synthesis, properties and microscopic applications of photochromic and photochromic fluorescent compounds

1. Synthesis of the new photochromic and fluorescent photochromic 1,2-bis(3-thienyl)perfluorocyclopentenes

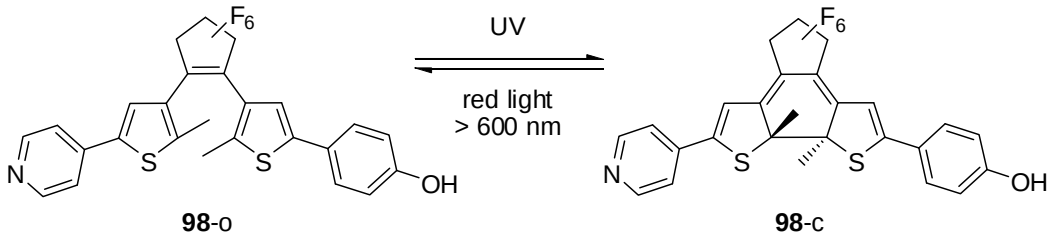
A need for compounds, which may be useful for optical nanoscopy, demands new photochromic systems with considerable absorption above 360 nm, a wavelength region, for which aberration-free lenses with high numerical apertures (1.35–1.45) are available.

Novel photochromic fluorescent diarylethenes that may improve the optical resolution with low light intensities have recently been developed in our group.[61,70]

Here we describe in detail the design and synthesis of interesting model compounds, which led to the construction of the optimized photochromic units.

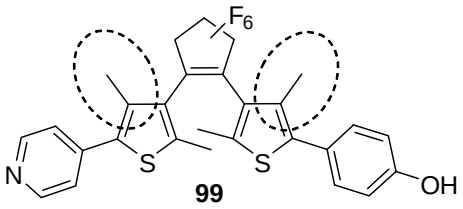
1.1. Model compounds

As a starting point, we used compound **98** described by the group of J.-M. Lehn (Scheme 1.1).[25]



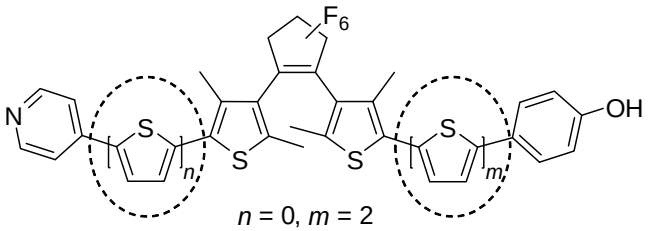
Scheme 1.1. Structure and main absorption bands (in benzene) of Lehn's photochromic compound.

It contains a 1,2-bis(2-methyl-3-thienyl)perfluorocyclopentene core with a 4-hydroxyphenyl group and a 4-pyridyl substituent at the 5- and 5'-positions of the thiophene rings, respectively. In benzene, its open and closed forms absorb at 296 nm and 602 nm, respectively. The conversion to the closed form in the photostationary state (α_{PS}) was reported to be larger than 98%. We decided to block each of the unsubstituted positions in the thiophene rings with a methyl group in order to increase the photochemical stability of this system.[71]



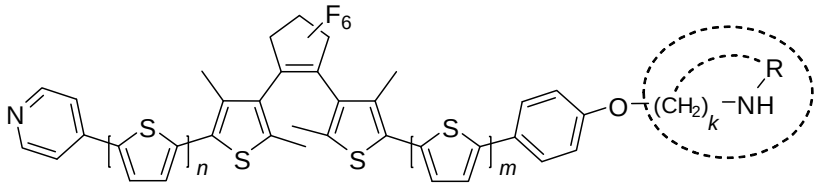
99

We added two additional thiophene rings to the conjugated system of the photochromic compound **99** in a symmetric and in a non-symmetric fashion to shift the positions of absorption bands of the open- and closed-ring isomers to the red spectral region.



n = 0, *m* = 2
n = 1, *m* = 1

We also constructed several saturated (non-conjugated) linkers, in order to regulate the fluorescence modulation by the FRET mechanism. A linker could either consist of a linear (flexible) chain, or a cyclic (rigid) system, and it should also contain an amino group, as an anchoring site for carboxylic acid residues in fluorescent dyes.



n = 0, *m* = 2
n = 1, *m* = 1

k = 0, 1, 2, 3
R = alkyl

The general structure of the adducts of the photochromic 1,2-bis(3-thienyl)perfluorocyclopentenes with fluorescent dyes (rhodamines) is represented in Figure 1.1. The choice of an appropriate rhodamine as a fluorophore is described in Section 1.3.

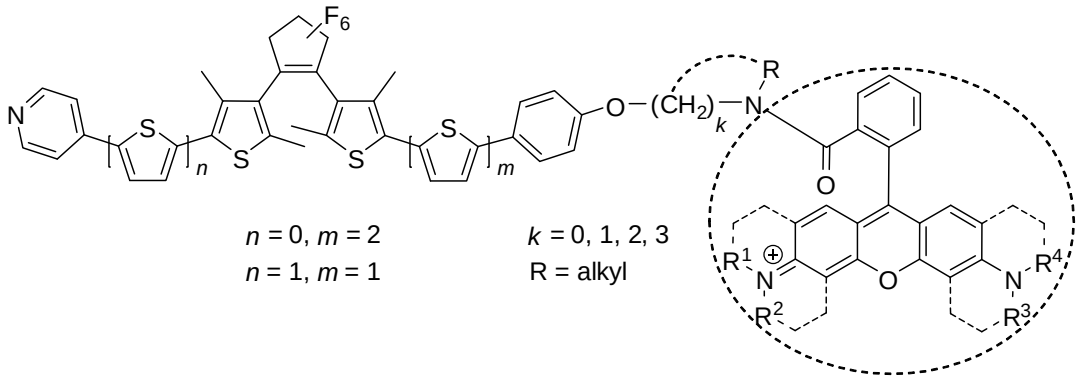


Figure 1.1. Photochromic unit and its connection with a rhodaminic fluorescent dye.

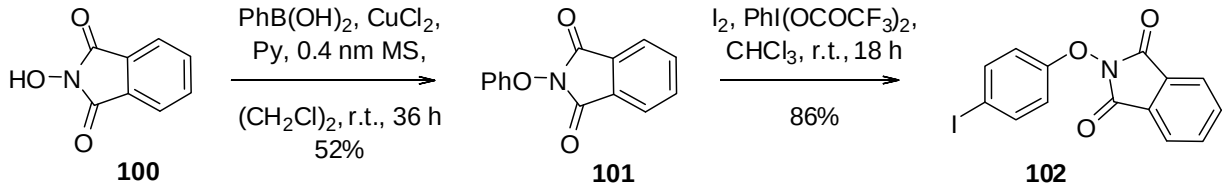
1.2. Search for an optimal linker to attach the photochromic part to a fluorescent dye

As was already mentioned above, an effective resonance energy transfer from the donor to the acceptor is possible, when the distance between the fluorophore and the photochromic unit is less than the Förster radius and there is no electronic conjugation between them.

1.2.1. One single bond as a "zero" linker between the oxygen and nitrogen atoms (*k* = 0)

We have chosen *N*-hydroxyphthalimide (**100**) as a precursor for the shortest ("zero") linker with $k = 0$. If the amino group in the fragment N–O is protected with the phthalimide residue, the hydroxy group can easily be derivatized.

Since the *O*-substituted phenoxy group can be introduced into the photochromic molecule via the corresponding iodide, we synthesized *N*-(4-iodophenoxy)phthalimide (**102**) (Scheme 1.2).

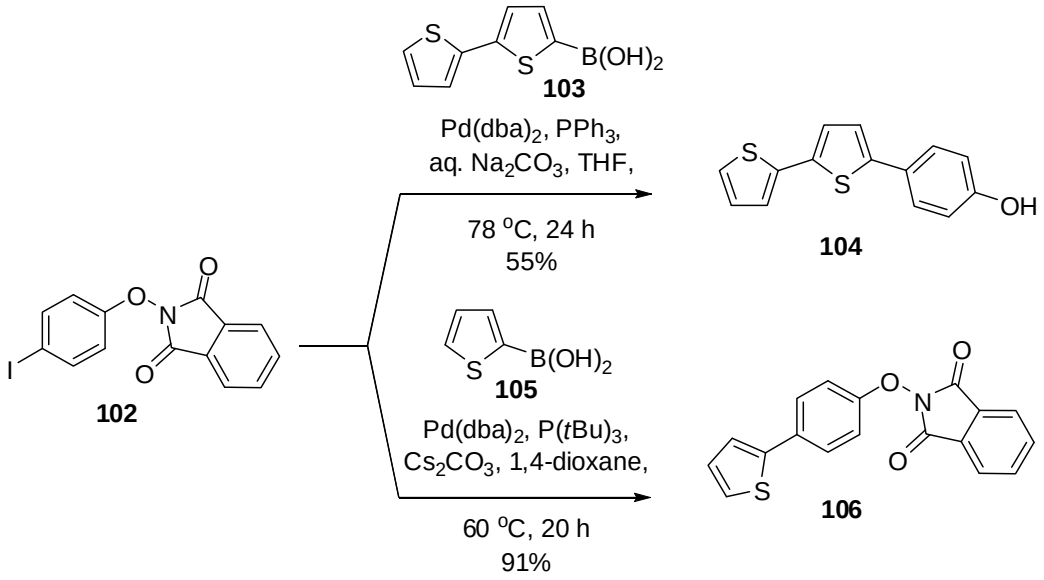


Scheme 1.2. Synthesis of *N*-(4-iodophenoxy)phthalimide (**102**).

The intermediate phenoxyphthalimide (**101**) was prepared from *N*-hydroxyphthalimide (**100**) as described in the literature.[72] Low conversions of *N*-phenoxyphthalimide (**101**) in the reactions with iodine and periodic acid in ethanol at 60 °C or in acetic acid under reflux were observed. Heating of **101** at 60 °C with ICl in acetic acid left the initial substance intact. Eventually, the reaction with iodine and $PhI(OCOCF_3)_2$ in chloroform at room temperature for 18 h gave the desired derivative **102** in good yield.

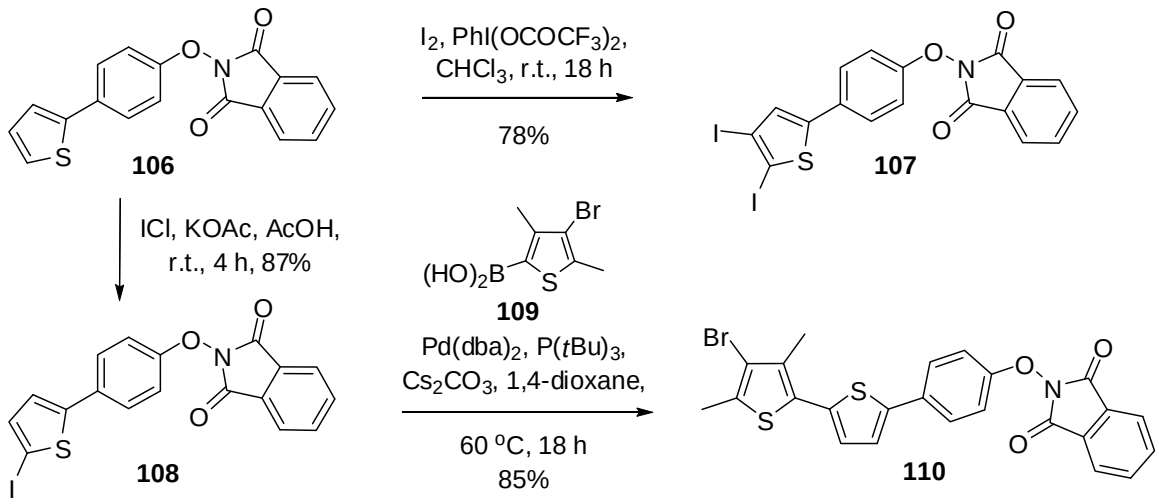
Then the aryl iodide **102** was cross coupled with the thiopheneboronic acids **103** and **105** under catalysis of $Pd(dba)_2$ (Scheme 1.3).[73] Under the standard conditions according to Suzuki

(20% aq. Na_2CO_3, THF, $Pd(dba)_2$, PPh_3, 78 °C, 24 h), the O–N bond was cleaved, and only 4-([2,2']bithiophen-5-yl)phenol (**104**) was isolated. Luckily, under anhydrous conditions with solid Cs_2CO_3 as a base and P(*t*Bu)$_3$ as a ligand[74] *N*-[4-(thiophen-2-yl)phenoxy]phtalimide (**106**) was obtained in 91% yield.



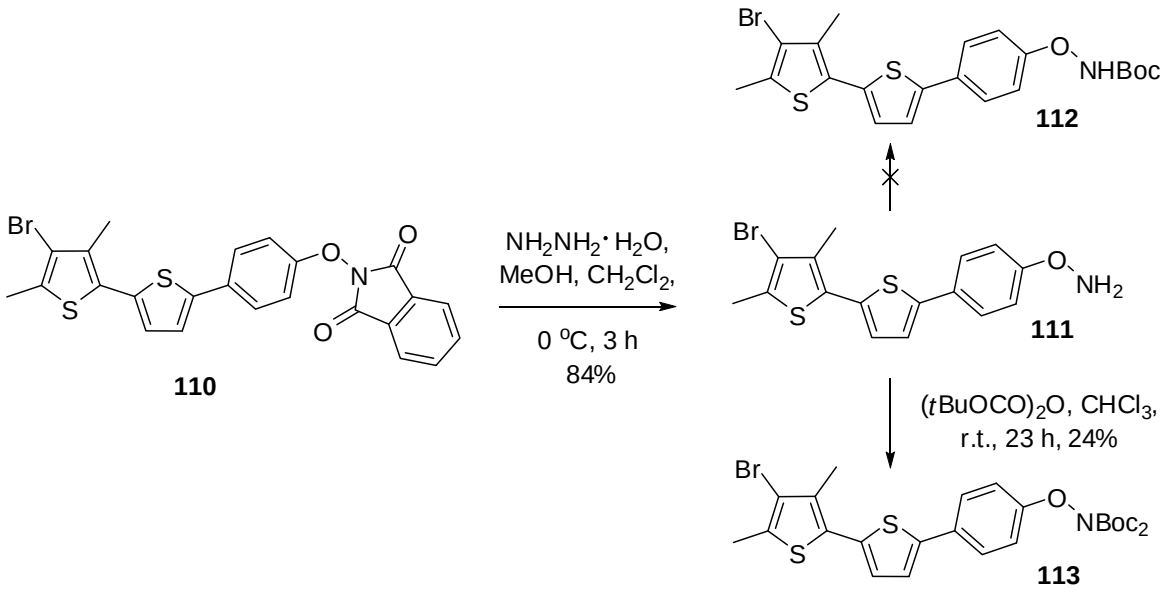
Scheme 1.3. Suzuki couplings of *N*-(4-iodophenoxy)phthalimide (**102**).

To avoid the formation of diiodothiophene **107**, the exact molar ratio of the iodine source and the substrate should be controlled during the iodination of compound **106**. The Suzuki coupling of the monoiodide **108** with 4-bromo-3,5-dimethylthiophene-2-boronic acid (**109**) under the anhydrous conditions mentioned above gave 85% of the "right side" precursor **110** with the protected amino group (Scheme 1.4).



Scheme 1.4. Synthesis of the building block **110**.

Since the phthalimide protective group turned out to be unstable under the conditions of the bromine-lithium exchange, we removed this group in compound **110** with hydrazine.[72a] The aryloxyamine **111** was isolated in 84% yield (Scheme 1.5). This compound had to be kept under an inert atmosphere, because it readily oxidized in air.

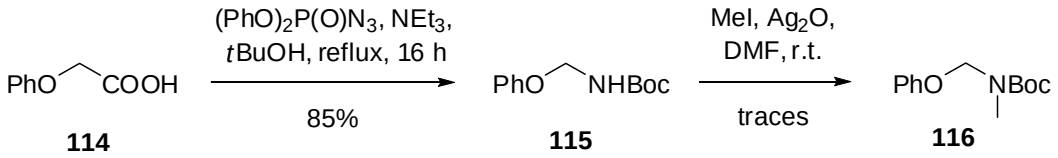


Scheme 1.5. An attempt to synthesize the mono-Boc-protected compound **112**.

Unfortunately, all attempts to obtain the mono-Boc-protected derivative **112** failed, and only the diprotected amine **113** was isolated in low yield. Therefore, this synthetic route was not pursued any further.

1.2.2. One-carbon linker between the oxygen and nitrogen atoms (*k* = 1)

To obtain the compound with two single bonds between the O- and N-atoms, we used phenoxyacetic acid (**114**) as a starting material (Scheme 1.6).



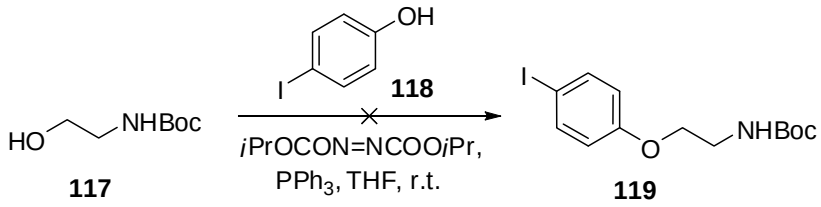
Scheme 1.6. Linker with two single bonds between the O- and N-atoms.

The reaction of compound **114** with *O*,*O*-diphenylphosphorylazide in *t*BuOH in the presence of NEt_3 gave the corresponding acyl azide, which, after elimination of nitrogen and Curtius rearrangement to the isocyanate, reacted with *tert*-butyl alcohol to furnish *tert*-butyl *N*-phenoxymethyl carbamate (**115**), which was isolated in 85% yield and subjected to methylation. Unfortunately, under standard conditions with methyl iodide and silver oxide in DMF at room

temperature,[75] we failed to obtain the desired compound **116**, and therefore we did not further pursue this synthetic route either.

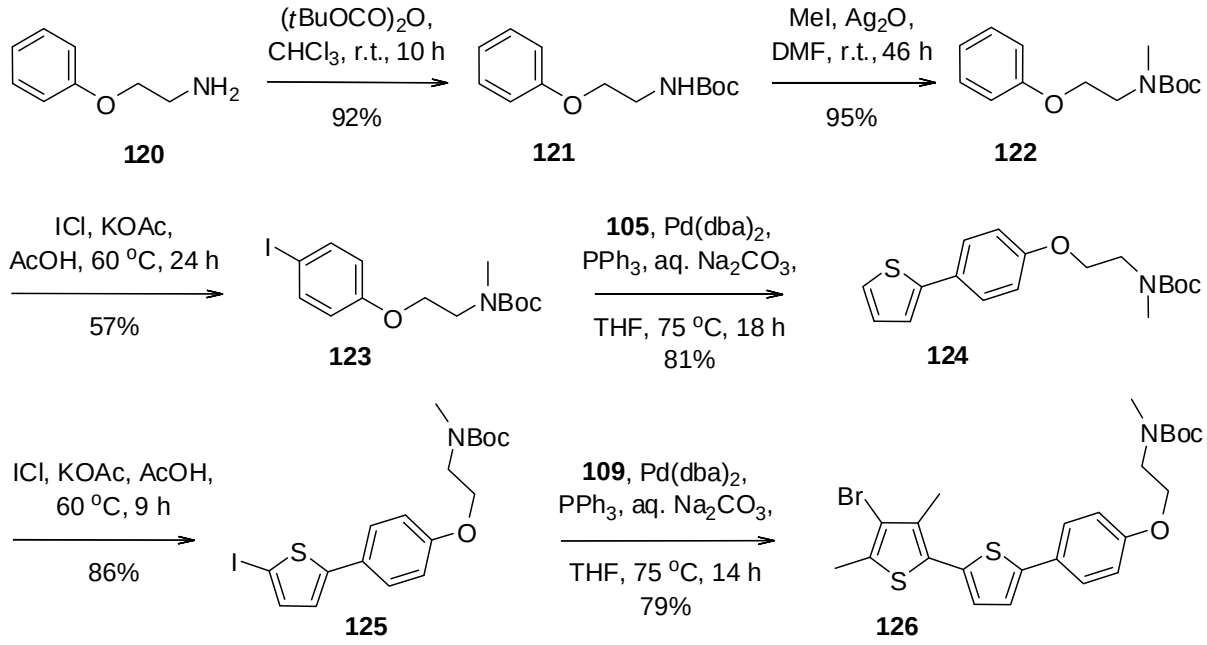
1.2.3. Two-carbon linker between the oxygen and nitrogen atoms (*k* = 2)

First we decided to prepare a compound with two carbon atoms between the phenoxy and the amino groups – *tert*-butyl 2-(4-iodophenoxy)ethyl carbamate (**119**) – by a Mitsunobu reaction[76] of *tert*-butyl *N*-(2-hydroxyethyl)carbamate (**117**) with 4-iodophenol (**118**). However, the expected product was not formed.



Scheme 1.7. An attempted Mitsunobu reaction towards compound **119**.

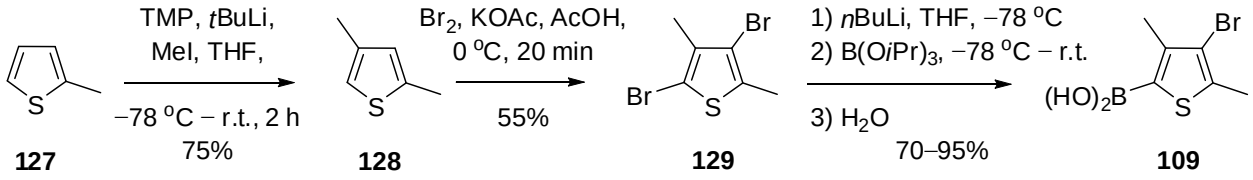
Probably, as in the case of *N*-Boc- and *N*-Z-serine derivatives, elimination of water and the formation of the corresponding enamide occurred instead of nucleophilic substitution.[77] Therefore, we decided to start the synthesis of the required photochromic compound from the commercially available amine **120** (Scheme 1.8).



Scheme 1.8. Synthesis of the "right hand" precursor **126** required for the construction of the photochromic compound.

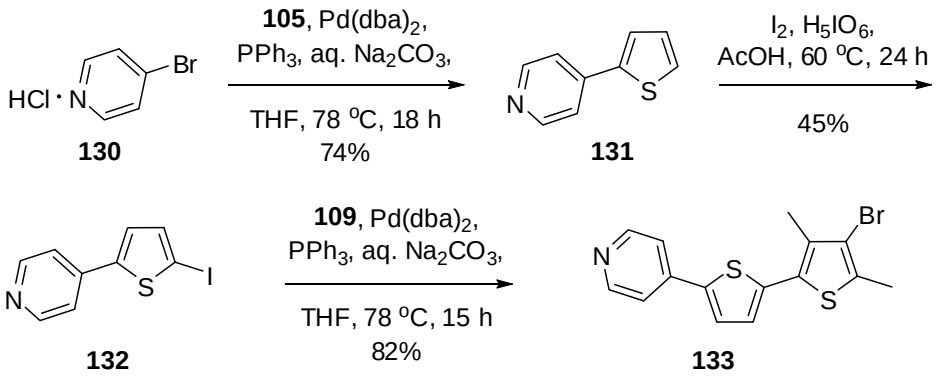
After protection of the amino group in compound **120** by the reaction with *tert*-butyl pyrocarbonate, the carbamate **121** was isolated by distillation in vacuo in high yield. The *N*-methyl group was introduced using the method mentioned above,[75] and *tert*-butyl *N*-(2-phenoxyethyl)-*N*-methylcarbamate (**122**) was obtained in 95% yield. Alkylation of the amino group not only removed the acidic proton, which could complicate further reactions with lithium-organic compounds, but it could also be used for the introduction of a fragment with a new (protected) reactive group for attaching the whole photochromic or even fluorescent photochromic assembly to an object of interest. Heating of compound **122** in an ethanolic solution with iodine and periodic acid led only to low conversion of the substrate (<50%). However, the treatment with ICl and potassium acetate in acetic acid at 60 °C gave the desired derivative **123** in good yield (57%). In the ^{13}C NMR spectrum, a doubling of the signals of the CH_2O and *N*-methyl groups (but not of the CH_2N group) was observed. It must be due to a slow (on the NMR time scale) rotation around the formally single OC(O)-N bond. The Suzuki coupling of **123** with thiophene-2-boronic acid (**105**) proceeded with high yield (91%). Compound **124** was iodinated with ICl in the 5-position of the thiophene ring, and the iodide **125** was isolated in 86% yield. Using the moist, freshly prepared 4-bromo-3,5-dimethylthiophene-2-boronic acid (**109**) in the next step, we managed to increase the yield of the "right hand" precursor **126** of the photochromic unit to 79%.

The boronic acid **109** was synthesized from 2-methylthiophene (**127**) (Scheme 1.9). The latter was selectively methylated in the 4-position of thiophene ring,[78] the resulting 2,4-dimethylthiophene (**128**) was brominated, and the product **129** was then transformed into the corresponding boronic acid **109**.



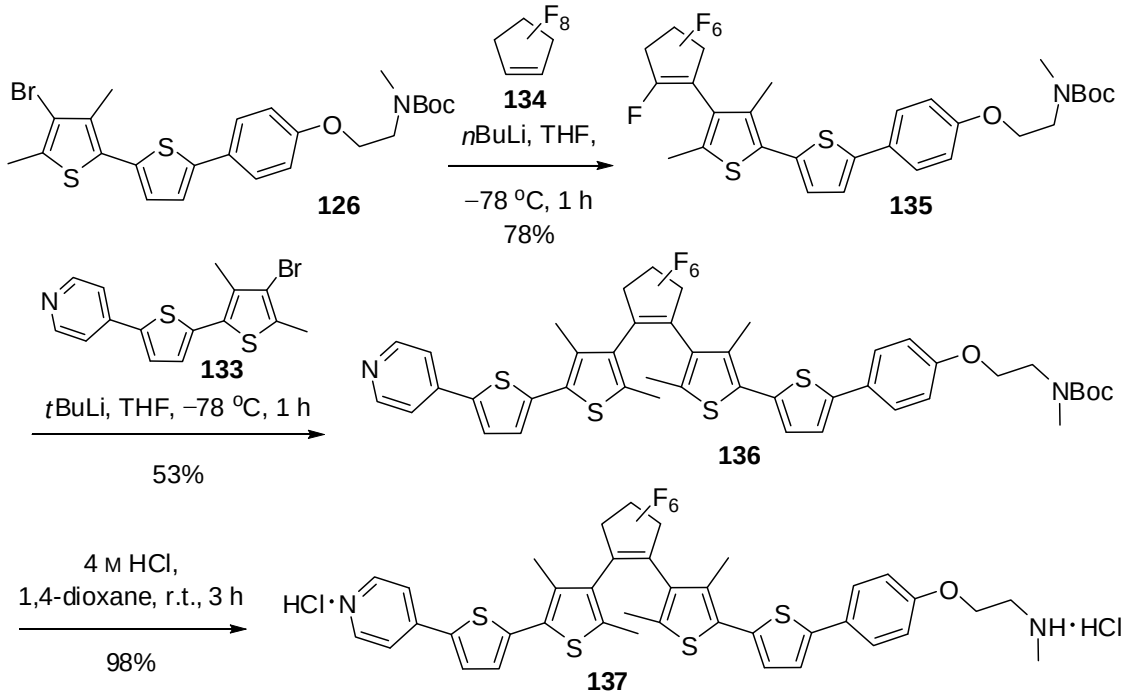
Scheme 1.9. Synthesis of 4-bromo-3,5-dimethylthiophene-2-boronic acid (**109**).

The "left hand" fragment **133** was prepared in a straightforward manner from 4-bromopyridinium hydrochloride (**130**), which was coupled with thiophene-2-boronic acid (**105**) and then iodinated with iodine and periodic acid.[79] The intermediate **132** was isolated in moderate yield and coupled with another thiopheneboronic acid (**109**) to give the compound **133** in 82% yield (Scheme 1.10).



Scheme 1.10. Synthesis of the "left hand" precursor **133** of the photochromic unit.

After bromine-lithium exchange at –78 °C in anhydrous THF, the "right" and "left hand" precursors **126** and **133** were treated with octafluorocyclopentene (**134**). Only in the case of **126** the reaction was efficient, and the corresponding coupling product **135** was isolated in good yield (Scheme 1.11).

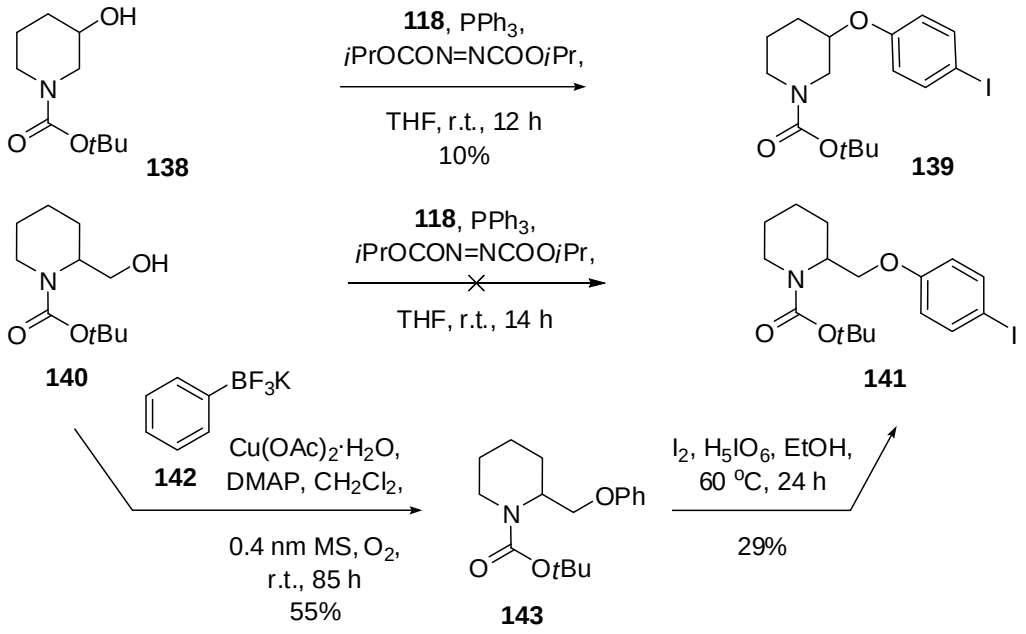


Scheme 1.11. Synthesis of the photochromic compound **136**.

To minimize the formation of the undesired symmetric photochromic compound, an excess of octafluorocyclopentene (**134**) was rapid added with a pre-cooled syringe to a solution of the *β*-lithiated thiophene. In the next step, the compound **133** was lithiated and coupled with the heptafluorocyclopentene derivative **135** to furnish the photochromic compound **136** in 53% yield. The reaction mixture was worked up quickly without exposure to direct sunlight, and the photochromic diarylethene **136** was isolated by column chromatography on silica gel mainly in

the open form (with traces of the closed form). Deprotection of compound **136** was carried out with 4 M HCl in dioxane, and the hydrochloride salt of the amine **137** was obtained in quantitative yield.

We also tried to synthesize more rigid linkers with three bonds between the O- and N-atoms (k = 2), in which the N-atom was part of a piperidine ring. Towards that, 3-hydroxy- and 2-hydroxymethyl-*N*-Boc-piperidines (**138** and **140**) were used as starting materials (Scheme 1.12).

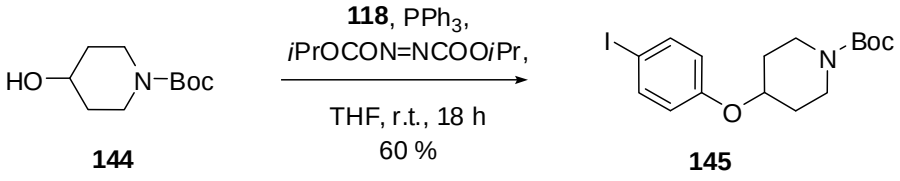


Scheme 1.12. An approach to compounds **139** and **141** with rigid C_2-linkers.

However, in these cases, the Mitsunobu reactions[76] with 4-iodophenol (**118**) did not provide satisfactory yields: in the first case only traces of the required product **139** were isolated (Scheme 1.12). In the second reaction the desired product **141** was not even detected. Eventually, it was obtained from *N*-Boc-2-(phenoxymethyl)piperidine (**143**), which, in turn, was synthesized in 55% yield from *N*-Boc-2-(hydroxymethyl)piperidine (**140**) and potassium phenyltrifluoroborate (**142**) in the presence of Cu(OAc)×H_2O, 4-(*N*,*N*-dimethylamino)pyridine and oxygen.[80] In a second step, iodination of **143** with iodine and periodic acid gave the compound **141**, but the yield was invariably low (29%). Because of the low overall yields, this method was considered to have no perspective and was not pursued any further.

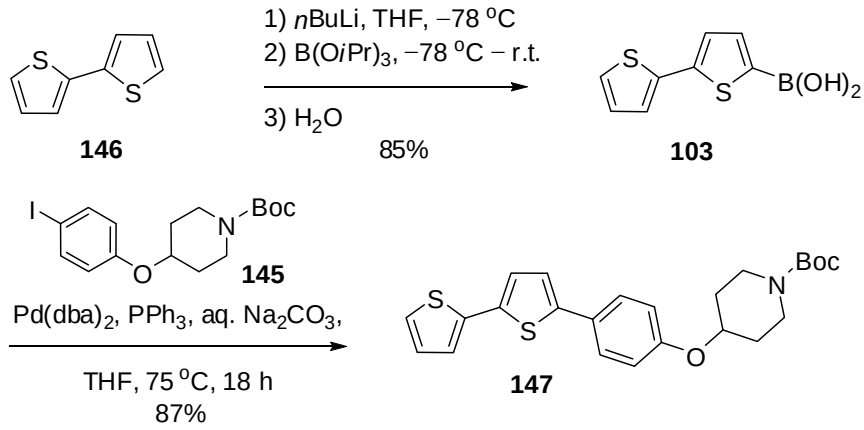
1.2.4. Three-carbon atom linker between the oxygen and nitrogen atoms (k = 3)

Predictably, the Mitsunobu reaction[76] with the similar substrate – *N*-Boc-4-hydroxypiperidine (**144**) – gave the required 4-(4-iodophenoxy)piperidine (**145**) (k = 3) in good yield (60%) (Scheme 1.13).



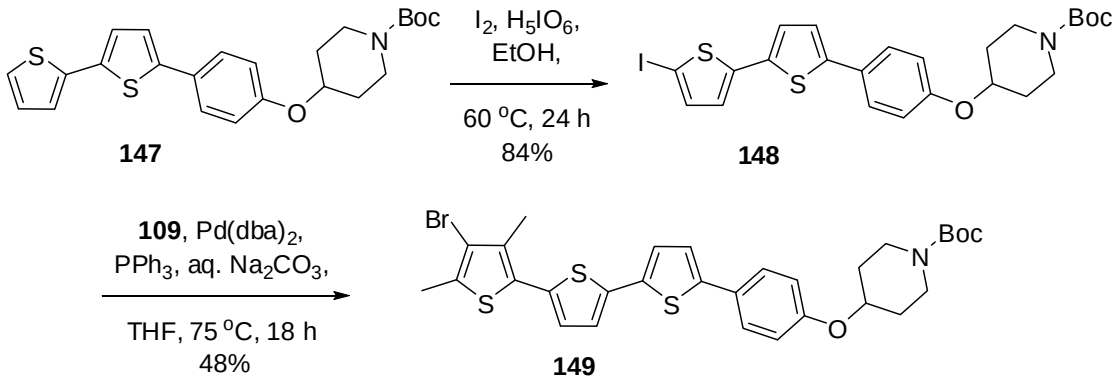
Scheme 1.13. The Mitsunobu reaction of *N*-Boc-4-hydroxypiperidine (**144**) with 4-iodophenol (**118**): synthesis of the intermediate **145** with C_3-linker.

The palladium catalyzed Suzuki reaction of compound **145** with [2,2']bithiophene-5-boronic acid (**103**) (obtained from bithiophene (**146**) by lithiation followed by substitution with tri(isopropyl)borate and hydrolysis[36b]) afforded compound **147** in good yield (Scheme 1.14).



Scheme 1.14. Synthesis of compound **147** with a long conjugation path.

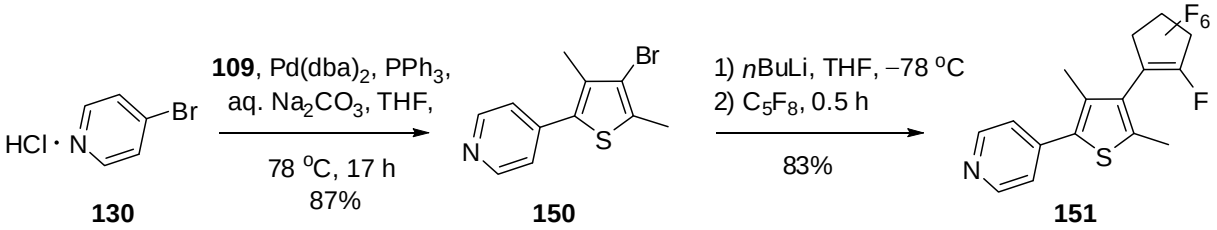
Iodination of **147** with iodine and periodic acid gave the monoiodide **148** in 84% yield, which was then coupled with 4-bromo-3,5-dimethylthiophene-2-boronic acid (**109**), and the yellowish crystalline compound **149** was isolated in 48% yield (Scheme 1.15).



Scheme 1.15. Synthesis of the "right hand" precursor **149** with a long conjugation path.

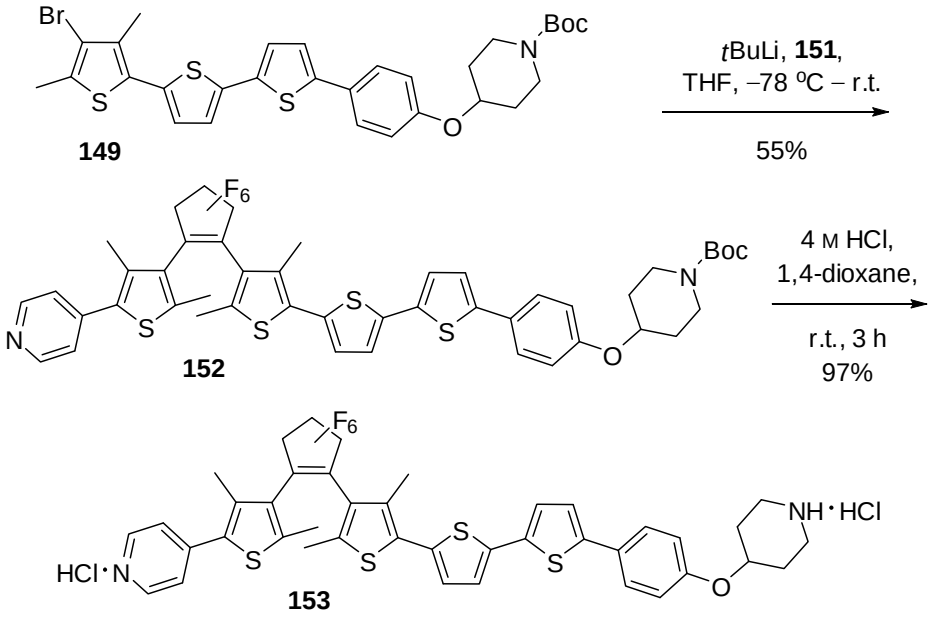
The "left hand" precursor **151** was obtained by cross coupling of 4-bromopyridinium hydrochloride (**130**) with thiopheneboronic acid **109** and subsequent reaction of the lithiated

derivative produced from the product **150** with octafluorocyclopentene (Scheme 1.16). The overal yield of compound **151** over 2 steps was 72%.



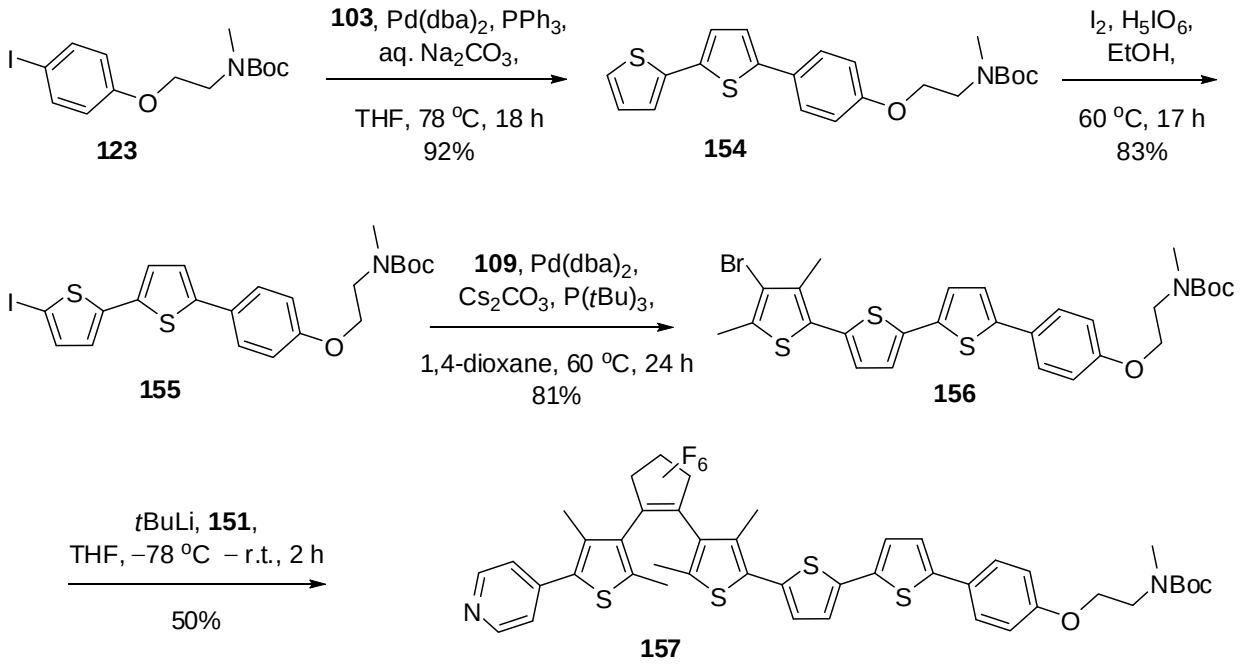
Scheme 1.16. Synthesis of the heptafluorocyclopentene derivative **151**.

The bromotristhiophene derivative **149** was subjected to bromine-lithium exchange, and the resulting lithio derivative was then treated with the heptafluorocyclopentene derivative **151** to provide the non-symmetric photochromic compound **152**, which was isolated in 55% yield. Deprotection of its amino group under acidic conditions afforded compound **153** as a hydrochloride (Scheme 1.17).



Scheme 1.17. Synthesis of the non-symmetric photochromic compound **152** with a rigid linker.

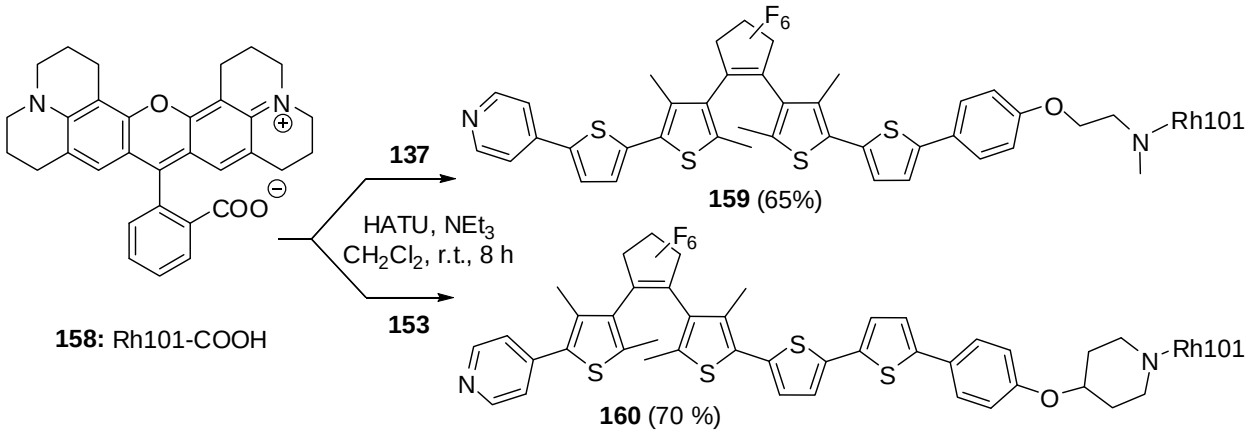
Similarly, the photochromic compound **157** with a flexible linker and a non-symmetric distribution of thiophene rings was prepared in four steps from compound **123** (Scheme 1.18) in an overall yield of 31%.



Scheme 1.18. Synthesis of the photochromic compound **157** with a flexible linker and a non-symmetric distribution of thiophene rings.

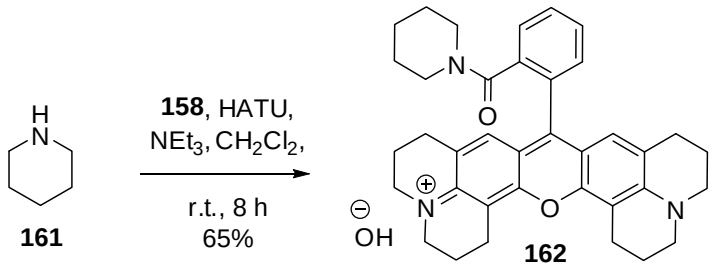
1.3. Synthesis of photochromic fluorescent compounds with rhodamine 101 as a fluorescent unit

Rhodamines are known to be good fluorescent dyes with large absorption coefficients and high fluorescence quantum yields, high photostability and low yield of the triplet-state. They are usually preferred in biological applications since the excitation in the range of 550–600 nm reduces the autofluorescence background, compared to UV and blue-light excitation. Due to the high degree of substitution and the planarity of the molecule, inexpensive rhodamine 101 (Rh101) is one of the most stable fluorophores with $\Phi_{fl} = 1$.[81] Its carboxy group is sterically congested, and therefore, coupling reactions with amines require special activation.[82] For activation of the carboxy group in rhodamine 101, we used a strong coupling agent – *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) – with addition of triethylamine as a base. The reactions of rhodamine 101 with photochromic compounds **137** and **153** were carried out in dichloromethane at room temperature under an inert atmosphere. The colored products were isolated by silica gel chromatography, and their purity was checked by analytical HPLC. The compounds **159** and **160** were obtained in 65 and 70% yield, respectively (Scheme 1.19).



Scheme 1.19. Photochromic fluorescent adducts **159** and **160**.

As a model compound, we prepared the amide **162**, which was obtained from Rh101 and piperidine (Scheme 1.20). The amidation of the carboxylic acid group in Rh101 leads to a red shift of the absorption (from 560 nm to 583 nm) and the emission bands (from 589 nm to 604 nm), without significant change in Φ_{fl} (Table 1.2). This reference compound helped to evaluate the properties of the amidated fluorescent dye in the absence of FRET.



Scheme 1.20. Synthesis of the model compound **162**, a secondary amide of Rh101-COOH.

Due to asymmetry of the absorption and emission bands of rhodamines and their amides (compound **162**), a photochromic system with an absorption maximum at about 620–640 nm for the closed form is suitable for an efficient overlapping with an emission band of amides derived from rhodamine 101.

1.4. Spectral properties of the new photochromic compounds and their adducts with rhodamine 101

The spectroscopic and photochromic properties of the novel molecular switches **136**, **152**, the fluorescent photochromic adducts **159**, **160**, and the model compound **162** were studied in ethanol at room temperature in the presence of air by irradiation of diluted solutions (~10^{-5} M) with UV and visible light, and are given in Tables 1.1 and 1.2.

Table 1.1. Spectral properties of the photochromic compounds **136** and **152**.

Compound	$\Phi_{OF\rightarrow CF}$ (λ, nm)	$\Phi_{CF\rightarrow OF}$ (λ, nm)	Conversion α_{PS}, %	$^{OF}\lambda_{max}$, nm ($\varepsilon\times10^{-4}$)	$^{CF}\lambda_{max}$, nm ($\varepsilon\times10^{-4}$)	Isobestic point λ, nm ($\varepsilon\times10^{-4}$)
136	0.124 (313)	1.9×10^{-4} (660)	99	345 (4.4)	641 (2.1)	370 (3.1)
152	0.113 (313)	–	–	380 (3.0)	608 (2.7)	410 (1.8)

$\Phi_{OF\rightarrow CF}$, cyclization quantum yield; $\Phi_{CF\rightarrow OF}$, quantum yield of cycloreversion; α_{PS}, conversion of the open to the closed form in the photostationary state. All ε values in M^{-1} cm^{-1}.

Table 1.2. Spectral parameters of the fluorescent photochromic adducts **159** and **160** and the model fluorescent compound **162**.

	Φ (λ, nm)		Φ_{fl}	α_{PS}, %	μ, %	E_{RET}	λ_{max}, nm ($\varepsilon\times10^{-4}$)		$^{Rh}\lambda_{abs}$ /λ_{em}, nm ($\varepsilon\times10^{-4}$)
	OF→CF	CF→OF					OF	CF	
159	0.061 (313)	2.2×10^{-4} (660)	0.29	98	92	0.93	345 (4.3)	641 (2.2)	585/603 (10.0)
160	0.026 (313)	–	–	95	44	0.60	380 (3.0)	608 (2.7)	583/601 (9.2)
162	–	–	0.97	–	–	–	–	–	583/604 (10.0)

Φ_{fl}, fluorescence quantum yield; μ, fluorescence modulation; E_{RET}, efficiency of the resonance energy transfer. All ε values in M^{-1} cm^{-1}.

It is not surprising, that the analogues of compounds **152** and **160** with symmetric distribution of the 3,4-unsubstituted thiophene rings prepared in this laboratory[70a] had the absorption spectra similar to those of the compounds **136** and **159**. By variation of the linker, we wanted to change the distance between the donor and acceptor and check, how this distance affects the properties of new compounds. Moreover, the synthetic approach to final compounds served as a model, which can further be used for assembling the optimized adduct and attaching it to an object. Placing the 3,4-unsubstituted thiophene units on "one side" of the

perfluorocyclopentene ring, we increased the conjugation length in the open-ring isomers of compounds **152** and **160**. Using these objects, we planned to investigate the changes in the absorption spectra of the open and closed forms, the fluorescence modulation and photochemical stability.

The photochromic systems in compounds **152** and **157** are identical, and the spectroscopic properties of these compounds are very similar. The open form of the photochromic compound **136** with symmetric distribution of the thiophene rings absorbs in ethanol at λ_{max} = 345 nm, while the photochromic compounds **152** and **157** absorb at longer wavelength with λ_{max} = 380 nm. Upon irradiation at 313 nm, the open-ring isomer **136** transforms to the closed-ring isomer and a new band with λ_{max} = 641 nm appears in the absorption spectrum. The color of the solution changes from light-yellow to dark-green.

Unfortunately, the photochromic performance of compound **152** was poor. Irradiation with 313 nm or 366 nm light produced the closed form of compound **152**, but also led to a shift of the absorption maximum (380 nm) of the unreacted material to shorter wavelengths, and the isobestic points changed their position (Figure 1.2). These effects can be explained by side-reactions and the formation of at least one by-product. Besides the expected peaks of the open- and closed-ring isomers, HPLC traces contained new intensive peaks, which indicated that the closed form was unstable even in the dark. Therefore, quantitative data for the conversion as well as the cyclization quantum yield for compound **152** cannot be measured.

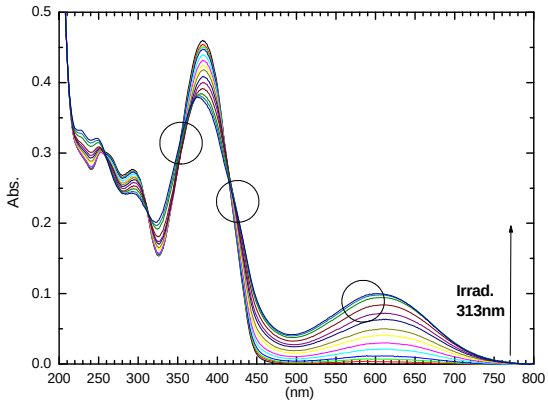


Figure 1.2. Changes in the absorption spectra of **152** during photoconversion between the open- and closed-ring isomers upon irradiation at 313 nm in ethanol.

Though the number and the nature of the aromatic rings in compounds **152**, **157** and **136** are the same, and they have formally equal conjugation paths in the closed-ring isomers, the absorption maximum of the closed form shifted from 608 nm in compounds **152** and **157** to 641 nm in compound **136**.

The photochromic unit **136** displayed a good photoconversion and was relatively photochemically stable. The absorption spectra of the open- and the closed-ring isomers of compound **136** are shown in Figure 1.3. The conversion between the two forms in the photostationary state upon irradiation at 313 nm was evaluated by HPLC. It was very high and reached 99%. Irradiation of compound **136** with visible light (>600 nm) restored the open-ring isomer and recovered the initial absorption spectra. The cyclization and cycloreversion quantum yields for this substance were found to be 0.124 и 1.9×10^{-4} upon irradiation at 313 nm and 660 nm light, respectively (Table 1.1).

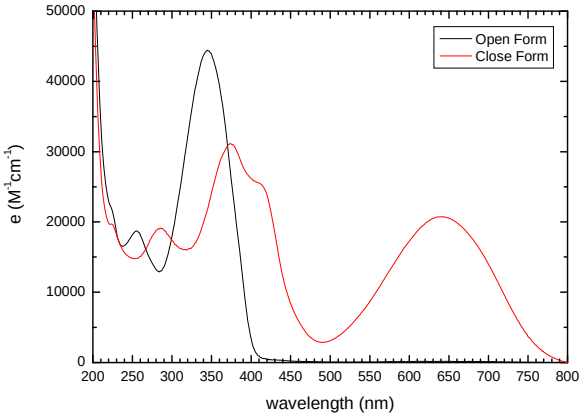


Figure 1.3. Absorption spectra of the open (black line) and closed (red line) forms of compound **136** in ethanol.

The absorption and emission spectra of the adduct **159** with rhodamine 101 in the open and closed forms are shown in Figure 1.4. The absorption maxima of the open- and closed-ring isomers were observed at 345 and 641 nm, respectively. The most intensive absorption band at 585 nm corresponded to rhodamine 101 amide. In comparison with the initial Rh101 (λ_{max} = 560 nm in MeOH), this band was considerably red-shifted both in the adduct **159** and the model amide **162** (582 nm). The emission maximum of the adduct **159** was found to be at 603 nm. Taking into account the typical asymmetry of the emission band, an excellent overlap of the absorption band of the acceptor (photochromic unit in the closed form) and the emission band of the donor (rhodamine 101 amide) was evident. In the photostationary state, upon irradiation with 313 nm light, the conversion of the adduct **159** reached 98% (measured by HPLC), and the residual fluorescence was only 8%. The good quenching of the fluorescence was also explained by the relatively high absorption coefficient of the closed form ($\varepsilon = 2.2\times10^4$ M^{-1} $см^{-1}$), which was comparable with the intensity of the main absorption band of the rhodamine amide ($\varepsilon = 1\times10^5$ M^{-1} cm^{-1}). The fluorescence quantum yield of the adduct **159** was reduced to 29% in comparison with that of the model amide **162** (97%). The efficiency of the energy transfer (E_{RET})

was estimated to be 93% from the comparison of the conversion between the open- to closed-ring isomer (98%) with the fluorescence modulation (91%).*

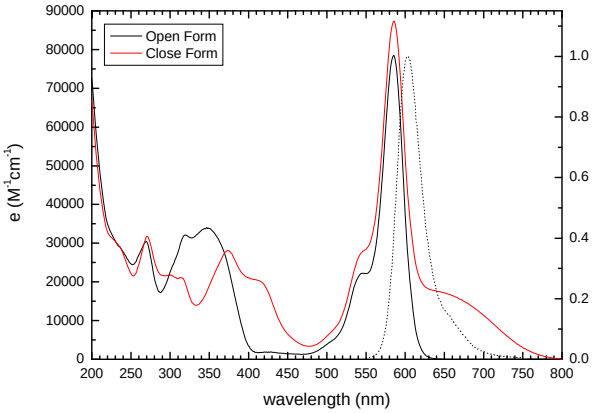


Figure 1.4. Absorption coefficients of the open and closed form (left axis) and normalized fluorescence emission (dotted line, right axis) of compound **159** in ethanol.

The emission maximum of compound **160** at 601 nm (Figure 1.5) is close to the emission band of the model adduct **162**. The cyclization quantum yield was reduced to 2.6% upon irradiation at 366 nm. Estimated by HPLC analysis, the content of the closed form of **160** in PSS (at 360 nm) was 95%, but the experimental value of the fluorescence modulation was only 44%.

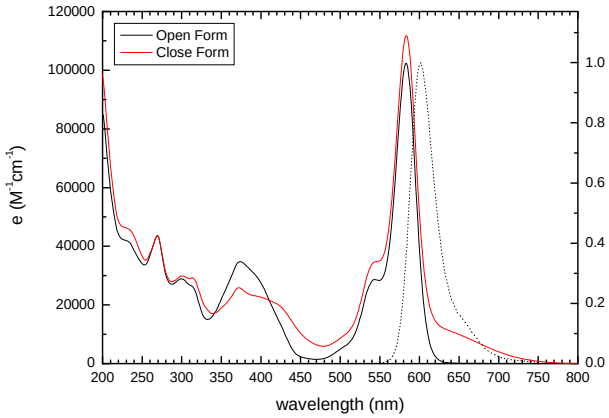


Figure 1.5. Absorption coefficients of the open and closed form (left axis) and normalized fluorescence emission (dotted line, right axis) of compound **160** in ethanol.

As was already mentioned, the photocyclization and photoreversion reactions of the photochromic compound **152** (which corresponded to the photochromic fluorescent substance **160**) were accompanied by formation of side-products detected by HPLC. Though the HPLC traces of the photochromic fluorescent compound **160** undergoing the ring-closing and ring-

* E_{RET} = 100% – (conversion – fluorescence modulation)

opening reactions looked better, additional peaks were also observed. Nevertheless, it is possible to conclude that presence of the rhodamine fragment reduced the rate of the side photoreactions and increased the stability of the closed form. Therefore, precise quantitative measurements for compound **160** were impossible, and the measured parameters given in Table 1.2 allow only qualitative comparison of adducts **159** and **160**.

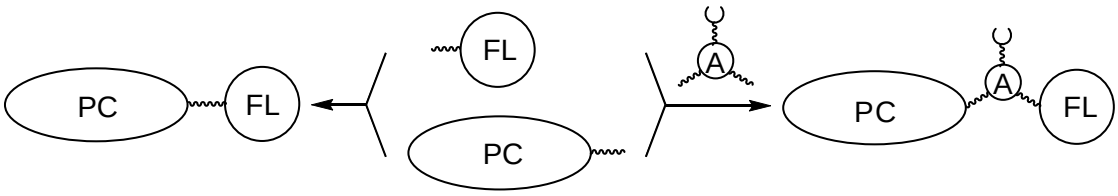
In all cases, a reduction in cyclization quantum yield ($\Phi_{OF\rightarrow CF}$) was observed for the final fluorescent adducts **159** and **160**, compared with the corresponding photochromic building blocks **136** and **152**. This tendency has previously been reported,[55b] and it could be attributed mainly to the fact that part of the irradiation light is absorbed by the fluorophore and cannot contribute to photocyclization. The efficiencies of the reversed reaction ($\Phi_{CF\rightarrow OF}$) are almost unaffected. The values obtained for conversions (α_{PS}) were slightly decreased, compared with the corresponding photochromic building blocks (**136** and **152**), but are still close to 100% for the adducts **159** and **160**.

2. Synthesis of fluorescent diarylethene derivatives with additional functionalities

It was interesting to try to attach the adduct **159** with a good photochromic performance to an object and study the switching of fluorescence in order to improve the optical resolution. An additional group is required for this connection.

2.1. Optimized fluorescent photochromic compounds with amino-reactive groups (NHS ester) and imaging of the silica-gel nanoparticles with an incorporated fluorescent photochromic compound

We extended the bridge between the photochromic unit and the fluorophore by inserting an aspartic and a glutamic acids that act as a branching point and possess an additional carboxylic group that may be easily transformed into an amino-reactive site.[70b]

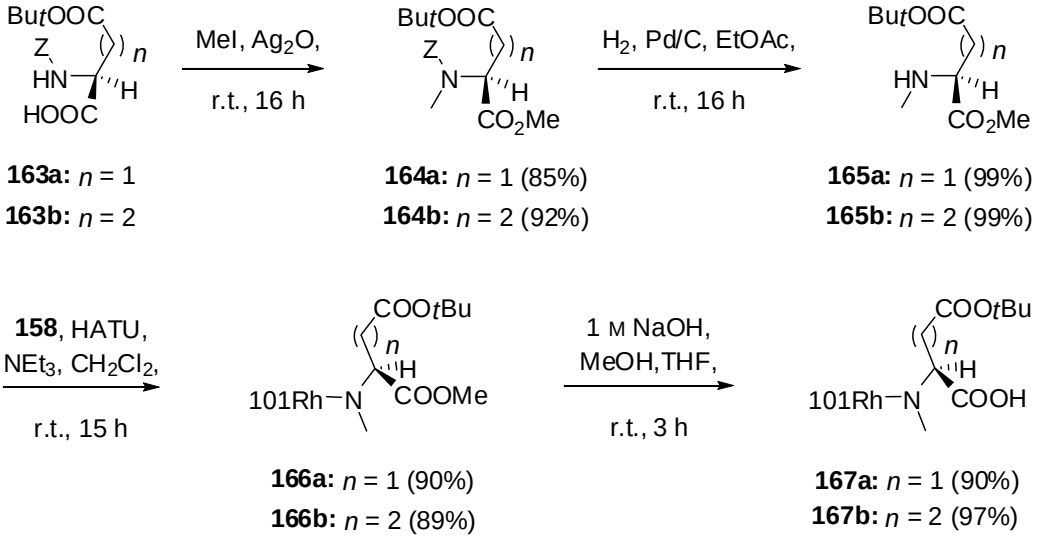


Scheme 2.1. Photochromic fluorescent adducts PC-FL, PC-A-FL and their fragments: PC – photochromic unit; FL – fluorophore; A = AA – aspartic acid or GA – glutamic acid.

This new functionality did not alter the individual properties of the fluorescent and photochromic building blocks. The additional amino acids only add three bonds to the linker, representing a small increase in the total length of the whole bridge, which is still much shorter

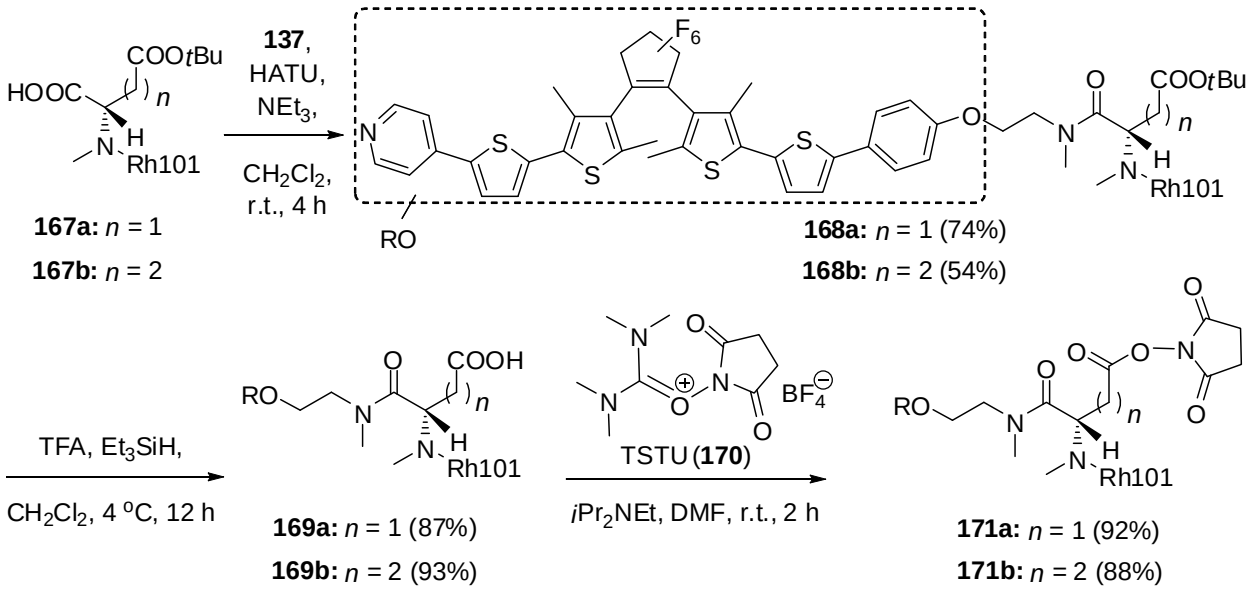
than the calculated Förster radius (ca. 50 Å). It is therefore expected that the efficiency of the resonance energy transfer (E_{RET}) between the donor and the acceptor in the final compounds will not be significantly reduced.

Both the aspartic and the glutamic acids have three functional groups, which should be initially orthogonally protected. Therefore, we started from the commercially available *tert*-butyl ester **163a** and in one step transformed it into compound **164a** by alkylation with MeI in the presence of Ag_2O (Scheme 2.2). An additional *N*-alkyl group was necessary for blocking the cyclization, which involves the NH residue of the rhodamine amide and the internal tetrasubstituted C=C bond of the aromatic rhodamine system. It prevents a self-closing of the rhodamine amide **166a** into the colorless spiro-form.



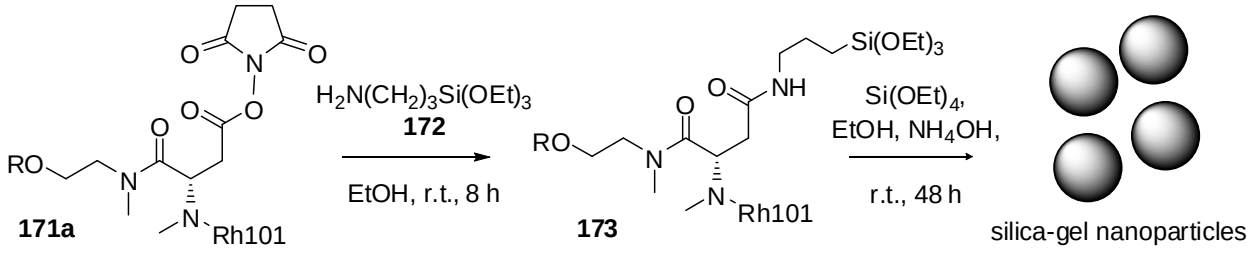
Scheme 2.2. Synthesis of the fluorescent photochromic compounds with additional free carboxylic acid groups.

The *N*-methylamino group was deprotected by hydrogenolysis, and compound **165a** was acylated with the carboxy group of rhodamine 101 using the strongly activating coupling agent HATU. The diester **166a** was saponificated with aqueous NaOH, and the monoacid **167a** was obtained. After coupling the photochromic unit **137** with monoacid **167a** using HATU and a base (Scheme 2.3), the *tert*-butyl ester group in the adduct **168a** was removed by treatment with trifluoroacetic acid and Et_3SiH. The new free carboxy group in compound **169a** was activated by the formation of the *N*-hydroxysuccinimidyl ester by reaction with TSTU (**170**).



Scheme 2.3. Synthesis of the amino-reactive fluorescent photochromic dithienylentenes **171a** and **171b**.

This functionalized fluorescent molecular switch **171a** was coupled with (3-aminopropyl)triethoxysilane (**172**) and then used for the synthesis of silica-gel nanoparticles by hydrolysis and condensation with tetraethyl orthosilicate in mixtures of water, ammonia, and ethanol (Scheme 2.4). In this way, the probe is chemically bonded to the silica network.[83] This avoids any leaking into the solvent or into standard microscopy immersion media, which is an undesirable effect usually observed with stained polymer beads, if the fluorophores are incorporated by swelling and not bound to the polymer backbone.



Scheme 2.4. Synthesis of silica-gel nanoparticles containing fluorescent photochromic diarylperfluorocyclopentene.

A compound with a glutamic instead of an aspartic acid residue between the photochromic and fluorescent units was prepared and activated by similar procedures (Schemes 2.2 and 2.3, *n* = 2) from the commercially available *Z*-*L*-glutamic acid 5-*tert*-butyl ester (**163b**). The longer alkyl chain in the glutamic acid makes the carboxy group in compound **169b** less sterically hindered,

and it may be used for labelling of more demanding amino groups. Moreover, glutamic acid diamides do not give cyclic (six-membered) imides as easily as the aspartic acid diamides form the corresponding five-membered cyclic imides. This kind of the side reaction leads to a losts of the photochromic unit.

2.1.1. Photochromic properties of the adducts 168a and 168b and their behavior inside the silica-gel network

The properties of compounds **168a** and **168b** (models for PC-AA-FL and PC-GA-FL) in ethanolic solutions were measured to evaluate the effect of the amino acid residues inserted between the photochromic unit and the fluorophore. It was not surprising that the addition of aspartic acid (AA) or glutamic acid (GA) residues did not have any effect on the spectral properties. The closed form of compounds **168a** and **168b** similar to the compound **159** (model PC-FL) absorbed light at 585 nm, while the emission band was found at 603 nm. The efficiency of the energy transfer between the rhodamine and both isomers of the photochromic unit was expected to be altered due to the different linkers used. However, only the RET efficiency for the open-ring isomer significantly changed, which resulted in a slightly improved fluorescence modulation (from 92% for **159** to 94%). Fortunately, the introduction of a additional bridge between photochromic unit and fluorophore increased the fluorescence quantum yield, which achieved 52 and 63% for the compounds **168a** and **168b**, respectively. All relevant measured parameters for compound **159** as well as compounds **168a** and **168b** are listed in Table 2.1.

Table 2.1. Absorption and fluorescent properties of the compounds **159**, **168a**, **168b** and fluorescent photochromic silica-gel nanoparticles (**NP**).

Compound	$^{Rh}\lambda$ max (nm)		Fluorescence quantum yield, Φ_{fl}	Conversion, α_{PS}	Fluorescence modulation, μ, %	Efficiency of the RET, E_{RET}	
	Abs.	Emiss.				OF	CF
159	585	603	0.29	0.98	92	0.72	0.98
168a	585	604	0.52	0.98	94	0.46	0.98
NP	590	610	0.52	0.59	94	–	–
168b	585	603	0.63	0.98	92	–	–

The spectroscopic properties of 30 nm particles containing the modified fluorescent switch **169a** in ethanolic suspension are shown in the Figure 2.1. Compared to the dye dissolved in ethanol, both the absorption and emission spectra are not substantially altered, except for a red shift of 5 nm in each spectrum. The figures display the changes of the absorption and fluorescence signal of the particles induced by irradiation with UV (375 nm) and red (671 nm) light. About 94% of the total fluorescence signal was reversibly modulated.

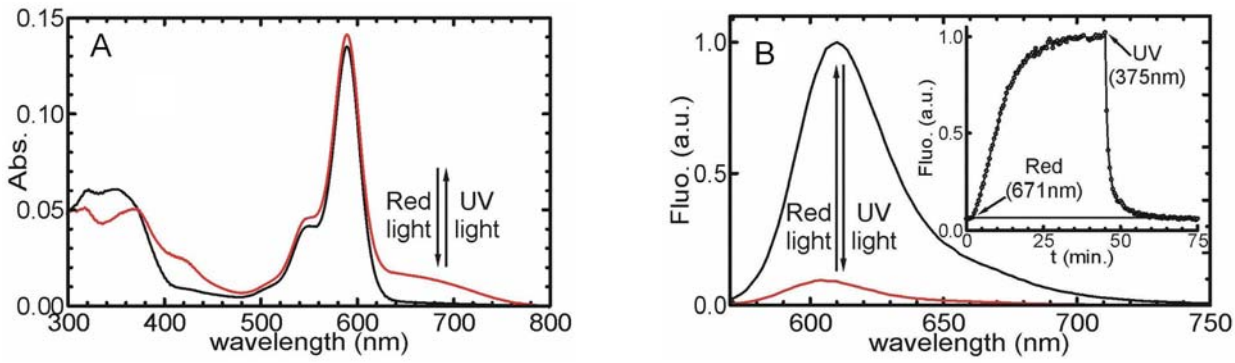


Figure 2.1. Absorption (A) and emission (B) spectra of 30 nm nanoparticles with incorporated **169a**, irradiated with UV (black line) and red light (red line), in ethanol suspension. The inset in (B) shows the kinetics of the emission modulation for a complete irradiation cycle with light of 671 nm (40 mW) and 375 nm (7 mW) in a total volume of 3 mL.

A conversion of 59% from the open to the closed form was estimated in nanoparticles from absorption measurements, assuming that the absorption coefficients are the same in the silica-gel and in solution. A lower conversion in constrained media has previously been reported for some diheteroarylethenes in polymers.[11e,84] However, the fluorescence modulation in the particles was as large as for compound **168a** in solution. This effect can be explained, if an average distance between fluorophores and photochromic units in particles is small enough to allow for an efficient intermolecular energy transfer.

In order to support this hypothesis, we measured the emission as a function of the conversion for the particles and for the free dye in ethanol (Figure 2.2). A linear dependence as expected for a one-to-one ratio between the donor and the acceptor was observed for the free dye **168a**, while particles presented a clear deviation from this linear behaviour. These intermolecular interactions are responsible for the high fluorescence modulation obtained in the particles despite of the lower conversion of the photochromic unit.

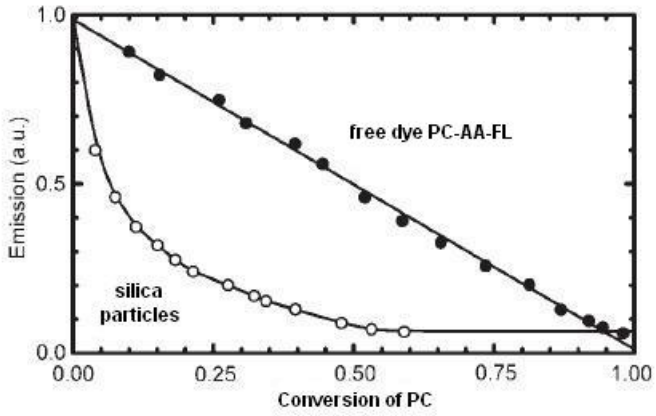


Figure 2.2. Emission at the maximum wavelength of the fluorophore (Rh101) as a function of the conversion of the photochromic moiety (PC) for the dye **168a** (PC-AA-FL) in ethanol solution (full symbols) and in silica-gel nanoparticles of 30 nm diameter (hollow symbols).

The switching properties of the particles were also tested in a confocal microscope (Figure 2.3). Single beads were centered in the focal spot and submitted to successive irradiation cycles with a 375-nm and a 671-nm laser. The Figure 2.3 shows the first 30 complete irradiation cycles, where no sign of fatigue is observed. Normally, the beads resisted up to 50–60 cycles before approximately 10% of the modulation ratio was lost. This fatigue was evidenced as an increase in the signal in the "off" state, with no significant alteration of the signal in the "on" state, indicating that the photochromic part of the molecule underwent irreversible photodamage.

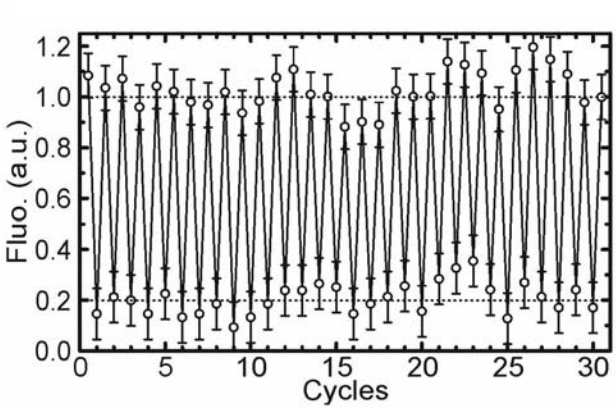


Figure 2.3. A series of 30 successive complete irradiation cycles (UV/red light) performed with a single silica-gel particle with attached compound **169a** in a confocal microscope.

In spite of the restricted number of photochromic cycles, using the RESOLFT-technique, we were able to improve the optical resolution along one axis. Figure 2.4 shows line scans in the direction over two particles separated by distance of 234 nm. Unresolved in the conventional

confocal mode, the two closely located nanoparticles were clearly separated in the RESOLFT mode. For improving the resolution in the second dimension, it is necessary to greatly increase the number of irradiation cycles, but it results in irreversible photodamage of the photochromic compound and a consequent loss of contrast.

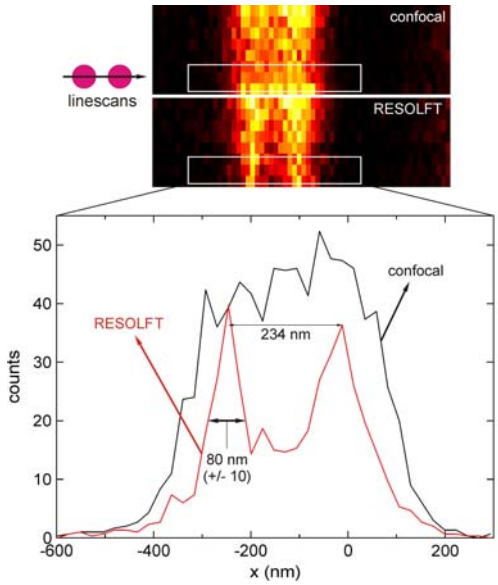
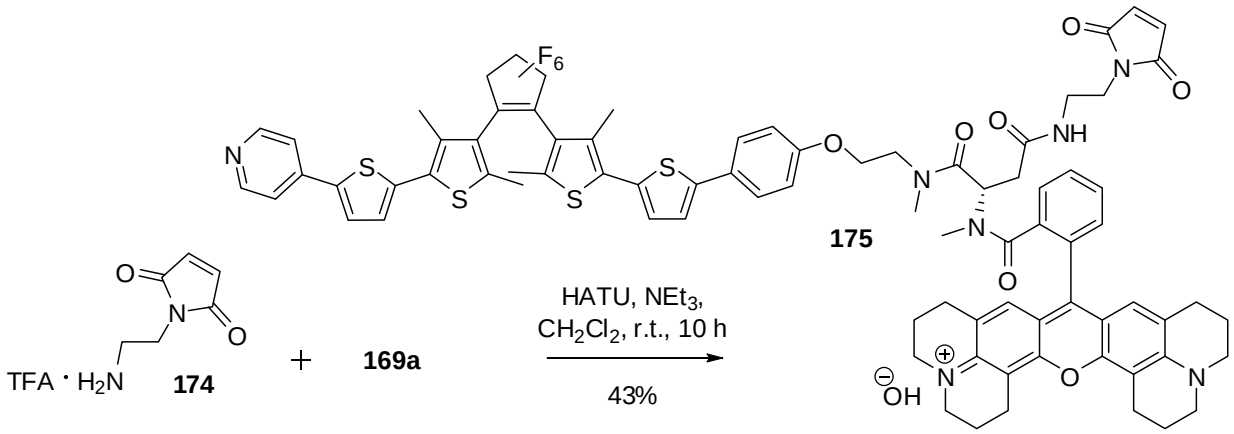


Figure 2.4. RESOLFT microscopy of switchable nanoparticles with attached compound **169a**.

The NHS ester of compound PC-AA-Rh **171a** may be used for attaching it to any amino-group containing object. The silica-gel core has some advantages for labelling biological samples. Biocompatibility and hydrophilic properties make the silica-gel nanoparticles promising for using in cells. The size of the constructed fluorescent photochromic silica-gel nanoparticles may be decreased to 20 nm (and even less) in diameter, which helps to improve the optical resolution with visible light. Moreover, the optical properties of nanoparticles are independent of their size and concentration.

2.2. A fluorescent photochromic compound with a maleimide fragment

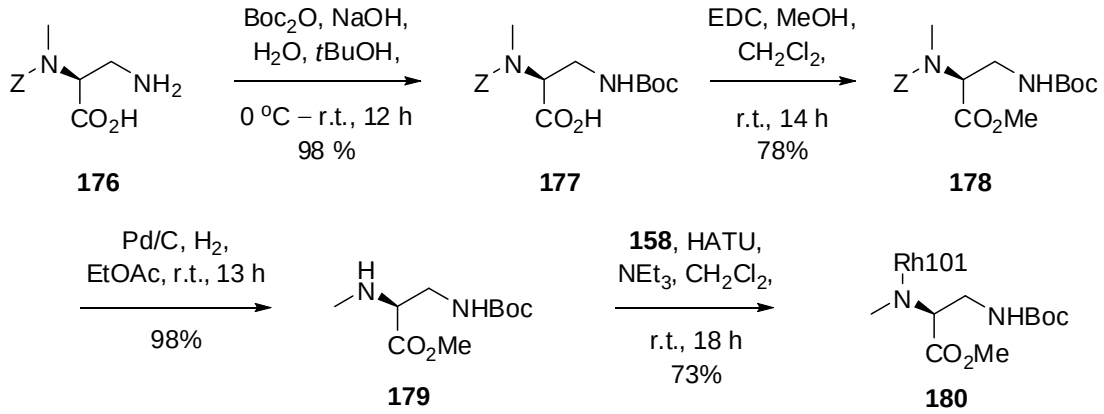
The maleimide group may serve for selective labelling of thiol-containing objects. Compound **175** was obtained by treatment of the acid **169a** with *N*-(2-aminoethyl)maleimide trifluoroacetate (**174**), HATU and NEt_3 (Scheme 2.5).



Scheme 2.5. Synthesis of the maleimide derivative **175**.

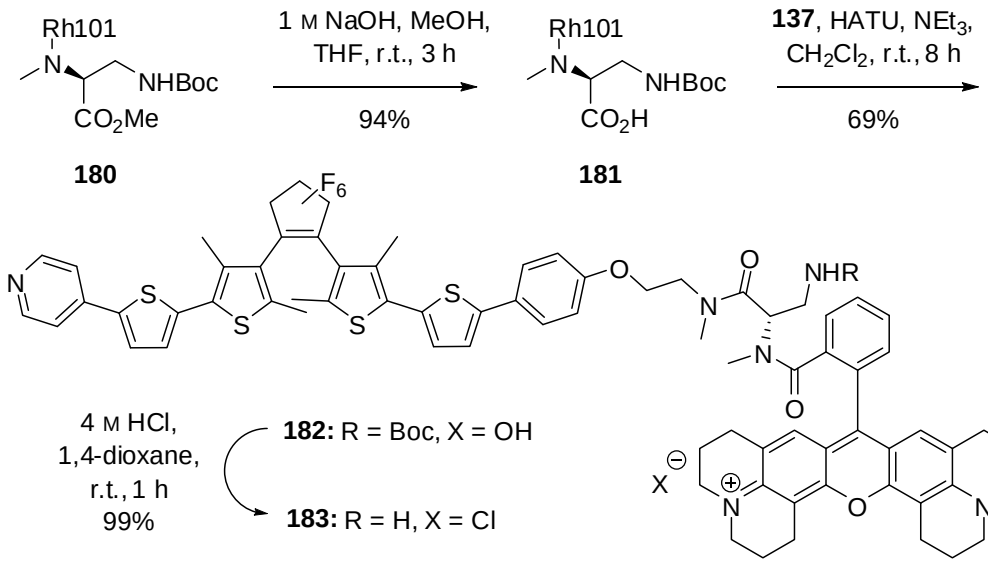
2.3. A fluorescent photochromic compound with an amino group

The diprotected compound **177** was prepared from the monoprotected diamino acid **176** as described,[85] and its carboxy group was transformed into the methyl ester with methanol in the presence of carbodiimide to provide the ester **178** in good yield (Scheme 2.6). The Z-protecting group in **178** was removed by hydrogenolysis on Pd/C, and amine **179** was obtained in quantitative yield. It was coupled with rhodamine 101 (**158**), and thus fluorescent compound **180** was prepared.



Scheme 2.6. The fluorescent compound **180**.

The methyl ester in compound **180** was saponificated with aq. NaOH, and the acid **181** was coupled with photochromic compound **137** (Scheme 2.7). After deprotection of the amino group in compound **182**, the fluorescent diarylethene **183** with a free amino group was produced quantitatively.

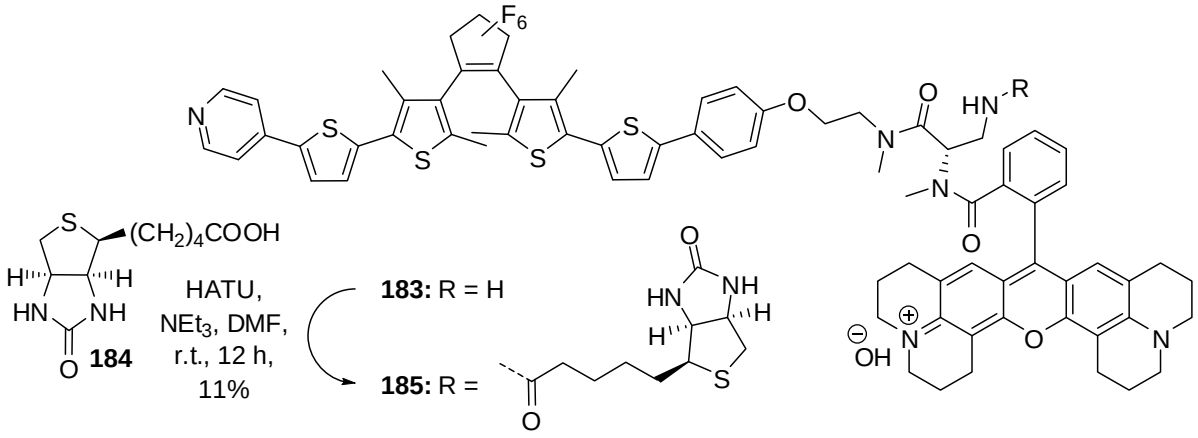


Scheme 2.7. Synthesis of the adduct **183** with a free amino group.

2.4. A fluorescent photochromic compound with a biotin fragment

We decided to decorate the fluorescent photochromic compound with a biotin fragment. Biotin is known to form strong complexes with streptavidin (or avidin), and these complexes may be used for labelling of various biologically relevant targets.

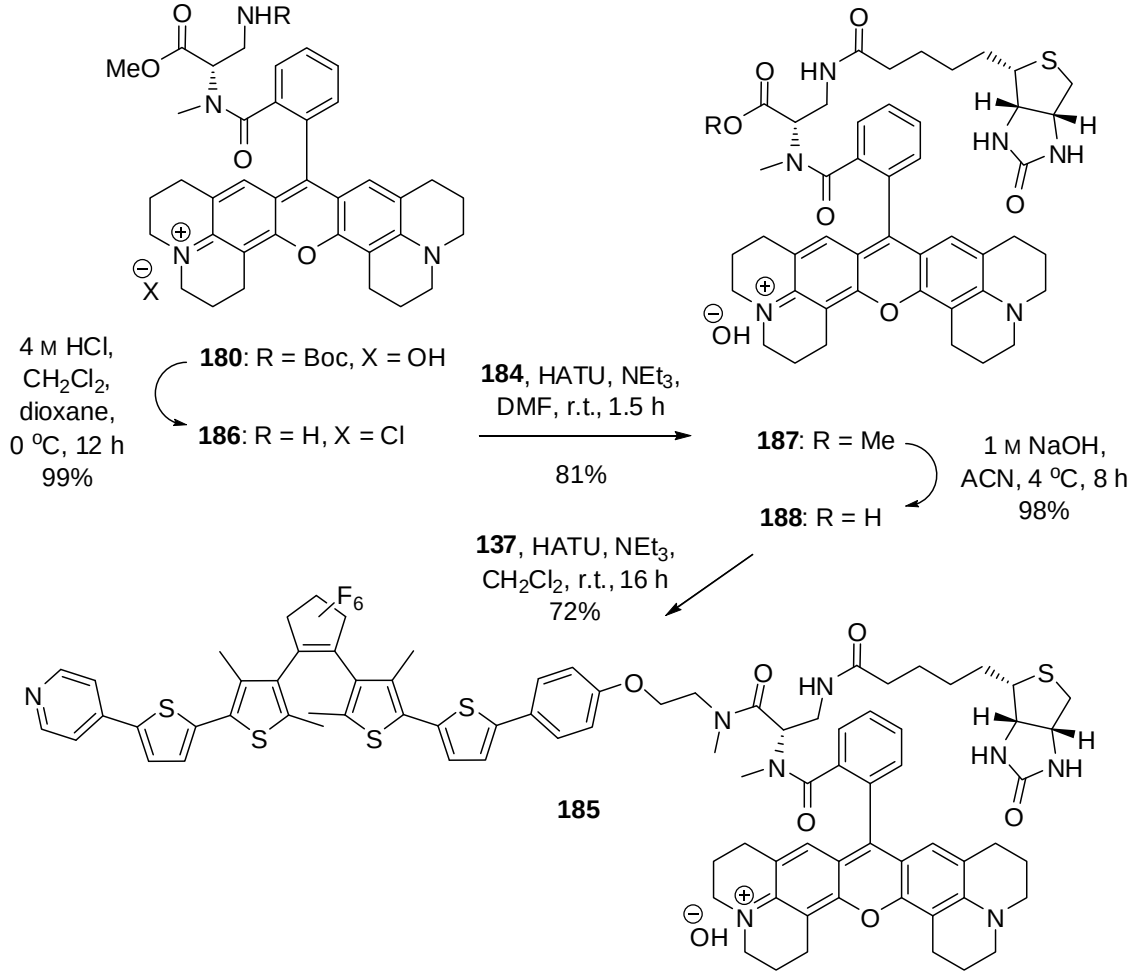
Unfortunately, a direct reaction of the amine **183** with the carboxylic acid group of biotin (**184**) in the presence of HATU and NEt_3 produced the desired product **185** only in low yield (Scheme 2.8).



Scheme 2.8. Synthesis of the biotinylated compound **185** from amine **183**.

Therefore, we changed the strategy, and cleaved off the Boc group in compound **180** under acidic conditions. Then compound **186** was coupled with biotin (**184**), and the methyl ester **187** was prepared in good yield (Scheme 2.9). After saponification of the ester group in compound **187** with NaOH in ACN, the acid **188** was isolated. Activation with HATU in the presence of a

base, and the reaction with the photochromic unit **137** afforded the fluorescent photochromic compound **185** with a biotin fragment in good yield.



Scheme 2.9 Synthesis of the biotinilated photochromic fluorescent dye **185** from the methyl ester **180**.

In ethanol, the absorption maxima of the open and closed forms of photochromic diarylethene **185** were observed at 347 and 636 nm, respectively. The absorption and emission of the fluorescent part was found at 586 nm and 604 nm, respectively. The fluorescence quantum yield was found to be 0.5, and the fluorescence modulation was very good (93%).

However, we did not manage to use this compound in living cells because it was found to be poorly soluble in water and did not penetrate through cell membranes.

3. Water-soluble photochromic and fluorescent photochromic compounds

Due to the good photochromic performance of perfluorocyclopentenes,[11e] their fluorescent adducts may help to improve the optical resolution and even visualize cell organells and track the processes occurring in them.

Diarylethenes are relatively hydrophobic, and even after attachment to a hydrophilic fluorescent dye their adducts are not soluble in aqueous buffers and require the use of an organic co-solvent (e. g. EtOH), which is not compatible with living cells.[69]

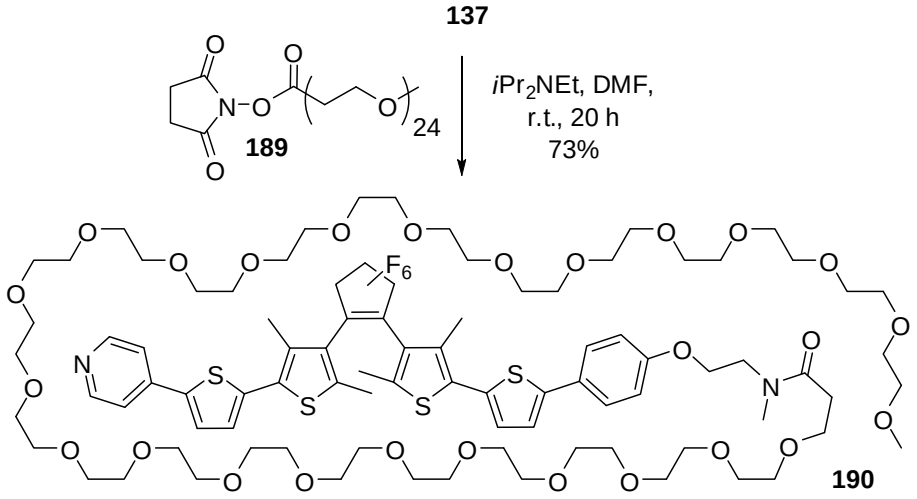
Big photoswitchable fluorescent proteins are soluble in water, but their fusion with studied proteins may change the natural cell components and perturb their functions. Thus, the synthesis of smaller hydrophilic and water-soluble molecular probes (e. g. fluorescent photochromic compounds) is an attractive task. Several obstacles may impede the successful implementation of this task.

For example, an amphiphilic diarylcyclopentene[86] with an improved cell permeability was found to produce big micelles in aqueous solutions, and its fluorescence intensity was very low.

Therefore, our goal was the design and synthesis of water-soluble diarylperfluorocyclopentenes with good photochromic performance in aqueous buffers. These compounds possess low molecular weight (in comparison with fluorescent proteins) and should have an additional binding site for attachment to a fluorescent dye and an object.

3.1. Photochromic unit with a poly(ethyleneglycol) side chain

The previously prepared adduct **159** was found to be insoluble in water, because its photochromic unit is very lipophilic. To increase the hydrophilic properties of a photochromic unit, we attached it to a long poly(ethyleneglycol) side chain. For that, we acylated the photochromic molecule **137** with the commercially available NHS ester **189**, and isolated the water-soluble compound **190** in a good yield (Scheme 3.1).



Scheme 3.1. Diarylethene **190** decorated with a poly(ethyleneglycol) side chain.

The compound **190** showed good photochromic properties in ethanol, but practically did not undergo ring-closing reaction in water. The conversion between the open and closed form in

water did not exceed 10%, and irradiation was accompanied by a spectral shift of the band of the closed form. This phenomenon could be attributed by photodecomposition.

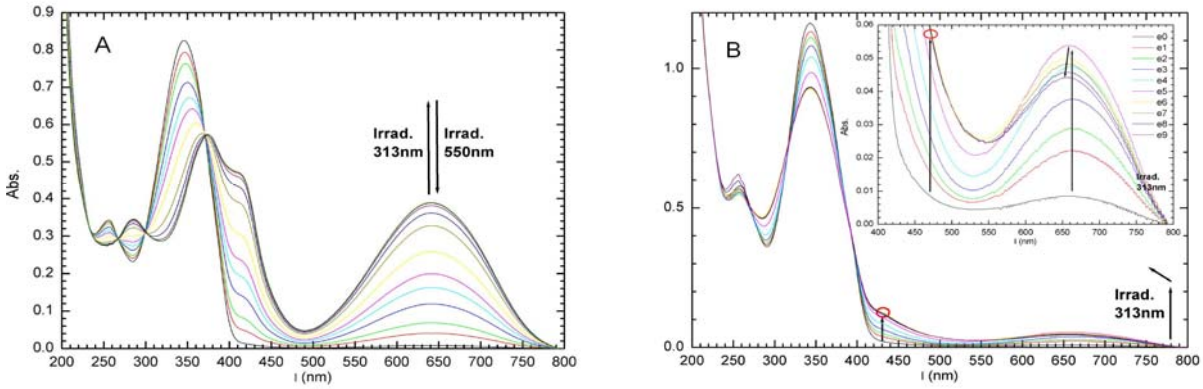
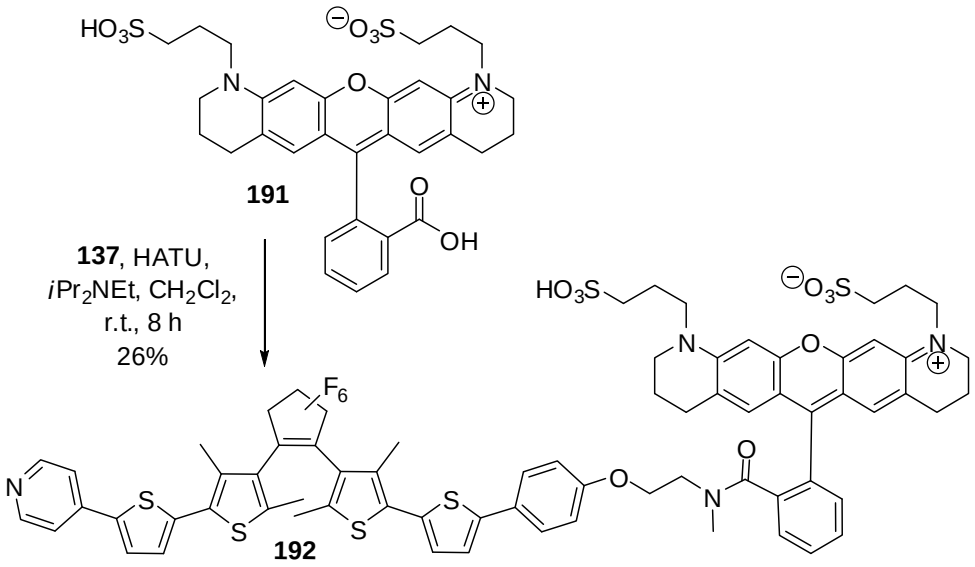


Figure 3.1. Absorption spectra of the compound **190** in ethanol (A) and in water (B).

3.2. Introduction of sulfonic acid residues into the fluorescent part of the photochromic assembly

In one step we connected the photochromic unit **137** and a new sulfo-rhodamine **191** with an excellent water-solubility (Scheme 3.2). Rhodamine **191** absorbs at 558 nm ($\varepsilon = 1.09 \times 10^5$ M^{-1} cm^{-1}), and emits at 582 nm ($\Phi_{fl} = 1$, water).



Scheme 3.2. Synthesis of compound **192** with a hydrophilic rhodamine moiety.

We intended to obtain a water-soluble substance with improved performance in aqueous solutions. The compound **192** could not be dissolved in pure water, but after addition of a small amount of a polar organic solvent (e. g. ethanol), it dissolved in water completely. Again, this compound showed a large fluorescence modulation and a fairly good fluorescence quantum yield

only in ethanol. Unexpectedly, it did not even emit light and did not show any photochromism in water.

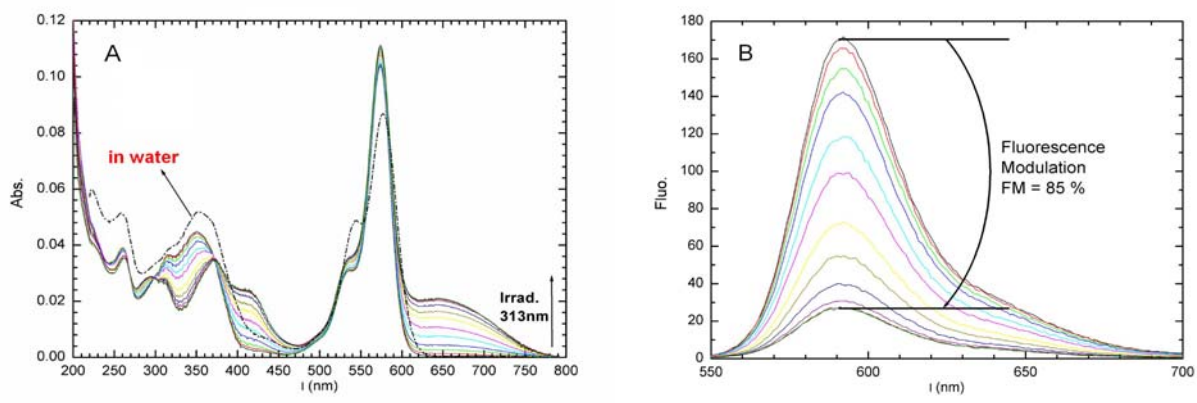
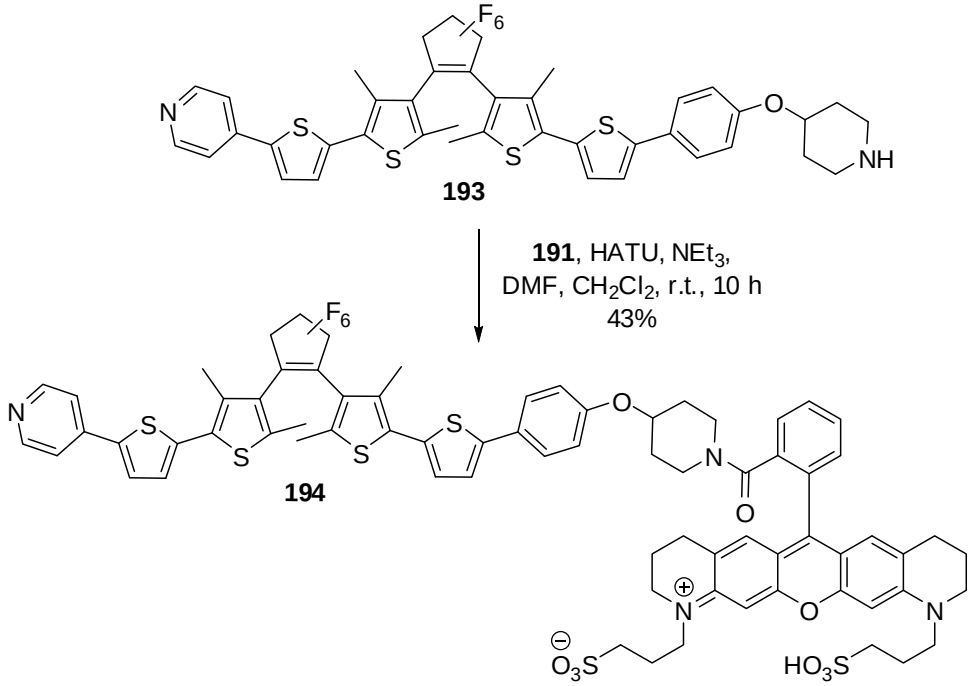


Figure 3.2. Absorption (A) and emission (B) spectra of the adduct **192** in ethanol. The dotted black line (A) corresponds to the absorption spectrum of **192** in water.

We thought that the flexible linker between the photochromic unit and the fluorophore enables the formation of a sandwich-like charge-transfer complex. In this case the planar fluorophore and the planar photochromic unit may form a complex by π-stacking, and it might quench the fluorescence. Therefore, we synthesized another adduct **194** with the same sulfo-rhodamine **192** and the photochromic unit, but with a rigid linker between them (Scheme 3.3). Unfortunately, in water this compound behaved similarly.



Scheme 3.3. Synthesis of compound **194** with a rigid linker.

3.3. Introduction of sulfonic acid residues into the photochromic unit

We planned to increase the water solubility of the photochromic unit by introducing sulfonic acid groups. Hydrophilic residues can be connected with the methyl groups adjacent to the perfluorocyclopentene fragment (priority 1 modification), or they may be attached to the central 3,4-unsubstituted thiophenes (priority 2 modification) (Figure 3.3).

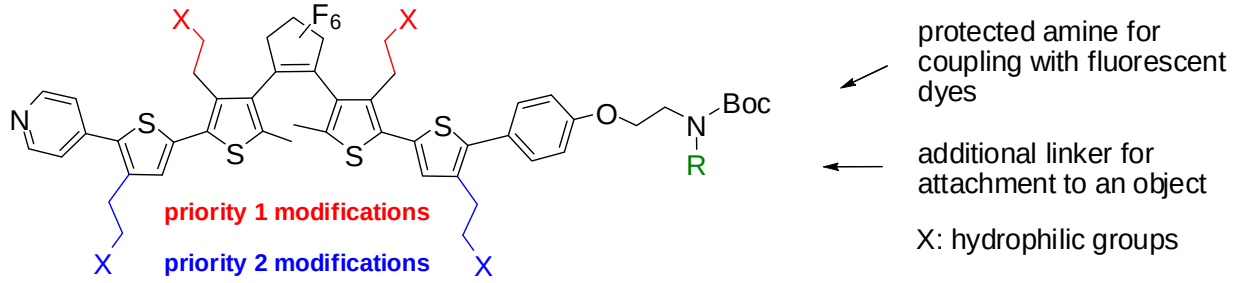
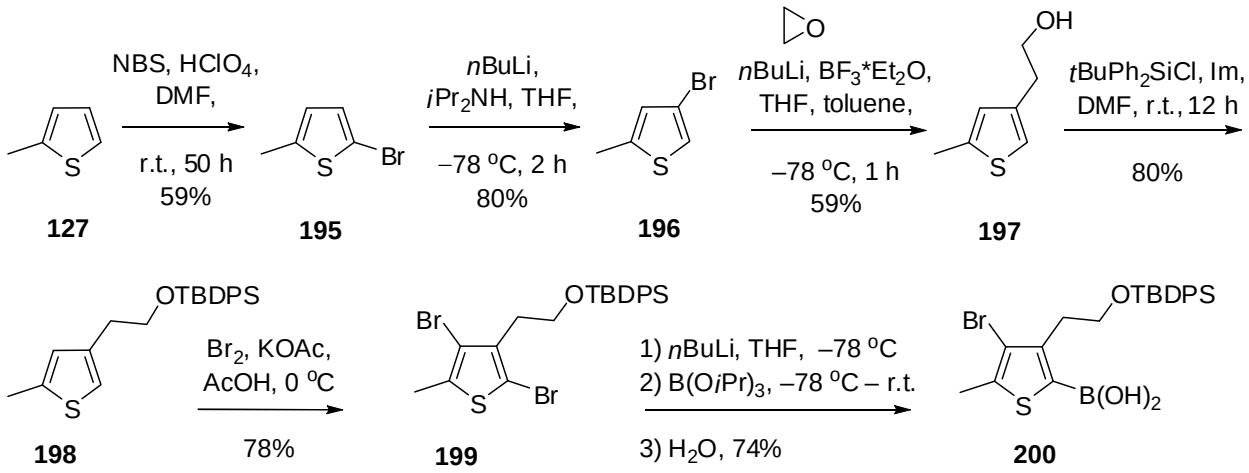


Figure 3.3. Photochromic compounds with hydrophilic groups.

Moreover, we decided to attach an additional *O*-protected 3-hydroxypropyl residue (R) to the amino group, having in mind that it could later link the whole molecule with an object. We planned to attach the required sulfonic acid groups to the hydroxy groups, and we needed to protect these hydroxyls, in order to proceed with the synthesis.

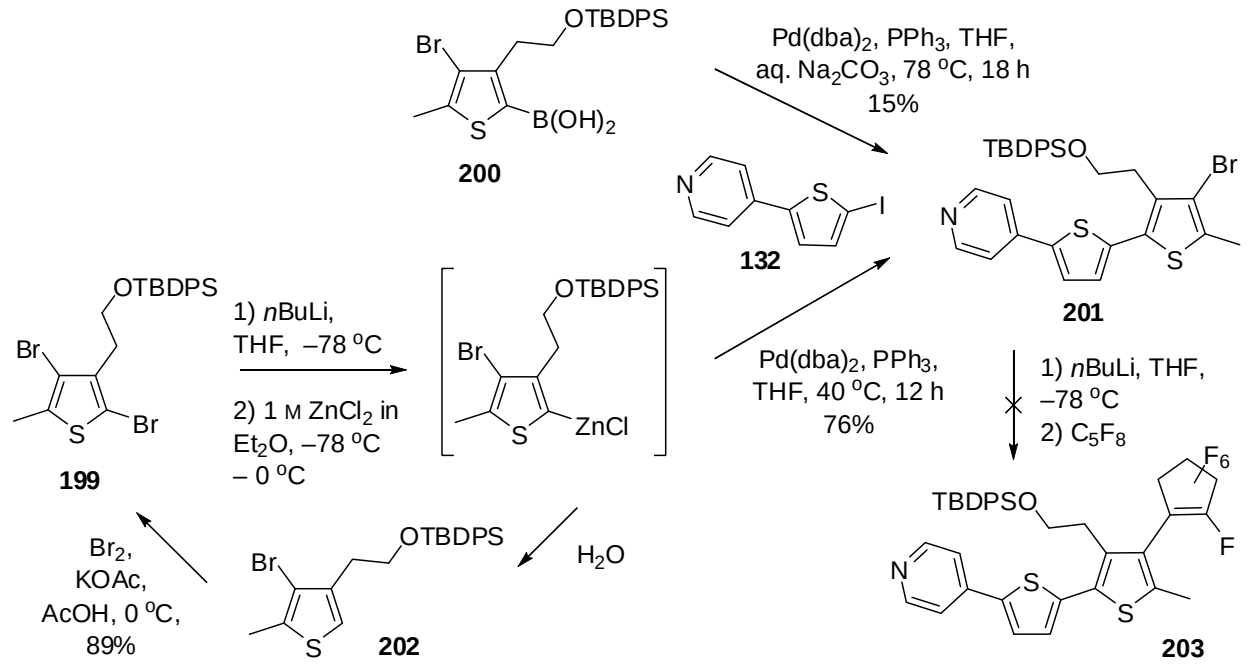
3.3.1. Target: priority 1 modifications

2-Methylthiophene (**127**) was used as a starting material for implemention of the priority 1 modifications. It was brominated with NBS in the presence of $HClO_4$,[87] and then bromide **195** was subjected to the "halogen dance" rearrangement to give 4-bromo-2-methylthiophene (**196**) in 80% yield (Scheme 3.4). The latter was subjected to bromine-lithium exchange and the lithio derivative was treated with oxirane.[88] The hydroxy group in compound **197** was protected with a *tert*-butyldiphenylsilyl residue, which was stable to hydrolysis during the subsequent synthetic procedures. The thiophene **198** was brominated with bromine in acetic acid in the presence of KOAc to afford the colorless crystalline compound **199**. The latter was transformed to the boronic acid **200** in good yield.



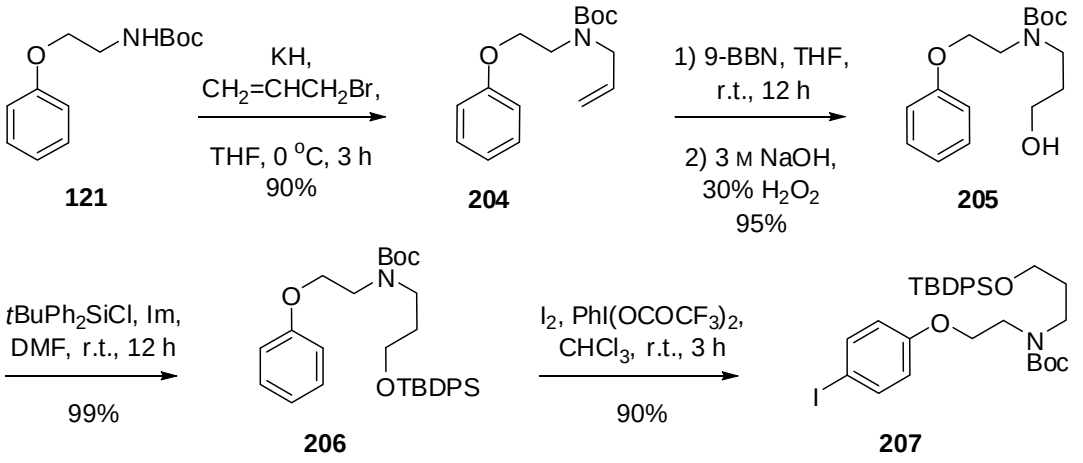
Scheme 3.4. Synthesis of the boronic acid **200** and its precursor **199** with a protected hydroxy group.

The Suzuki coupling of the iodide **132** with the boronic acid **200** gave compound **201** in low yield. To reduce the number of steps, the coupling reaction was performed with the zink-derivative of compound **199** (Scheme 3.5). Towards that end, a selective lithiation of **199** followed by addition of $ZnCl_2$ was performed, and the reaction mixture was added into the flask containing the iodide **132** and a palladium catalyst. The coupling product – the "left hand" precursor **201** – was isolated in good yield. The dehalogenated derivative **202** obtained as a side-product by hydrolysis of the zink derivative was also isolated and successively rebrominated to substrate **199**. Unfortunately, the required heptafluorocyclopentene derivative **203** could not be obtained from the iodide **201** and perfluorocyclopentene.



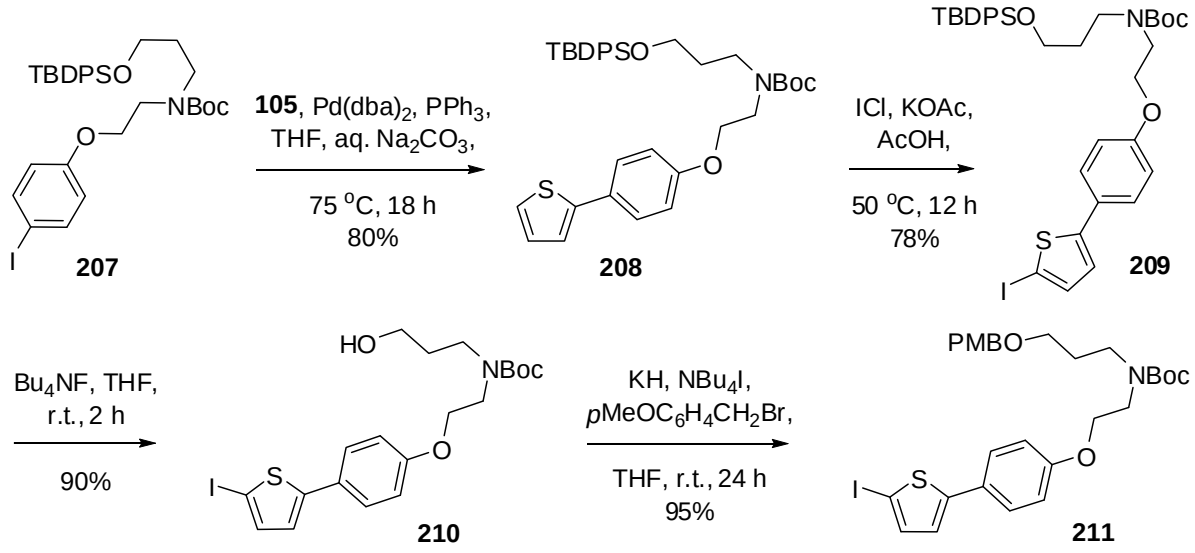
Scheme 3.5. Synthesis of the "left hand" precursor **201** of the photochromic unit.

Synthesis of the precursor of the "right hand" part of the photochromic unit is depicted in Scheme 3.6. The phenoxyethylamine derivative **121** was alkylated with allyl bromide, and the terminal double bond of **204** was subjected to hydroboration with 9-BBN followed by oxidation.[89] The alcohol **205** was protected with a TBDPS group, and the compound **206** was iodinated with bis(trifluoroacetoxy)iodobenzene.



Scheme 3.6. Transformations towards the intermediate **207**.

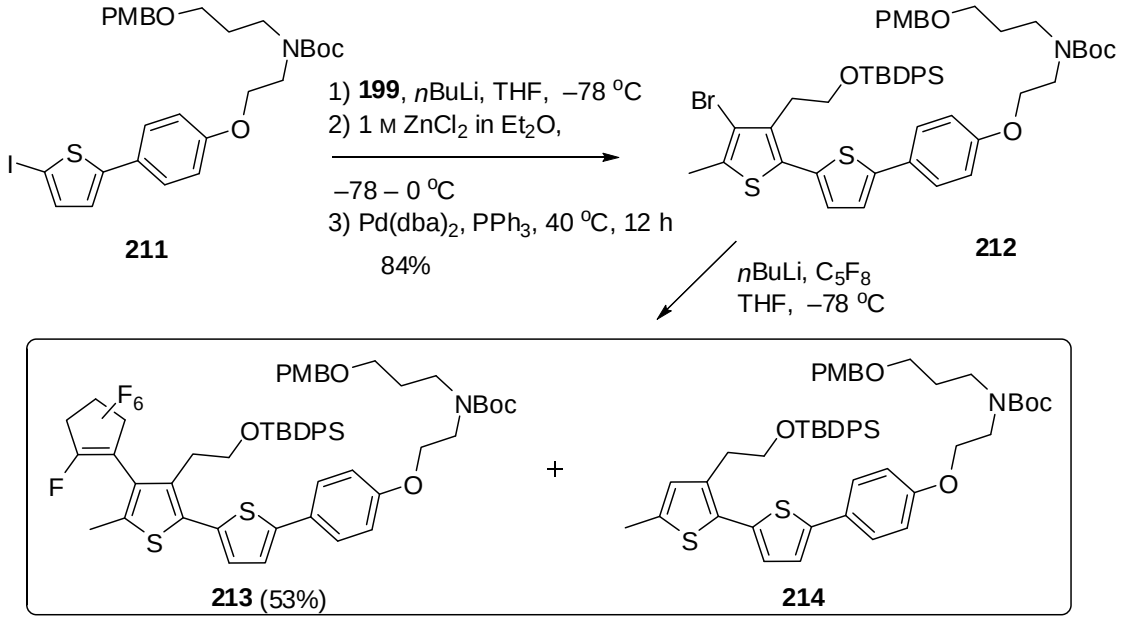
The iodide **207** was coupled with thiophene-2-boronic acid (**105**), and compound **208** was further iodinated with ICl in acetic acid to afford **209** in good yield (Scheme 3.7). At this stage, the hydroxy group was deblocked in order to change the protecting group and purify the intermediate substance, which turned to be a solid.



Scheme 3.7. Transformations towards the "right hand" precursor of the photochromic unit.

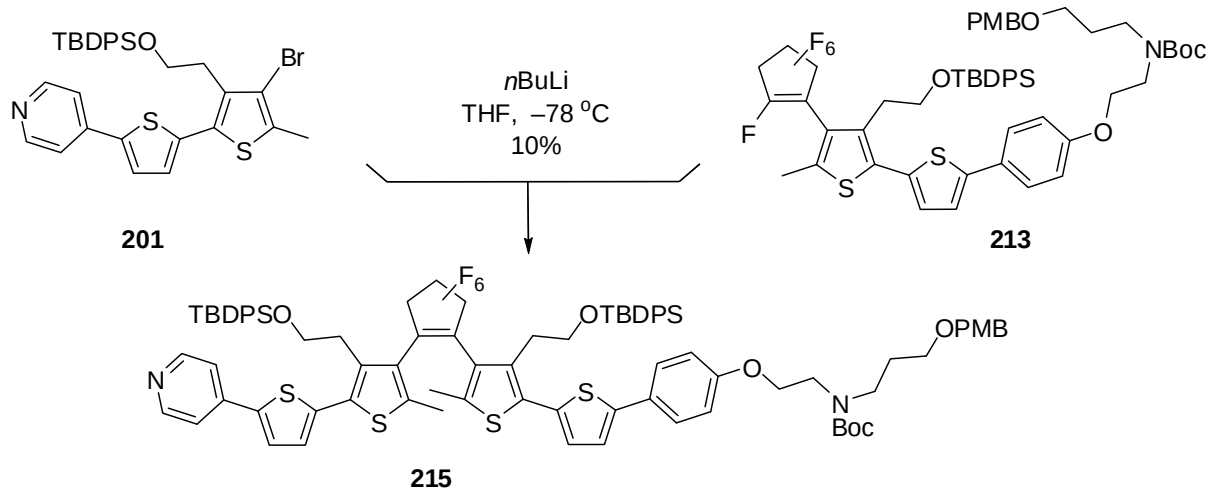
The hydroxy group in compound **210** was protected with *p*-methoxybenzyl chloride, and the biaryl **211** was coupled with the Zn derivative of **199** in the presence of a palladium catalyst.

The triaryl compound **212** was isolated in good yield (Scheme 3.8). Unfortunately, after lithiation of **212** and reaction with an excess of perfluorocyclopentene, the required heptafluorocyclopentene derivative **213** was obtained as an inseparable mixture with the dehalogenated derivative **214**.



Scheme 3.8. Towards the heptafluorocyclopentene derivative **213** – the "right hand" precursor of the photochromic compound.

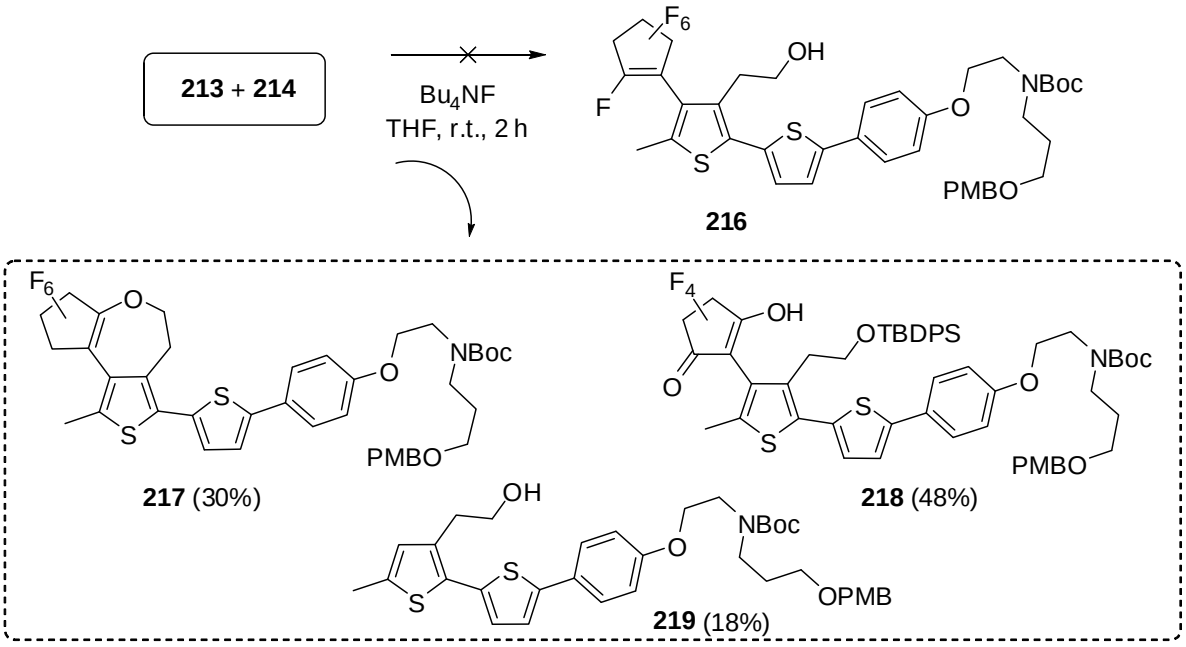
The reaction of the lithiated derivative of **201** with this mixture gave the required photochromic compound **215** in very low yield (Scheme 3.9).



Scheme 3.9. Photochromic compound **215** with protected hydroxy groups.

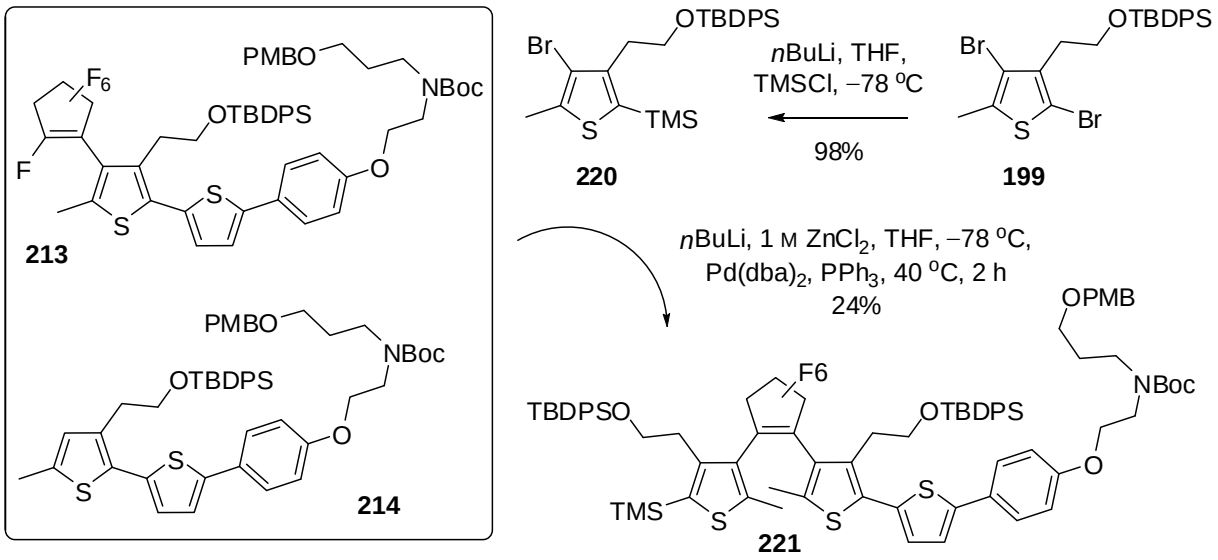
Deprotection of the hydroxy groups might help to separate the corresponding alcohols and obtain the compound **216** (Scheme 3.10). Unfortunately, deprotection was accompanied by the

loss of one or three fluorine atoms and formation of a mixture of compounds **217**, **218**, and **219**. A fluorine atom was substituted by the adjacent hydroxy group, and a seven-membered ring was formed to give compound **217**.



Scheme 3.10. Cleavage of the TBDPS-protecting group in compound **213**.

The same mixture of **213** plus **214** and the new bromide **220** (which was obtained by lithiation of compound **199** followed by substitution with trimethylsilyl chloride) gave the photochromic adduct **221** in low yield (Scheme 3.11). These synthetic difficulties forced us to undertake the priority 2 modifications.

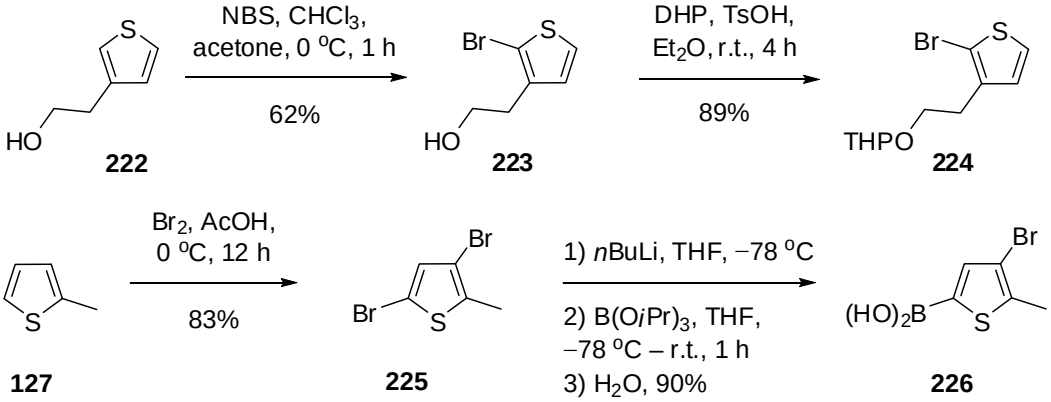


Scheme 3.11. Synthesis of the photochromic compound **221**.

3.3.2. Synthesis of the photochromic unit according to priority 2 modifications

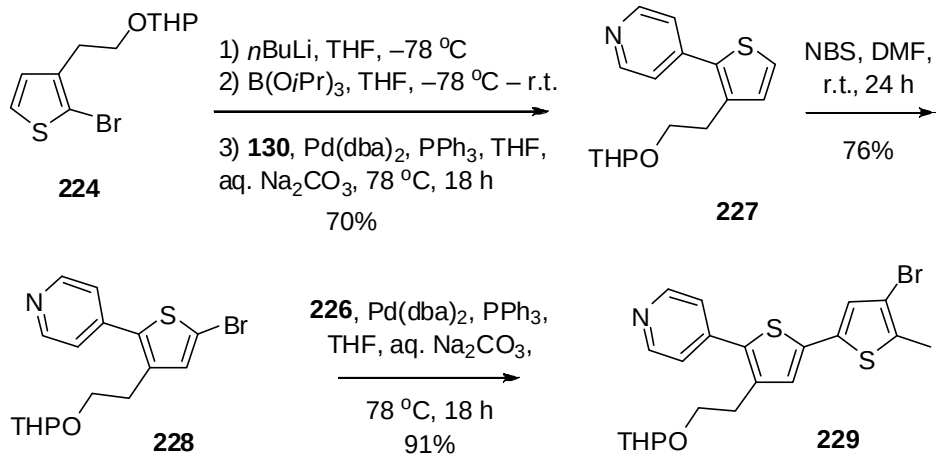
The modifications of the second type are free from the drawbacks mentioned above. The hydroxy groups are now far away from the central fluorinated part of the photochromic unit.

As a starting compound with an additional functional group, we used 3-(2-hydroxyethyl)thiophene (**222**), which was selectively brominated with NBS in the second position of the thiophene ring. Then the hydroxy group in compound **223** was protected with a tetrahydropyranyl residue (Scheme 3.12). The reversed order of reactions (protection of hydroxy groups followed by bromination according to known procedures[90]) in our case led to lower overall yields. 2-Methylthiophene (**127**) was used as a second thiophene fragment, which after bromination in acetic acid and monolithiation followed by addition of $B(OiPr)_3$, afforded the boronic acid **226** in good yield.



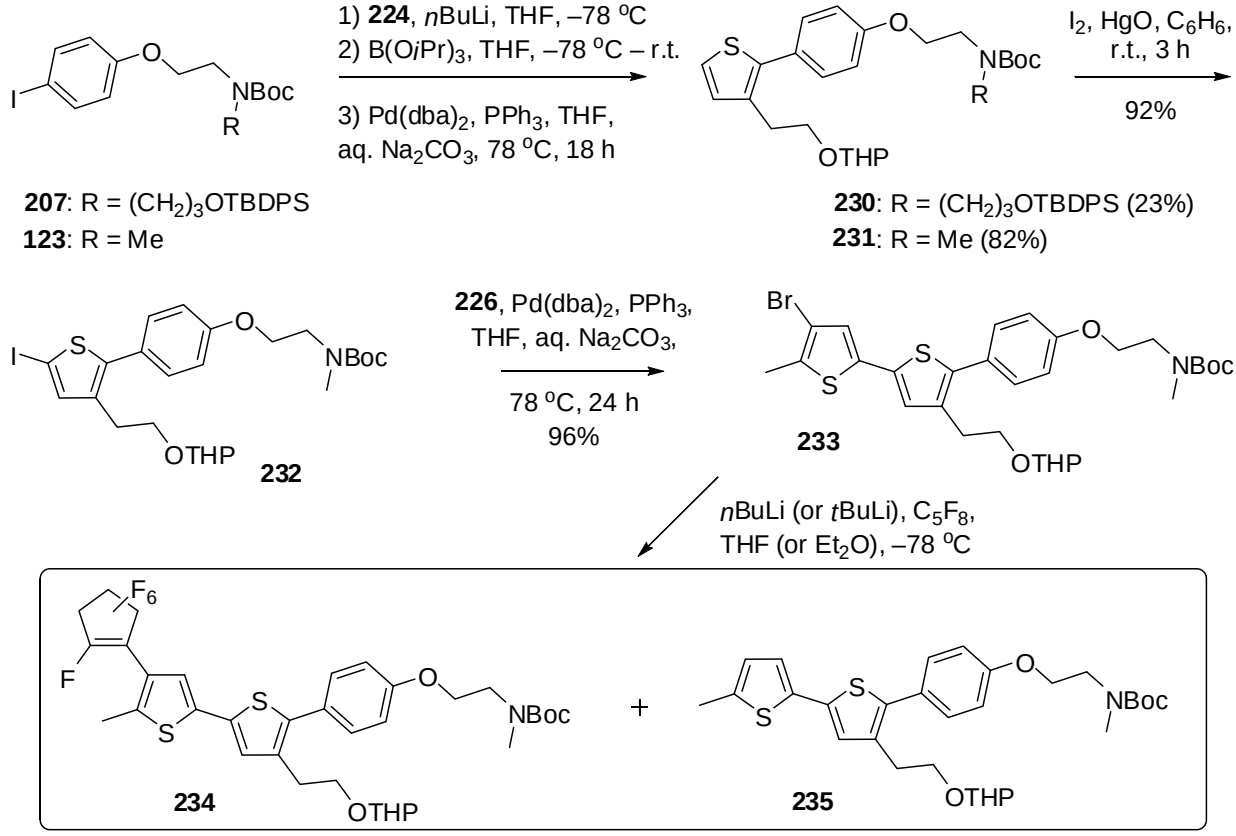
Scheme 3.12. Thiophene derivatives **224** and **226** as small building blocks.

The bromothiophene **224** was also transformed into the corresponding boronic acid and used in the Suzuki coupling with 4-bromopyridine (**130**) (Scheme 3.13). Attempted iodination of the isolated compound **227** with iodine with either periodic acid in ethanol or bis(trifluoroacetoxy)iodobenzene in chloroform or 80% aq. acetic acid was unsuccessful and gave only the starting material or the deprotected compound (in the last case). The reaction of **227** with BuLi in the presence of TMEDA followed by addition of CBr_4[91] did not lead to the desired compound **228**. Fortunately, compound **227** could be brominated with NBS, and the bromide **228** was coupled with thiopheneboronic acid **226**. The pure building block **229** was isolated by silica gel chromatography.



Scheme 3.13. The"left hand" building block **229** of the photochromic unit.

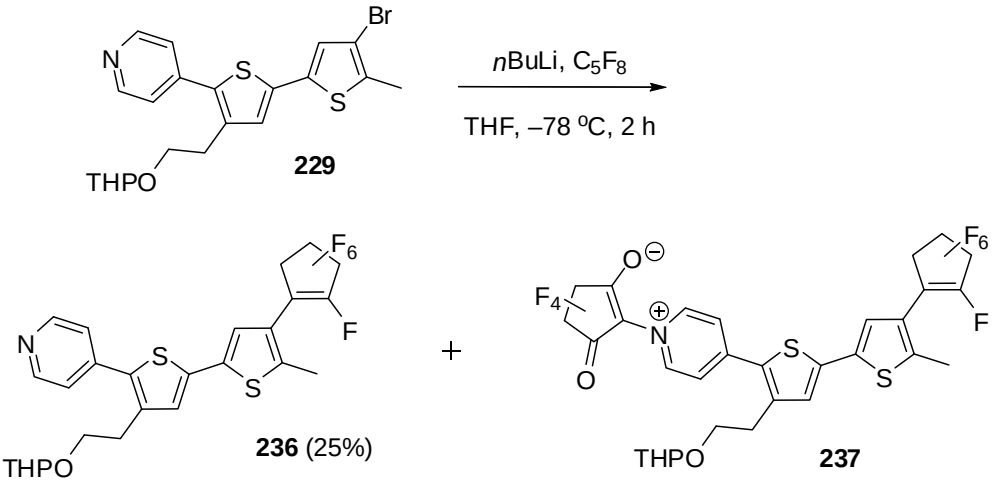
First, we coupled the aryl iodide **207** with the bromothiophene **224**, but the yield of compound **230** was low. Therefore, the aryl iodide **123** was first transformed to compound **231** by a Suzuki coupling with the boronic acid obtained from bromothiophene **224** (Scheme 3.14). Then compound **231** was iodinated with I_2 and HgO in benzene, and iodide **232** was isolated in high yield. After that, it was coupled with 3-bromo-2-methylthiophene-5-boronic acid (**226**) to give the "right hand" precursor **233** in very good yield.



Scheme 3.14. "Right hand" precursor **233** and the products isolated after reaction with perfluorocyclopentene.

Both the "right" and the "left hand" precursors **229** and **233** were subjected to halogen-lithium exchange followed by treatment with an excess of perfluorocyclopentene. In the first case, heptafluorocyclopentene derivative **234** could not be separated from the dehalogenated compound **235** (Scheme 3.14). In the second case, the yield of the required heptafluorocyclopentene **236** was reduced due to a side reaction, which furnished the highly fluorescent compound **237** (Scheme 3.15). The by-product **237** absorbs at 440 nm (chloroform), and emits at 502 nm (with a shoulder at 530 nm, exited at 420 nm). The fluorescence quantum yield was found to be as high as 0.51 in chloroform.

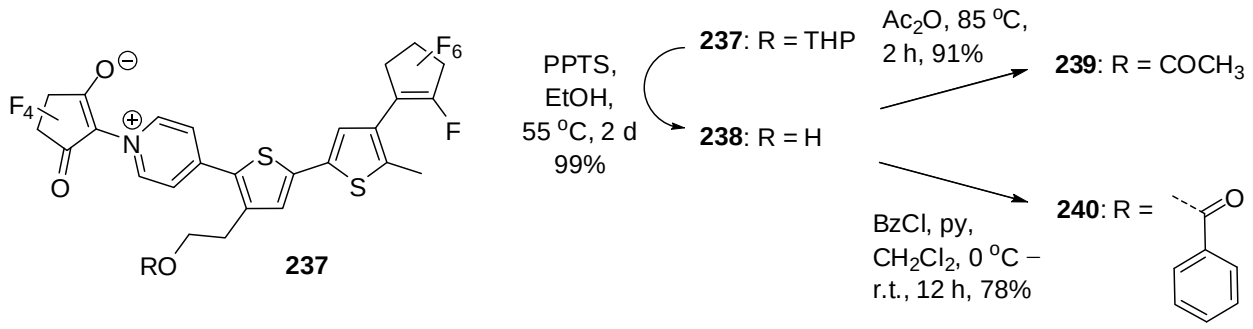
Although this side process was not observed at –78 °C, and the reaction was quenched at this temperature, the same by-product was obtained during the work-up procedure or isolation of the compound **236** (on SiO_2), or even when the isolated compound was stored at temperatures higher than 0 °C. Therefore, the compound **236** was used immediately after isolation or kept under argon at –18 °C.



Scheme 3.15. Synthesis of the heptafluorocyclopentene derivative **236** (and the side-product **237**).

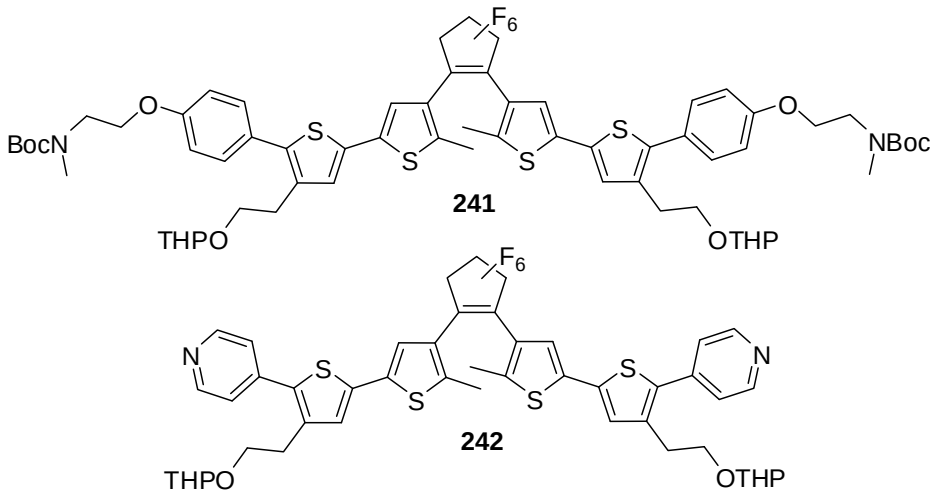
The formation of the side-product **237** with betaine structure may easily be explained by the presence of an excess of perfluorocyclopentene in the reaction mixture. This perfluoroalkene adds the pyridine fragment, and the following step-wise hydrolytic substitution of the fluorine atoms at the activated positions (in the presence of water during the work-up and isolation procedures) leads to the final betaine.[92]

Trying to prove the structure of the interesting substance **237** by an X-ray analysis, we deprotected the hydroxy group of compound **237**, and prepared the acetic and benzoic acid esters **239** and **240** from the alcohol **238** (Scheme 3.16).

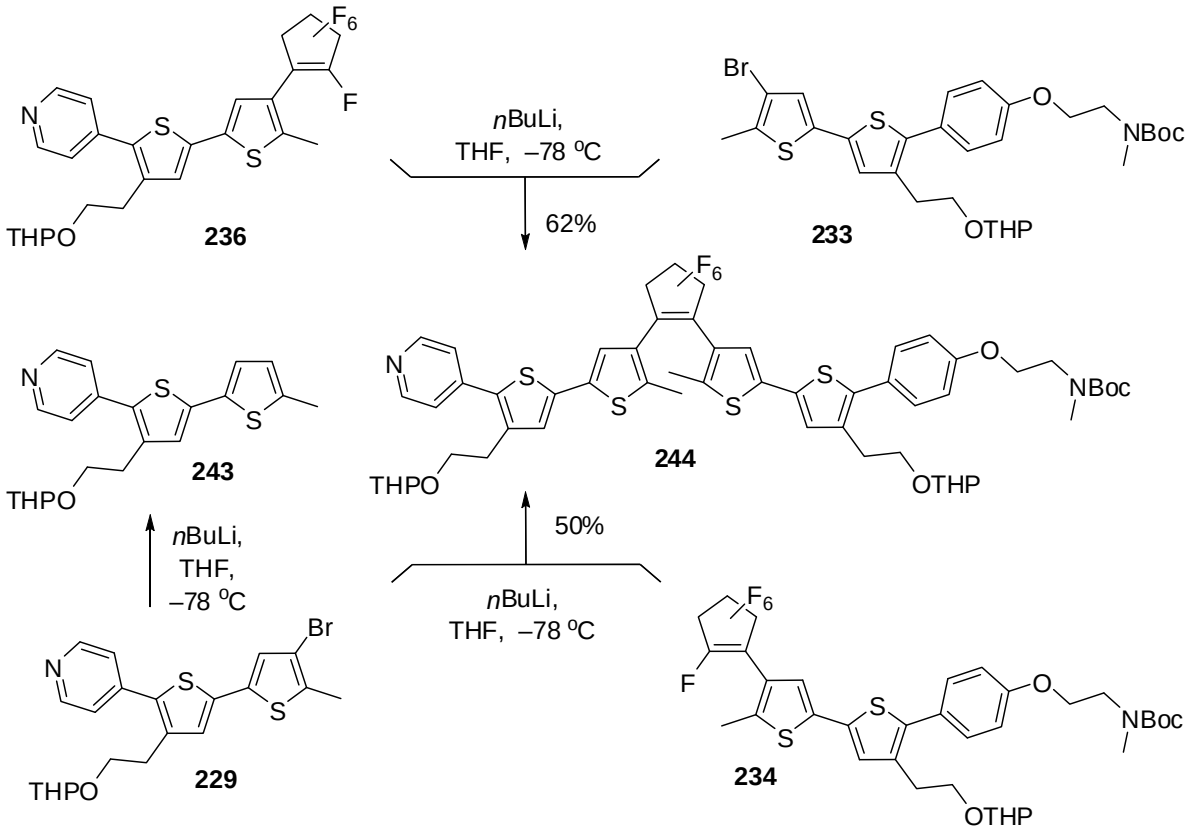


Scheme 3.16. Modifications of compound **238**.

In both reactions towards heptafluorocyclopentenes **234** (Scheme 3.14) and **236** (Scheme 3.15), symmetric photochromic compounds **241** and **242** were also isolated as by-products.

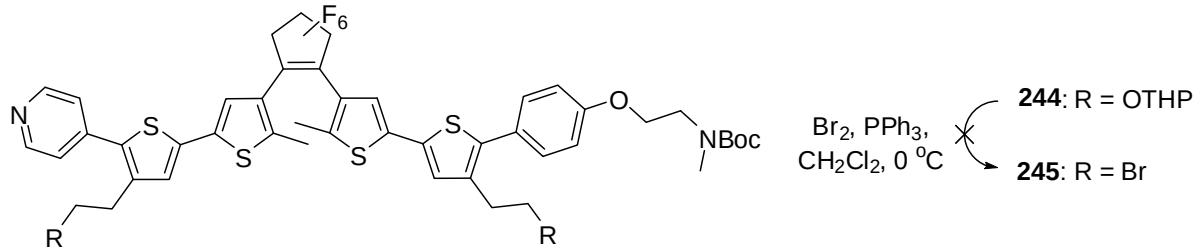


Coupling of the heptafluorocyclopentene **236** with the lithiated derivative of compound **233** gave the required photochromic compound **244** in good yield (Scheme 3.17), while the reaction of heptafluorocyclopentene **234** with bromide **229** (after its lithiation) resulted in a mixture of the photochromic compound **244** and the degalogenated derivative **243** with a very similar R_f value.



Scheme 3.17. Synthesis of the non-symmetric photochromic compound **244** with protected hydroxy groups.

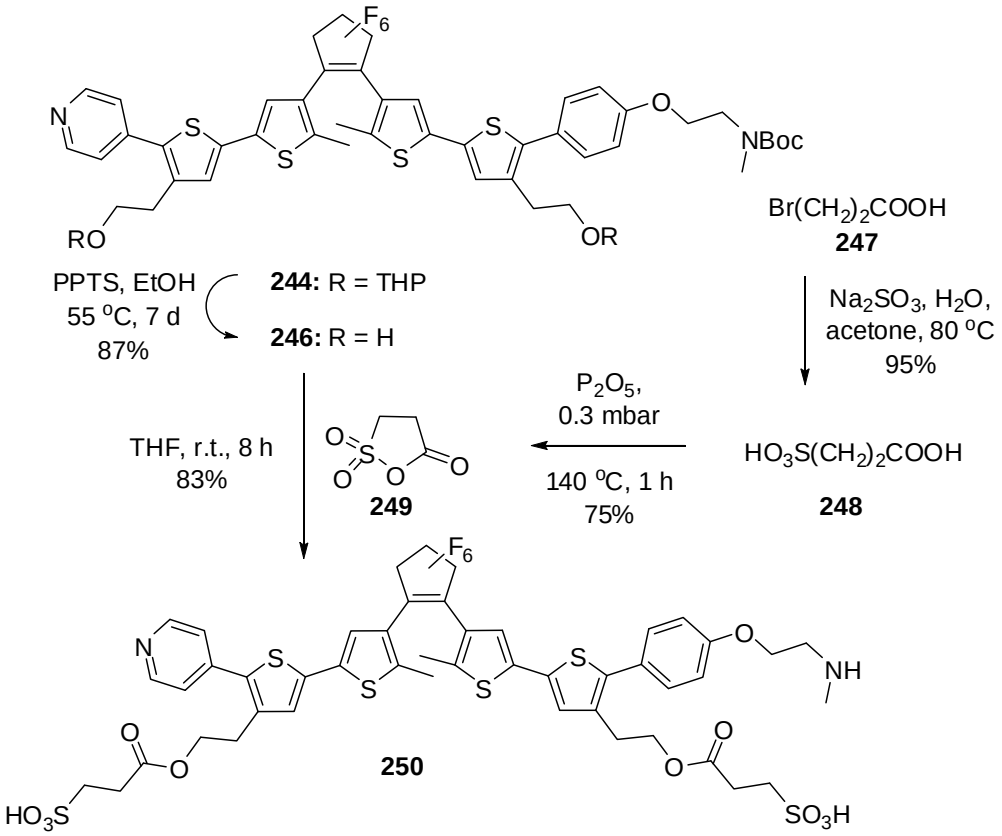
In trying to introduce sulfonic acid residues by nucleophilic substitution of bromine atoms, the THP-protecting groups in compound **244** were subjected to the reaction with bromine and PPh_3 (Scheme 3.18). Unfortunately, the required dibromide **245** could not be isolated from the reaction mixture.



Scheme 3.18. An attempt to synthesize the dibromo derivative **245**.

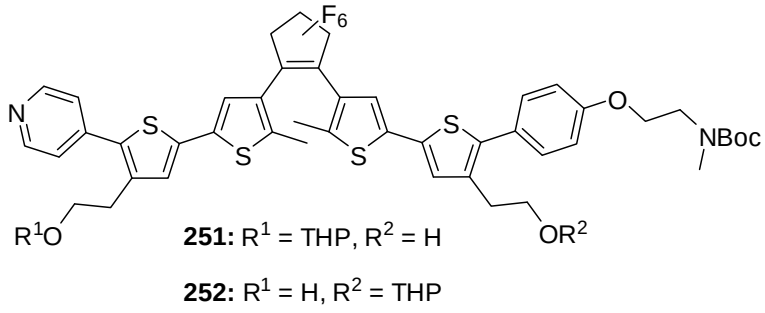
Therefore, the THP-protecting groups in the photochromic compound **244** were cleaved off under mild conditions using PPTS in ethanol. Then the liberated hydroxy groups in compound **246** were acylated with β-sulfopropionic anhydride (**249**) (Scheme 3.19). The latter was obtained by dehydration of β-sulfopropionic acid (**248**) with phosphorus pentoxide,[93] which was obtained from 3-bromopropionic acid (**247**) by stirring with Na_2SO_3 in aqueous acetone. The

Boc-protecting group in compound **246** was also cleaved off at this stage, and the water-soluble photochromic compound **250** was isolated in good yield.

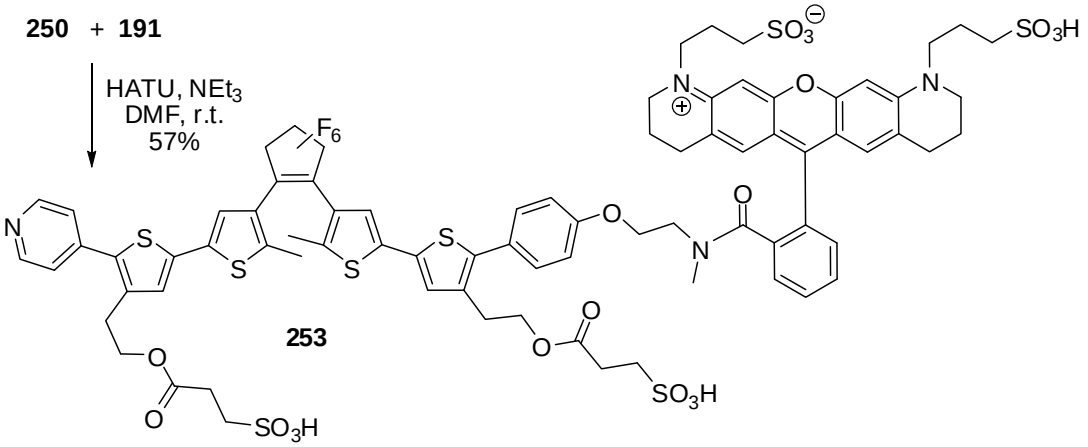


Scheme 3.19. Synthesis of the final photochromic and water-soluble building block **250** with a free amino group.

Shortening of the reaction time during deprotection of compound **244** led to a lower conversion of the substrate, and two intermediate products **251** and **252** were isolated.



The photochromic compound **250** was coupled with the hydrophilic fluorescent dye **191** (after its activation with HATU in the presence of NEt_3) to provide the fluorescent photochromic compound **253** with a highly enhanced water solubility and good performance in aqueous media (Scheme 3.20).



Scheme 3.20. Water-soluble fluorescent photochromic compound **253**.

3.3.3. Properties of the water-soluble photochromic diarylethenes prepared in this study

The highly water-soluble photochromic compound **250** may be switched to the colored closed form by irradiation with UV or violet light (365–375 nm) (Figure 3.4). The colorless open-ring isomer of compound **250** has an absorption maximum at 342 nm (MeOH or water). Irradiation with 366 nm or 375 nm light transforms it to the blue-colored closed form with a broad absorption band around 630 nm in methanol and water. Irradiation with visible light (>500 nm) restores the initial absorption spectrum of the photochromic compound.

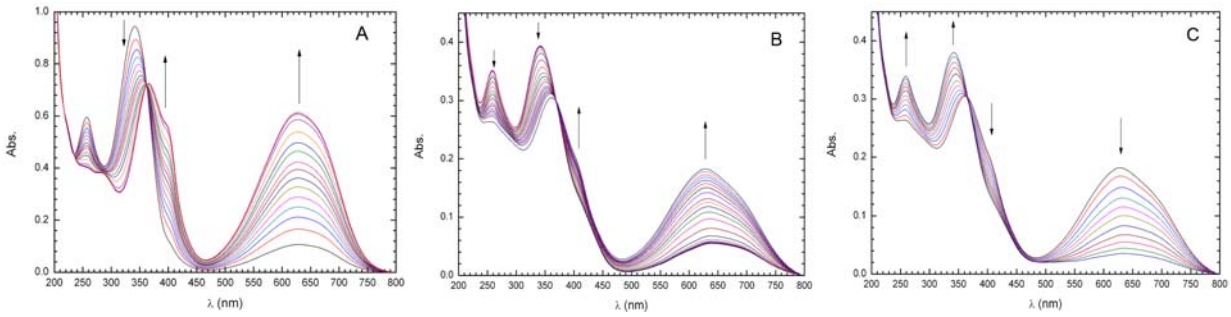


Figure 3.4. Absorption spectra of compound **250** undergoing photoconversion between the open- and closed-ring isomers upon irradiation at 366 nm in MeOH (A) and in water (B). The reverse reaction upon irradiation at 550 nm in water (C). The arrows indicate changes upon UV (A and B) and VIS (C) irradiation.

It is interesting, that the cyclization of the photochromic compound **250** proceedes in methanol and water with very different rates. The kinetics of the ring-closing reaction in methanol and water upon irradiation with UV light is given in Figure 3.5. In methanol the full conversion to the closed form was achieved in 5 min, but in water the rate was very slow, and the absorption signal at 630 nm was still "growing up" during irradiation for 1.5 h. However, in

future applications the irradiation cycles will be performed with two lasers (not with a lamp), so that their rates are expected to be enchanced.

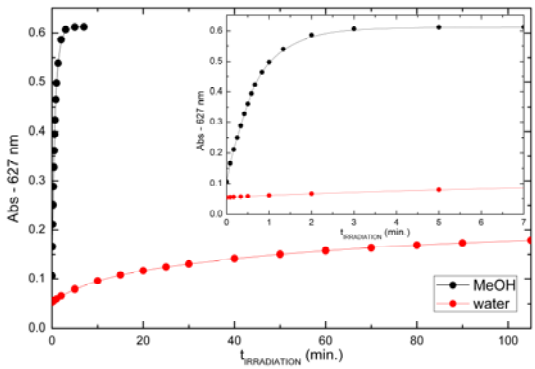


Figure 3.5. Kinetics of the cyclization reaction of compound **250** in MeOH (black spots) and water (red spots) under irradiation with 366 nm light.

The compound **246** with two hydroxy groups was found to be soluble in water to some extent. In methanol, this compound displayed normal photochromic behaviour (Scheme 3.6). Absorption maxima of compound **246** in methanol are the same as those observed for compound **250**: 342 nm and 630 nm for open and closed forms, respectively. In water, the open-ring isomer of **246** absorbed at 350 nm, and irradiation with 366 nm light converts it to the closed form with a broad absorption band at 630 nm. However, irradiation of the closed form with visible light (550 nm) in water led to precipitation of compound **246** as a blue solid.

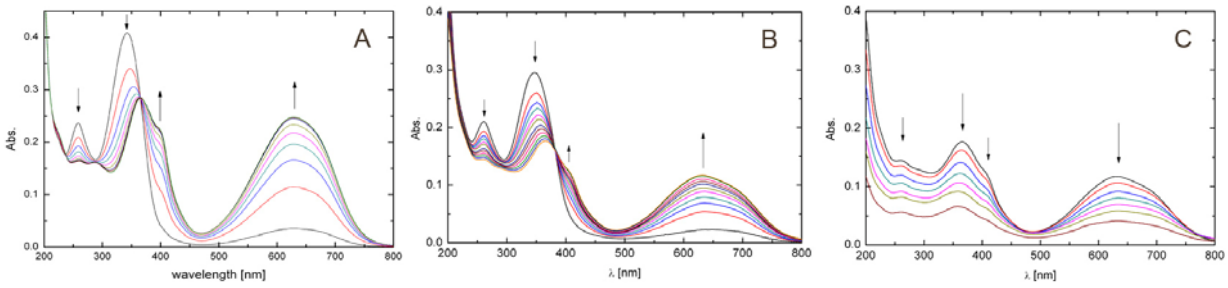


Figure 3.6. Absorption spectra of compound **246** undergoing photoconversion between the open- and closed-ring isomers upon irradiation at 366 nm in MeOH (A) and in water (B). The irradiation at 550 nm in water (C) was accompanied by precipitation of the compound **246**. The arrows indicate changes upon UV (A and B) and VIS (C) irradiation.

Chapter 3. Fluorescent derivatives of the GM1 ganglioside and their use in single molecule studies

1. Structure of ganglioside GM1 and the fluorescent ganglioside GM1 derivatives

Gangliosides[94] are glycosphingolipids with sialic (*N*-acetylneuraminic) acid residues. They are components of the plasma membranes of mammalian cells and are particularly abundant in neuronal membranes. Gangliosides consist of a hydrophobic ceramide moiety and hydrophilic oligosaccharide chain. Ceramide moiety contains the sphingosine residue acylated with a long fatty acid chain (mainly C18). Carbohydrate chain is structurally very variable and contains at least one *N*-acetylneuraminic (sialic) acid residue. The structure of the representative ganglioside GM1 is shown in Figure 1.1.*

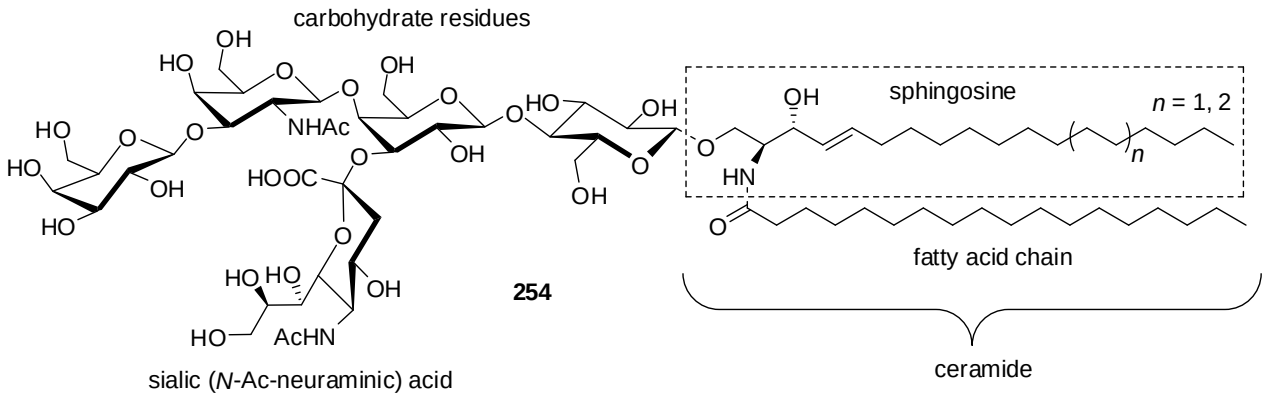


Figure 1.1. Ganglioside GM1

Ganglioside derivatives with radioactive,[95] photoreactive,[96] paramagnetic[97] and fluorescent labels are of great importance and have been used in studies of cell surfaces as well as processes occurring on them.

The single-molecule based studies of lipid dynamics such as fluorescence correlation spectroscopy (FCS) or its combination with super-resolution stimulated emission depletion (STED) far-field fluorescence microscopy[4] require bright and photostable fluorescent labels. Therefore, it is of utmost importance to develop a ganglioside-labeling stategy that provides high flexibility and an optimized procedure with a minimal bias.

Various parts of the GM1 molecule have previously been decorated with different fluorescent dyes (Figure 1.2). Possible labelling sites include the sialic acid residue (a,b), the

* The starting natural ganglioside GM1 we used is a mixture of two compounds which differ in their lipid composition. Their long chain base consists of C_{18} and C_{20} *erythro*-sphingosines in approximately equal amounts.

amino group of the sphingosine fragment (c), and the fatty acid chain (d,e). Chemical modifications of the GM1 molecule are required for the introduction of labelles.

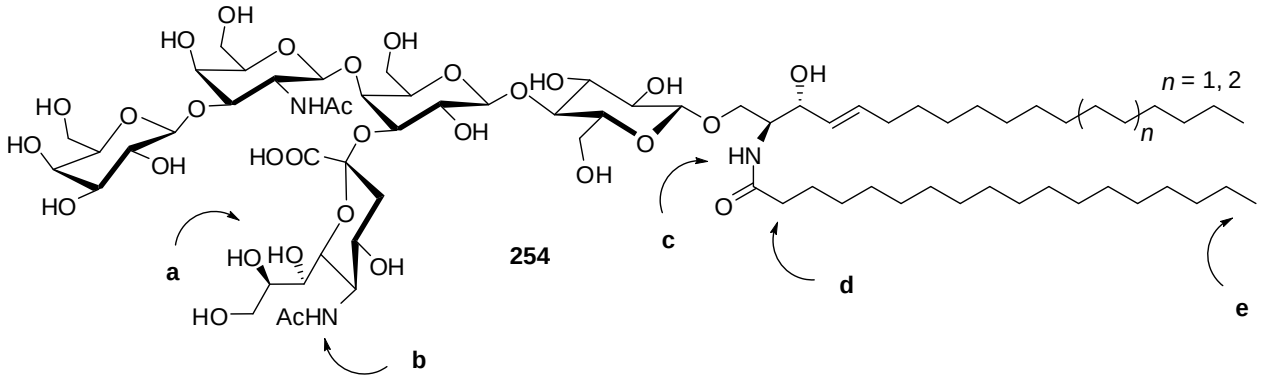
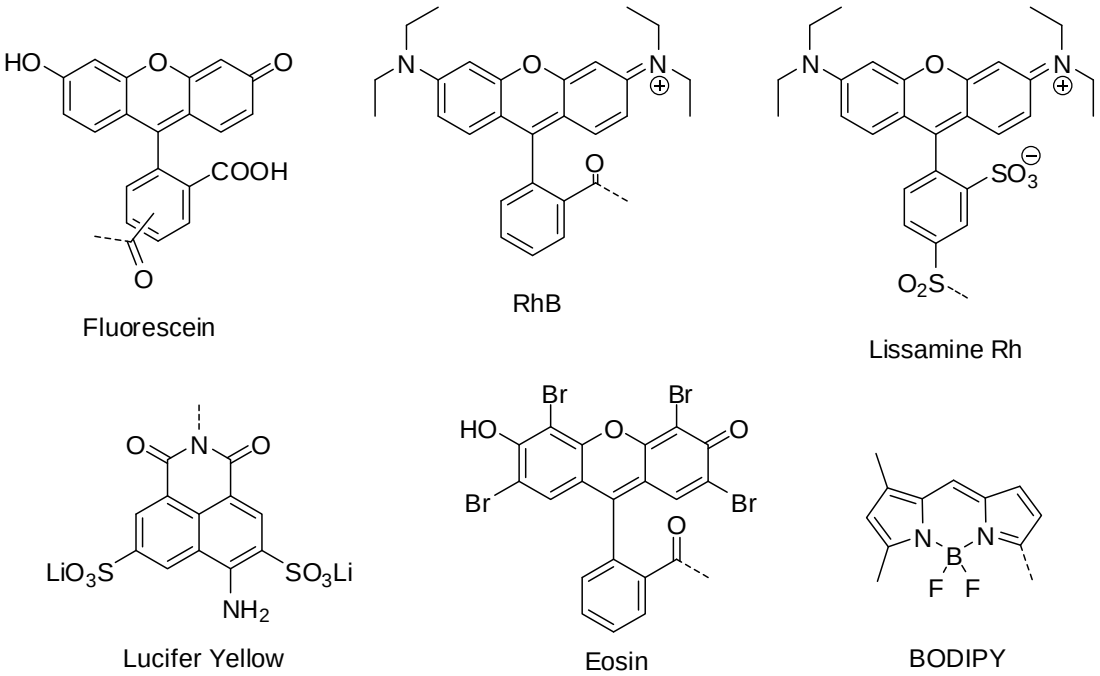


Figure 1.2. Positions for labelling the ganglioside with fluorescent probes.

The sialic acid residue is a convenient labelling site in gangliosides. It is possible to chemically modify the sialic acid residues directly on the surfaces of living cells for attaching the markers. Numerous early reports deal with an oxidative cleavage of the CH(OH)CH(OH) group in the sialic acid residue to an aldehyde followed by the reaction with hydrazides of fluoresceine and rhodamine B,[98] lissamine rhodamine,[98a,99] Lucifer yellow CH,[99,100] and eosin.[101]

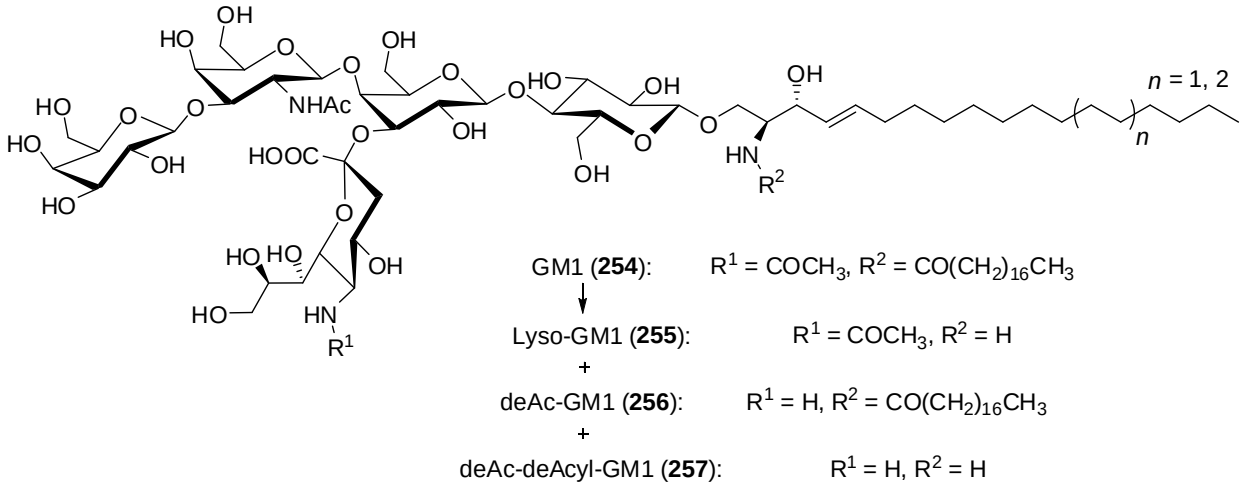


The ganglioside lacking one acetyl group at the sialic acid moiety (with the free amino group of the neuraminic acid) has been labelled with the BODIPY dye.[102]

Another possibility is to modify or even to replace the natural ceramide residue of gangliosides. The lysoganglioside GM1 (lyso-GM1) was used as a starting material for these

(and other) modifications. It lacks the natural fatty acid chain and has a free amino group in the sphingosine residue (Scheme 1.1). Lyso-GM1 could be prepared from the natural ganglioside GM1 either by enzymatic methods,[103] or chemically.[95a,104]

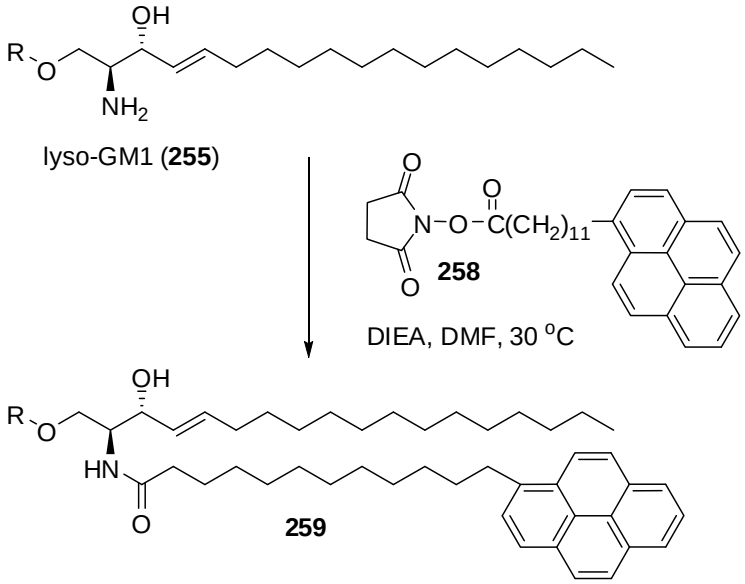
In 1992 Sonnino *et al.* reported the one-step reaction for the synthesis of lyso-GM1. Alkaline hydrolysis of the GM1 ganglioside with KOH in *n*-propanol in the absence of oxygen results in the cleavage of the long fatty acid acyl chain with a high degree of selectivity[104b] (Scheme 1.1). Two minor products – deAc-GM1 (**256**) with free amino group in neuraminic acid and deAc-deAcyl-GM1 (**257**) with free amino groups in both neuraminic acid and sphingosine – were also isolated.



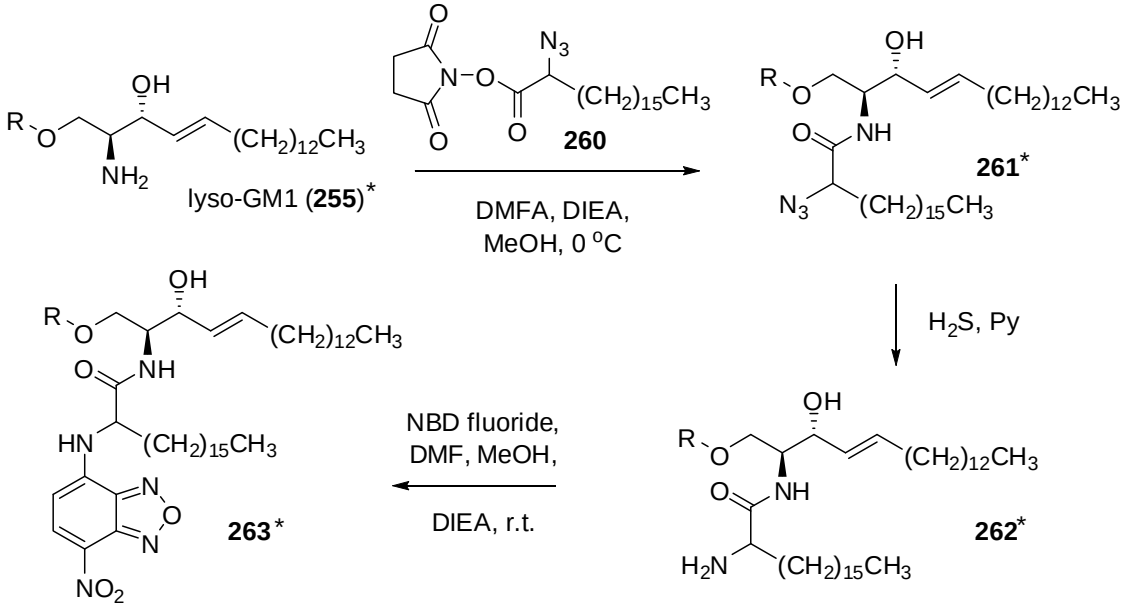
Scheme 1.1. Products of the alkaline hydrolysis of the GM1 ganglioside according to Sonnino.[104b]

Lyso-GM1 (**255**) was used for the synthesis of the GM1 derivatives with fluorescent labels directly attached to the free amino group in the sphingosine residue.[105] In this case a fluorophore mimics the native long acyl chain.

Another option is to decorate the fatty acid chain of the ganglioside molecule with a fluorescent dye. Though the latter approach is more technically sophisticated, it is more flexible on respect of positioning the fluorescent dye, and it formally keeps the global structure of the natural GM1 intact. Obviously, the final goal can be achieved by two different approaches: either the selected position of the fatty acid is first labelled with a fluorophore, and then the whole intermediate is coupled with the amino group in lyso-GM1 (Scheme 1.2), or, alternatively, an activated fatty acid with a binding site is coupled with lyso-GM1 followed by reaction with an activated fluorescent dye (Scheme 1.3).



Scheme 1.2. Synthesis of the pyrene-labelled GM1 ganglioside **259** according to Schwarzmann.[95a]

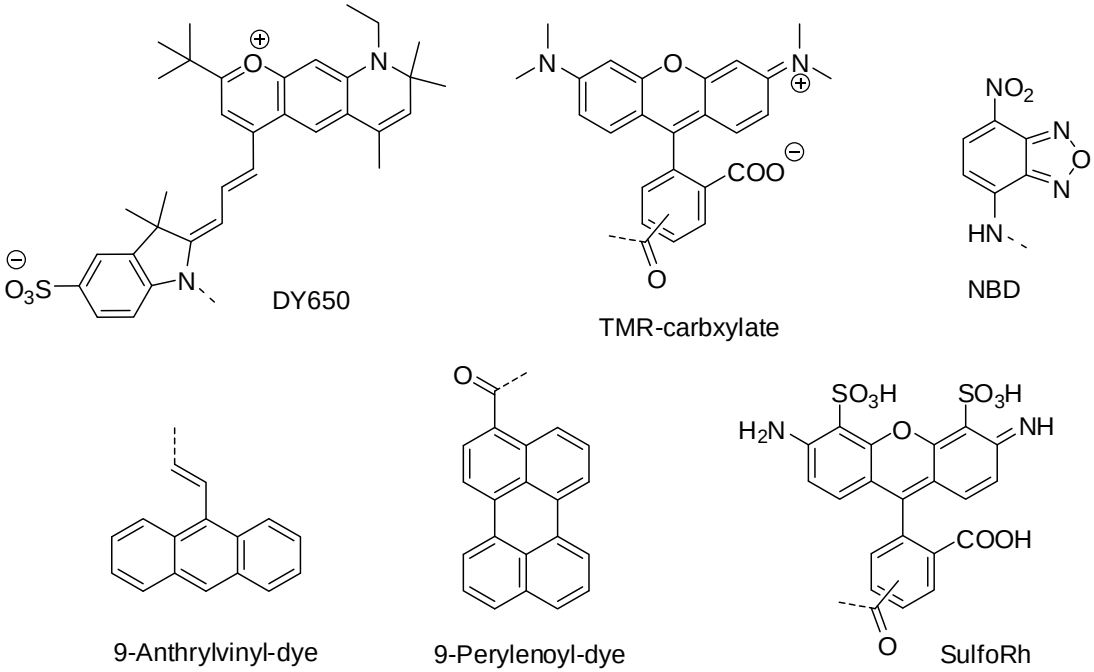


Scheme 1.3. Synthesis of the NBD-labelled GM1 ganglioside **263*** according to Schwarzmann.[106]

High cost of lyso-GM1 makes the first approach attractive, as this precious compound is used only at the last crucial step. Lyso-GM1 has been bound via a linker with up to 12 carbon atoms with the following fluorescent dyes: pyrene,[95a] tetramethylrhodamine,[107] NBD,[108] "dark-red" fluorophore (DY650),[109] 9-anthrylvinyl- and 3-perylenoyl-fluorophores[110] as well

* Ganglioside derivatives contains ^{14}C-labelled acetyl group in the sialic acid residue.

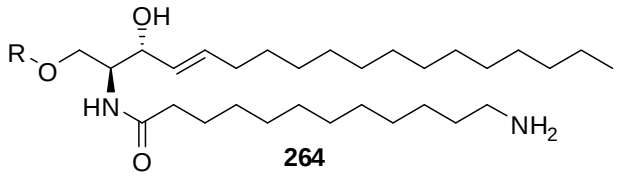
as various BODIPY-derivatives.[111] In these compounds a linker is important fragment, as it mimics the natural fatty acid chain.



In similar manner, compound deAc-deAcyl-GM1 has been selectively labelled with activated pyrene-decanoic acid[97] or BODIPY-FL-pentanoic acid[102b] followed by the reacylation of the neuraminic acid with acetic anhydride in order to obtain the fluorescent GM1 derivatives.

Another approach was reported in 2005, when Schwarzmann *et al.* coupled activated 2-azidostearic acid (**260**) with lyso-GM1, reduced the azido group with H_2S and treated the corresponding amine with the reactive NBD fluoride, thus creating the NBD fluorophore in the α-position of the stearic acid residue in the native GM1 (Scheme 1.3). In this procedure, the valuable lyso-GM1 was used in an earlier step, after which two more steps followed.

Using 12-aminodecanoic acid protected with the Fmoc-group[96] or CF_3CO- group[112] in the coupling reaction with lyso-GM1 (or deAc-deAcyl-GM1) followed by deprotection (after reacylation in the case of deAc-deAcyl-GM1) resulted in the ganglioside derivative **264** with the free amino group in the ω-position of the fatty acid chain. This compound has been decorated with sulforhodamine.[113]



All reported modifications (except [102] and [106]) did not leave the GM1 molecule intact, but either changed the sialic acid residue, or shortened the natural C18 acyl chain. Moreover, most of the above mentioned fluorescent dyes are not photostable enough for the super high-resolution microscopy in cells.

2. Synthesis of ganglioside derivatives with α- or ω-amino- and ω-mercaptostearic acid residues

The goal of this part of work was to develop the synthesis of the nowel ganglioside GM1 derivatives labelled with photo- and chemically stable fluorescent dyes, suitable for the harsh STED/FCS irradiation conditions. For that, it was neccessary first to synthesize the suitable precursors and to work-out optimized labelling strategy.

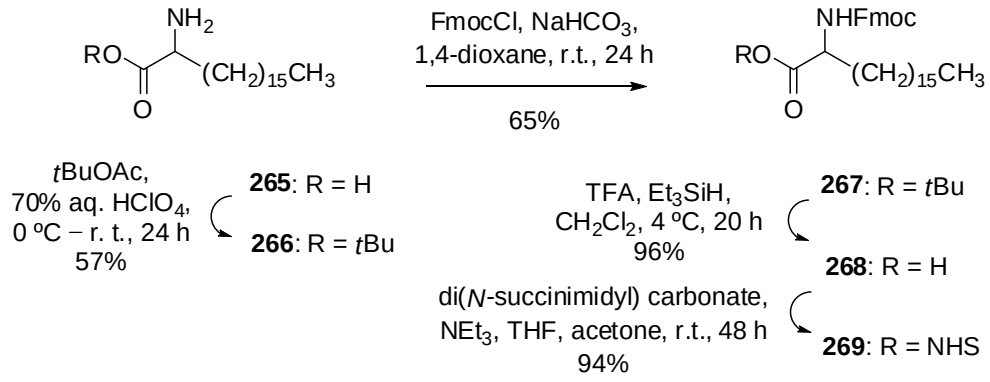
We embarked on the synthesis of the ganglioside GM1 derivatives, which contain its natural structure, with an only slightly modified ceramide residue. Supplementary amino and thiol groups at the α- or ω-positions of the stearic acid chain allow the simple one-step labelling of the ganglioside, using activated fluorescent dyes of different polarities.

The α-position of the stearic acid residue is favorable both for the attachment of hydrophilic and lipophilic dyes. The former may be embedded into the polar domain containing the hydrated "head" groups that are not far away from the beginning of the acyl chains. To provide a certain orientational freedom, the polar hydrophilic labels should be attached to the α-position with a linker. A lipophilic label at the α-position of the stearic acid residue is expected to be incorporated into the non-polar domain of the lipid bilayer without perturbation. Derivatization of the α-position creates an additional stereogenic center, and therefore two diastereomeric GM1 derivatives are generally obtained. They may differ in their chromatographic mobility, and the interpretation of the analytical data may be not as easy as in the case of a single diastereomer. Shifting the labelling position to the end of the C_{18} chain does not only remove all complications associated with the presence of two diastereomers, but also secures the position of a fluorophore within the non-polar lipid domain.

In the present study, we introduce ganglioside GM1 derivatives with the *N*-protected α- and ω-aminostearic acids, as well as with the *S*-protected ω-mercaptostearic acid that, after deblocking, may provide additional labelling sites for the amino- and thiol-reactive fluorophores. However, polar dyes are not recommended for the "ω-labelling" of the ceramide moiety, as they may cause "looping" and perturbation of the lipid bilayer.

The *N*-(9-fluorenylmethyl)oxycarbonyl (Fmoc) group is a good protection for that purpose, as it may be removed by treatment with diluted solutions of piperidine or NH_3 in DMF or methanol, and these reagents do not react with any other functional groups in GM1.[96]

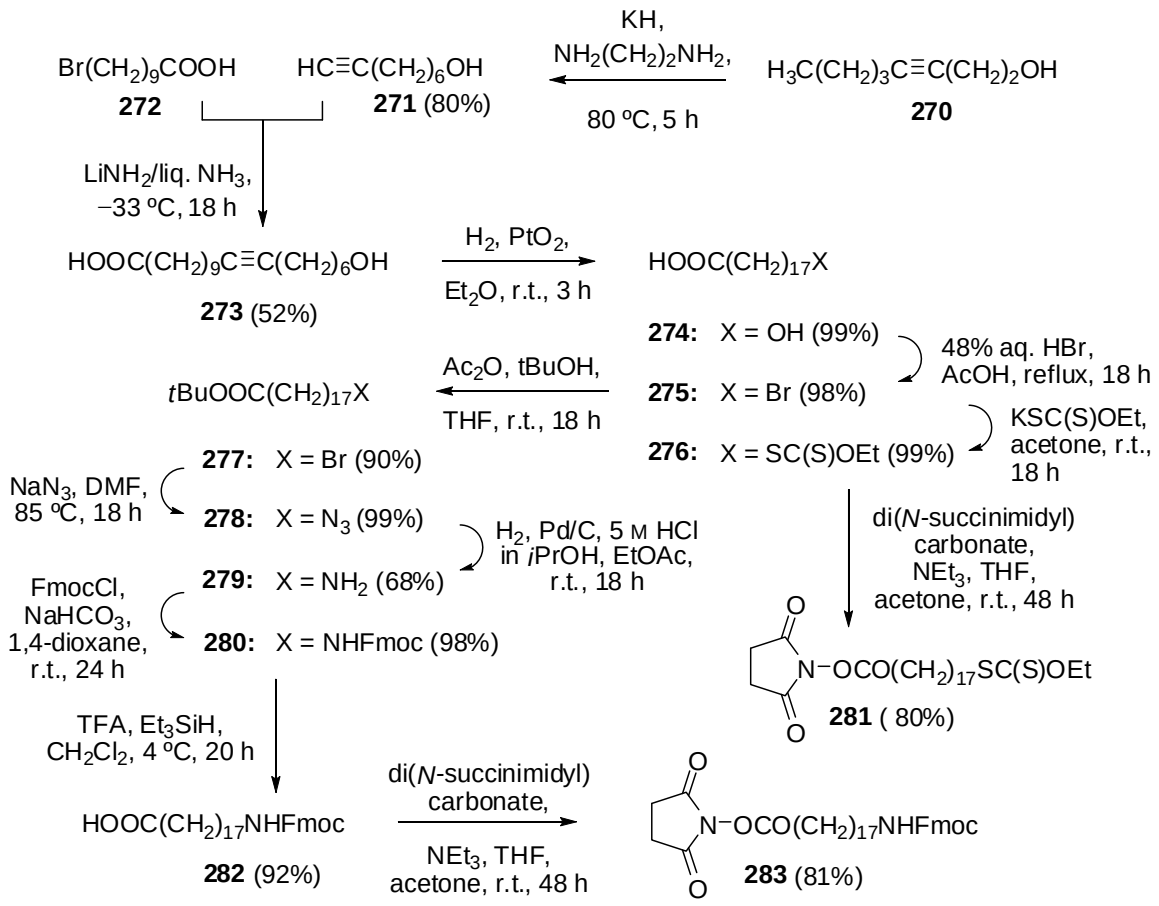
The synthesis of the NHS ester of the Fmoc-protected α-aminostearic acid **269** is shown in Scheme 2.1. Aminostearic acid **265** synthesized in our laboratory in sufficient amounts was subjected to the reaction with *tert*-butyl acetate in the presence of $HClO_4$. The *tert*-butyl ester **266** obtained was treated with FmocCl in dioxane, then the α-carboxy group in compound **267** was deprotected with TFA in CH_2Cl_2, and the acid **268** was activated with di(*N*-succinimidyl) carbonate to yield the desired active ester **269** in good overall yield.



Scheme 2.1. Synthesis of the activated α-aminostearic acid **269**.

An ω-aminostearic acid was required with an *N*-protective group, which could easily be removed without destroying other functionalities of GM1. As in the case of α-aminostearic acid (**265**), an Fmoc-protection which is compatible with the *tert*-butyl ester, was chosen.[114]

The synthesis of active esters of the *N*-protected ω-amino- and *S*-protected ω-mercaptostearic acids is shown in Scheme 2.2. The target compound – *tert*-butyl ω-aminostearate (**279**) – may be obtained from the corresponding azido ester **278**, which, in turn, is easily prepared from the bromo or the hydroxy ester. As a precursor the ω-hydroxy- or ω-bromostearic acid was required. The synthesis of the ω-hydroxystearic acid **274** (Scheme 2.2) was started with the "acetylene zipper" reaction[115] of 3-octyn-1-ol (**270**) in which the triple bond was moved to the end of the carbon-carbon chain. The resulting 7-octyn-1-ol (**271**) was coupled with 10-bromodecanoic acid (**272**) in liquid ammonia in the presence of lithium amide to give 18-hydroxyoctadec-11-ynoic acid (**273**) in 50% yield.[116] It turned out to be necessary to use freshly distilled ammonia, otherwise the impurities dramatically inhibited the coupling reaction. The triple bond in **273** was hydrogenated over PtO_2 to yield ω-hydroxyoctadecanoic acid (**274**).

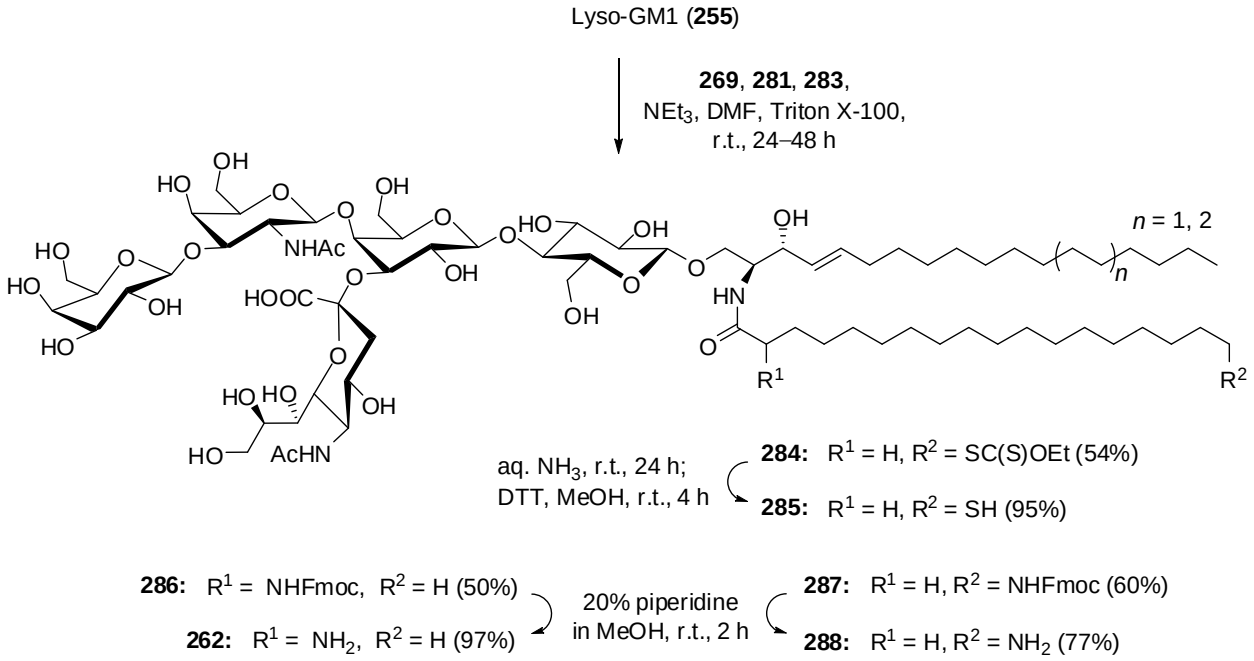


Scheme 2.2. Active esters **283** and **281** of *N*-protected ω-amino- and *S*-protected ω-mercaptostearic acids.

Direct conversion of the ω-hydroxy to the corresponding ω-bromo acid **275** with aq. HBr in acetic acid furnished the common precursor for both the ω-amino- and the ω-mercaptostearic acids. Toward those, **275** was converted to the *tert*-butyl ester **277**, in which the *tert*-butyl group not only protects the carboxy function, but also improves the solubility. The bromo ester **277** was transformed into the azido ester **278**, which was hydrogenated over Pd/C to afford the amino ester **279** as the hydrochloride. To avoid an undesired transesterification, alcohols should not be used as solvents for this step. The amino group in **279** readily reacted with FmocCl to yield the urethane **280**, and then the *tert*-butyl ester was cleaved with TFA in the presence of Et_3SiH as a scavenger of *tert*-butyl cations. The Fmoc-protected amino acid **282** was treated with di(*N*-succinimidyl) carbonate, and the active ester **283** was isolated and kept at –20 ºC. A synthesis of the ω-mercaptostearic acid has not been reported so far. To introduce a protected thiol group, the ω-bromostearic acid (**275**) was treated with potassium xanthogenate in anhydrous acetone at room temperature, and the resulting acid **276** was converted to the NHS ester **281** (Scheme 2.2).

With three protected NHS esters at the disposal, the synthesis of the corresponding GM1 derivatives with additional "anchoring" sites was initiated. Towards that, the lyso-GM1 was

treated with an excess of the respective active ester (**269**, **281**, **283**) in anhydrous DMF/THF solution in the presence of NEt_3 as a base and the non-ionic detergent Triton X-100 at room temperature (Scheme 2.3).



Scheme 2.3. Analogues of GM1 ganglioside with α-amino-, ω-amino- and ω-mercaptostearic acid residues **262**, **288** and **285**.

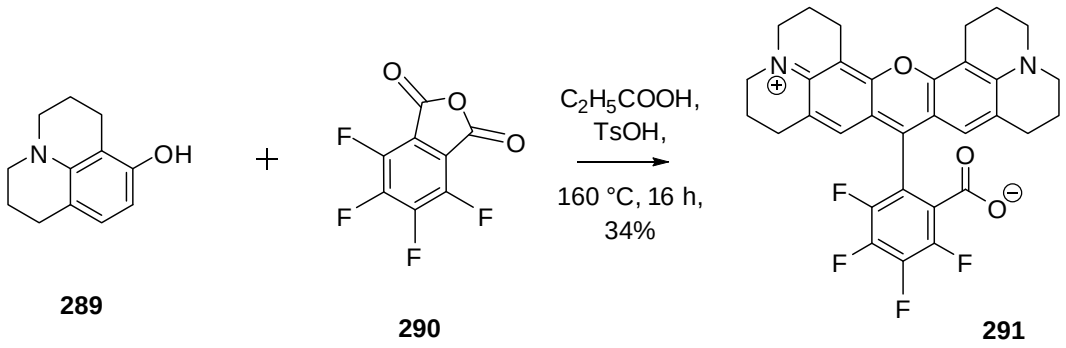
Treatment of the products, *N*-Fmoc-protected 2- and 18-amino derivatives **286** and **288** with 20% piperidine in methanol at room temperature was sufficient for complete deprotection of the amino groups. Compound **262** with a ^{14}C-labelled acetyl group in the sialic acid residue was first synthesized by Schwarzmann *et al*. from ^{14}C-labelled lyso-GM1 and the NHS ester of 2-azidostearic acid **260** (Scheme 1.3).[106] In this current investigation, the new amino group was introduced into the GM1 molecule not by reducing the azido ester, but by removal of an Fmoc-protective group. This facilitated the detection and chromatographic separation of the intermediate **286**, and it did not require the use of poisonous and noxious hydrogen sulfide. The *S*-protective group from the mercapto-modified ganglioside **284** was cleaved off by stirring with aqueous ammonia at room temperature (Scheme 2.3), and the obtained disulfide dimer of the thiol **285** was reduced by stirring with dithiotreitol in methanol followed by dialysis against water and lyophilization to furnish pure **285**.

3. Synthesis of GM1 derivatives labelled with new fluorescent dyes

Several potential labelling sites in the natural GM1 molecule provide an additional possibility in search for the labelled glycosphingolipids, which in a certain assay behave just like their unlabelled counterparts.

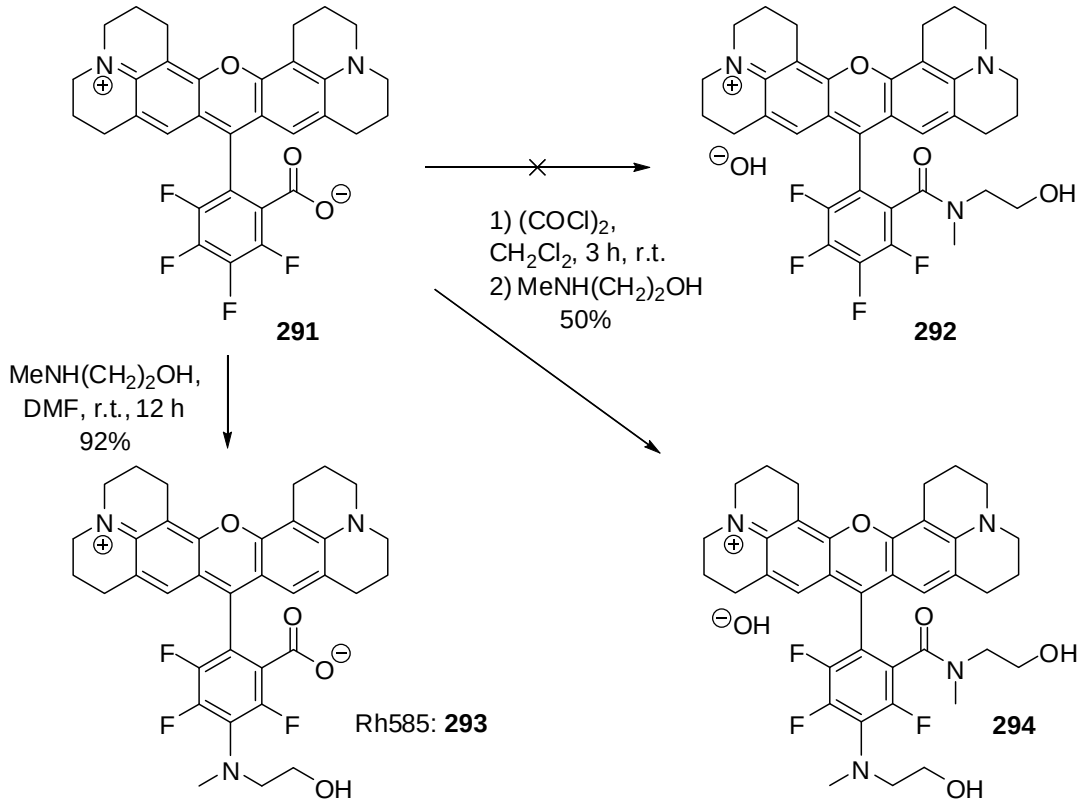
The applicability of compounds **262** and **288** for the labelling reactions involving amino-reactive fluorophores was tested under the following conditions. To a solution of a modified ganglioside in anhydrous DMSO were added an activated dye (1–2 equiv.) along with a base, and the reaction mixture was stirred at room temperature in the dark under inert gas for 1–2 days. The colored product that was also developed with anisaldehyde reagent was isolated on HPTLC plates. The mass spectra (with electrospray ionization) confirmed the constitutions of the adducts, while their purities and homogenities were tested by HPTLC.

As an example of a non-polar lipophilic dye, an analogue of rhodamine 101 with a tetrafluoro-substituted benzoic acid residue, synthesized from julolidine (**289**) and tetrafluorophthalic anhydride (**290**),[117] was chosen (Scheme 3.1). Its emission maximum was found to be shifted into the red spectral region in comparison with the non-fluorinated rhodamine 101 (611 nm vs. 589 nm in MeOH).



Scheme 3.1. Synthesis of the fluorinated rhodamine 101 (**291**).

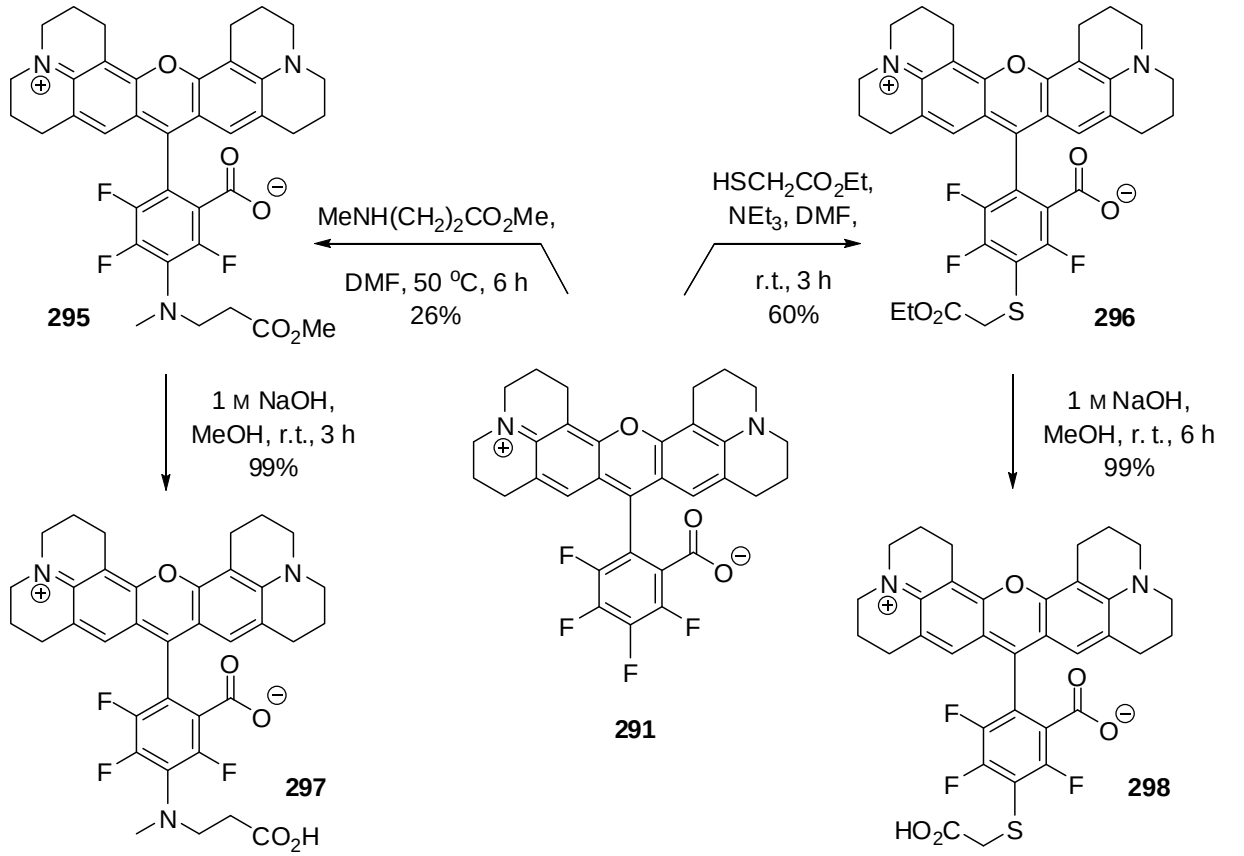
To deliver this fluorophore with a linker for further modifications, the carboxilic acid of the fluorinated rhodamine **291** was activated with oxalyl chloride followed by treatment with 2-(*N*-methylamino)ethanol (Scheme 3.2).



Scheme 3.2. Synthesis of the fluorinated rhodamine 101 with a linker (**293**).

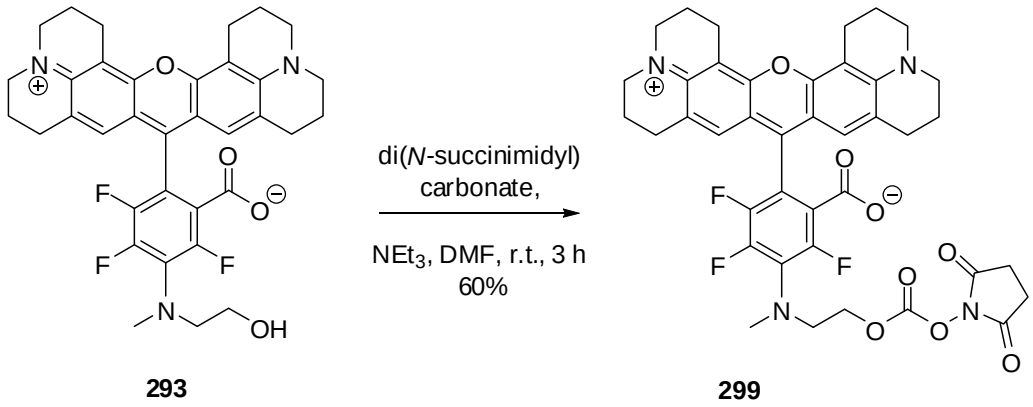
Unexpected, instead of compound **292**, the twofold substituted compound **294**, which lost one fluorine atom, was obtained. Thus, the fluorinated rhodamine **291** without preliminary activation, upon treatment with an excess of 2-(*N*-methylamino)ethanol in DMF at room temperature, gave the mono-substituted compound **293**. Only one fluorine atom was found to be changed by a secondary amino group. This substitution results in a small blue shift of both the absorption and the emission bands of compound **293** (585 and 604 nm, respectively).

The fluorinated rhodamine **291** was subjected to the reactions with other nucleophiles (Scheme 3.3). While reaction with an excess of methyl 3-(*N*-methylamino)propionate gave compound **295** in low yield, reaction with an excess of ethyl thioglycolate resulted in the mixture of mono-, di- and three-substituted compounds. Using equimolar ratio of the reaktants, the compound **296** was isolated in good yield. Both compounds **295** and **296** were saponificated, but further reactions of the acids **297** and **298** with NHS-OH and DCC or TSTU gave the mixture of the mono- and di-NHS esters.



Scheme 3.3. Synthesis of the substituted fluorenated rhodamines 101.

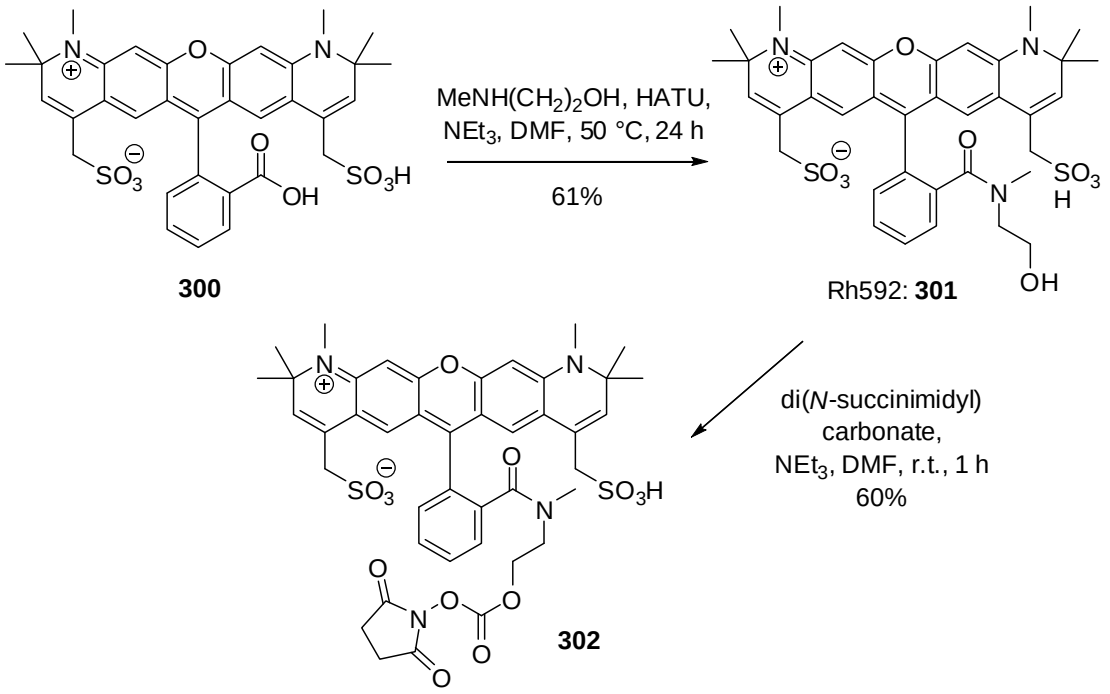
The hydroxy group in alcohol **293** was transformed to the NHS carbonate by treatment with di(*N*-succinimidyl) carbonate and a base in DMF (Scheme 3.4), and the activated dye **299** was isolated by preparative HPLC.



Scheme 3.4. The amino-reactive NHS carbonate **299**.

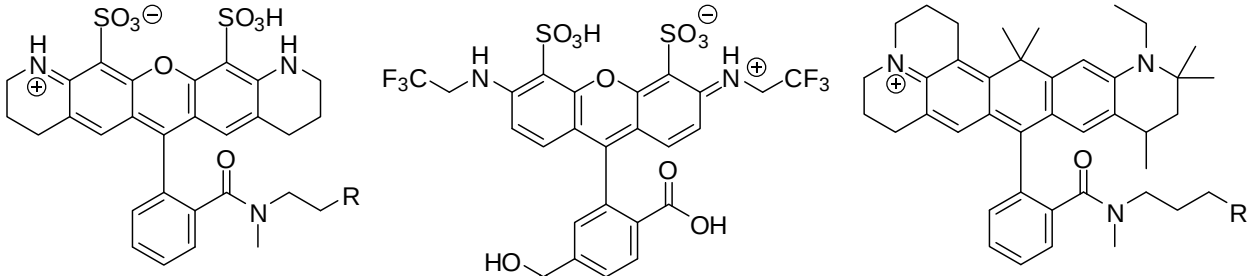
Towards the synthesis of the amino-reactive water-soluble dye **302** (Scheme 3.5), the carboxy group of the sulfonated rhodamine **300** was activated with HATU in the presence of NEt_3, and condensed with 2-(*N*-methylamino)ethanol. The resulting hydroxyethylamide **301** showed an absorption maximum at 592 nm and emitted at 614 nm. Compound **301**, upon

reaction with di(*N*-succinimidyl) carbonate, gave the activated carbonate **302**, which was isolated by preparative HPLC.



Scheme 3.5. Synthesis of activated fluorescent dye **302**.

Several amino-reactive compounds based on the highly photostable fluorescent dyes have been prepared in our laboratory (Rh539[118] and Rh501[119]).



303: R = CONHS (Rh539)

304: R = OCONHS

λ_{abs} = 539 nm
λ_{em} = 560 nm

305 (Rh501)

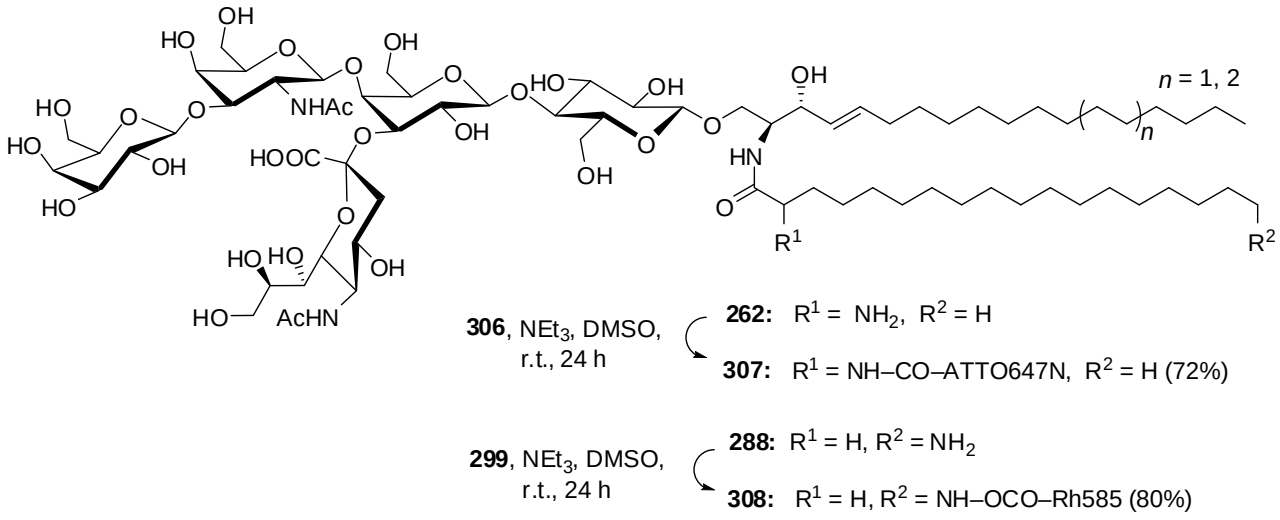
λ_{abs} = 501 nm
λ_{em} = 524 nm

306 : R = CONHS

(ATTO647N)

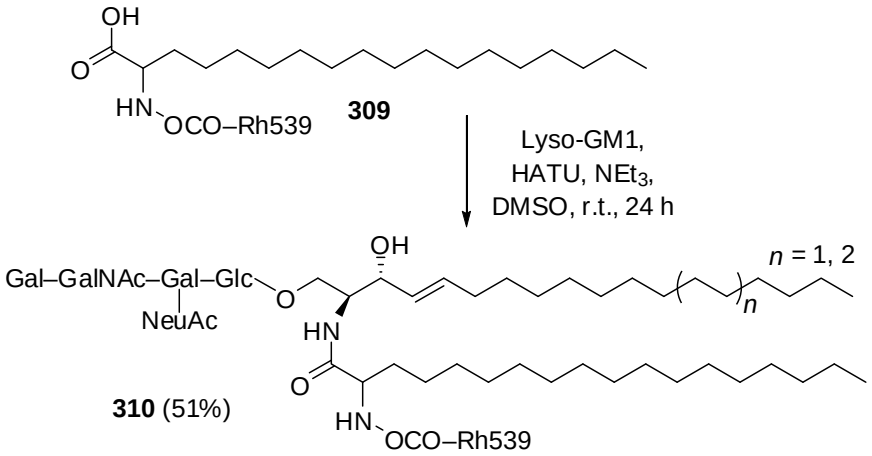
λ_{abs} = 644 nm
λ_{em} = 669 nm

Along with synthesized fluorophores we used a commercial fluorescent dye (ATTO647N NHS ester) for labelling of the GM1 derivatives **262** and **288** (Scheme 3.6). The yields of the labelled compounds, after purification by the HPTLC, were found to be good (70–80%), although the applied excess of the activated fluorescent probes was not high (up to 2 equiv.).



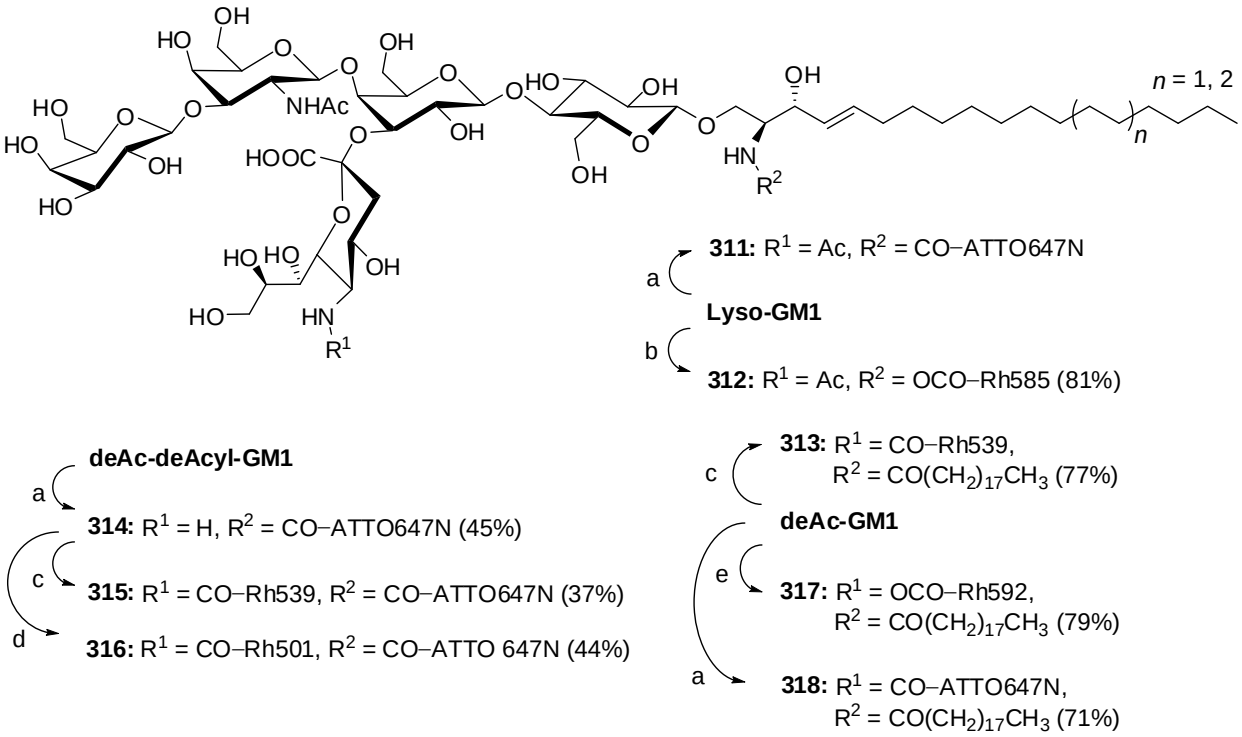
Scheme 3.6. Compounds **262**, **288** and their conjugation with fluorescent dyes.

As alternative we changed the strategy and prepared the fluorescent labelled stearic acid **309**, and it was coupled with lyso-GM1 using the activating agent HATU. Thus, the ganglioside derivatives **310** with the hydrophilic fluorescent label at α-position of the stearic acid chain was obtained (Scheme 3.7).



Scheme 3.7. Synthesis of the fluorescent ganglioside GM1 derivative **310**.

Each of the compounds deAc-GM1, lyso-GM1 has one free amino group in the neuraminic acid residue or in the sphingosine moiety, respectively. Amino groups are known to readily react with *N*-hydroxysuccinimidyl esters or NHS carbonates. The relatively polar water-soluble sulfonated rhodamines (Rh501, Rh539, Rh592) were attached to the polar "head" groups of ganglioside derivatives, while non-polar dyes (Rh585, ATTO647N) were used for labelling of lyso-GM1 (Scheme 3.8).



Scheme 3.8. GM1 derivatives with fluorescent labels: a) **306**, DMSO, NEt_3, r.t., 24 h; b) **299**, DMSO, NEt_3, r.t., 24 h; c) **303**, DMSO, NEt_3, r.t., 24 h; d) **305**, HATU, NEt_3, r.t., 24 h; e) **302**, DMSO, NEt_3, r.t., 24 h.

Chromatographic behavior of some labelled ganglioside GM1 derivatives is presented in Figure 3.1.

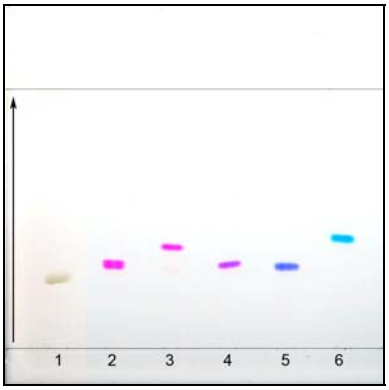


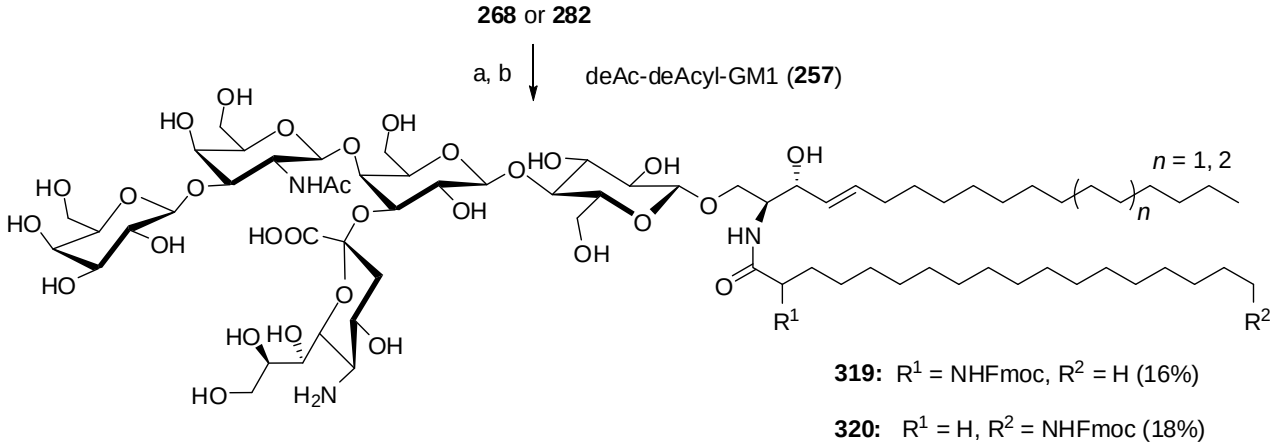
Figure 3.1. High performance thin layer chromatography (HPTLC) of fluorescent labelled gangliosides presented in the Schemes 3.6–3.8. Lane 1: natural ganglioside GM1; lane 2: product **310**; lane 3: product **313**; lane 4: product **308**; lane 5: product **317**; lane 6: product **307**. Solvent system: $CHCl_3$/MeOH/15 mM $CaCl_2$ (60:35:8, v/v). Lane 1 was visualized by treatment with an anisaldehyde reagent followed by heating at 150 °C for 5 min.

It is possible to selectively introduce two different dyes into the compound deAc-deAcyl-GM1 with two amino groups in the neuraminic acid and sphingosine residues. The most active amino group is located in the sphingosine residue, and therefore the compound deAc-deAcyl-GM1 was first labelled with a lipophilic fluorescent dye (ATTO647N) to give the derivative **314** (Scheme 3.8). Treatment with the second hydrophilic activated fluorophore (**303** or **305**) gave the required products **315** and **316** with violet or greenish colors, which looked like a superposition of the two colors used. STED and multicolor FCS experiments of labelled GM1 deivatives **315** and **316** incorporated in the plasma membrane of living PtK2 cells were performed (see below).[120]

4. Novel intermediates for selective twofold labelling of the natural GM1 skeleton

If it is necessary to label the amino group in the neuraminic acid first (e. g. with a hydrophilic dye) and then introduce the second marker into the sphingosine part, a more complicated approach should be used.[104a] It is based on the temporary protection of the more reactive amino group in the sphingosine residue of the compound deAc-deAcyl-GM1, labelling of the free amino group in the neuraminic acid, and finally deprotection of the amino group in the sphingosine fragment.

As more convenient alternative, one can first selectively reacylate deAc-deAcyl-GM1 with an activated *N*-protected α- and ω-aminostearic acids (**268** or **282**) in order to be able to label the amino group at the "head" of the GM1 derivative with a polar fluorescent dye, deprotect the stearic acid amino group and then label it with a second dye.



Scheme 4.1. Analogues of GM1 ganglioside **319** and **320** with a free amino group in the sialic acid residue and protected amino groups in the α- or ω-positions of the stearic acid chain: a) $SOCl_2$, 50 °C, 4 h; b) 0.1% aq. $NaHCO_3$, Et_2O, 4 °C, 24 h.

Acids **268** and **282** were treated with thionyl chloride and then coupled with deAc-deAcyl-GM1 to afford compounds **319** and **320** (Scheme 4.1). Their compositions were confirmed by mass spectrometry, and the structure assignments rest on their ^{1}H NMR spectra. The signals of the aromatic protons of the fluorenyl residue were observed for both compounds. The characteristic signal of the 5-H at the carbon atom connected with the free amino group in the neuraminic acid fragment of compound **319** was found to occur at 2.80 ppm in [D_4]methanol. Compound **320** was not completely soluble in MeOH, and the ^{1}H NMR spectra were recorded in [D_6]DMSO solution, in which all signals were shifted to higher field. Therefore, the signal of 5-H in compound **320** was observed at 2.55 ppm, where it overlapped with the signal of the solvent. Acylation of the free amino group in the sphingosine residue with functionalized stearic acid residues resulted in shifts of the signal at δ = 2.95 ppm for 2-H in deAc-deAcyl-GM1 to lower fields for compounds **319** and **320**. The presence of only one signal of an acetyl group (GalNAc) in the ^{1}H NMR spectra of compounds **319** and **320** was also in accordance with previous assignments.

5. STED-FCS measurements of fluorescent GM1 lipid analogs

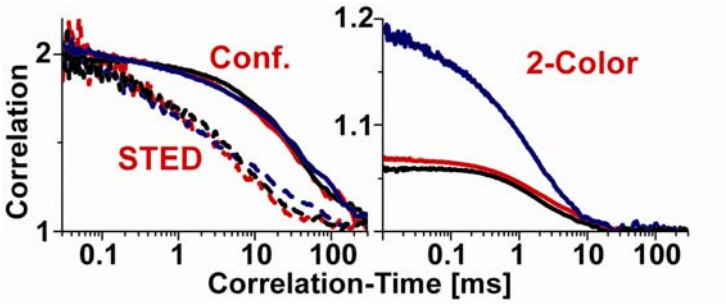


Figure 5.1. STED-FCS measurements of fluorescent GM1 lipid analogues in living cells (left panel, all data normalized to 2) and multicolor FCS measurements of doubly-labelled GM1 in model membranes (right panel). Left panel: ATTO647N-labelled GM1 (black: compound **311** (Scheme 3.8) with "acyl-chain replacement", blue: compound **307** (Scheme 3.6) with "acyl-chain addition", red: compound **318** (Scheme 3.8) with head-group labelling) with confocal (solid lines 'Conf.') and STED recording (dotted lines 'STED'), i.e., focal spots of ~240 nm and ~40 nm in diameter. Right panel: FCS data for the doubly labelled compound **316** (Scheme 3.8) with lipophilic ATTO647N dye replacing the acyl chain and a new hydrophilic "blue" dye (Rh501) at the carbohydrate "head" of GM1 with confocal recording (blue: autocorrelation of Rh501 fluorescence, red: autocorrelation of ATTO647N fluorescence, black: cross-correlation of the simultaneous excited Rh501 and ATTO647N fluorescence).

STED and multicolor FCS experiments of GM1 derivatives labelled with different fluorescent dyes were performed.[120] The left panel of Figure 5.1 shows FCS data of ATTO647N-labelled GM1 analogues incorporated in the plasma membrane of living PtK2 cells for conventional confocal and high-resolution STED microscopy recordings. Diffusion of the fluorescent lipids through the focal spots with a diameter of ~240 nm (confocal) and ~40 nm (STED), which were placed on the plasma membrane, resulted in dropping off FCS correlation data with a decay time characteristic of the lipid transit time. Due to the smaller focal spot diameter, the correlation data of the STED recordings are shifted to shorter times. However, this shift is less than that expected from free-diffusing species, rendering transient trapping of these GM1 lipids on the nanoscale (for details see ref. [4]). Most surprisingly, the FCS data recorded for the GM1 analogues labelled with the ATTO647N dye show no significant differences for the various labelling positions ("acyl-chain replacement" in compound **311**, "acyl-chain addition" in **307** or head-group labelling in **318**), rendering (at least in this case) that the influence of the dye on the trapping characteristics is negligible.[4]

The right panel of Figure 5.1 shows FCS data for compound **316** (Scheme 3.8) with a confocal recording for this GM1 derivative doubly labelled with a hydrophilic dye Rh501 (at the polar carbohydrate "head") and the non-polar ATTO647N dye replacing the long fatty acid chain. The cross-correlation data of the simultaneously excited fluorescence of Rh501 and ATTO647N confirm the double labelling[121] and demonstrate that this lipid analog is a suitable control sample for multicolor FCS experiments. Interestingly, the similarly marked compound **315** is inappropriate as a standard, because of the pronounced FRET effect between the donor fluorescent Rh539 and ATTO647N performing as an acceptor. All correlation data render the fluorescent GM1 lipid analogues as appropriate probes for studying lipid dynamics in living cells and shed new light on long-standing biological questions such as the details of lipid rafts.[4]

Experimental Part

1. General Remarks

Reagents obtained from commercial sources were used without purification. Ganglioside GM1 was purchased from ALEXIS Biochemicals (AXXORA, Lörrach, Germany). ATTO647N NHS ester was purchased from ATTO-Tec GmbH (Siegen, Germany, www.atto-tec.com).

NMR spectra were recorded on a *Varian Unity-200* and a *Varian Mercury-200* (200 MHz for ^{1}H and 50.3 MHz for ^{13}C NMR), a *Bruker AM 250* (250 MHz for ^{1}H and 62.9 MHz for ^{13}C NMR), a *Varian Unity-300* and a *Varian Mercury-300* (300 MHz for ^{1}H, 75.5 MHz for ^{13}C, and 282.4 MHz for ^{19}F NMR), a *Varian Inova 500* (500 MHz for ^{1}H and 125.7 MHz for ^{13}C NMR) or a *Varian INOVA 600* (600 MHz for ^{1}H NMR) spectrometers in $CHCl_3$ if not otherwise specified. Multiplicities were determined by APT (Attached Proton Test) measurements. Chemical shifts (δ) are given in ppm. All spectra are referenced to tetramethyl silane as an internal standard (δ = 0 ppm) using the signals of the residual protons of deuterated solvents: δ = 7.26 ppm for $CHCl_3$, 3.31 ppm for CHD_2OD and 2.50 ppm for [D_5]DMSO. Multiplicities of signals are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad. Coupling constants (J) are given in Hz.

Melting points were determined in capillaries using a *SMP 10* apparatus (Bibby Sterling LTD, UK), values are uncorrected.

For dialysis the Spectra/Por Dialysis Membrane (6–8 KDa MWCO) from Spectrum (www.spectrapor.com) was used.

Electron ionization mass spectra (EI-MS, 70 eV) and chemical ionisation mass spectra (CI-MS, 200 eV, reactant gas NH_3) were measured with a *Finnigan MAT 95* spectrometer. Electrospray ionization mass spectra (ESI-MS) were recorded with a *LCQ Finnigan* spectrometer. High-resolution mass spectra (HR-MS) were measured with a *Bruker APEX IV 7T FT-ICR* instrument.

Absorption and fluorescence stationary measurements were carried out in a *Varian Cary 4000* UV-Vis spectrophotometer, and in a *Varian Cary Eclipse* fluorescence spectrophotometer, respectively. Sealed quartz cuvettes of 1 cm path length were used. Emission spectra were corrected for instrument response. Irradiation of the samples to drive the photochromic reactions was performed with a 200W Mercury lamp (LOT-Oriel GmbH & Co. KG, Darmstadt, Germany) equipped with a monochromator and a system of filters to select the appropriate wavelengths, or with a CW diode laser of 375 nm (iPulse-375, Toptica Photonics AG, Gräfelfing, Germany) and a diode-pumped solid-state laser at 671 nm (DPSSL Monolas-671-300MM, Alphalas, Göttingen,

Germany).

HPLC-System (Knauer): Smartline pump 1000 (2×), UV-detector 2500, column thermostat 4000, mixing chamber, injection valve with 20 and 100 µL loop for the analytical and preparative columns, respectively; 6-port-3-channel switching valve; analytical column: Eurosphere-100 C18, 5 µm, 250×4 mm; preparative column: Eurosphere-100 C18, 5 µm, 250×8 mm; solvent A: MeCN + 0.1% v/v TFA, solvent B: H_2O + 0.1% v/v TFA; temperature 25 °C.

Analytical TLC was performed on precoated aluminium silica gel 60/F_{254} plates (Merck Darmstadt, Germany), and on precoated silica gel RP–18W/F_{254} glass plates (Macherey-Nagel, Düren, Germany). TLC plates were visualized under ultraviolet light (254 nm) and (or) developed by treatment with molybdenum phosphoric acid solution (5% in EtOH), ninhydrine, anisaldehyde reagents, aq. $KMnO_4$ or iodine. Preparative TLC was performed on precoated silica gel glass plates. High performance silica gel thin-layer plates (HPTLC, 0.2 mm Kieselgel 60, 10×10 cm) were purchased from VWR International (Darmstadt, Germany). Flash chromatography was performed using Merck silica gel, grade 60, 0.04–0.063 mm, and reversed phase chromatography was performed using Polygoprep 60–50 C18 (Macherey-Nagel, Düren, Germany).

For the optical rotation measurements a *Perkin-Elmer 241* digital polarimeter with 1 dm cell was used; optical rotation values $[\alpha]_D^{20}$ are given in 10^{-1} deg cm^2 g^{-1}; concentrations (*c*) are given in g/100 mL for chloroform solutions.

Elemental analyses were carried out at Mikroanalytisches Laboratorium des Instituts für Organische und Biomolekulare Chemie der Georg-August-Universität Göttingen.

Anhydrous THF and diethyl ether were destilled over sodium benzophenone ketyl. Organic solutions were dried with $MgSO_4$ or Na_2SO_4. All reactions were carried out with magnetic stirring under positive argon or nitrogen atmosphere using the standard technique with vacuum – inert gas manifold, unless stated otherwise.

2. Preparation of the Known Compounds

2-Bromo-5-methylthiophene (**195**) was prepared according to the published procedure,[87] 4-bromo-2-methylthiophene (**196**) was synthesized by a procedure of a "halogene dance".[122] 2,4-Dimethylthiophene (**128**) was prepared according to Smith.[78] 2,4-Dibromo-3,5-dimethylthiophene (**129**) was synthesized according to the published bromination method.[20] 4-Bromo-3,5-dimethylthiophene-2-boronic acid (**109**) was synthesized as described for the similar compound.[32] 2-Phenoxyisoindoline-1,3-dion (**101**) was prepared according to Johnson.[72b] 4-

(Thiophen-2-yl)pyridine (**131**) was prepared according to the published procedure,[123] 4-(5-iodothiophen-2-yl)pyridine (**132**) was prepared according to Nakajima.[79] [2,2']Bithiophene-5-boronic acid (**103**) was prepared as described.[36b] 2-Bromo-3-(2-hydroxyethyl)thiophene (**223**) was synthesized as described for the similar compound,[124] 2-bromo-3-[2-(tetrahydro-*2H*-pyran-2-yloxy)ethyl]thiophene (**224**) was obtained as described for the similar compound.[90] 2,4-Dibromo-5-methylthiophene (**225**) and 4-bromo-5-methylthiophene-2-boronic acid (**226**) were synthesized according to Kawai.[23] Lyso-GM1 (**255**) was prepared according to Sonnino.[104b] *tert*-Butyl (2*R*/*S*)-[(9-fluorenylmethoxycarbonyl)amino]octadecanoate (**267**) and (2*R*/*S*)-[(9-fluorenylmethoxycarbonyl)amino]octadecanoic acid (**268**) were prepared according to Papini.[125] 7-Octyn-1-ol (**271**) was prepared by a procedure of an "acetylene zipper" reaction.[115] 18-Hydroxyoctadecanoic acid (**274**) was prepared according to Riche.[116] 18-Bromooctadecanoic acid (**275**) was synthesized as described for the similar compound.[126]

3. Experimental Procedures

General procedure for the Suzuki coupling (GP 1.1): An aromatic halide (1 mmol), thiopheneboronic acid (1–2 mmol), $Pd(dba)_2$ (1–4 mol%) and Ph_3P (4–16 mol%) were loaded into the Schlenk-flask with a reflux condenser and a bubble-counter. This set-up was evacuated and flushed with argon several times. Then THF (5–7 mL) and 20% aq. Na_2CO_3 (5–7 mL) were added, and the mixture was refluxed (bath temp. 78 °C) for 12–24 h. The mixture was diluted with EtOAc (Et_2O, CH_2Cl_2 or $CHCl_3$), washed with brine, dried, and evaporated under reduced pressure. The product was isolated by chromatography on silica gel or by crystallization from an appropriate solvent.

General procedure for the Suzuki coupling (GP 1.2): To a Schlenck flask filled with N_2 or Ar were placed an aromatic iodide (1 mmol), boronic acid (1.2–1.4 mmol), $Pd(dba)_2$ (3 mol%), and Cs_2CO_3 (1 mmol). 1,4-Dioxane (1–6 mL) was added followed by the solution of P(*t*Bu)$_3$ (3.6 mol%) in dioxane (c = 0.26 mmol/mL), and the mixture was stirred at 40–60 °C for 12–24 h. Then it was diluted with CH_2Cl_2, washed with water, brine, and dried. After evaporation of the solvents under reduced pressure, the product was isolated by chromatography on silica gel or by crystallization from an appropriate solvent.

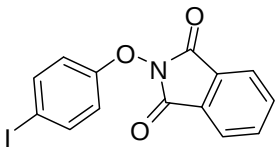
General procedure for iodination (GP 2.1): To a solution of a substrate (1 mmol) and iodine (0.55–2 mmol) in $CHCl_3$ (3–10 mL), bis(trifluoroacetoxy)iodobenzene (0.55–2 mmol) was added in small portions at 0 °C, and the mixture was stirred at room temperature for 18–24 h. After evaporation of the solvent, MeOH (5 mL) was added, and the precipitate was washed with 5% aq. Na_2SO_3, water, and dried in air. The oily product was diluted with CH_2Cl_2, washed with

5% aq. Na_2SO_3, water, brine, dried and evaporated under reduced pressure. The product was isolated by chromatography on silica gel or by crystallization from an appropriate solvent.

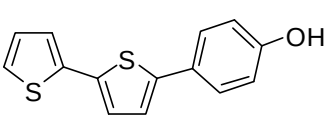
General procedure for iodination (GP 2.2): To a mixture of a substrate (1 mmol) and KOAc (1.1 mmol) in AcOH (4 mL), a 0.5 M solution of ICl in AcOH (1.1 mmol) was added at 0 °C, and the mixture was stirred at room temperature for 4 h or at 50–60 °C for 6–24 h. After cooling, AcOH was evaporated under reduced pressure, and the semi-solid residue was diluted with cold 5% aq. Na_2SO_3. The precipitated product was collected by filtration, washed with water and dried in air. The oily product was diluted with CH_2Cl_2, washed with water, brine, dried and evaporated under reduced pressure. The product was isolated by chromatography on silica gel or by crystallization from an appropriate solvent.

General procedure for synthesis of 1-(3-thienyl)heptafluorocyclopentenes (GP 3): To a virgosly stirred mixture of 3-thienyl bromide (1 mmol) in anhydrous THF or Et_2O (5–15 mL), *n*BuLi (2.5 M in hexane, 1.2–1.5 mmol) was added dropwise at –78 °C, and the mixture was stirred for 0.5–1 h at this temperature. Then cooled octafluorocyclopentene (2–10 mmol) was quickly added, the mixture was stirred for 1 h at –78 °C, and then quenched with brine. The reaction mixture was allowed to warm up to room temperature and diluted with EtOAc (15 mL), washed with brine, dried, and evaporated under reduced pressure. The product was isolated by chromatography on silica gel or by crystallization from an appropriate solvent.

2-(4-Iodophenoxy)isoindoline-1,3-dione (102): According to GP 2.1, compound **101** (1.10 g, 4.6 mmol), iodine (2.33 g, 9.2 mmol), and bis(trifluoroacetoxy)iodobenzene (3.96 g, 9.2 mmol) gave 1.44 g (86%) of the title compound as a colorless solid after recrystallization; m. p. 161 °C (MeOH) (Lit.[127] 158–160 °C).



4-(2,2'-Bithiophen-5-yl)phenol (104): According to GP 1.1, iodide **102** (0.37 g, 1.0 mmol), thiopheneboronic acid **103** (0.42 g, 2.0 mmol), $Pd(dba)_2$ (23 mg, 0.04 mmol), and PPh_3 (42 mg, 0.16 mmol) gave 0.14 g (55%) of the title compound as a colorless solid after purification on SiO_2 (50 g) eluting with hexane/EtOAc (2:1); R_f = 0.31. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.76–6.84 (m, 2H, CH), 7.06–7.10 (m, 1H, CH), 7.22–7.30 (m, 3H, CH), 7.44–7.54 (m, 3H, CH), 9.74 ppm (s, 1H, OH). CI-MS (NH_3), positive mode, *m/z* (rel. int., %): 259.0 (100) $[M+H]^+$.

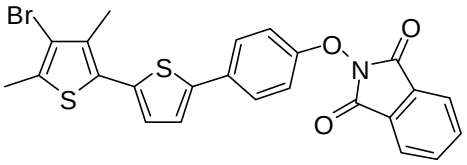


2-[4-(Thiophen-2-yl)phenoxy]isoindoline-1,3-dion (106): According to GP 1.2, iodide **102** (2.19 g, 6.00 mmol), thiophene-2-boronic acid (**105**) (0.92 g, 7.20 mmol), $Pd(dba)_2$ (104 mg, 0.18 mmol), Cs_2CO_3 (1.96 g, 6.00 mmol), and P(*t*Bu)$_3$ (0.82 mL, 0.22 mmol) gave 1.76 g (91%) of the title compound as a grey solid, decomp. >138 °C (EtOH). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.11 (dd, *J* = 3.6, 5.1 Hz, 1H, CH), 7.27–7.33 (m, 2H, CH), 7.44 (dd, *J* = 1.1, 3.6 Hz, 1H, CH), 7.50 (dd, *J* = 1.1, 5.1 Hz, 1H, CH), 7.60–7.67 (m, 2H, CH), 7.88–7.99 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): δ = 114.0 (2×CH), 123.5 (CH), 123.6 (2×CH), 125.3 (CH), 126.9 (2×CH), 128.3 (CH), 128.6 (2×C), 129.9 (C), 135.0 (2×CH), 142.2 (C), 157.9 (CO), 162.6 ppm (2×CO). $C_{18}H_{11}NO_3S$ (321), EI-MS, positive mode, *m/z* (rel. int., %): 321 (100) [$M^{+\cdot}$], 277 (20), 176 (85), 175 (90), 147 (84), 130 (80), 104 (46), 102 (28), 90 (20), 77 (24), 76 (85), 63 (24), 50 (46), 45 (25).

Diiodide 107: According to GP 2.1, compound **106** (0.32 g, 1.0 mmol), iodine (0.28 g, 1.1 mmol), and bis(trifluoroacetoxy)iodobenzene (0.47 g, 1.1 mmol) gave 0.40 g (78%) of the title compound as a grey solid after recrystallization; decomp. >198 °C (MeOH). ^{1}H NMR (300 MHz, [D$_6$]DMSO, 35 °C): δ = 7.35–7.45 (m, 3H, CH), 7.50–7.57 (m, 2H, CH), 7.90–8.01 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, [D$_6$]DMSO, 35 °C): δ = 77.9 (C-I), 80.8 (C-I), 113.4 (2×CH), 123.6 (2×CH), 128.6 (2×C), 128.8 (C), 130.5 (2×CH), 135.0 (2×CH), 144.5 (CH), 146.3 (C), 158.7 (CO), 162.6 ppm (2×CO). $C_{18}H_9I_2NO_3S$ (572.8), EI-MS, positive mode, *m/z* (rel. int., %): 573 (65) [$M^{+\cdot}$], 428 (55), 427 (100), 147 (23), 76 (37).

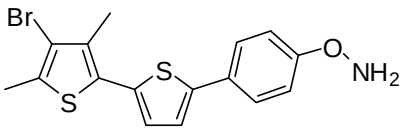
2-[4-(5-Iodothiophen-2-yl)phenoxy]isoindoline-1,3-dion (108): According to GP 2.2, compound **106** (1.29 g, 4.0 mmol), KOAc (0.43 g, 4.4 mmol), and 0.5 M ICl in AcOH (8.8 mL, 4.4 mmol) gave 1.55 g (87%) of the title compound as a grey solid after recrystallization, decomp. >160 °C (CH_2Cl_2/MeOH). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.18 (d, *J* = 3.7 Hz, 1H, CH), 7.27–7.36 (m, 3H, CH), 7.57–7.63 (m, 2H, CH), 7.90–8.00 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): δ = 74.9 (C-I), 114.0 (2×CH), 123.6 (2×CH), 125.4 (CH), 126.9 (2×CH), 128.6 (2×C), 129.0 (C), 135.0 (2×CH), 138.0 (CH), 148.0 (C), 158.2 (CO), 162.7 ppm (2×CO). EI-MS, positive mode, *m/z* (rel. int., %): 447 (60) [$M^{+\cdot}$], 302 (40), 301 (100), 273 (14), 147 (14), 102 (14). Elemental analysis: found: C 48.41, H 2.12, N 3.08; calcd. for $C_{18}H_{10}INO_3S$ (447.2): C 48.34, H 2.25, N 3.13.

2-[4-(4'-Bromo-3',5'-dimethyl-2,2'-bithiophen-5-yl)phenoxy]isoindoline-1,3-dione (110):



According to GP 1.2, iodide **108** (2.68 g, 6.00 mmol), boronic acid **109** (1.69 g, 7.20 mmol), $Pd(dba)_2$ (0.10 g, 0.18 mmol), Cs_2CO_3 (1.96 g, 6.00 mmol), and P(*t*Bu)$_3$ (0.82 mL, 0.22 mmol) gave 2.6 g (85%) of the title compound as a grey solid; decomp. >157 °C (CH_2Cl_2/MeOH). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 2.34 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 7.21 (d, J = 3.8 Hz, 1H, CH), 7.30–7.36 (m, 2H, CH), 7.49 (d, J = 3.8 Hz, 1H, CH), 7.65–7.71 (m, 2H, CH), 7.91–8.00 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): δ = 14.8 (CH_3), 15.6 (CH_3), 108.6 (C-Br), 113.6 (C), 114.1 (2×CH), 123.7 (2×CH), 124.3 (CH), 126.9 (2×CH), 127.2 (CH), 128.6 (2×C), 129.3 (C), 132.3 (C), 132.5 (C), 134.1 (C), 135.1 (2×CH), 142.3 (C), 158.2 (CO), 162.7 ppm (2×CO). EI-MS, positive mode, *m/z* (rel. int., %): 511 (58), 509 (48) [$M^{+\cdot}$], 366 (76), 365 (20), 364 (70), 147 (100), 104 (72), 76 (78), 50 (26). Elemental analysis: found: C 56.25, H 2.96, N 2.99; calcd. for $C_{24}H_{16}BrNO_3S_2$ (510.4): C 56.47, H 3.16, N 2.74.

***O*-[4-(4'-Bromo-3',5'-dimethyl-2,2'-bithiophen-5-yl)phenyl]hydroxylamine (111):** Hydrazine hydrate (78 µL, 2.5 mmol) in MeOH (1 mL) was added to a suspension of compound **110** (0.51 g, 1.0 mmol) in CH_2Cl_2 (15 mL) at 0 °C, and the mixture was stirred at this temperature for 3 h. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (50 g SiO_2) eluting with hexane/EtOAc (4:1) to give 0.32 g (84%) of a colorless solid, which easily oxidized in air. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.38 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 5.89 (s, 2H, NH_2), 7.02 (d, J = 3.8 Hz, 1H, CH), 7.12–7.19 (m, 3H, CH), 7.49–7.55 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 15.2 (CH_3), 15.9 (CH_3), 113.6 (2×CH), 113.9 (C-Br), 122.3 (CH), 126.6 (CH), 126.7 (2×CH), 127.2 (C), 127.9 (C), 132.3 (C), 132.8 (C), 134.4 (C), 144.2 (C), 161.0 ppm (C-O). $C_{16}H_{14}BrNOS_2$ (379), EI-MS, positive mode, *m/z* (rel. int., %): 381/379 (37) [$M^{+\cdot}$], 364 (56), 365 (15), 366 (53), 76 (100).



***tert*-Butyl *N*-phenoxymethyl carbamate (115):**[128] Into a two-necked flask (250 mL) with a refluxed condenser and a bubble-counter, phenoxyacetic acid (**114**) (0.76 g, 5.0 mmol), DPPA (1.65 g, 6.0 mmol), and NEt_3 (0.67 g, 6.6 mmol) were placed, then *t*BuOH (50 mL) was added, and the mixture was refluxed for 16 h. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (50 g SiO_2) eluting with hexane/EtOAc (2:1) to yield 0.94 g (85%) of a colorless solid; R_f = 0.33. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.43 (s, 9H, *t*Bu), 4.62 (d, J = 10

Hz, 2H, CH_2), 5.20 (br s, 1H, NH), 6.81–6.89 (m, 2H, CH), 6.90–6.95 (m, 1H, CH), 7.18–7.26 ppm (m, 2H, CH). $C_{12}H_{17}NO_3$ (223.1), ESI-MS, positive mode, *m/z* (rel. int., %): 469 (58) $[2M+Na]^+$, 246 (100) $[M+Na]^+$, 224 (17) $[M+H]^+$.

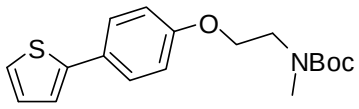
***tert*-Butyl *N*-2-phenoxyethyl carbamate (121)**: A solution of *tert*-butyl pyrocarbonat (7.96 g, 36.4 mmol) in $CHCl_3$ (5 mL) was added dropwise to the solution of 2-phenoxyethylamine (**120**) (5.00 g, 36.4 mmol) in $CHCl_3$ (10 mL) with cooling by ice-water. After stirring for 10 h at room temperature, the solvent was evaporated under reduced pressure, and the residue was distilled in vacuo to give 7.96 g (92%) of the title compound as a colorless solid; b. p. 92 °C (0.08 mbar), m. p. 30 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.45 (s, 9H, *t*Bu), 3.54 (q, J = 5.2 Hz, 2H, CH_2N), 4.02 (t, J = 5.2 Hz, 2H, CH_2O), 5.04 (br. s, 1H, NH), 6.84–6.90 (m, 2H, CH), 6.91–7.00 (m, 1H, CH), 7.28–7.34 ppm (m, 2H, CH). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 28.4 (3×CH_3), 40.1 (CH_2), 67.0 (CH_2), 79.5 (C-O), 114.3 (2×CH), 121.0 (CH), 129.5 (2×CH), 156.1 (CO), 158.4 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 237 (5) [$M^{+\cdot}$], 120 (15), 94 (53), 88 (100), 77 (15), 57 (68), 44 (17). Elemental analysis: found: C 65.72, H 7.77, N 5.69; calcd. for $C_{13}H_{19}NO_3$ (237.3): C 65.80, H 8.07, N 5.90.

***tert*-Butyl *N*-methyl-*N*-2-phenoxyethyl carbamate (122):** To a solution of compound **121** (7.8 g, 32.9 mmol) and methyl iodide (32.7 g, 0.23 mol) in DMF (60 mL), Ag_2O (11.4 g, 49.3 mmol) was added with cooling by ice-water bath. After vigorous stirring for 46 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and filtered through a Celite® pad. The flilter-cake was washed with CH_2Cl_2 (2×50 mL); the combined filtrates were washed with sat. aq. $Na_2S_2O_3$ (2×50 mL), water (6×50 mL), brine (100 mL) and dried. Solvents were evaporated under reduced pressure, and the residue was kept at high vacuo to yield the title compound (7.85 g, 95%) as a yellowish solid; m. p. 48 °C (hexane). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.45 (s, 9H, *t*Bu), 2.98 (s, 3H, CH_3N), 3.54–3.65 (m, 2H, CH_2N), 4.02–4.14 (m, 2H, CH_2O), 6.84–7.00 (m, 3H, CH), 7.23–7.33 ppm (m, 2H, CH). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 28.4 (3×CH_3), 35.0 (CH_3N), 48.3 (CH_2N), 65.3 (CH_2O), 79.6 (C-O), 114.3 (2×CH), 120.8 (CH), 129.5 (2×CH), 156.1 (CO), 158.4 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 274 $[M+Na]^+$ (100). Elemental analysis: found: C 66.92, H 8.58, N 5.37; calcd. for $C_{14}H_{21}NO_3$ (251.3): C 66.91, H 8.42, N 5.57.

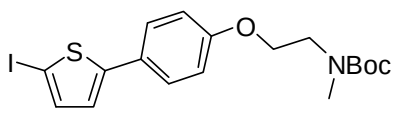
***tert*-Butyl *N*-[2-(4-iodophenoxy)ethyl]-*N*-methyl carbamate (123):** According to GP 2.2, compound **122** (2.51 g, 10.0 mmol), KOAc (1.03 g, 10.5 mmol), and 0.5 M ICl in AcOH (21 mL, 10.5 mmol) gave 2.15 g (57%) of the

title compound as a colorless solid after recrystallization; m.p. 75 °C (hexane). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.43 (s, 9H, *t*Bu), 2.94 (s, 3H, CH_3N), 3.48–3.65 (m, 2H, CH_2N), 3.92–4.15 (m, 2H, CH_2O), 6.61–6.68 (m, 2H, CH), 7.49–7.56 ppm (m, 2H, CH). ^{13}C NMR (62.9 MHz, $CDCl_3$, 2 rotamers): δ = 28.4 (3×CH_3), 35.4/36.2 (CH_3N), 48.2 (CH_2N), 66.2/66.8 (CH_2O), 79.7 (C-O), 82.9 (C-I), 116.8 (2×CH), 138.2 (2×CH), 155.9 (CO), 158.5 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 377 (2) [$M^{+\cdot}$], 220 (20), 102 (95), 57 (100), 44 (66), 41 (16). Elemental analysis: found: C 44.39, H 5.09, N 3.94; calcd. for $C_{14}H_{20}INO_3$ (377.2): C 44.58, H 5.34, N 3.71.

***tert*-Butyl *N*-methyl-*N*-[2-[4-(thiophen-2-yl)phenoxy]ethyl] carbamate (124):** According to GP 1.1, iodide **123** (4.5 g, 12.0 mmol), thiophen-2-boronic acid (**105**) (1.84 g, 14.4 mmol), $Pd(dba)_2$ (0.28 g, 0.5 mmol) and Ph_3P (0.50 g, 1.9 mmol) gave after purification by silica gel chromatography (100 g SiO_2) eluting with hexane/EtOAc (2:1) the title compound (3.26 g, 81%) as a colorless solid; m. p. 90 °C (aq. MeOH), R_f = 0.4. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 2.99 (s, 3H, CH_3N), 3.56–3.66 (m, 2H, CH_2N), 4.04–4.18 (m, 2H, CH_2O), 6.86–6.94 (m, 2H, CH), 7.05 (dd, *J* = 3.6, 5.1 Hz, 1H, CH), 7.20 (dd, *J* = 1.1, 3.6 Hz, 1H, CH), 7.22 (dd, *J* = 1.1, 5.1 Hz, 1H, CH), 7.50–7.56 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 28.4 (3×CH_3), 35.4/36.2 (CH_3N), 48.3 (CH_2N), 66.2/66.8 (CH_2O), 79.7 (C-O), 114.7 (2×CH), 122.1 (CH), 123.9 (CH), 127.2 (2×CH), 127.5 (C), 127.9 (CH), 144.2 (C), 155.7 (CO), 158.2 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 331 (8) [$M^{+\cdot}$], 176 (100), 102 (52), 57 (25). Elemental analysis: found: C 64.59, H 6.79, N 4.07; calcd. for $C_{18}H_{23}NO_3S$ (333.4): C 64.84, H 6.95, N 4.20.

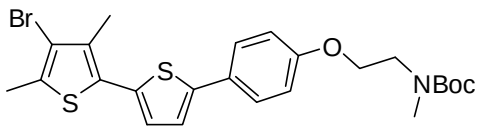


***tert*-Butyl *N*-[2-[4-(5-iodothiophen-2-yl)phenoxy]ethyl]-*N*-methyl carbamate (125):** According to GP 2.2, compound **124** (2.66 g, 8.0 mmol), KOAc (0.86 g, 8.8 mmol), and 0.5 M ICl in AcOH (17.6 mL, 8.8 mmol) gave 3.15 g (86%) of the title compound as a colorless solid after recrystallization; m. p. 118–119 °C (aq. MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 2.98 (s, 3H, CH_3N), 3.52–3.68 (m, 2H, CH_2N), 4.01–4.21 (m, 2H, CH_2O), 6.86 (d, *J* = 3.8 Hz, 1H, CH), 6.86–6.92 (m, 2H, CH), 7.18 (d, *J* = 3.8 Hz, 1H, CH), 7.39–7.44 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 28.4 (3×CH_3), 35.4/36.2 (CH_3N), 48.3 (CH_2N), 66.2/66.9 (CH_2O), 71.0 (C-I), 79.7 (C-O), 114.8 (2×CH), 123.6 (CH), 126.6 (C), 127.1 (2×CH), 137.8 (CH), 150.2 (C), 155.5/155.7 (CO), 158.6 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 459 (11) [$M^{+\cdot}$], 302 (88), 210 (10), 102 (100), 57 (25). Elemental

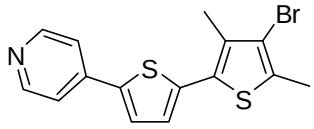


analysis: found: C 46.87, H 4.57, N 4.09; calcd. for $C_{18}H_{22}INO_3S$ (459.3): C 47.07, H 4.83, N 3.95.

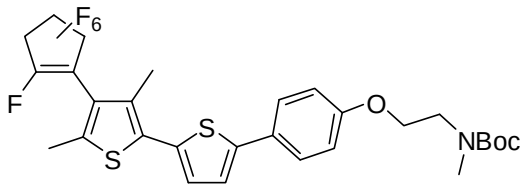
***tert*-Butyl *N*-[2-[4-(4'-bromo-3',5'-dimethyl-[2,2']bithiophen-5-yl)phenoxy]ethyl]-*N*-methyl carbamate (126):** According to GP 1.1, iodide **125** (1.79 g, 3.90 mmol), $Pd(dba)_2$ (90 mg, 0.16 mmol), PPh_3 (164 mg, 0.62 mmol) and thiopheneboronic acid **109** (1.10 g, 4.68 mmol) gave after purification by silica gel chromatography (100 g SiO_2) eluting with hexane/EtOAc (8:1) the title compound (1.6 g, 79%) as a yellow solid; m. p. 80–81 °C (aq. EtOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 2.38 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.99 (s, 3H, CH_3N), 3.55–3.68 (m, 2H, CH_2N), 4.04–4.21 (m, 2H, CH_2O), 6.87–6.95 (m, 2H, CH), 7.02 (d, J = 3.8 Hz, 1H, CH), 7.14 (d, J = 3.8 Hz, 1H, CH), 7.49–7.55 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 15.2 (CH_3), 15.9 (CH_3), 28.4 (3×CH_3), 35.3/36.1 (CH_3N), 48.3 (CH_2N), 66.2/66.9 (CH_2O), 77.2 (C), 79.7 (C-O), 114.8 (2×CH), 122.4 (CH), 126.6 (CH), 127.0 (2×CH), 127.8 (C), 132.3 (C), 132.9 (C), 134.5 (C), 144.1 (C), 150.2 (C), 155.5/155.7 (CO), 158.4 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 523/521 (8) [$M^{+\cdot}$], 366 (40), 364 (36), 102 (100), 58 (14), 57 (54), 41 (18). Elemental analysis: found: C 54.93, H 5.13, N 2.89; calcd. for $C_{24}H_{28}BrNO_3S_2$ (522.5): C 55.17, H 5.40, N 2.68.



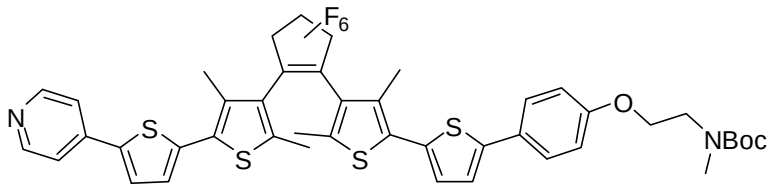
4-(4'-Bromo-3',5'-dimethyl-[2,2']bithiophen-5-yl)pyridine (133): According to GP 1.1, compound **132** (0.74 g, 2.60 mmol) and 3-bromo-2,5-dimethylthiophene-2-boronic acid **109** (0.73 g, 3.10 mmol) in the presence of $Pd(dba)_2$ (59 mg, 0.10 mmol) and Ph_3P (105 mg, 0.40 mmol) after purification on SiO_2 (130 g) eluting with CH_2Cl_2 (+ 1% v/v Et_3N) followed by CH_2Cl_2/acetone mixture (8:2 + 1% v/v Et_3N) gave 0.75 g (82%) of yellow needles; m. p. 143–144 °C (aq. MeOH). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 2.02 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 7.08 (d, J = 4.3 Hz, 1H, CH), 7.43 (m, 3H, CH), 8.59 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 15.3 (CH_3), 16.0 (CH_3), 114.3 (C-Br), 119.4 (2×CH), 125.7 (CH), 126.4 (CH), 127.1 (C), 133.3 (C), 133.7 (C), 138.1 (C), 140.4 (C), 140.9 (C), 150.4 ppm (2×CH). EI-MS, positive mode, *m/z* (rel. int., %): 351 (100) $[M+H]^+$, 350 (22) [$M^{+\cdot}$], 349 (94). Elemental analysis: found: C 51.67, H 3.26, N 3.79; calcd. for $C_{15}H_{12}BrNS_2$ (350.3): C 51.43, H 3.45, N 4.00.



***tert*-Butyl *N*-[2-[4-[3',5'-dimethyl-4'-(heptafluorocyclopent-1-enyl)-2,2'-bithiophen-5-yl]phenoxy]ethyl]-*N*-methyl carbamate (135):** According to GP 3, bromide **126** (1.05 g, 2.0 mmol), *n*BuLi (2.5 M in hexane, 1.2 mL, 3.0 mmol), and C_5H_8 (0.85 g, 4.0 mmol) gave 1.0 g (78%) of the title compound as a grey crystalline substance after purification on SiO_2 (120 g) eluting with hexane/EtOAc (4:1); R_f = 0.23, decomp. >80 °C (MeOH). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 2.22 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.99 (s, 3H, CH_3N), 3.56–3.65 (m, 2H, CH_2N), 4.05–4.18 (m, 2H, CH_2O), 6.86–6.95 (m, 2H, CH), 7.05 (d, J = 3.8 Hz, 1H, CH), 7.16 (d, J = 3.8 Hz, 1H, CH), 7.48–7.55 ppm (m, 2H, CH). ^{13}C NMR (62.9 MHz, $CDCl_3$, 2 rotamers):* δ = 13.9 (CH_3), 14.1 (CH_3), 28.4 (3×CH_3), 34.0/36.1 (CH_3N), 48.1 (CH_2N), 63.6/66.2 (CH_2O), 79.6 (C-O), 114.7 (2×CH), 121.0 (C), 122.3 (CH), 126.9 (C), 127.0 (2×CH), 129.9 (C), 132.5 (C), 133.4 (C), 137.5 (C), 139.6 (C), 155.7 (CO), 158.5 ppm (CO). CI-MS (NH_3), positive mode, *m/z* (rel. int., %): 653.3 (100) $[M+NH_4]^+$, 636.3 (26) $[M+H]^+$, 134.1 (24). HR-MS (ESI, positive mode): found: 636.1472; calcd. for $C_{29}H_{28}F_7NO_3S_2$: 636.1477 $[M+H]^+$.



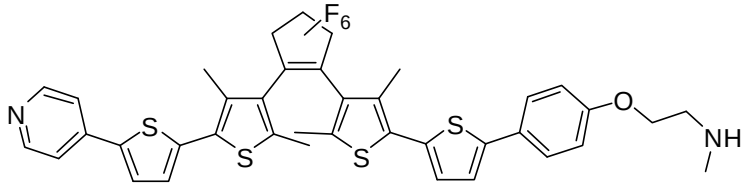
Photochromic compound 136: Bromide **135** (79 mg, 0.23 mmol) in THF (4 mL) was subjected to halogene–lithium exchange at –78 °C with *t*BuLi (0.15 mL of 1.5 M solution in pentane, 0.23 mmol). After 30 min compound **133** (95 mg, 0.15 mmol) in THF (1 mL) was added dropwise. After keeping the reaction mixture for an hour at –78 °C, the cold bath was removed, and the reaction was left for warming-up to ca. 0 °C. It was quenched by addition of brine (1 mL), diluted with EtOAc (15 mL); organic layer was separated, dried, and concentrated under reduced pressure. The title compound was isolated by chromatography (70 g SiO_2), eluting with hexane/EtOAc (2:3) to give 70 mg (53%) of a yellow foam. HPLC: 80 → 100% A (20 → 0% B) for 0–15 min, 100% ACN from 15–20 min, 1 mL/min, 25 °C, t_R (OF) = 11.8 min, t_R (CF) = 16.3 min, detection at the isobestic point (369 nm). ^{1}H NMR (300 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.46 (s, 9H, *t*Bu), 2.19–2.56 (br. s, 3H, CH_3), 2.32–2.39 (br. s, 3H, CH_3), 2.98 (s, 3H, CH_3N), 3.56–3.65 (m, 2H, CH_2N), 4.05–4.18 (m, 2H, CH_2O), 6.86–6.93 (m, 2H, CH), 7.01 (d, J = 3.8 Hz, 1H, CH), 7.09 (m, 1H, CH), 7.13 (m, 1H, CH), 7.44 (d, J = 3.8 Hz, 1H, CH), 7.44–7.47 (m, 2H, CH), 7.47–7.53 (m, 2H, CH), 8.56–8.60 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, open form, mixture of



* Due to low intensities, signals of the fluorinated carbons were not detected.

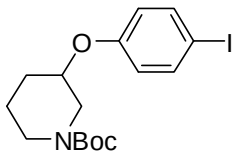
rotamers): δ = 14.9 (CH_3), 15.3 (CH_3), 28.0 (CH_3), 28.4 (3×CH_3), 29.7 (CH_3), 35.5/36.2 (CH_3N), 48.3 (CH_2N), 66.2/66.9 (CH_2O), 79.7 (C-O), 114.8 (2×CH), 119.5 (2×CH), 122.4 (CH), 125.7 (CH), 125.9/126.0 (C), 126.2/126.3 (C), 126.6 (CH), 126.8 (CH), 126.9 (2×CH), 129.2 (C), 130.0 (C), 132.3 (C), 133.4 (C), 133.9 (C), 134.0 (C), 137.6 (C), 138.4/138.5 (C), 139.4/139.5 (C), 140.5 (C), 140.9 (C), 144.1 (C), 150.4 (2×CH), 155.6 (CO), 158.5 ppm (CO). ^{19}F NMR (282 MHz, $CDCl_3$): δ = –110.5 (m, 4F), –133.0 ppm (m, 2F). ESI-MS, positive mode, *m/z* (rel. int., %): 887.2 (100) $[M+H]^+$. HR-MS (ESI, positive mode): found: 887.1899; calcd. for $C_{44}H_{40}N_2O_3S_4F_6$: 887.1904 $[M+H]^+$.

Photochromic compound 137: Starting compound **136** (0.22 g, 0.25 mmol) was dissolved in



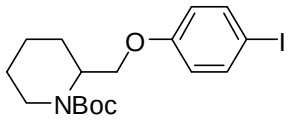
CH_2Cl_2 (0.5 mL), and 4 M solution of HCl in dioxane (2 mL) was added at 0 °C. The reaction mixture was left for warming-up to room temperature with stirring. After keeping at room temperature for 3 h, TLC displayed the full conversion of the starting material. Solvents were evaporated in vacuo, and the residue was triturated with anhydrous Et_2O. Ether was decanted from the precipitated HCl-salt; it was dried in vacuo to yield the title compound (0.21 g, 99%) as a yellow powder, which was used in the final coupling step without further purification. ESI-MS, positive mode, *m/z* (rel. int., %): 787.1 (100) $[M+H]^+$, 394.1 (80) $[M+2H]^{2+}$. HR-MS (ESI, positive mode): found: 787.1371; calcd. for $C_{39}H_{32}F_6N_2OS_4$: 787.1374 $[M+H]^+$.

***tert*-Butyl 3-(4-iodophenoxy)piperidine-1-carboxylate (139):** To a mixture of compound **138**



(0.10 g, 0.5 mmol), 4-iodophenol (**118**) (0.11 g, 0.5 mmol), and PPh_3 (0.16 g, 0.6 mmol) in anhydrous THF (2.5 mL), a solution of DIAD (Fluka, 0.12 mL, 0.6 mmol) in anhydrous THF (0.5 mL) was added at 0 °C. The mixture was stirred at room temperature for 12 h, diluted with Et_2O (30 mL), washed with 1 M NaOH, water, dried, and evaporated under reduced pressure. The title compound was purified by silica gel chromatography (30 g SiO_2) eluting with hexane/EtOAc (2:1) to give 30 mg (15%) of a colorless solid; R_f = 0.36. $C_{16}H_{22}INO_3$ (403), EI-MS, positive mode, *m/z* (rel. int., %): 426.0 (100) $[M+Na]^+$.

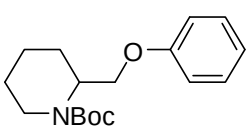
***tert*-Butyl 2-[(4-iodophenoxy)methyl]piperidine-1-carboxylate (141):** According to GP 2.2,



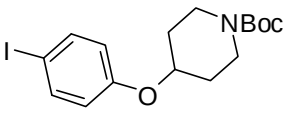
compound **143** (0.15 g, 0.50 mmol), KOAc (70 mg, 0.75 mmol), and 0.5 M ICl in AcOH (1.5 mL, 0.75 mmol) gave 60 mg (29%) of the title compound as a colorless solid after recrystallization; m. p. 115 °C (aq.

MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9H, *t*Bu), 1.55–1.72 (m, 5H, 2×CH_2 and C*H*H), 1.80–1.94 (m, 1H, CH*H*), 2.80 (t, J = 12.9 Hz, 1H, NC*H*H), 4.00 (d, J = 7.2 Hz, 2H, CH_2O), 3.98–4.12 (m, 1H, NCH*H*), 4.50–4.63 (m, 1H, CH), 6.63–6.74 (m, 2H, CH), 7.48–7.60 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 19.2 (CH_2), 25.0 (CH_2), 25.2 (CH_2), 28.4 (3×CH_3), 39.9 (CH_2), 48.9 (CH), 65.6 (CH_2O), 79.6 (C), 82.8 (C-I), 117.0 (2×CH), 138.1 (2×CH), 155.1 (CO), 158.5 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 417 [$M^{+\cdot}$] (1), 184 (20), 142 (38), 128 (100), 84 (58), 57 (46). Elemental analysis: found: C 49.27, H 5.58, N 3.19; calcd. for $C_{17}H_{24}INO_3$ (417.3): C 48.93, H 5.80, N 3.36.

***tert*-Butyl 2-(phenoxymethyl)piperidine-1-carboxylate (143):**[80] A suspension of potassium phenyltrifluoroborate (0.55 g, 3.0 mmol), $Cu(OAc)_2×H_2O$ (30 mg, 0.15 mmol), DMAP (40 mg, 0.3 mmol), and 4 Å molecular sieves (1.2 g) in CH_2Cl_2 (15 mL) was stirred at room temperature for 5 min, then compound **140** (0.32 g, 1.5 mmol) was added, and the mixture was stirred with a free access of air at room temperature for 85 h. The mixture was filtered through a Celite® pad, washed with CH_2Cl_2 (80 mL), and the filtrate was evaporated under reduced pressure. The title compound was isolated by silica gel chromatography (50 g SiO_2) eluting with hexane/EtOAc (4:1) to yield 0.24 g (55%) of a colorless solid; m. p. 75 °C (aq. MeOH), R_f = 0.37. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9H, *t*Bu), 1.52–1.72 (m, 5H, 2×CH_2 and C*H*H), 1.86–1.96 (m, 1H, CH*H*), 2.83 (t, J = 12.8 Hz, 1H, NC*H*H), 4.03 (d, J = 9 Hz, 2H, CH_2O), 4.00–4.14 (m, 1H, NCH*H*), 4.52–4.64 (m, 1H, CH), 6.86–6.99 (m, 3H, CH), 7.22–7.32 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 19.2 (CH_2), 25.1 (CH_2), 25.2 (CH_2), 28.4 (3×CH_3), 39.8 (CH_2), 49.0 (CH), 65.4 (CH_2O), 79.5 (C), 114.5 (2×CH), 129.4 (CH), 120.7 (2×CH), 155.2 (CO), 158.7 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 605 (12) $[2M+Na]^+$, 314 (100) $[M+Na]^+$. HR-MS (ESI, positive mode): found: 314.1727; calcd. for $C_{17}H_{25}NO_3$: 314.1727 $[M+Na]^+$.

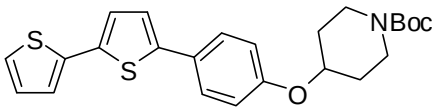


***tert*-Butyl 4-(4-iodophenoxy)piperidine-1-carboxylate (145):** *p*-Iodophenol (**118**) (0.88 g, 4.0 mmol), PPh_3 (1.26 g, 4.8 mmol), and *N*-Boc-4-hydroxypiperdine (**144**) (Acros Organics, 0.80 g, 4.0 mmol) were dissolved in anhydrous THF (20 mL), and the solution of DIAD (0.97 g, 4.8 mmol) in THF (5 mL) was added dropwise at 0 °C. The mixture was stirred overnight at room temperature, then the solvent was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (100 g SiO_2) eluting with hexane/EtOAc (6:1) to give the title compound (0.72 g, 60%) as a colorless solid; m. p 89 °C (aq. MeOH), R_f = 0.20. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.46 (s, 9H, *t*Bu), 1.66–1.78 (m, 2H, 2×C*H*H), 1.84–1.94 (m, 2H, 2×CH*H*), 3.27–3.38 (m, 2H,



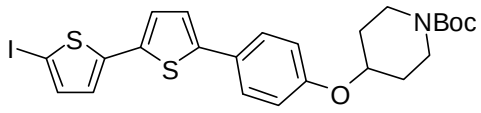
2×NC*H*H), 3.62–3.70 (m, 2H, 2×NCH*H*), 4.42 (m, 1H, CH-O), 6.67 (m, 2H, CH), 7.46 ppm (m, 2H, CH). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 28.4 (3×CH_3), 30.3 (2×CH_2), 40.8 (2×CH_2N), 72.2 (CH-O), 79.6 (C), 83.0 (C-I), 118.4 (2×CH), 138.3 (2×CH), 154.7 (CO), 157.0 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 403 (28) [$M^{+\cdot}$], 220 (36), 184 (73), 128 (32), 84 (82), 57 (100). Elemental analysis: found: C 47.54, H 5.50, N 3.47; calcd. for $C_{16}H_{22}INO_3$ (403.3): C 47.66, H 5.50, N 3.47.

***tert*-Butyl 4-[4-([2,2']bithiophen-5-yl)phenoxy]piperidine-1-carboxylate (147):** According to



GP 1.1, iodide **145** (0.73 g, 1.81 mmol), [2,2']bithiophene-5-boronic acid (**103**) (0.76 g, 3.62 mmol), $Pd(dba)_2$ (41 mg, 0.07 mmol) and PPh_3 (76 mg, 0.28 mmol) gave the title compound (0.69 g, 87%) as a yellowish solid after recrystallization; m. p. 146 °C (MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 1.68–1.81 (m, 2H, 2×C*H*H), 1.85–1.98 (m, 2H, 2×CH*H*), 3.27–3.40 (m, 2H, 2×NC*H*H), 3.62–3.75 (m, 2H, 2×NCH*H*), 4.48 (m, 1H, CH-O), 6.86–6.92 (m, 2H, CH), 7.00 (dd, *J* = 3.7, 5.1 Hz, 1H, CH), 7.05 (d, *J* = 3.8 Hz, 1H, CH), 7.09 (d, *J* = 3.8 Hz, 1H, CH), 7.17 (dd, *J* = 1.2, 3.7 Hz, 1H, CH), 7.21 (dd, *J* = 1.2, 5.1 Hz, 1H, CH), 7.46–7.52 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 28.7 (3×CH_3), 30.7 (2×CH_2), 40.8 (2×CH_2N), 72.5 (CH-O), 79.9 (C), 116.7 (2×CH), 123.0 (CH), 123.6 (CH), 124.4 (CH), 124.8 (CH), 127.2 (2×CH), 127.5 (C), 128.1 (CH), 136.0 (C), 137.8 (C), 143.2 (C), 155.1 (CO), 157.1 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 441 [$M^{+\cdot}$] (46), 262 (14), 259 (18), 258 (100), 84 (29), 57 (38). Elemental analysis: found: C 65.45, H 6.11, N 3.10; calcd. for $C_{24}H_{27}NO_3S_2$ (441.6): C 65.42, H 5.95, N 3.18.

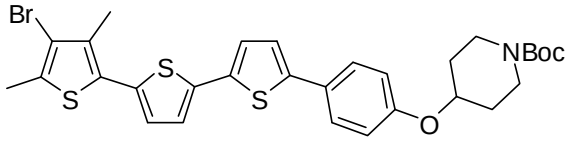
***tert*-Butyl 4-[4-(5'-iodo[2,2']bithiophen-5-yl)phenoxy]piperidine-1-carboxylate (148):**[129] A



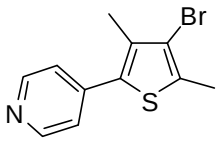
solution of the initial substance **147** (0.54 g, 1.3 mmol), periodic acid (52 mg, 0.23 mmol) and crystalline iodine (0.14 g, 0.56 mmol) in EtOH (15 mL) and AcOH (5 mL) was stirred at 60 °C for 24 h. The main part of the solvent was evaporated under reduced pressure, the precipitate was filtered, washed with 5% aq. Na_2SO_3, water, dried in air to give, after recrystallization, 0.62 g (84%) of the title compound as a yellow solid; m. p. 150 °C (MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 1.68–1.81 (m, 2H, 2×C*H*H), 1.85–1.97 (m, 2H, 2×CH*H*), 3.28–3.38 (m, 2H, 2×NC*H*H), 3.42–3.53 (m, 2H, 2×NCH*H*), 4.47 (m, 1H, CH-O), 6.82 (d, *J* = 3.8 Hz, 1H, CH), 6.86–6.94 (m, 2H, CH), 7.05 (d, *J* = 3.8 Hz, 1H, CH), 7.09 (d, *J* = 3.8 Hz, 1H, CH), 7.13 (d, *J* = 3.8 Hz, 1H, CH), 7.44–7.52 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 28.4 (3×CH_3), 30.4 (2×CH_2), 40.5 (2×CH_2N), 71.5 (C-I), 72.3

(CH-O), 79.6 (C), 116.4 (2×CH), 122.7 (CH), 124.7 (CH), 125.0 (CH), 126.9 (C), 127.0 (2×CH), 134.5 (C), 137.7 (CH), 143.4 (C), 143.5 (C), 154.8 (CO), 157.1 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 568 (20), 567 [$M^{+\cdot}$] (72), 384 (90), 277 (19), 257 (18), 213 (20), 128 (34), 84 (70), 57 (100), 41 (22). Elemental analysis: found: C 50.81, H 4.40, N 2.39; calcd. for $C_{24}H_{26}INO_3S_2$ (567.5): C 50.89, H 4.45, N 2.47.

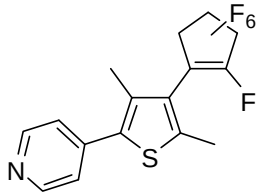
***tert*-Butyl 4-[4-(4''-bromo-3'',5''-dimethyl-[2,2',5',2'']trithiophene-5-yl)phenoxy]piperidine-1-carboxylate (149):** According GP 1.1, iodide **148** (0.45 g, 0.80 mmol), 4-bromo-3,5-dimethylthiophene-2-boronic acid (**109**) (0.23 g, 0.96 mmol), $Pd(dba)_2$ (18 mg, 32 μmol) and PPh_3 (34 mg, 0.13 mmol) gave 0.44 g (87%) of the title compound as a yellow solid after recrystallization; m. p. 198 °C (MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 9H, *t*Bu), 1.70–1.82 (m, 2H, 2×C*H*H), 1.86–1.98 (m, 2H, 2×CH*H*), 2.36 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.28–3.38 (m, 2H, 2×NC*H*H), 3.63–3.73 (m, 2H, 2×NCH*H*), 4.47 (m, 1H, CH-O), 6.85–6.93 (m, 2H, CH), 6.96 (d, *J* = 8.0 Hz, 1H, CH), 7.04–7.12 (m, 3H, CH), 7.45–7.53 ppm (m, 2H, CH). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 15.3 (CH_3), 16.0 (CH_3), 28.4 (3×CH_3), 30.5 (2×CH_2), 40.5 (2×CH_2N), 72.3 (CH-O), 79.6 (C), 114.0 (C-Br), 116.4 (2×CH), 122.8 (CH), 123.6 (CH), 124.6 (CH), 126.3 (CH), 127.0 (2×CH), 127.1 (C), 127.5 (C), 132.6 (C), 133.1 (C), 134.8 (C), 135.3 (C), 137.3 (CH), 143.2 (C), 154.8 (CO), 156.9 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 631/629 [$M^{+\cdot}$] (8), 448/447 (38), 441 (29), 258 (69), 84 (23), 57 (50), 56 (40), 55 (24), 44 (62), 41 (100). Elemental analysis: found: C 57.34, H 4.78, N 2.31; calcd. for $C_{30}H_{32}BrNO_3S_3$ (630.7): C 57.13, H 4.95, N 2.22.



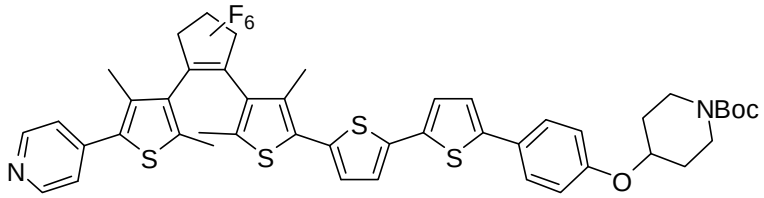
4-(4-Bromo-3,5-dimethylthiophen-2-yl)pyridine (150): According to GP 1.1, thiopheneboronic acid **109** (3.08 g, 13.2 mmol), 4-bromopyridine hydrochloride (**130**) (4.25 g, 21.9 mmol), $Pd(dba)_2$ (0.30 g, 0.53 mmol), and PPh_3 (0.55 g, 2.1 mmol) gave 3.0 g (87%) of a colorless solid after purification by silica gel chromatography (100 g SiO_2) eluting with CH_2Cl_2 + 1% v/v Et_3N and acetone (0 → 10% v/v of acetone); m. p. 93 °C (aq. MeOH). ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 2.33 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 7.28–7.36 (m, 2H, CH), 8.58–8.67 ppm (m, 2H, CH). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 15.3 (CH_3), 15.9 (CH_3), 114.6 (C-Br), 122.9 (CH), 131.5 (C), 134.5 (C), 134.6 (C), 142.0 (C), 150.1 ppm (CH). $C_{11}H_{10}BrNS$ (267.0), EI-MS, positive mode, *m/z* (rel. int., %): 269 (100) [M(^{81}Br)]$^+$, 268 (26), 267 (100) [M(^{79}Br)]$^+$, 266 (15), 188 (76).



4-[3,5-Dimethyl-4-(heptafluorocyclopent-1-enyl)-thiophen-2-yl]pyridine (151): According to GP 3, bromide **150** (3.2 g, 12.0 mmol), 2.5 M *n*BuLi in hexane (5.0 mL, 12.5 mmol), and C_5F_8 (3.5 g, 16.5 mmol) gave 3.8 g (83%) of the title compound as a brown solid after purification by silica gel chromatography (100 g SiO_2) eluting with cyclohexane/EtOAc (2:1→1:1); m. p. 96–97 °C (aq. MeOH). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 2.18 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 7.30–7.34 (m, 2H, CH), 8.61–8.65 ppm (m, 2H, CH). ^{13}C NMR (62.9 MHz, $CDCl_3$):* δ = 14.1 (2×CH_3), 121.6 (C), 123.2 (2×CH), 133.7 (C), 134.2 (C), 141.4 (C), 142.1 (C), 150.2 ppm (2×CH). EI-MS, positive mode, *m/z* (rel. int., %): 382 (18) $[M+H]^+$, 381 (100) $[M^{+\cdot}]$, 380 (19) $[M–H]^+$, 366 (14) $[M–CH_3]^+$. Elemental analysis: found: C 50.60, H 2.51, N 3.83; calcd. for $C_{16}H_{10}F_7NS$ (381.3): C 50.40, H 2.64, N 3.67;.



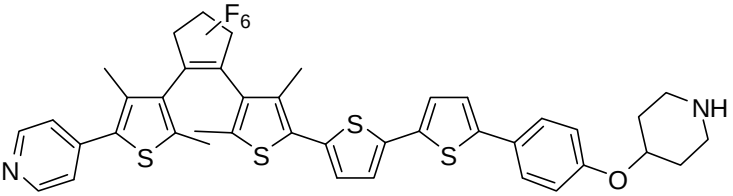
Photochromic compound 152: To a solution of compound **149** (0.19 g, 0.30 mmol) in anhydrous THF (4 mL), a 1.5 M solution of *t*BuLi in pentane (0.27 mL, 0.40 mmol) was added dropwise at –78 °C under inert atmosphere, and the mixture was stirred for 20 min. Then a solution of compound **151** (0.12 g, 0.33 mmol) in anhydrous THF (0.5 mL) was added dropwise, and the mixture was stirred at room temperature overnight. It was diluted with EtOAc (20 mL), washed with brine, and dried over Na_2SO_4. The solvents were evaporated under reduced pressure, and the residue was purified by silica gel chromatography (100 g SiO_2) eluting with hexane/EtOAc (1:2) to yield 0.15 g (55%) of the title compound as a yellow foam; R_f = 0.39. HPLC: 70 → 100% A (30 → 0% B) for 0–15 min, 100% ACN from 15–20 min, 1 mL/min, 25 °C, t_R (OF) = 19.5 min, detection at 410 nm. ^{1}H NMR (300 MHz, $CDCl_3$ open form, mixture of rotamers): δ = 1.47 (s, 9H, *t*Bu), 1.69–1.84 (m, 2H, CH*H*), 1.86–2.00 (m, 2H, CH*H*), 2.13–2.19 (m, 3H, CH_3), 2.19–2.32 (m, 3H, CH_3), 2.32–2.37 (m, 3H, CH_3), 2.37–2.43 (m, 3H, CH_3), 3.29–3.42 (m, 2H, NC*H*H), 3.64–3.77 (m, 2H, NCH*H*), 4.47 (m, 1H, CH-O), 6.88–6.95 (m, 2H, CH), 6.98 (d, *J* = 3.9 Hz, 1H, CH), 7.09–7.12 (m, 3H, CH), 7.26–7.30 (m, 2H, CH_{py}), 7.48–7.54 (m, 2H, CH), 8.58–8.63 ppm (m, 2H, CH_{py}). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.8 (CH_3), 15.0 (2×CH_3), 15.1 (CH_3), 28.4 (3×CH_3), 30.4 (2×CH_2), 40.6 (2×CH_2N), 72.3 (CH-O), 79.6 (C), 116.4 (2×CH), 122.8 (CH), 123.1 (2×CH), 123.5 (CH), 124.6 (CH), 126.1 (C), 126.4 (CH), 127.0 (2×CH), 129.7 (C), 132.5 (C), 132.6 (C), 133.4 (C), 134.2



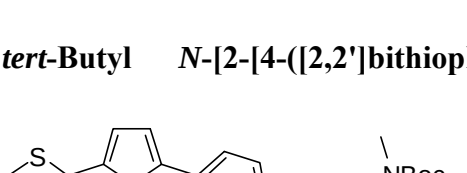
* Due to low intensities, signals of the fluorinated carbons were not detected.

(C), 135.1 (C), 137.5 (C), 138.6 (C), 138.9 (C), 141.0 (C), 141.6 (C), 143.3 (C), 150.0 (2×CH), 150.1 (C), 154.8 (CO), 157.0 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 912.2 (23) [$M^{+\cdot}$], 812.2 (42), 729.1 (100), 56.0 (32), 40.9 (50). HR-MS (ESI, positive mode): found: 913.2056; calcd. for $C_{46}H_{42}F_6N_2O_3S_4$: 913.2061 $[M+H]^+$.

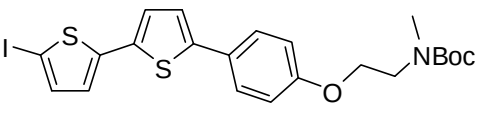
Photochromic compound 153: To a solution of compound **152** (40 mg, 0.044 mmol) in CH_2Cl_2 (0.5 mL), 4 M HCl in 1,4-dioxane (2 mL) was added, and the mixture was stirred at room temperature for 3 h. Then the solvents were evaporated under reduced pressure, Et_2O was added, and the precipitated product was filtered to yield 34 mg (97%) of the title compound as a yellow powder. $C_{41}H_{34}F_6N_2OS_4$ (812), EI-MS, positive mode, *m/z* (rel. int., %): 813 (21), 812 [$M^{+\cdot}$] (48), 731 (22), 730 (38), 729 (100).



***tert*-Butyl *N*-[2-[4-([2,2']bithiophen-5-yl)phenoxy]ethyl]-*N*-methyl carbamate (154):** According to GP 1.1, iodide **123** (0.38 g, 1.0 mmol), [2,2']bithiophene-5-boronic acid (**103**) (0.42 g, 2.0 mmol), $Pd(dba)_2$ (23 mg, 0.04 mmol) and PPh_3 (42 mg, 0.16 mmol) gave 0.38 g (92%) of the title compound as a yelowish solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 3.00 (s, 3H, CH_3N), 3.57–3.66 (m, 2H, CH_2N), 4.06–4.18 (m, 2H, CH_2O), 6.88–6.94 (m, 2H, CH), 7.02 (d, J = 3.6 Hz, 1H, CH), 7.10–7.13 (m, 2H, CH), 7.17–7.22 (m, 2H, CH), 7.48–7.55 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 28.4 (3×CH_3), 36.4/35.2 (CH_3N), 48.2 (CH_2N), 66.1/66.9 (CH_2O), 79.7 (C), 114.8 (2×CH), 122.6 (CH), 123.3 (CH), 124.1 (CH), 124.5 (CH), 126.9 (2×CH), 127.8 (CH), 135.7 (C), 137.5 (2×C), 143.0 (C), 155.8 (CO), 158.3 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 415.2 (12) [$M^{+\cdot}$], 258.0 (100), 102.0 (46), 57 (20). Elemental analysis: found: C 63.30, H 5.83, N 3.29; calcd. for $C_{22}H_{25}NO_3S_2$ (415.6): C 63.58, H 6.06, N 3.37.

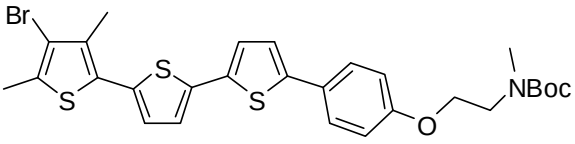


***tert*-Butyl *N*-[2-[4-(5'-iodo-[2,2']bithiophen-5-yl)phenoxy]ethyl]-*N*-methyl carbamate (155):**[129] A solution of the compound **154** (0.29 g, 0.70 mmol), periodic acid (30 mg, 0.13 mmol) and crystalline iodine (80 mg, 0.30 mmol) in EtOH (7 mL) was stirred at 60 °C for 17 h. The mixture was allowed cooling down, and the precipitate was filtered off, washed with 5% aq. Na_2SO_3, water, dried in air and gave after recrystallization 0.30 g (83%) of the title compound as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 2.99 (s, 3H, CH_3), 3.56–3.66 (m, 2H, CH_2N), 4.05–4.17 (m, 2H, CH_2O), 6.84 (d, J = 3.8 Hz, 1H, CH),

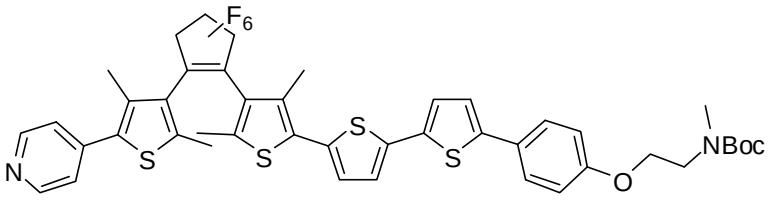


6.87–6.94 (m, 2H, CH), 7.05 (d, J = 3.8 Hz, 1H, CH), 7.09 (d, J = 3.8 Hz, 1H, CH), 7.15 (d, J = 3.8 Hz, 1H, CH), 7.46–7.54 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 28.4 (3×CH_3), 36.4/35.2 (CH_3N), 48.2 (CH_2N), 66.2/66.8 (CH_2O), 71.5 (C-I), 79.7 (C), 114.8 (2×CH), 122.7 (CH), 124.7 (CH), 125.0 (CH), 127.0 (2×CH), 134.5 (C), 137.6 (CH), 143.4 (C), 143.6 (2×C), 155.8 (CO), 158.5 ppm (CO). EI-MS, positive mode, *m/z* (rel. int., %): 541.1 (18) [$M^{+\cdot}$], 384.0 (100), 258.0 (14), 102.0 (96), 57.0 (32), 41.0 (16). Elemental analysis: found: C 48.65, H 4.37, N 2.71; calcd. for $C_{22}H_{24}INO_3S_2$ (541.5): C 48.80, H 4.47, N 2.59.

Compound 156: According to GP 1.2, iodide **155** (0.2 g, 0.37 mmol), 4-bromo-3,5-dimethylthiophene-2-boronic acid (**109**) (0.13 g, 0.55 mmol), $Pd(dba)_2$ (12 mg, 0.02 mmol), Cs_2CO_3 (0.12 g, 0.37 mmol), and P(tBu)$_3$ (0.1 mL, 0.026 mmol) gave 0.18 g (81%) of the title compound as a yellow solid; hexane/EtOAc 2:1, R_f = 0.43. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, tBu), 2.38 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 3.00 (s, 3H, NCH_3), 3.56–3.64 (m, 2H, NCH_2), 4.04–4.18 (m, 2H, OCH_2), 6.84–6.92 (m, 2H, CH), 6.94–7.02 (m, 1H, CH), 7.08–7.18 (m, 3H, CH), 7.46–7.56 ppm (m, 2H, CH). EI-MS, positive mode, *m/z* (rel. int., %): 605/603 (5) [$M^{+\cdot}$], 505/503 (18), 448/446 (60), 102 (56), 58 (42), 44 (100). HR-MS (ESI, positive mode): found: 626.0457, 628.0449; calcd. for $C_{28}H_{30}BrNO_3S_3$: 626.0463, 628.0443 $[M+Na]^+$.

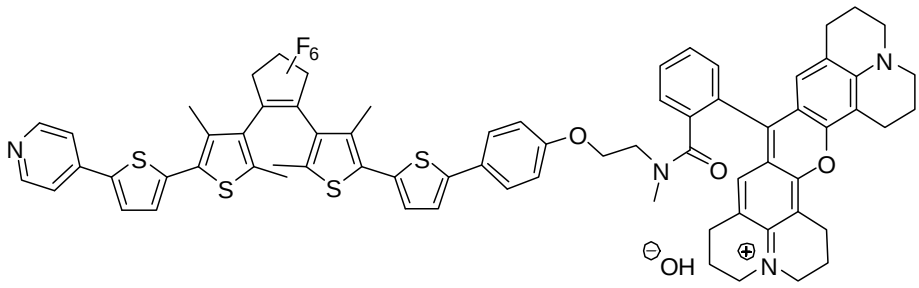


Photochromic compound 157: To a solution of bromide **156** (54 mg, 0.089 mmol) in anhydrous THF (2.5 mL), 1.5 M solution of tBuLi in pentane (1.5 mL, 0.10 mmol) was dropwise added at –78 °C, stirred for 20 min followed by the addition of heptafluorocyclopentene **151** (35 mg, 0.10 mmol) in anhydrous THF (0.5 mL), and the mixture was stirred at room temperature for 2 h. The mixture was diluted with EtOAc (20 mL), washed with brine, and dried with Na_2SO_4. After evaporation of the solvent, the title compound was purified by silica gel chromatography (50 g SiO_2) eluting with hexane/EtOAc (1:1) to yield 43 mg (50%) of a bright yellow solid; R_f = 0.27. HPLC: 90 → 100% A (10 → 0% B) for 0–15 min, 100% ACN from 15–20 min, 1 mL/min, 25 °C, t_R (OF) = 7.3 min, t_R (CF) = 14.0 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.46/1.47 (s, 9H, tBu), 2.10–2.24 (m, 6H, CH_3), 2.31–2.44 (m, 6H, CH_3), 2.98/2.99 (s, 3H, CH_3N), 3.54–3.67 (m, 2H, CH_2N), 4.03–4.19 (m, 2H, CH_2O), 6.86–6.94 (m, 2H, CH), 6.98–7.04 (m, 1H, CH), 7.06–7.16 (m, 2H, CH), 7.21–7.26 (m, 1H, CH), 7.39–7.43



(m, 1H, CH), 7.45–7.58 (m, 3H, CH), 8.57–8.70 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, open form, mixture of rotamers):* δ =14.0 (2×CH_3), 14.8 (CH_3), 15.0 (CH_3), 28.4 (3×CH_3), 36.0/36.1 (CH_3N), 48.3 (CH_2N), 66.2/66.8 (CH_2O), 79.7 (C), 114.9 (2×CH), 119.5 (C), 122.8 (2×C), 123.0 (C), 123.1 (2×CH), 123.4 (2×C), 123.5 (CH), 124.6 (2×C), 126.3 (CH), 126.9 (2×CH), 127.1 (2×CH), 133.2 (2×C), 134.1 (C), 134.3 (2×C), 134.5 (C), 137.5 (C), 141.9 (C), 156.6/156.7 (CO), 158.4/158.8 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 887.2 (49) $[M+H]^+$, 777.2 (100) $[M+H–Boc]^+$. HR-MS (ESI, positive mode): found: 887.1898; calcd. for $C_{44}H_{40}F_6N_2O_3S_4$: 887.1899 $[M+H]^+$.

Adduct 159: Compound **137** (9 mg, 12 μmol), rhodamine 101 (**158**) (5 mg, 12 μmol) and HATU



(9 mg, 24 μmol) were mixed in CH_2Cl_2 (3 mL), and Et_3N was added (4 mg, 48 μmol). The mixture was left overnight at room temperature with stirring under Ar. Then it was diluted with CH_2Cl_2 (20 mL), washed with 0.1 M aq. H_2SO_4, water, 0.5 M aq. $NaHCO_3$ (5 mL each time), and dried. After evaporation of the solvent under reduced pressure, the residue was purified on silica gel (30 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to yield 12 mg (80%) of the title compound as a violet solid; R_f = 0.1. Purity of the colored fractions was controlled by HPLC: 80 → 100% A (20 → 0% B) for 0–15 min, 100% ACN from 15–20 min, 1 mL/min, 25 °C, t_R (OF) = 18.0 min, t_R (CF) = 18.8 min, detection at 368 nm (isobestic point). ^{1}H NMR (300 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.85–1.97 (m, 4H, CH_2 in Rh), 1.97–2.14 (m, 4H, CH_2 in Rh), 2.21–2.28 (br. s, 6H, CH_3), 2.34–2.41 (br. s, 6H, CH_3), 2.62–2.73 (m, 4H, CH_2 in Rh), 2.82–2.94 (m, 4H, CH_2 in Rh), 3.11–3.14 (m, 3H, CH_3N), 3.25–3.58 (m, 10H, 4×CH_2 in Rh and CH_2N), 3.95–3.98 (m, 2H, CH_2O), 6.62–6.68 (m, 2H, CH), 6.72–6.76 (m, 2H, CH), 7.05 (d, J = 3.8 Hz, 1H, CH), 7.11 (d, J = 3.8 Hz, 1H, CH), 7.18 (m, 1H, CH), 7.32–7.37 (m, 1H, CH), 7.44–7.52 (m, 5H, CH), 7.55–7.60 (m, 1H, CH), 7.64–7.70 (m, 2H, CH), 8.56–8.62 ppm (m, 2H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 1259.4 (100) $[M]^+$. HR-MS (ESI, positive mode): found: 1259.3514; calcd. for $C_{71}H_{61}N_4O_3S_4F_6^+$: 1259.3531 $[M]^+$.

* Due to low intensities, signals of the fluorinated carbons were not detected.

Adduct 160: To a solution of compound **153** (21 mg, 24 μmol) and rhodamine 101 (**158**) (12 mg, 24 μmol) in CH_2Cl_2 (3 mL), were added HATU (19 mg, 50 μmol) and NEt_3 (7 μL, 50 μmol) at 0 °C under inert atmosphere, and the mixture was stirred at room temperature overnight. It was diluted with CH_2Cl_2 (10 mL), washed with water, 0.1 M aq. H_2SO_4, 0.5 M aq. $NaHCO_3$, water, brine (5 mL each time), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the title compound was isolated by silica gel chromatography (20 g SiO_2) eluting with $CHCl_3$/MeOH (10:1) to give 21 mg (70%) of the title compound as a dark violet powder; R_f = 0.1. HPLC: 90 → 100% A (10 → 0% B) for 0–15 min, 100% ACN from 15–20 min, 1 mL/min, 25 °C, t_R (OF) = 17.2 min, t_R (CF) = 18.0 min, detection at 370 nm. ^{1}H NMR (300 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.73–1.87 (m, 2H, 2×C*H*H), 1.88–2.03 (m, 6H, 2×CH*H* and 2×CH_2 in Rh), 2.04–2.16 (m, 4H, CH_2 in Rh), 2.14/2.15 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.39/2.40 (s, 3H, CH_3), 2.63–2.78 (m, 4H, 2×CH_2 in Rh), 2.96–3.08 (m, 4H, 2×CH_2 in Rh), 3.24–3.40 (m, 2H, 2×NC*H*H), 3.40–3.62 (m, 8H, 4×CH_2N in Rh), 3.62–3.76 (m, 2H, 2×NCH*H*), 4.55–4.67 (m, 1H, CH-O), 6.65 (s, 1H, CH), 6.72 (s, 1H, CH), 6.87–6.93 (m, 2H, CH), 6.96 (d, J = 3.8 Hz, 1H, CH), 7.07–7.10 (m, 3H, CH), 7.26–7.32 (m, 3H, CH_{py} and CH), 7.45–7.51 (m, 2H, CH), 7.51–7.56 (m, 1H, CH), 7.60–7.68 (m, 2H, CH), 8.58–8.62 ppm (m, 2H, CH_{py}). ESI-MS, positive mode, *m/z* (rel. int., %): 1285.4 (100) $[M]^+$. HR-MS (ESI, positive mode): found: 1285.3687; calcd. for $C_{73}H_{64}F_6N_4O_3S_4^+$: 1285.3687 $[M]^+$.

1-Methyl 4-*tert*-butyl *N*-benzyloxycarbonyl-*N*-methyl-*L*-aspartate (164a):[85] To a solution of *N*-Z-Asp(O*t*Bu)OH (**163a**) (Fluka, 1.66 g, 5.0 mmol) and MeI (10.2 g, 72.0 mmol) in anhydrous DMF (15 mL), Ag_2O (4.3 g, 19.0 mmol) was added, and the black suspension was vigorously stirred at room temperature for 16 h. Dichloromethane (100 mL) was added to the reaction mixture, and it was filtered through a Celite® pad. The filter-cake was washed with CH_2Cl_2 (2×50 mL), and the combined organic solutions were washed with sat. aq. $Na_2S_2O_3$ (2×50 mL), water (8×50 mL), brine (50 mL), and dried. The mixture was concentrated under reduced pressure, and the residue was purified by chromatography on SiO_2 (100 g) eluting with hexane/EtOAc (1:1) to yield the title compound (1.5 g, 85%) as a colorless oil; R_f = 0.6. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.42 (s, 9H, *t*Bu), 2.58–2.73 (m, 1H, CH), 2.91/2.94 (s, 3H,

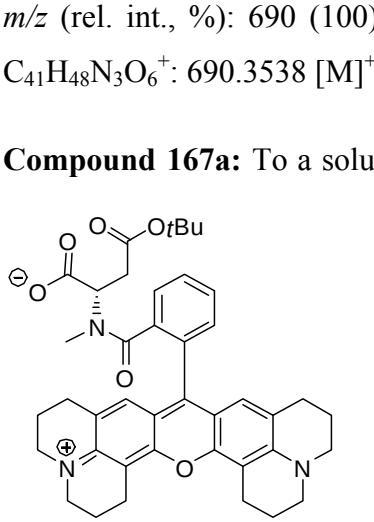
CH_3N), 2.90–3.03 (m, 1H, CH), 3.56/3.71 (s, 3H, OCH_3), 4.75–4.97 (m, 1H, CH), 5.12 (m, 2H, CH_2), 7.33 ppm (m, 5H, CH). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 27.9 (3×CH_3), 32.7/33.7 (CH_3N), 35.8/36.4 (CH_2), 52.3/52.4 (CH), 57.0 (CH_3O), 67.4 (CH_2), 81.1/81.3 (C), 127.7 (CH), 127.9 (2×CH), 128.4 (2×CH), 136.2/136.4 (C), 155.7/156.2 (NC=O), 169.6 (C=O), 170.7 ppm (C=O). $[\alpha]_D^{20}$ = –27.0 (*c* = 1.33, $CHCl_3$). $C_{18}H_{25}NO_6$ (351.2), ESI-MS, positive mode, *m/z* (rel. int., %): 725 (48) $[2M+Na]^+$, 374 (100) $[M+Na]^+$.

1-Methyl 4-*tert*-butyl *N*-methyl-*L*-aspartate (*N*-MeAsp(O*t*Bu)OMe) (165a): A solution of compound **164a** (0.64 g, 1.82 mmol) in EtOAc (20 mL) was flushed with argon and then vigorously stirred with 76 mg of 10% Pd/C (MERCK, oxidized form) in the atmosphere of H_2 at a normal pressure and room temperature for 16 h. Then the mixture was flushed with argon, filtered through Celite® to remove the charcoal with Pd, the filter-cake was washed with EtOAc (3×10 mL), and the filtrate was evaporated in vacuo to give the title compound as a colorless oil (0.4 g, 99%), which was used in the next step without further purification. ^1H NMR (250 MHz, $CDCl_3$): δ = 1.40 (s, 9H, *t*Bu), 2.37 (s, 3H, CH_3N), 2.49–2.67 (m, 2H, CH_2), 3.42–3.48 (m, 1H, CH), 3.71 ppm (s, 3H, CH_3O). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 27.9 (3×CH_3), 34.6 (CH_3N), 38.7 (CH_2), 51.9 (CH), 59.4 (CH_3O), 81.0 (C), 170.0 (CO), 174.0 ppm (CO). $[\alpha]_D^{20}$ = –0.5 (*c* = 1.21, $CHCl_3$). $C_{10}H_{19}NO_4$ (217.1), ESI-MS, positive mode, *m/z* (rel. int., %): 457 (6) $[2M+Na]^+$, 435 (4) $[2M+H]^+$, 240 (30) $[M+Na]^+$, 218 (100) $[M+H]^+$.

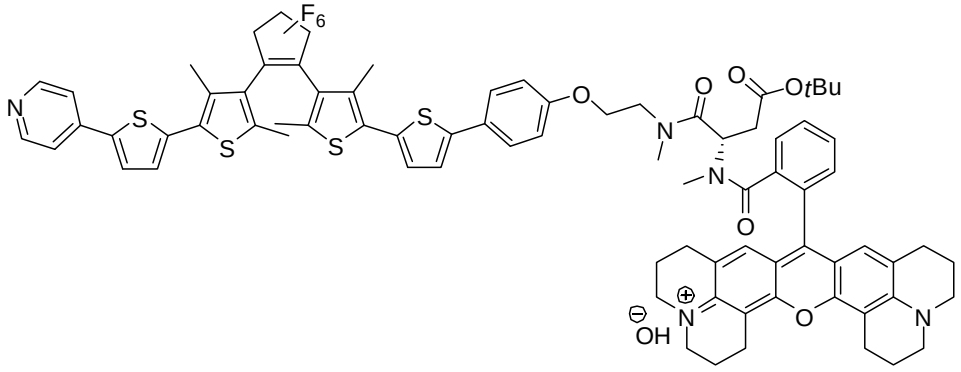
Compound 166a: To a solution of compound **165a** (0.10 g, 0.47 mmol), rhodamin 101 (**158**) (0.22 g, 0.44 mmol) and HATU (0.33 g, 0.88 mmol) in CH_2Cl_2 (2 mL), NEt_3 (3 drops) was added. The mixture was stirred at room temperature overnight. Then it was diluted with CH_2Cl_2 (30 mL), washed with water, 0.1 M H_2SO_4, water, 0.5 M $NaHCO_3$, water, brine (10 mL each time) and dried. After evaporation of the solvent under reduced pressure, the residue was purified on silica gel (100 g) eluting with CH_2Cl_2/MeOH (10:1) to obtain 0.28 g (90%) of the title compound as a dark violet powder. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 1 mL/min, 25 °C, t_R = 6.5 min, detection at 254 nm. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.38 (s, 9H, *t*Bu), 1.87–2.02 (m, 4H, CH_2 in Rh), 2.02–2.15 (m, 4H, CH_2 in Rh), 2.31–2.42 (m, 1H, C*H*H), 2.58–2.72 (m, 4H, CH_2 in Rh), 2.74–2.83 (m, 1H, CH*H*), 2.91 (s, 3H, CH_3N), 2.93–3.04 (m, 4H, CH_2 in Rh), 3.37 (s, 3H, CH_3O), 3.39–3.59 (m, 8H, CH_2N in Rh), 4.65–4.73 (m, 1H, CH), 6.64–6.72 (m, 2H, CH), 7.27–7.33 (m, 1H, CH), 7.45–7.53 (m, 1H, CH), 7.58–7.69 ppm (m, 2H, CH). ESI-MS, positive mode,

m/z (rel. int., %): 690 (100) $[M]^+$. HR-MS (ESI, positive mode): found: 690.3538; calcd. for $C_{41}H_{48}N_3O_6^+$: 690.3538 $[M]^+$.

Compound 167a: To a solution **166a** (0.21 g, 0.3 mmol) in MeOH (1 mL) and THF (1 mL), 1 M NaOH (0.5 mL) was added at 0 °C. The mixturre was stirred at room temperature for 3 h. Then 1 M HCl (0.7 mL) was added to pH 2.0, and the mixture was extracted with CH_2Cl_2 (3×5 mL). The extract was washed with brine, dried over $MgSO_4$, filtred and evaporated to give the title compound (0.2 g, 90%) as a dark violet solid. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 1 mL/min, 25 °C, t_R = 5.0 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38 (s, 9H, *t*Bu), 1.85–1.98 (m, 4H, CH_2 in Rh), 1.98–2.10 (m, 4H, CH_2 in Rh), 2.32–2.44 (m, 1H, C*H*H), 2.60–2.70 (m, 4H, CH_2 in Rh), 2.72–2.84 (m, 1H, CH*H*), 2.92 (s, 3H, CH_3N), 2.92–3.02 (m, 4H, CH_2 in Rh), 3.30–3.56 (m, 8H, CH_2 in Rh), 4.82–4.92 (m, 1H, CH), 6.58–6.76 (m, 2H, CH), 7.26–7.32 (m, 1H, CH), 7.48–7.62 ppm (m, 3H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 676.3 (100) $[M+H]^+$. HR-MS (ESI, positive mode): found: 676.3380; calcd. for $C_{41}H_{45}N_3O_6$: 676.3381 $[M+H]^+$.



Compound 168a: Compound **137** (150 mg, 0.175 mmol), **167a** (118 mg, 0.175 mmol) and HATU (133 mg, 0.350 mmol) were mixed in CH_2Cl_2 (1 mL), and NEt_3 (100 µL) was added. The mixture was stirred at room temperature for 4 h, then it was diluted with CH_2Cl_2 (20 mL), washed with water, 0.1 M H_2SO_4, water, 0.5 M $NaHCO_3$, water, brine, and dried. After evaporation of the solvent under reduced pressure, the residue was purified by silica gel chromatography (100 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to give the title compound (0.19 g, 74%) as a dark violet solid. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 100% A for 20–25 min, 1 mL/min, 25 °C, t_R (OF) = 21.0 min, t_R (CF) = 22.1 min, detection at 370 nm (isobestic point). ^{1}H NMR (300 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.30/1.35 (s, 9H, *t*Bu), 1.87–2.02 (m, 4H, CH_2 in Rh), 2.02–2.15 (m, 4H, CH_2 in Rh), 2.17–2.26 (m, 6H, CH_3), 2.32–2.39 (m, 6H, CH_3), 2.60–2.73 (m, 5H, CH_2 in Rh and C*H*H), 2.78/2.86 (s,



3H, CH_3N), 2.91/2.92 (s, 4H, CH_3N and CH*H*), 2.94–3.06 (m, 4H, CH_2 in Rh), 3.38–3.61 (m, 10H, 4×CH_2 in Rh and CH_2N), 4.05–4.17 (m, 2H, OCH_2), 5.79/5.51 (m, 1H, CH), 6.62/6.68 (s, 1H, CH in Rh), 6.71/6.72 (s, 1H, CH in Rh), 6.83–6.90 (m, 2H, CH), 6.98–7.02 (m, 1H, CH), 7.08–7.10 (m, 1H,CH), 7.10–7.15 (m, 1H, CH), 7.33–7.40 (m, 1H, CH in Rh), 7.40–7.54 (m, 6H, CH), 7.58–7.69 (m, 2H, CH), 8.54–8.60 ppm (m, 2H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 1444 (100) $[M]^+$. HR-MS (ESI, positive mode): found: 1444.4581, 722.7328; calcd. for $C_{80}H_{76}F_6N_5O_6S_4^+$: 1444.4577 $[M]^+$, 722.7325 $[M+H]^{2+}$.

Compound 169a: To a solution **168a** (127 mg, 87 μmol) in CH_2Cl_2 (0.2 mL) were added Et_3SiH (25 mg, 0.22 mmol) and TFA (87 μL, 1.1 mmol) at 0 °C under argon atmosphere, and the mixture was kept overnight at 4 °C. Then solvent and TFA were evaporated, and the residue was purified on silica gel RP C18 (50 g) eluting with ACN/water (9:1 + 0.1% v/v TFA) to yield 105 mg (87%) of the title compound as a dark violet solid. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 100% A from 20–25 min, 1 mL/min, 25 °C, t_R (OF) = 14.1 min, t_R (CF) = 21.3 min, detection at 370 nm (isobestic point). ^{1}H NMR (600 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.85–2.00 (m, 4H, CH_2 in Rh), 2.00–2.12 (m, 4H, CH_2 in Rh), 2.15–2.26 (m, 6H, CH_3), 2.28–2.40 (m, 6H, CH_3), 2.60–2.73 (m, 5H, 2×CH_2 in Rh and C*H*H), 2.74–2.80 (m, 3H, CH_3N), 2.82–2.95 (s, 4H, CH_3N and CH*H*), 2.98–3.08 (m, 4H, 2×CH_2 in Rh), 3.38–3.52 (m, 8H, 4×CH_2 in Rh), 3.50–3.58 (m, 2H, CH_2N), 4.01–4.10 (m, 2H, OCH_2), 5.44/5.70 (m, 1H, CH), 6.60–6.74 (m, 2H, CH in Rh), 6.76–6.84 (m, 2H, CH), 6.92–7.00 (m, 1H, CH), 7.02–7.10 (m, 1H, CH), 7.15–7.20 (m, 1H, CH), 7.30–7.38 (m, 2H, CH), 7.40–7.48 (m, 2H, CH), 7.56–7.64 (m, 2H, CH), 7.68–7.74 (m, 1H, CH), 7.80–7.86 (m, 2H, CH), 8.60–8.70 ppm (m, 2H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 1388 (100) $[M+H]^+$. HR-MS (ESI, positive mode): found: 1388.3951, calcd. for $C_{76}H_{67}F_6N_5O_6S_4$: 1388.3957 $[M+H]^+$.

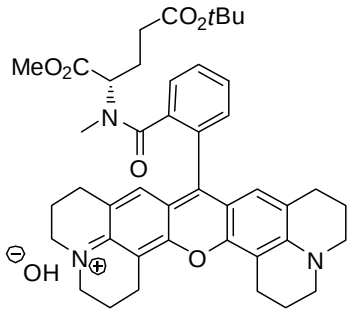
Compound 171a: TSTU (**170**) (Alfa Aesar, 1.3 mg, 4.4 μmol) and DIEA (1.0 μL, 5.4 μmol) were added to a solution of **169a** (5.0 mg, 3.6 μmol) in anhydrous DMF (0.2 mL). The mixture was stirred in the dark at room temperature for 2 h under argon, and the solvent was evaporated to yield 5 mg (92%) of the title compound as a dark violet solid. ^{1}H NMR (600 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.93–2.03 (m, 4H, 2×CH_2 in Rh), 2.04–2.15 (m, 4H, 2×CH_2 in Rh), 2.16–2.26 (m, 6H, CH_3), 2.28–2.41 (m, 6H, CH_3), 2.76 (s, 4H, 2×CH_2 in NHS), 2.60–3.15 (m, 16H, 4×CH_2 in Rh, 2×CH_3N, CH_2), 3.42–3.65 (m, 8H, 4×CH_2 in Rh), 3.50–3.70 (m, 2H, CH_2N), 4.00–4.20 (m, 2H, OCH_2), 5.64/5.94 (m, 1H, CH), 6.58–6.76 (m, 2H, CH in Rh), 6.80–6.93 (m, 2H, CH), 6.97–7.03 (m, 1H, CH), 7.06–7.17 (m, 2H, CH), 7.37–7.54 (m, 6H, CH), 7.54–7.74 (m, 3H, CH), 8.53–8.62 ppm (m, 2H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 1485 (100) $[M]^+$, 743 (12) $[M+H]^{2+}$. HR-MS (ESI, positive mode): found: 743.2094, calcd. for $C_{80}H_{71}F_6N_6O_8S_4^+$: 743.2094 $[M+H]^{2+}$.

(*S*)-5-*tert*-Butyl 1-methyl 2-(*N*-benzyloxycarbonyl-*N*-methylamino)pentanedioate (164b): Compound **164b** was prepared as describe for compound **164a** from compound **163b** (1.68 g, 5.0 mmol) in amount of 1.68 g (92%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 2 rotamers): δ = 1.41/1.43 (s, 9H, *t*Bu), 1.89–2.07 (m, 1H, C*H*H), 2.18–2.35 (m, 3H, CH_2CO and CH*H*), 2.87/2.88 (s, 3H, NCH_3), 3.64/3.71 (s, 3H, OCH_3), 4.61/4.79 (dd, J = 4.3, 10.6 Hz, 1H, CH), 5.13/5.15 (s, 2H, CH_2), 7.27–7.42 ppm (m, 5H, CH). ^{13}C NMR (50 MHz, $CDCl_3$, 2 rotamers): δ = 23.8/24.1 (*C*H_2CH), 27.9 (3×CH_3), 30.6 (CH), 31.5/31.8 (*C*H_2CO), 52.1 (NCH_3), 58.1/58.3 (OCH_3), 67.4 (CH_2Ph), 80.6 (C), 127.9 (2×CH), 128.1 (CH), 128.6 (2×CH), 136.5/136.6 (C), 156.4/157.1 (CO), 171.5/171.7 (CO), 172.1 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 753.4 (42) $[2M+Na]^+$, 388.2 (100) $[M+Na]^+$. HR-MS (ESI, positive mode): found: 388.1729; calcd. for $C_{19}H_{27}NO_6$: 388.1731 $[M+Na]^+$.

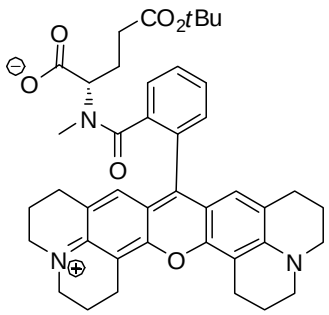
(*S*)-5-*tert*-Butyl 1-methyl 2-(*N*-methylamino)pentanedioate (165b): Compound **165b** was prepared as describe for compound **165a** from compound **164b** (1.6 g, 4.4 mmol) in amount of 1.0 g (99%) as a colorless oil. ^{1}H NMR (300 MHz,

$CDCl_3$): δ = 1.37 (s, 9H, *t*Bu), 1.68–1.95 (m, 2H, CH_2), 2.26 (t, J = 7.5 Hz, 2H, CH_2CO), 2.29 (s, 3H, NCH_3), 3.10 (dd, J = 6.0, 7.6 Hz, 1H, CH), 3.67 ppm (s, 3H, OCH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 27.9 (3×CH_3), 28.1 (*C*H_2CH), 31.7 (*C*H_2CO), 34.6 (CH), 51.7 (NCH_3), 62.3 (OCH_3), 80.3 (C), 172.5 (CO), 175.6 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 485.3 (100) $[2M+Na]^+$, 254.2 (37) $[M+Na]^+$, 232.2 (32) $[M+H]^+$. HR-MS (ESI, positive mode): found: 232.1544; calcd. for $C_{11}H_{21}NO_4$: 232.1543 $[M+H]^+$.

Compound 166b: To a solution of **165b** (0.14 g, 0.61 mmol), rhodamin 101 (**158**) (0.20 g, 0.40 mmol) and HATU (0.30 g, 0.80 mmol) in CH_2Cl_2 (1 mL), NEt_3 (80 µL) was added at 0 °C. The mixture was stirred at room temperature for 7 h. Then it was diluted with CH_2Cl_2 (30 mL), washed with water, 0.1 M H_2SO_4, water, 0.5 M $NaHCO_3$, water, brine (10 mL each time) and dried over Na_2SO_4. After evaporation of the solvent under reduced pressure, the residue was purified on silica gel (100 g) eluting with CH_2Cl_2/MeOH (20:1 → 10:1) to give 0.26 g (89%) of the title compound as a dark violet solid. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 1 mL/min, 25 °C, t_R = 6.8 min, detection at 254 nm. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.39 (s, 9H, *t*Bu), 1.65–1.90 (m, 2H, CH_2), 1.90–2.00 (m, 4H, CH_2 in Rh), 2.04–2.18 (m, 4H, CH_2 in Rh), 2.70 (t, J = 6.0 Hz, 4H, CH_2 in Rh), 2.80 (s, 3H, NCH_3), 2.85 (s, 2H, CH_2CO), 3.00 (t, J = 6.3 Hz, 4H, CH_2 in Rh), 3.51 (s, 3H, OCH_3), 3.45–3.55 (m, 8H, CH_2 in Rh), 4.68–4.78 (m, 1H, CH), 6.71 (d, J = 2.7 Hz, 2H, CH), 7.32–7.40 (m, 1H, CH), 7.54–7.60 (m, 1H, CH), 7.64–7.71 ppm (m, 2H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 704.4 (100) $[M^+]$. HR-MS (ESI, positive mode): found: 704.3696, calcd. for $C_{43}H_{50}N_3O_6^+$ (704.4): 704.3694 $[M^+]$.



Compound 167b: To a solution **166b** (0.22 g, 0.3 mmol) in MeOH (1 mL) and THF (1 mL), 1 M NaOH (0.5 mL) was added at 0 °C. The mixturre was stirred at room temperature for 3 h. Then 1 M HCl (0.7 mL) was added to pH 2.0, and the mixture was extracted with CH_2Cl_2 (3×5 mL). The extract was washed with brine, dried over $MgSO_4$, filtred and evaporated under reduced pressure to give 0.20 g (97%) of the title compound as a dark violet powder. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 1 mL/min, 25 °C, t_R = 5.2 min, detection at 254 nm. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 9H, *t*Bu), 1.74–2.00 (m, 5H, 2×CH_2 in Rh and C*H*H), 2.00–2.24 (m, 5H, 2×CH_2 in Rh and CH*H*), 2.57–2.73 (m, 4H, CH_2 in Rh), 2.79 (s, 3H, NCH_3), 2.83 (s, 2H, CH_2CO), 2.89–3.05 (m, 4H, CH_2 in Rh), 3.36–3.59 (m, 8H, CH_2 in Rh), 4.71–4.83 (m, 1H, CH),



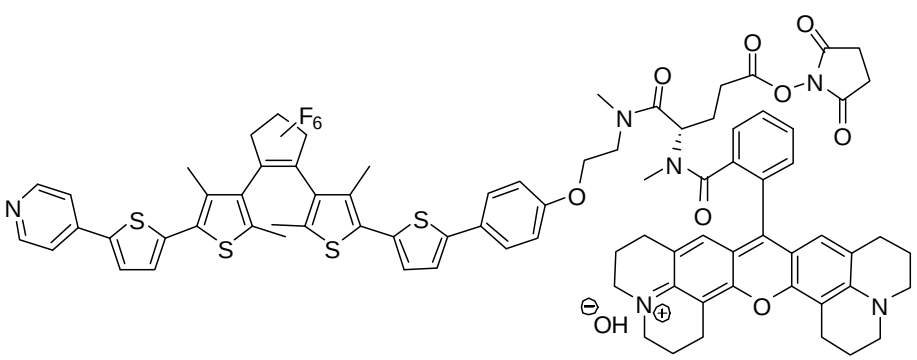
6.63–6.75 (m, 2H, CH), 7.29–7.41 (m, 1H, CH), 7.53–7.61 (m, 1H, CH), 7.61–7.71 ppm (m, 2H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 690.4 (100) $[M+H]^+$. HR-MS (ESI, positive mode): found: 690.3537, calcd. for $C_{42}H_{47}N_3O_6$ (689.3): 690.3538 $[M+H]^+$.

Compound 168b: Photochromic compound **137** (50 mg, 58 μmol), compound **167b** (40 mg, 58 μmol) and HATU (44 mg, 0.12 mmol) were mixed in CH_2Cl_2 (0.5 mL), and NEt_3 (25 μL) was added. The mixture was stirred at room temperature for 4 h, then it was diluted with CH_2Cl_2 (20 mL), washed with water, 0.1 M H_2SO_4, water, 0.5 M $NaHCO_3$, water, brine, and dried. After evaporation of the solvent under reduced pressure, the residue was purified by silica gel chromatography (50 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to yield 45 mg (54%) of the title compound as a dark violet powder. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 100% A for 20–25 min, 1 mL/min, 25 °C, t_R (OF) = 22.6 min, t_R (CF) = 23.7 min, detection at 370 nm (isobestic point). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26–1.33 (m, 9H, *t*Bu), 1.80–1.95 (m, 6H, 2×CH_2 in Rh and CH_2), 1.95–2.08 (m, 4H, CH_2 in Rh), 2.09–2.22 (m, 6H, CH_3), 2.22–2.33 (m, 6H, CH_3), 2.57–2.66 (m, 4H, CH_2 in Rh), 2.73 (s, 3H, NCH_3), 2.78/2.81 (s, 3H, NCH_3), 2.83–2.96 (m, 6H, CH_2 in Rh and CH_2CO), 3.35–3.50 (m, 8H, CH_2 in Rh), 3.49–4.10 (m, 4H, CH_2O and CH_2N), 5.09/5.32 (t, J = 7.0 Hz, 1H, CH), 6.53–6.58 (m, 1H, CH), 6.65 (d, J = 5.6 Hz, 1H, CH), 6.71–6.84 (m, 2H, CH), 6.90–6.97 (m, 1H, CH), 6.99–7.09 (m, 2H, CH), 7.27–7.40 (m, 5H, CH), 7.40–7.64 (m, 4H, CH), 8.44–8.57 ppm (m, 2H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 1458.5 (100) $[M^+]$. HR-MS (ESI, positive mode): found: 729.7401, calcd. for $C_{81}H_{78}F_6N_5O_6S_4^+$ (1458.5): 729.7403 $[M+H]^{2+}$.

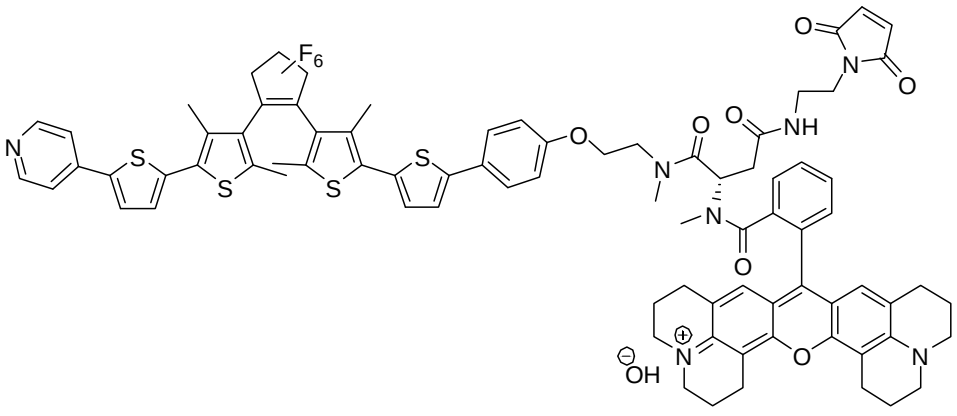
Compound 169b: To a solution of **168b** (37 mg, 25 μmol) in CH_2Cl_2 (1 mL) were added Et_3SiH (10 μL, 62.5 μmol) and TFA (25 μL, 0.33 mmol) at 0 °C under argon atmosphere, and the mixture was kept overnight at 4 °C. Then solvent and TFA were evaporated, and the residue was purified on silica gel RP C18 (50 g)

eluting with ACN/water (9:1 + 0.1% v/v TFA) to yield 33 mg (93%) of the title compound as a dark violet solid. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 100% A for 20–25 min, 1 mL/min, 25 °C, t_R (OF) = 17.5 min, detection at 370 nm (isobestic point). ESI-MS, positive mode, *m/z* (rel. int., %): 1402.4 (100) $[M+H]^+$, 701.7 (78) $[M+2H]^{2+}$. HR-MS (ESI, positive mode): found: 1402.4111, calcd. for $C_{77}H_{69}F_6N_5O_6S_4$ (1401.4): 1402.4108 $[M+H]^+$.

Compound 171b: TSTU (**170**) (2.6 mg, 8.7 μmol) and DIEA (10 μL) were added to a solution of compound **169b** (10 mg, 7.2 μmol) in anhydrous DMF (0.4 mL). The mixture was stirred in the dark at room temperature for 2 h under argon, and the solvent was evaporated. The title compound was purified by silica gel chromatography (20 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to give 9.5 mg (88%) of a dark violet powder. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 100% A for 20–25 min, 1 mL/min, 25 °C, t_R (OF) = 18.4 min, detection at 370 nm (isobestic point). ESI-MS, positive mode, *m/z* (rel. int., %): 1499 (100) $[M]^+$. HR-MS (ESI, positive mode): found: 750.2171, calcd. for $C_{81}H_{73}F_6N_6O_8S_4^+$ (1499): 750.2172 $[M+H]^{2+}$.

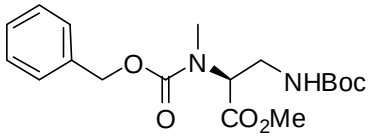


Compound 175: In an anhydrous flask under inert atmosphere to a mixture of acid **169a** (8.5 mg, 6.1 μmol) and *N*-(2-aminoethyl)maleimide trifluoroacetate (**174**) (Fluka, 3.1 mg, 12.3 μmol) in CH_2Cl_2 (0.5 mL), HATU (4.7 mg, 12.3 μmol) and NEt_3 (2.5 μL, 18.4 μmol) were added at 0 °C, and the mixture was stirred at room temperature for 10 h. After evaporation of the solvent under reduced pressure, the title compound was isolated by silica gel (20 g SiO_2) eluting with CH_2Cl_2/MeOH (15:1) to yield 4.1 mg (43%) of a dark violet powder. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 100% A for 20–25 min, 1 mL/min, 25 °C, t_R (OF) = 18.6 min, detection at 370 nm (isobestic point). ESI-

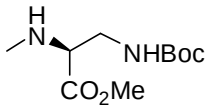


MS, positive mode, *m/z* (rel. int., %): 1510.5 (8) $[M]^+$, 755.7 (100) $[M+H]^{2+}$. HR-MS (ESI-MS, positive mode): found: 755.7253; calcd. for $C_{82}H_{75}F_6N_7O_7S_4^{2+}$: 755.7252 $[M+H]^{2+}$.

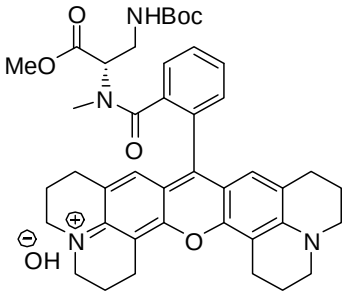
Compound 178: To a solution of **177** (0.74 g, 2.1 mmol) in CH_2Cl_2 (5 mL), MeOH (0.1 mL, 2.5 mmol) was added followed by EDC (0.36 g, 2.3 mmol) at 0 °C, and the mixture was stirred at room temperature for 8 h. It was diluted with Et_2O (30 mL), washed with 0.5 M citric acid, water, 0.5 M $NaHCO_3$, water, brine, and dried. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (70 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to yield 0.6 g (78%) of a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 (s, 9H, *t*Bu), 2.96 (s, 3H, NCH_3), 3.59 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 4.39–4.58 (m, 1H, CH), 4.75–4.90 (m, 1H, NH), 5.05–5.20 (m, 2H, CH_2O), 7.34 ppm (m, 5H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 28.3 (3×CH_3), 33.4/34.0 (NCH_3), 39.3/39.7 (NCH_2), 52.33 (OCH_3), 59.7/60.0 (CH), 67.5/67.7 (OCH_2), 79.6/79.7 (C), 127.8 (CH), 128.0 (CH), 128.5 (3×CH), 136.1/136.4 (C), 155.8 (NHC=O), 156.7 (NC=O), 170.3 ppm (C=O). $C_{18}H_{26}N_2O_6$ (366), ESI-MS, positive mode, *m/z* (rel. int., %): 755 (100) $[2M+Na]^+$, 389 (45) $[M+Na]^+$.



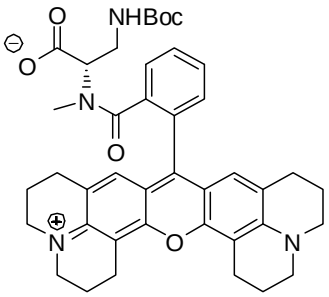
Compound 179: A solution of **178** (0.29 g, 0.79 mmol) in EtOAc (15 mL) was vigorously stirred with 100 mg of 10% Pd/C (Merck, oxidized form) in the atmosphere of H_2 under a normal pressure and at room temperature for 16 h. Then the mixture was flushed with argon, filtered through a Celite® pad to remove the charcoal with Pd, the filter-cake was washed with EtOAc (3×10 mL), and the filtrate was evaporated in vacuo to give the title compound (0.18 g, 98%) as a colorless oil, which was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 (s, 9H, *t*Bu), 2.38 (s, 3H, NCH_3), 3.19–3.29 (m, 2H, CH_2), 3.38–3.48 (m, 1H, CH), 3.73 (s, 3H, OCH_3), 5.05 ppm (s, 1H, NH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 28.3 (3×CH_3), 34.6 (NCH_3), 42.0 (CH_2), 52.1 (OCH_3), 62.6 (CH), 79.5 (C), 155.8 (NHC=O), 173.5 ppm (C=O). ESI-MS, positive mode, *m/z* (rel. int., %): 487.3 (100) $[2M+Na]^+$, 255.2 (25) $[M+Na]^+$, 233.2 (30) $[M+H]^+$. HR-MS (ESI, positive mode): found: 233.1495, 255.1317; calcd. for $C_{10}H_{20}N_2O_4$: 233.1496 $[M+H]^+$, 255.1315 $[M+Na]^+$.



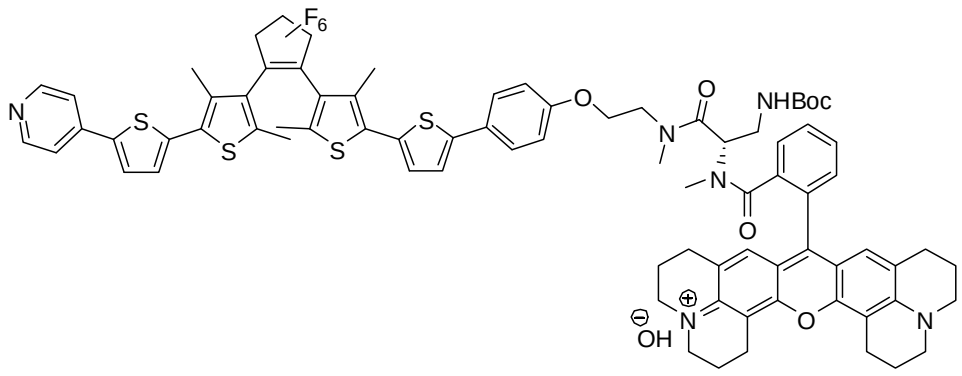
Compound 180: To a mixture of amine **179** (0.10 g, 0.43 mmol) and rhodamine 101 (**158**) (0.14 g, 0.29 mmol) in CH_2Cl_2 (1 mL), NEt_3 (60 µL, 0.43 mmol) and HATU (0.22 g, 0.57 mmol) were added at 0 °C, and the mixture was stirred at room temperature overnight. After evaporation of the solvent under reduced pressure, the title compound was isolated by silica gel chromatography (100 g SiO_2) eluting with CH_2Cl_2/MeOH (15:1 → 10:1) to yield 0.15 g (73%) of a dark violet solid. $C_{42}H_{49}N_4O_6^+$ (705.4), ESI-MS, positive mode, *m/z* (rel. int., %): 705 (100) $[M]^+$.



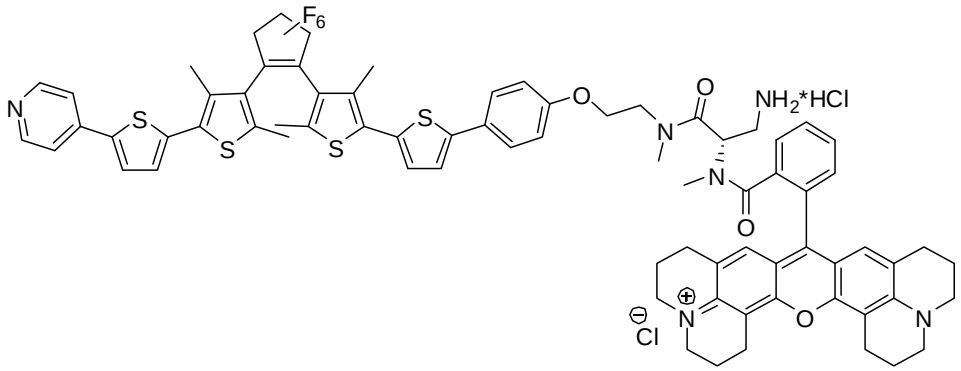
Compound 181: The compound **180** (0.14 g, 0.2 mmol) was dissolved in 1 mL MeOH/THF mixture (1:1), and 1 M NaOH (0.4 mL) was added. The mixture was stirred at room temperature for 3 h, diluted with CH_2Cl_2 (10 mL), washed with 0.5 M H_2SO_4, water, dried over Na_2SO_4, and evaporated under reduced pressure to give 0.13 g (94%) of the title compound as a dark violet solid. $C_{41}H_{46}N_4O_6$ (690.3), ESI-MS, positive mode, *m/z* (rel. int., %): 691 (100) $[M+H]^+$.



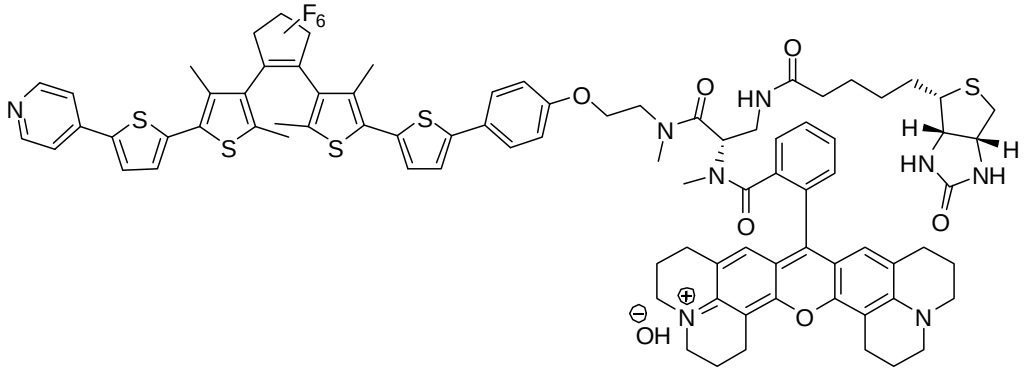
Compound 182: Rhodamine derivative **181** (0.12 g, 0.18 mmol) and photochromic compound **137** (0.15 g, 0.18 mmol) were mixed in CH_2Cl_2 (2 mL), and HATU (0.14 g, 0.35 mmol) and NEt_3 (75 µL, 0.53 mmol) were added under inert atmosphere, and the mixture was stirred at room temperature for 8 h. It was diluted with CH_2Cl_2 (20 mL), washed with water, 0.1 M H_2SO_4, water, 0.5 M $NaHCO_3$, water, brine (5 mL each time), and dried. After evaporation of the solvent, the title compound was purified by silica gel chromatography (100 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to give 0.18 g (69%) of a dark violet powder. $C_{80}H_{77}F_6N_6O_6S_4^+$ (1459.5), ESI-MS, positive mode, *m/z* (rel. int., %): 1459 (100) $[M]^+$.



Compound 183: To a solution of compound **182** (0.17 g, 0.11 mmol) in CH_2Cl_2 (0.5 mL), 4 M HCl in 1,4-dioxane (1 mL) was added followed by stirring at room temperature for 1 h. To this mixture ACN (5 mL) was added, and the precipitate was filtered off, washed with cold ACN, and dried to yield the title compound (150 mg, 99%) as a violet solid. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 1 mL/min, 25 °C, t_R (OF) = 7.7 min, detection at 370 nm. $C_{75}H_{69}F_6N_6O_4S_4^+$ (1359.4), ESI-MS, positive mode, *m/z* (rel. int., %): 1359 (31) $[M]^+$, 680 (100) $[M+H]^+$.

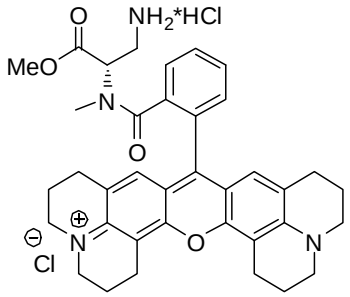


Compound 185: To a mixture of compound **188** (32 mg, 0.039 mmol) and **137** (34 mg, 0.04 mmol) in CH_2Cl_2 (4 mL), HATU (30 mg, 0.08 mmol) and NEt_3 (5 µL) were added at 0 °C, and the mixture was stirred at room temperature for 16 h. It was diluted with CH_2Cl_2 (30 mL), washed with 0.1 M HCl, water, 0.5 M $NaHCO_3$, water (10 mL each time), and dried over Na_2SO_4. After evaporation of the solvent under reduced pressure, the title compound was isolated by silica gel chromatography (30 g SiO_2) eluting with CH_2Cl_2/MeOH (8:1 + 0.1% NEt_3) to obtain 31 mg (49%) of a dark-violet powder. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 100% A for 20–30 min, 1 mL/min, 25 °C, t_R (OF) = 25.0 min, detection at 590 nm. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.21–1.28 (m, 2H, CH_2), 1.41–1.59 (m, 2H, CH_2), 1.59–1.82 (m, 2H, CH_2), 1.82–1.92 (m, 2H, CH_2CO), 1.92–2.13 (m, 8H, CH_2 in Rh), 2.13–2.26 (m, 6H, CH_3), 2.26–2.39 (m, 6H, CH_3), 2.39–2.58 (m, 4H, CH_2 in Rh), 2.58–2.75 (m, 4H, CH_2 in Rh), 2.75–3.08 (m, 10H, 2×NCH_3, CH_2S, CH_2N), 3.08–3.30 (m, 1H, CHS), 3.30–3.54 (m, 8H, CH_2 in Rh), 3.54–3.76 (m, 2H, CH_2N), 3.80–4.45 (m, 4H, CH_2O and 2×CH), 5.44–5.64 (m, 1H, CH), 6.65–6.90 (m, 5H, CH), 6.95–7.00 (m, 1H, CH), 7.04–7.11 (m, 2H, CH), 7.38–7.48 (m, 5H, CH), 7.53–7.65 (m, 2H,

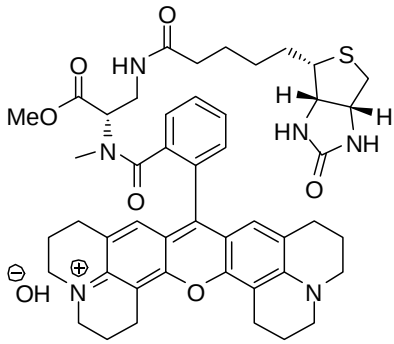


CH), 7.63–7.82 (m, 1H, CH), 8.51–8.61 ppm (m, 2H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 1585.5 (2) $[M]^+$, 793.3 (100) $[M+H]^{2+}$. HR-MS (ESI, positive mode): found: 793.2504, calcd. for $C_{85}H_{83}F_6N_8O_6S_5^+$: 793.2505 $[M+H]^{2+}$.

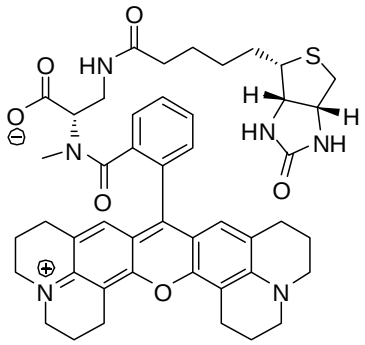
Compound 186: Compound **180** (36 mg, 0.05 mmol) in CH_2Cl_2 (1.5 mL) was reacted with 4 M HCl in 1,4-dioxane (0.8 mL) at 4 °C overnight. The solvents were evaporated to obtain 33 mg (99%) of the title compound as a violet powder, which was washed with Et_2O and used in the next step without additional purification. $C_{37}H_{41}ClN_4O_4^+Cl^-$ (605.3), ESI-MS, positive mode, *m/z* (rel. int., %): 605 (100) $[M]^+$.



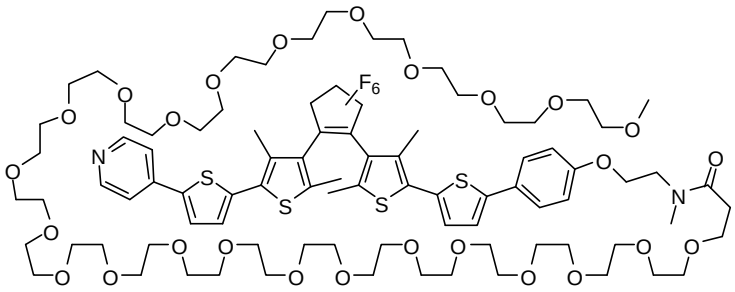
Compound 187: To a solution of biotin (**184**) (12 mg, 0.05 mmol) in DMF (1 mL), HATU (38 mg, 0.10 mmol) and NEt_3 (10 µL) were added at 0 °C followed by compound **186** (33 mg, 0.05 mmol), and the mixture was stirred at room temperature for 1.5 h. After evaporation of the solvent in vacuo, the product was isolated by silica gel chromatography (30 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to obtain 33 mg (79%) of the title compound as a violet powder. HPLC: 70 → 100% A (30 → 0% B) for 0–20 min, 1 mL/min, 25 °C, t_R = 5.1 min, detection at 254 nm. $C_{47}H_{55}N_6O_6S^+$ (831.4), ESI-MS, positive mode, *m/z* (rel. int., %): 831 (100) $[M]^+$.



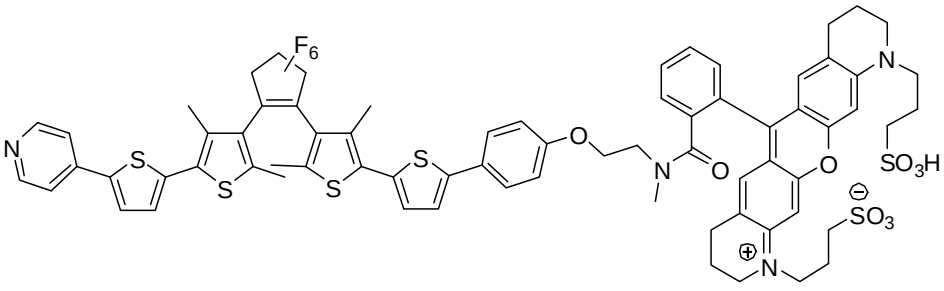
Compound 188: A solution of **187** (33 mg, 0.039 mmol) in ACN (0.5 mL) and 1 M NaOH (10 drops) was kept at 4 °C for 12 h, and evaporated under reduced pressure to get 32 mg (99%) of the title compound as a violet powder. Compound was used in the next step without further purification. HPLC: 70 → 100% A (30 → 0% B) for 0–20 min, 1 mL/min, 25 °C, t_R = 4.1 min, detection at 254 nm. $C_{46}H_{52}N_6O_6S$ (816.4), ESI-MS, positive mode, *m/z* (rel. int., %): 839 (44) $[M+Na]^+$, 817 (100) $[M+H]^+$.



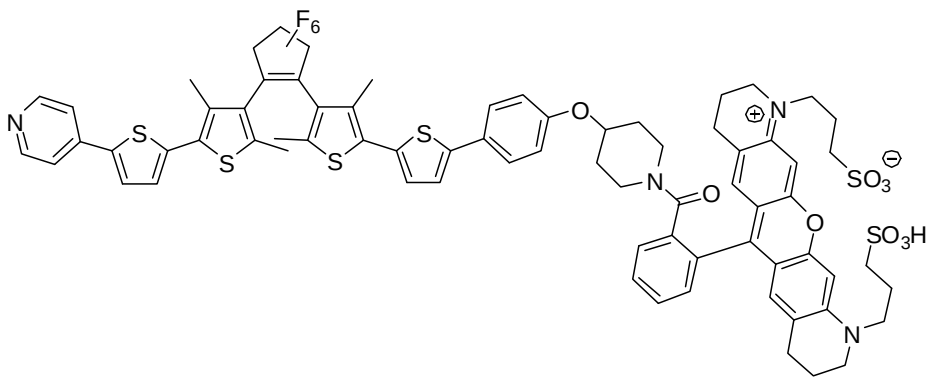
Adduct 190: In a dry flask under inert atmosphere, to a solution of compound **137** (5 mg, 5.8 µmol) and m-dPEG™24-NHS ester (**189**) (Quanta BioDesign, 7 mg, 5.8 µmol) in anhydrous DMF (25 µL), DIEA (2 µL, 11.6 µmol) was added, and the mixture was stirred at room temperature for 12 h. After evaporation of the solvent in vacuo, the title compound was isolated by silica gel chromatography (20 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to yield 8 mg (73%) of a yellow solid. HPLC: 80 → 100% A (20 → 0% B) for 0–20 min, 100% A for 20–30 min, 1 mL/min, 25 °C, t_R (OF) = 24.8 min, detection at 370 nm (isobestic point). ESI-MS, positive mode, *m/z* (rel. int., %): 1885.8 (2) $[M+H]^+$, 943.4 (100) $[M+2H]^{2+}$, 629.3 (40) $[M+3H]^{3+}$. HR-MS (ESI, positive mode): found: 943.3918, 629.2637; calcd. for $C_{89}H_{130}F_6N_2O_{26}S_4$: 943.3922 $[M+2H]^{2+}$, 629.2639 $[M+3H]^{3+}$.



Adduct 192: To a stirred mixture of photochromic compound **137** (17 mg, 0.02 mmol) and dye **191** (13 mg, 0.02 mmol) in anhydrous DMF (0.5 mL), HATU (16 mg, 0.04 mmol) and DIEA (3 drops) were added, and the mixture was stirred at room temperature for 8 h. After evaporation of the solvent in vacuo, the title compound was isolated by silica gel chromatography (20 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to give 7.5 mg (26%) of a dark violet powder. HPLC: 50 → 100% A (50 → 0% B) for 0–25 min, 1 mL/min, 25 °C, t_R (OF) = 15.3 min, detection at 360 nm. ESI-MS, positive mode, *m/z* (rel. int., %): 1423.3 (60) $[M+H]^+$, 712.2 $[M+2H]^{2+}$. HR-MS (ESI, positive mode): found: 712.1523; calcd. for $C_{71}H_{64}F_6N_4O_9S_6$: 712.1524 $[M+2H]^{2+}$.

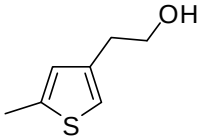


Adduct 194: To a mixture of compounds **193** (5.9 mg, 6.7 μmol), **191** (4.0 mg, 6.4 μmol) and



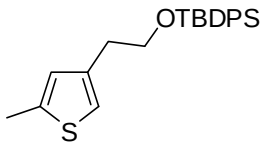
NEt_3 (5 μL) in DMF (0.1 mL) and CH_2Cl_2 (0.1 mL), HATU (5.2 mg, 12.7 μmol) was added at 0 °C under nitrogen atmosphere, and the mixture was stirred at room temperature for 10 h. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (20 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to afford 4.2 mg (43%) of a dark violet powder. HPLC: 20 → 80% A (80 → 20% B) for 0–25 min, 1 mL/min, 25 °C, t_R (OF) = 12.2 min, t_R (CF) = 13.3 min, detection at 360 nm. ESI-MS, negative mode, *m/z* (rel. int., %): 1448.4 (100) $[M–H]^-$. HR-MS (ESI, positive mode): found: 1449.3125; calcd. for $C_{73}H_{64}F_6N_4O_9S_6$: 1449.3131 $[M+H]^+$.

2-(5-Methylthiophen-3-yl)ethanol (197): To a solution of 4-bromo-2-methylthiophene (**196**)



(8.86 g, 50.0 mmol) in anhydrous THF (30 mL) and toluene (300 mL), 2.5 M solution of *n*BuLi in hexane (24 mL, 60.0 mmol) was added dropwise at –78 °C under inert atmosphere. The mixture was stirred for 30 min at this temperature, then ethylene oxide (8.8 mL) was added followed by $BF_3×Et_2O$ (8.8 mL), which was added after 3 min. The mixture was stirred at –78 °C for 1 h, then MeOH (20 mL) was added at 0 °C, and the mixture was washed with sat. aq. NH_4Cl (100 mL), and dried over Na_2SO_4. The solvents were evaporated under reduced pressure, and the title compound was distilled in vacuo to obtain 5.32 g (75%) of a colorless oil, b.p. 60 °C (0.7 mbar). ^1H NMR (200 MHz, $CDCl_3$): δ = 2.45 (s, 3H, CH_3), 2.80 (t, J = 6.3 Hz, 2H, CH_2), 3.81 (dt, J = 6.0, 6.3 Hz, 2H, CH_2O), 6.64 (s, 1H, CH), 6.78 ppm (s, 1H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 15.1 (CH_3), 33.7 (CH_2), 62.8 (CH_2O), 119.5 (CH), 126.6 (CH), 138.7 (C), 140.5 ppm (C). $C_7H_{10}OS$ (142), EI-MS, positive mode, *m/z* (rel. int., %): 142.0 $[M^{+\cdot}]$ (40), 111.0 (100).

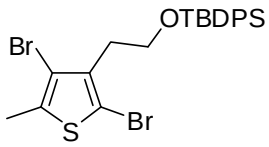
4-[2-(*tert*-Butyldiphenylsilyloxy)ethyl]-2-methylthiophene (198): To a mixture of compound



197 (3.15 g, 22.2 mmol) and imidazole (3.02 g, 44.4 mmol) in anhydrous DMF (2 mL), *tert*-butyldiphenylsilyl chloride (7.32 g, 26.6 mmol) was added at 0 °C under inert atmosphere, and the mixture was stirred at room temperature for 24 h. Then the mixture was washed with 0.5 M aqueous citric acid up to pH =

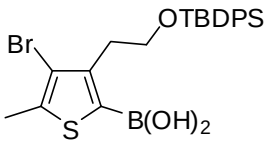
5.0, diluted with Et_2O (100 mL), washed with water (50 mL), 5% aq. Na_2CO_3, water (3×50 mL), brine, and dried over $MgSO_4$. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (100 g SiO_2) eluting with hexane/EtOAc (30:1) to yield 7.49 g (89%) of a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.05 (s. 9H, *t*BuSi), 2.44 (s, 3H, CH_3), 2.80 (t, J = 6.9 Hz, 2H, CH_2), 3.83 (t, J = 6.9 Hz, 2H, CH_2O), 6.56 (s, 1H, CH), 6.70 (s, 1H, CH), 7.31–7.48 (m, 6H, CH), 7.59–7.68 ppm (m, 4H, CH). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 15.1 (CH_3), 19.0 (C-Si), 26.7 (3×CH_3), 33.7 (CH_2), 64.4 (CH_2O), 119.1 (CH), 127.3 (CH), 127.7 (4×CH), 129.6 (2×CH), 133.9 (2×C-Si), 135.7 (4×CH), 139.3 (C), 139.4 ppm (C). ESI-MS, positive mode, *m/z* (rel. int., %): 403 (16) $[M+Na]^+$, 381 (100) $[M+H]^+$. Elemental analysis: found: C 72.61, H 7.15; calcd. for $C_{23}H_{28}OSSi$ (380.6): C 72.58, H 7.41.

3-[2-(*tert*-Butyldiphenylsilyloxy)ethyl]-2,4-dibromo-5-methylthiophene (199): A solution of



bromine (2.1 mL, 41.3 mmol) in AcOH (10 mL) was added dropwise to a stirring mixture of compound **198** (7.49 g, 19.7 mmol) and KOAc (4.87 g, 49.6 mmol) in AcOH (25 mL) at 0 °C, and the mixture was stirred at room temperature for 20 min. Then it was poured into the ice-water mixture contained Na_2CO_3, neutralized with aq. Na_2CO_3, and extracted with $CHCl_3$ (200 mL). The organic phase was washed with water (3×100 mL), brine, and dried over Na_2SO_4. After evaporation of the solvent under reduced pressure, the title compound was isolated by silica gel chromatography (150 g SiO_2) eluting with hexane/EtOAc (70:1) to give 7.06 g (67%) of a colorless powder; m. p. 60–61 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.03 (s, 9H, *t*BuSi), 2.31 (s, 3H, CH_3), 2.93 (t, J = 7.2 Hz, 2H, CH_2), 3.77 (t, J = 7.2 Hz, 2H, CH_2O), 7.33–7.42 (m, 6H, CH), 7.61–7.66 ppm (m, 4H, CH). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 15.2 (CH_3), 19.0 (C-Si), 26.6 (3×CH_3), 33.1 (CH_2), 61.7 (CH_2O), 106.7 (C-Br), 111.3 (C-Br), 127.7 (4×CH), 129.6 (2×CH), 133.8 (2×C-Si), 134.2 (C), 135.7 (4×CH), 136.9 ppm (C). ESI-MS, positive mode, *m/z* (rel. int., %): 539.0 (100) $[M+H]^+$, 282.9 (92) $[M+H+Na]^{2+}$. Elemental analysis: found: C 51.57, H 4.63; calcd. for $C_{23}H_{26}Br_2OSSi$ (538.4): C 51.31, H 4.87.

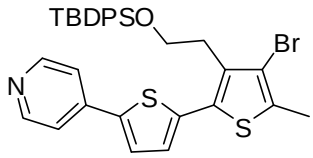
4-Bromo-3-[2-(*tert*-butyldiphenylsilyloxy)ethy]-5-methylthiophene-2-boronic acid (200): To



a solution of compound **199** (0.81 g, 1.5 mmol) in anhydrous Et_2O (6 mL), 2.5 M solution of *n*BuLi in hexane (0.63 mL, 1.58 mmol) was added dropwise at –78 °C under inert atmosphere, and the mixture was stirred for 1 h at this temperature. Then $B(OiPr)_3$ (0.42 g, 2.25 mmol) was added, and the mixture was stirred for 10 min at –78 °C and 2 h at room temperature. Then water (1 mL) was added at 0 °C, the organic layer was extracted with 1 M NaOH (3×20 mL), the aqueous solution was acidified

with citric acid at 0 °C. The precipitate was filtered, and dried to give 0.55 g (74%)* of title compound as a colorless powder. ^{1}H NMR (300 MHz, [D$_6$]DMSO, boronic acid anhydride): δ = 0.92/0.96 (s, 18H, *t*BuSi), 2.25/2.35 (s, 6H, CH$_3$), 3.19 (t, J = 7.1 Hz, 2H, part of CH$_2$), 3.32 (t, J = 7.4 Hz, 2H, part of CH$_2$), 3.73 (t, J = 7.1 Hz, 2H, part of CH$_2$), 3.80 (t, J = 7.3 Hz, 2H, part of CH$_2$), 7.25–7.47 (m, 6H, CH), 7.51–7.59 (m, 4H, CH), 7.92 ppm (s, 2H, OH). ^{13}C NMR (75.5 MHz, [D$_6$]DMSO, boronic acid anhydride): δ = 15.1 (CH$_3$), 18.6 (C-Si), 26.5 (3×CH$_3$), 32.7 (CH$_2$), 63.6 (CH$_2$O), 113.7 (C-Br), 127.5/127.7 (4×CH), 129.5/129.6 (2×CH), 132.9 (2×C-Si), 133.1 (C), 134.9/135.0 (4×CH), 138.0 (C), 144.1 ppm (C). $C_{46}H_{54}B_2Br_2O_5S_2Si_2$, 986 ($C_{23}H_{28}BBrO_3SSi$, 502). ESI-MS, positive mode, *m/z* (rel. int., %): 987.2 (100) $[2M–H_2O+H]^+$, 527.1/525.1 (59) $[M+Na]^+$.

4-[4'-Bromo-3'-[2-(*tert*-butyldiphenylsilyloxy)ethyl]-5'-methyl-[2,2']bithiophen-5-yl]pyridine (201): Into a Schlenk-flask under inert atmosphere were placed 4-(5-iodothiophen-2-yl)pyridine (**132**) (0.51 g, 1.77 mmol), PPh_3 (0.19 g, 0.71 mmol) and $Pd(dba)_2$ (0.10 g, 0.18 mmol). Anhydrous THF (20 mL) was added, and the mixture was stirred at room temperature for 10 min. In the second Schlenk-flask, to a solution of **199** (1.91 g, 3.54 mmol) in anhydrous THF (10 mL), 2.5 M solution of *n*BuLi in hexane (1.42 mL, 3.54 mmol) was added dropwise at –78 °C under inert atmosphere, and the mixture was stirred for 1 h at this temperature followed by the dropwise addition of 1 M $ZnCl_2$ in Et_2O (3.9 mL). This mixture was warmed up to room temperature, added to the solution in the first Schlenk-flask, and resulted yellow mixture was stirred at 40 °C for 18 h. Then the reaction mixture was quenched with aq. NH_4Cl (5 mL), washed with water, brine, and dried over Na_2SO_4. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (50 g SiO_2) eluting with CH_2Cl_2/MeOH (100:1) to give 0.83 g (76%) of a yellow powder; m. p. 90 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.00 (s, 9H, *t*BuSi), 2.40 (s, 3H, CH$_3$), 3.15 (t, J = 7.1 Hz, 2H, CH$_2$), 3.89 (t, J = 7.1 Hz, 2H, CH$_2$O), 7.16 (d, J = 3.8 Hz, 1H, CH), 7.28 (d, J = 3.8 Hz, 1H, CH), 7.31–7.44 (m, 8H, CH and CH$_{py}$), 7.59–7.63 (m, 4H, CH), 8.57–8.62 ppm (m, 2H, CH$_{py}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 15.2 (CH$_3$), 19.2 (C-Si), 26.8 (3×CH$_3$), 32.7 (CH$_2$), 62.7 (CH$_2$O), 113.9 (C-Br), 119.5 (2×CH), 125.7 (CH), 127.4 (CH), 127.6 (4×CH), 129.0 (2×CH), 129.5 (C), 133.6 (2×C-Si), 134.4 (C), 135.6 (4×CH), 137.5 (C), 140.3 (C), 140.7 (C), 141.0 (C), 150.4 ppm (2×CH). ESI-MS, positive mode, *m/z* (rel. int., %): 618.1 and 620.1 $[M+H]^+$ (100). HR-MS (ESI, positive mode): found: 618.0949; calcd. for $C_{32}H_{32}BrNOS_2Si$: 618.0951 $[M+H]^+$.



* as boronic acid anhydride

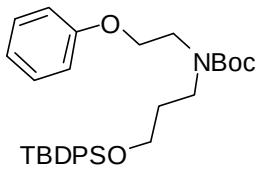
3-Bromo-4-[2-(*tert*-butyldiphenylsilyloxy)ethyl]-2-methylthiophene (202): The title compound was isolated from the reaction mixtures of the synthesis of compound **201** as a hydrolysis product of the Zn-derivative obtained from the starting material **199**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.06 (s, 9H, *t*BuSi), 2.40 (s, 3H, CH_3), 2.87 (t, J = 6.8 Hz, 2H, CH_2), 3.87 (t, J = 6.8 Hz, 2H, CH_2O), 6.88 (s, 1H, CH), 7.45–7.34 (m, 6H, CH), 7.67–7.62 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 15.2 (CH_3), 19.1 (C-Si), 26.8 (3×CH_3), 33.8 (CH_2), 62.9 (CH_2O), 112.2 (C-Br), 118.8 (CH), 127.6 (4×CH), 129.5 (2×CH), 133.7 (2×C-Si), 135.6 (4×CH), 137.7 ppm (2×C). $C_{23}H_{27}BrOSSi$ (459), CI-MS (NH_3), positive mode, *m/z* (rel. int., %): 476.2 and 478.2 $[M+NH_4]^+$ (100).

***tert*-Butyl *N*-(2-phenoxyethyl)-*N*-prop-2-enyl carbamate (204):** To a stirred suspension of KH (1.85 g, 45.0 mmol) in anhydrous THF (75 mL), a solution of **121** (7.11 g, 30.0 mmol) in anhydrous THF (30 mL) was added under inert atmosphere, and the mixture was stirred at room temperature for 10 min. Then allyl bromide (5.45 g, 45.0 mmol) was added at 0 °C, and the mixture was stirred for 3 h at this temperature followed by quenching of the reaction mixture with sat. aq. NH_4Cl (18 mL). The mixture was diluted with Et_2O (100 mL), washed with water, brine, and dried. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (150 g SiO_2) eluting with hexane/EtOAc (16:1) to give 7.40 g (89%) of a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 9H, *t*Bu), 3.50–3.65 (m, 2H, CH_2N), 3.88–4.02 (m, 2H, NC*H*$_2$CH), 4.02–4.16 (m, 2H, CH_2O), 5.04–5.23 (m, 2H, CH=C*H*$_2$), 5.71–5.89 (m, 1H, CH), 6.85–7.00 (m, 3H, CH), 7.23–7.34 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 28.4 (3×CH_3), 45.7/46.3 (CH_2N), 50.3/51.3 (CH_2N), 66.0/66.6 (CH_2O), 79.8 (C), 114.4 (2×CH), 116.0/116.7 (CH_2=), 120.8 (CH), 129.4 (2×CH), 134.1 (CH=), 155.5 (CO), 158.6 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 300.0 $[M+Na]^+$ (100). Elemental analysis: found: C 69.06, H 8.33, N 5.29; calcd. for $C_{16}H_{23}NO_3$ (277.4): C 69.29, H 8.36, N 5.05.

***tert*-Butyl *N*-(3-hydroxypropyl)-*N*-(2-phenoxyethyl) carbamate (205):** To a solution of **204** (7.20 g, 26 mmol) in anhydrous THF (12 mL), 0.5 M 9-BBN in THF (72.8 mL, 36.4 mmol) was added at 0 °C under inert atmosphere, the mixture was stirred at 0 °C for 3 h and further at room temperature for 18 h. Then 3 M aq. NaOH (12 mL) was added followed by the slow addition of 30% aq. H_2O_2 (12 mL), and the mixture was stirred at 50 °C for 2 h. The reaction mixture was saturated with Na_2CO_3, the aqueous phase was extracted with Et_2O (2×50 mL), and combined organic

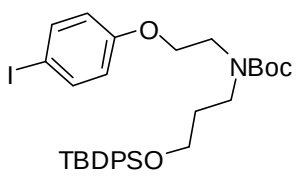
fractions were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The title compound was isolated by silica gel chromatography (150 g SiO_2) eluting with hexane/EtOAc (2:1) to obtain 7.28 g (95%) of a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 9H, *t*Bu), 1.67–1.80 (m, 2H, CH_2), 3.39–3.70 (m, 6H, 2×CH_2N and CH_2OH), 3.76 (t, J = 6.9 Hz, 1H, OH), 4.03–4.14 (m, 2H, CH_2O), 6.84–6.92 (m, 2H, CH), 6.96 (t, J = 7.3 Hz, 1H, CH), 7.27–7.34 ppm (m, 2H, CH). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.2 (3×CH_3), 30.5 (CH_2), 43.7 (CH_2N), 46.6 (CH_2N), 58.2 (CH_2OH), 65.9 (CH_2O), 80.5 (C), 114.4 (2×CH), 121.2 (CH), 129.6 (2×CH), 157.1 (CO), 158.6 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 318.2 $[M+Na]^+$ (100). Elemental analysis: found: C 64.84, H 8.21, N 4.58; calcd. for $C_{16}H_{25}NO_4$ (295.4): C 65.06, H 8.53, N 4.74.

***tert*-Butyl *N*-[3-(*tert*-butyldiphenylsilyloxy)propyl]-*N*-(2-phenoxyethyl) carbamate (206):** To

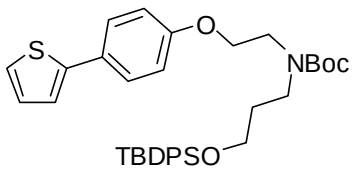


a mixture of **205** (6.55 g, 22.2 mmol) and imidazole (3.02 g, 44.4 mmol) in anhydrous DMF (10 mL), *tert*-butyl diphenylsilyl chloride (7.33 g, 26.6 mmol) was added at 0 °C under inert atmosphere, and the mixture was stirred at room temperature for 18 h. Then 0.5 M aq. citric acid was added until pH-value became 5.0. The reaction mixture was diluted with Et_2O (100 mL), washed with water (50 mL), 5% aq. Na_2CO_3, water (3×50 mL), brine, and dried over $MgSO_4$. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (150 g SiO_2) eluting with CH_2Cl_2 to give 11.7 g (99%) of a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.07 (s, 9H, *t*BuSi), 1.42 (s, 9H, *t*Bu), 1.75–1.89 (m, 2H, CH_2), 3.45 (t, J = 7.0 Hz, 2H, NC$H_2$$CH_2$$CH_2$O), 3.51–3.63 (m, 2H, O$CH_2CH_2$N), 3.65–3.75 (m, 2H, N$CH_2$$CH_2CH_2$O), 4.00–4.14 (m, 2H, OC$H_2$$CH_2$N), 6.86–7.00 (m, 3H, CH), 7.26–7.33 (m, 2H, CH), 7.33–7.47 (m, 6H, CH), 7.63–7.70 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 19.2 (C-Si), 26.8 (3×CH_3), 28.4 (3×CH_3), 31.2/31.8 (CH_2), 45.4/46.2 (CH_2N), 46.9/47.2 (CH_2N), 61.6 (CH_2OSi), 66.1/66.6 (CH_2O), 79.5 (C), 114.4 (2×CH), 120.7 (CH), 127.6 (4×CH), 129.4 (2×CH), 129.6 (2×CH), 133.7 (2×C), 135.5 (4×CH), 155.6 (CO), 158.7 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 556.3 $[M+Na]^+$ (100). Elemental analysis: found: C 72.22, H 7.82, N 2.80; calcd. for $C_{32}H_{43}NO_4Si$ (533.8): C 72.00, H 8.12, N 2.62.

***tert*-Butyl *N*-[3-(*tert*-butyldiphenylsilyloxy)propyl]-*N*-[2-(4-iodophenoxy)ethyl] carbamate (207):** According to GP 2.1, compound **206** (11.7 g, 22.0 mmol), iodine (3.07 g, 12.1 mmol), and bis(trifluoroacetoxy)iodobenzene (5.20 g, 12.1 mmol) gave 13.6 g (89%)* of the title compound as a yellow oil after filtration through SiO_2 (300 mL) eluting with CH_2Cl_2. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.05 (s, 9H, *t*BuSi), 1.41 (s, 9H, *t*Bu), 1.73–1.89 (m, 2H, CH_2), 3.36–3.48 (m, 2H, NCH_2CH$_2$CH$_2$O), 3.48–3.62 (m, 2H, OCH$_2$CH_2N), 3.62–3.74 (m, 2H, NCH$_2$CH$_2$CH_2O), 3.95–4.13 (m, 2H, OCH_2CH$_2$N), 6.61–6.69 (m, 2H, CH), 7.32–7.46 (m, 6H, CH), 7.49–7.57 (m, 2H, CH), 7.62–7.73 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 19.2 (C-Si), 26.9 (3×CH_3), 28.4 (3×CH_3), 31.2/31.8 (CH_2), 45.5/46.2 (CH_2N), 46.8/47.1 (CH_2N), 61.6 (CH_2OSi), 66.6/66.8 (CH_2N), 79.6 (C), 82.8 (C-I), 116.8 (2×CH), 127.6 (4×CH), 129.6 (2×CH), 133.7 (2×C), 135.5 (4×CH), 138.2 (2×CH), 155.6 (CO), 158.6 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 682.2 (100) $[M+Na]^+$. HR-MS (ESI, positive mode): found: 682.1819; calcd. for $C_{32}H_{42}INO_4Si$: 682.1820 $[M+Na]^+$.

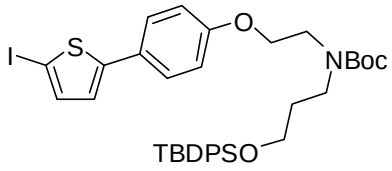


***tert*-Butyl *N*-[3-(*tert*-butyldiphenylsilyloxy)propyl]-*N*-[2-[4-(thiophen-2-yl)phenoxy]ethyl] carbamate (208):** According to GP 1.1, iodide **207** (12.9 g, 19.5 mmol), thiophen-2-boronic acid (**105**) (3.0 g, 23.4 mmol), Pd(dba)$_2$ (0.2 g, 0.4 mmol) and PPh_3 (0.4 g, 1.6 mmol) yielded the title compound (10.6 g, 88%) as a yellow oil after purification by silica gel chromatography (200 g SiO_2) eluting with hexane/EtOAc (8:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.06 (s, 9H, *t*BuSi), 1.42 (s, 9H, *t*Bu), 1.74–1.90 (m, 2H, CH_2), 3.39–3.51 (m, 2H, NCH_2CH$_2$CH$_2$O), 3.52–3.64 (m, 2H, OCH$_2$CH_2N), 3.64–3.76 (m, 2H, NCH$_2$CH$_2$CH_2O), 4.02–4.16 (m, 2H, OCH_2CH$_2$N), 6.86–6.93 (m, 2H, CH), 7.05 (dd, *J* = 3.6, 5.0 Hz, 1H, CH), 7.18–7.24 (m, 2H, CH), 7.33–7.47 (m, 6H, CH), 7.48–7.55 (m, 2H, CH), 7.64–7.70 ppm (m, 4H, CH). ^{13}C NMR (50 MHz, $CDCl_3$, 2 rotamers): δ = 19.1 (C-Si), 26.7 (3×CH_3), 28.3 (3×CH_3), 31.1/31.7 (CH_2), 44.2–47.1 (2×CH_2N), 61.6 (CH_2OSi), 66.4/66.8 (CH_2O), 79.6 (C), 114.8 (2×CH), 122.2 (CH), 123.9 (CH), 127.3 (2×CH), 127.8 (4×CH), 128.0 (CH), 129.7 (2×CH), 133.9 (2×C-Si), 135.7 (4×CH), 143.5 (C), 144.4 (C), 155.8 (CO), 158.5 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 616.3 $[M+H]^+$ (100). Elemental analysis: found: C 70.48, H 7.08, N 2.33; calcd. for $C_{36}H_{45}NO_4SSi$ (615.9): C 70.20, H 7.36, N 2.27.

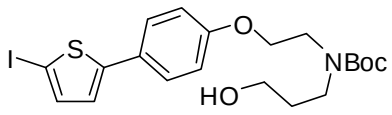


* contains 5% of the initial substance (^{1}H NMR).

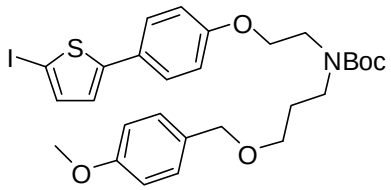
***tert*-Butyl *N*-[3-(*tert*-butyldiphenylsilyloxy)propyl]-*N*-[2-[4-(5-iodothiophen-2-yl)phenoxy] ethyl] carbamate (209):** According to GP 2.2, compound **208** (10.4 g, 16.9 mmol), KOAc (1.8 g, 18.6 mmol), and 0.5 M ICl in AcOH (37 mL, 18.6 mmol) gave 9.5 g (78%) of the title compound as a yellow oil after purification by silica gel chromatography (200 g SiO_2) eluting with hexane/EtOAc (30:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.06 (s, 9H, *t*BuSi), 1.42 (s, 9H, *t*Bu), 1.75–1.89 (m, 2H, CH_2), 3.37–3.50 (m, 2H, NC$H_2$$CH_2CH_2O$), 3.52–3.64 (m, 2H, OCH_2CH_2N), 3.64–3.75 (m, 2H, $NCH_2CH_2CH_2O$), 4.01–4.17 (m, 2H, OC$H_2$$CH_2$N), 6.84–6.92 (m, 2H, CH), 7.18 (d, J = 3.8 Hz, 1H, CH), 7.33–7.46 (m, 8H, CH), 7.63–7.69 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 19.2 (C-Si), 26.8 (3×CH_3), 28.4 (3×CH_3), 31.3/31.8 (CH_2), 45.4/46.21 (CH_2N), 46.8/47.1 (CH_2N), 61.6 (CH_2OSi), 66.4/66.8 (CH_2O), 71.0 (C-I), 79.6 (C), 114.8 (2×CH), 123.5 (CH), 126.5 (C), 127.1 (2×CH), 127.6 (4×CH), 129.6 (2×CH), 133.7 (2×C-Si), 135.5 (4×CH), 137.8 (CH), 150.3 (C), 155.6 (CO), 158.7 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 1505.4 $[2M+Na]^+$ (35), 764.1 $[M+Na]^+$ (100), 741.8 $[M+H]^+$ (22). HR-MS (ESI, positive mode): found: 764.1691; calcd. for $C_{36}H_{44}INO_4SSi$: 764.1697 $[M+Na]^+$.



***tert*-Butyl *N*-(3-hydroxypropyl)-*N*-[2-[4-(5-iodothiophen-2-yl)phenoxy]ethyl] carbamate (210):** To a solution of iodide **209** (9.23 g, 12.5 mmol) in THF (40 mL), 1 M solution of *t*Bu_4NF in THF (18.8 mL) was added, and the mixture was stirred at room temperature for 2 h. Then it was diluted with Et_2O (100 mL), washed with water, brine, and dried over Na_2SO_4. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (200 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to give 6.0 g (95%) of a colorless powder; m. p. 88–89 °C (hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 1.63–1.87 (m, 2H, CH_2), 3.39–3.79 (m, 6H, CH_2), 4.03–4.17 (m, 2H, OC$H_2$$CH_2$N), 6.86 (d, J = 3.8 Hz, 1H, CH), 6.84–6.92 (m, 2H, CH), 7.18 (d, J = 3.8 Hz, 1H, CH), 7.40–7.47 ppm (m, 2H, CH). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.3 (3×CH_3), 30.5 (CH_2), 43.8 (CH_2N), 46.6 (CH_2N), 58.2 (CH_2OH), 66.2 (CH_2O), 71.1 (C-I), 80.7 (C), 114.9 (2×CH), 123.7 (CH), 126.9 (C), 127.2 (2×CH), 137.9 (CH), 150.3 (C), 157.0 (CO), 158.5 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 1029.1 $[2M+Na]^+$ (100), 526.1 $[M+Na]^+$ (99). Elemental analysis: found: C 47.73, H 5.05, N 3.00; calcd. for $C_{20}H_{26}INO_4S$ (503.4): C 47.72, H 5.21, N 2.78.

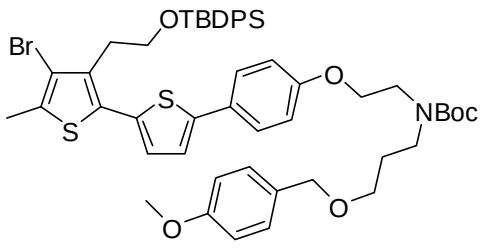


***tert*-Butyl *N*-[2-[4-(5-iodothiophen-2-yl)phenoxy]ethyl]-*N*-[3-(4-methoxybenzyloxy)propyl] carbamate (211):** To a suspension of KH (0.57 g, 14.0 mmol) in anhydrous THF (40 mL),



compound **210** (3.52 g, 7.0 mmol) was added at 0 °C, and the mixture was stirred for 20 min at this temperature. Then 4-methoxybenzyl bromide (2.11 g, 10.5 mmol) was added at 0 °C followed by NBu_4I (0.52 g, 1.4 mmol), and stirring at room temperature was continued for 12 h. The mixture was quenched with aq. NH_4Cl, and diluted with EtOAc (80 mL). Organic layer was separated, washed with water, brine, and dried over Na_2SO_4. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (100 g SiO_2) eluting with hexane/EtOAc (4:1) to yield 4.0 g (92%) of a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 (s, 9H, *t*Bu), 1.80–1.93 (m, 2H, CH_2), 3.34–3.43 (m, 2H, $NCH_2CH_2CH_2O$), 3.43–3.51 (m, 2H, OCH_2CH_2N), 3.52–3.62 (m, 2H, $NCH_2CH_2CH_2O$), 3.79 (s, 3H, OCH_3), 4.01–4.15 (m, 2H, OCH_2CH_2N), 4.42 (s, 2H, OCH_2), 6.83–6.92 (m, 5H, CH), 7.18 (d, J = 3.7 Hz, 1H, CH), 7.22–7.28 (m, 2H, CH), 7.38–7.45 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 28.4 (3×CH_3), 29.2 (CH_2), 45.6/46.2 (CH_2N), 46.8/47.2 (CH_2N), 55.2 (OCH_3), 66.3/66.6 (CH_2O), 67.5 (CH_2OPh), 71.0 (C-I), 72.6 ($PhCH_2O$), 79.7 (C), 113.7 (2×CH), 114.8 (2×CH), 123.5 (CH), 126.5 (C), 127.0 (2×CH), 129.2 (2×CH), 130.4 (C), 137.8 (CH), 150.3 (C), 155.2/155.6 (CO), 158.6 (CO), 159.1 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 1269.2 (55) $[2M+Na]^+$, 662.1 (27) $[M+K]^+$, 646.1 (52) $[M+Na]^+$, 624.1 (100) $[M+H]^+$. HR-MS (ESI, positive mode): found: 624.1274, 646.1092, 662.0831; calcd. for $C_{28}H_{34}INO_5S$: 624.1275 $[M+H]^+$, 646.1095 $[M+Na]^+$, 662.0834 $[M+K]^+$.

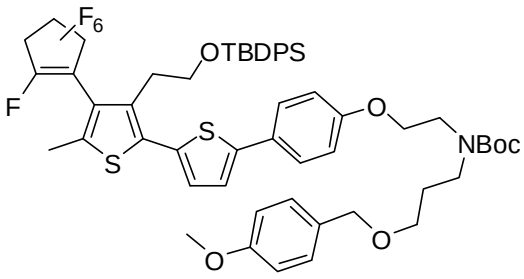
***tert*-Butyl *N*-[2-[4-[4'-bromo-3'-(2-*tert*-butyldiphenylsilyloxy)ethyl-5'-methyl-[2,2']bithiophen-5-yl]phenoxy]ethyl]-*N*-[3-(4-methoxybenzyloxy)propyl] carbamate (212):** Iodide **211**



(3.86 g, 6.2 mmol), PPh_3 (0.65 g, 2.5 mmol) and $Pd(dba)_2$ (0.36 g, 0.6 mmol) were placed to a Schlenk-flask. Then anhydrous THF (37.5 mL) was added, and the mixture was stirred at room temperature for 10 min. In the second Schlenk-flask to a solution of **199** (5.0 g, 9.3 mmol) in anhydrous THF (37.5 mL), 2.5 M solution of *n*BuLi in hexane (3.7 mL, 9.3 mmol) was added dropwise at –78 °C under inert atmosphere. The mixture was stirred for 1 h at this temperature, and then 1 M $ZnCl_2$ in Et_2O (10.2 mL) was added dropwise. The latter solution was warmed up to room temperature and added to the first Schlenk-flask. The resulted yellow mixture was stirred at 40 °C for 18 h. Then it was quenched with aq. NH_4Cl (5 mL), washed with water,

brine, and dried over Na_2SO_4. After evaporation of the solvents under reduced pressure, the title compound was isolated by silica gel chromatography (100 g SiO_2) eluting with hexane/EtOAc (8:1) to yield 4.9 g (84%) of a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.02 (s, 9H, *t*BuSi), 1.47 (s, 9H, *t*Bu), 1.83–1.97 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 3.15 (t, J = 7.3 Hz, 2H, CH_2CH$_2$OSi), 3.37–3.53 (m, 4H, NCH_2CH$_2$CH$_2$O and OCH$_2$CH_2N), 3.54–3.66 (m, 2H, NCH$_2$CH$_2$CH_2O), 3.79 (s, 3H, OCH_3), 3.88 (t, J = 7.3 Hz, 2H, CH$_2$CH_2OSi), 4.04–4.18 (m, 2H, OCH_2CH$_2$N), 4.44 (s, 2H, OCH_2), 6.84–6.94 (m, 4H, CH), 7.06 (d, J = 3.8 Hz, 1H, CH), 7.09 (d, J = 3.8 Hz, 1H, CH), 7.24–7.29 (m, 2H, CH), 7.30–7.40 (m, 6H, CH), 7.46–7.52 (m, 2H, CH), 7.61–7.67 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 15.2 (CH_3), 19.1 (C-Si), 26.8 (3×CH_3), 28.4 (3×CH_3), 29.2 (CH_2), 32.6 (*C*H$_2$C), 45.6/46.3 (CH_2N), 46.8/47.2 (CH_2N), 55.2 (OCH_3), 62.7 (CH_2OSi), 66.3/66.7 (CH_2O), 67.5 (CH_2OPh), 72.6 (PhCH_2O), 79.7 (C), 113.5 (C-Br), 113.7 (2×CH), 114.8 (2×CH), 122.4 (CH), 127.0 (2×CH), 127.1 (CH), 127.6 (4×CH), 129.2 (2×CH), 129.5 (2×CH), 130.5 (C), 132.6 (2×C), 133.4 (2×C), 133.7 (2×C-Si), 133.8 (C), 135.6 (4×CH), 144.3 (C), 155.7 (CO), 158.5 (CO), 159.1 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 976.2 and 978.2 [M+Na]$^+$ (100). Elemental analysis: found: C 64.16, H 6.07, N 1.53; calcd. for $C_{51}H_{60}BrNO_6S_2Si$ (955.1): C 64.13, H 6.33, N 1.47.

***tert*-Butyl *N*-[2-[4-[3'-[2-(*tert*-butyldiphenylsilyloxy)ethyl]-5'-methyl-4'-(heptafluoro-cyclopent-1-enyl)-[2,2']bithiophen-5-yl]phenoxy]ethyl]-*N*-[3-(4-methoxybenzyloxy)propyl] carbamate (213):** According to GP 3, bromide **212** (1.9 g, 2.0 mmol), *n*BuLi (2.5 M in hexane, 2.0 mL, 3.0 mmol), and C_5F_8 (1 mL, 6.0 mmol) gave 1.7 g (78%)* of the title compound as a grey oil after purification by silica gel chromatography (100 g SiO_2) eluting with EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.99 (s, 9H, *t*BuSi), 1.46 (s, 9H, *t*Bu), 1.81–1.85 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 3.00 (t, J = 7.7 Hz, 2H, CH_2CH$_2$OSi), 3.36–3.53 (m, 4H, NCH_2CH$_2$CH$_2$O and OCH$_2$CH_2N), 3.53–3.63 (m, 2H, NCH$_2$CH$_2$CH_2O), 3.66 (t, J = 7.7 Hz, 2H, CH$_2$CH_2OSi), 3.78 (s, 3H, OCH_3), 4.03–4.20 (m, 2H, OCH_2CH$_2$N), 4.43 (s, 2H, OCH_2), 6.83–6.95 (m, 4H, CH), 7.00 (d, J = 3.7 Hz, 1H, CH), 7.08 (d, J = 3.7 Hz, 1H, CH), 7.23–7.41 (m, 8H, CH), 7.44–7.52 (m, 2H, CH), 7.55–7.65 ppm (m, 4H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 1090.3 [M+Na]$^+$ (100). HR-MS (ESI, positive mode): found: 1068.3586; calcd. for $C_{56}H_{60}F_7NO_6S_2Si$: 1068.3592 [M+H]$^+$.



* contains ca. 20% of the debrominated derivative **214** formed from bromide **212** (^{1}H NMR)

Deprotection of compound 213: To a solution of **213** (0.21 g, 0.2 mmol) in THF (1 mL), 1 M solution of tBu$_4$NF in THF (0.3 mL, 0.3 mmol) was added, and the mixture was stirred overnight at room temperature. Then it was diluted with EtOAc, washed with water, brine, dried and evaporated under reduced pressure. The residue was filtered through SiO_2 eluting with hexane/EtOAc (2:1) to give compounds **217**, **218** and **219**.

Compound 217: Yield: 50 mg (30%) of a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44 (s, 9H, tBu), 1.78–1.93 (m, 2H, CH_2), 2.55 (s, 3H, CH_3), 3.22–3.32 (m, 2H, CH_2), 3.32–3.42 (m, 2H, CH_2), 3.42–3.51 (m, 2H, CH_2), 3.51–3.62 (m, 2H, CH_2), 3.77 (s, 3H, CH_3O), 3.99–4.19 (m, 2H, CH_2O), 4.41 (s, 2H, CH_2), 4.45–4.55 (m, 2H, OCH_2), 6.81–6.92 (m, 4H, CH), 6.94 (d, J = 3.7 Hz, 1H, CH), 7.13 (d, J = 3.7 Hz, 1H, CH), 7.19–7.30 (m, 2H, CH), 7.43–7.54 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 15.6 (CH_3), 28.4 (3×CH_3), 28.6/29.2 (CH_2), 30.0 (CH_2), 45.6/46.2 (CH_2N), 46.8/47.2 (CH_2N), 55.2 (CH_3O), 66.4/66.6 (CH_2OPh), 67.5 ($PhCH_2O$), 72.6 (2×CH_2O), 79.7 (C), 113.7 (2×CH), 114.9 (2×CH), 122.5 (CH), 124.1 (C), 126.5 (C), 127.0 (2×CH), 127.5 (C), 128.2 (CH), 129.2 (2×CH), 130.4 (C), 131.9 (C), 138.6 (C), 138.9 (C), 145.4 (C), 155.3/155.6 (CO), 158.7 (CO), 159.1 ppm (CO). ^{19}F NMR (282 MHz, $CDCl_3$): δ = –102.2 (s, 2F), –118.4 (s, 2F), –132.0 ppm (s, 2F). ESI-MS, positive mode, *m/z* (rel. int., %): 1640.7 (100) $[2M+Na]^+$, 832.1 (84) $[M+H]^+$. HR-MS (ESI, positive mode): found: 810.2352; calcd. for $C_{40}H_{41}F_6NO_6S_2$: 810.2352 $[M+H]^+$.

Compound 218: Yield: 100 mg (48%) of a grey powder. ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 0.92 (s, 9H, tBuSi), 1.38 (s, 9H, tBu), 1.70–1.84 (m, 2H, CH_2), 2.10 (s, 3H, CH_3), 2.96 (m, 2H, CH_2), 3.31 (m, 2H, CH_2), 3.41 (t, J = 6.2 Hz, 2H, CH_2), 3.52 (t, J = 5.7 Hz, 2H, CH_2N), 3.60 (m, 2H, CH_2), 3.72 (s, 3H, CH_3O), 4.12 (t, J = 5.7 Hz, 2H, CH_2O), 4.36 (s, 2H, CH_2), 6.84–6.91 (m, 2H, CH), 6.94–7.02 (m, 3H, CH), 7.19–7.26 (m, 2H, CH), 7.28–7.43 (m, 7H, CH), 7.49–7.59 ppm (m, 6H, CH). ^{13}C NMR (75 MHz, [D_6]DMSO, 2 rotamers): δ =14.0 (CH_3), 18.6 (C-Si), 26.4 (3×CH_3), 27.9 (3×CH_3), 28.6 (CH_2), 31.7 (CH_2), 44.6/45.4 (CH_2N), 46.1 (CH_2N), 54.9 (CH_3O), 63.2 (CH_2O), 65.9 (CH_2OPh), 66.9 ($PhCH_2O$), 71.5 (CH_2O), 78.6 (C), 113.5 (2×CH), 115.0 (2×CH), 122.8 (CH), 126.2 (CH), 126.4 (2×CH), 127.0 (C), 127.6 (4×CH), 129.0 (2×CH), 129.5 (2×CH), 130.4

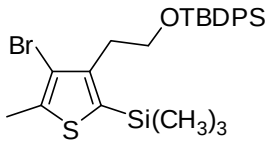
(2×C), 131.9 (C), 133.2 (2×C-Si), 134.1 (C), 134.8 (4×CH), 135.3 (C), 142.4 (2×C), 154.6 (CO), 157.9 (CO), 158.6 ppm (CO). ^{19}F NMR (282 MHz, [D$_6$]DMSO): δ = –125.7 ppm (s, 4F). ESI-MS, negative mode, *m/z* (rel. int., %): 1042.5 (100) $[M–H]^-$. HR-MS (ESI, positive mode): found: 1044.3610; calcd. for $C_{56}H_{61}F_4NO_8S_2Si$: 1044.3622 $[M+H]^+$.

Compound 219: Yield: 23 mg (18%) of a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9H, *t*Bu), 2.44 (s, 3H, CH_3), 2.99 (t, *J* = 6.6 Hz, 2H, CH_2), 3.33–3.42 (m, 2H, CH_2), 3.42–3.49 (m, 2H, CH_2), 3.49–3.61 (m, 2H, CH_2), 3.77 (s, 3H, CH_3O), 3.87 (t, *J* = 6.6 Hz, 2H, CH_2), 4.00–4.14 (m, 2H, CH_2), 4.40 (s, 2H, CH_2O), 6.64 (s, 1H, CH), 6.81–6.90 (m, 4H, CH), 7.01 (d, *J* = 3.7 Hz, 1H, CH), 7.10 (d, *J* = 3.7 Hz, 1H, CH), 7.20–7.26 (m, 3H, CH), 7.45–7.51 ppm (m, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 15.2 (CH_3), 28.4 (3×CH_3), 28.6/29.2 (CH_2), 32.5 (CH_2), 45.6/46.2 (CH_2N), 46.9/47.2 (CH_2N), 55.2 (CH_3O), 62.8 (CH_2O), 66.3/66.6 (CH_2OPh), 67.5 ($PhCH_2O$), 72.6 (CH_2O), 79.7 (C), 113.7 (2×CH), 114.8 (2×CH), 122.3 (CH), 126.7 (CH), 126.9 (2×CH), 128.3 (CH), 129.2 (2×CH), 130.0 (C), 130.4 (C), 134.4 (C), 134.8 (2×C), 138.6 (C), 143.7 (C), 155.3/155.6 (CO), 158.3 (CO), 158.7 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 1296.8 (56) $[2M+Na]^+$, 660.2 (100) $[M+Na]^+$. $C_{35}H_{43}NO_6S_2$, 637.3.

Photochromic compound 215: To a stirred solution of compound **201** (0.10 g, 0.17 mmol) in anhydrous THF (8 mL), 1.5 M solution of *t*BuLi (0.11 mL) was added dropwise at –78 °C, and the mixture was stirred for 30 min. Then a solution of **213** (0.15 g, 0.14 mmol) in THF (2 mL) was added, and the reaction mixture was kept at –78 °C for 1 h, and then at room temperature overnight. After dilution with EtOAc (20 mL), washing with brine, drying and evaporation under reduced pressure, the residue was filtered through silica gel (50 g SiO_2) eluting with EtOAc giving 22 mg (10%) of the title compound as a green foam. HPLC: 100% ACN, 1 mL/min, 25 °C, t_R (OF) = 7.5 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$, mixture of rotamers): δ = 0.90–1.20 (m, 18H, *t*BuSi), 1.30–1.55 (m, 9H, *t*Bu), 1.71–1.85 (m, 2H, CH_2), 2.23–2.51 (m, 6H, CH_3), 2.92–3.05 (m, 2H, C*H*$_2$CH$_2$OSi), 3.12–3.27 (m, 2H, C*H*$_2$CH$_2$OSi), 3.28–3.60 (m, 6H, NC*H*$_2$CH$_2$CH$_2$O, NCH$_2$CH$_2$C*H*$_2$O and OCH$_2$C*H*$_2$N), 3.60–3.68 (m, 2H, CH_2OSi), 3.78 (s, 3H, OCH_3), 3.85–3.92 (m, 2H, CH_2OSi), 4.01–4.20 (m, 2H, OC*H*$_2$CH$_2$N), 4.40 (s, 2H, OCH_2), 6.80–6.93 (m, 4H, CH), 7.00–7.80 (m, 30H, CH), 8.42–8.58

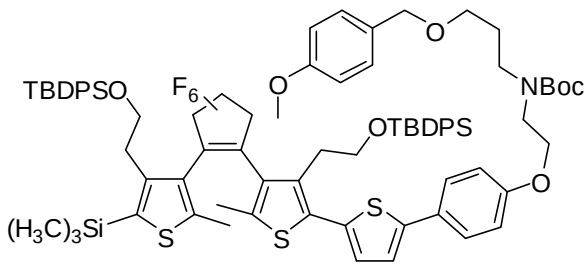
ppm (m, 2H, CH_{py}). $C_{88}H_{92}F_6N_2O_7S_4Si_2$ (1586.5), ESI-MS, positive mode, *m/z* (rel. int., %): 1609.4 $[M+Na]^+$ (100).

4-Bromo-3-(2-(*tert*-butyldiphenylsilyloxy)ethyl)-5-methyl-3-trimethylsilylthiophene (220):



The bromide **199** (0.54 g, 1.0 mmol) in anhydrous THF (4 mL) was subjected to bromine-lithium exchange with 2.5 M solution of *n*BuLi in hexane (0.4 mL, 1.0 mmol) at –78 °C during 30 min followed by the addition of trimethylsilyl chloride (1.3 mL, 10.0 mmol). The mixture was stirred at room temperature overnight, then quenched with brine, diluted with Et_2O (20 mL), the organic layer was separated, and dried. After evaporation of the solvents under reduced pressure, the title compound was purified by silica gel chromatography (50 g SiO_2) eluting with hexane/EtOAc (4:1) to yield 0.52 g (98%) of a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.21 (s, 9H, $SiCH_3$), 1.01 (s, 9H, *t*BuSi), 2.30 (s, 3H, CH_3), 2.90–3.05 (m, 2H, CH_2), 3.63–3.76 (m, 2H, CH_2O), 7.23–7.42 (m, 6H, CH), 7.54–7.68 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 0.0 (3×CH_3), 15.2 (CH_3), 19.0 (C-Si), 26.7 (3×CH_3), 34.5 (CH_2), 63.5 (CH_2O), 114.5 (C-Br), 127.5 (4×CH), 129.4 (2×CH), 131.9 (C), 133.7 (2×C-Si), 135.5 (4×CH), 138.4 (C), 143.1 ppm (C). $C_{26}H_{35}BrOSSi_2$ (530.1), CI-MS (NH_3), positive mode, *m/z* (rel. int., %): 550 (100) and 548 (90) $[M+NH_4]^+$, 533 (56) and 531 (50) $[M+H]^+$.

Photochromic compound 221: To a solution of **220** (0.48 g, 0.9 mmol) in anhydrous THF (5 mL), 2.5 M solution of *n*BuLi in hexane (0.4 mL, 1.0 mmol) was added at –78 °C, and the mixture was stirred for 30 min at –78 °C. Then a solution of heptafluorocyclopentene **213** (0.19 g, 0.18 mmol) in THF (2 mL) was added. After 2 h, the reaction mixture was quenched with brine, diluted with EtOAc (20 mL), washed with brine, and dried. After evaporation of the solvents, the residue was applied on silica gel, and eluted with hexane/EtOAc (4:1 → 2:1) to yield 65 mg (24%)* of the title compound as a green foam. $C_{82}H_{95}F_6NO_7S_3Si_3$ (1499.5), ESI-MS, positive mode, *m/z* (rel. int., %): 1522.3 (100) $[M+Na]^+$.



* contains the debrominated derivative **214** (20% by ^{1}H NMR)

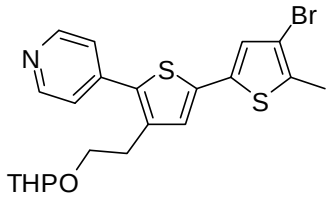
4-[3-[2-(Tetrahydro-*2H*-pyran-2-yloxy)ethyl]thiophen-2-yl]pyridine (227): To a solution of **224** (8.73 g, 30.0 mmol) in anhydrous THF (150 mL), 2.5 M solution of *n*BuLi in hexane (13.2 mL, 33.0 mmol) was added dropwise at –78 °C, and the mixture was stirred for 1 h at this temperature. Then $B(O\textit{i}Pr)_3$ (8.46 g, 45.0 mmol) was added, and the mixture was stirred for 1 h at –78 °C and then 2 h at room temperature. After the addition of water (2 mL), 4-bromopyridine hydrochloride (**130**) (7.0 g, 36.0 mmol), Ph_3P (315 mg, 1.2 mmol), $Pd(dba)_2$ (173 mg, 0.3 mmol), and 20% aq. Na_2CO_3 (150 mL) were added, and the mixture was heated at 78 °C with a reflux condenser and a bubble-counter for 18 h. Then it was diluted with EtOAc (100 mL), the organic layer was separated, washed with brine (50 mL), dried, and evaporated under reduced pressure. The title compound was isolated by silica gel chromatography (200 g SiO_2) eluting with hexane/EtOAc (4:1 → 1:1) to yield 6.1 g (70%) of a colorless oil; R_f = 0.14 (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38–1.85 (m, 6H, 3×CH_2, H-3/4/5), 3.10 (t, J = 6.9 Hz, 2H, CH_2), 3.41–3.49 (m, 1H, C*H*H-6), 3.60–3.70 (dt, J = 6.9, 9.7 Hz, 1H, C*H*HO), 3.67–3.76 (m, 1H, CH*H*-6), 3.94–4.04 (dt, J = 6.9, 9.7 Hz, 1H, CH*H*O), 4.59 (t, J = 3.3 Hz, 1H, CHO), 7.08 (d, J = 5.2 Hz, 1H, CH), 7.34 (d, J = 5.2 Hz, 1H, CH), 7.45 (dd, J = 1.7, 4.5 Hz, 2H, CH), 8.62 ppm (dd, J = 1.7, 4.5 Hz, 2H, CH). ^{13}C NMR (125.5 MHz, $CDCl_3$): δ = 19.4 (CH_2-4), 25.4 (CH_2-5), 29.3 (CH_2), 30.5 (CH_2-3), 62.0 (CH_2-6), 67.2 (CH_2O), 98.6 (CHO), 123.4 (2×CH), 125.4 (CH), 130.2 (CH), 135.9 (C), 136.9 (C), 142.1 (C), 149.8 ppm (2×CH). EI-MS, positive mode, *m/z* (rel. int., %): 289.2 [$M^{+\cdot}$] (26), 187.2 (100), 175.1 (72), 85 (74). Elemental analysis: found: C 66.65, H 6.44, N 5.04; calcd. for $C_{16}H_{19}NO_2S$ (289.4): C 66.41, H 6.62, N 4.84.

4-[5-Bromo-3-[2-(tetrahydro-*2H*-pyran-2-yloxy)ethyl]thiophen-2-yl]pyridine (228): To a solution of **227** (5.78 g, 20.0 mmol) in anhydrous DMF (20 mL), a solution of NBS (4.27 g, 24.0 mmol) in anhydrous DMF (20 mL) was added dropwise, and the mixture was stirred at room temperature for 24 h in the dark. Then the mixture was poured into ice, extracted with $CHCl_3$ (2×200 mL), washed with 10% aq. KOH (2×100 mL), water (3×100 mL), and dried over $MgSO_4$. The solvents were evaporated under reduced pressure, and the residue was filtered through SiO_2 (200 mL) eluting with hexane/EtOAc (1:1) to give the title compound (5.2 g, 76%)* as a yellow oil; R_f = 0.12. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43–1.84 (m, 6H, 3×CH_2, H-3/4/5), 2.94 (t, J = 6.6 Hz, 2H, CH_2), 3.42–3.51 (m, 1H, C*H*H-6), 3.56–3.67 (dt, J = 6.7, 9.7 Hz, 1H, C*H*HO), 3.67–3.76 (m, 1H, CH*H*-6), 3.91–4.01 (dt, J = 6.7, 9.7 Hz, 1H, CH*H*O), 4.58 (t, J = 3.2 Hz, 1H, CHO), 7.05 (s, 1H, CH), 7.39 (dd, J = 1.7, 4.5 Hz, 2H, CH), 8.62 ppm (dd, J = 1.7, 4.5 Hz, 2H, CH). ^{13}C NMR

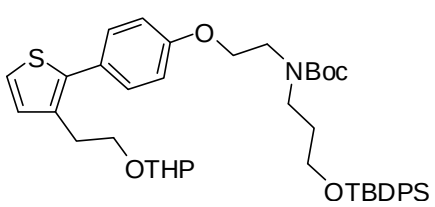
* contains ca. 7% of the initial substance (by HPLC).

(125.5 MHz, $CDCl_3$): δ = 19.4 (CH_2-4), 25.4 (CH_2-5), 29.2 (CH_2), 30.5 (CH_2-3), 62.1 (CH_2-6), 67.0 (CH_2O), 98.7 (CHO), 112.5 (C-Br), 123.2 (2×CH), 132.9 (CH), 137.4 (C), 137.7 (C), 141.0 (C), 149.9 ppm (2×CH). CI-MS (NH_3), positive mode, *m/z* (rel. int., %): 370.3 and 368.3 $[M+H]^+$ (100). HR-MS (ESI, positive mode): found: 368.0315; calcd. for $C_{16}H_{18}BrNO_2S$: 368.0314 $[M+H]^+$.

4-[4'-Bromo-5'-methyl-4-[2-(tetrahydro-*2H*-pyran-2-yloxy)ethyl]-[2,2']bithiophen-5-yl]pyridine (229): According to GP 1.1, bromide **228** (5.52 g, 15.0 mmol), $Pd(dba)_2$ (173 mg, 0.3 mmol), Ph_3P (315 mg, 1.2 mmol), and thiopheneboronic acid **226** (3.65 g, 16.5 mmol) gave the title compound (6.35 g, 91%) as a yellow oil after silica gel chromatography (200 g SiO_2) eluting with hexane/EtOAc (2:1 → 1:1); R_f = 0.11 (hexane/EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42–1.86 (m, 6H, 3×CH_2, H-3/4/5), 2.40 (s, 3H, CH_3) 2.98 (t, *J* = 6.7 Hz, 2H, CH_2), 3.42–3.52 (m, 1H, C*H*H-6), 3.60–3.78 (m, 2H, C*H*HO and CH*H*-6), 3.95–4.06 (dt, *J* = 6.7, 9.6 Hz, 1H, CH*H*O), 4.61 (t, *J* = 3.3 Hz, 1H, CHO), 7.00 (s, 1H, CH), 7.09 (s, 1H, CH), 7.46 (dd, *J* = 1.7, 4.5 Hz, 2H, CH), 8.62 ppm (dd, *J* = 1.7, 4.5 Hz, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.8 (CH_3), 19.3 (CH_2-4), 25.3 (CH_2-5), 29.4 (CH_2), 30.5 (CH_2-3), 62.0 (CH_2-6), 67.0 (CH_2O), 98.7 (CHO), 109.7 (C), 123.3 (2×CH), 126.2 (CH), 126.7 (CH), 133.8 (2×C), 134.9 (C), 136.3 (C), 138.1 (C), 141.7 (C), 149.9 ppm (2×CH). ESI-MS, positive mode, *m/z* (rel. int., %): 466.0 and 464.0 $[M+H]^+$ (100). HR-MS (ESI, positive mode): found: 464.0358; calcd. for $C_{21}H_{22}BrNO_2S_2$: 464.0348 $[M+H]^+$.



***tert*-Butyl *N*-[3-(*tert*-butyldiphenylsilyloxy)propyl]-*N*-[2-[4-[3-[2-(tetrahydro-*2H*-pyran-2-yloxy)ethyl]thiophen-2-yl]phenoxy]ethyl] carbamate (230):** To a solution of **224** (0.44 g, 1.5 mmol) in anhydrous THF (15 mL), 2.5 M solution of *n*BuLi in hexane (0.66 mL, 1.65 mmol) was added dropwise at –78 °C under inert atmosphere, and the mixture was stirred for 1 h at this temperature. Then $B(OiPr)_3$ (0.42 g, 2.25 mmol) was added, and the mixture was stirred for 1 h at at –78 °C and 2 h at room temperature. After addition of water (0.5 mL), compound **207** (0.66 g, 1.0 mmol), Ph_3P (42 mg, 0.16 mmol), $Pd(dba)_2$ (23 mg, 0.04 mmol), and 20% aq. Na_2CO_3 (15 mL) were placed, and the mixture was heated at 78 °C with a reflux condenser and a bubble-counter for 18 h. Then the mixture was diluted with EtOAc (50 mL), the organic phase was separated, washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The title compound was isolated by silica gel chromatography (50 g SiO_2) eluting with hexane/EtOAc (8:1) to yield 0.17 g (23%) of a yellow

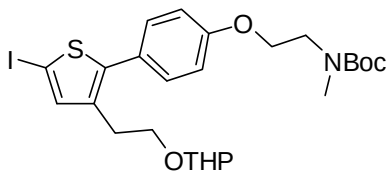


oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.06 (s, 9H, *t*BuSi), 1.42 (s, 9H, *t*Bu), 1.48–1.75 (m, 6H, 3×CH_2, H-3/4/5), 1.75–1.89 (m, 2H, $NCH_2CH_2CH_2O$), 2.94 (t, J = 7.0 Hz, 2H, CH_2), 3.39–3.51 (m, 3H, $NCH_2CH_2CH_2O$ and C*H*H-6), 3.54–3.66 (m, 3H, OCH_2CH_2N and C*H*HO), 3.67–3.81 (m, 3H, $NCH_2CH_2CH_2O$ and CH*H*-6), 3.95 (dt, J = 7.1, 9.6 Hz, 1H, CH*H*O), 4.03–4.16 (m, 2H, OCH_2CH_2N), 4.59 (t, J = 3.3 Hz, 1H, CHO), 6.88–6.94 (m, 2H, CH), 7.03 (d, J = 5.2 Hz, 1H, CH), 7.19 (d, J = 5.2 Hz, 1H, CH), 7.36–7.42 (m, 8H, CH), 7.64–7.69 ppm (m, 4H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$, 2 rotamers): δ = 19.2 (C-Si), 19.4 (CH_2-4), 25.4 (CH_2-5), 26.8 (3×CH_3, *t*BuSi), 28.4 (3×CH_3, *t*BuO), 29.1 (CH_2CH_2O), 30.6 (CH_2-3), 31.2/31.8 ($NCH_2CH_2CH_2O$), 45.4/46.3 (CH_2N), 46.9/47.2 (CH_2N), 61.6 (CH_2OSi), 62.0 (CH_2-6), 66.4/66.8 (OCH_2CH_2N), 67.6 (CH_2CH_2O), 79.6 (C), 98.6 (CHO), 114.4 (2×CH), 123.1 (CH), 126.9 (C), 127.6 (4×CH), 129.5 (CH), 129.6 (2×CH), 130.6 (2×CH), 133.7 (2×C), 134.3 (C), 135.5 (4×CH), 139.0 (C), 155.6 (CO), 158.2 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 766.4 $[M+Na]^+$ (100). HR-MS (ESI, positive mode): found: 766.3578; calcd. for $C_{43}H_{57}NO_6SSi$: 766.3568 $[M+Na]^+$.

***tert*-Butyl *N*-methyl-*N*-[2-[4-[3-[2-(tetrahydro-*2H*-pyran-2-yloxy)ethyl]thiophen-2-yl]phenoxy]ethyl] carbamate (231):** To a solution of **224** (3.93 g, 13.5 mmol) in anhydrous THF (70 mL), 2.5 M solution of *n*BuLi in hexane (5.94 mL, 14.9 mmol) was added dropwise at –78 °C under inert atmosphere, and the mixture was stirred for 1 h at this temperature. Then $B(OiPr)_3$ (3.81 g, 20.3 mmol) was added, and the mixture was stirred for 1 h at –78 °C and 2 h at room temperature. After addition of water (0.5 mL), compound **123** (3.39 g, 9.0 mmol), Ph_3P (189 mg, 0.72 mmol), $Pd(dba)_2$ (104 mg, 0.18 mmol) and 20% aq. Na_2CO_3 (70 mL) were added, and the mixture was heated at 78 °C with a reflux condenser and a bubble-counter for 18 h. Then it was diluted with EtOAc (100 mL), organic phase was separated, washed with brine (50 mL), dried, and evaporated under reduced pressure. The title compound was isolated by silica gel chromatography (150 g SiO_2) eluting with hexane/EtOAc (8:1 → 4:1) to yield 3.7 g (90%) of a colorless oil; R_f = 0.12 (hexane/EtOAc 4:1). HPLC: 70 → 100% A (30 → 0% B) for 0–20 min, 1 mL/min, 25 °C, t_R = 10.9 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 9H, *t*Bu), 1.48–1.89 (m, 6H, 3×CH_2, H-3/4/5), 2.93 (t, J = 7.0 Hz, 2H, CH_2CH_2O), 2.99 (s, 3H, CH_3N), 3.40–3.49 (m, 1H, C*H*H-6), 3.54–3.67 (m, 3H, OCH_2CH_2N and CH_2CHHO), 3.70–3.79 (m, 1H, CH*H*-6), 3.94 (dt, J = 7.0, 9.6 Hz, 1H, CH_2CHHO), 4.06–4.17 (m, 2H, OCH_2CH_2N), 4.58 (t, J = 3.3 Hz, 1H, CHO), 6.88–6.96 (m, 2H, 2×CH), 7.02 (d, J = 5.2 Hz, 1H, CH), 7.18 (d, J = 5.2 Hz, 1H, CH), 7.36–7.43 ppm (m, 2H, CH). ^{13}C NMR (125.5 MHz, $CDCl_3$, 2 rotamers): δ = 19.5 (CH_2-4), 25.5 (CH_2-5), 28.5 (3×CH_3), 29.1 (CH_2), 30.6 (CH_2-3),

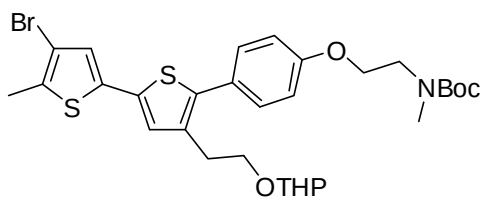
35.4/36.3 (CH_3N), 48.3 (CH_2N), 62.0 (CH_2-6), 66.1/66.9 (OCH_2), 67.6 (CH_2OTHP), 79.7 (C), 98.5 (CHO), 114.3 (2×CH), 123.0 (CH), 126.9 (C), 129.5 (2×CH), 130.6 (CH), 134.2 (C), 138.8 (C), 155.4/155.7 (CO), 157.9/158.1 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 484.2 $[M+Na]^+$ (100), 462.2 $[M+H]^+$ (6). Elemental analysis: found: C 65.33, H 7.39, N 2.90; calcd. for $C_{25}H_{35}NO_5S$ (461.6): C 65.05, H 7.64, N 3.03.

***tert*-Butyl *N*-[2-[4-[5-iodo-3-[2-(tetrahydro-*2H*-pyran-2-yloxy)ethyl]thiophen-2-yl]phenoxy] ethyl]-*N*-methyl carbamate (232):** To a solution of **231** (3.55 g, 7.7 mmol) in benzene (30 mL),



HgO (2.0 g, 9.2 mmol) and iodine (2.37 g, 9.2 mmol) were added in small portions. The mixture was stirred at room temperature for 4 h, and then filtered through a Celite® pad, washing with toluene (100 mL). The combined organic solutions were washed with sat. aq. $Na_2S_2O_3$ (2×50 mL), water (3×50 mL), and dried. After evaporation of the solvents under reduced pressure, the residue was filtered through SiO_2 (100 g) eluting with hexane/EtOAc (4:1) to give 4.19 g (92%) of a yellowish oil; R_f = 0.09. HPLC: 70 → 100% A (30 → 0% B) for 0–20 min, 1 mL/min, 25 °C, t_R = 15.5 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 1.49–1.86 (m, 6H, 3×CH_2, H-3/4/5), 2.87 (t, J = 6.9 Hz, 2H, C*H*$_2$CH$_2$O), 2.99 (s, 3H, CH_3N), 3.42–3.50 (m, 1H, C*H*H-6), 3.52–3.60 (m, 1H, CH_2C*H*HO), 3.62 (t, J = 5.0 Hz, 3H, OCH_2CH_2N), 3.69–3.78 (m, 1H, CH*H*-6), 3.90 (dt, J = 6.9, 9.7 Hz, 1H, CH_2CH*H*O), 4.07–4.17 (m, 2H, OC*H*$_2$CH$_2$N), 4.57 (t, J = 3.4 Hz, 1H, CHO), 6.90–6.95 (m, 2H, 2×CH), 7.17 (s, 1H, CH), 7.31–7.36 ppm (m, 2H, CH). ^{13}C NMR (125.5 MHz, $CDCl_3$, 2 rotamers): δ = 19.4 (CH_2-4), 25.5 (CH_2-5), 28.5 (3×CH_3), 28.7 (CH_2), 30.6 (CH_2-3), 35.4/36.2 (CH_3N), 48.3 (CH_2N), 62.0 (CH_2-6), 66.1/66.8 (OCH_2), 67.3 (CH_2OTHP), 70.6 (C-I), 79.6 (C), 98.5 (CHO), 114.4 (2×CH), 125.8 (C), 130.5 (2×CH), 136.4 (C), 139.3 (CH), 145.0 (C), 155.3/155.6 (CO), 158.2/158.4 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 610.1 $[M+Na]^+$ (100). HR-MS (ESI, positive mode): found: 610.1095; calcd. for $C_{25}H_{34}INO_5S$: 610.1100 $[M+Na]^+$.

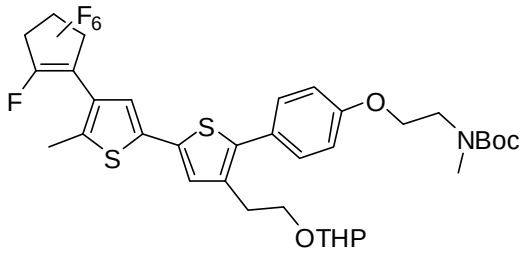
***tert*-Butyl *N*-[2-[4-[4'-bromo-5'-methyl-4-[2-(tetrahydro-*2H*-pyran-2-yloxy)ethyl]-[2,2']bi-thiophen-5-yl]phenoxy]ethyl]-*N*-methyl carbamate (233):** According GP 1.1, iodide **232** (4.0



g, 6.8 mmol), $Pd(dba)_2$ (78 mg, 0.14 mmol), Ph_3P (142 mg, 0.54 mmol), and thiophene boronic acid **226** (1.8 g, 8.2 mmol) gave the title compound (4.15 g, 96%) as a yellow oil after silica gel chromatography (150 g SiO_2) eluting with hexane/EtOAc (4:1); R_f = 0.12. HPLC: 70

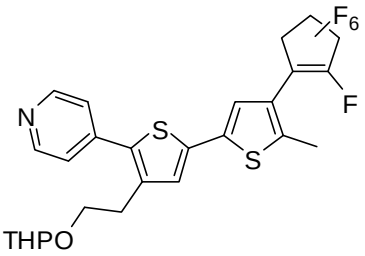
→ 100% A (30 → 0% B) for 0–20 min, 100% A for 20–25 min, 1 mL/min, 25 °C, t_R = 22.9 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 1.49–1.87 (m, 6H, 3×CH_2, H-3/4/5), 2.39 (s, 3H, CH_3), 2.90 (t, J = 6.9 Hz, 2H, CH_2CH_2O), 3.00 (s, 3H, CH_3N), 3.42–3.52 (m, 1H, C*H*H-6), 3.56–3.66 (m, 3H, OCH_2CH_2N, CH_2C*H*HO), 3.70–3.80 (m, 1H, CH*H*-6), 3.95 (dt, J = 7.0 Hz, 9.6, 1H, CH_2CH*H*O), 4.06–4.17 (m, 2H, OCH_2CH_2N), 4.60 (t, J = 3.3 Hz, 1H, CHO), 6.89–6.96 (m, 2H, CH), 6.93 (s, 1H, CH), 7.05 (s, 1H, CH), 7.38–7.43 ppm (m, 2H, CH). ^{13}C NMR (125.5 MHz, $CDCl_3$, 2 rotamers): δ = 14.8 (CH_3), 19.5 (CH_2-4), 25.5 (CH_2-5), 28.5 (3×CH_3), 29.2 (CH_2), 30.6 (CH_2-3), 35.4/36.2 (CH_3N), 48.3 (CH_2N), 62.0 (CH_2-6), 66.1/66.9 (OCH_2), 67.3 (CH_2OTHP), 79.7 (C), 98.5 (CHO), 109.4 (C-Br), 114.5 (2×CH), 125.3 (CH), 126.2 (CH), 130.4 (2×CH), 132.7 (C), 133.6 (C), 134.5 (C), 135.1 (C), 137.7 (C), 138.3 (C), 155.4/155.7 (CO), 158.1/158.2 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 660.1 and 658.1 $[M+Na]^+$ (100). HR-MS (ESI, positive mode): found: 658.1268 and 660.1246; calcd. for $C_{30}H_{38}BrNO_5S_2$: 658.1267 and 660.1248 $[M+Na]^+$.

***tert*-Butyl *N*-methyl-*N*-[2-[4-[5'-methyl-4'-(heptafluorocyclopent-1-enyl)-4-[2-(tetrahydro-*2H*-pyran-2-yloxy)ethyl]-2,2'-bithiophen-5-yl]phenoxy]ethyl] carbamate (234):** According to GP 3, bromide **233** (0.32 g, 0.50 mmol), 2.5 M solution of *n*BuLi in hexane (0.22 mL, 0.55 mmol), and perfluorocyclopenthene (0.7 mL, 5.0 mmol) after isolation by silica gel chromatography (100 g SiO_2) eluting with hexane/EtOAc (4:1) gave 0.25 g (66%)* of the title compound as a yellowish oil. HPLC: 70 → 100% A (30 → 0% B) for 0–20 min, 100% A for 20–25 min, 1 mL/min, 25 °C, t_R = 22.4 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 9H, *t*Bu), 1.49–1.89 (m, 6H, 3×CH_2, H-3/4/5), 2.45/2.46 (s, 3H, CH_3), 2.91 (t, J = 6.9 Hz, 2H, CH_2CH_2O), 3.00 (s, 3H, CH_3N), 3.42–3.52 (m, 1H, C*H*H-6), 3.56–3.68 (m, 3H, OCH_2CH_2N, CH_2C*H*HO), 3.71–3.82 (m, 1H, CH*H*-6), 3.96 (dt, J = 6.9, 9.5 Hz, 1H, CH_2CH*H*O), 4.05–4.19 (m, 2H, OCH_2CH_2N), 4.61 (t, J = 3.3 Hz, 1H, CHO), 6.89–6.97 (m, 2H, 2×CH), 7.07 (s, 1H, CH), 7.11 (s, 1H, CH), 7.38–7.44 ppm (m, 2H, 2×CH). ESI-MS, positive mode, *m/z* (rel. int., %): 772.2 $[M+Na]^+$ (100). HR-MS (ESI, positive mode): found: 772.1972; calcd. for $C_{35}H_{38}F_7NO_5S_2$: 772.1972 $[M+Na]^+$.

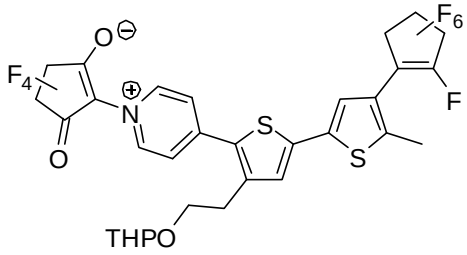


* contains ca. 20% of the debrominated derivative **235** formed from bromide **233** (HPLC).

4-[5'-Methyl-4'-(heptafluorocyclopent-1-enyl)-4-[2-(tetrahydro-*2H*-pyran-2-yloxy)ethyl]-2,2'-bithiophen-5-yl]pyridine (236): According to GP 3, bromide **229** (0.93 g, 2.0 mmol), 2.5 M solution of *n*BuLi in hexane (0.88 mL, 2.2 mmol), and perfluorocyclopenthene (2.7 mL, 20.0 mmol) after isolation by silica gel chromatography (100 g SiO_2) eluting with hexane/EtOAc (1:1, R_f = 0.13) gave 0.30 g (25%) of the title compound as a yellowish oil.* HPLC: 30 → 100% A (70 → 0% B) for 0–25 min, 1 mL/min, 25 °C, t_R = 19.4 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42–1.86 (m, 6H, 3×CH_2, H-3/4/5), 2.46/2.47 (s, 3H, CH_3), 2.99 (t, J = 6.6 Hz, 2H, CH_2), 3.42–3.52 (m, 1H, C*H*H-6), 3.62–3.78 (m, 2H, C*H*HO and CH*H*-6), 4.01 (dt, J = 6.6, 9.6 Hz, 1H, CH*H*O), 4.62 (t, J = 3.2 Hz, 1H, CHO), 7.13 (s, 1H, CH), 7.15 (s, 1H, CH), 7.44 (dd, J = 1.7, 4.6 Hz, 2H, CH), 8.63 ppm (dd, J = 1.7, 4.6 Hz, 2H, CH). ^{13}C NMR (75.5 MHz, $CDCl_3$):** δ = 14.6/14.7 (CH_3), 19.3 (CH_2-4), 25.4 (CH_2-5), 29.4 (CH_2), 30.5 (CH_2-3), 62.1 (CH_2-6), 67.7 (CH_2O), 98.7 (CHO), 120.4 (C), 122.9 (CH), 123.3 (2×CH), 127.5 (CH), 135.1 (C), 135.5 (C), 135.6 (C), 138.1 (C), 141.5 (C), 143.0 (C), 150.1 ppm (2×CH). ESI-MS, positive mode, *m/z* (rel. int., %): 578.1 $[M+H]^+$ (100). HR-MS (ESI, positive mode): found: 578.1063; calcd. for $C_{26}H_{22}F_7NO_2S_2$: 578.1058 $[M+H]^+$.



Compound 237: The compound was obtained as by-product in the synthesis of compound **236** in an almoust equal amount as an orange powder. ^{1}H NMR (300 MHz, [D_6]DMSO, 70 °C): δ = 1.33–1.53 (m, 4H, CH_2), 1.53–1.69 (m, 2H, CH_2), 2.51/2.52 (s, 3H, CH_3), 3.16 (t, J = 6.3 Hz, 2H, CH_2), 3.34–3.47 (m, 1H, C*H*HO), 3.57–3.68 (m, 1H, C*H*HO), 3.69–3.82 (m, 1H, CH*H*O), 3.91–4.06 (m, 1H, CH, CH*H*O), 4.58–4.62 (m, 1H, CHO), 7.47 (s, 1H, CH), 7.59 (s, 1H, CH), 8.29 (d, J = 7.2 Hz, 2H, CH), 9.30 ppm (d, J = 7.2 Hz, 2H, CH). ^{13}C NMR (75.5 MHz, [D_6]DMSO, 70 °C): δ = 13.8 (CH_3), 18.7 (CH_2-4), 24.6 (CH_2-5), 29.4 (CH_2), 29.8 (CH_2-3), 32.0/32.4 (C), 61.1 (CH_2-6), 65.9 (CH_2O), 97.8 (CHO), 109.2 (3×C, C and 2×CF_2), 109.6 (CF_2), 110.2 (CF_2), 114.55 (CF_2), 119.6 (C), 124.3 (2×CH), 129.3 (2×CH), 131.5 (C), 133.4 (C), 138.7 (C), 141.0 (2×CH), 144.5 (C), 144.7 (C), 147.12 (C), 150.5 (=CF), 171.3 ppm (2×CO). ^{19}F NMR (282 MHz, [D_6]DMSO, 70 °C): δ = –106.4 (d, J = 12.6 Hz, 2F), –117.2 (d, J = 16.4 Hz, 2F), –126.2 (s, 5F), –129.2 ppm (s, 2F). ESI-MS, positive mode, *m/z* (rel.

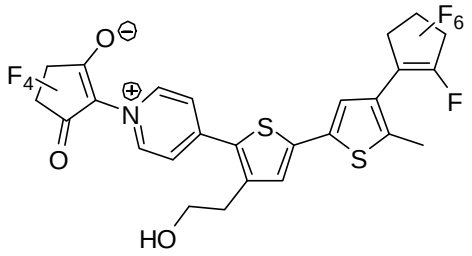


* The compound is unstable at room temperature (or even at +4 °C), either in the reaction mixture or after isolation: it transforms to compound **237** with higher R_f on TLC.

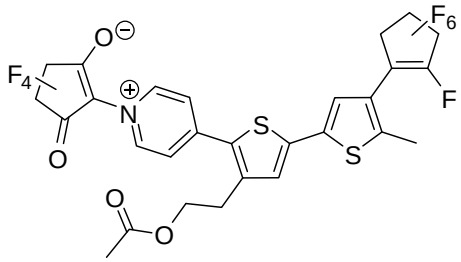
** Due to low intensities, signals of the fluorinated carbons were not detected.

int., %): 768.1 (100) $[M+Na]^+$. HR-MS (ESI, positive mode): found: 768.0696; calcd. for $C_{31}H_{22}F_{11}NO_4S_2$: 768.0707 $[M+Na]^+$.

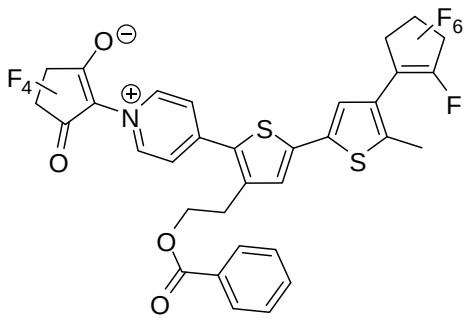
Compound 238: A solution of **237** (38 mg, 0.05 mmol) and PPTS (5 mg, 0.02 mmol) in EtOH (6 mL) was stirred at 55 °C for 2 days. After evaporation of the solvent under reduced pressure, the compound was filtred through SiO_2 (80 mL) eluting with hexane/EtOAc (2:1) to get 30 mg (99%) of the title compound as an orange powder; decomp. >240 °C (MeOH). ^{1}H NMR (300 MHz, [D$_6$]DMSO, 35 °C): δ = 2.50 (s, 3H, CH_3), 3.02 (s, 2H, CH_2), 3.80 (s, 2H, CH_2O), 4.91 (s, 1H, OH), 7.50 (s, 1H, CH), 7.60 (s, 1H, CH), 8.32 (d, J = 7.2 Hz, 2H, CH), 9.25 ppm (d, J = 7.2 Hz, 2H, CH). ^{13}C NMR (125.7 MHz, [D$_6$]DMSO, 35 °C): δ = 14.2 (CH_3), 32.6 (CH_2), 41.3 (C), 60.5 (CH_2O), 109.4 (2×CF_2), 109.8 (CF_2), 110.4 (CF_2), 114.8 (CF_2), 115.5 (C), 119.7 (C), 124.3 (2×CH), 124.4 (CH), 129.5 (CH), 131.5 (C), 133.5 (C), 138.8 (C), 141.2 (2×CH), 144.8 (C), 145.4 (C), 147.2 (C), 150.6 (CF), 171.5 ppm (2×CO). ESI-MS, positive mode, *m/z* (rel. int., %): 1344.5 (50) $[2M+Na]^+$, 662.3 (100) $[M+H]^+$. Elemental analysis: found: C 47.89, H 2.11, N 2.06; calcd. for $C_{26}H_{14}F_{11}NO_3S_2$ (661.5): C 47.21, H 2.13, N 2.12.



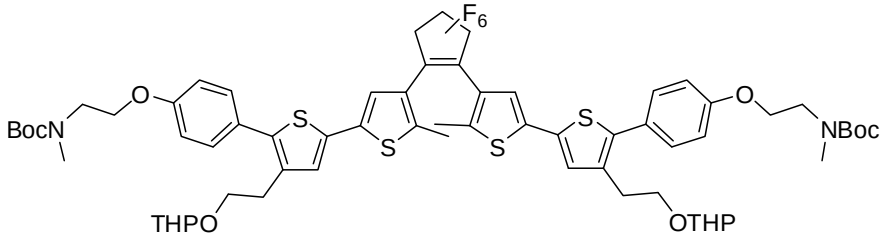
Compound 239:[130] The compound **238** (20 mg, 0.03 mmol) was stirred in Ac_2O (1 mL) at 85 °C for 2 h, diluted with Et_2O (50 mL), washed with 5% aq. Na_2CO_3 (20 mL), brine and dried over Na_2SO_4. After evaporation of the solvent under reduced pressure, the residue was filtred through SiO_2 (20 g) eluting with hexane/EtOAc (4:1) to afford 20 mg (91%) of the title compound as an orange powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.07 (s, 3H, CH_3CO), 2.51/2.52 (s, 3H, CH_3), 3.18 (t, J = 7.1 Hz, 2H, CH_2), 4.40 (t, J = 7.1 Hz, 2H, CH_2O), 7.20 (s, 1H, CH), 7.27 (s, 1H, CH), 8.01 (d, J = 7.4 Hz, 2H, CH), 9.76 ppm (d, J = 7.4 Hz, 2H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 726.0 (100) $[M+Na]^+$, 704.0 (13) $[M+H]^+$. HR-MS (ESI, positive mode): found: 726.0256; calcd. for $C_{28}H_{16}F_{11}NO_4S_2$: 726.0237 $[M+Na]^+$.



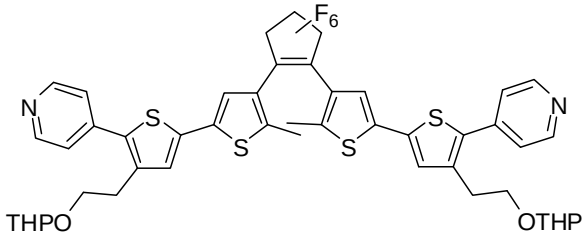
Compound 240: To a solution of **238** (13 mg, 0.02 mmol) in CH_2Cl_2 (10 mL), pyridine (7 µL, 0.08 mmol) and BzCl (5 µL, 0.04 mmol) were added at 0 °C, and the mixture was stirred at room temperature overnight. After evaporation of the solvent under reduced pressure, the residue was filtred through SiO_2 (20 g) eluting with hexane/EtOAc (2:1) to yield 12 mg (78%) of the title compound as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.51/2.52 (s, 3H, CH_3), 3.35 (t, J = 6.6 Hz, 2H, CH_2), 4.63 (t, J = 6.6 Hz, 2H, CH_2O), 7.24–7.28 (m, 2H, CH), 7.41 (t, J = 7.3 Hz, 2H, CH), 7.57 (t, J = 7.3 Hz, 1H, CH), 7.92 (d, J = 7.3 Hz, 2H, CH), 7.98 (d, J = 6.8 Hz, 2H, CH_{py}), 9.71 ppm (d, J = 6.8 Hz, 2H, CH_{py}). ESI-MS, positive mode, *m/z* (rel. int., %): 1553.2 (23) $[2M+Na]^+$, 788.1 (100) $[M+Na]^+$. HR-MS (ESI, positive mode): found: 788.0394; calcd. for $C_{33}H_{18}F_{11}NO_4S_2$: 788.0394 $[M+Na]^+$.



Photochromic compound 241: The title compound was isolated as a green foam in the synthesis of compound **234**. HPLC: 50 → 100% A (50 → 0% B) for 0–25 min, 1 mL/min, 25 °C, t_R (OF) = 8.5 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45 (s, 18H, *t*Bu), 1.47–1.86 (m, 12H, CH_2), 1.89–2.16 (m, 6H, CH_3), 2.89 (s, 4H, CH_2), 2.98 (s, 6H, CH_3N), 3.38–3.52 (m, 2H, 2×C*H*H-6), 3.61 (s, 6H, 2×CH_2N and 2×CH*H*OTHP), 3.68–3.80 (m, 2H, 2×CH*H*-6), 3.87–4.00 (m, 2H, 2×CH*H*OTHP), 4.03–4.18 (m, 4H, CH_2O), 4.59 (s, 2H, CH), 6.85–6.97 (m, 4H, CH), 7.07 (s, 2H, CH), 7.09 (s, 2H, CH), 7.34–7.46 ppm (m, 4H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 1268.1 (26) $[M+H]^+$, 1309.2 (100) $[M+Na]^+$. HR-MS (ESI, positive mode): found: 1309.4225; calcd. for $C_{65}H_{76}F_6N_2O_{10}S_4$: 1309.4179 $[M+Na]^+$.



Photochromic compound 242: The title compound was isolated as a green foam in the synthesis of compound **236**. HPLC: 30 → 100% A (70 → 0% B) for 0–25 min, 1 mL/min, 25 °C, t_R (OF) = 21.1 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.45–1.85 (m, 12H, CH_2), 1.96/2.16 (s, 6H, CH_3), 2.98 (t, J = 6.6 Hz, 4H, CH_2), 3.41–3.52 (m, 2H, 2×C*H*H-6), 3.60–3.80 (m, 4H, 2×CH*H*-6 and 2×C*H*HO),



3.93–4.07 (m, 2H, 2×CH*H*O), 4.57–4.63 (m, 2H, CH), 7.13 (s, 2H, CH), 7.15 (s, 2H, CH), 7.42–7.48 (m, 4H, CH), 8.58–8.66 ppm (m, 4H, CH). ^{19}F NMR (282 MHz, $CDCl_3$): δ = –110.08 (t, J = 5.0 Hz, 4F), –131.80 ppm (quint, J = 5.0 Hz, 2F). ESI-MS, positive mode, *m/z* (rel. int., %): 943.2 (100) $[M+H]^+$. HR-MS (ESI, positive mode): found: 943.2167; calcd. for $C_{47}H_{44}F_6N_2O_4S_4$: 943.2161 $[M+H]^+$.

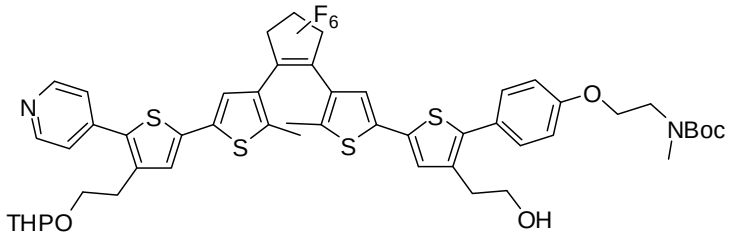
Photochromic compound 244: To a solution of bromide **233** (0.64 g, 1.0 mmol) in anhydrous THF (15 mL), 2.5 M solution of *n*BuLi in hexane (0.4 mL, 1.0 mmol) was added dropwise at –78 °C, and the mixture was stirred for 1 h at this temperature. Then a solution of heptafluorocyclopentene **236** (0.29 g, 0.5 mmol) in THF (9 mL) was added, and the mixture was stirred for 2 h at –78 °C and further 1 h at room temperature followed by the addition of brine (1 mL). The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. The title compound was isolated by silica gel chromatography (100 g SiO_2) eluting with hexane/EtOAc (1:1 → 1:3) to give 0.35 g (62%) as a green foam. HPLC: 30 → 100% A (70 → 0% B) for 0–25 min, 1 mL/min, 25 °C, t_R (OF) = 13.9 min, detection at 254 nm. ^{1}H NMR (600 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.47 (s, 9H, *t*Bu), 1.49–1.86 (m, 12H, 6×CH_2), 1.95 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 2.91 (t, J = 6.9 Hz, 2H, CH_2), 2.99 (t, J = 6.8 Hz, 2H, CH_2), 3.00 (s, 3H, CH_3N), 3.44–3.50 (m, 2H, 2×C*H*H-6), 3.59–3.65 (m, 3H, CH_2N and C*H*HOTHP), 3.65–3.69 (m, 1H, C*H*HOTHP), 3.72–3.80 (m, 2H, 2×CH*H*-6), 3.95 (dt, J = 6.9, 9.5 Hz, 1H, CH*H*OTHP), 4.01 (dt, J = 6.8, 9.6 Hz, 1H, CH*H*OTHP), 4.08–4.17 (m, 2H, CH_2O), 4.59–4.64 (m, 2H, 2×CHO), 6.91–6.95 (m, 2H, 2×CH), 7.09 (s, 1H, CH), 7.10 (s, 1H, CH), 7.14 (s, 1H, CH), 7.16 (s, 1H, CH), 7.34–7.42 (m, 2H, 2×CH), 7.45 (dd, J = 1.5, 4.5 Hz, 2H, CH), 8.63 ppm (dd, J = 1.5, 4.5 Hz, 2H, CH). ^{13}C NMR (125.7 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 14.2 (CH_3), 14.5/14.6 (CH_3), 19.5 (2×CH_2-4), 25.4/25.5 (2×CH_2-5), 29.2/29.4 (2×CH_2), 30.6 (2×CH_2-3), 35.4/36.3 (CH_3N), 48.3 (CH_2N), 62.1 (2×CH_2-6), 66.2/66.9 (OCH_2), 67.1 (CH_2OTHP), 67.4 (CH_2OTHP), 79.7 (C), 98.6/98.7 (2×CHO), 114.0 (C), 114.5 (2×CH), 122.0 (CH), 123.0 (CH), 123.2 (3×CH), 125.4 (C), 125.6 (C), 126.7/127.1 (2×CH), 130.4 (2×CH), 133.3 (C), 134.8 (C), 135.2 (2×C), 135.8 (C), 135.9 (C), 138.0 (2×C), 138.6 (C), 139.1 (CH), 140.3 (C), 141.2 (C), 141.5 (C), 149.9 (2×CH), 155.4/155.7 (CO), 158.2/158.3 ppm (CO). ^{19}F NMR (282 MHz, $CDCl_3$): δ = –110.04 (s, 2F), –110.14 (s, 2F), –131.82 ppm (quint, J = 5.7 Hz, 2F). ESI-MS, positive mode, *m/z* (rel. int., %): 1115.3 (100) $[M+H]^+$. HR-MS (ESI, positive mode): found: 1115.3259; calcd. for $C_{56}H_{60}F_6N_2O_7S_4$: 1115.3260 $[M+H]^+$.

Photochromic compound 246: A solution of **244** (0.22 g, 0.20 mmol) and PPTS (10 mg, 0.04 mmol) in EtOH (10 mL) was stirred at 55 °C for 7 days under inert atmosphere. After evaporation of the solvent, the title compound was isolated by silica gel chromatography (100 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to yield 0.165 g (87%) as a blue foam. ^{1}H NMR (300 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.47 (s, 9H, *t*Bu), 1.97 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 2.88 (t, J = 6.5 Hz, 2H, CH_2), 2.97 (t, J = 6.5 Hz, 2H, CH_2), 2.99 (s, 3H, CH_3N), 3.58–3.66 (m, 2H, CH_2N), 3.85 (t, J = 6.5 Hz, 2H, C*H*$_2$OH), 3.93 (t, J = 6.5 Hz, 2H, C*H*$_2$OH), 4.07–4.17 (m, 2H, CH_2O), 6.90–6.97 (m, 2H, 2×CH), 7.05 (s, 1H, CH), 7.09 (s, 1H, CH), 7.11 (s, 1H, CH), 7.15 (s, 1H, CH), 7.35–7.41 (m, 2H, 2×CH), 7.41–7.45 (m, 2H, CH), 8.56–8.68 ppm (m, 2H, CH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.5 (CH_3), 14.6 (CH_3), 28.5 (3×CH_3), 31.9 (CH_3N), 32.1 (2×CH_2), 48.3 (CH_2N), 62.5 (2×CH_2OH), 62.8 (CH_2O), 79.8 (C), 114.6 (2×CH), 122.3 (CH), 123.3 (3×CH), 125.4 (C), 125.6 (C), 126.2 (CH), 126.7 (CH), 130.5 (2×CH), 133.8 (C), 134.6 (2×C), 134.7 (C), 135.4 (C), 135.5 (C), 136.4 (2×C), 137.6 (2×C), 139.1 (C), 140.5 (C), 141.4 (C), 141.6 (C), 149.8 (2×CH), 158.3 (C=O), 158.4 ppm (C). ^{19}F NMR (282 MHz, $CDCl_3$): δ = –109.96 (s, 2F), –110.04 (s, 2F), –131.79 ppm (t, J = 5.2 Hz, 2F). ESI-MS, positive mode, *m/z* (rel. int., %): 947.1 $[M+H]^+$ (100). HR-MS (ESI, positive mode): found: 947.2114; calcd. for $C_{46}H_{44}F_6N_2O_5S_4$: 947.2110 $[M+H]^+$.

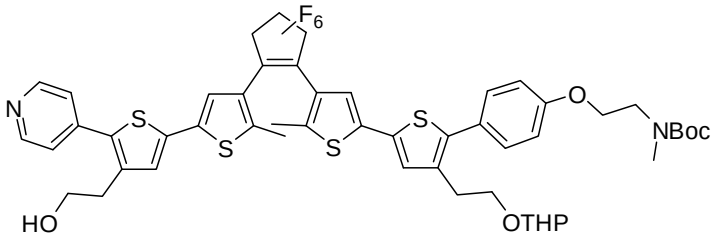
Photochromic compound 250: Compound **246** (14 mg, 0.015 μmol) was stirred with β-sulfopropionic acid anhydride (**249**) (4 mg, 0.03 μmol) in anhydrous THF at room temperature for 12 h under argon. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (20 g SiO_2) eluting with CH_2Cl_2/MeOH (2:1) to give 15 mg (83%) of a green foam. HPLC: 30 → 90% A (70 → 10% B) for 0–25 min, 1 mL/min, 25 °C, t_R (OF) = 14.0 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 2.03–2.18 (m, 6H, CH_3), 2.59–2.87 (m, 4H, CH_2CO), 2.85 (s, 3H, CH_3N), 2.95–3.14 (m, 6H, 2×CH_2, CH_2N), 3.45–3.50 (m, 4H, 2×CH_2S), 4.16–4.26 (m, 2H, CH_2O), 4.27–4.40 (m, 4H, CH_2OCO), 7.03–7.16 (m, 4H, CH), 7.22 (s, 1H, CH), 7.25 (s, 1H, CH), 7.34–7.50 (m, 2H, CH), 7.53–7.63 (m, 2H, CH), 8.52–8.68 ppm (m, 2H,

CH). ESI-MS, negative mode, *m/z* (rel. int., %): 1117.2 (100) $[M–H]^–$, 558.1 (91) $[M–2H]^{2–}$. HR-MS (ESI, negative mode): found: 558.0520; calcd. for $C_{47}H_{44}F_6N_2O_{11}S_6$: 558.0514 $[M–2H]^{2–}$.

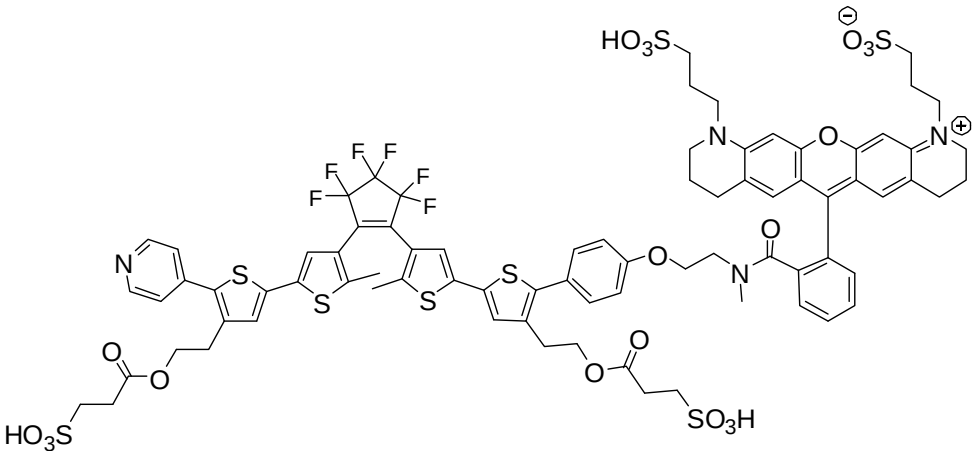
Photochromic compound 251: The title compound was isolated as a green foam in the synthesis of the compound **246**. ^{1}H NMR (600 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.47 (s, 9H, *t*Bu), 1.49–1.85 (m, 6H, 3×CH_2), 1.97/2.16 (s, 6H, CH_3), 2.89 (t, J = 6.5 Hz, 2H, C$H_2$$CH_2$OH), 3.00 (m, 5H, CH_3N and C$H_2$$CH_2$OTHP), 3.42–3.53 (m, 1H, C*H*H-6), 3.57–3.80 (m, 4H, CH_2N, C*H*HOTHP, and CH*H*-6), 3.86 (t, J = 6.5 Hz, 2H, CH_2OH), 4.01 (dt, J = 6.9, 9.7 Hz, 1H, CH*H*OTHP), 4.07–4.19 (m, 2H, CH_2O), 4.62 (t, J = 3.2 Hz, 1H, CHO), 6.91–6.98 (m, 2H, 2×CH), 7.06 (s, 1H, CH), 7.11 (s, 1H, CH), 7.14 (s, 1H, CH), 7.15 (s, 1H, CH), 7.36–7.42 (m, 2H, 2×CH), 7.43–7.49 (m, 2H, 2×CH), 8.57–8.71 ppm (m, 2H, 2×CH). ESI-MS, positive mode, *m/z* (rel. int., %): 1053.3 (100) $[M+Na]^+$, 1031.3 (89) $[M+H]^+$. HR-MS (ESI, positive mode): found: 1031.2685; calcd. for $C_{51}H_{52}F_6N_2O_6S_4$: 1031.2685 $[M+H]^+$.



Photochromic compound 252: The title compound was isolated as a green foam in the synthesis of the compound **246**. ^{1}H NMR (600 MHz, $CDCl_3$, open form, mixture of rotamers): δ = 1.47 (s, 9H, *t*Bu), 1.49–1.87 (s, 6H, 3×CH_2), 1.97/2.16 (s, 6H, CH_3), 2.90 (t, J = 6.4 Hz, 2H, C$H_2$$CH_2$OTHP), 2.97 (t, J = 6.5 Hz, 2H, C$H_2$$CH_2$OH), 3.00 (s, 3H, CH_3N), 3.41–3.52 (m, 1H, C*H*H-6), 3.56–3.68 (m, 3H, CH_2N and C*H*HOTHP), 3.70–3.81 (m, 1H, CH*H*-6), 3.94 (t, J = 6.5 Hz, 3H, CH_2OH and CH*H*OTHP), 4.06–4.18 (m, 2H, CH_2O), 4.60 (s, 1H, CHO), 6.89–6.98 (m, 2H, 2×CH), 7.08 (s, 1H, CH), 7.09 (s, 1H, CH), 7.11 (s, 1H, CH), 7.16 (s, 1H, CH), 7.35–7.48 (m, 4H, CH), 8.60–8.70 ppm (m, 2H, CH). ESI-MS, positive mode, *m/z* (rel. int., %): 1053.3 (100) $[M+Na]^+$, 1031.3 (34) $[M+H]^+$. HR-MS (ESI, positive mode): found: 1031.2691; calcd. for $C_{51}H_{52}F_6N_2O_6S_4$: 1031.2685 $[M+H]^+$.

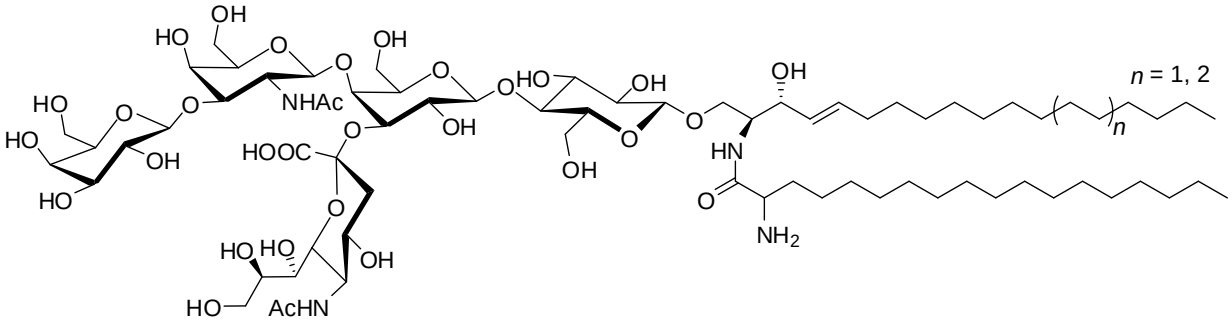


Adduct 253: To a mixture of amine **250** (1.1 mg, 0.98 μmol), dye **191** (0.6 mg, 0.95 μmol), and NEt_3 (10 μL) in DMF (0.2 mL), HATU (0.76 mg, 2.0 μmol) was added. The mixture was stirred under inert atmosphere at room temperature



for 12 h. After evaporation of the solvent, the residue was purified by preparative TLC eluting with $CHCl_3$/MeOH/H_2O (60:35:5) to give 1.0 mg (57%) of a violet powder. ESI-MS, negative mode, *m/z* (rel. int., %): 1753.4 (23) $[M–H]^–$, 876.5 (100) $[M–2H]^{2–}$, 584.2 (86) $[M–3H]^{3–}$. HR-MS: found: 887.1224; calcd. for $C_{79}H_{76}F_6N_4O_{19}S_8$: 887.1220 $[M–3H+Na]^{2–}$.

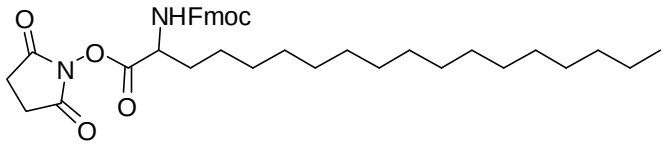
GM1-2-NH_2 (262): Compound **286** (6 mg, 3.3 μmol) was stirred with a 20% solution of



piperidine in MeOH (0.4 mL) at room temperature for 2 h, and the mixture was concentrated under reduced pressure. The title product was isolated by chromatography on SiO_2 (10 g) eluting with $CHCl_3$/MeOH/H_2O (60:35:5), then dissolved in water and freeze-dried to give two compounds with different R_f-values (0.31 and 0.19) and the same mass spectra: 2.7 mg (52%) and 2.3 mg (44%) as a colorless powder. ^{1}H NMR (600 MHz, CD_3OD): δ = 0.90 (t, 6H, CH_3), 1.29 (s, 48H, CH_2), 1.39 (m, 2H, CH_2-7), 1.51/1.64 (m, 2H, CH_2-3'), 1.91 (m, 1H, CH*a*), 1.99 (s, 3H, CH_3, GalNAc), 2.01 (s, 3H, CH_3, Neu5Ac), 2.03 (m, 2H, CH_2-6), 2.73 (m, 1H, CH*e*), 3.53 (m, 1H, CH-2'), 3.25–4.20 (m, 31H, 21×CH and 5×CH_2O), 3.41 (m, 1H, CH-1a), 4.00 (m, 1H, CH-2), 4.10 (m, 1H, CH-3), 4.16 (m, 1H, CH-1b), 4.30 (d, *J* = 7.8 Hz, 1H, Glc(I)-H1), 4.41 (d, *J* = 7.9 Hz, 1H, Gal(II)-H1), 4.45 (d, *J* = 7.6 Hz, 1H, Gal(IV)-H1), 4.92 (d, *J* = 8.6 Hz, 1H, GalNAc-H1), 5.46 (m, 1H, CH-4), 5.72 ppm (m, 1H, CH-5). ESI-MS, negative mode, *m/z*:

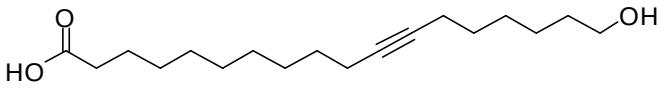
1560.1 $[M_1–H]^-$, 1588.0 $[M_2–H]^-$. HR-MS (ESI, positive mode): found: 781.4510, 795.4667; calcd. for $C_{73}H_{132}N_4O_{31}$ and $C_{75}H_{136}N_4O_{31}$: 781.4511 $[M_1+2H]^{2+}$, 795.4667 $[M_2+2H]^{2+}$.

***N*-Hydroxysuccinimidyl (2*R*/*S*)-[(9-fluorenylmethoxycarbonyl)amino]octadecanoate (269):**



A solution of **268** (45 mg, 86 µmol) and di(*N*-succinimidyl) carbonate (33 mg, 0.13 mmol) in anhydrous acetone (4 mL) was added to a solution of NEt_3 (25 µL) in anhydrous THF (6 mL), and the mixture was stirred at room temperature for 48 h. Evaporation under reduced pressure and filtration through a pad of SiO_2 (15 g) eluting with EtOAc gave the title compound (50 mg, 94%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, J = 7.0 Hz, 3H, CH_3), 1.25 (m, 28H, CH_2), 1.75–2.13 (m, 2H, H-3), 2.85 (s, 4H, CH_2 in NHS), 4.24 (t, J = 7.4 Hz, 1H, H-9'), 4.45 (m, 2H, CH_2O), 4.75 (m, 1H, H-2), 5.21 (m, 1H, NH), 7.32 (m, 2H, H-2'/7'), 7.40 (m, 2H, H-3'/6'), 7.60 (d, J = 7.4 Hz, 2H, H-1'/8'), 7.77 ppm (d, J = 7.4 Hz, 2H, H-4'/5'). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.1 (CH_3), 22.7 (CH_2, C-17), 24.9 (CH_2, C-4), 25.5 (2×CH_2 in NHS), 29.0–29.7 (11×CH_2, C-5 to C-15), 31.9 (CH_2, C-3), 32.7 (CH_2, C-16), 47.0 (CH, C-9'), 52.3 (CH, C-2), 67.2 (CH_2), 119.9 (2×CH, C-1'/8'), 125.0 (2×CH, C-2'/7'), 127.1 (2×CH, C-4'/5'), 127.7 (2×CH, C-3'/6'), 141.3 (2×C), 143.6 (C), 143.8 (C), 155.5 (CO), 168.4 (CO), 168.5 ppm (2×CO in NHS). ESI-MS, positive mode, *m/z* (rel. int., %): 641.3 $[M+Na]^+$, 1259.1 $[2M+Na]^+$. HR-MS (ESI, positive mode): found: 641.3569; calcd. for $C_{37}H_{50}N_2O_6$: 641.3561 $[M+Na]^+$.

18-Hydroxyoctadec-11-ynoic acid (273): To a solution of $LiNH_2$ (prepared from 0.3 g Li, 27 mg of $Fe(NO_3)_3$×$9H_2O$ in 200 mL of liquid ammonia under Ar) was added within 15 min a solution of 7-octyn-1-ol (**271**) (2.08 g, 16.5 mmol) in anhydrous THF (13 ml), and the mixture was stirred for 1 h. A solution of 10-bromdecanoic acid (**272**) (1.60 g, 6.4 mmol) in anhydrous THF (26 mL) was added, and the reaction mixture was stirred for 18 h. After evaporation of NH_3, the reaction was quenched with ice, acidified up to pH 3 (conc. aq. HCl), extracted with Et_2O (3×150 mL), and dried over Na_2SO_4. The title product was isolated by chromatography on SiO_2 (150 g) eluting with hexane/EtOAc (2:1 + 1% AcOH) to yield 1.0 g (52%) of a colorless solid; m. p. 69 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.18–1.50 (m, 18H, CH_2), 1.50–1.66 (m, 4H, CH_2), 2.05–2.17 (m, 4H, $CH_2C\equiv$), 2.30 (t, J = 7.5 Hz, 2H, CH_2CO), 3.62 (t, J = 6.6 Hz, 2H, CH_2O), 6.49 ppm (br s, 1H, OH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 18.7 (2×CH_2, C-10/13), 24.7 (CH_2, C-16), 25.2 (CH_2,



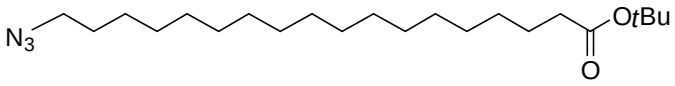
C-3), 28.6 (CH_2), 29.0 (5×CH_2), 29.1 (CH_2, C-5), 29.2 (CH_2, C-6), 32.5 (CH_2, C-17), 34.0 (CH_2, C-2), 62.9 (CH_2O), 80.1 (C), 80.3 (C), 179.3 ppm (CO). CI-MS (NH_3), *m/z* (rel. int., %): 314.5 $[M+NH_4]^+$ (100). Elemental analysis: found: C 72.67, H 10.87; calcd. for $C_{18}H_{32}O_3$ (296.4): C 72.93, H 10.88.

18-(Ethoxycarbonothioylthio)octadecanoic acid (276): To a solution of **275** (0.20 g, 0.55 mmol) in anhydrous acetone (15 mL), was added a solution of potassium xanthogenate (0.12 g, 0.77 mmol) in acetone (10 mL), and the mixture was stirred at room temperature for 18 h under an inert atmosphere. The solvent was evaporated under reduced pressure; the residue was dissolved in Et_2O (50 mL), and 1 M aq. HCl (10 mL) was added. The organic layer was washed with water (2×20 mL), brine (20 mL), dried and concentrated in vacuo to give the title compound (0.22 g, 99%) as a colorless solid; m. p. 79 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.18–1.37 (m, 26H, CH_2), 1.42 (t, J = 7.1 Hz, 3H, CH_3), 1.55–1.75 (m, 4H, CH_2, H-3/17), 2.34 (t, J = 7.5 Hz, 2H, CH_2-2), 3.10 (t, J = 7.5 Hz, 2H, CH_2-18), 4.64 ppm (q, J = 7.1 Hz, 2H, CH_2O). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 13.8 (CH_3), 24.7 (CH_2, C-3), 28.3 (CH_2), 28.9 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.4 (2×CH_2), 29.5 (CH_2), 29.6 (6×CH_2), 34.0 (CH_2, C-17), 35.9 (CH_2 C-2), 69.7 (CH_2O), 180.1 (CO), 215.3 ppm (C=S). ESI-MS, negative mode, *m/z* (rel. int., %): 403.0 $[M–H]^–$ (100), 807.9 $[2M–H]^–$ (76); positive mode, *m/z* (rel. int., %): 405.2 $[M+H]^+$ (27), 427.2 $[M+Na]^+$ (100), 831.5 $[2M+Na]^+$ (24). HR-MS (ESI, positive mode): found: 427.2312; calcd. for $C_{21}H_{40}O_3S_2$: 427.2311 $[M+Na]^+$.

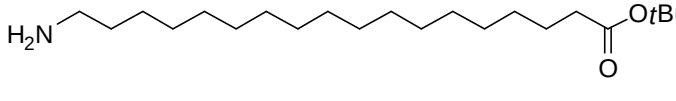
***tert*-Butyl 18-bromooctadecanoate (277):** To a solution of **275** (0.9 g, 2.5 mmol) in THF (7.5 mL), trifluoroacetic anhydride (0.67 mL, 4.8 mmol) was slowly added at 0 ºC. After 1 h, *t*BuOH (2.8 mL) was added, and the solution was stirred at room temperature overnight. The reaction mixture was poured into sat. aq. $NaHCO_3$ (30 mL), extracted with CH_2Cl_2 (3×30 mL), washed with brine (30 mL) and dried. The residue was purified by flash chromatography on SiO_2 (50 g) eluting with hexane/EtOAc (16:1) to give 0.94 g (90%) of the title compound as a colorless solid; m. p. 46 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.17–1.33 (m, 26H, CH_2), 1.41 (s, 9H, *t*Bu), 1.48–1.61 (m, 2H, CH_2), 1.76–1.89 (m, 2H, CH_2), 2.17 (t, J = 7.5 Hz, 2H, CH_2CO), 3.38 ppm (t, J = 6.9 Hz, 2H, CH_2Br). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 25.1 (CH_2, C-3), 28.1 (3×CH_3), 28.2 (CH_2, C-16), 28.8 (CH_2, C-4), 29.1 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (2×CH_2), 29.6 (6×CH_2), 32.8 (CH_2, C-17), 34.0 (CH_2Br),

35.6 (CH_2, C-2), 79.8 (C), 173.3 ppm (CO). CI-MS (NH_3), *m/z* (rel. int., %): 436.4 and 438.4 $[M+NH_4]^+$ (100). Elemental analysis: found: C 62.83, H 10.69; calcd. for $C_{22}H_{43}BrO_2$ (419.5): C 62.99, H 10.33.

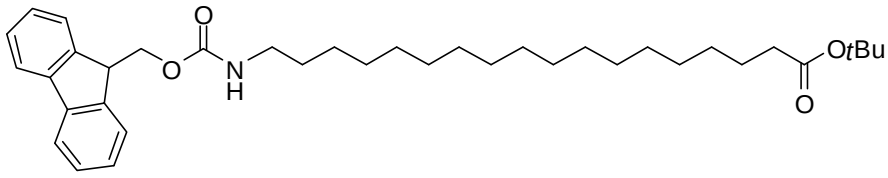
***tert*-Butyl 18-azidooctadecanoate (278):** Compound **277** (0.84 g, 2.0 mmol) was stirred with NaN_3 (0.16 g, 2.4 mmol) in anhydrous DMF (13 mL) at 85 ºC for 18 h. The solvent was evaporated in vacuo, the mixture was diluted with CH_2Cl_2 (50 mL), the solution was washed with water (3×20 mL), dried over Na_2SO_4, and evaporated to give the title compound (0.76 g, 99%) as a colorless solid; m. p. 42 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.23 (m, 26H, CH_2), 1.42 (s, 9H, *t*Bu), 1.45–1.65 (m, 4H, CH_2, H-3/17), 2.17 (t, J = 7.5 Hz, 2H, CH_2CO), 3.23 ppm (t, J = 7.0 Hz, 2H, CH_2N). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 25.1 (CH_2, C-3), 26.7 (CH_2), 28.1 (3×CH_3), 28.8 (CH_2), 29.1 (2×CH_2), 29.3 (CH_2), 29.5 (3×CH_2), 29.6 (6×CH_2), 35.6 (CH_2, C-2), 51.5 (CH_2N), 79.8 (C), 173.3 ppm (CO). CI-MS (NH_3), *m/z* (rel. int., %): 399 $[M+NH_4]^+$ (100). Elemental analysis: found: C 69.08, H 11.94, N 10.75; calcd. for $C_{22}H_{43}N_3O_2$ (381.6): C 69.24, H 11.36, N 11.01.



***tert*-Butyl 18-aminooctadecanoate hydrochloride (279):** To a suspension of 10% Pd/C (0.11 g, Merck, oxidized form) in EtOAc (10 mL), was added a solution of **278** (0.57 g, 1.5 mmol) in EtOAc (60 mL) with 5–6 M HCl in *i*PrOH (0.75 mL). The mixture was stirred under H_2 (1 atm) at room temperature overnight, diluted with EtOAc (100 mL), filtered through Celite® and concentrated in vacuo to give 0.4 g (68%) of the title compound as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.22 (m, 26H, CH_2), 1.42 (s, 9H, *t*Bu), 1.47–1.63 (m, 2H, CH_2), 1.65–1.82 (m, 2H, CH_2), 2.17 (t, J = 7.5 Hz, 2H, CH_2CO), 2.94 ppm (m, J = 7.5 Hz, 2H, CH_2N). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 25.1 (CH_2, C-3), 26.7 (CH_2, C-16), 28.1 (3×CH_3), 28.9 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.5 (2×CH_2), 29.6 (5×CH_2), 29.7 (2×CH_2), 35.6 (CH_2, C-2), 39.7 (CH_2NH_2), 79.9 (C), 173.4 ppm (CO). CI-MS (NH_3), *m/z* (rel. int., %): 711 $[2M+H]^+$ (8), 356.4 $[M+H]^+$ (100). HR-MS (ESI, positive mode): found: 356.3524; calcd. for $C_{22}H_{45}NO_2$: 356.3523 $[M+H]^+$.



***tert*-Butyl 18-[(9-fluorenylmethoxycarbonyl)amino]octadecanoate (280):** To a cold solution of **279** (0.37 g, 0.95 mmol) in 1,4-dioxane (60 mL), was added in small portions FmocCl (0.30 g,



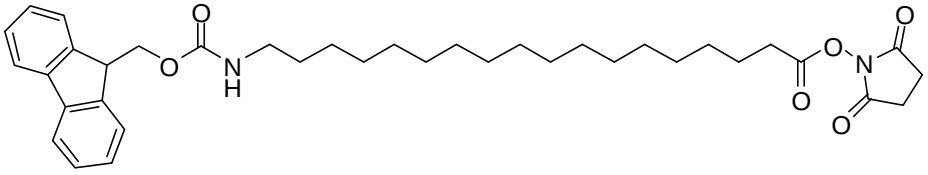
1.14 mmol) followed by $NaHCO_3$ (0.44 g). The reaction mixture was stirred at room temperature for 24 h, then diluted with $CHCl_3$ (100 mL), washed with water (2×50 mL) and dried. After evaporation of the solvents, the residue was filtered through silica gel (150 mL) eluting with CH_2Cl_2 to yield 0.53 g (97%) of the title compound as a colorless solid; m. p. 91–93 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.24 (m, 26H, CH_2), 1.42 (s, 9H, *t*Bu), 1.44–1.63 (m, 4H, CH_2, H-3/17), 2.18 (t, J = 7.5 Hz, 2H, CH_2CO), 3.16 (dt, J = 6.8, 7.4 Hz, 2H, CH_2N), 4.20 (t, J = 6.9 Hz, 1H, CH), 4.38 (d, J = 6.9 Hz, 2H, CH_2), 4.76 (m, 1H, NH), 7.29 (t, J = 7.2 Hz, 2H, H-2'/7'), 7.38 (t, J = 7.2 Hz, 2H, H-3'/6'), 7.58 (d, J = 7.4 Hz, 2H, H-1'/8'), 7.75 ppm (d, J = 7.4 Hz, 2H, H-4'/5'). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 25.1 (CH_2, C-3), 25.8 (CH_2), 28.1 (3×CH_3), 29.1 (CH_2), 29.3 (2×CH_2), 29.5 (3×CH_2), 29.6 (4×CH_2), 29.7 (2×CH_2), 30.0 (CH_2), 35.7 (CH_2, C-2), 41.2 (CH_2NH), 47.4 (CH), 66.5 (CH_2), 79.8 (C), 119.8 (2×CH, C-1'/8'), 125.0 (2×CH, C-2'/7'), 127.0 (2×CH, C-4'/5'), 127.6 (2×CH, C-3'/6'), 141.4 (2×C), 144.1 (2×C), 156.4 (CO), 173.3 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 1177.2 $[2M+Na]^+$ (100), 600 $[M+Na]^+$ (45). Elemental analysis: found: C 76.80, H 9.96, N 2.26; calcd. for $C_{37}H_{55}NO_4$ (577.8): C 76.91, H 9.59, N 2.42.

***N*-Hydroxysuccinimidyl 18-(ethoxycarbonothioylthio)octadecanoate (281):** The title compound was prepared from compound **276** (0.10 g, 0.25 mmol) as described above for compound **269** to yield 0.10 g (80%) of a colorless solid; m. p. 79 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.20–1.32 (m, 26H, CH_2), 1.40 (t, J = 7.1 Hz, 3H, CH_3), 1.60–1.78 (m, 4H, CH_2, H-3/17), 2.58 (t, J = 7.5 Hz, 2H, CH_2-2), 2.82 (s, 4H, CH_2), 3.09 (t, J = 7.5 Hz, 2H, CH_2-18), 4.62 ppm (q, J = 7.1 Hz, 2H, CH_2O). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 13.8 (CH_3), 24.6 (CH_2, C-3), 25.6 (2×CH_2), 28.4 (CH_2), 28.8 (CH_2), 28.9 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (2×CH_2), 29.7 (5×CH_2), 31.0 (CH_2, C-17), 35.9 (CH_2, C-2), 69.7 (CH_2O), 168.5 (CO), 170.0 (2×CO), 215.0 ppm (C=S). ESI-MS, positive mode, *m/z* (rel. int., %): 502.3 $[M+H]^+$ (27), 524.2 $[M+Na]^+$ (100), 1025.5 $[2M+Na]^+$ (35). HR-MS (ESI, positive mode): found: 524.2471; calcd. for $C_{25}H_{43}NS_2O_5$: 524.2475 $[M+Na]^+$.

18-[(9-Fluorenylmethoxycarbonyl)amino]octadecanoic acid (282): To a solution of **280** (0.144 g, 0.25 mmol) in CH_2Cl_2 (0.8 mL), were added Et_3SiH (50 µL) and TFA (0.5 mL) at 0 °C, and

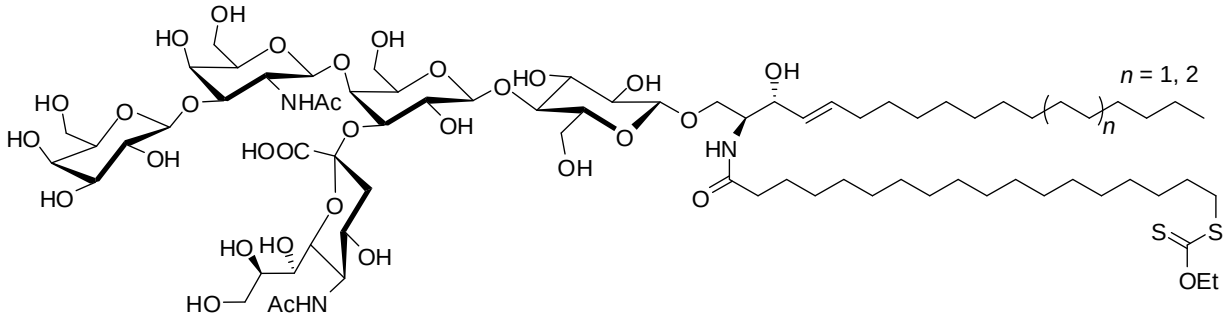
the mixture was kept at 4 °C for 20 h. After evaporation in vacuo, the residue was purified by silica gel chromatography (50 g SiO_2) eluting with CH_2Cl_2/MeOH (40:1) to yield 0.12 g (92%) of the title compound as a colorless solid; m. p. 122 ºC. ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.21 (m, 26H, CH_2), 1.32–1.54 (m, 4H, CH_2, H-3/17), 2.17 (t, J = 7.3 Hz, 2H, CH_2CO), 2.97 (dt, J = 5.6, 6.0 Hz, 2H, CH_2N), 4.21 (t, J = 6.6 Hz, 1H, H-9'), 4.29 (d, J = 6.6 Hz, 2H, CH_2O), 7.20 (m, 1H, NH), 7.32 (t, J = 7.4 Hz, 2H, H-2'/7'), 7.41 (t, J = 7.4 Hz, 2H, H-3'/6'), 7.68 (d, J = 7.3 Hz, 2H, H-1'/8'), 7.88 ppm (d, J = 7.4 Hz, 2H, H-4'/5'). ^{13}C NMR (75.5 MHz, [D_8]THF): δ = 24.3 (CH_2, C-3), 24.8 (CH_2, C-17), 27.6 (CH_2, C-16), 29.8 (CH_2), 30.0 (CH_2), 30.3 (2×CH_2), 30.4 (CH_2), 30.5 (5×CH_2), 30.9 (CH_2), 34.0 (CH_2), 34.2 (CH_2, C-2), 41.5 (CH_2NH), 48.3 (CH), 67.5 (CH_2), 120.5 (2×CH, C-1'/8'), 125.8 (2×CH, C-2'/7'), 127.5 (2×CH, C-4'/5'), 128.1 (2×CH, C-3'/6'), 142.2 (2×C), 145.4 (2×C), 156.9 (CO), 174.2 ppm (CO). ESI-MS, positive mode, *m/z* (rel. int., %): 1087.4 $[2M–H+2Na]^+$ (10), 1065.3 $[2M+Na]^+$ (100), 544.5 $[M+Na]^+$ (10), 522.2 $[M+H]^+$ (2); negative mode, *m/z* (rel. int., %): 1063.6 $[2M–2H+Na]^–$ (30), 1041.3 $[2M–H]^–$ (100), 520.0 $[M–H]^–$ (2). HR-MS (ESI, positive mode): found: 522.3580; calcd. for $C_{33}H_{47}NO_4$: 522.3578 $[M+H]^+$.

***N*-Hydroxysuccinimidyl 18-[(9-fluorenylmethoxycarbonyl)amino]octadecanoate (283):** The



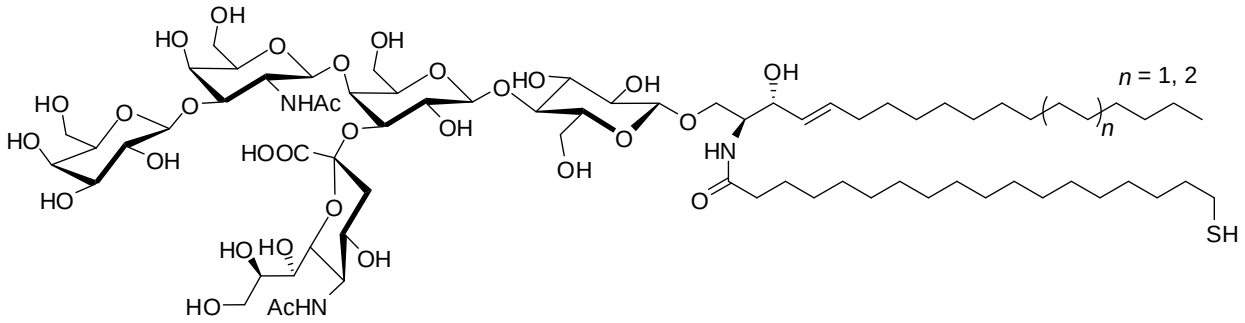
title compound was prepared from compound **282** (0.10 g, 0.2 mmol) as described above for compound **269**, yield 0.10 g (81%) of a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.17–1.33 (m, 26H, CH_2), 1.34–1.54 (m, 2H, CH_2), 1.65–1.80 (m, 2H, CH_2), 2.58 (t, J = 7.5 Hz, 2H, CH_2CO), 2.81 (s, 4H, CH_2 in NHS), 3.17 (m, 2H, CH_2N), 4.20 (t, J = 6.9 Hz, 1H, CH), 4.38 (d, J = 6.9 Hz, 2H, CH_2), 4.73 (m, 1H, NH), 7.29 (t, J = 7.4 Hz, 2H, H-2'/7'), 7.38 (t, J = 7.4 Hz, 2H, H-3'/6'), 7.58 (d, J = 7.4 Hz, 2H, H-1'/8'), 7.75 ppm (d, J = 7.4 Hz, 2H, H-4'/5'). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 24.5 (CH_2, C-3), 25.6 (2×CH_2), 26.7 (CH_2, C-17), 28.8 (CH_2), 29.1 (CH_2), 29.3 (2×CH_2), 29.5 (2×CH_2), 29.6 (5×CH_2), 30.0 (CH_2), 30.3 (CH_2), 30.9 (CH_2), 41.1 (CH_2NH), 47.3 (CH), 66.4 (CH_2), 119.9 (2×CH, C-1'/8'), 125.0 (2×CH, C-2'/7'), 127.0 (2×CH, C-4'/5'), 127.6 (2×CH, C-3'/6'), 141.3 (2×C), 144.0 (2×C), 156.4 (CO), 168.7 (CO), 169.2 ppm (2×CO in NHS). ESI-MS, positive mode, *m/z* (rel. int., %): 1259.3 $[2M+Na]^+$ (100), 641.5 $[M+Na]^+$ (67). HR-MS (ESI, positive mode): found: 641.3569; calcd. for $C_{37}H_{50}N_2O_6$: 641.3561 $[M+Na]^+$.

Compound GM1-18-SC(S)OEt (284): The title compound was obtained using the



procedure described above for compound **262**, starting from lyso-GM1 (10 mg, 7.7 μmol) and **281** (39 mg, 77 μmol) to yield 6.9 mg (54%) of a colorless solid. ESI-MS, negative mode, *m/z* (rel. int., %): 831.9 $[M_1–2H]^{2–}$ (40), 845.9 (57) $[M_2–2H]^{2–}$, 1664.8 $[M_1–H]^–$ (67), 1692.9 $[M_2–H]^–$ (100). HR-MS (ESI, positive mode): found: 831.9160, 845.9322; calcd. for $C_{76}H_{135}N_3O_{32}S_2$ and $C_{78}H_{139}N_3O_{32}S_2$: 831.9162 $[M_1–2H]^{2–}$, 845.9319 $[M_2–2H]^{2–}$.

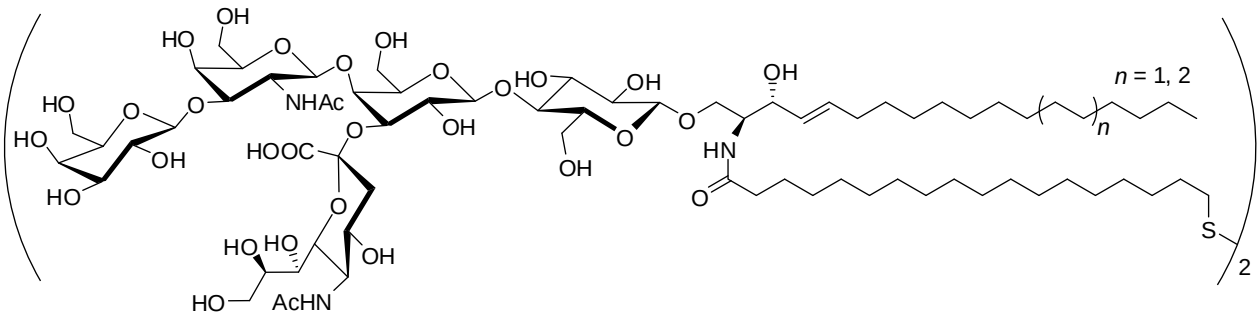
Compound GM1-18-SH (285): The compound **285**-dimer (2.0 mg, 0.63



μmol) was stirred with DTT (4.8 mg, 0.03 mmol) in MeOH (0.1 mL) at room temperature for 4 h, concentrated in vacuo, dialyzed against water with reduced content of oxygen* and addition of a small amount of EDTA, and lyophilized to give the title compound in quantitative yield. ESI-MS, negative mode, *m/z*: 1576.8 $[M_1–H]^–$, 1604.9 $[M_2–H]^–$. HR-MS (ESI, negative mode): found: 1576.8439, 1604.8734; calcd. for $C_{73}H_{131}N_3O_{31}S$ and $C_{75}H_{135}N_3O_{31}S$: 1576.8414 $[M_1–H]^–$, 1604.8727 $[M_2–H]^–$.

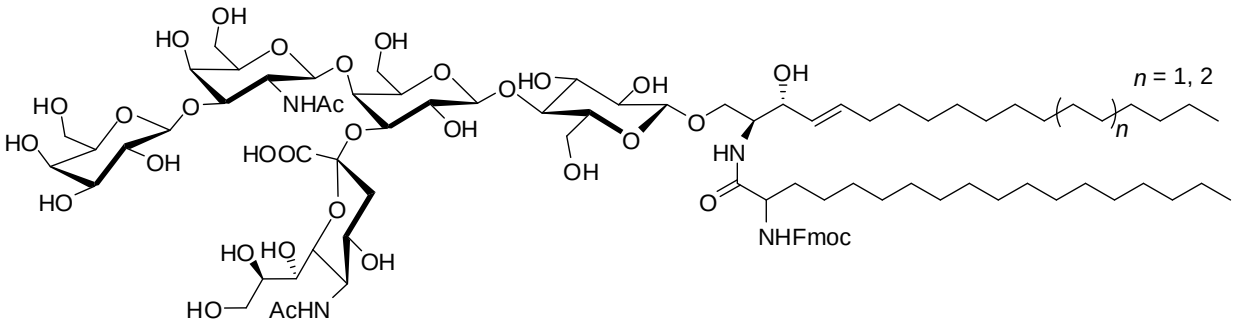
* obtained by continuous refluxing of distilled water in argon atmosphere.

Compound (GM1-18-S)$_2$ (285-dimer): The compound **284** (4.8 mg, 2.9 μmol) was stirred in



25% aqueous ammonia solution (0.5 mL) at room temperature for 24 h, then lyophilized and purified by chromatography on SiO_2 (10 g) eluting with $CHCl_3$/MeOH/H_2O (60:35:5) to give the title compound (4.3 mg, 96%). ^{1}H NMR (600 MHz, [D_6]DMSO, 35 °C): δ = 0.85 (t, 6H, CH_3), 1.24 (s, 96H, CH_2), 1.43 (m, 4H, CH_2-3'), 1.60 (m, 4H, CH_2-17'), 1.62 (m, 2H, CHa), 1.74 (s, 6H, GalNAc), 1.86 (s, 6H, Neu5Ac), 1.93 (m, 4H, CH_2-6), 2.01 (m, 4H, CH_2-2'), 2.52 (m, 2H, CHe), 2.67 (m, 4H, CH_2-18'), 3.0–4.75 (m, 62H, 42×CH and 10×CH_2O), 3.42 (m, 2H, CH-1a), 3.77 (m, 2H, CH-2), 3.87 (m, 2H, CH-3), 3.98 (m, 2H, CH-1b), 4.15 (d, *J* = 8.5 Hz, 2H, Glc(I)-H1), 4.20 (d, *J* = 7.1 Hz, 2H, Gal(IV)-H1), 4.26 (d, *J* = 7.9 Hz, 2H, Gal(II)-H1), 4.89 (d, *J* = 8.6 Hz, 2H, GalNAc-H1), 5.35 (m, 2H, CH-4), 5.53 (m, 2H, CH-5), 7.41 (m, 2H, NH), 7.65 (m, 2H, NH), 7.98 ppm (m, 2H, NH). ESI-MS, negative mode, *m/z* (rel. int., %): 1604.8 (81) $[M_1–2H]^{2–}$, 1590.4 (100) $[M_2–2H]^{2–}$, 1576.8 (31) $[M_3–2H]^{2–}$. HR-MS (ESI, negative mode): found: 3209.7485, 3181.7223, 3153.6886; calcd. for $C_{150}H_{268}N_6O_{62}S_2$, $C_{148}H_{264}N_6O_{62}S_2$, $C_{146}H_{260}N_6O_{62}S_2$: 3209.7440, 3181.7131, 3153.6818 $[M–H]^–$.

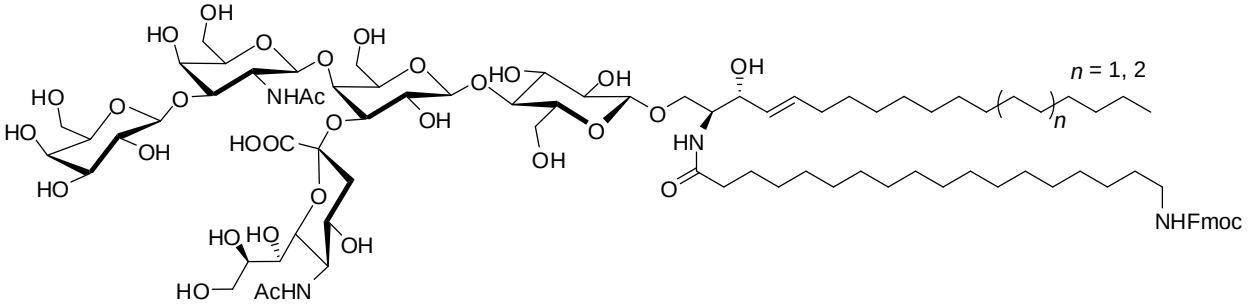
Compound GM1-2-NHFmoc (286): Compound **269** (48 mg, 78.0 μmol) in anhydrous



THF (0.3 mL) was added to a solution of lyso-GM1 (10 mg, 7.8 μmol), Triton X-100 (0.13 mL) and NEt_3 (2 μL) in anhydrous DMF (0.2 mL). The mixture was stirred at room temperature for 24 h and then concentrated under reduced pressure. The product was isolated by chromatography on SiO_2 (10 g) eluting with $CHCl_3$/MeOH/H_2O (60:35:5), yield 7 mg (50%) of a colorless powder after lyophilization. ESI-MS, negative mode, *m/z*: 1781.9 $[M_1–H]^–$, 1809.9 $[M_2–H]^–$.

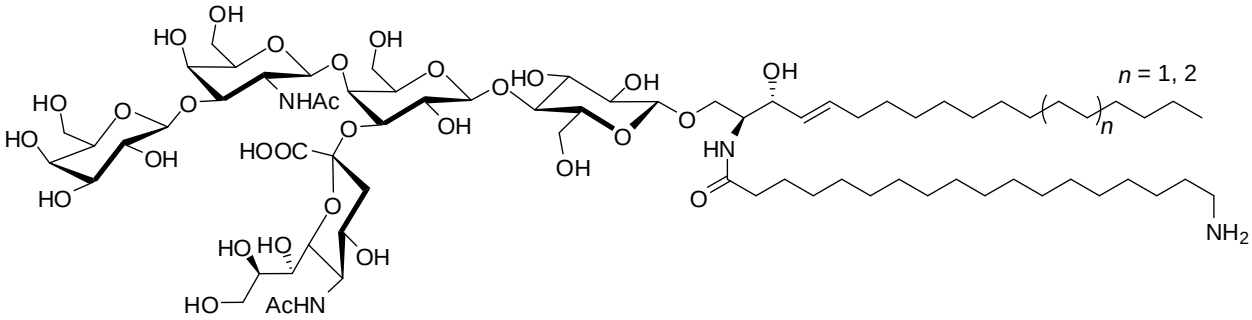
HR-MS (ESI, negative mode): found: 1781.9476, 1809.9780; calcd. for $C_{88}H_{142}N_4O_{33}$ and $C_{90}H_{146}N_4O_{33}$: 1781.9484 $[M_1–H]^-$, 1809.9797 $[M_2–H]^-$.

Compound GM1-18-NHFmoc (287): The title compound was obtained using the



procedure described above for the compound **286**, starting from lyso-GM1 (10 mg, 7.7 μmol) and **283** (47 mg, 77 μmol) to yield 8.4 mg (60%) of a colorless solid. ESI-MS, negative mode, *m/z*: 1781.8 $[M_1–H]^-$, 1810.0 $[M_2–H]^-$. HR-MS (ESI, positive mode): found: 892.4850, 906.5009; calcd. for $C_{88}H_{142}N_4O_{33}$ and $C_{90}H_{146}N_4O_{33}$: 892.4851 $[M_1+2H]^{2+}$ and 906.5007 $[M_2+2H]^{2+}$.

Compound GM1-18-NH$_2$ (288): The title compound was obtained from compound

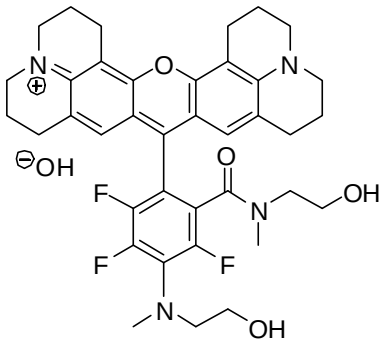


287 (8 mg, 4.4 μmol) using the procedure described above for the compound **262**, yield 5.4 mg (77%) of a colorless powder. ^{1}H NMR (600 MHz, CD_3OD): δ = 0.90 (t, 3H, CH_3), 1.29 (s, 46H, CH_2), 1.37 (m, 2H, CH_2-7), 1.59 (m, 2H, CH_2-3'), 1.63 (m, 2H, CH_2-17'), 1.90 (m, 1H, CH*a*), 1.99 (s, 3H, CH_3, GalNAc), 2.01 (s, 3H, CH_3, Neu5Ac), 2.02 (m, 2H, CH_2-6), 2.17 (m, 2H, CH_2-2'), 2.73 (m, 1H, CH*e*), 2.87 (m, 2H, CH_2-18'), 3.25–4.25 (m, 31H, 21×CH and 5×CH_2O), 3.54 (m, 1H, CH-1a), 3.96 (m, 1H, CH-2), 4.07 (m, 1H, CH-3), 4.21 (m, 1H, CH-1b), 4.30 (d, *J* = 7.8 Hz, 1H, Glc(I)-H1), 4.43 (d, *J* = 8.0 Hz, 1H, Gal(II)-H1), 4.45 (d, *J* = 7.5 Hz, 1H, Gal(IV)-H1), 4.90 (d, *J* = 8.8 Hz, 1H, GalNAc-H1), 5.44 (m, 1H, CH-4), 5.68 ppm (m, 1H, CH-5). ESI-MS, negative mode, *m/z*: 1560.1 $[M_1–H]^-$, 1588.1 $[M_2–H]^-$. HR-MS (ESI, positive mode): found: 792.4416, 806.4573; calcd. for $C_{73}H_{132}N_4O_{31}$ and $C_{75}H_{136}N_4O_{31}$: 792.4420 $[M_1+H+Na]^{2+}$, 806.4577 $[M_2+H+Na]^{2+}$.

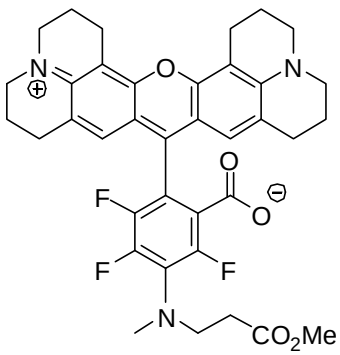
Rhodamine 291: A mixture of **289** (1.3 g, 6.82 mmol) and **290** (1.0 g, 4.55 mmol) in propionic acid (5 mL) with *p*-toluenesulfonic acid monohydrate (0.1 g) was stirred at 160 °C for 16 h. The solvent was evaporated under reduced pressure, and the residue was separated on Polygoprep 60–50 C18 (100 g) eluting with MeOH/water (1:1 → 9:1, with 0.1% v/v TFA) to provide the title compound (0.87 g, 34%) as a shiny dark-green solid. HPLC: 50 → 100% A (50 → 0% B) for 0–25 min, 1 mL/min, 25 °C, t_R = 13.6 min, detection at 254 nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.90–1.98 (m, 4H, CH_2), 1.99–2.12 (m, 4H, CH_2), 2.62–2.89 (m, 4H, CH_2C), 2.90–3.00 (m, 4H, CH_2C), 3.36–3.49 (m, 8H, CH_2N), 6.99 ppm (s, 2H, CH). ^{19}F NMR (282 MHz, $CDCl_3$): δ = –138.4 (ddd, J = 3, 13, 24 Hz, 1F), –140.4 (ddd, J = 2, 13, 22 Hz, 1F), –153.0 (ddd, J = 3, 20, 24 Hz, 1F), –159.3 ppm (dt, J = 2, 21 Hz, 1F). ESI-MS, positive mode, *m/z* (rel. int., %): 585 (100) $[M+Na]^+$. HR-MS (ESI, positive mode): found: 563.1954; calcd. for $C_{32}H_{26}F_4N_2O_3$: 563.1952 $[M+H]^+$.

Compound 293: To a stirred solution of **291** (5.6 mg, 0.01 mmol) in anhydrous DMF (0.2 mL), was added *N*-metylaminoethanol (20 μL), and the mixture was stirred at room temperature for 12 h. The solvent was evaporated in vacuo, and the title product was isolated by chromatography on SiO_2 (10 g) eluting with CH_2Cl_2/MeOH (10:1) to yield 5.7 mg (92%) of a violet solid. HPLC: 50 → 100% A (50 → 0% B) for 0–25 min, 1 mL/min, 25 °C, t_R = 8.9 min, detection at 254 nm. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.90–2.02 (m, 4H, CH_2), 2.03–2.15 (m, 4H, CH_2), 2.66–2.88 (m, 4H, CH_2C), 2.98–3.12 (m, 7H, CH_3N and 2×CH_2C), 3.25–3.34 (m, 4H, CH_2N), 3.46–3.58 (m, 6H, CH_2N), 3.72 (t, J = 5.8 Hz, 2H, CH_2O), 6.88 ppm (s, 2H, CH). ^{19}F NMR (282.4 MHz, CD_3OD): δ = –120.1 (t, J = 10 Hz, 1F), –143.1 (dd, J = 5, 20 Hz, 1F), –144.8 ppm (dd, J = 12, 20 Hz, 1F). ESI-MS, positive mode, *m/z* (rel. int., %): 618.3 $[M+H]^+$ (100). HR-MS (ESI, positive mode): found: 618.2580; calcd. for $C_{35}H_{34}F_3N_3O_4$: 618.2574 $[M+H]^+$.

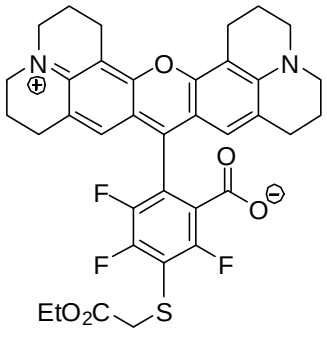
Compound 294: Compound **291** (5.6 mg, 0.01 mmol) in CH_2Cl_2 (0.5 mL) was stirred with $(COCl)_2$ (6 µL, 0.07 mmol) at room temperature for 3 h, the solvent was evaporated in vacuo, then CH_2Cl_2 (0.2 mL) and *N*-metylaminoethanol (20 µL) were added, and the mixture was stirred at room temperature overnight. The title compound was isolated by silica gel chromatography (15 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to yield 3.4 mg (50%) of a violet powder. ESI-MS, positive mode, *m/z* (rel. int., %): 675.7 (100) $[M]^+$.



Compound 295: Compound **291** (34 mg, 0.06 mmol) in DMF (1 mL) was stirred with methyl 3-(*N*-methylamino)propionate (0.14 g, 1.20 mmol) at 50 °C for 6 h. After evaporation of the solvent in vacuo, the residue was purified by silica gel chromatography (50 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to yield 10 mg (26%) of the title compound as a violet powder. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.90–2.01 (m, 4H, 2×CH_2), 2.01–2.14 (m, 4H, 2×CH_2), 2.60 (t, J = 6.5 Hz, 2H, CH_2CO), 2.71–2.85 (m, 4H, 2×CH_2), 2.96 (s, 3H, CH_3N), 2.99–3.08 (m, 4H, 2×CH_2), 3.43–3.58 (m, 10H, 4×CH_2 and CH_2N), 6.93 ppm (s, 2H, 2×CH). ^{19}F NMR (282.4 MHz, CD_3OD): δ = –125.1 (dd, J = 7.7, 13.5 Hz, 1F), –142.8 (dd, J = 7.7, 22.5 Hz, 1F), –144.9 ppm (dd, J = 13.5, 22.5 Hz, 1F). $C_{37}H_{36}F_3N_3O_5$ (659), ESI-MS, positive mode, *m/z* (rel. int., %): 660.5 (100) $[M+H]^+$.

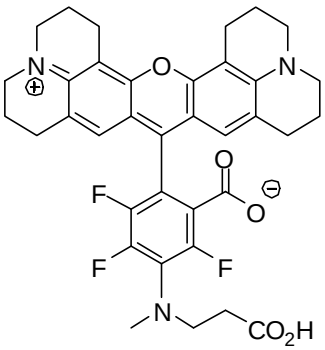


Compound 296: To a mixture of **291** (5.6 mg, 0.01 mmol) and NEt_3 (5 µL) in DMF (0.1 mL), a 0.01 M DMF solution of ethyl thioglycolate (0.1 mL, 0.01 mmol) was added, and the mixture was stirred at room temperature for 3 h. After evaporation of the solvent in vacuo, the residue was purified by silica gel chromatography (10 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1) to yield 4 mg (60%) of the title compound as a violet powder. HPLC: 70 → 100% A (30 → 0% B) for 0–20 min, 1 mL/min, 25 °C, t_R = 7.7 min, detection at 254 nm. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.18 (t, J = 7.1 Hz, 3H, CH_3), 1.88–2.01 (m, 4H, 2×CH_2), 2.01–2.15 (m, 4H, 2×CH_2), 2.70–2.85 (m, 4H, 2×CH_2), 2.99–3.08 (m, 4H, 2×CH_2), 3.46–3.58 (m, 8H, 4×CH_2), 3.63 (s, 2H, SCH_2), 4.09 (q, J = 7.1 Hz, 2H, CH_2O), 6.90 ppm (s, 2H, 2×CH). ^{19}F NMR (282.4 MHz, CD_3OD): δ = –110.3 (d, J = 15.0 Hz, 1F), –126.8 (dd, J = 2.5, 24.5 Hz, 1F), –144.8 ppm (dd, J = 15.0, 24.5 Hz, 1F). ESI-MS,

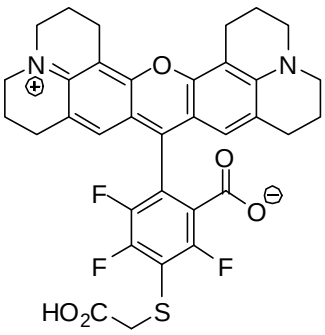


positive mode, *m/z* (rel. int., %): 663.4 (100) $[M+H]^+$. HR-MS (ESI, positive mode): found: 663.2134; calcd. for $C_{36}H_{33}F_3N_2O_5S$: 663.2135 $[M+H]^+$.

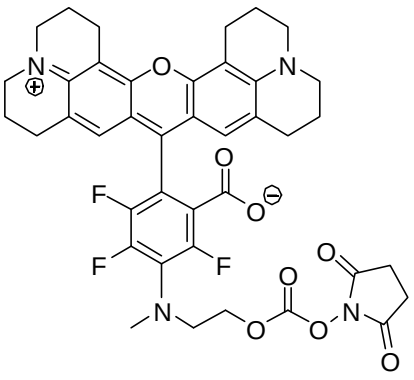
Compound 297: To a solution of **291** (3.2 mg, 4.9 mmol) in MeOH (0.1 mL), 1 M aq. NaOH (0.1 mL) was added, and the mixture was kept at 4 °C for 3 h. After evaporation of the solvents in vacuo, the residue was purified by silica gel chromatography (10 g SiO_2) eluting with CH_2Cl_2/MeOH (10:1 → 2:1) to yield 3.1 mg (99%) of the title compound as a violet powder. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.89–2.01 (m, 4H, 2×CH_2), 2.01–2.14 (m, 4H, 2×CH_2), 2.49–2.61 (m, 2H, CH_2CO), 2.72–2.84 (m, 4H, 2×CH_2), 2.98 (s, 3H, CH_3N), 3.00–3.08 (m, 4H, 2×CH_2), 3.44–3.57 (m, 10H, 4×CH_2, CH_2N), 6.92 ppm (s, 2H, 2×CH). ^{19}F NMR (282.4 MHz, CD_3OD): δ = –125.0 (m, 1F), –142.2 (m, 1F), –144.7 ppm (m, 1F). $C_{36}H_{34}F_3N_3O_5$ (645), ESI-MS, positive mode, *m/z* (rel. int., %): 646.6 (100) $[M+H]^+$.



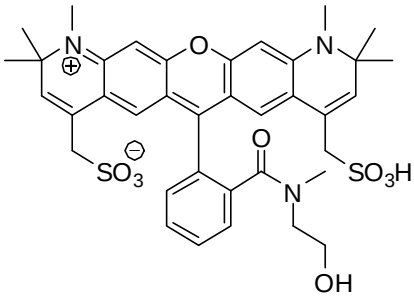
Compound 298: The title compound was obtained from compound **296** (4.0 mg, 6.0 μL) as described for compound **297** to yield 3.8 mg (99%) of a violet powder after purification by silica gel chromatography (10 g SiO_2) eluting with CH_2Cl_2/MeOH (5:1 → 2:1). $C_{34}H_{30}F_3N_2O_5S$ (634), ESI-MS, positive mode, *m/z* (rel. int., %): 673.2 (56) $[M+K]^+$, 657.3 (100) $[M+Na]^+$, 635.5 (34) $[M+H]^+$.



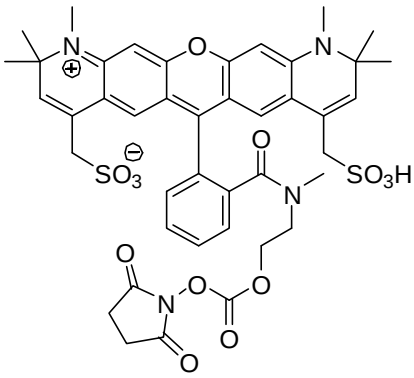
Compound 299: Compound **291** (6.2 mg, 0.01 μmol) was stirred with di(*N*-succinimidyl) carbonate (25.6 mg, 0.10 μmol) and NEt_3 (25 μL) in anhydrous DMF (1 mL) at room temperature for 3 h. The title product was isolated by preparative HPLC (A/B: 60/40 → 100/0 in 25 min, 4 mL/min, 25 °C, t_R = 6.5 min, detection at 254 nm) and lyophilized to give 4.7 mg (60%) of a dark-violet powder. ESI-MS, positive mode, *m/z* (rel. int., %): 759.3 $[M+H]^+$ (100). HR-MS (ESI, positive mode): found: 759.2634; calcd. for $C_{40}H_{37}F_3N_4O_8$: 759.2636 $[M+H]^+$.



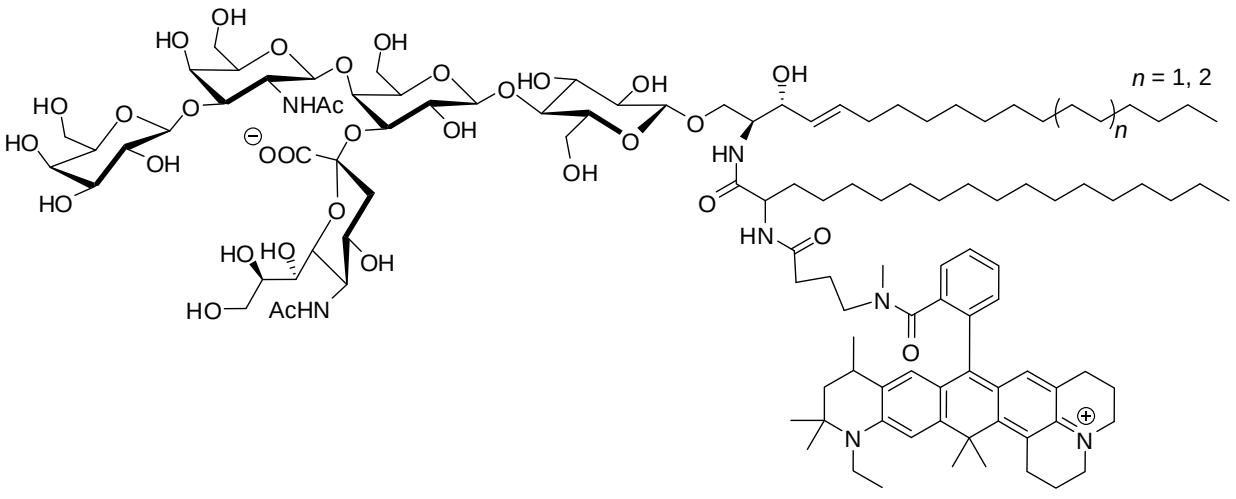
Compound 301: To a solution of compound **300** (65.4 mg, 0.1 mmol) and NEt_3 (50 µL) in anhydrous DMF (1 mL), was added HATU (76.0 mg, 0.2 mmol) followed by 2-(*N*-methylamino)ethanol (80 µL, 1.0 mmol), and the mixture was stirred at 50 ºC for 24 h. After evaporation of the solvent under reduced pressure, the title compound was isolated by chromatography on SiO_2 (50 g) eluting with MeOH/Et_2O (1:1 + 0.5% NEt_3) to yield 45 mg (61%) of a violet powder. HPLC: 20 → 50% A (80 → 50% B) for 0–25 min, 1 mL/min, 25 °C, t_R = 17.5 min, detection at 254 nm. ^{1}H NMR (300 MHz, D_2O): δ = 1.62 (s, 6H, CH_3), 1.68 (s, 6H, CH_3), 2.71/2.79 (s, 3H, NCH_3), 3.05–3.11 (m, 2H, NCH_2), 3.26 (s, 6H, NCH_3), 3.38 (m, 2H, CH_2O), 3.80–3.91 (m, 2H, CH_2S), 3.99–4.10 (m, 2H, CH_2S), 6.07 (s, 2H, CH=), 6.85 (s, 2H, CH), 7.28 (s, 2H, CH), 7.62–7.70 (s, 1H, CH), 7.76–7.83 (s, 1H, CH), 7.90–8.00 ppm (s, 2H, CH). ESI-MS, negative mode, *m/z* (rel. int., %): 734.4 $[M–H]^-$ (100). HR-MS (ESI, positive mode): found: 736.2356; calcd. for $C_{37}H_{41}N_3O_9S_2$: 736.2357 $[M+H]^+$.



Compound 302: Compound **301** (37 mg, 0.05 mmol) was stirred with di(*N*-succinimidyl) carbonate (51 mg, 0.2 mmol) and NEt_3 (0.1 mL) in anhydrous DMF (0.6 mL) at room temperature for 1 h. The title compound was isolated by preparative HPLC (A/B: 20/80 → 50/50 in 25 min, 4 mL/min, 25 °C, t_R = 11.5 min, detection at 254 nm) to give after lyophilization 26.3 mg (60%) of a violet powder. ESI-MS, negative mode, *m/z* (rel. int., %): 875.3 $[M–H]^-$ (100). HR-MS (ESI, positive mode): found: 877.2416; calcd. for $C_{42}H_{44}N_4O_{13}S_2$: 877.2419 $[M+H]^+$.

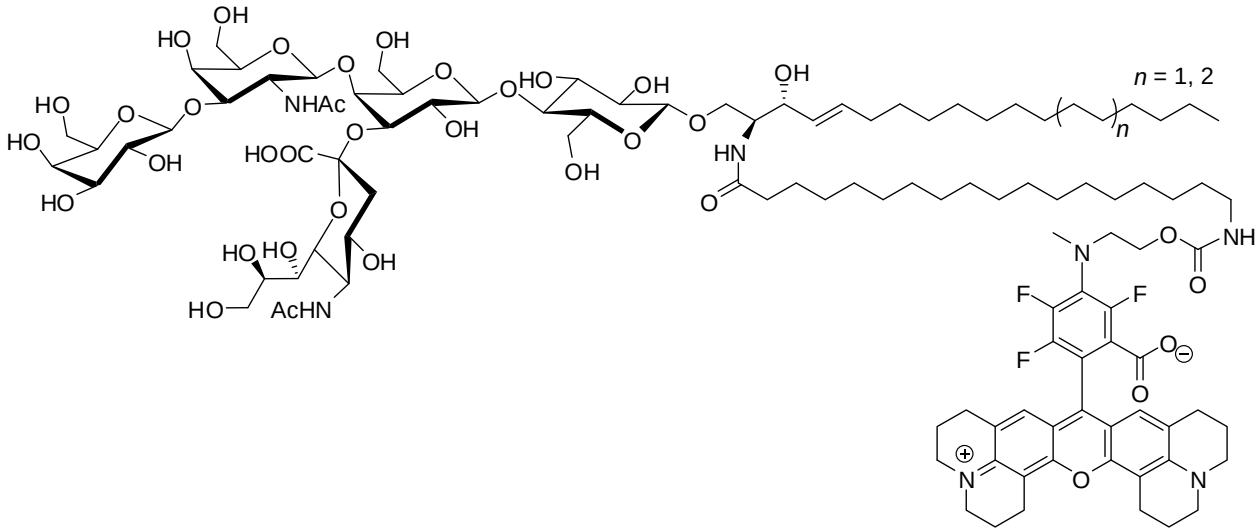


Compound 307: One of the isomers of compound **262** (1 mg, 0.63 µmol) and ATTO647N NHS



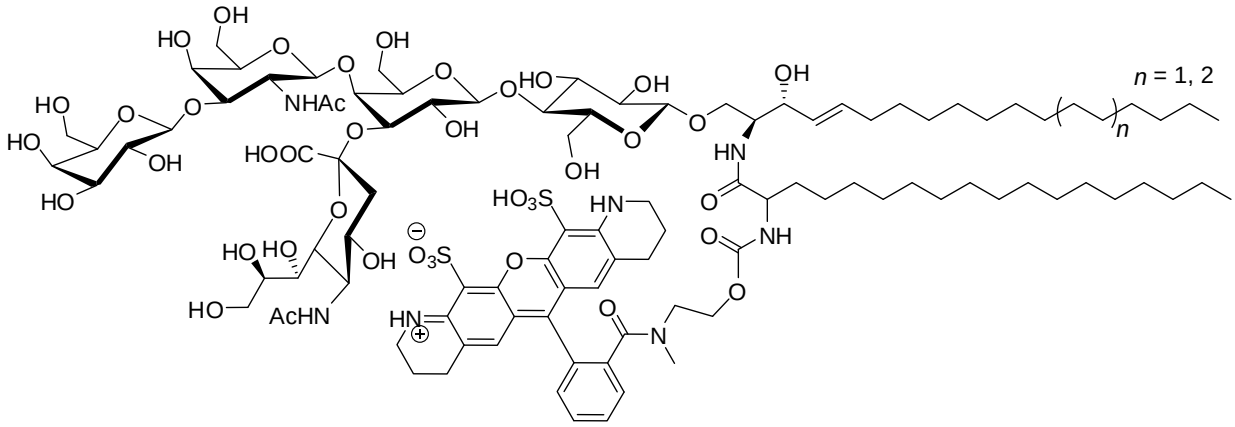
ester (1 mg, 1.26 μmol) were dissolved in anhydrous DMSO (0.1 mL); NEt_3 (5 μL) was added, and the solution was stirred at room temperature for 24 h. After evaporation of the solvent under reduced pressure, the title compound was isolated by HPTLC eluting with $CHCl_3$/MeOH/H_2O (60:35:5) to yield 1.0 mg (72%) of a blue powder. ESI-MS, positive mode, *m/z*: 2188.27 $[M_1+H]^+$, 2216.31 $[M_2+H]^+$, 1095.15 $[M_1+2H]^{2+}$, 1109.16 $[M_2+2H]^{2+}$. HR-MS (ESI, positive mode): found: 1106.1312, 1120.1484; calcd. for $C_{115}H_{181}N_7O_{33}$ and $C_{117}H_{185}N_7O_{33}$: 1106.1333 $[M_1+H+Na]^{2+}$, 1120.1489 $[M_2+H+Na]^{2+}$.

Compound 308: The title compound was obtained using the procedure described above for



compound **307**, starting from **288** (1 mg, 0.63 μmol) and **299** (1 mg, 1.28 μmol), yield 1.1 mg (80%) of a violet powder. ESI-MS, positive mode, *m/z*: 2205.1 $[M_1+H]^+$, 2227.1 $[M_1+Na]^+$, 2233.2 $[M_2+H]^+$, 2255.1 $[M_2+Na]^+$. HR-MS (ESI, positive mode): found: 1114.0566, 1128.0715; calcd. for $C_{109}H_{164}F_3N_7O_{36}$ and $C_{111}H_{168}F_3N_7O_{36}$: 1114.0657 $[M_1+H+Na]^{2+}$, 1128.0724 $[M_2+H+Na]^{2+}$.

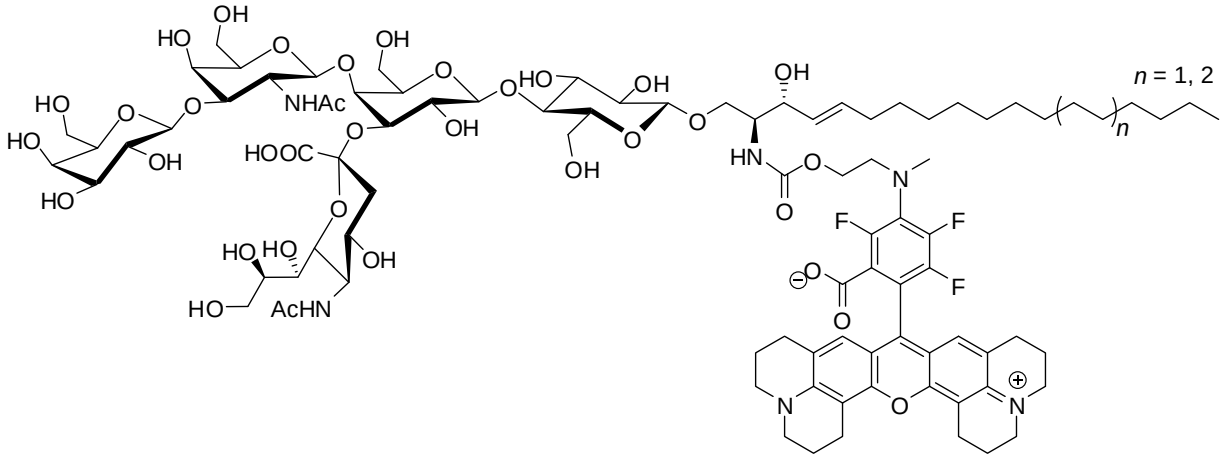
Compound 310: To a solution of compound **309** (5.5 mg, 5.75 μmol) and NEt_3 (10 μL) in



DMSO (0.1 mL), was added HATU (3.3 mg, 8.60 μmol) followed by lyso-GM1 (3 mg, 2.30

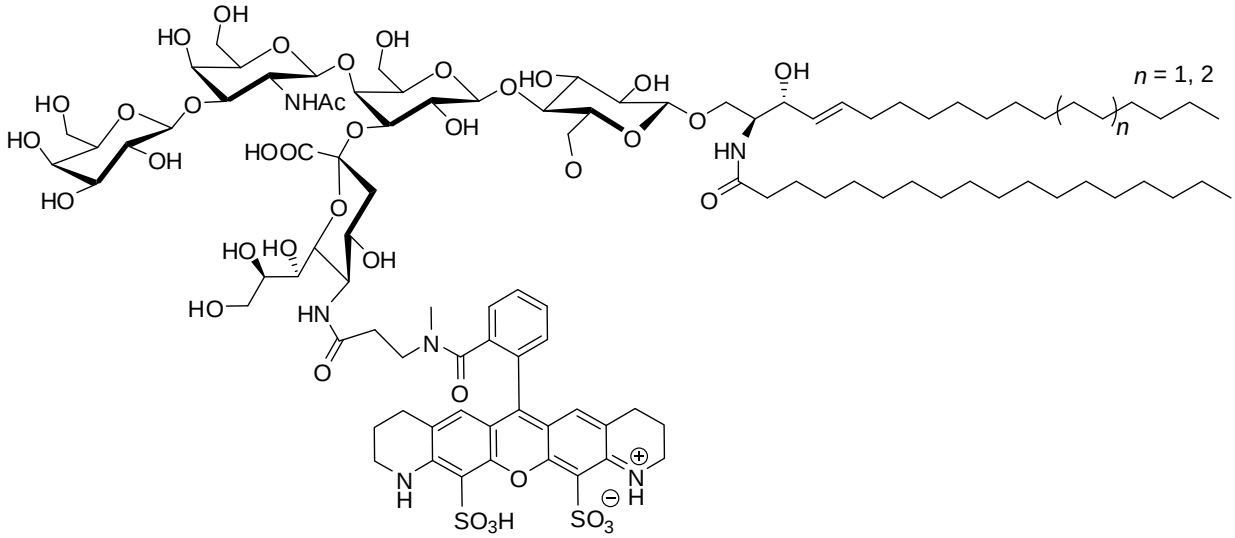
μmol) after 20 min. The mixture was stirred at room temperature for 24 h and concentrated under reduced pressure. The title product was isolated by preparative TLC (silica gel) eluting with $CHCl_3$/MeOH/H_2O (60:35:5) to yield 2.6 mg (51%) of a red powder. ESI-MS, positive mode, *m/z*: 2214.03 $[M_1+H]^+$, 2242.07 $[M_2+H]^+$. HR-MS (ESI, negative mode): found 1105.9927, 1120.0092; calcd. for $C_{103}H_{159}N_7O_{41}S_2$ and $C_{105}H_{163}N_7O_{41}S_2$: 1105.9934 $[M_1–2H]^{2–}$, 1120.0090 $[M_2–2H]^{2–}$.

Compound 312: Lyso-GM1 (1.0 mg, 0.77 μmol) and **299** (0.9 mg, 1.15 μmol) were stirred with



NEt_3 (5 μL) in DMSO (0.2 mL) at room temperature for 24 h, and the solvent was evaporated under reduced pressure. The title product was isolated by preparative TLC (silica gel) eluting with $CHCl_3$/MeOH/H_2O (60:35:5), yield 1.2 mg (81%) of a violet powder. ESI-MS, negative mode, *m/z*: 1922.9 $[M_1–H]^–$, 1950.9 $[M_2–H]^–$. HR-MS (ESI, positive mode): found: 970.9431, 987.4361; calcd. for $C_{91}H_{129}N_6O_{35}$ and $C_{93}H_{133}N_6O_{35}$: 970.9431 $[M_1+H+Na]^{2+}$, 987.4365 $[M_2+H+Na]^{2+}$.

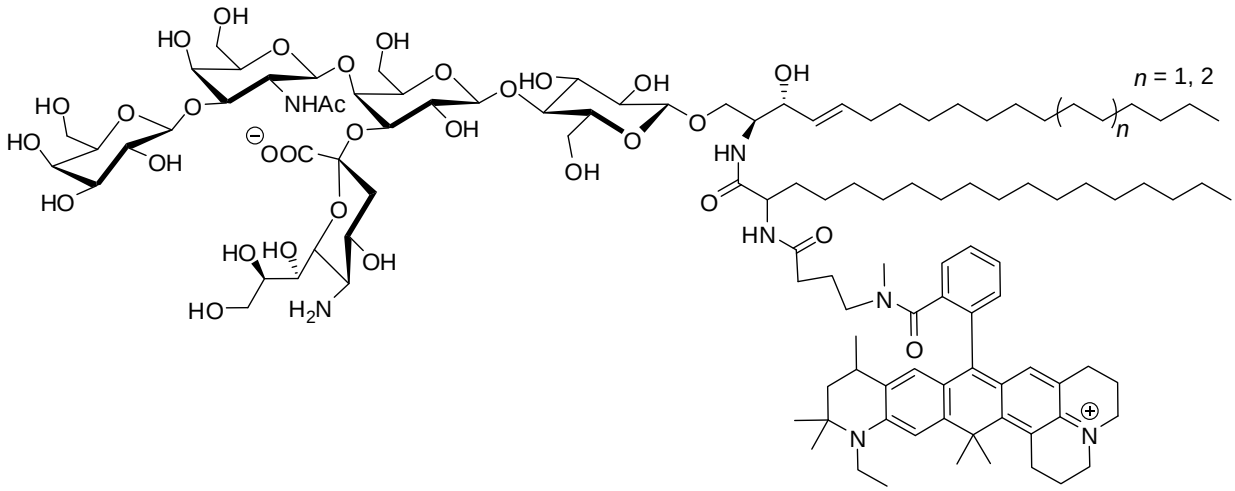
Compound 313: The title compound was obtained using the procedure described above for



compound **312**, starting from deAc-GM1 (1.0 mg, 0.7 μmol) and **303** (0.75 mg, 1.0 μmol), yield

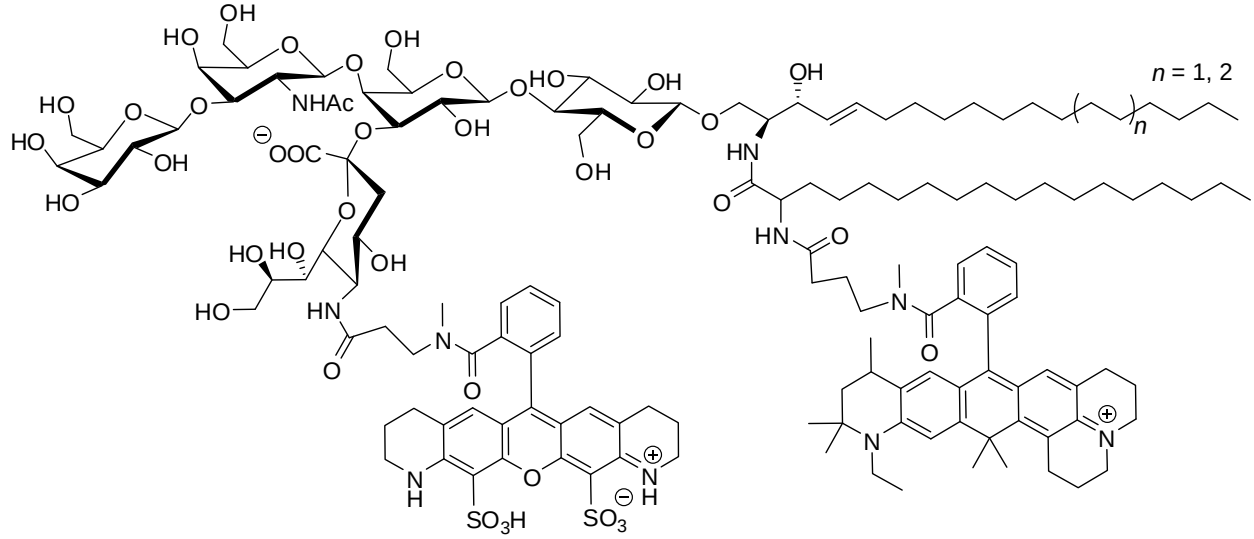
1.1 mg (77%) of a red powder. ESI-MS, negative mode, *m/z*: 1070 $[M_1–2H]^{2–}$, 1084 $[M_2–2H]^{2–}$. HR-MS (ESI, negative mode): found: 1083.5034 $[M–2H]^{2–}$; calcd. for $C_{103}H_{160}N_6O_{39}S_2$: 1083.5009.

Compound 314: The title product was obtained using the procedure described above for



compound **312**, starting from ATTO647N NHS ester (1.0 mg, 1.19 µmol) and deAc-deAcyl-GM1 (3.0 mg, 2.37 µmol), yield 1.0 mg (45%) of a blue powder. ESI-MS, positive mode, *m/z*: 945.0 $[M_1+H+Na]^{2+}$, 959.1 $[M_2+H+Na]^{2+}$. HR-MS (ESI, positive mode): found: 933.5005, 944.4913, 958.5072; calcd. for $C_{95}H_{144}N_6O_{31}$ and $C_{97}H_{148}N_6O_{31}$: 933.5011 $[M_1+2H]^{2+}$, 944.4921 $[M_1+H+Na]^{2+}$, 958.5077 $[M_2+H+Na]^{2+}$.

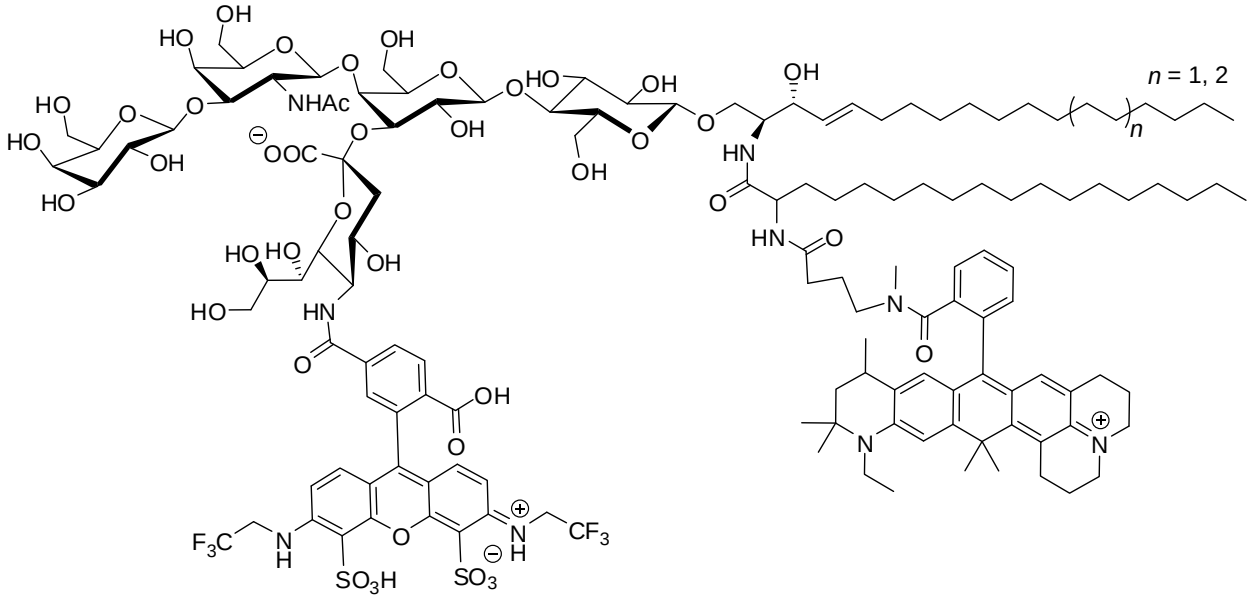
Compound 315: The title product was obtained using the procedure described above for



compound **312**, starting from **314** (1.0 mg, 0.54 µmol) and **303** (1.0 mg, 1.33 µmol), yield 0.5 mg (37%) of a violet powder. ESI-MS, positive mode, *m/z*: 2503.13 $[M_1+H]^+$, 2531.16 $[M_2+H]^+$. HR-MS (ESI, positive mode): found: 1252.0609, 1263.0525, 1247.0433; calcd. for

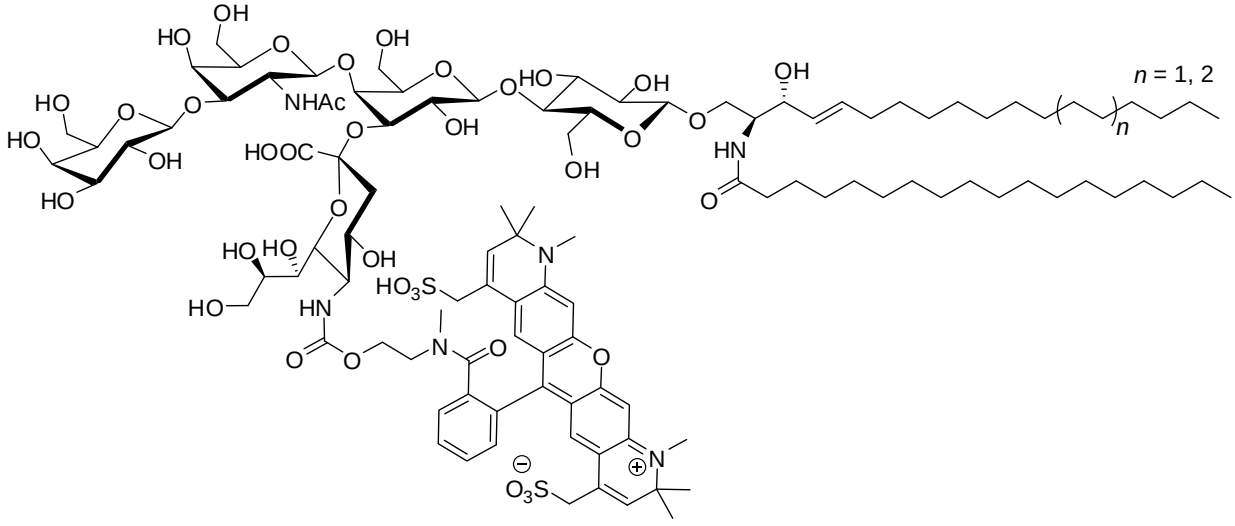
$C_{125}H_{171}N_9O_{40}S_2$, $C_{127}H_{175}N_9O_{40}S_2$: 1252.0605 $[M_1+2H]^{2+}$, 1263.0515 $[M_1+2Na]^{2+}$, 1247.0425 $[M_2+2Na]^{2+}$.

Compound 316: The title product was obtained using the procedure described above for



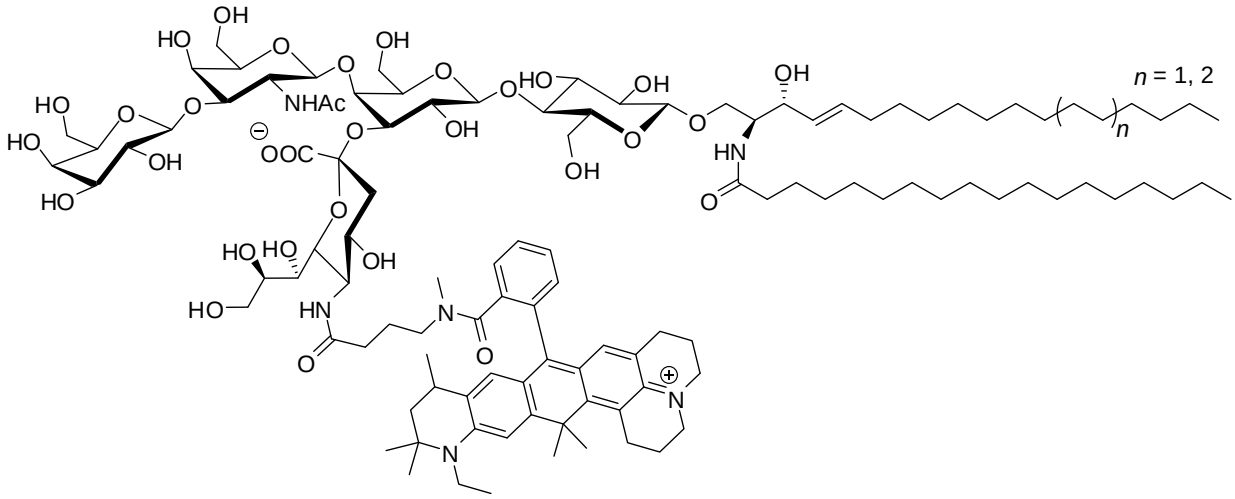
compound **312**, starting from **314** (0.5 mg, 0.27 μmol) and **305** (1.0 mg, 1.43 μmol), yield 0.3 mg (44%) of a greenish powder. ESI-MS, negative mode, *m/z*: 1272 $[M_1–2H]^{2–}$, 1286 $[M_2–2H]^{2–}$.

Compound 317 was obtained using the procedure described above for compound **312**, starting



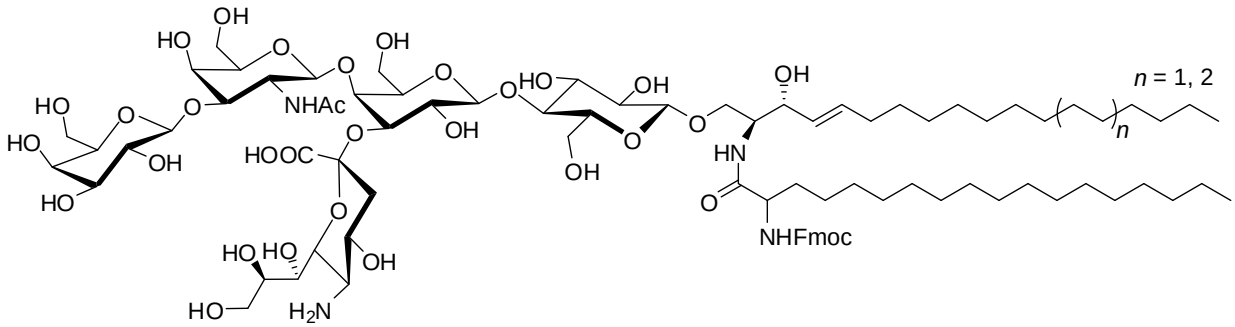
from deAc-GM1 (1.5 mg, 1.0 μmol) and **302** (1.75 mg, 2.0 μmol), yield 1.7 mg (79%) of a violet powder. ESI-MS, negative mode, *m/z* (rel. int., %): 1132 (64) $[M_1–2H]^{2–}$, 1146 (100) $[M_2–2H]^{2–}$. HR-MS (ESI, negative mode): found: 1131.5294, 1145.5453; calcd. for $C_{109}H_{168}N_6O_{40}S_2$ and $C_{111}H_{172}N_6O_{40}S_2$: 1131.5296 $[M_1–2H]^{2–}$, 1145.5453 $[M_2–2H]^{2–}$.

Compound 318 was obtained using the procedure described above for compound **312**, starting



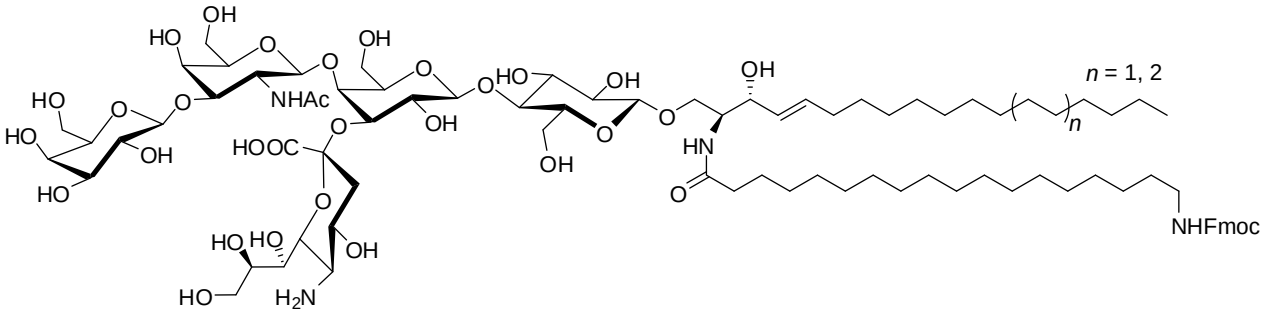
from deAc-GM1 (1.0 mg, 0.67 μmol) and ATTO647N NHS ester (1.1 mg, 1.33 μmol), yield 1.0 mg (71%) of a blue powder. ESI-MS, positive mode, *m/z* (%): 1066.63 (80) $[M_1+2H]^{2+}$, 1080.65 (100) $[M_2+2H]^{2+}$; HR-MS (ESI, positive mode): found: 1066.6311, 1080.6469; calcd for $C_{113}H_{178}N_6O_{32}$ and $C_{115}H_{182}N_6O_{32}$: 1066.6316 $[M_1+2H]^{2+}$, 1080.6472 $[M_2+2H]^{2+}$.

Compound 319: The compound **268** (2.1 mg, 3.95 μmol) was stirred with $SOCl_2$ (0.2



mL) at 50 °C for 4 h, and the excess of $SOCl_2$ was evaporated in vacuo to give the corresponding chloroanhydride. To a solution of deAc-deAcyl-GM1 (5.0 mg, 3.95 μmol) in 0.1% aq. $NaHCO_3$ (0.4 mL) and Et_2O (0.4 mL) was added at 0 °C a solution of chloranhydride in Et_2O (0.2 mL), and the mixture was stirred at 4 °C for 24 h. After evaporation of the solvents in vacuo, the title compound was isolated by chromatography on SiO_2 (10 g) eluting with $CHCl_3$/MeOH/H_2O (60:35:8) to yield 1.1 mg (16%) of a colorless powder after lyophilization. ESI-MS, negative mode, *m/z*: 1740 $[M_1–H]^-$, 1769 $[M_2–H]^-$. HR-MS (ESI, positive mode): found: 893.4617, 907.4771; calcd. for $C_{86}H_{140}N_4O_{32}$, $C_{88}H_{144}N_4O_{32}$: 893.4618 $[M_1+2Na]^{2+}$, 907.4774 $[M_2+2Na]^{2+}$.

Compound 320: The title compound was prepared as described for compound **319**



starting from compound **282** (3.4 mg, 6.48 µmol) and deAc-deAcyl-GM1 (8.2 mg, 6.48 µmol) to yield 2.1 mg (18%) of a colorless powder. ESI-MS, negative mode, *m/z*: 1740 $[M_1–H]^-$, 1769 $[M_2–H]^-$. HR-MS (ESI, positive mode): found: 882.4701, 896.4865; calcd. for $C_{86}H_{140}N_4O_{32}$, $C_{88}H_{144}N_4O_{32}$: 882.4708 $[M_1+H+Na]^{2+}$, 896.4864 $[M_2+H+Na]^{2+}$.

Summary

In the present work, photochromic 1,2-bis(3-thienyl)perfluorocyclopentenes, which can be switched with high efficiency with visible or near-UV light, have been synthesized and their photochromic behavior has been studied. The coupling of heptafluorocyclopentenes **135** and **151** with 3-bromothiophenes **133**, **149** and **156** afforded the desired photochromic compounds **136**, **152** and **157** in 50–55% yields. The open form of the photochromic compound **136** absorbs at 345 nm in ethanol, and it is possible to use a diode laser emitting at 375 nm for the ring-closing reaction. The photochromic units in **152** and **157** absorb even at longer wavelength (λ_{max} = 380 nm). Upon irradiation at 313 nm or 366 nm, the open-ring isomers may be transformed to the closed-ring isomers with new broad bands at 641 nm and 608 nm for **136** and **152** (**157**), respectively.

The photochromic fluorescent adducts **159** and **160** with improved properties in the visible region were prepared, and their photophysical properties have been studied. The photocyclization reaction responsible for quenching the fluorescence can be activated by focused light. Compounds **159** and **160** possess large fluorescence modulation (92% and 44%, respectively) even under irradiation at 375 nm, where many photostable diarylperfluorocyclopentenes do not absorb.[11e] By using rhodamine 101 as a fluorophore, the fluorescence signal of the adducts **159** and **160** can be probed at 550–600 nm and detected between 600 and 700 nm, thereby minimizing the generation of a potential background signal caused by the autofluorescence of the biological object.

The molecular switches were equipped with an additional linkers to attach different functional groups, such as NHS esters (compounds **171a**, **171b**), maleimide residues (compound **175**), biotin (compound **185**) or amino groups (compound **183**), which can be used for selective labelling of the studied objects. After incorporation into silica nanoparticles, the fluorescent photochromic compound **171a** displayed good photochromic behavior with a large fluorescent quantum yield (52%) and an efficient fluorescence modulation (94%) between two states, which may be interconverted with near-UV and red light. Properties of these nanoparticles relevant for potential applications in fluorescence microscopy were studied. In particular, subdiffraction images of nanoparticles were obtained with an improved optical resolution in one dimention of the focal plane of a confocal microscope.

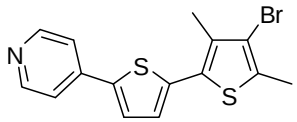
Non-symmetric 1,2-bis(3-thienyl)perfluorocyclopenthenes **246** and **250** with hydrophilic groups and an additional binding site were synthesized. The switching behavior of the prepared compounds was studied in alcoholic and aqueous solutions. Compound **250** with enhanced water

solubility showed a good photochromic performance in water, though the switching velocity was slower then in methanol.

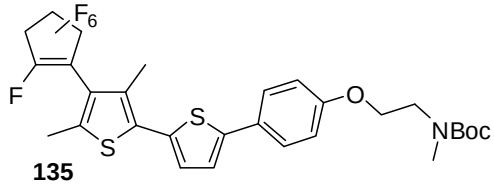
For evaluation of the dynamic processes occurring in biological membranes by means of fluorescence correlation spectroscopy (FCS) and stimulated emission depletion (STED), a number of ganglioside GM1 derivatives labelled with one or two different fluorescent dyes in various positions were synthesized. Analogues of GM1 labelled with two different dyes were found to be applicable in multicolor experiments.

The novel ganglioside GM1 derivatives with protected and free amino and thiol groups in the α- and ω-positions of the stearic acid residues were prepared and characterized. The compounds **262** and **288** were used for the synthesis of GM1 derivatives bearing fluorescent dyes.

The ganglioside derivatives **319** and **320** retaining the natural structure of GM1 with two anchoring sites – with one free amino group in the neuraminic acid and a second protected one in the fatty acid residue, either in the α- or in the ω-position, – were synthesized. These compounds may serve as precursors for GM1 derivatives labelled with two different (fluorescent) probes.



133



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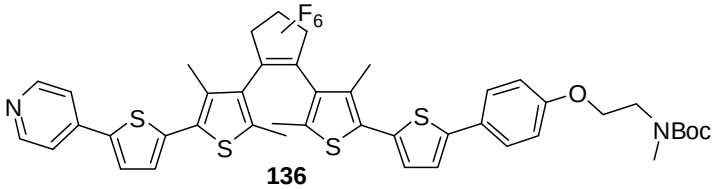
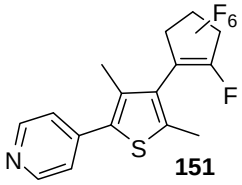
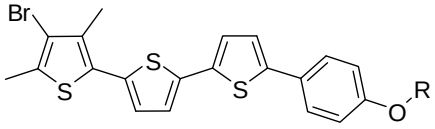
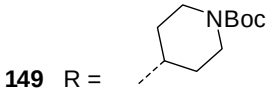


136



151





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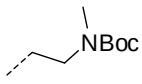

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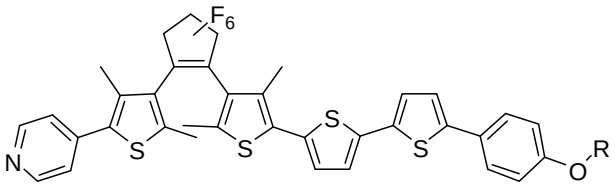

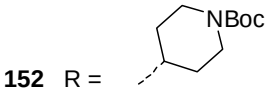

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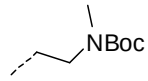

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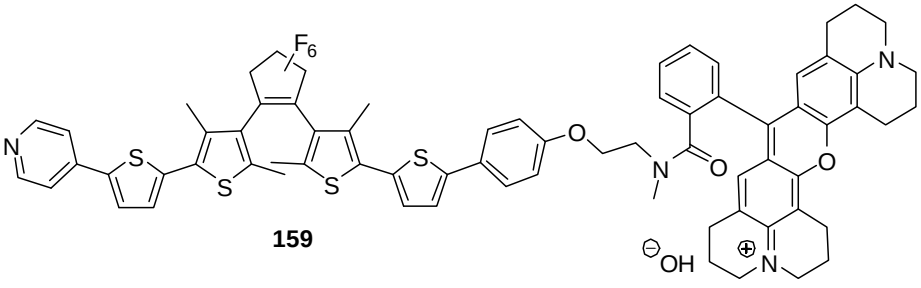

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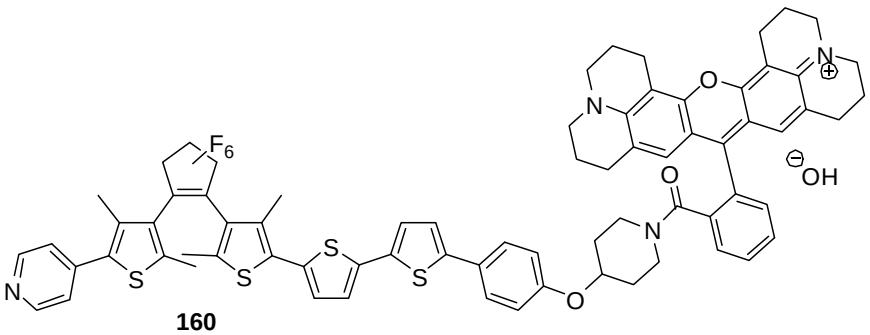
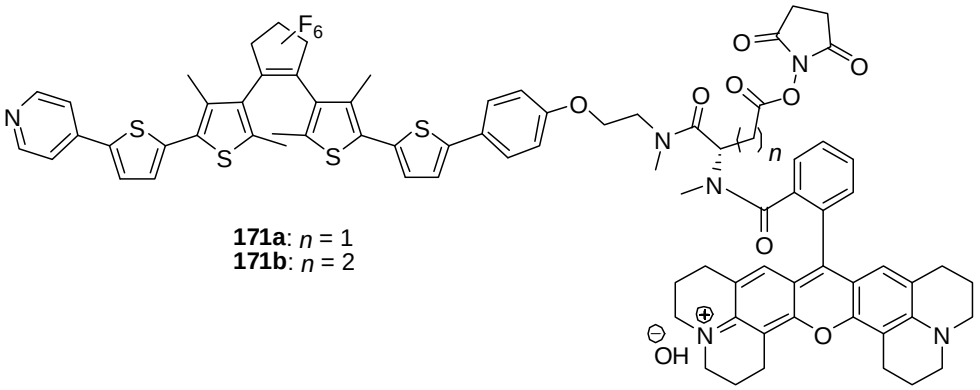
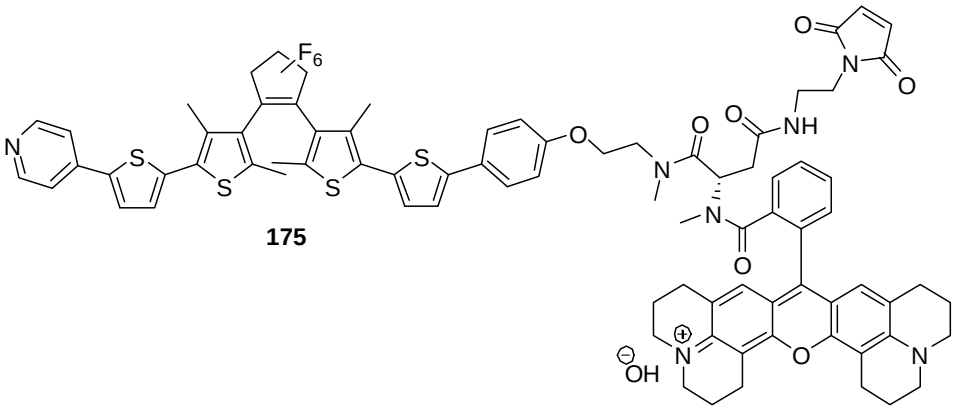


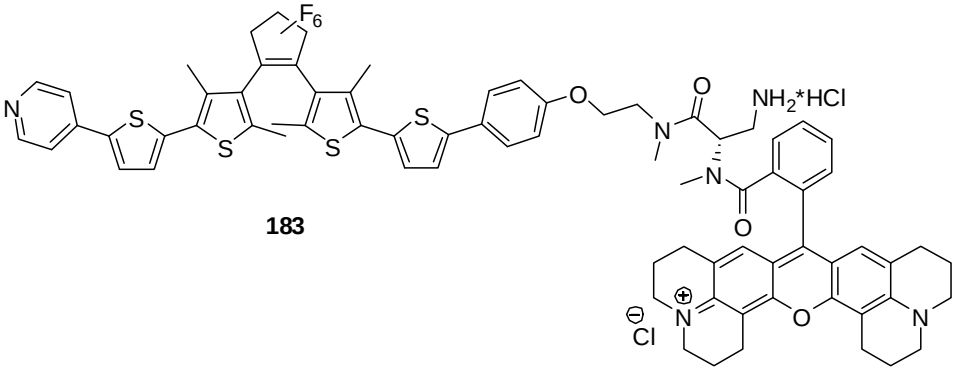
160



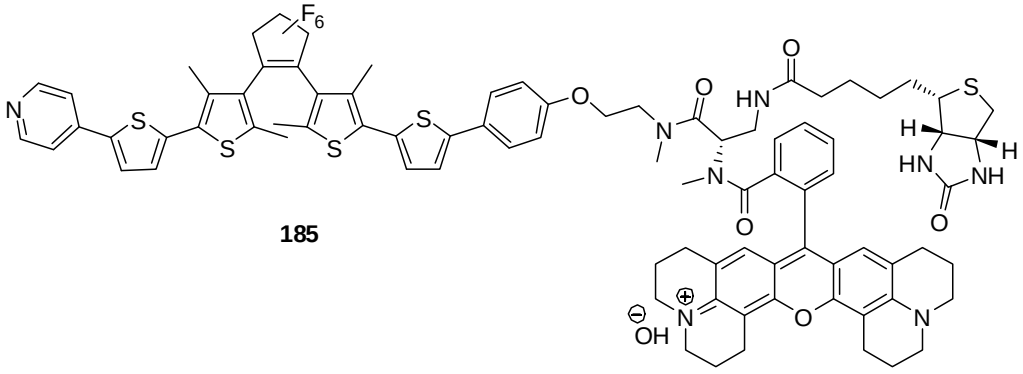
171a: *n* = 1
171b: *n* = 2



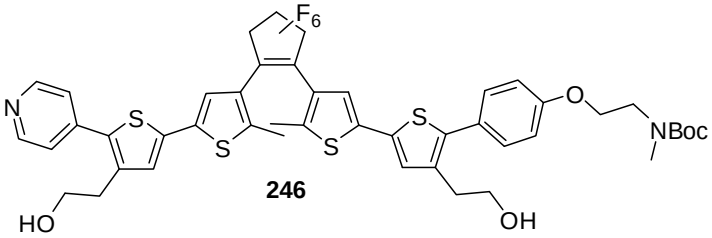
175



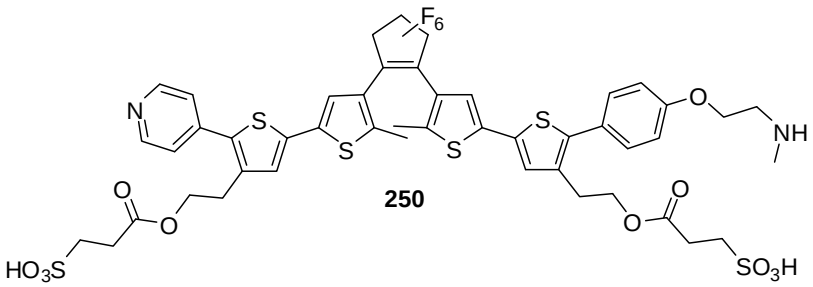
183



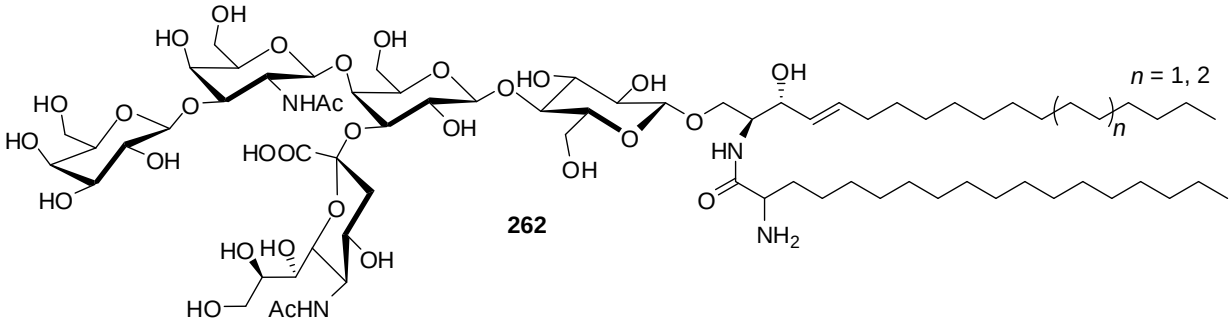
185



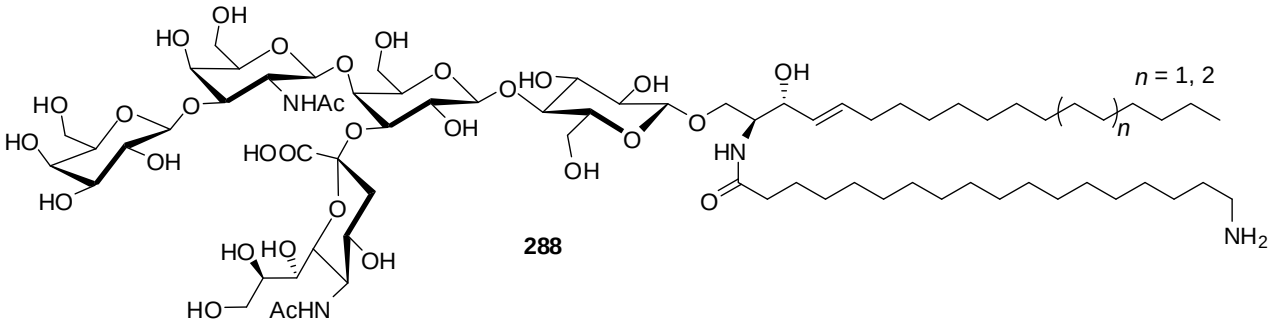
246



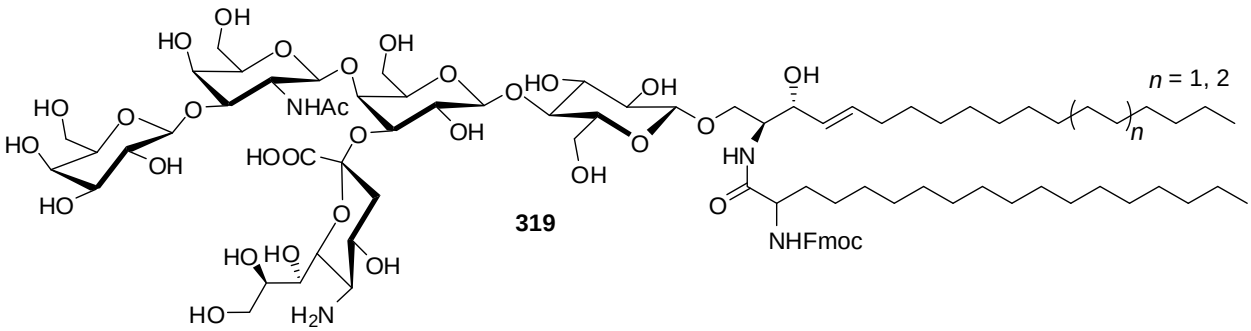
250



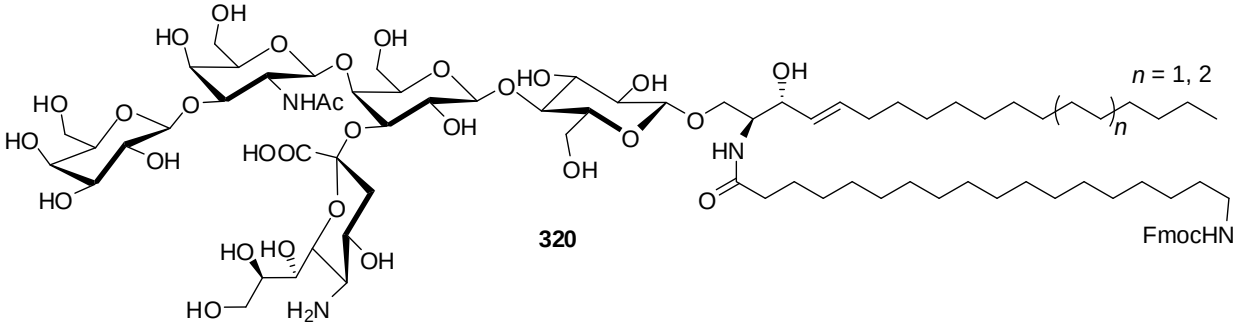
262



288



319



320

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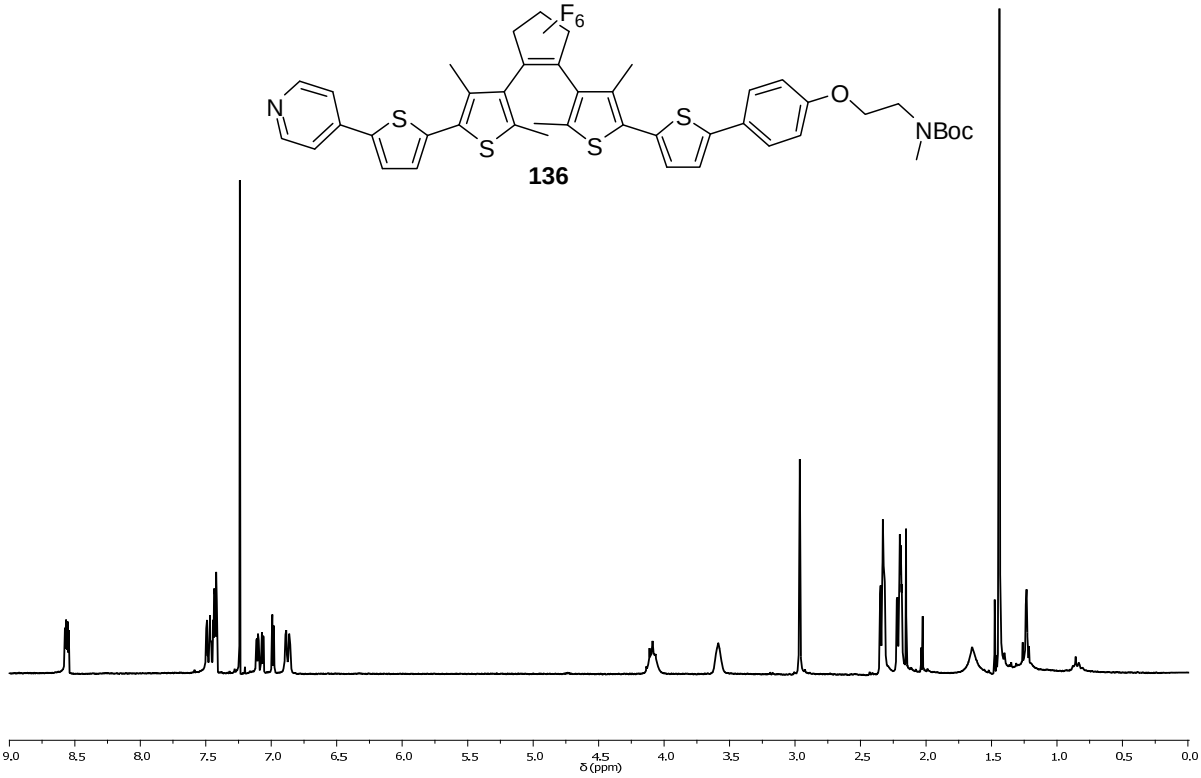
Spectral Data

1. NMR Spectra



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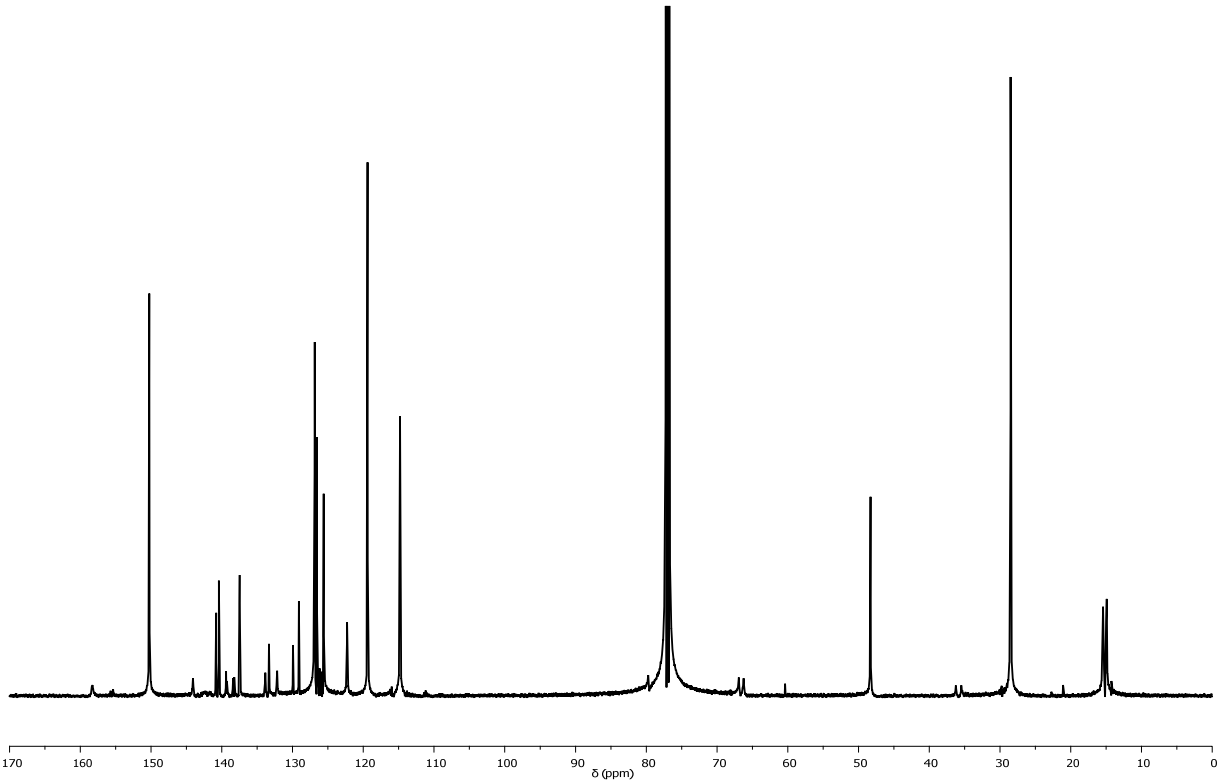

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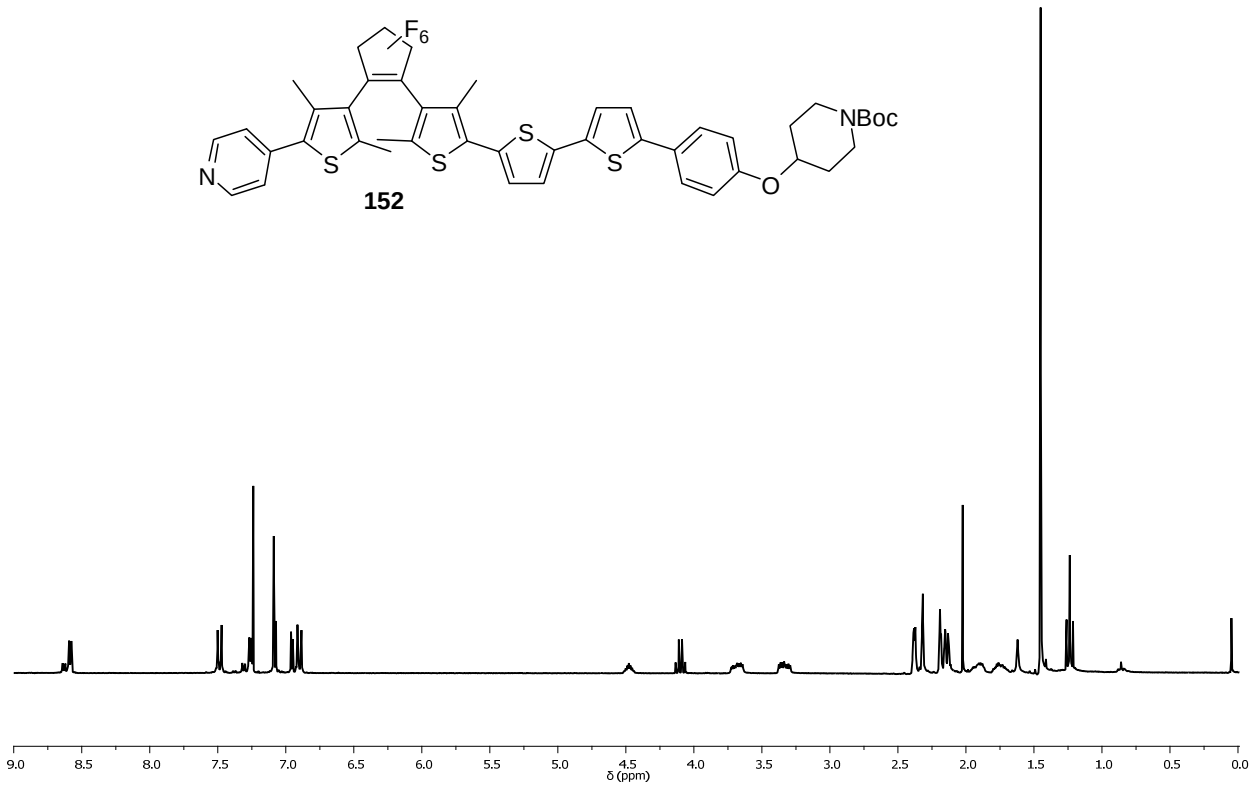

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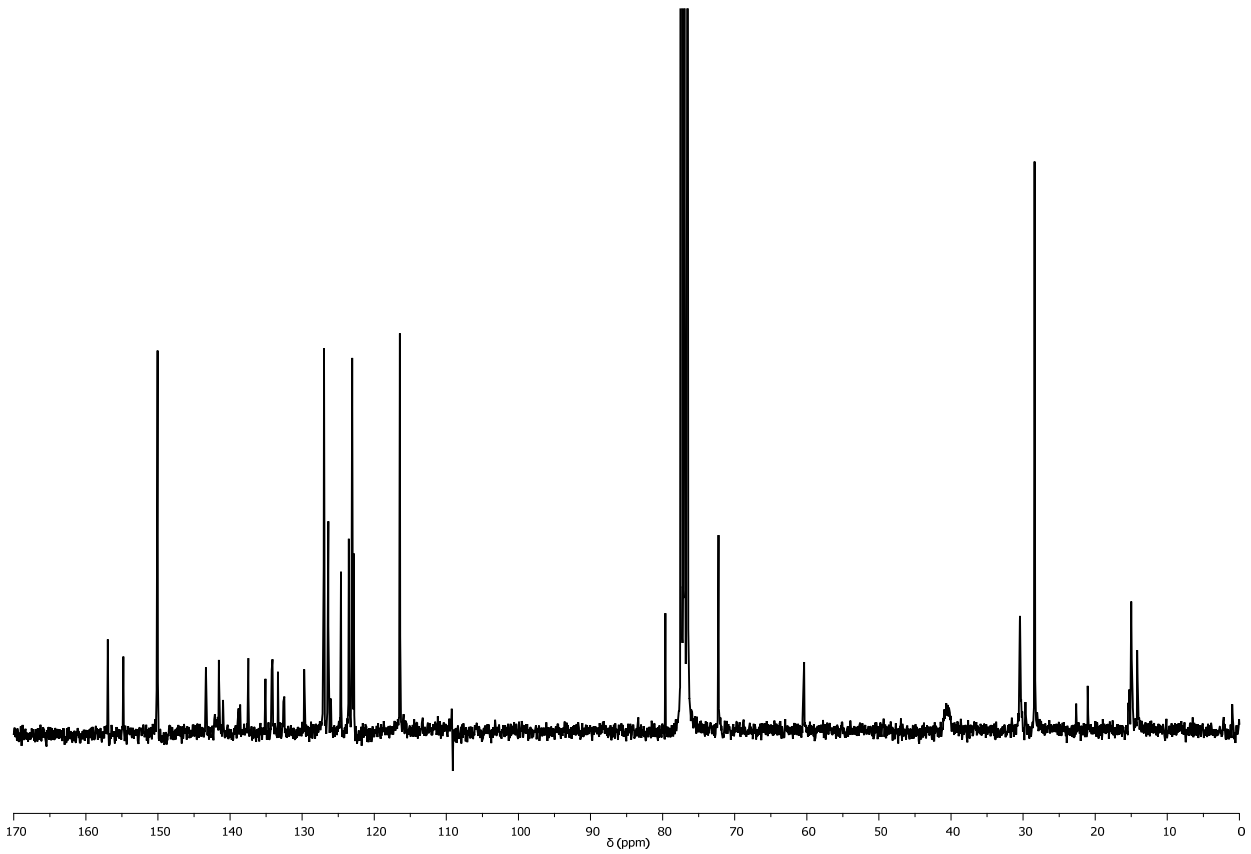

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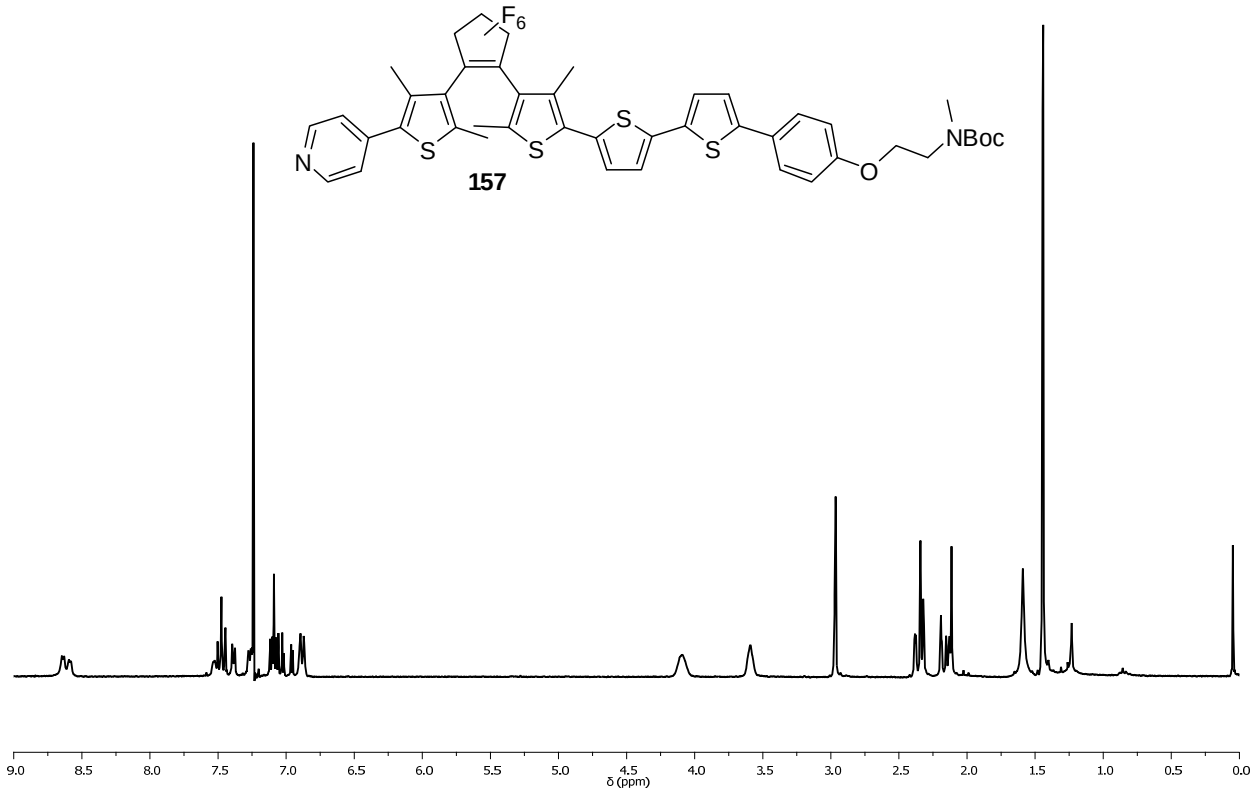

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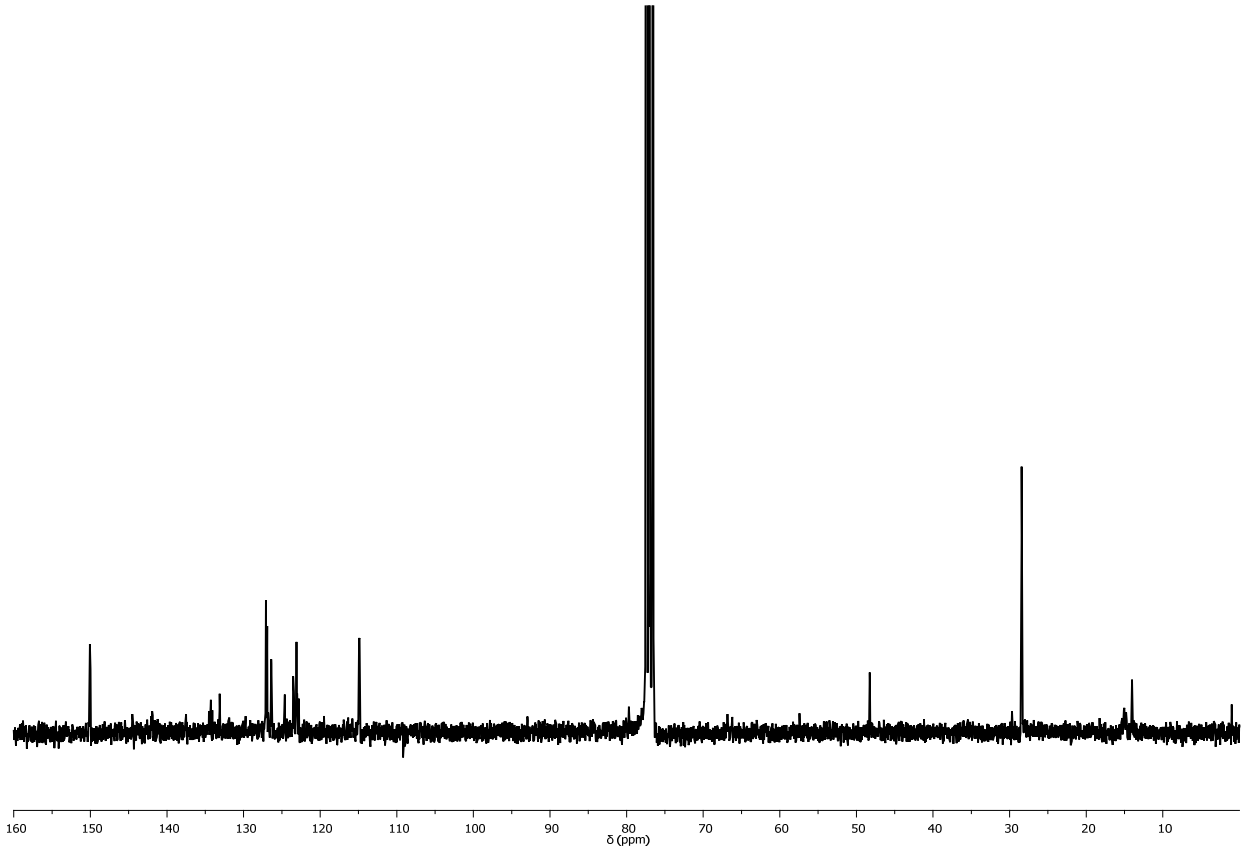

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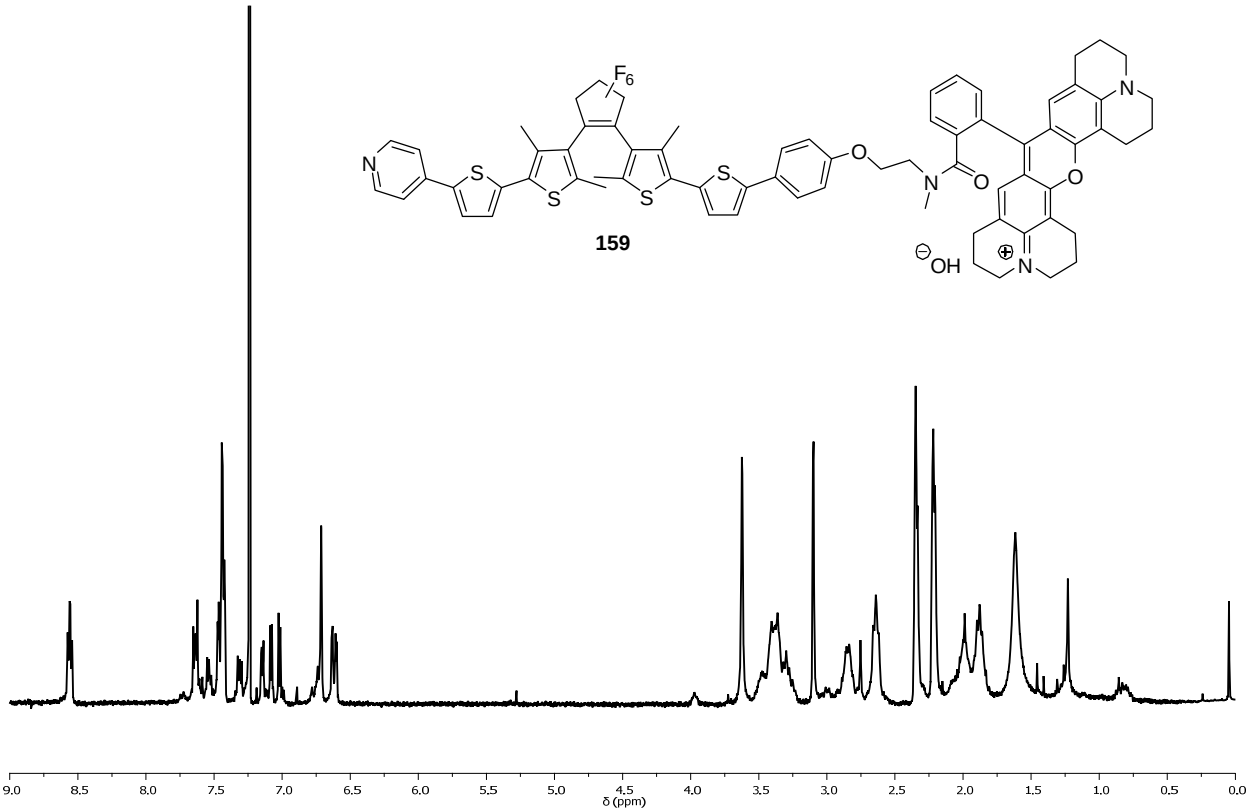

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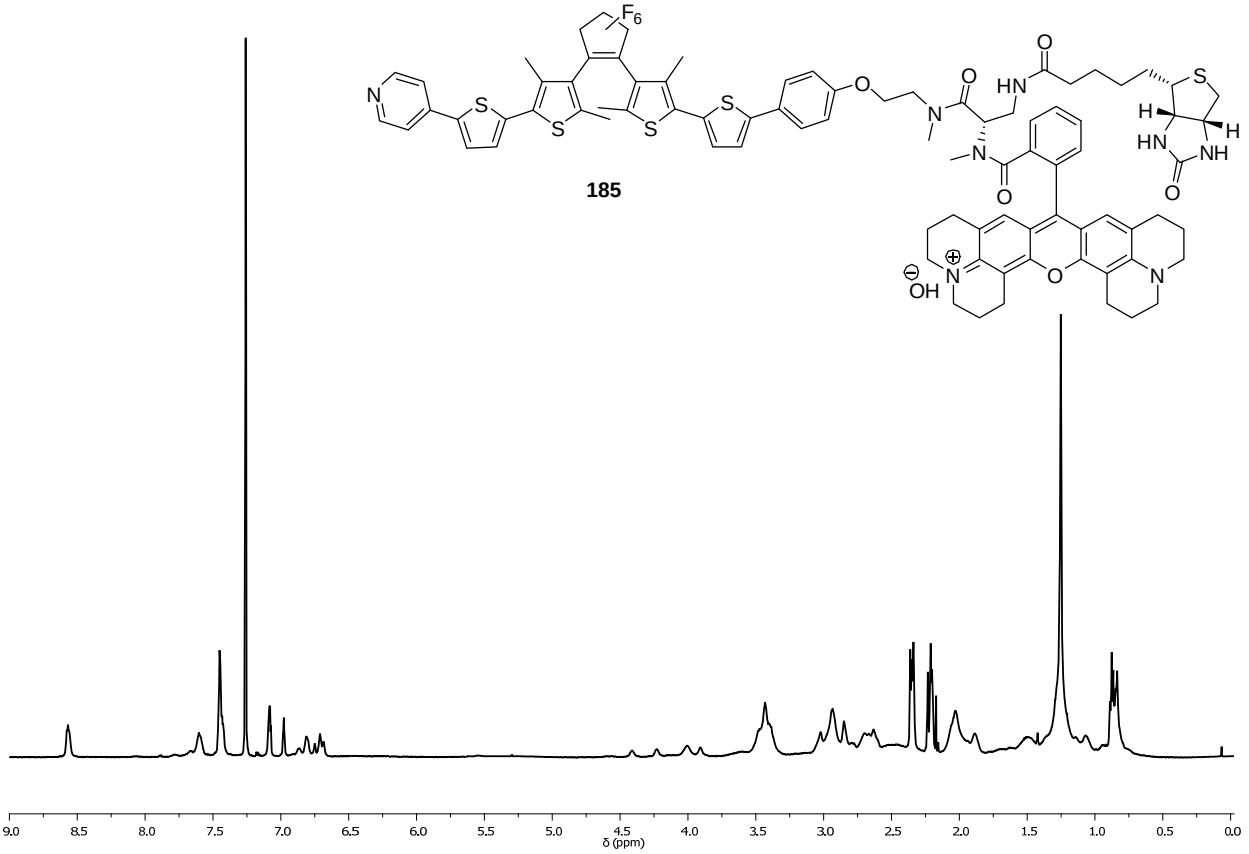

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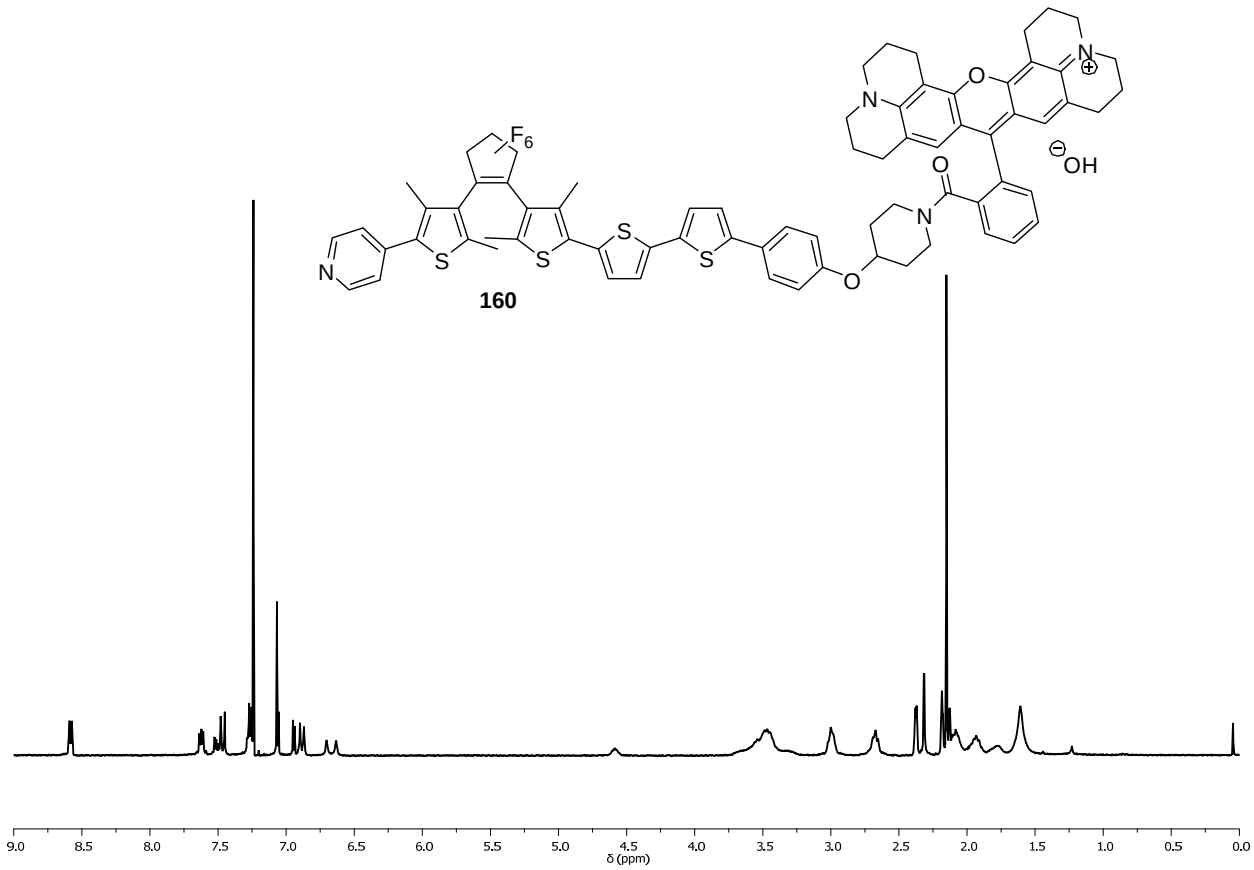

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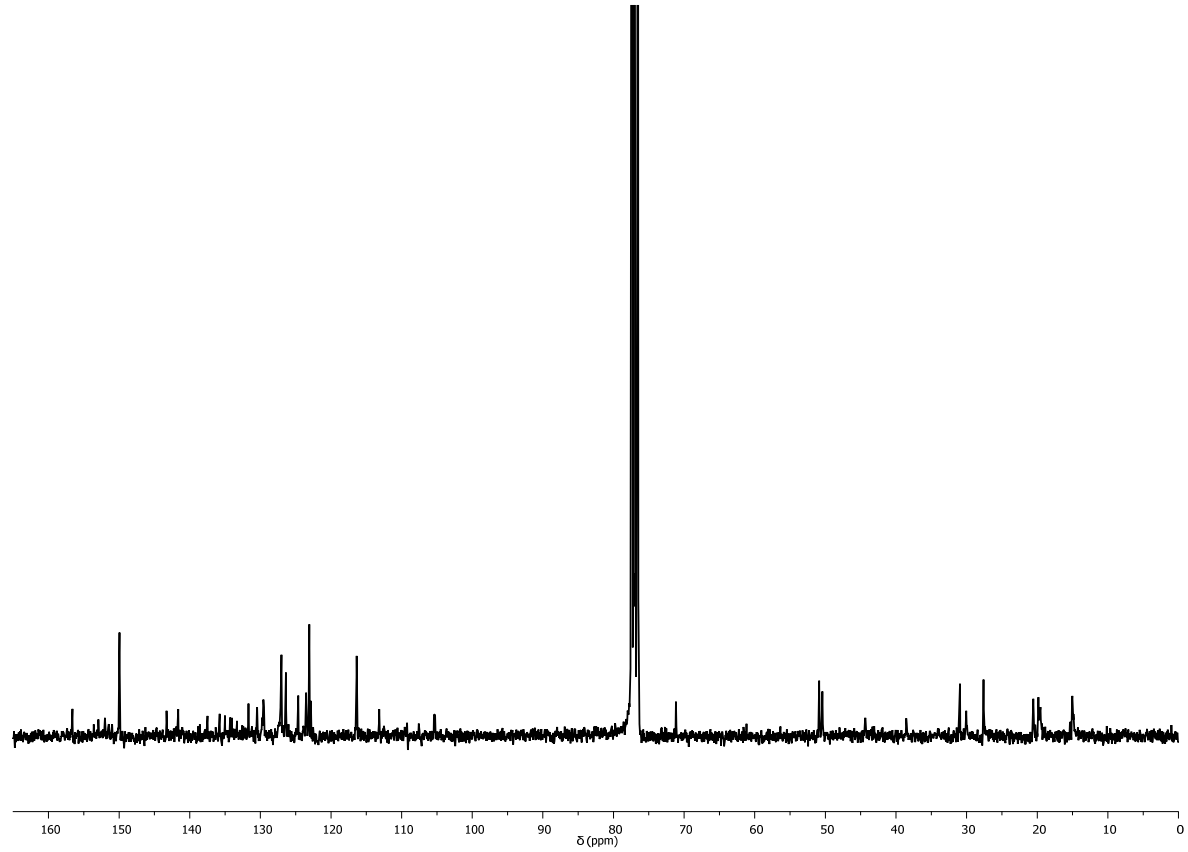

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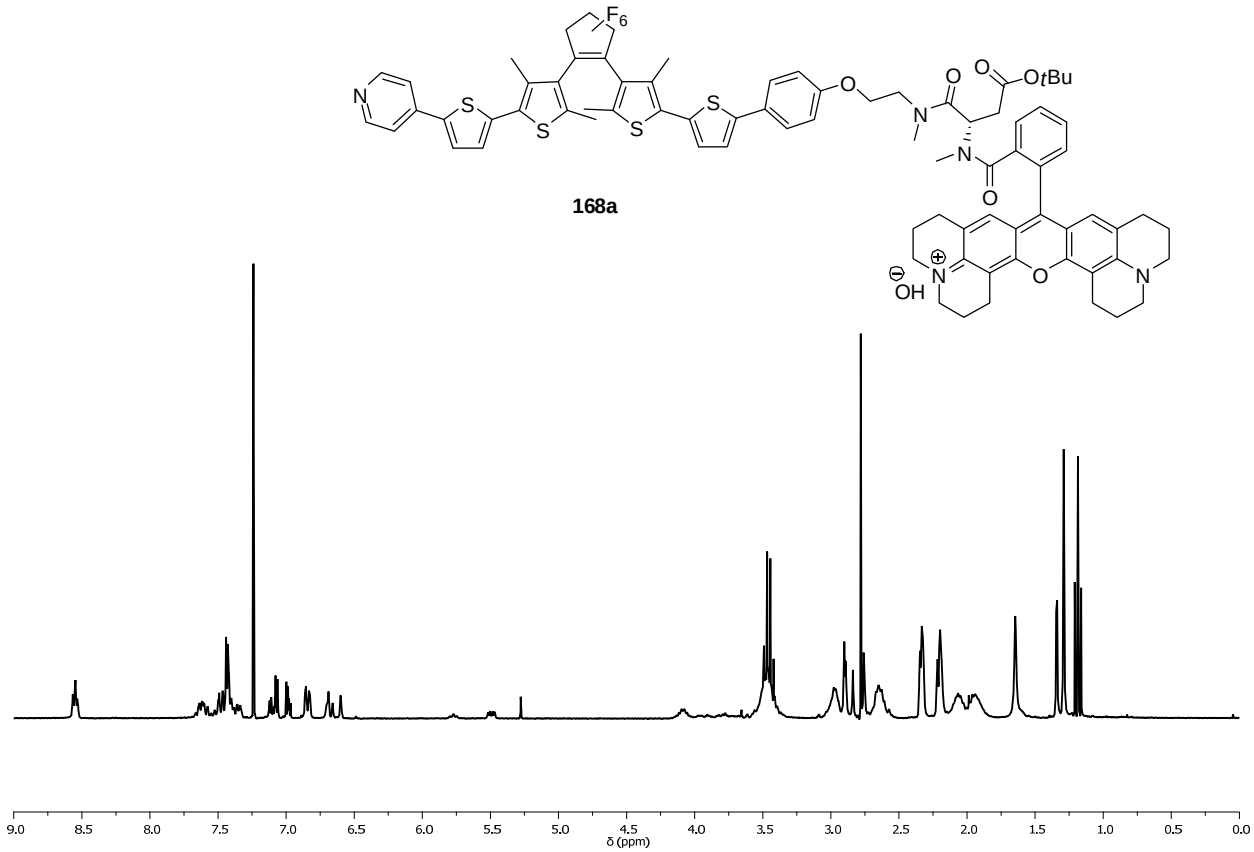

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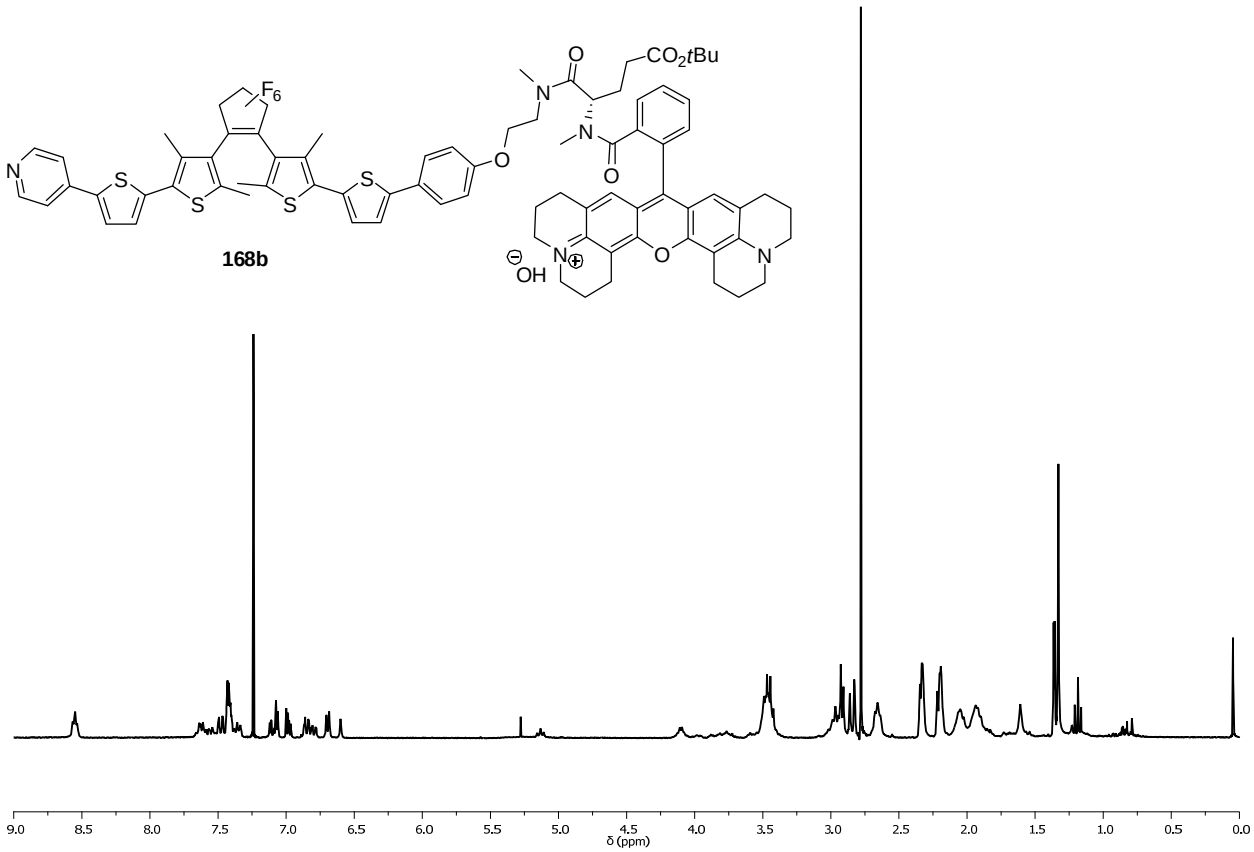
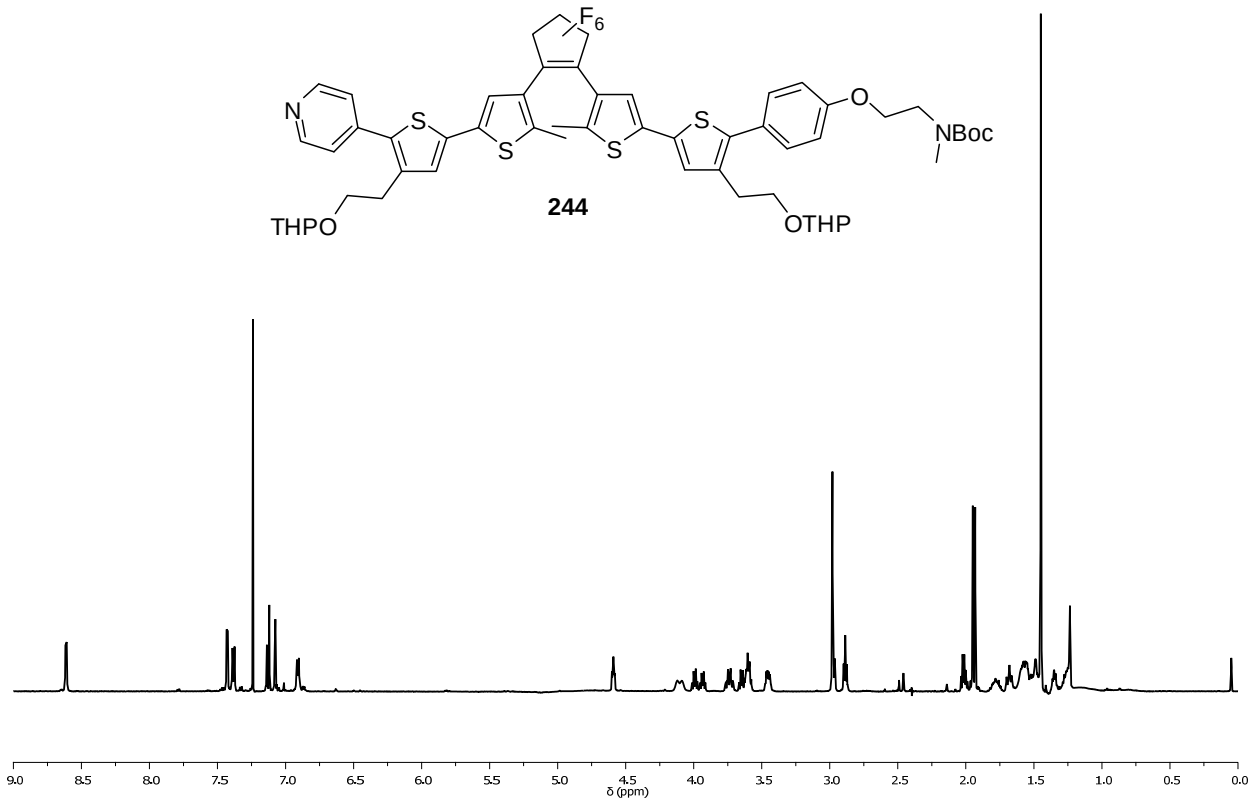
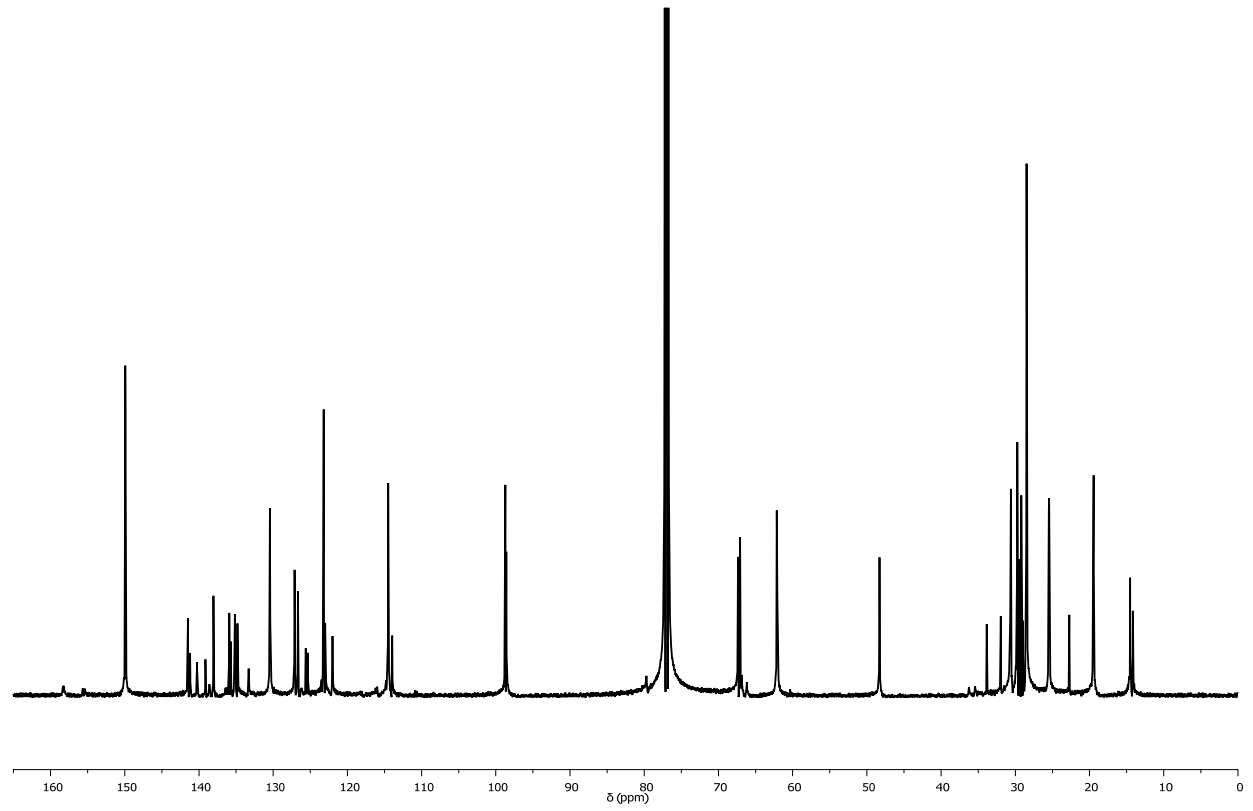


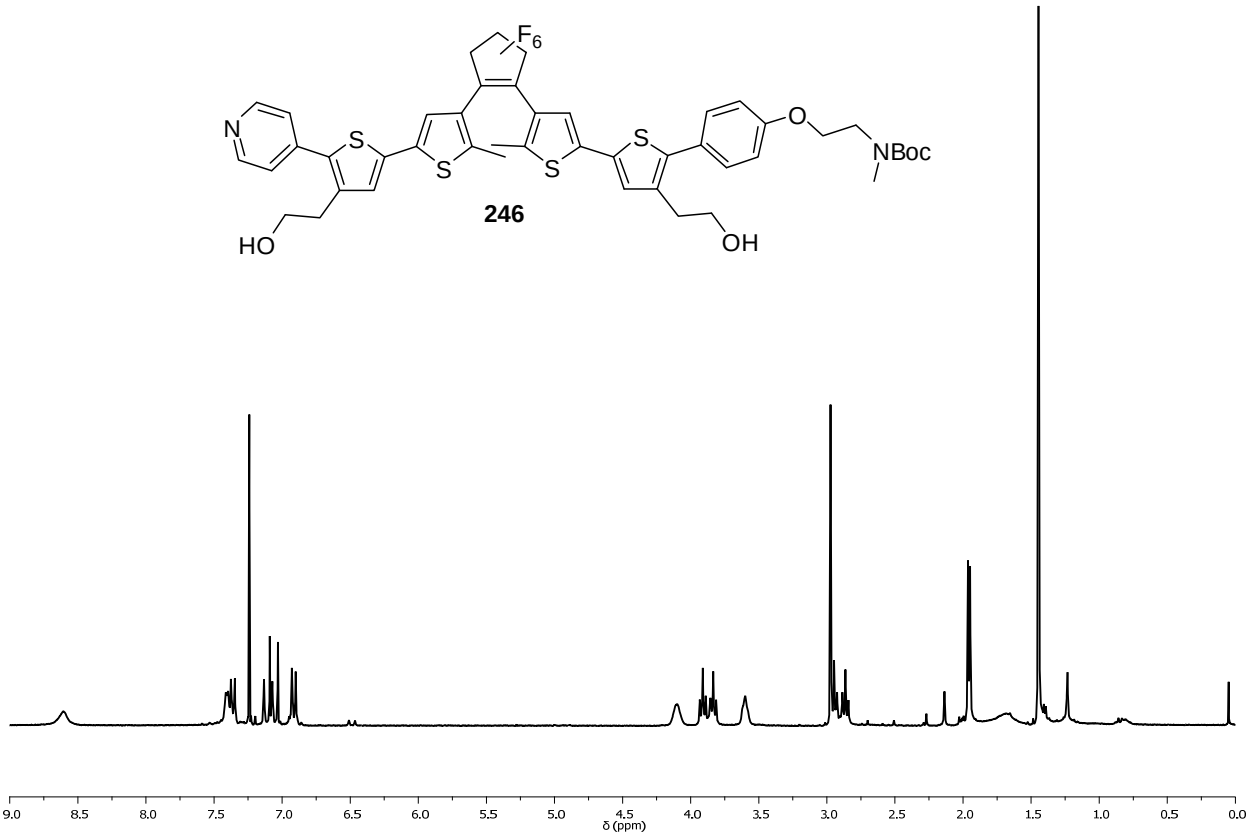
^{1}H NMR (300 MHz, $CDCl_3$)



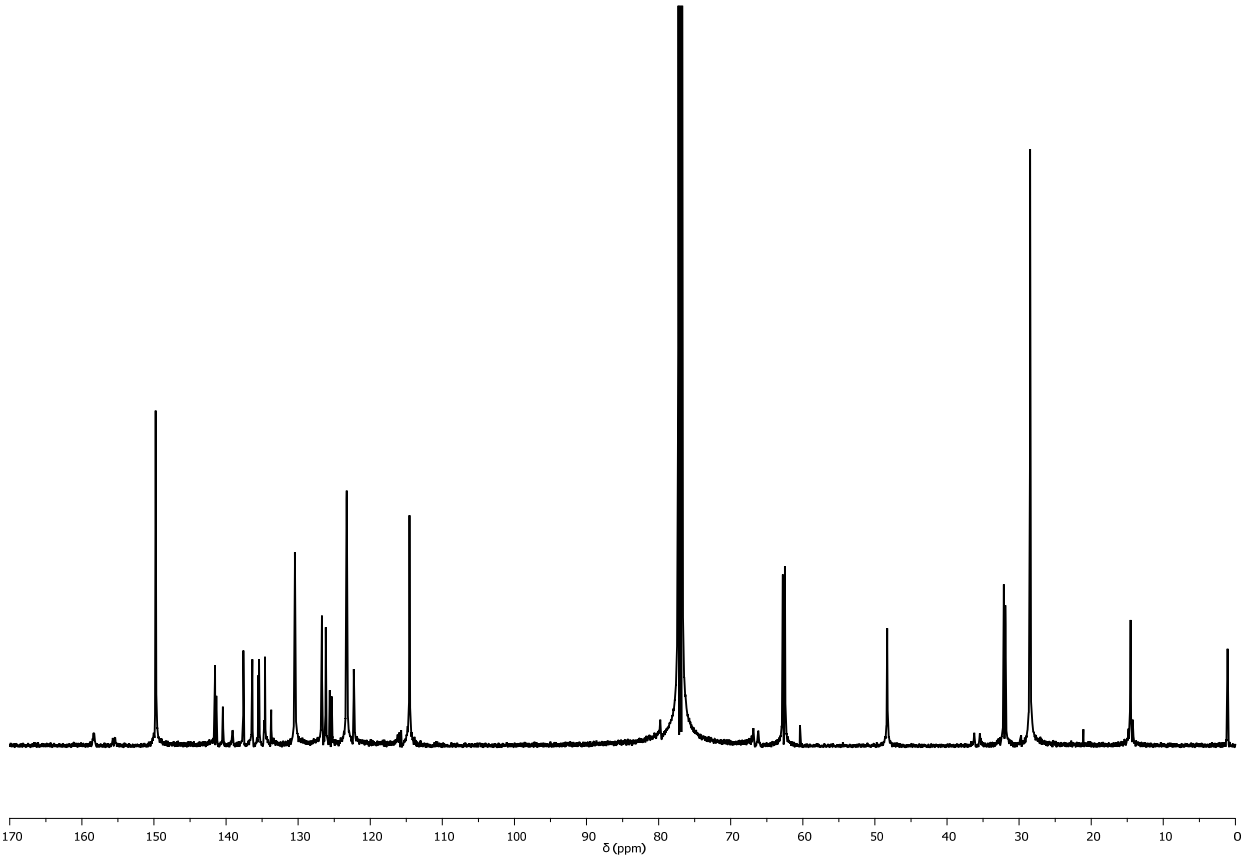
^{1}H NMR (600 MHz, $CDCl_3$)



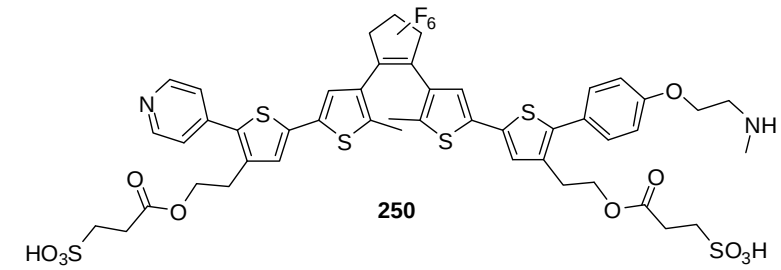
^{13}C NMR (126 MHz, $CDCl_3$)



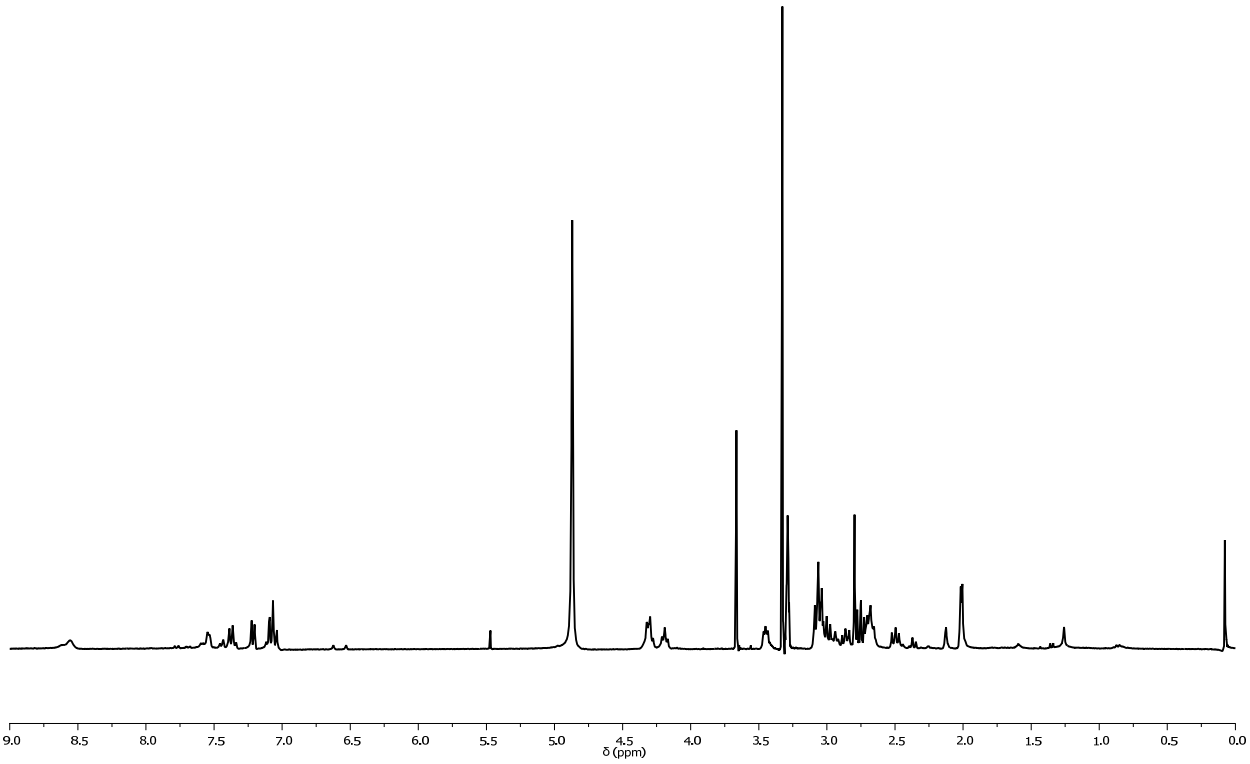
^{1}H NMR (300 MHz, $CDCl_3$)



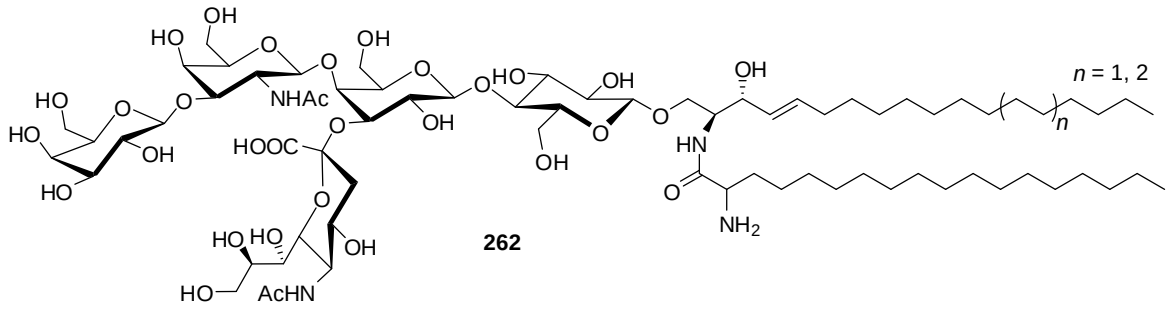
^{13}C NMR (126 MHz, $CDCl_3$)



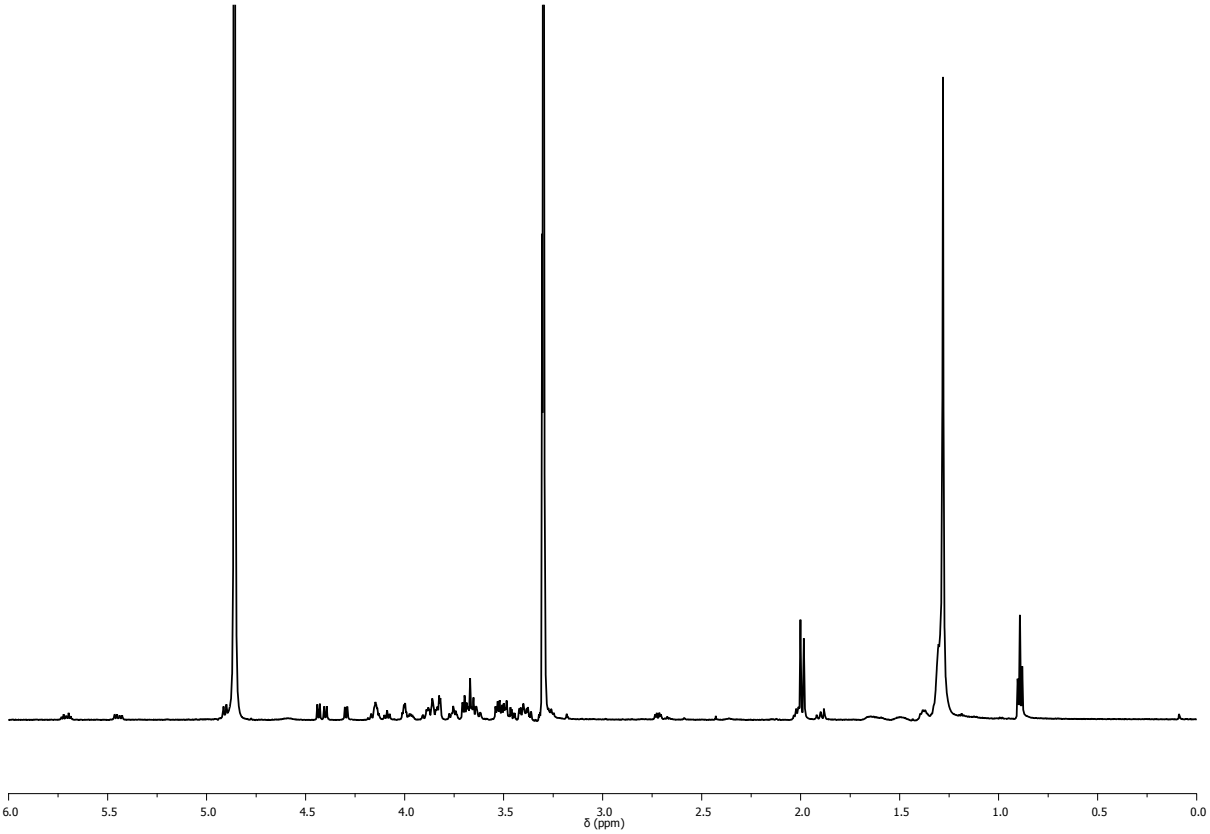
250



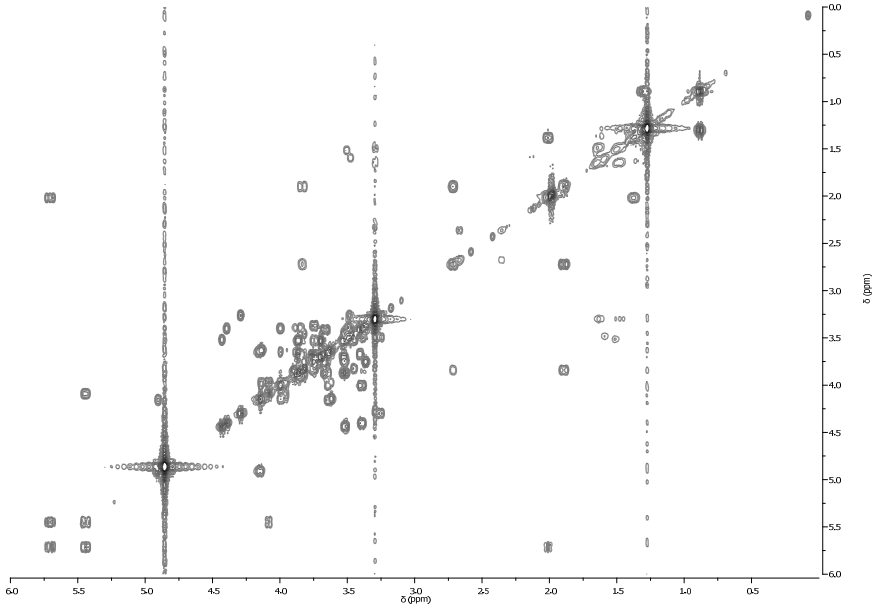
^{1}H NMR (300 MHz, CD_3OD)



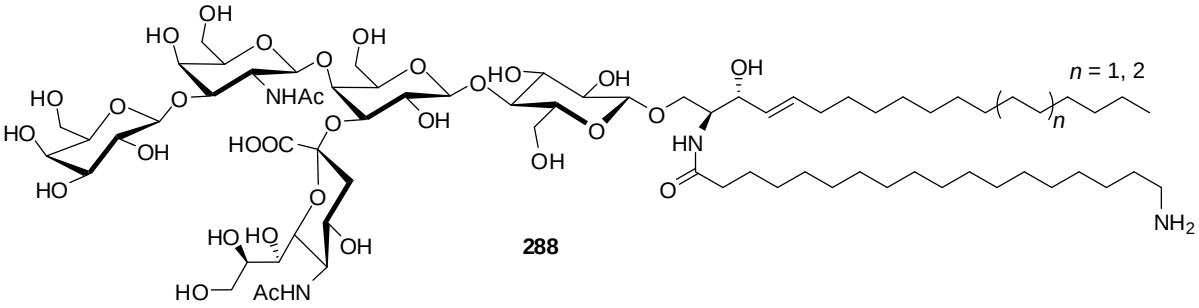
262



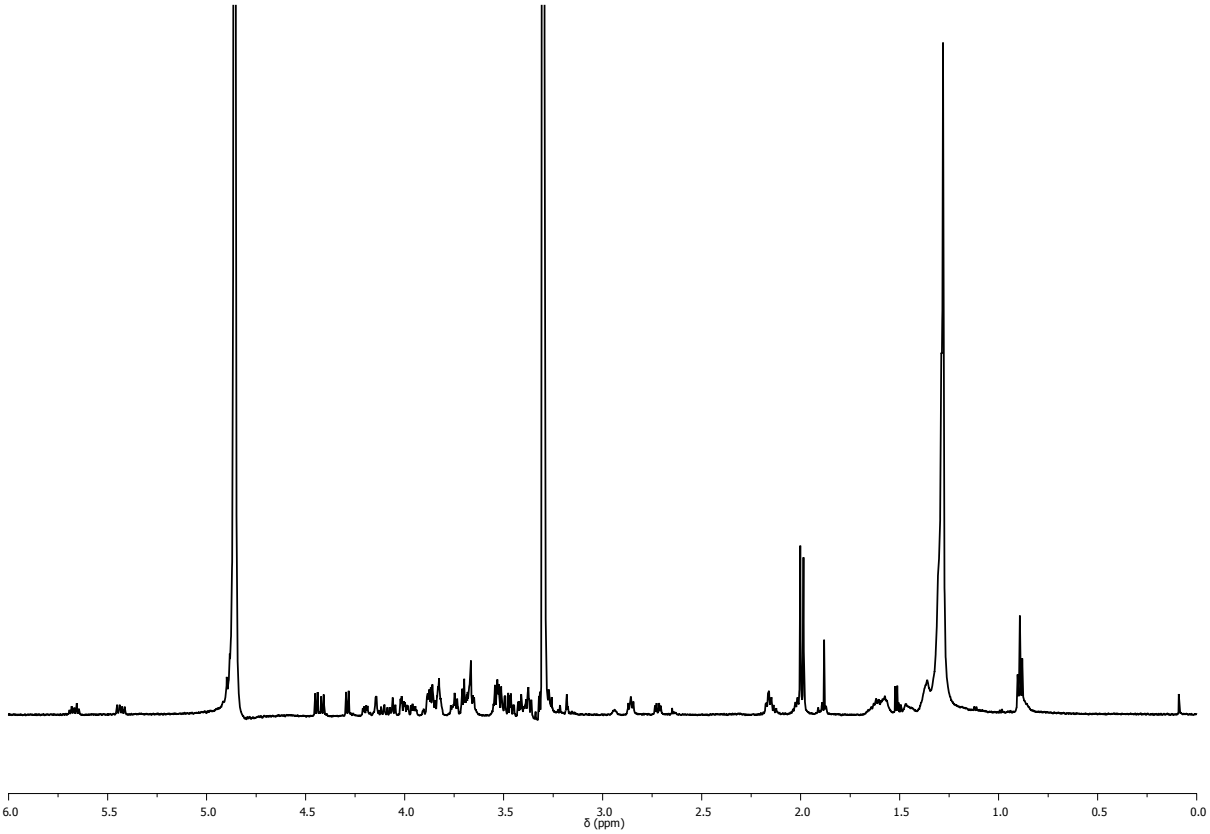
^{1}H NMR (600 MHz, CD_3OD)



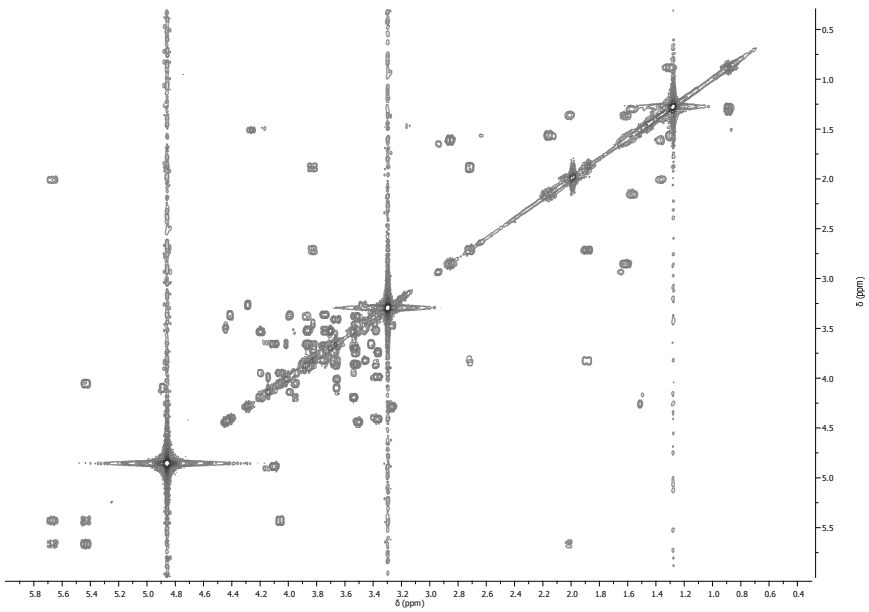
^{1}H–^{1}H COSY NMR (600 MHz, CD_3OD)



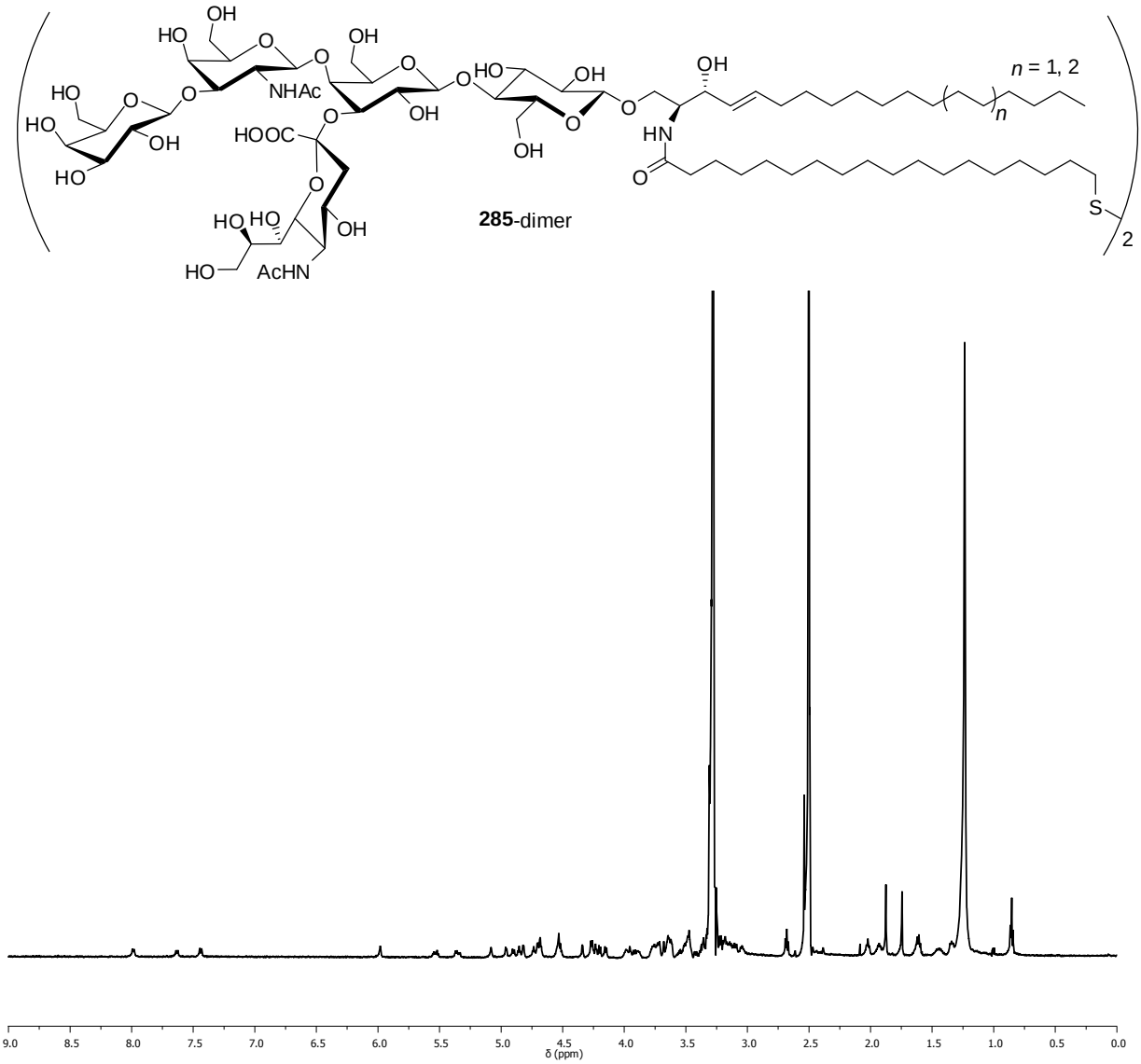
288



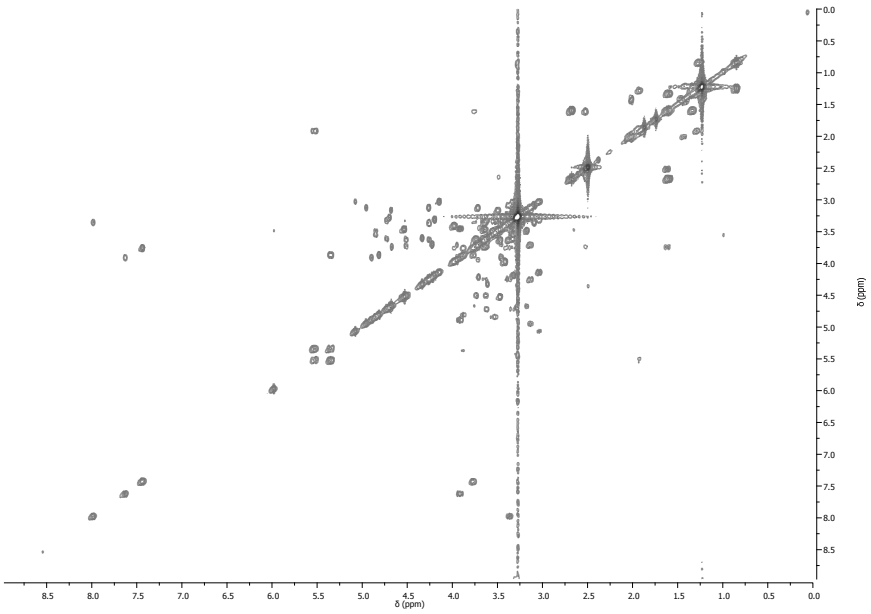
^{1}H NMR (600 MHz, CD_3OD)



^{1}H–^{1}H COSY NMR (600 MHz, CD_3OD)



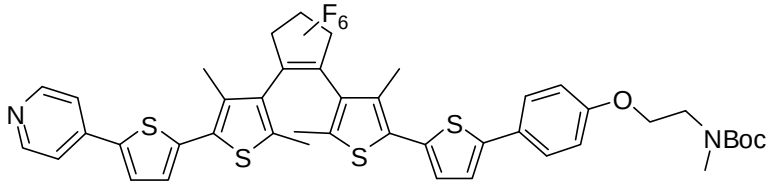
^{1}H NMR (600 MHz, [D_6]DMSO)



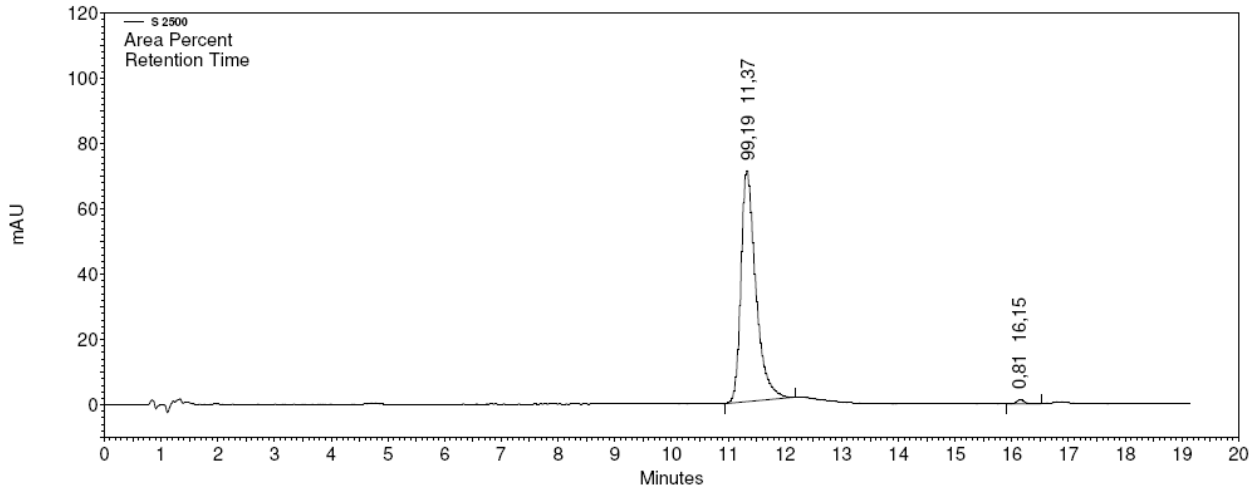
^{1}H–^{1}H COSY NMR (600 MHz, [D_6]DMSO)

2. HPLC Spectra

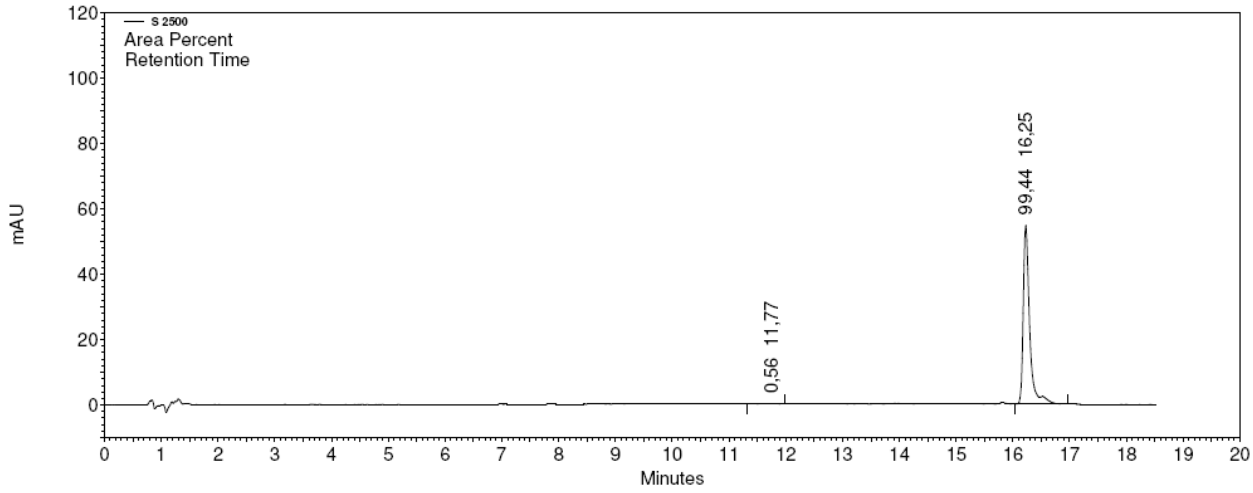
Photochromic Compound **136**:



ACN/water: 80/20 → 100/0 for 0–20 min, 25 °C, flow rate 1mL/min, detection at 369 nm

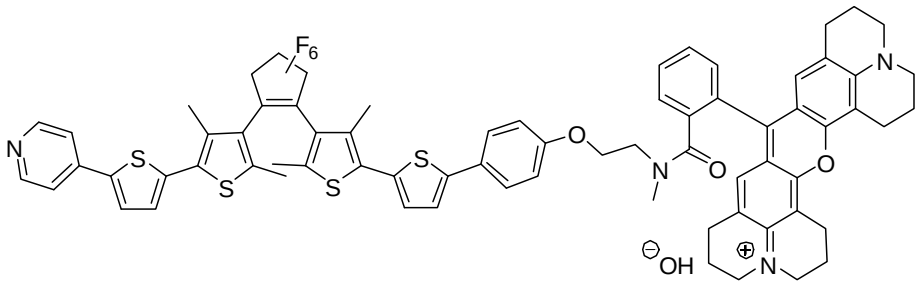


a) open form

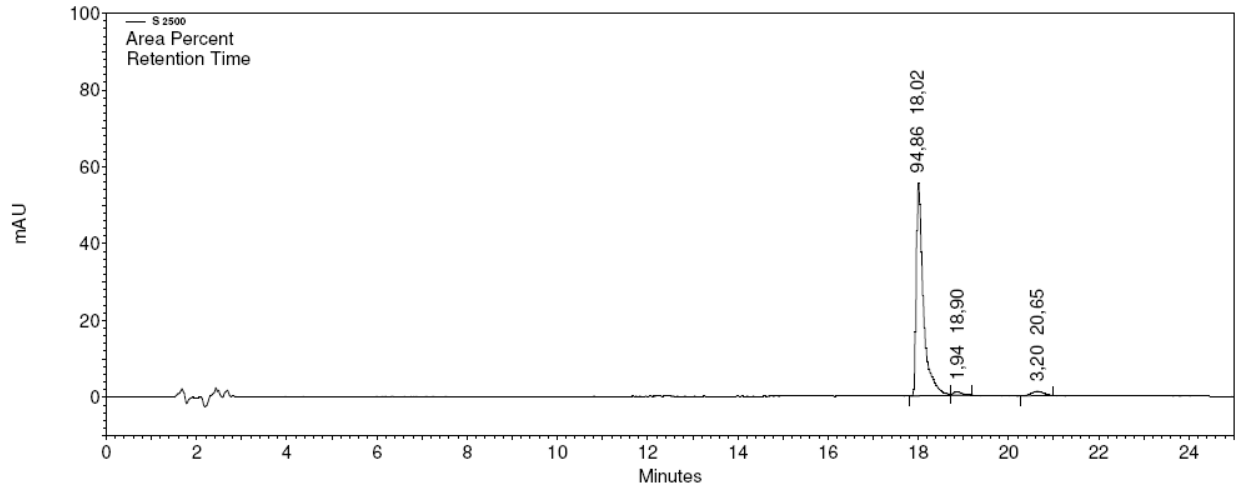


b) closed form (in photostationary state)

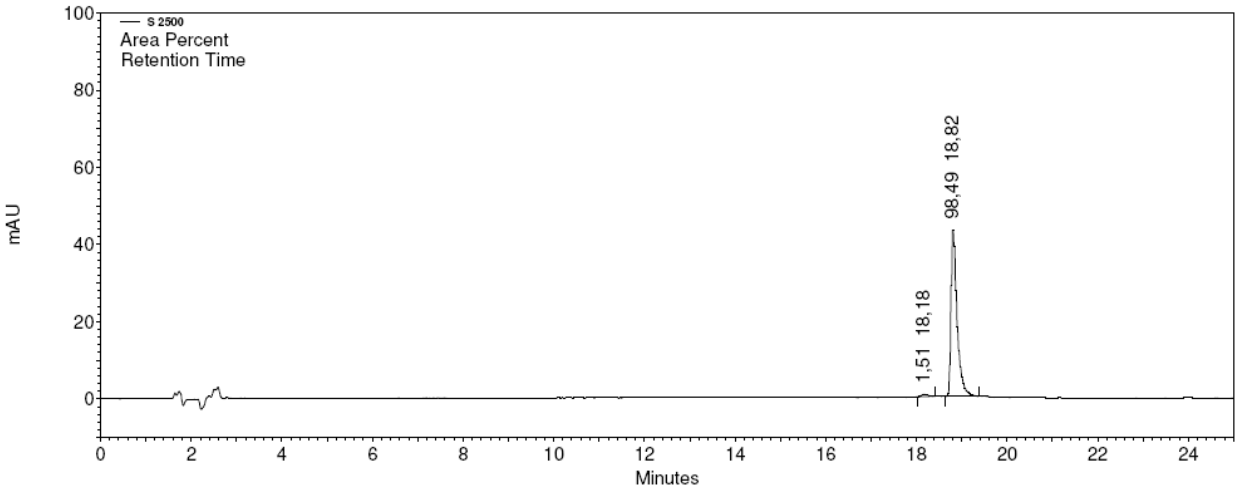
Photochromic Fluorescent Compound **159**:



ACN/water: 80/20 → 100/0 for 0–20 min, 100% ACN for 20–25 min, 25 °C, flow rate 1mL/min, detection at 369 nm



a) open form



b) closed form (in photostationary state)

Crystallographic Data

The X-Ray diffraction data obtained for a single crystall of compound **239** (preliminary results):

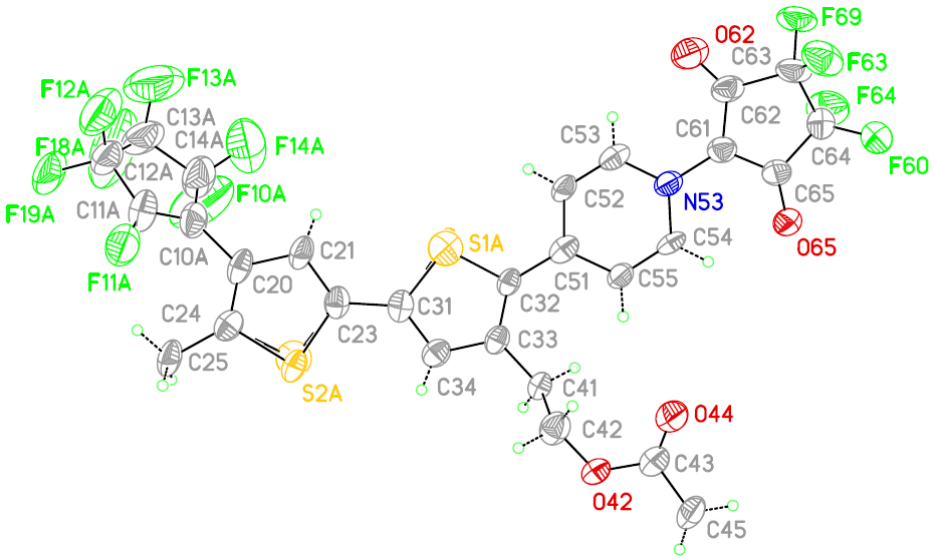


Table 1. Crystal data and structure refinement for compound **239**.

Identification code	atx031_3	
Empirical formula	C10 H10 F1.67 N2 O2 S1.67	
Formula weight	275.30	
Temperature	293(2) K	
Wavelength	1.54178 Å	
Crystal system	triclinic	
Space group	P-1	
Unit cell dimensions	a = 7.2393(14) Å	α= 75.24(3)°.
	b = 12.815(3) Å	β= 89.92(3)°.
	c = 17.513(4) Å	γ = 75.11(3)°.
Volume	1514.8(5) $Å^3$	
Z	6	
Density (calculated)	1.811 Mg/m^3	
Absorption coefficient	4.346 mm^{-1}	
F(000)	850	
Crystal size	0.025 x 0.015 x 0.300 mm^3	
Theta range for data collection	2.62 to 65.17°.	
Index ranges	-8<=h<=8, -13<=k<=15, -20<=l<=20	
Reflections collected	18504	
Independent reflections	4906 [R(int) = 0.0863]	

Completeness to theta = 65.17°	94.6 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4906 / 28 / 454
Goodness-of-fit on F^2	2.222
Final R indices [I>2sigma(I)]	R1 = 0.1414, wR2 = 0.3611
R indices (all data)	R1 = 0.1803, wR2 = 0.3728
Largest diff. peak and hole	1.484 and -0.809 e.Å^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for compound **239**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
S(2A)	1145(8)	13349(2)	8680(1)	42(1)
S(2B)	270(160)	13310(30)	8610(30)	70(30)
S(1A)	2980(20)	9701(3)	9508(2)	39(2)
S(1B)	3400(200)	9750(40)	9560(40)	60(30)
O(42)	2569(11)	10516(6)	12848(4)	52(2)
C(34)	1506(14)	11224(9)	10205(5)	41(2)
N(53)	4484(12)	5791(7)	11630(4)	41(2)
C(51)	3338(13)	8093(9)	10914(5)	39(2)
C(33)	1929(13)	10195(8)	10808(5)	36(2)
C(32)	2767(13)	9284(8)	10521(4)	35(2)
C(52)	3500(14)	7296(9)	10482(5)	39(2)
C(25)	450(17)	14968(10)	7221(6)	54(3)
C(21)	1767(15)	11798(10)	7955(5)	46(3)
C(20)	1377(15)	12821(10)	7364(5)	46(3)
C(61)	5165(14)	4618(9)	11994(6)	42(2)
C(53)	4061(14)	6188(10)	10833(5)	43(3)
C(24)	1002(15)	13735(10)	7653(5)	46(3)
C(23)	1672(13)	11941(8)	8712(6)	41(2)
C(31)	1979(13)	11105(8)	9461(6)	41(2)
C(45)	3000(20)	9721(12)	14217(6)	69(4)
C(42)	3000(17)	10530(11)	12055(6)	53(3)
O(44)	4467(14)	8782(8)	13244(5)	73(3)
C(43)	3459(18)	9581(11)	13407(6)	57(3)
C(54)	4352(14)	6531(9)	12080(5)	38(2)
C(41)	1449(14)	10194(9)	11650(5)	38(2)
F(69)	6399(11)	1889(6)	11916(4)	70(2)
F(63)	8728(9)	2413(6)	12351(5)	72(2)

O(62)	6640(11)	3964(8)	10911(5)	63(2)
O(65)	4219(12)	4534(6)	13320(4)	54(2)
F(64)	4293(13)	2347(7)	13018(5)	82(2)
C(64)	5752(19)	2869(10)	12949(7)	57(3)
C(62)	6195(15)	3835(10)	11602(6)	49(3)
C(65)	4943(15)	4098(9)	12805(6)	42(2)
C(63)	6829(17)	2707(10)	12207(7)	54(3)
C(55)	3800(14)	7659(8)	11736(5)	38(2)
F(60)	6926(13)	2405(6)	13613(4)	81(2)
F(13A)	820(30)	11701(14)	5014(9)	205(8)
F(11A)	3452(11)	13993(6)	6053(4)	79(2)
F(19A)	1211(14)	14222(7)	4595(4)	105(3)
F(12A)	3471(14)	12679(9)	4872(4)	117(4)
F(14A)	610(20)	11209(10)	6496(6)	165(6)
C(11A)	2257(18)	13385(11)	5947(6)	65(3)
C(10A)	1318(17)	12872(10)	6507(6)	60(3)
C(12A)	1900(20)	13233(11)	5146(6)	72(4)
C(14A)	150(20)	12295(14)	6180(7)	85(4)
C(13A)	420(30)	12559(15)	5277(8)	94(5)
F(10A)	-1713(15)	12656(15)	6302(7)	161(6)
F(18A)	-1260(20)	13185(19)	4904(7)	212(9)
O(100)	240(30)	13750(20)	10451(12)	181(8)
C(100)	1670(30)	13777(19)	10866(14)	121(7)
C(110)	1520(90)	14540(50)	11230(50)	450(60)

Table 3. Bond lengths [Å] and angles [°] for compound **239**.

S(2A)-C(23)	1.731(9)	N(53)-C(61)	1.429(14)
S(2A)-C(24)	1.736(9)	C(51)-C(52)	1.401(14)
S(2B)-C(24)	1.750(17)	C(51)-C(55)	1.411(13)
S(2B)-C(23)	1.748(17)	C(51)-C(32)	1.455(14)
S(1A)-C(32)	1.737(8)	C(33)-C(32)	1.385(13)
S(1A)-C(31)	1.739(10)	C(33)-C(41)	1.513(12)
S(1B)-C(32)	1.743(17)	C(52)-C(53)	1.348(15)
S(1B)-C(31)	1.747(17)	C(25)-C(24)	1.515(16)
O(42)-C(43)	1.348(14)	C(21)-C(23)	1.385(14)
O(42)-C(42)	1.420(12)	C(21)-C(20)	1.410(15)
C(34)-C(31)	1.383(13)	C(20)-C(24)	1.357(16)
C(34)-C(33)	1.425(14)	C(20)-C(10A)	1.486(14)
N(53)-C(53)	1.365(13)	C(61)-C(62)	1.414(14)
N(53)-C(54)	1.366(12)	C(61)-C(65)	1.435(14)

C(23)-C(31)	1.442(13)	C(34)-C(33)-C(41)	119.9(9)
C(45)-C(43)	1.501(15)	C(33)-C(32)-C(51)	130.8(7)
C(42)-C(41)	1.534(15)	C(33)-C(32)-S(1A)	110.9(7)
O(44)-C(43)	1.195(14)	C(51)-C(32)-S(1A)	118.1(7)
C(54)-C(55)	1.367(14)	C(33)-C(32)-S(1B)	109.7(19)
F(69)-C(63)	1.376(12)	C(51)-C(32)-S(1B)	119.1(13)
F(63)-C(63)	1.335(13)	S(1A)-C(32)-S(1B)	11(6)
O(62)-C(62)	1.233(13)	C(53)-C(52)-C(51)	121.8(9)
O(65)-C(65)	1.227(11)	C(23)-C(21)-C(20)	112.7(10)
F(64)-C(64)	1.378(15)	C(24)-C(20)-C(21)	113.8(9)
C(64)-F(60)	1.348(14)	C(24)-C(20)-C(10A)	124.1(10)
C(64)-C(65)	1.486(17)	C(21)-C(20)-C(10A)	122.1(10)
C(64)-C(63)	1.546(16)	C(62)-C(61)-N(53)	123.7(9)
C(62)-C(63)	1.518(18)	C(62)-C(61)-C(65)	112.0(10)
F(13A)-C(13A)	1.265(18)	N(53)-C(61)-C(65)	124.3(8)
F(11A)-C(11A)	1.345(15)	C(52)-C(53)-N(53)	121.4(9)
F(19A)-C(12A)	1.356(15)	C(20)-C(24)-C(25)	130.1(8)
F(12A)-C(12A)	1.338(15)	C(20)-C(24)-S(2A)	110.9(8)
F(14A)-C(14A)	1.313(18)	C(25)-C(24)-S(2A)	119.0(8)
C(11A)-C(10A)	1.325(16)	C(20)-C(24)-S(2B)	106(2)
C(11A)-C(12A)	1.496(17)	C(25)-C(24)-S(2B)	119.4(11)
C(10A)-C(14A)	1.460(19)	S(2A)-C(24)-S(2B)	22(4)
C(12A)-C(13A)	1.52(2)	C(21)-C(23)-C(31)	129.0(9)
C(14A)-F(10A)	1.343(19)	C(21)-C(23)-S(2A)	110.5(7)
C(14A)-C(13A)	1.554(19)	C(31)-C(23)-S(2A)	120.5(7)
C(13A)-F(18A)	1.34(2)	C(21)-C(23)-S(2B)	106(2)
O(100)-C(100)	1.28(2)	C(31)-C(23)-S(2B)	121.8(12)
C(100)-C(110)	1.28(4)	S(2A)-C(23)-S(2B)	22(4)
C(23)-S(2A)-C(24)	92.0(5)	C(34)-C(31)-C(23)	129.5(9)
C(24)-S(2B)-C(23)	90.9(10)	C(34)-C(31)-S(1A)	110.0(7)
C(32)-S(1A)-C(31)	92.7(5)	C(23)-C(31)-S(1A)	120.5(7)
C(32)-S(1B)-C(31)	92.3(10)	C(34)-C(31)-S(1B)	108.7(19)
C(43)-O(42)-C(42)	116.2(9)	C(23)-C(31)-S(1B)	121.3(12)
C(31)-C(34)-C(33)	114.1(9)	S(1A)-C(31)-S(1B)	11(6)
C(53)-N(53)-C(54)	119.2(9)	O(42)-C(42)-C(41)	109.8(8)
C(53)-N(53)-C(61)	121.2(8)	O(44)-C(43)-O(42)	122.1(10)
C(54)-N(53)-C(61)	119.5(8)	O(44)-C(43)-C(45)	127.6(11)
C(52)-C(51)-C(55)	115.6(9)	O(42)-C(43)-C(45)	110.4(10)
C(52)-C(51)-C(32)	120.9(8)	N(53)-C(54)-C(55)	120.4(8)
C(55)-C(51)-C(32)	123.5(8)	C(33)-C(41)-C(42)	109.6(8)
C(32)-C(33)-C(34)	112.4(8)	F(60)-C(64)-F(64)	107.6(10)
C(32)-C(33)-C(41)	127.7(9)	F(60)-C(64)-C(65)	113.2(9)

F(64)-C(64)-C(65)	110.0(10)	C(11A)-C(10A)-C(20)	128.3(11)
F(60)-C(64)-C(63)	111.2(10)	C(14A)-C(10A)-C(20)	121.0(10)
F(64)-C(64)-C(63)	109.2(9)	F(12A)-C(12A)-F(19A)	106.7(10)
C(65)-C(64)-C(63)	105.6(9)	F(12A)-C(12A)-C(11A)	112.9(11)
O(62)-C(62)-C(61)	130.6(11)	F(19A)-C(12A)-C(11A)	112.4(11)
O(62)-C(62)-C(63)	121.7(9)	F(12A)-C(12A)-C(13A)	110.2(12)
C(61)-C(62)-C(63)	107.7(9)	F(19A)-C(12A)-C(13A)	110.9(12)
O(65)-C(65)-C(61)	129.0(10)	C(11A)-C(12A)-C(13A)	103.7(10)
O(65)-C(65)-C(64)	122.8(9)	F(14A)-C(14A)-F(10A)	105.2(15)
C(61)-C(65)-C(64)	108.2(8)	F(14A)-C(14A)-C(10A)	113.6(12)
F(63)-C(63)-F(69)	106.5(8)	F(10A)-C(14A)-C(10A)	111.7(13)
F(63)-C(63)-C(62)	110.0(10)	F(14A)-C(14A)-C(13A)	110.5(13)
F(69)-C(63)-C(62)	110.4(9)	F(10A)-C(14A)-C(13A)	109.9(13)
F(63)-C(63)-C(64)	112.3(9)	C(10A)-C(14A)-C(13A)	105.9(12)
F(69)-C(63)-C(64)	112.6(10)	F(13A)-C(13A)-F(18A)	104.9(17)
C(62)-C(63)-C(64)	105.1(9)	F(13A)-C(13A)-C(12A)	115.2(15)
C(54)-C(55)-C(51)	121.6(9)	F(18A)-C(13A)-C(12A)	110.1(15)
C(10A)-C(11A)-F(11A)	125.5(11)	F(13A)-C(13A)-C(14A)	113.5(16)
C(10A)-C(11A)-C(12A)	113.9(12)	F(18A)-C(13A)-C(14A)	107.3(14)
F(11A)-C(11A)-C(12A)	120.5(10)	C(12A)-C(13A)-C(14A)	105.6(11)
C(11A)-C(10A)-C(14A)	110.7(11)	C(110)-C(100)-O(100)	120(4)

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Lebenslauf

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Seit September 2006 arbeite ich an meiner Dissertation unter der wissenschaftlichen Anleitung von Prof. Dr. Armin de Meijere in der Abteilung von Prof. Stefan W. Hell im Max-Planck-Institut für biophysikalische Chemie, Göttingen.

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