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SUPPORTING INFORMATION

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Title: Functionalization of the *meso*-Phenyl Ring of Rhodamine Dyes Through S_NAr with Sulfur Nucleophiles: Synthesis, Biophysical Characterizations, and Comprehensive NMR Analysis
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Functionalization of meso-Phenyl Ring of Red-emitting Rhodamine Dyes through S_NAr with Sulfur Nucleophiles: Synthesis, Biophysical Characterizations and Comprehensive NMR analysis

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Supporting Information

Figure S1. Comparison of the imaging performance of antibody conjugates obtained from fluorescent dye 5-H (Abberior Star 600) and Alexa Fluor® 594. To compare the imaging performance of the conjugates, subunits of the nuclear pore complexes were labeled by indirect immunofluorescence staining. Then confocal (left) and STED images (right) of the samples were recorded. The performance of the dyes under conventional an STED microscopy conditions were similar. The optical resolutions were found to be ~55 nm for compound 5-H and Alexa Fluor® 594, under the applied imaging parameters.

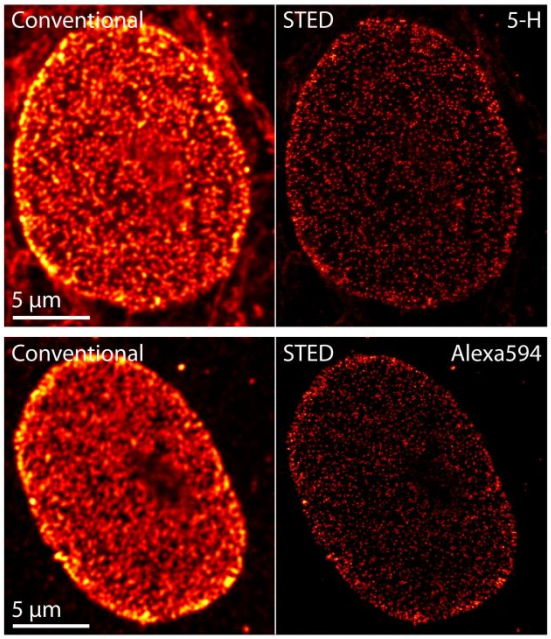


Figure S2. Spectral crosstalk and specificity of the labeling. (A) To analyze the spectral crosstalk of the dyes used for two-color nanoscopy, subunits of the nuclear pore complexes were labeled with the reference fluorescent dye KK114 (top) and Abberior Star™ 600 (compound **5**-NHS in Scheme 1) by indirect immunofluorescence staining (see main text for details). The color table was kept constant for the individual color channels. Very low crosstalk was detected. (B) Note that in addition to the specific nuclear pore complex labeling, *both* dyes show some incorporation into vesicles and membrane bound cell organelles. In order to minimize the unspecific binding, the structure of KK114 dye was decorated with two sulfonic acid residues (see references 6a, 8d, 12, and 18 in the main text). Many applications of KK114 dye showed that it provides fluorescent images with negligible background (see references 24 in the main text). Therefore, it is not surprising that in the case of KK114 dye, this effect is less pronounced than for Abberior Star™ 600 dye.

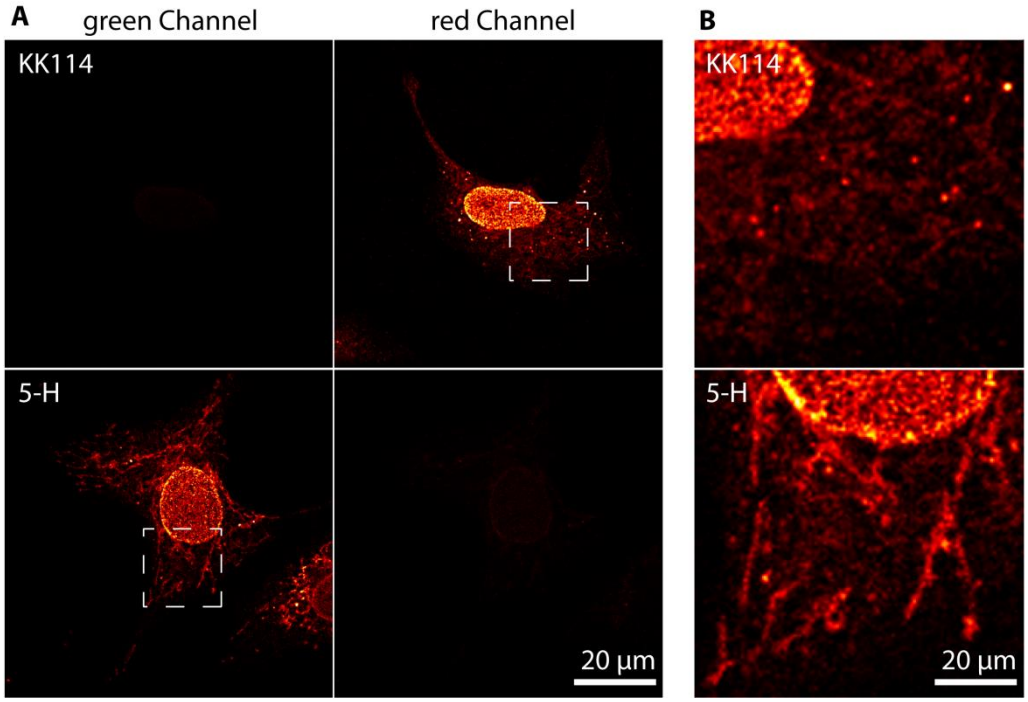
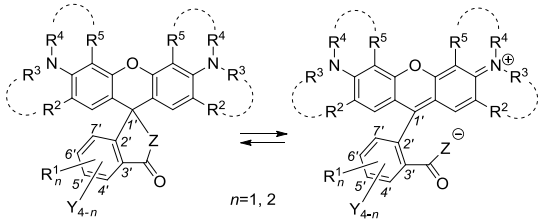


Table S1. Degrees of labeling (DOL) of antibody conjugates used in immunofluorescence

Conjugates	DOL
Star 600 Goat anti-Rabbit IgG	3.4 (fluorescence quantum yield 33%)
KK 114 Goat anti-Rabbit IgG	2.6
Star 600 Sheep anti-Mouse IgG	9.7 (fluorescence quantum yield 20%)
KK 114 Sheep anti-Mouse IgG	5.2
Alexa Fluor® 594 Sheep anti-Mouse IgG	1.7

Table S2. Structures of rhodamine derivatives of the present study. In some cases (if Z = O), the equilibrium between the "closed" (lactone) and "open" (zwitterionic) forms may take place (see footnotes to table).



a **b**

Compound	R^1	R^2	R^3	R^4	R^5	Y	Z
6	COOH	H	H	CF_3CH_2	H	H	O
7	COOH	H	H	CF_3CH_2	SO_3H	H	O
8	COOH	F	H	CF_3CH_2	SO_3H	H	O
9	$COOCH_3$	H	H	CF_3CH_2	H	H	O
13	$COOCH_2CH_3$	-C(CH_3)=CH-C(CH_3)$_2$-		CH_3	H	H	O
14	$COOCH_2CH_3$	-C(CH_2SO_3H)=CH-C(CH_3)$_2$-		CH_3	H	H	O
15	COOH	-C(CH_3OH)=CH-C(CH_3)$_2$-		-$(CH_2)_3$-		H	O
16	$COOCH_2CH_3$	-C(CH_3OH)=CH-C(CH_3)$_2$-		-$(CH_2)_3$-		H	O
17	SCH_2COOH	-C(CH_3)=CH-C(CH_3)$_2$-		CH_3	H	F	O
18	$SCH_2COOCH_2CH_3$	-C(CH_2SO_3H)=CH-C(CH_3)$_2$-		-$(CH_2)_3$-		F	OAll[a]
19	2×$SCH_2COOCH_2CH_3$[b]	-C(CH_2SO_3H)=CH-C(CH_3)$_2$-		-$(CH_2)_3$-		F	OAll[a]
20	$SCH_2COOCH_2CH_3$	-C($CH_2SO_3CH_3$)=CH-C(CH_3)$_2$-		-$(CH_2)_3$-		F	OAll[a]
21	$S(CH_2)_2SO_3H$	-C(CH_2OH)=CH-C(CH_3)$_2$-		-$(CH_2)_3$-		F	O
22	$SCH_2COOCH_2CH_3$	-$(CH_2)_3$-		-$(CH_2)_3$-		F	O
23	$NMe(CH_2)_2OH$	-$(CH_2)_3$-		-$(CH_2)_3$-		F	O
24	$NMe(CH_2)_2CO_2CH_3$	-$(CH_2)_3$-		-$(CH_2)_3$-		F	O
25	$NMe(CH_2)_2CO_2H$	-$(CH_2)_3$-		-$(CH_2)_3$-		F	O
26	F	-C(CH_2SO_3H)=CH-C(CH_3)$_2$-		-$(CH_2)_3$-		F	$NMe(CH_2)_2CO_2H$[a]

[a] allyl esters (Z = OAll) and amides (Z = NR_2) exist only in the "open" form (b); [b] disubstituted compound.

Table S3. Chemical shifts (δ, ppm) and coupling constants (J, Hz) of aromatic protons H-4′–H-7′ in compounds **6**-**9**, **13**-**16**.

Compound, solvent	H-4′ (J)	H-5′ (J)	H-6′ (J)	H-7′ (J)
6 (5′)	7.76 dd	–	7.62 dd	6.64 dd
[D_6]DMSO-	*(1.5, 0.7)*		*(8.0, 1.5)*	*(8.0, 0.7)*
6 (6′)	7.82 d *(7.9)*		–	7.53 s
[D_6]DMSO-	8.12 d *(7.9)*			
7 (5′)	8.63 br. s	–	8.34 dd	7.58 d
[D_6]DMSO-			*(8.0, 1.6)*	*(8.0)*
7 (6′)	8.40–8.49 m		–	8.08 d
[D_4]methanol				*(0.9)*
8 (5′)	8.44 s	–	8.14 d	7.56 d
[D_6]DMSO-			*(8.0)*	*(8.0)*
8 (6′)	8.26 – 8.34 m		–	7.84 s
[D_6]DMSO-				
9 (5′)	8.87 dd	–	8.39 dd	7.52 d
[D_4]methanol	*(1.8, 1.3)*		*(8.0, 1.8)*	*(8.0)*
9 (6′)	8.35 – 8.42 m		–	7.98 d
[D_4]methanol				*(1.0)*
13 (5′)	8.72 d	–	8.27 dd	7.23 d
[D_4]methanol	*(1.5, 0.7)*		*(8.0, 1.5)*	*(8.0)*
13 (6′)	8.10 d	8.23 dd	–	7.78 dd
[D_4]methanol	*(8.0)*	*(8.0, 1.4)*		*(1.4, 0.7)*
14 (5′)	8.85 d	–	8.42 d	7.52 d
[D_4]methanol	*(1.3)*		*(8.0)*	*(8.0)*
14 (6′)	8.32–8.35 m		–	7.97 d
[D_4]methanol				*(1.4)*
15 (5′)	8.71 s	–	8.14 d	7.23 d
[D_4]methanol			*(7.7)*	*(7.7)*
15 (6′)	8.31-8.33 m		–	7.91 br. s
[D_3]acetonitrile				
16 (5′)	8.70 d	–	8.19 dd	7.35 d
[D_4]methanol	*(1.7)*		*(8.0, 1.7)*	*(8.0)*
16 (6′)	8.10 d	8.23 dd	–	7.88 d
[D_4]methanol	*(8.1)*	*(8.1, 1.7)*		*(1.7)*

Table S4. ^{19}F NMR data of red emitting rhodamines **5, 17-27**.[a]

Compound, solvent	F-4´ (*J*)	F-7´ (*J*)	F-5´ (*J*)	F-6´ (*J*)
5-H[b]	-141.8 dd	-108.8 d *(13.5)*	-124.5 d	
[D_6]DMSO	*(26.6, 13.5)*		*(26.6)*	
17	-143.4 dd	-112.4 d	-125.8 dd	–
[D_6]DMSO	*(25.5, 17.3)*	*(17.3)*	*(25.5, 3.6)*	
18	-140.16 dd	-107.7 dd	-124.1 dd	–
[D_4]methanol	*(23.0, 14.2)*	*(14.2, 3.8)*	*(23.0,3.8)*	
19	-107.0 *(15.5)*		–	–
[D_4]methanol	-107.5 *(15.5)*			
20	-137.5 dd	-108.2 dd	-125.5 dd	–
[D_6]acetone	*(23.0, 13.9)*	*(13.9, 3.6)*	*(23.0, 3.6)*	
21	-142.7 m	-109.4 dd	-125.1 dd	–
$CDCl_3$		*(15.2)*	*(22.5)*	
22	-144.8 dd	-110.3 d	-126.8 dd	–
[D_4]methanol	*(24.4, 14.6)*	*(14.6)*	*(24.4, 2.0)*	
23	-144.8 dd	-125.0 m	-143.1 dd	–
[D_4]methanol	*(20.5,12.3)*		*(20.5, 5.3)*	
24	-144.9 dd	-125.1 dd	-142.8 dd	–
[D_4]methanol	*(23, 14)*	*(14, 8)*	*(23, 8)*	
25	-144.7 m	-125.0 m	-142.2 d	–
[D_4]methanol			*(20.7)*	
26	-136.0 m		-150.8 ddd *(22.2, 21.2, 5.8)*	
D_2O	-138.7 ddd *(22.2, 11.3, 5.8)*		-149.3 ddd *(22.2, 21.2, 5.8)*	
27	-138.1 m[c]	- 138.2 m[c]	-152.7 m	-151.1 dd
[D_4]methanol				*(18.9, 4.4)*

[a] Chemical shifts are given in ppm, coupling constants (*J*) in Hz; [b] second set of signals of lower intensity was observed ; [c] overlapping multiplets.

Figure S3. Diagnositic regions of the ^{1}H NMR spectra of the two isomers of compound **11** in $CDCl_3$. a) 5´-isomer with the signals of H-7´, H-6´ and H-4´, and b) 6´-isomer with the signals of H-7´, H-4´ and H-5´.

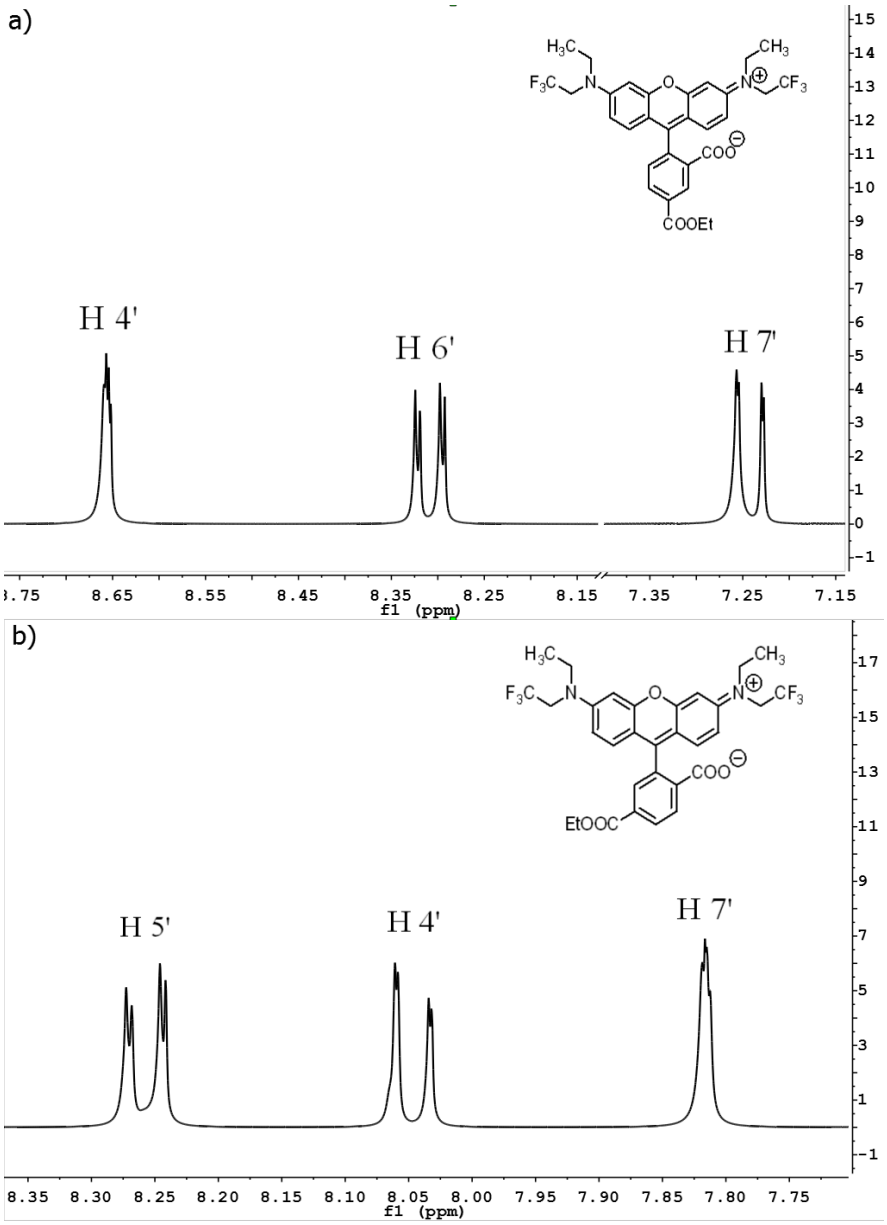


Figure S4. Aromatic regions of the 2D NOESY spectra of the two isomers of **11** in $CDCl_3$. a) 5´ isomer; b) 6´ isomer.

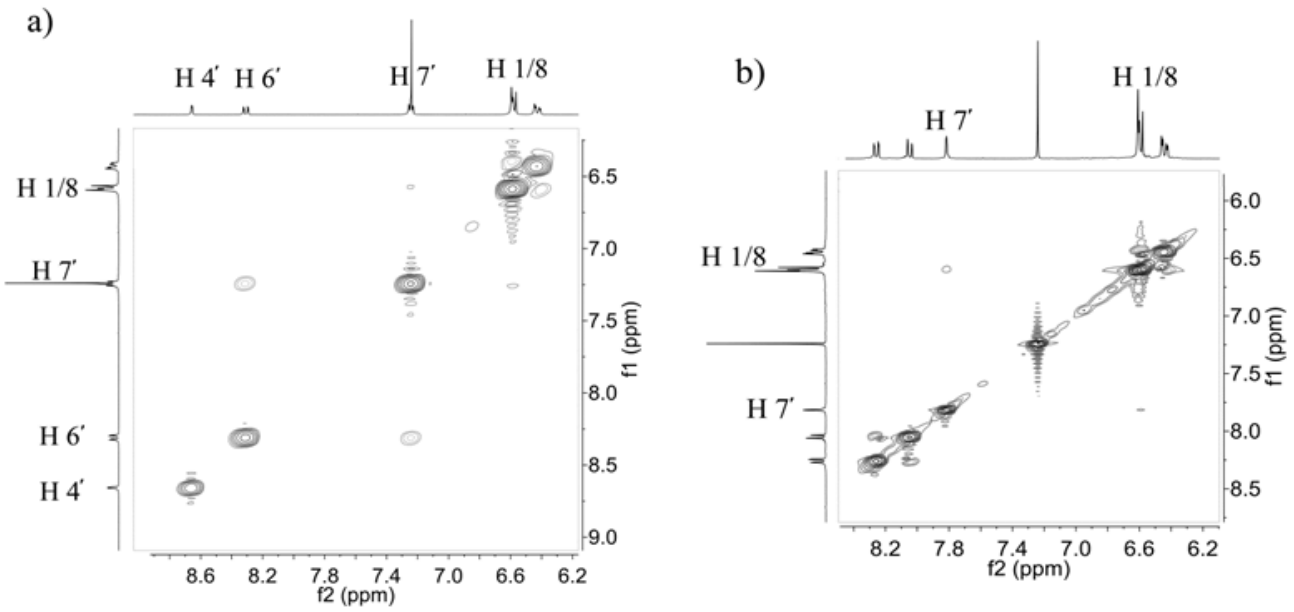
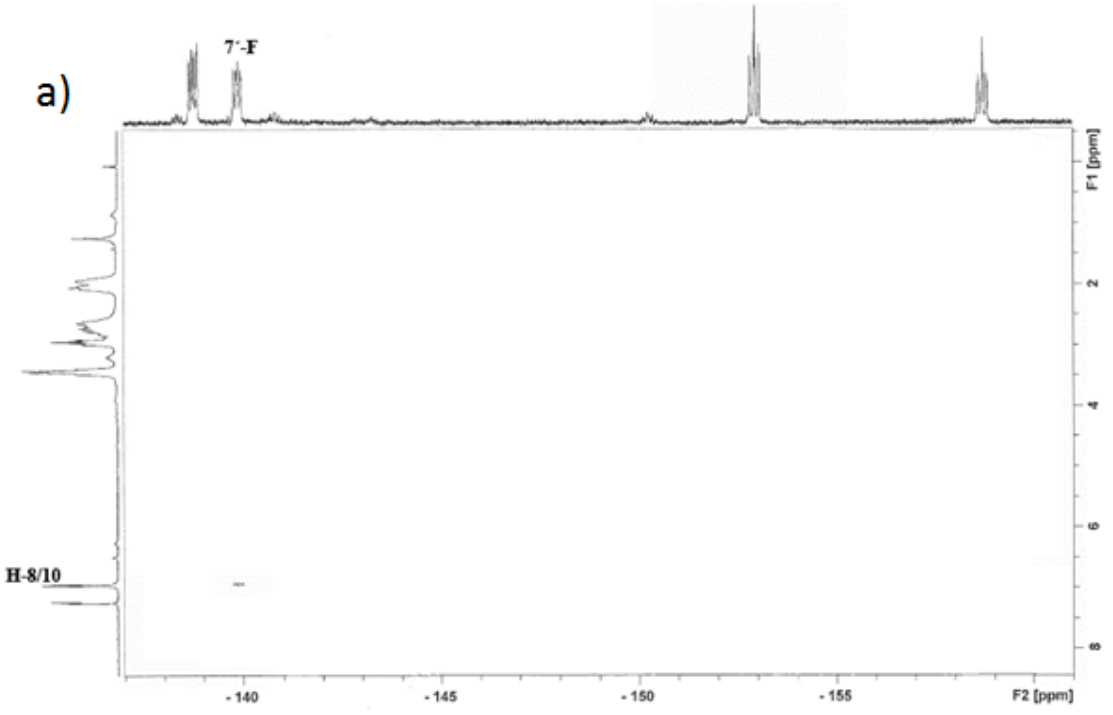
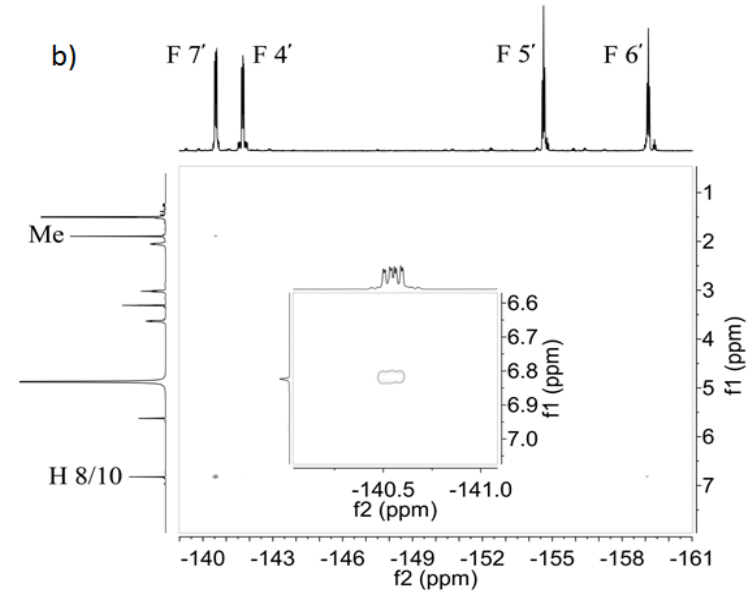
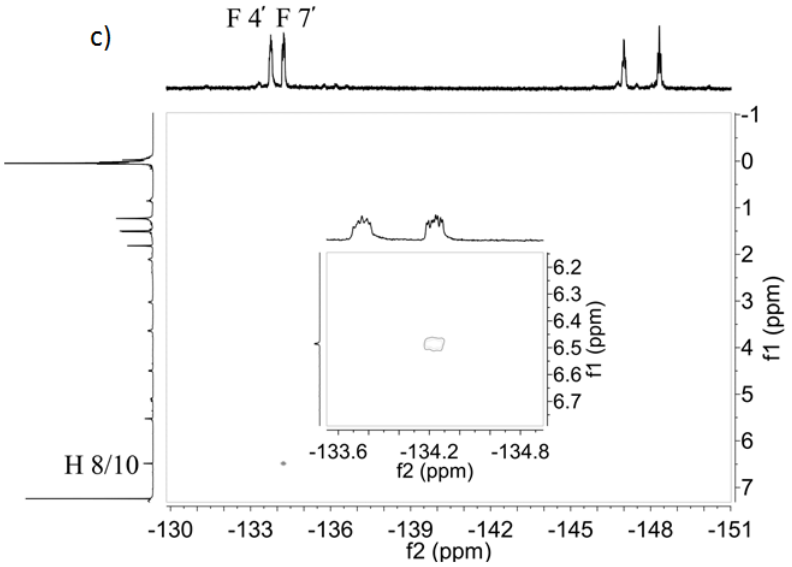
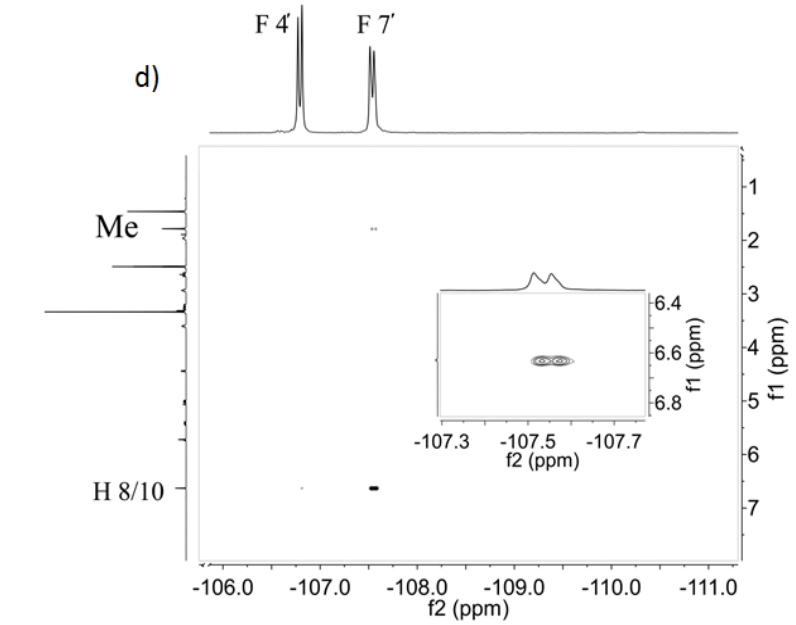


Figure S5. $^{1}H,^{19}F$-HOESY spectrum of a) compound **1,** b) compound **2**, c) compound **27**, d) compound **28,** e) compound **4**.




b)
F 7′
F 4′
F 5′
F 6′
Me
H 8/10
f1 (ppm)
f2 (ppm)
-140 -143 -146 -149 -152 -155 -158 -161
-140.5 -141.0
6.6 6.7 6.8 6.9 7.0


c)
F 4′ F 7′
H 8/10
f1 (ppm)
f2 (ppm)
-130 -133 -136 -139 -142 -145 -148 -151
-133.6 -134.2 -134.8
6.2 6.3 6.4 6.5 6.6 6.7


d)
F 4′
F 7′
Me
H 8/10
-106.0
-107.0
-108.0
-109.0
-110.0
-111.0
f2 (ppm)
f1 (ppm)
-107.3
-107.5
-107.7
6.4
6.6
6.8

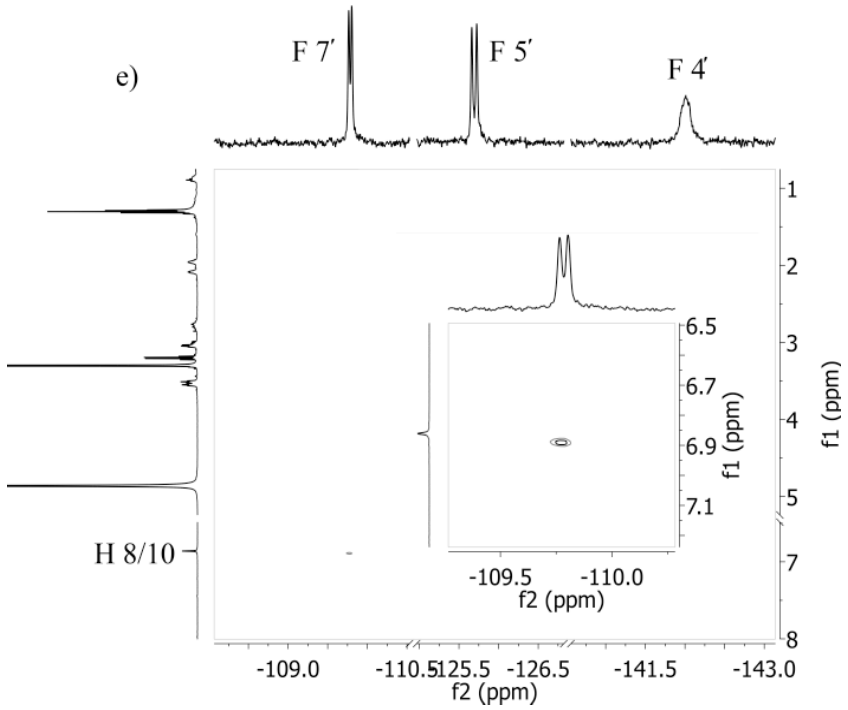

e)
F 7′
F 5′
F 4′
H 8/10
-109.0
-110.5
-125.5
-126.5
-141.5
-143.0
f2 (ppm)
f1 (ppm)
-109.5
-110.0
6.5
6.7
6.9
7.1

Figure S6a. $^{1}H,^{1}H$-NOESY spectrum of the compound **28**.

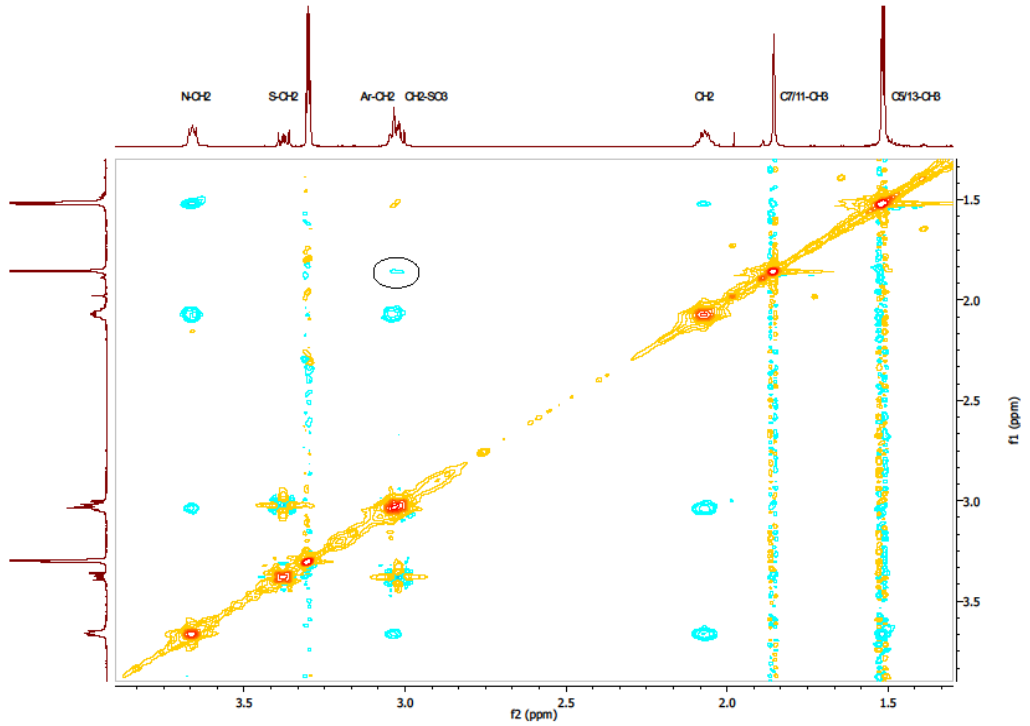


Figure S6b. $^{1}H,^{1}H$-NOESY spectrum of the compound **29**

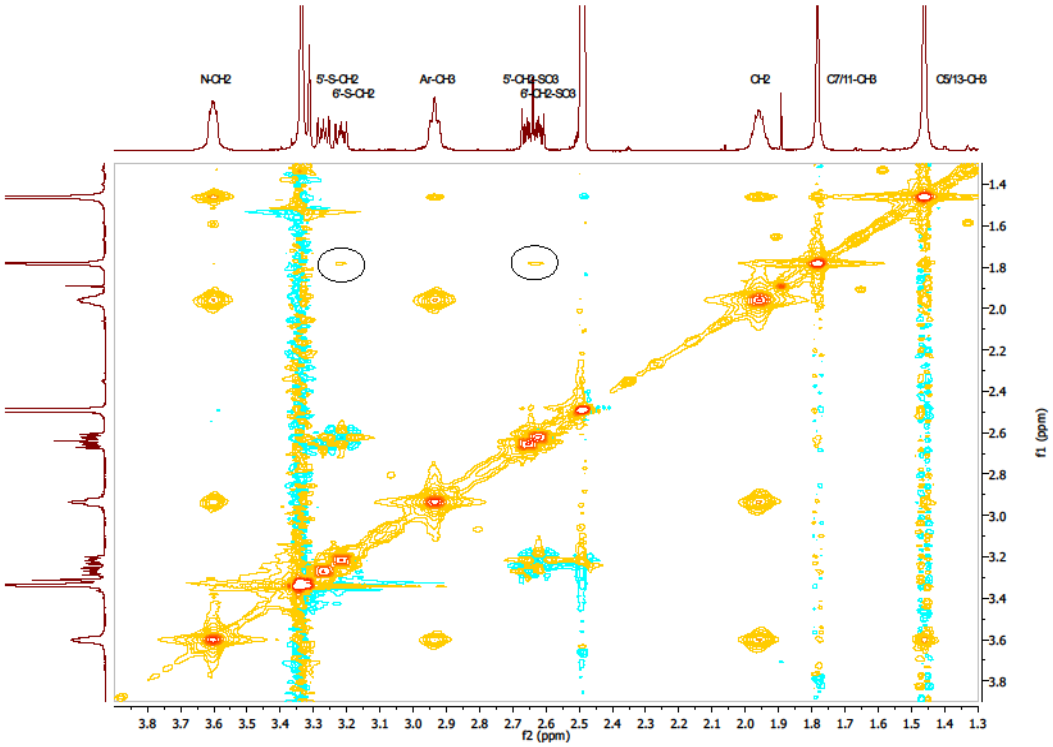


Figure S7a. $^{1}H,^{13}C$-HMBC spectrum of the compound **28**

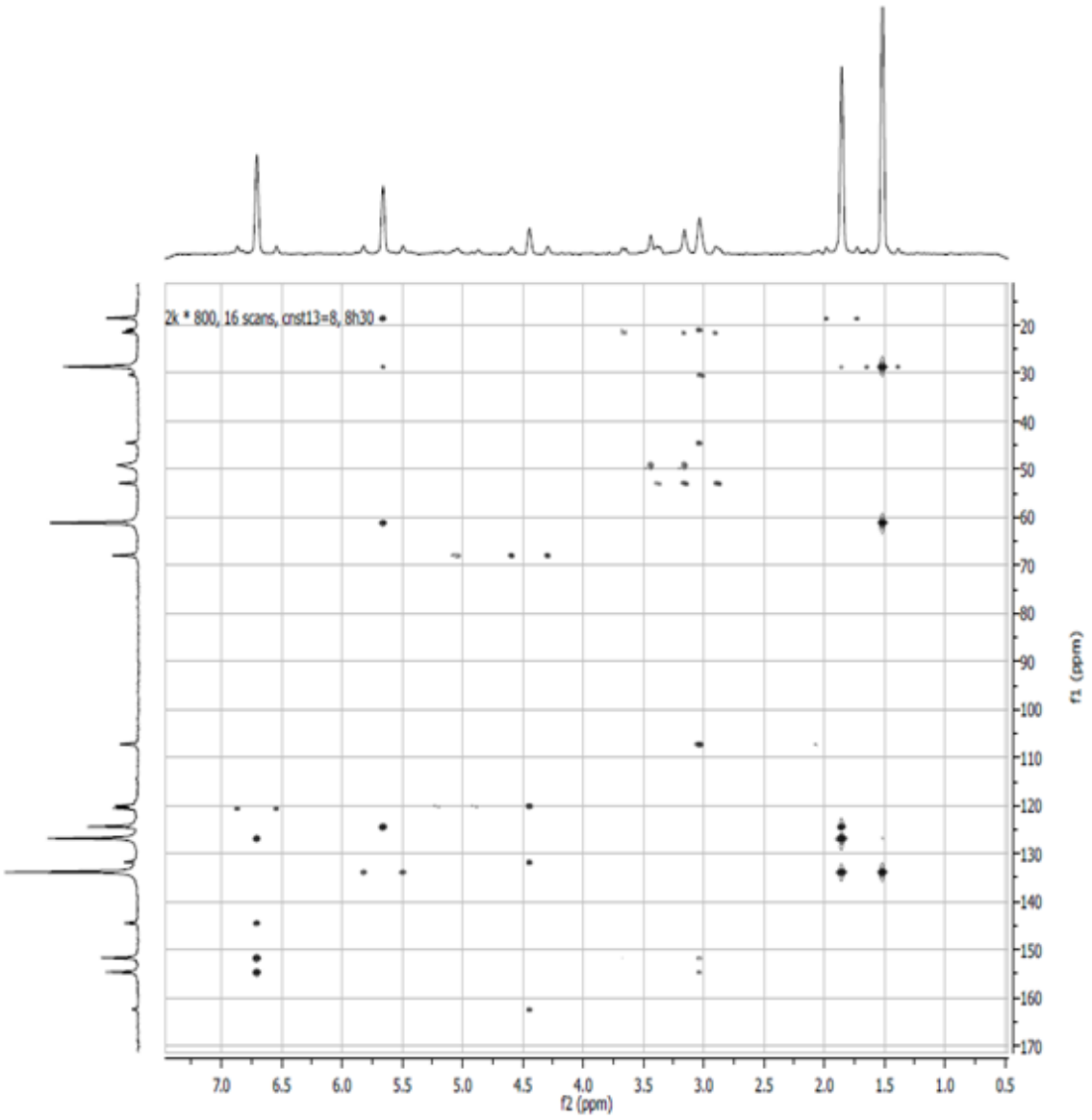


Figure S7b. $^{1}H,^{13}C$-HMBC spectrum of the compound **29**

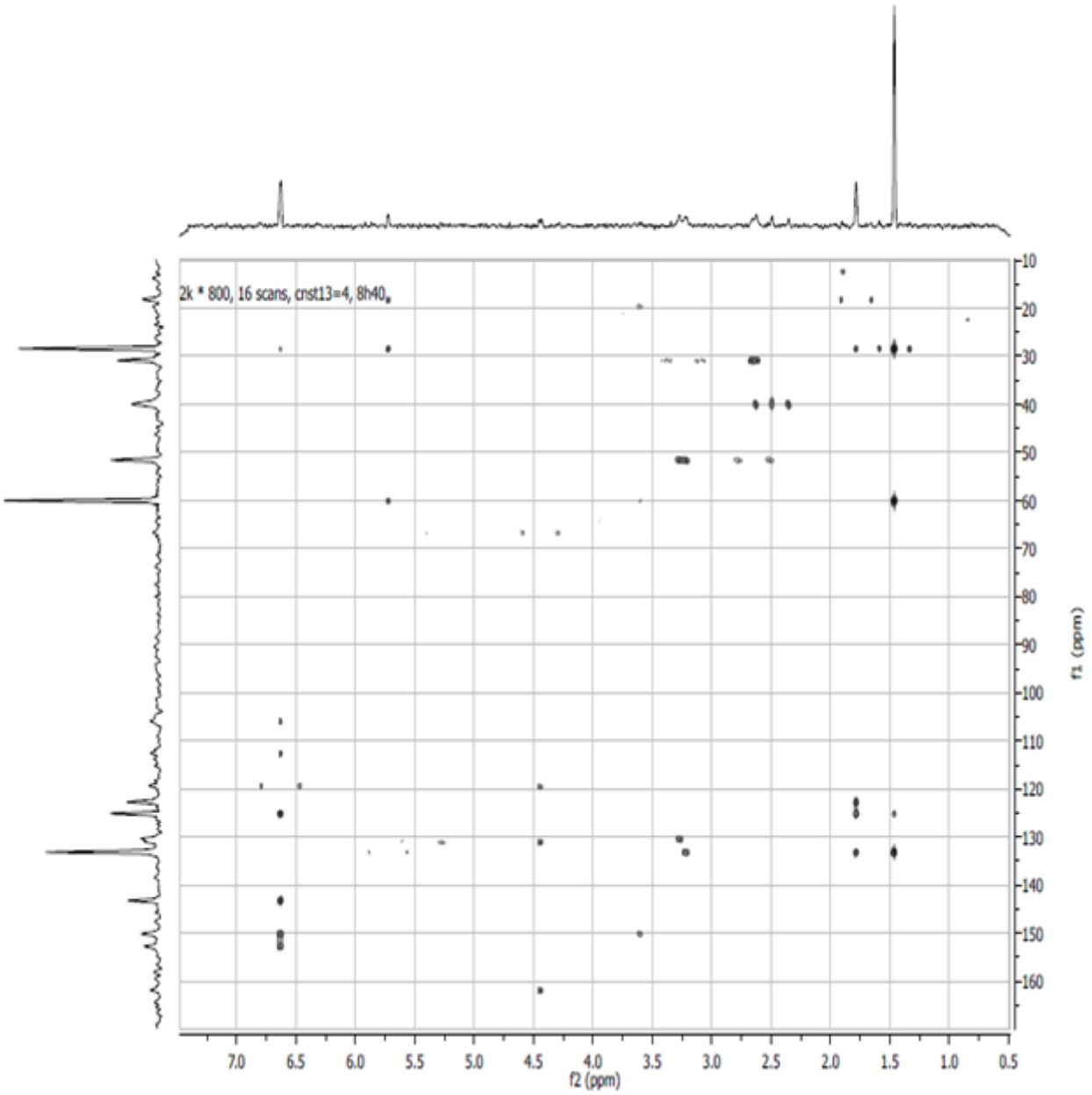
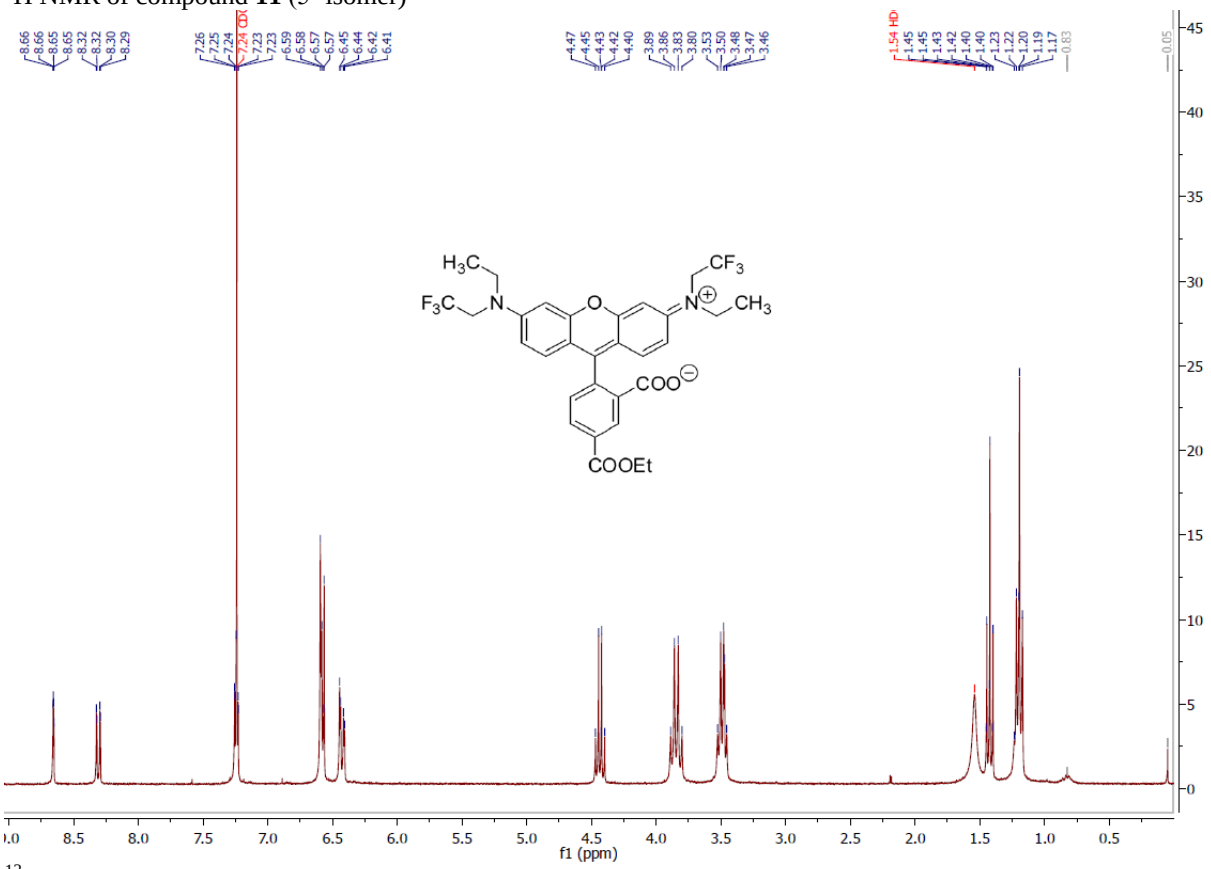
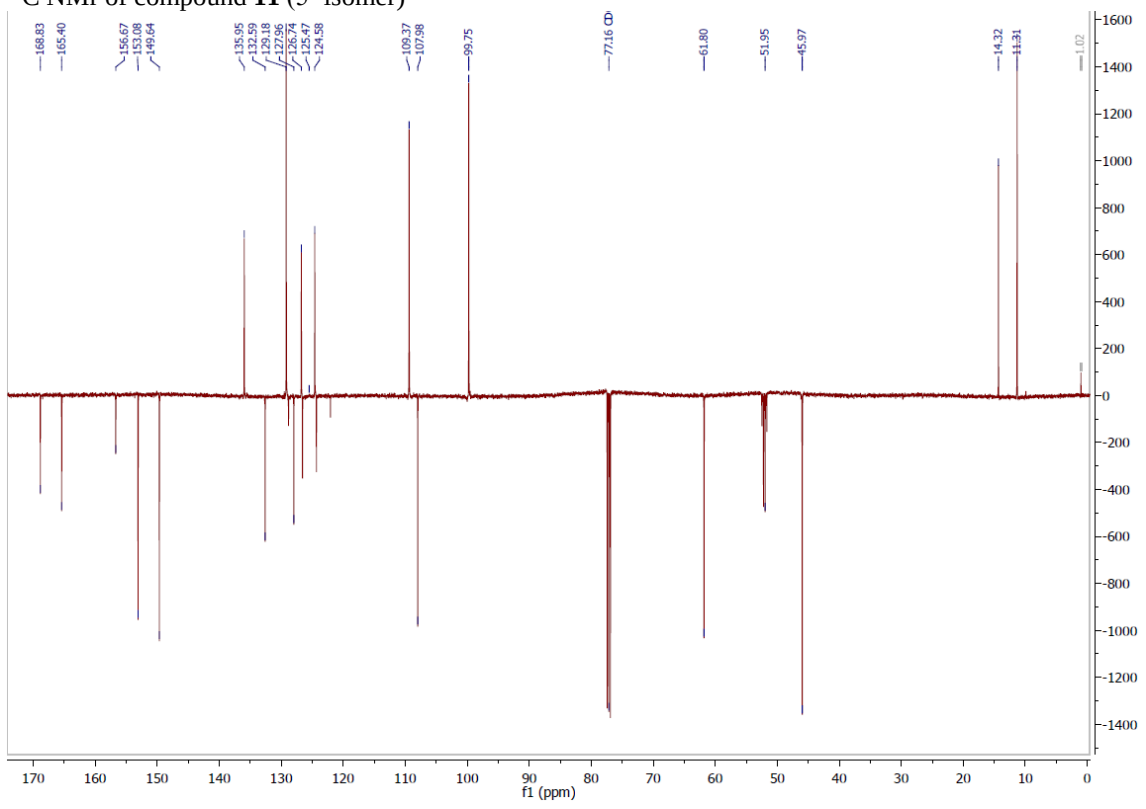


Figure S8. ^{1}H, ^{13}C NMR spectra and IR spectra

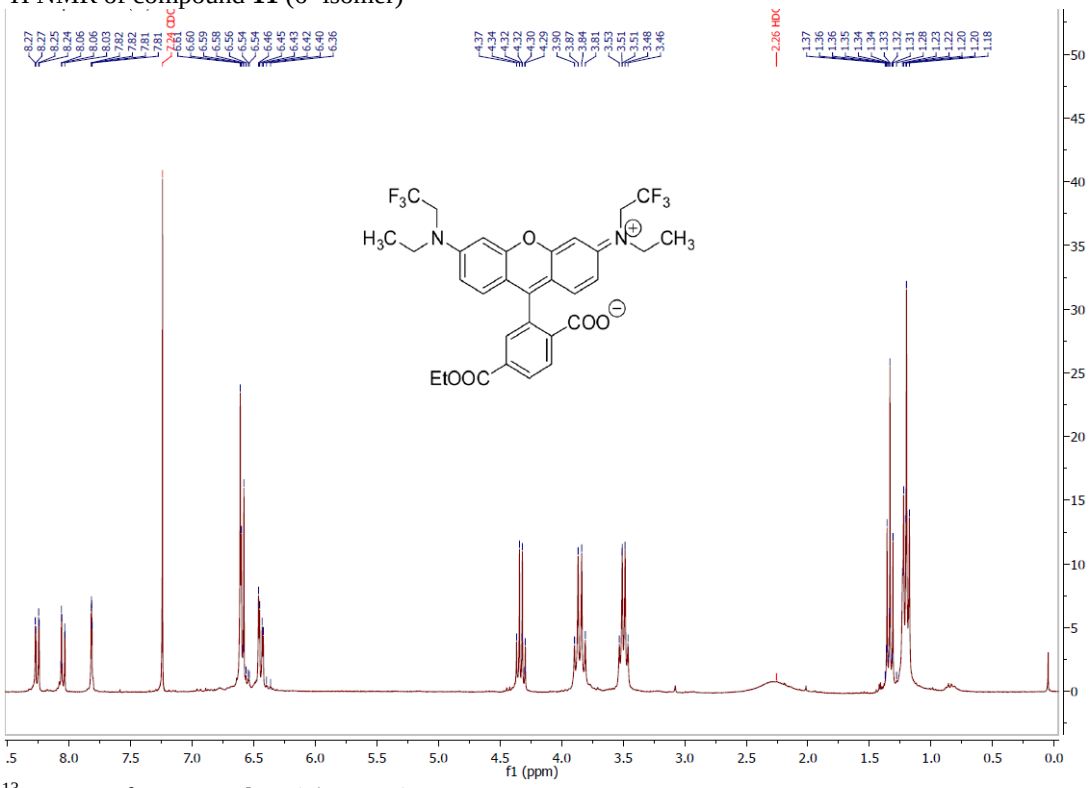
^{1}H NMR of compound **11** (5′-isomer)



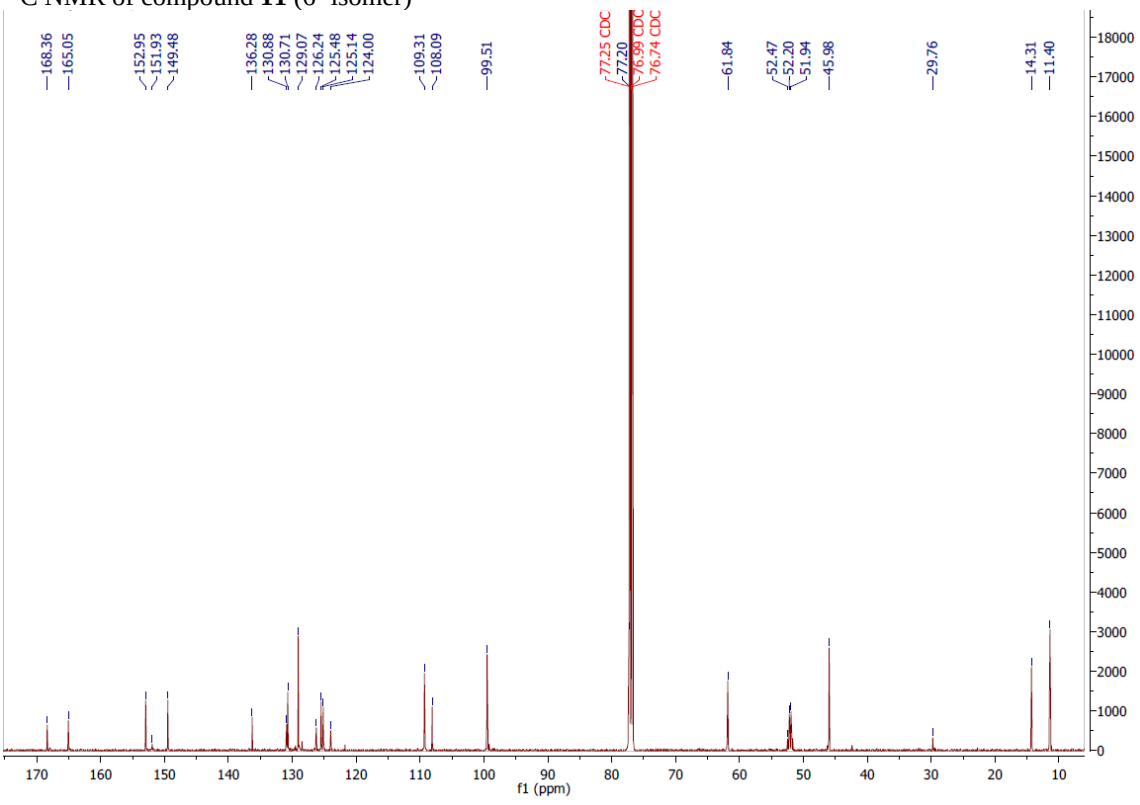
^{13}C NMr of compound **11** (5′-isomer)



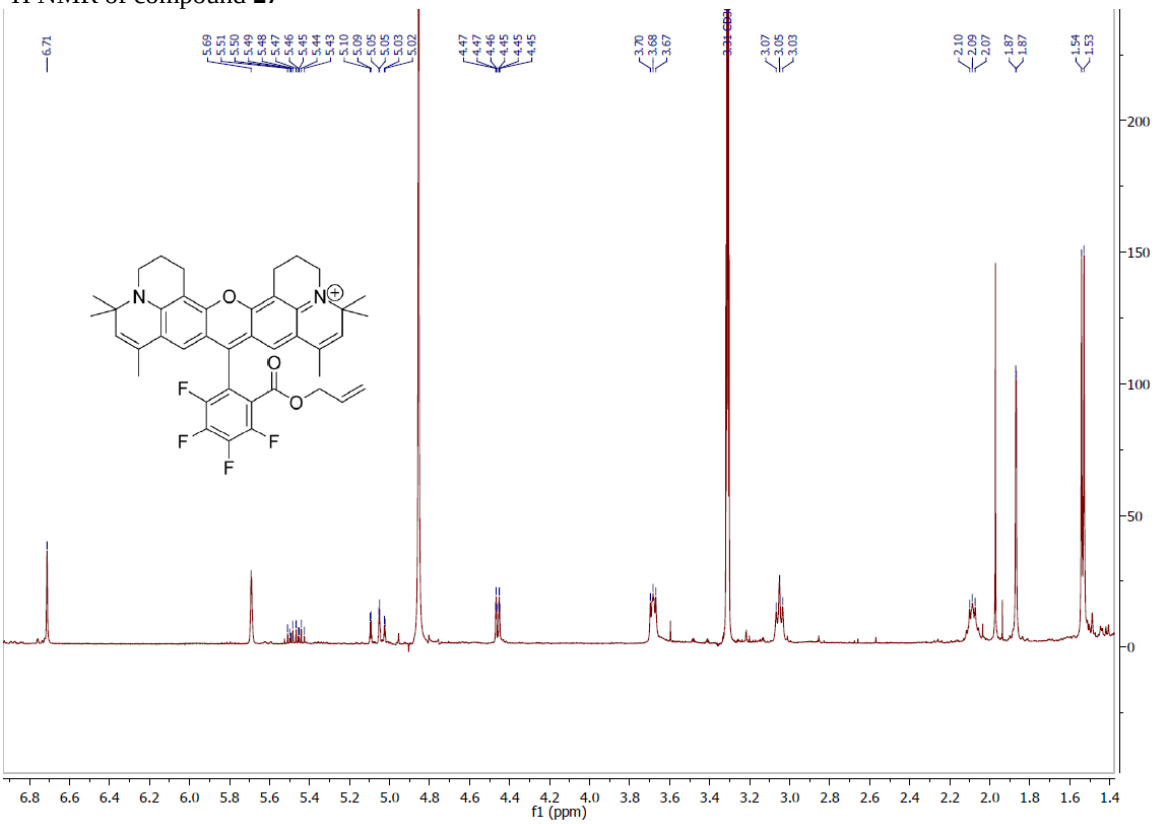
^{1}H NMR of compound **11** (6′-isomer)



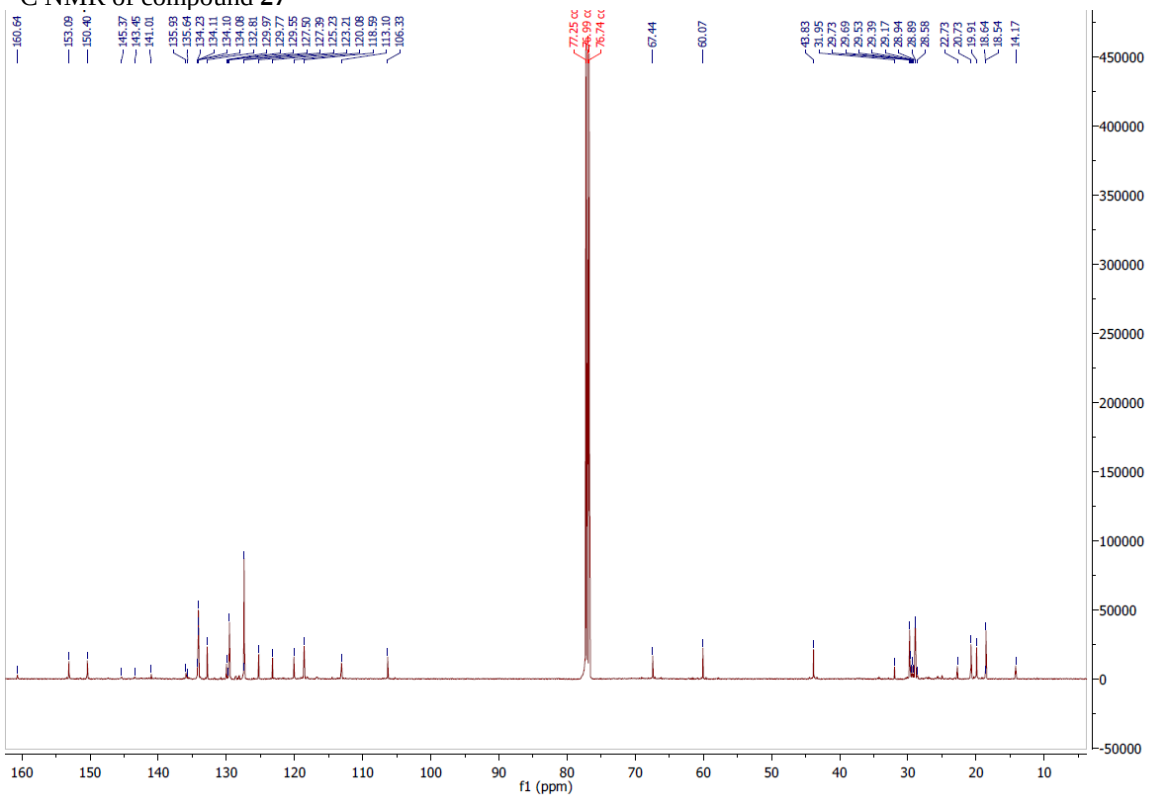
^{13}C NMR of compound **11** (6′-isomer)



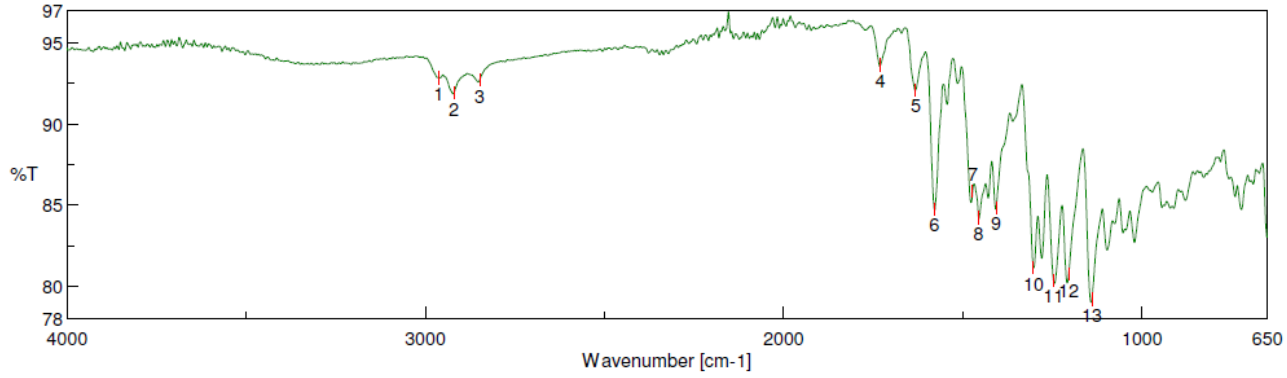
^{1}H NMR of compound **27**



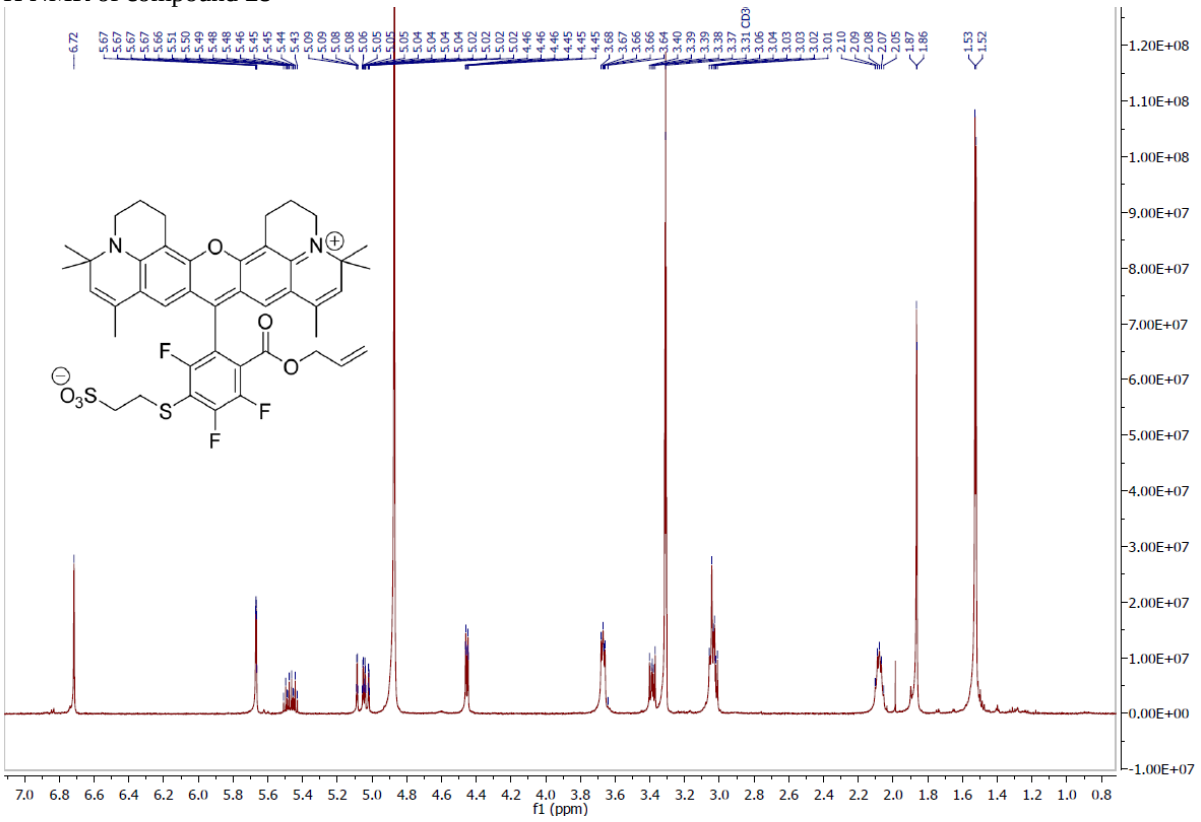
^{13}C NMR of compound **27**



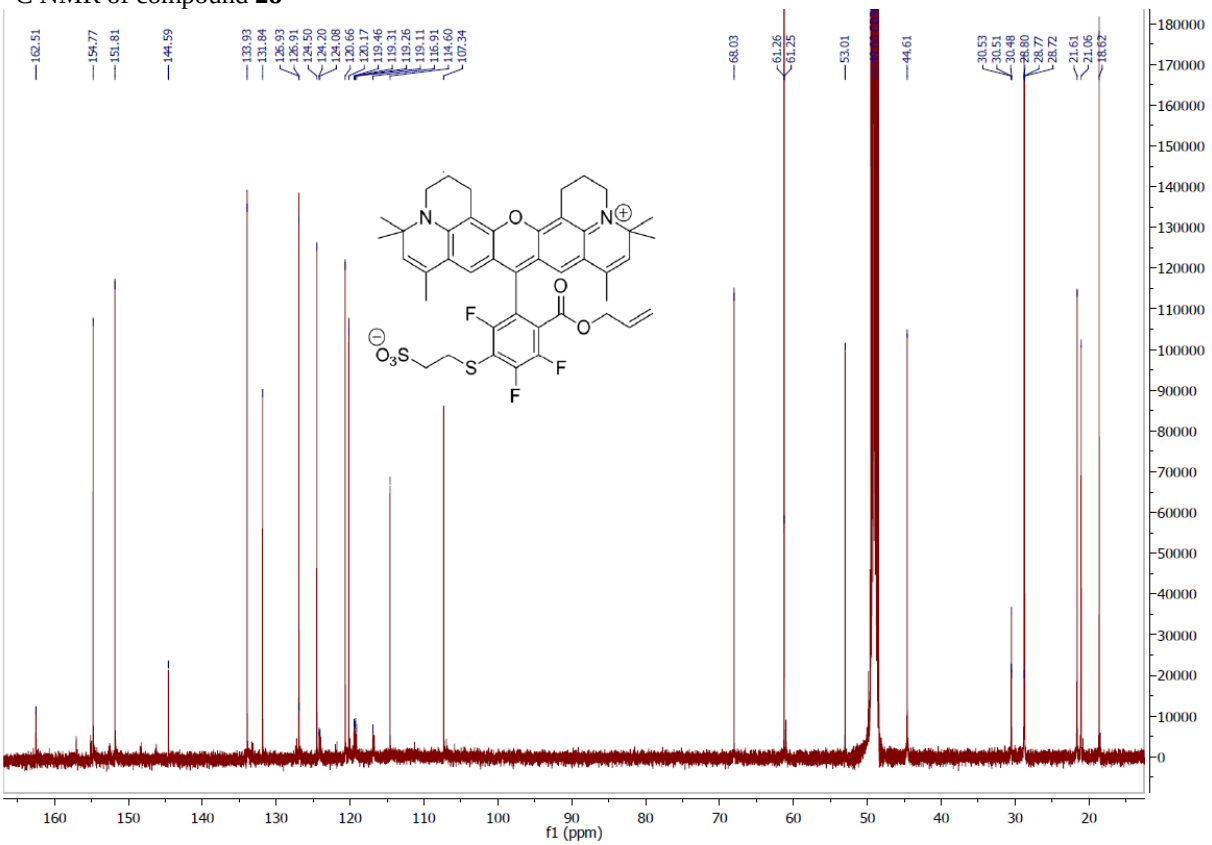
FT-IR of compound **27**



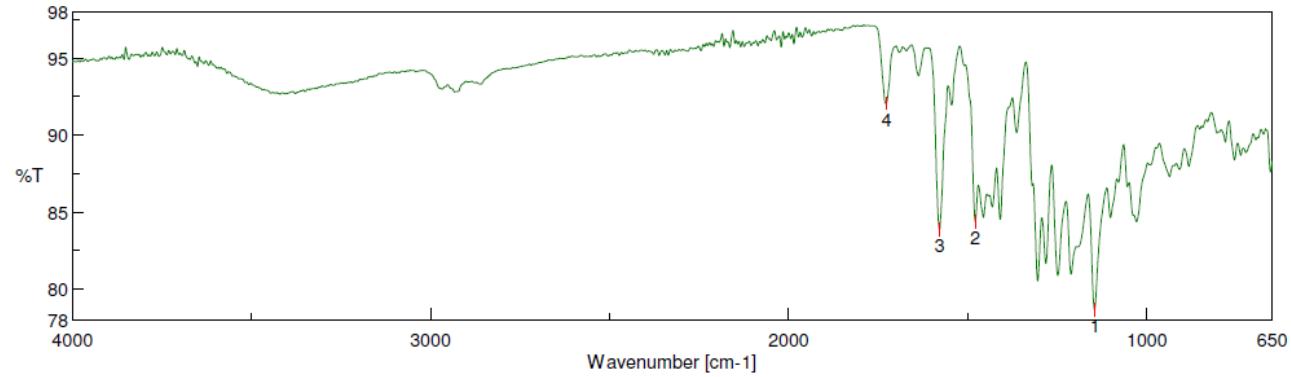
^{1}H NMR of compound **28**



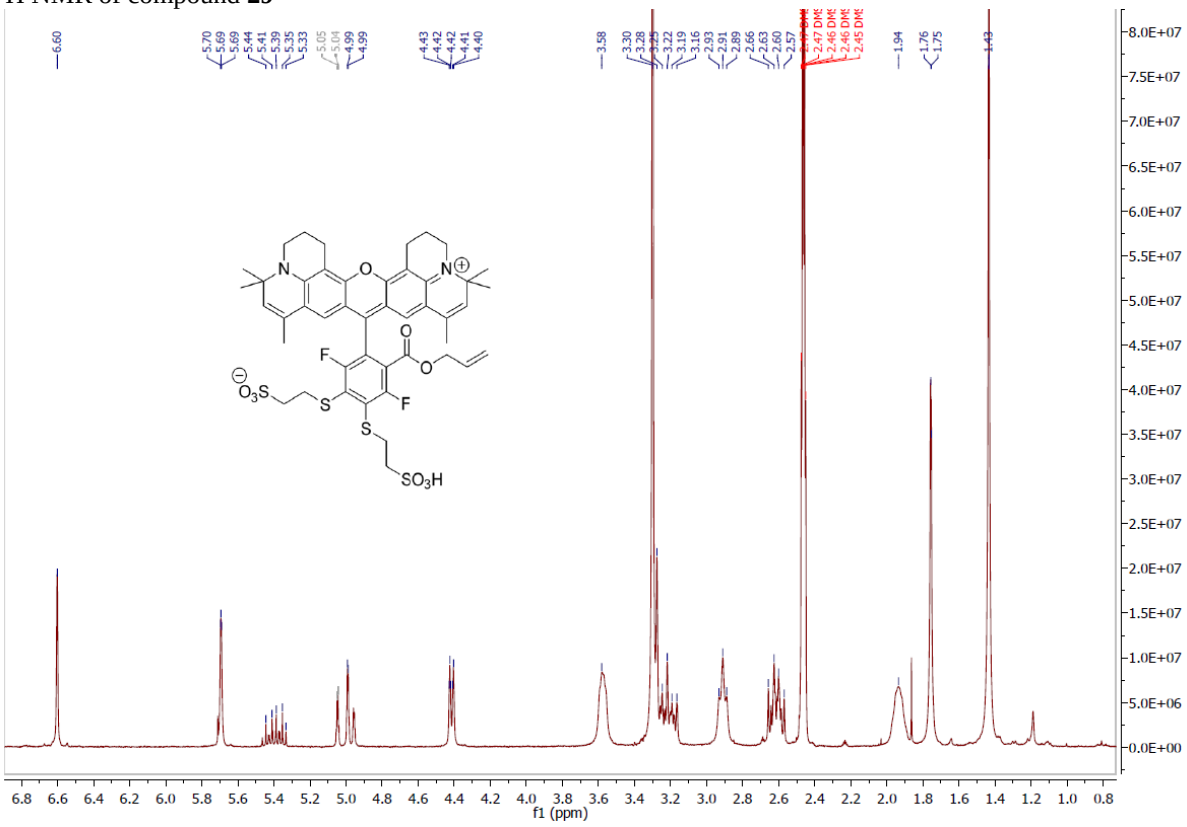
^{13}C NMR of compound **28**



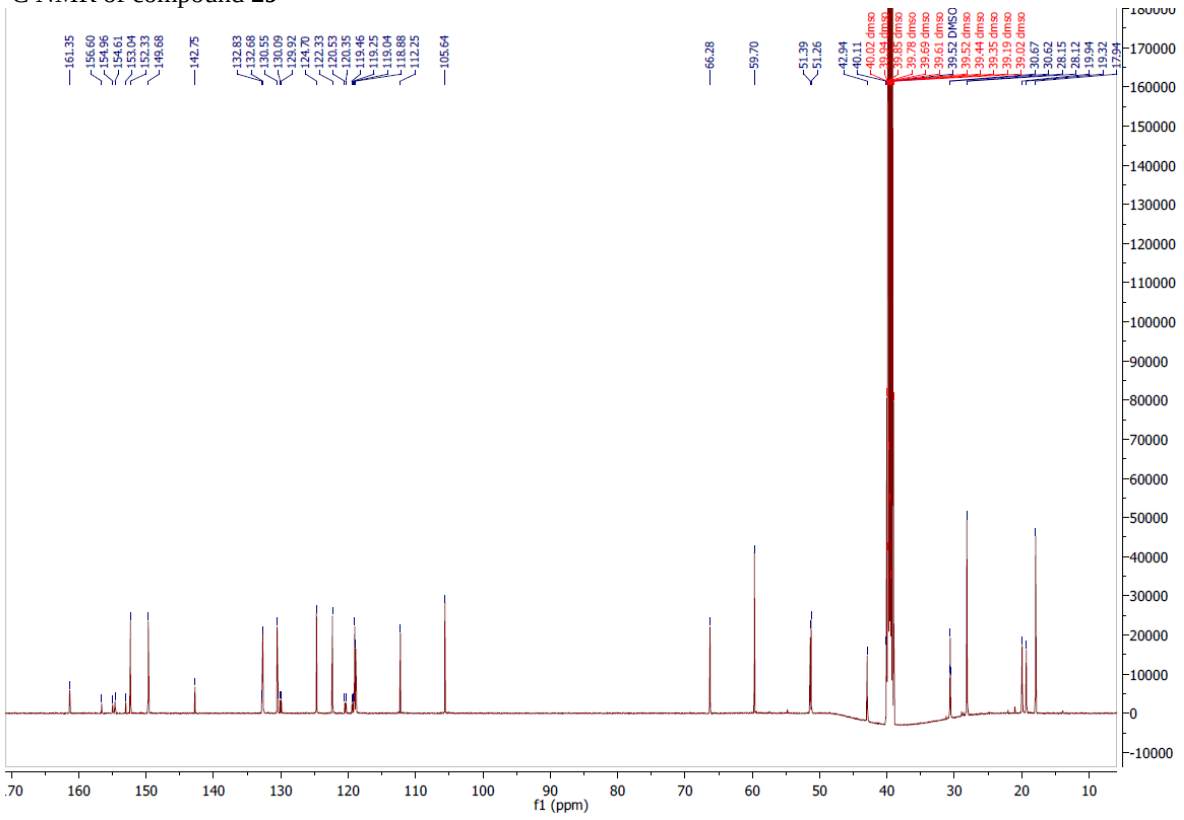
FT-IR compound **28**



^{1}H NMR of compound **29**



^{13}C NMR of compound **29**



FT-IR compound **29**