

Supplementary informations

Clickable C-glycosyl scaffold for the development of a dual fluorescent and [¹⁸F]fluorinated cyanine-containing probe and preliminary in vitro/vivo evaluation by fluorescence imaging

Julen Ariztia, Kamal Jouad, Valérie Jouan-Hureaux, Julien Pierson, Charlotte Collet, Bertrand Kuhnast, Katalin Selmeczi, Cédric Boura, Sandrine Lamandé-Langle, Nadia Pellegrini Moïse

Preparation of compounds **4**, **5**, **21**

Copy of ¹H NMR spectra for compounds **6-20**, **22**, **25-27**

Copy of ¹³C NMR spectra for compounds **6-20**, **26-27**

Copy of ¹⁹F NMR spectra for compounds **6**, **8**, **10**, **12**, **14**, **17**, **26**, **27**

Copy of HRMS for compounds **21-22** and **23-24**

Analytical HPLC profile of compound **19** (Figures S1 and S2)

References

3,6-anhydro-2-deoxy-4,5-*O*-(1-methylethylidene)-7-*O*-acetyl-D-ribo-heptanoic acid methyl ester 4¹

To a solution of **3-*E*** and **3-*Z*** (1 g, 3.50 mmol) in ethyl acetate (100 mL), 400 mg of Pd/C 10% w (40% w/w) were added under inert atmosphere. The reaction mixture was stirred at 40 psi of hydrogen atmosphere for 48 h (Paar apparatus). After 48 h, the catalyst was filtered off through a Celite[®] pad. 400 mg Pd/C 10% w (40% w/w) were added to the filtrate and the mixture was stirred for another 48 h at 40 psi of hydrogen atmosphere. The catalyst was filtered off through a Celite[®] pad and the filtrate was concentrated under *vacuum*. The crude product was purified by flash chromatography on silica gel (eluent: cyclohexane/EtOAc 100/0 to 50/50) to afford the compound to afford the compound **4**. Quantitative yield, colorless oil, R_f = 0.48 (Cycl/EtOAc: 6/4); ¹H NMR (CDCl₃, 400 MHz): δ (ppm) = 1.34 (s, 3H, CH₃), 1.49 (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 2.72 (dd, 1H, $J_{2a,2b}$ = 16.0, $J_{2,3}$ = 5.0 Hz, *H*-2a), 2.81 (dd, 1H, $J_{2b,3}$ = 6.0 Hz, *H*-2b), 3.71 (s, 3H, OCH₃), 4.05 (dd, 1H, $J_{7a,7b}$ = 11.0, $J_{7a,6}$ = 4.5 Hz, *H*-7a), 4.13-4.28 (m, 2H, *H*-7b and *H*-6), 4.36 (m, 1H, *H*-3), 4.67 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.80 (dd, 1H, *H*-4).

3,6-anhydro-2-deoxy-4,5-*O*-(1-methylethylidene)-7-hydroxy-D-ribo-heptanoic acid methyl ester 5¹

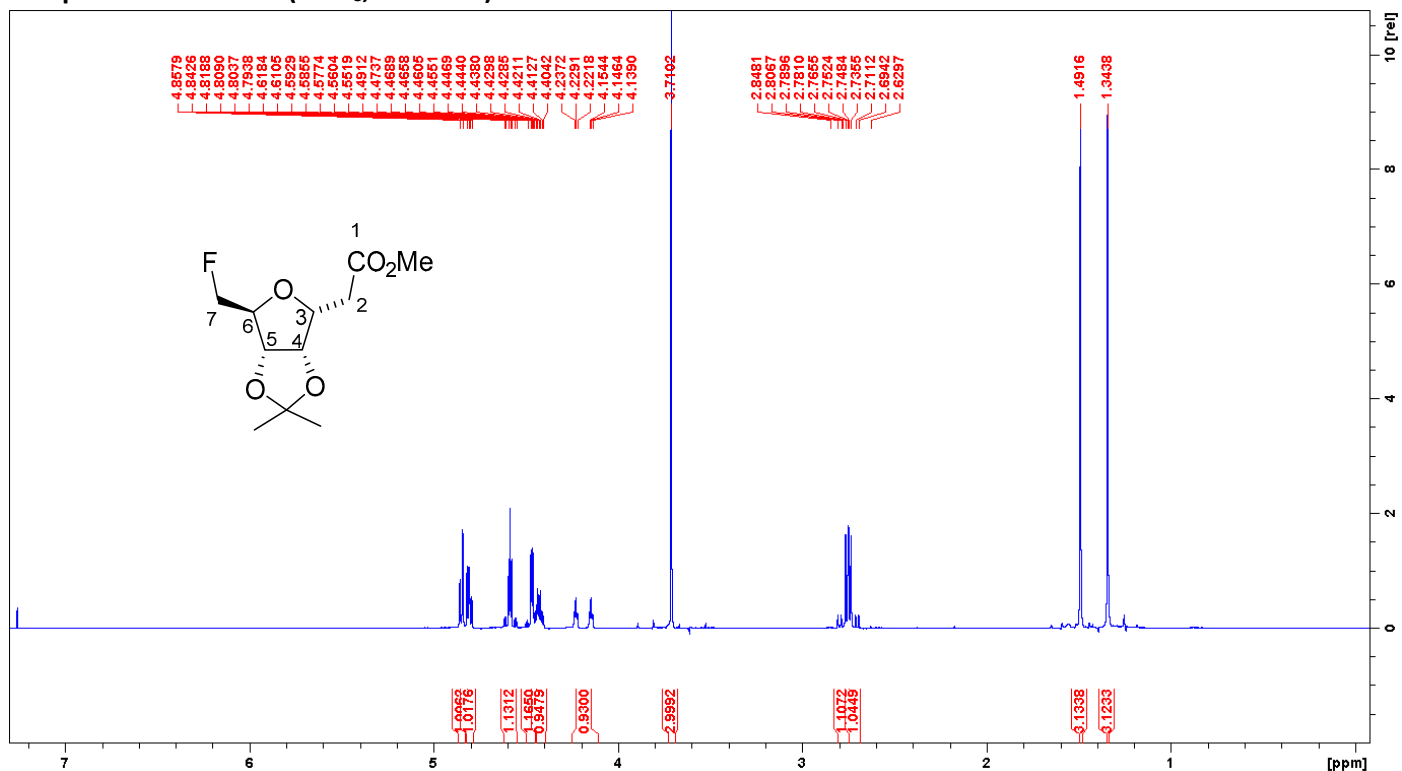
To a solution of **4** (500 mg, 1.70 mmol) in dry methanol (20 mL), a catalytic amount of sodium was added under inert atmosphere. The reaction mixture was stirred for 3 h at room temperature. The mixture was neutralized by addition of Amberlite IR-120 (and filtered). The solvent was removed under *vacuum*, the crude product was purified by flash chromatography on silica gel (eluent: cyclohexane/EtOAc 100/0 to 40/60) to afford the compound **5**. Yield: 85% as a yellow oil, R_f = 0.10 (Cycl/EtOAc: 6/4); ¹H NMR (CD₃OD, 400 MHz): δ (ppm) = 1.32 (s, 3H, CH₃), 1.44 (s, 3H, CH₃), 2.66 (dd, 1H, $J_{2a,2b}$ = 16.5 Hz, $J_{2a,3}$ = 7.0 Hz, *H*-2a), 2.72 (dd, 1H, $J_{2b,2a}$ = 16.5 Hz, $J_{2b,3}$ = 7.0 Hz, *H*-2b), 3.53 (dd, 1H, $J_{7a,7b}$ = 12.0 Hz, $J_{7a,6}$ = 5.5 Hz, *H*-7a), 3.58 (dd, 1H, $J_{7b,7a}$ = 12.0 Hz, $J_{7b,6}$ = 5.5 Hz, *H*-7b), 3.68 (s, 3H, OCH₃), 4.00 (app td, 1H, $J_{6,7b}$ = $J_{6,7a}$ = 5.5 Hz, $J_{6,5}$ = 1.0 Hz, *H*-6), 4.40 (app td, 1H, $J_{3,2a}$ = $J_{3,2b}$ = 7.0 Hz, $J_{3,4}$ = 4.0 Hz, *H*-3), 4.74 (dd, 1H, $J_{5,4}$ = 6.0 Hz, $J_{5,6}$ = 1.0 Hz, *H*-5), 4.77 (dd, 1H, $J_{4,5}$ = 6.0 Hz, $J_{4,3}$ = 4.0 Hz, *H*-4).

Compound 21²

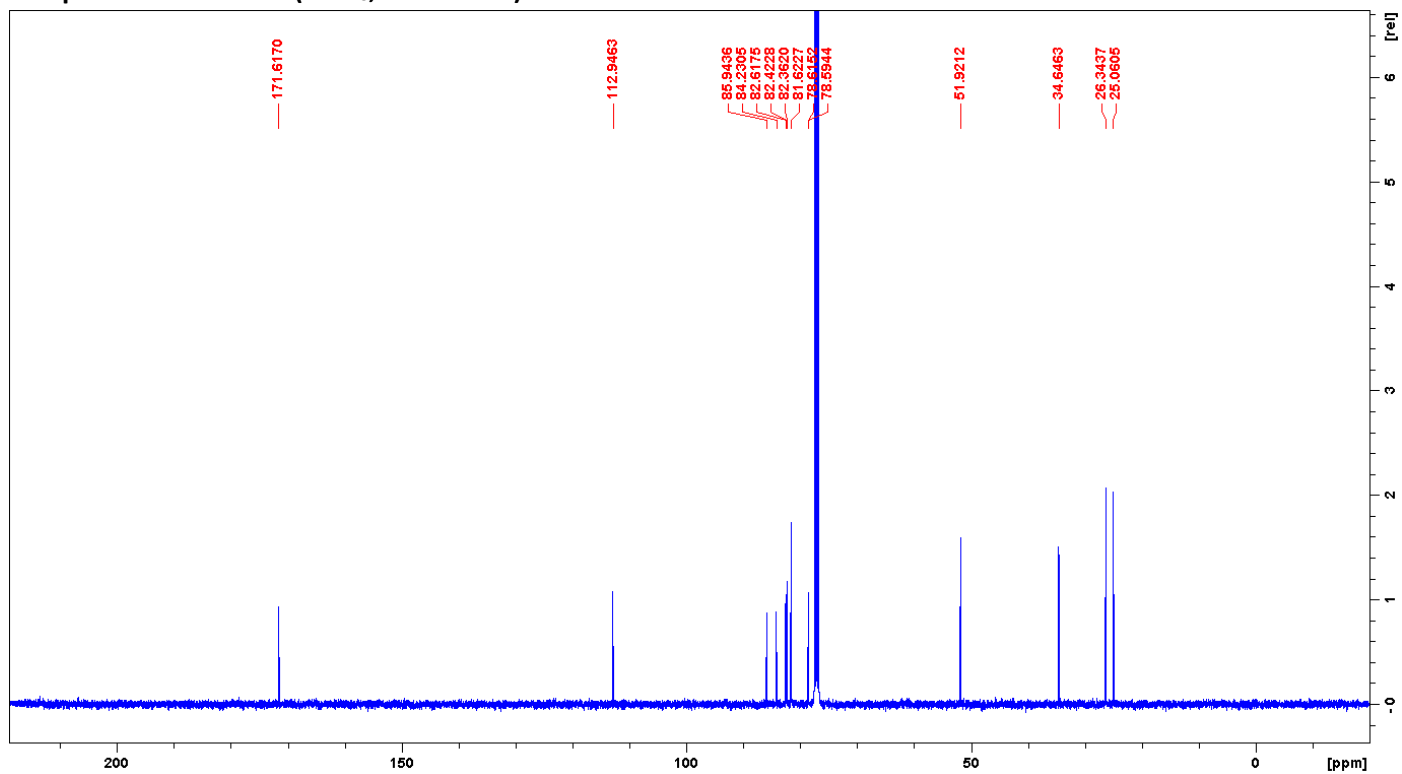
To a solution of c(RGDfK) (10.0 mg, 14.0 μ mol, 1.0 eq.) in DMF (1 mL), 7.1 mg of 6-azidohexanoic acid (28.0 μ mol, 2.0 eq.) and 5 μ L of Et₃N (35.0 μ mol, 2.5 eq.) were added and the mixture was stirred at 30 °C for 16 h. The organic solvent was evaporated under *vacuum* and the solid residue was washed with diethyl ether. The obtained solid was dried under *vacuum*.

to afford the compound **21**. Yield: 75% as white powder. **HRMS** $[M]^+$ $m/z = 743.3979$ (calculated for $C_{33}H_{51}N_{12}O_8$: 743.3947).

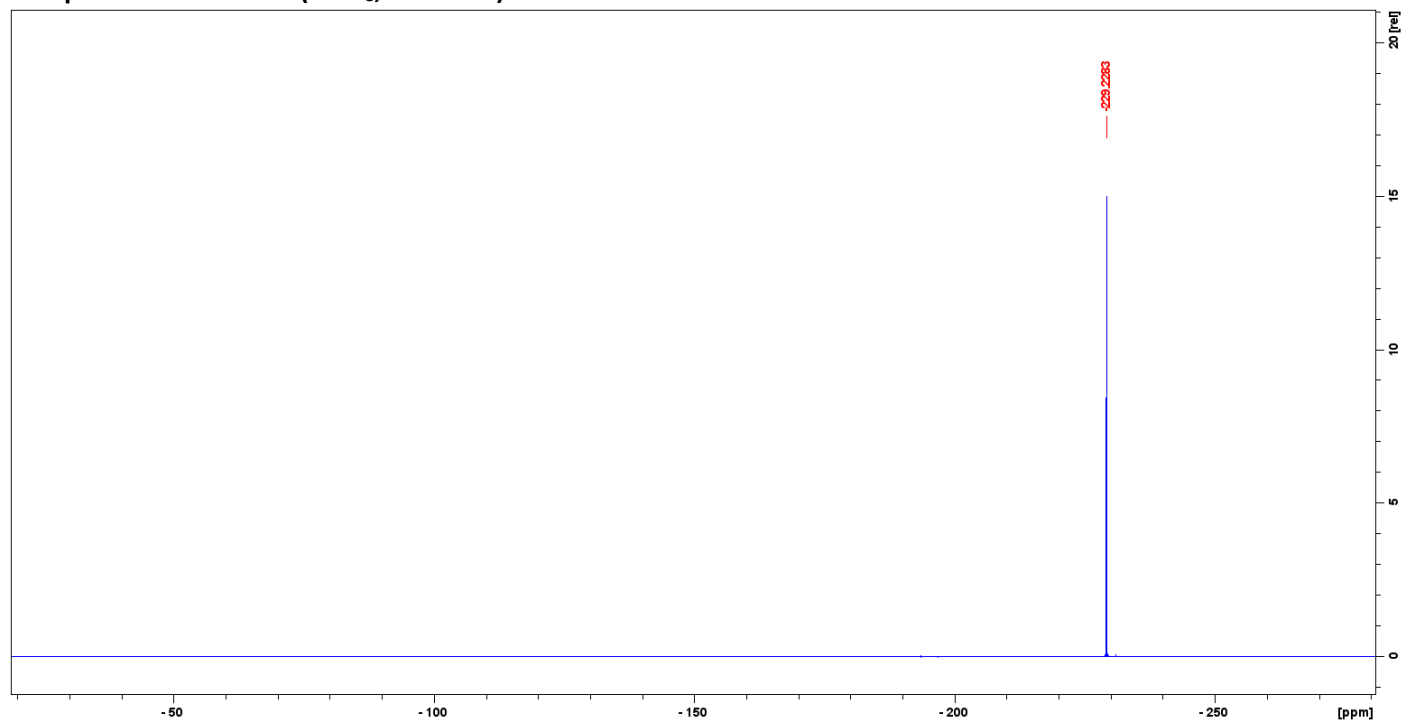
Compound 6 - ^1H NMR (CDCl_3 , 400 MHz)



Compound 6 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



Compound 6 – ^{19}F NMR (CDCl_3 , 376 MHz)

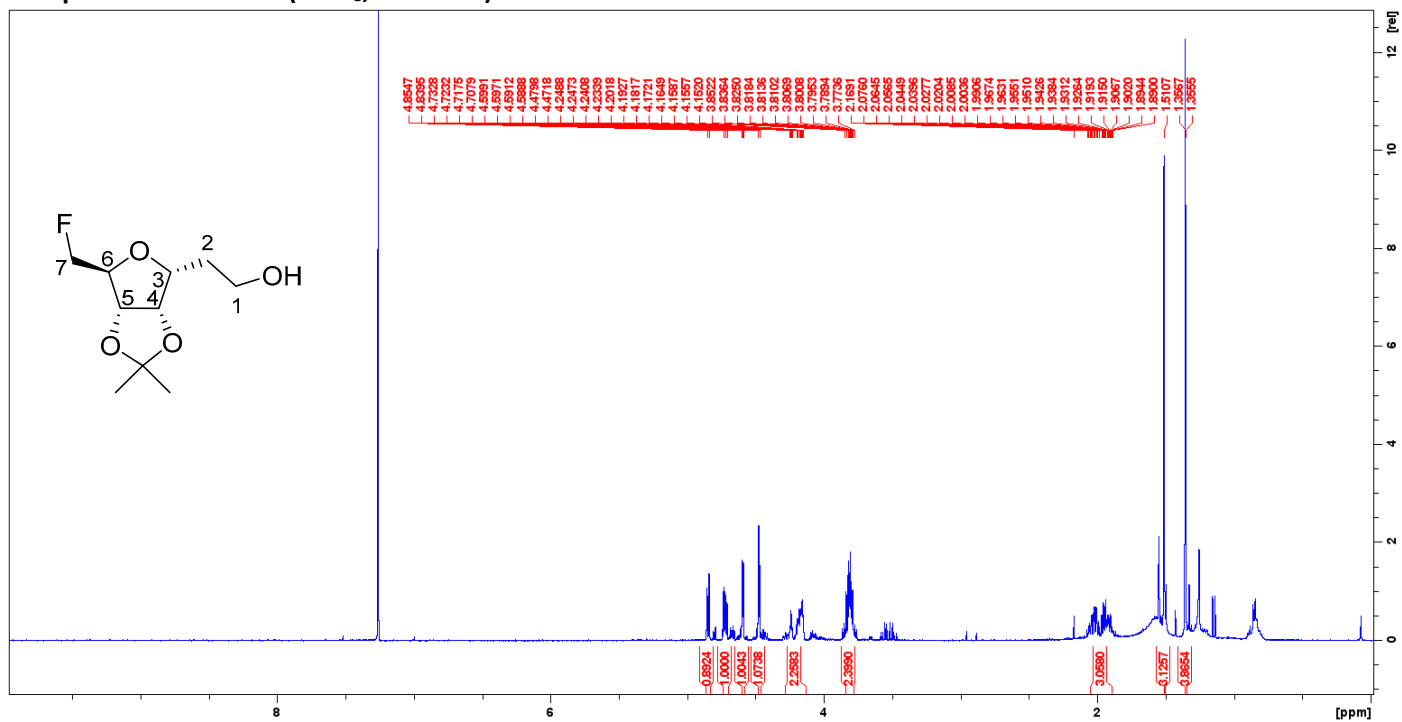


Chemical structure of compound 1 is shown above the spectrum. The structure is a furanose derivative with a TBDPSO group at C7, a CO₂Me group at C2, and a 1,3-dioxolane ring at C4. The peaks are labeled with their chemical shifts and integrations.

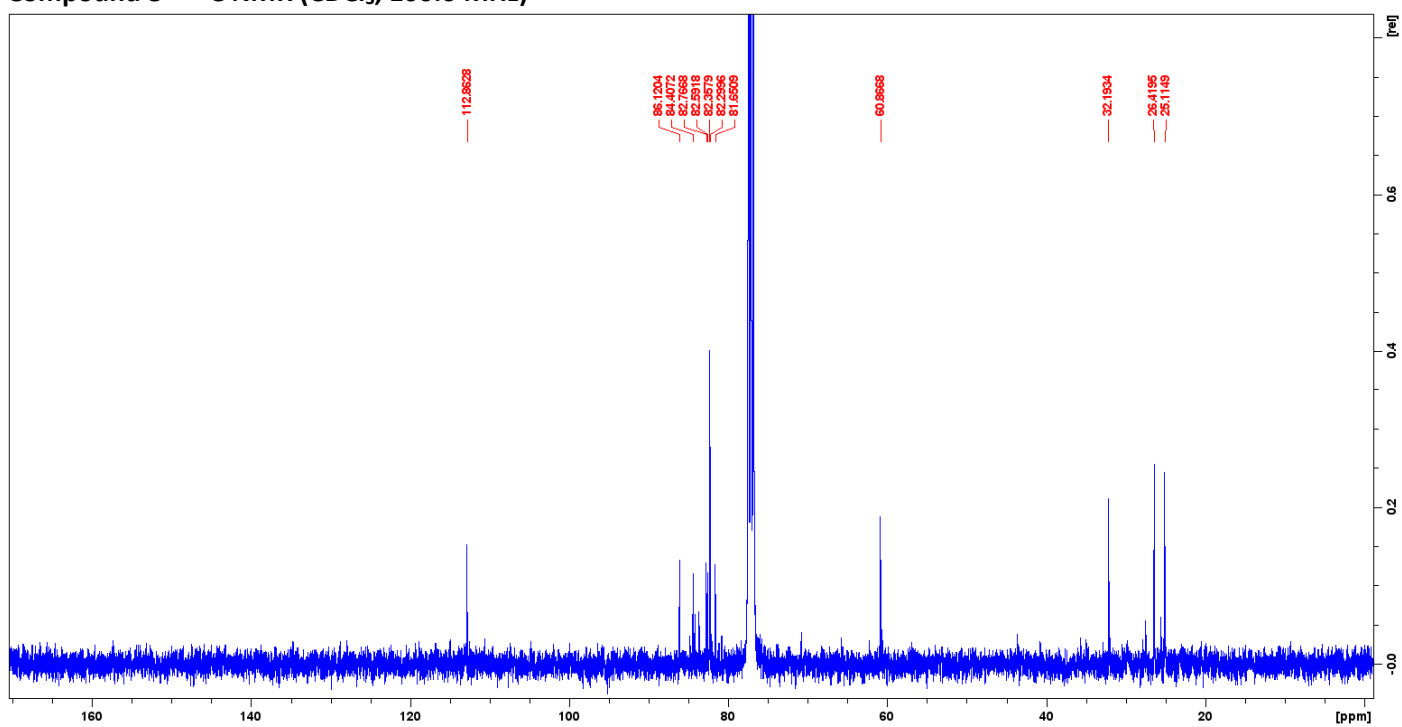
Chemical Shift (ppm)	Integration
7.6779, 7.6740, 7.6709, 7.6644, 7.6595, 7.6545, 7.6505, 7.6451, 7.6416, 7.6377, 7.4337, 7.4355, 7.4317, 7.4273, 7.4221, 7.4088, 7.4048, 7.3992, 7.3945, 7.3911, 7.3828, 7.3750	4.0078, 6.0031
4.8723, 4.8569, 4.8335, 4.8135, 4.7985, 4.7882, 4.6105, 4.6003, 4.5934, 4.5833, 4.5764, 4.5553, 4.5206, 4.1111, 4.1016, 3.7851, 3.7758, 3.7585, 3.7482, 3.7089, 3.6831, 3.6652, 3.6559	0.5849, 1.0014, 1.0000, 1.0062, 0.5937, 1.0069
2.7916, 2.7747, 2.7565, 2.7334, 2.7158, 2.6921, 2.6748	2.0203
1.4875, 1.3474	3.0016, 3.0051
1.6636	6.0018

13C NMR spectrum of compound 10. The x-axis is chemical shift in ppm [ppm] from 0 to 180. The y-axis is intensity in arbitrary units [rel] from 0 to 8. The spectrum shows several peaks, with the most intense at 78.5903 ppm. Other labeled peaks include 171.7627, 135.7495, 135.7330, 134.9377, 132.9850, 132.8771, 132.8351, 129.9640, 127.9690, 112.4909, 84.3006, 84.3095, 81.9650, 78.5903, 65.4135, 51.8350, 34.9432, 26.9511, 26.3877, 25.1697, and 19.2084.

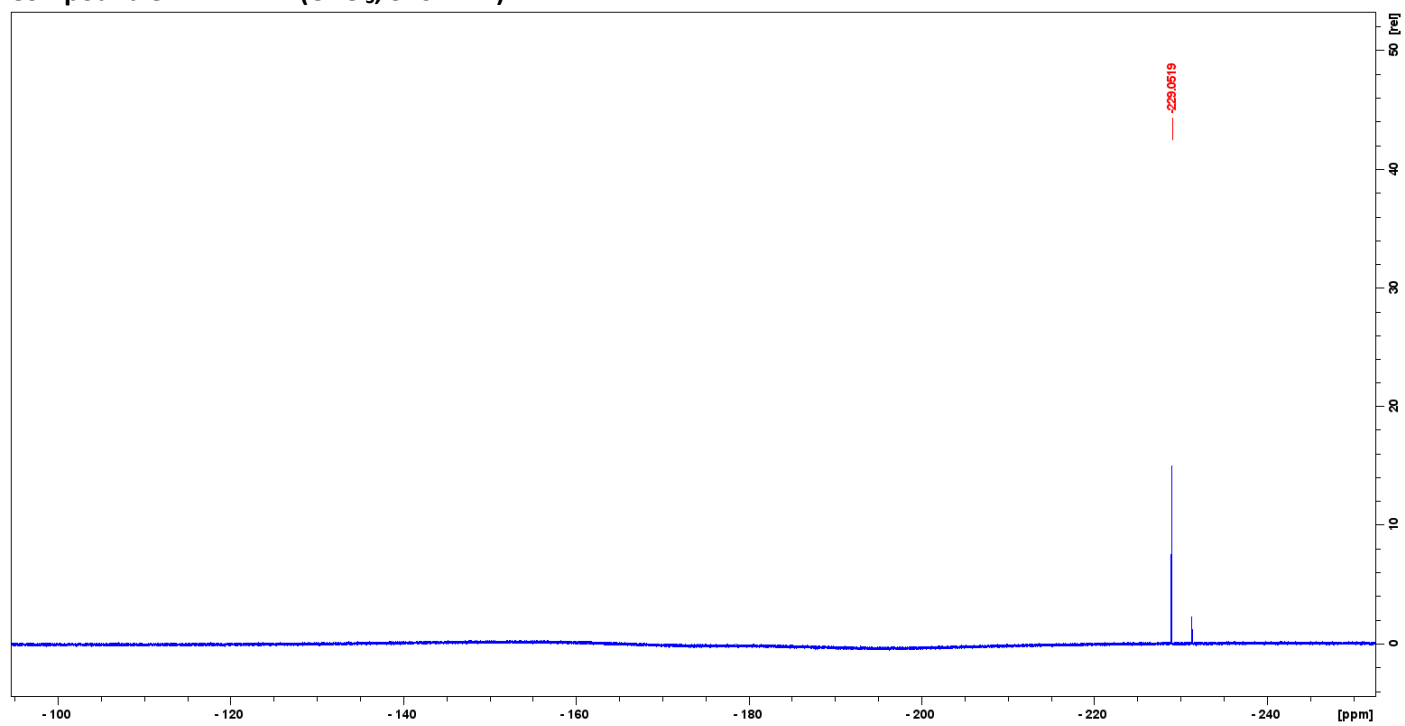
Compound 8 - ^1H NMR (CDCl_3 , 400 MHz)



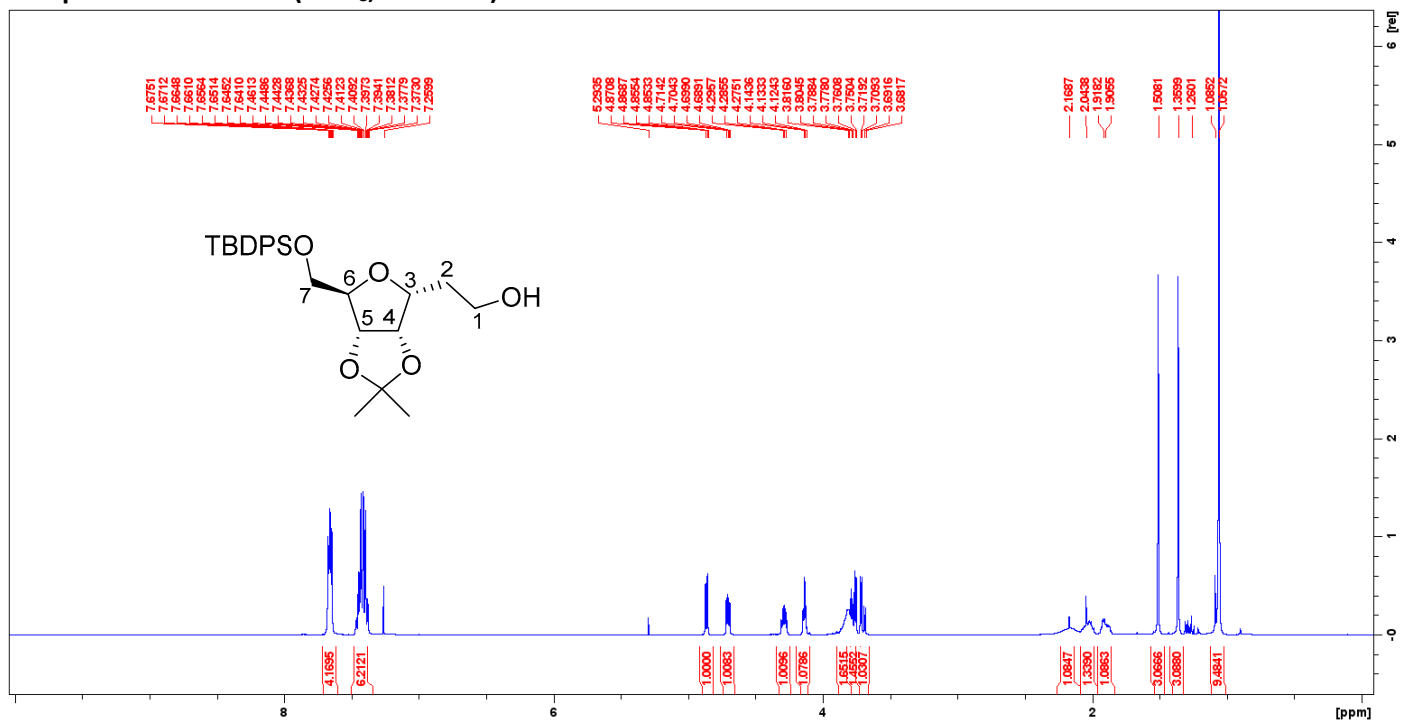
Compound 8 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



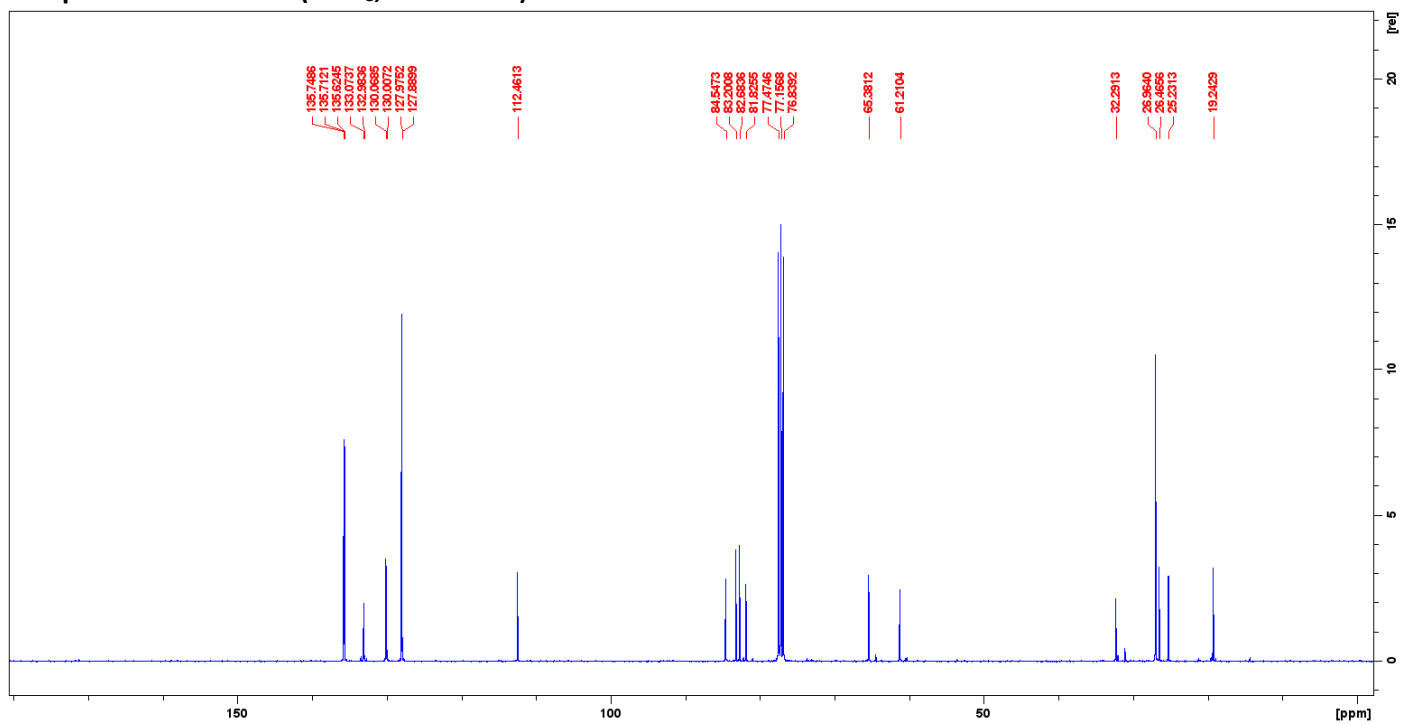
Compound 8 – ^{19}F NMR (CDCl_3 , 376 MHz)



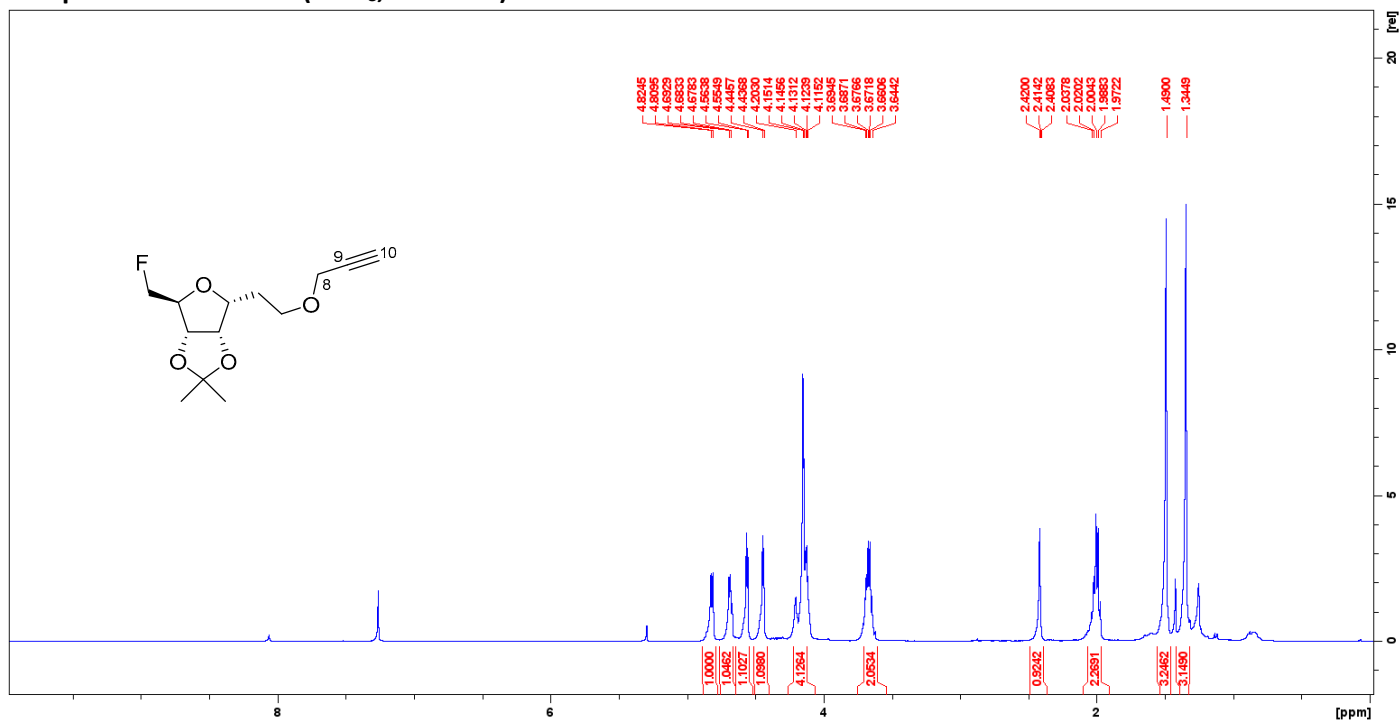
Compound 9 – ^1H NMR (CDCl_3 , 400 MHz)



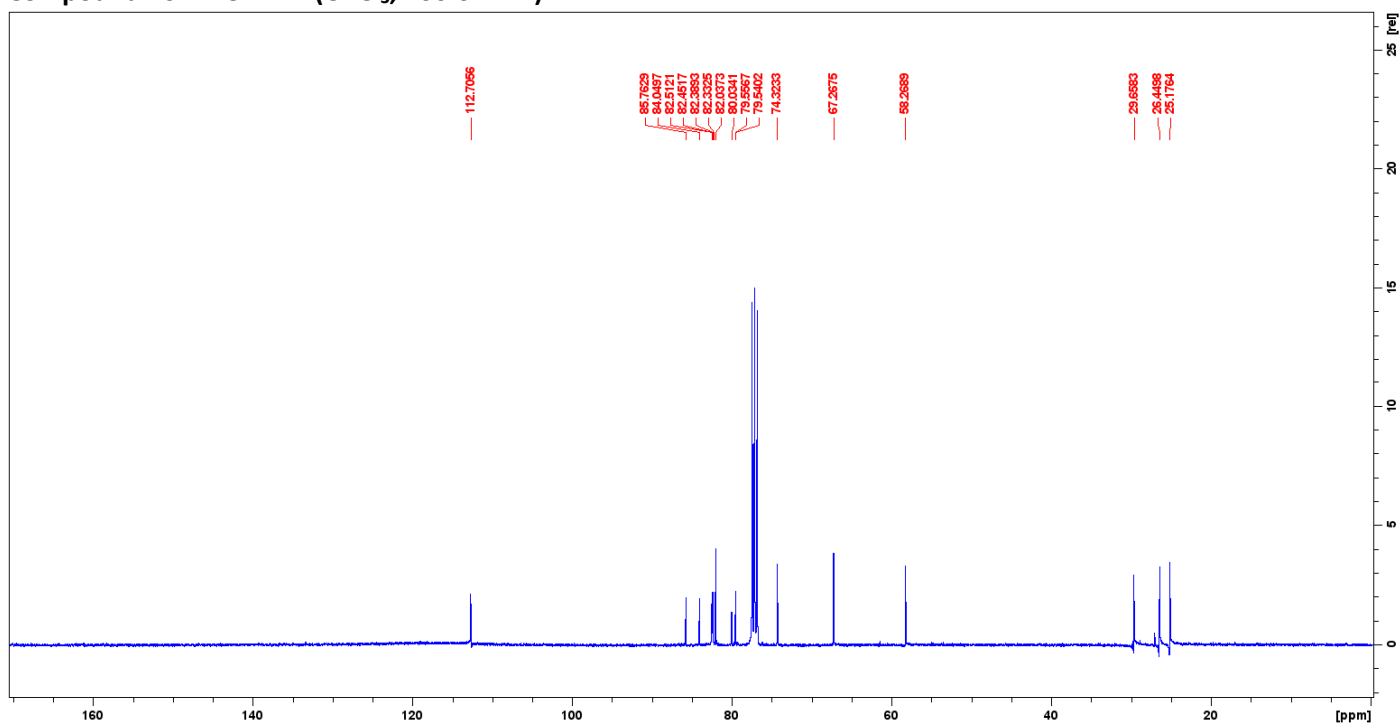
Compound 9 – ^{13}C NMR (CDCl_3 , 100.6 MHz)



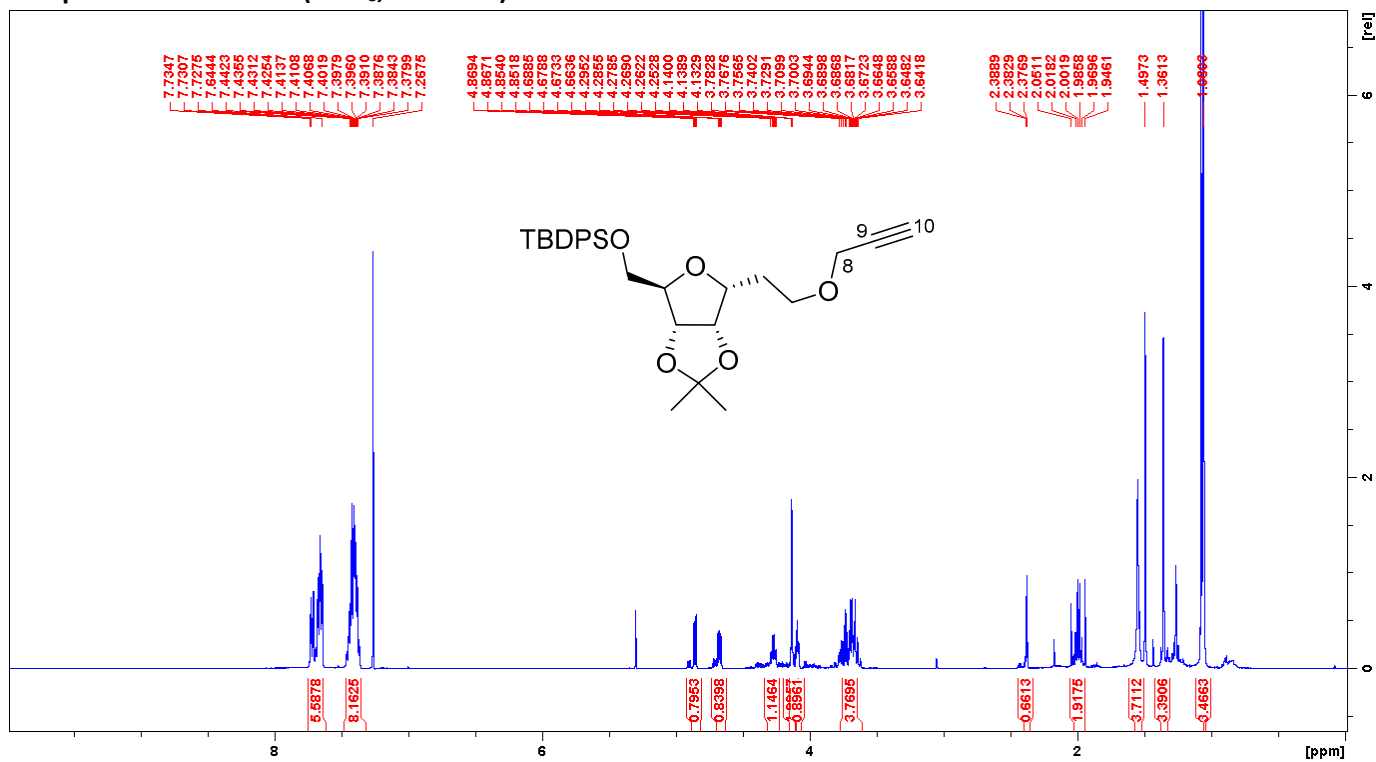
Compound 10 - ^1H NMR (CDCl_3 , 400 MHz)



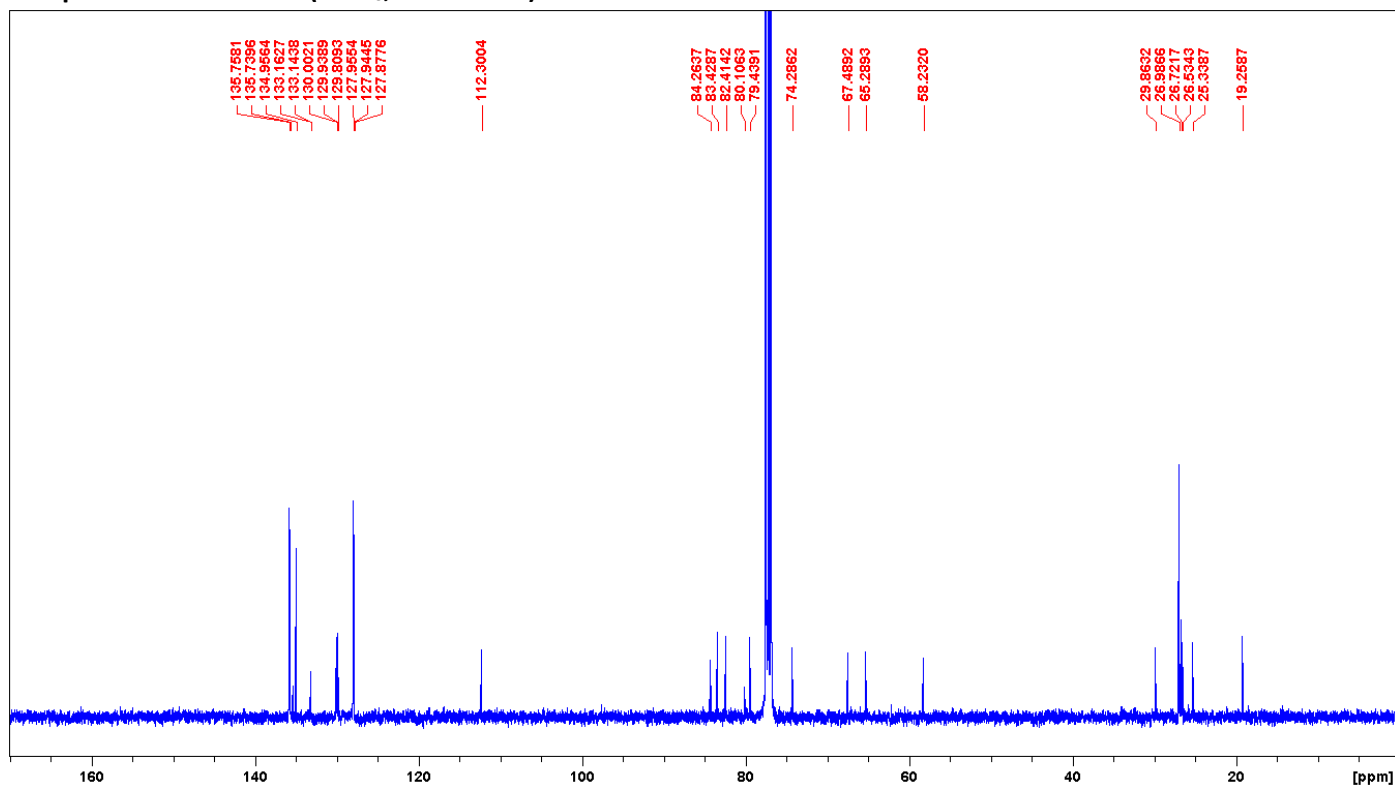
Compound 10 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



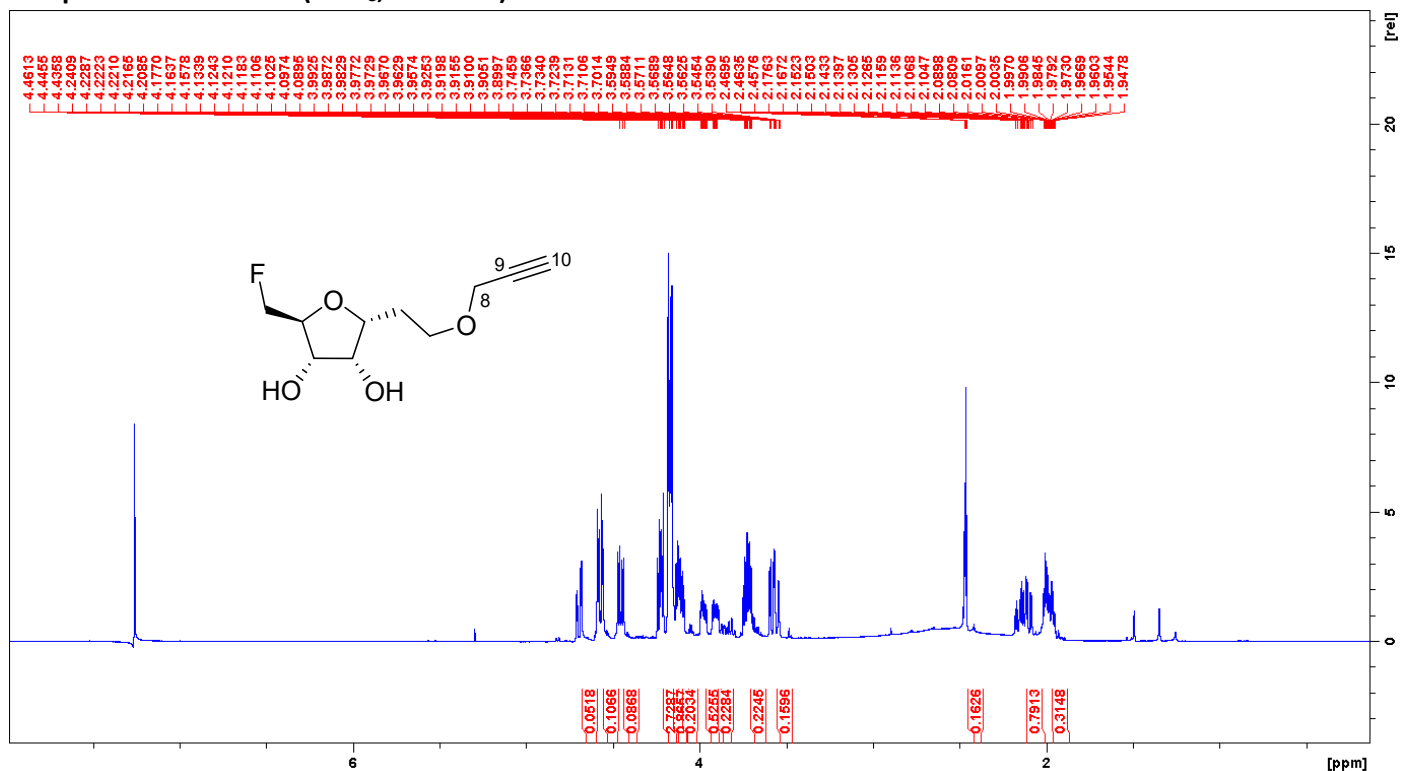
Compound 11 - ^1H NMR (CDCl_3 , 400 MHz)



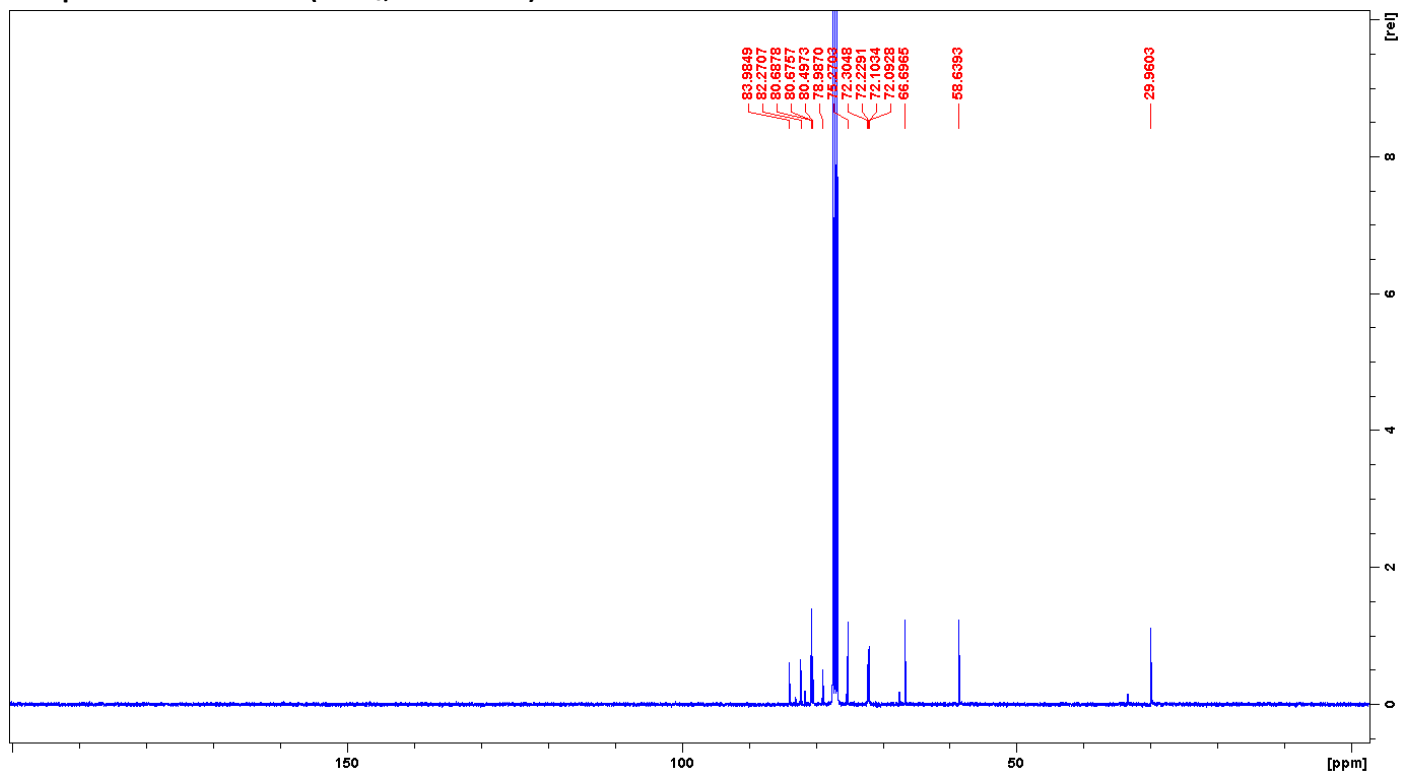
Compound 11 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



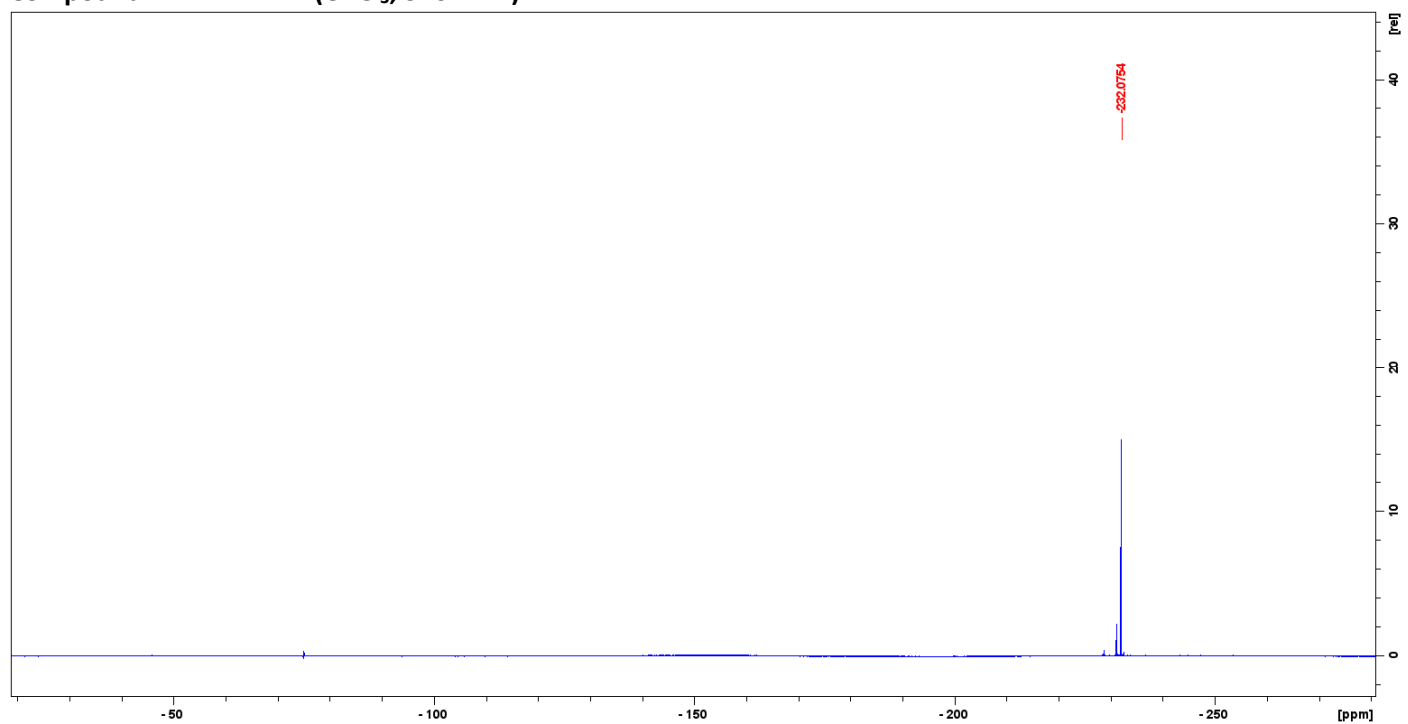
Compound 12 - ^1H NMR (CDCl_3 , 400 MHz)



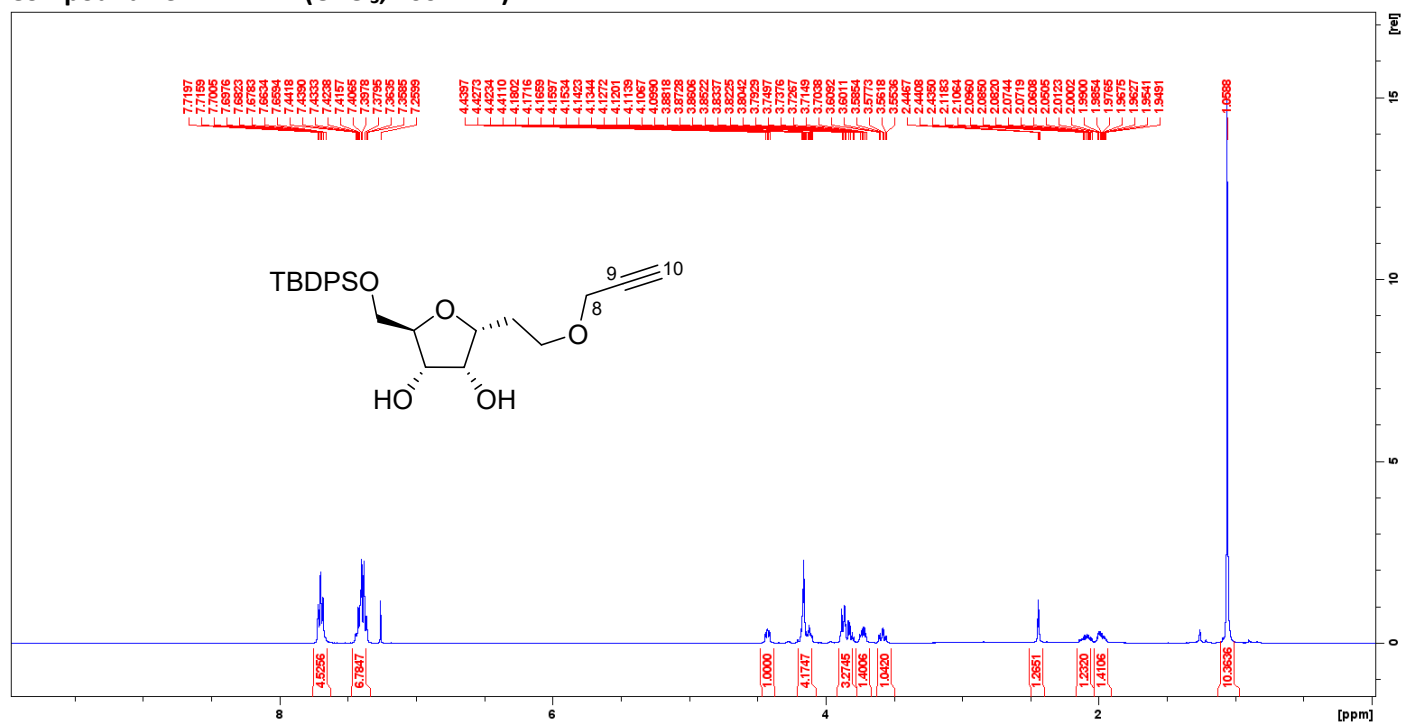
Compound 12 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



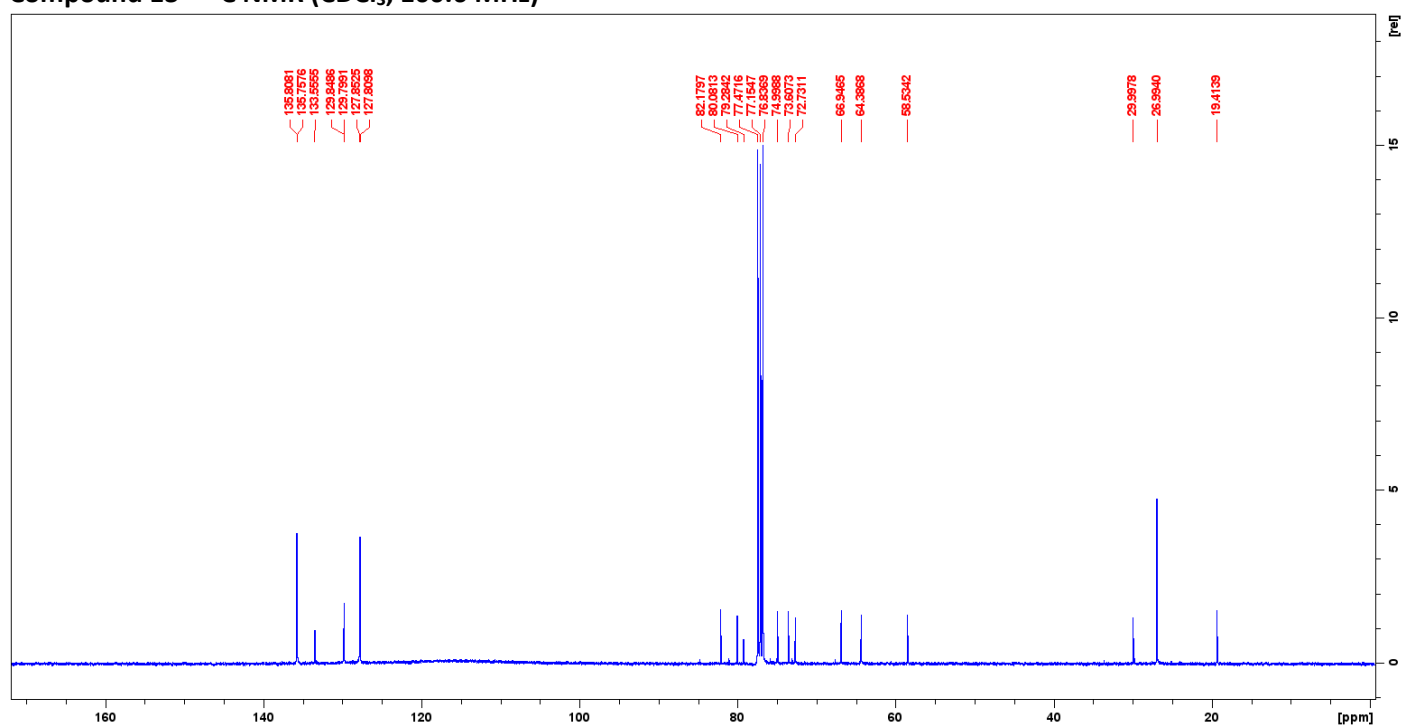
Compound 12 – ^{19}F NMR (CDCl_3 , 376 MHz)



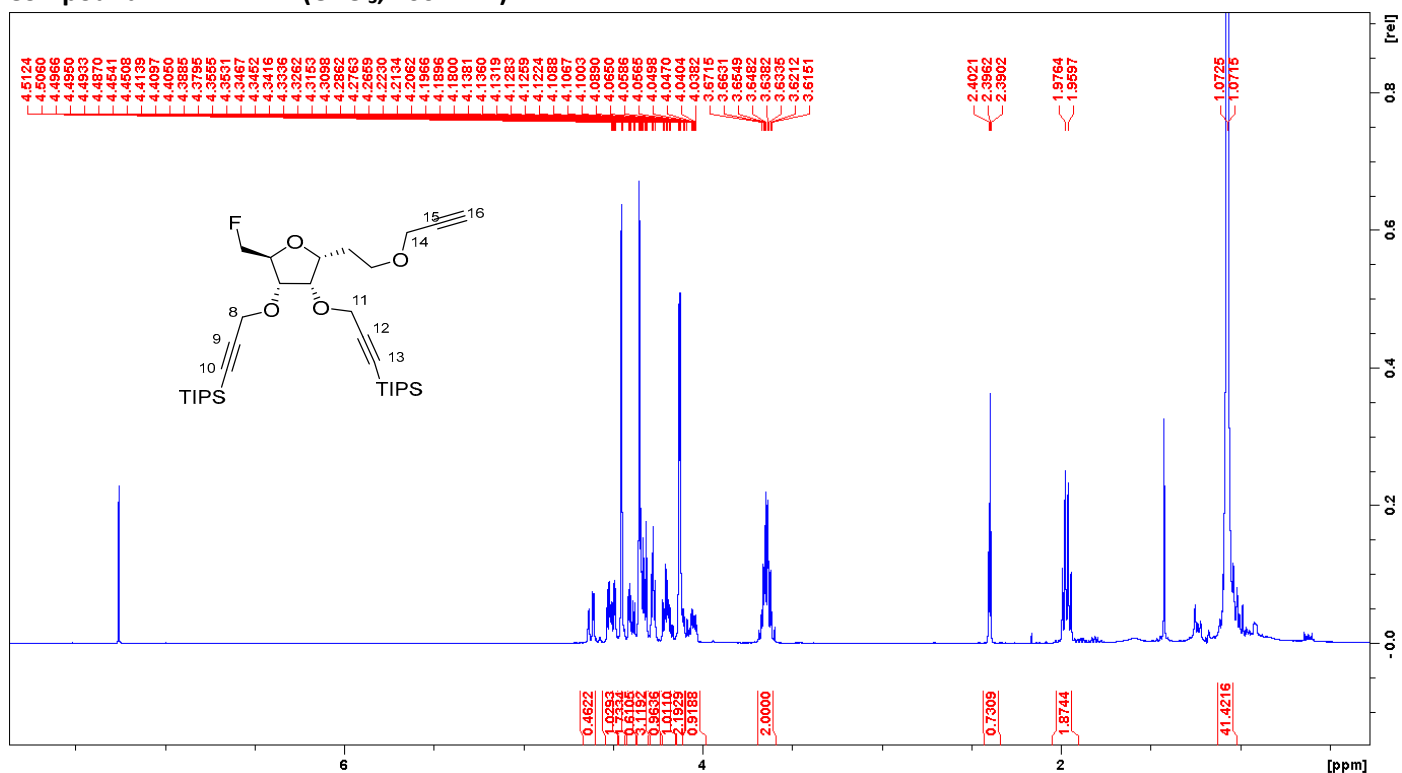
Compound 13 - ^1H NMR (CDCl_3 , 400 MHz)



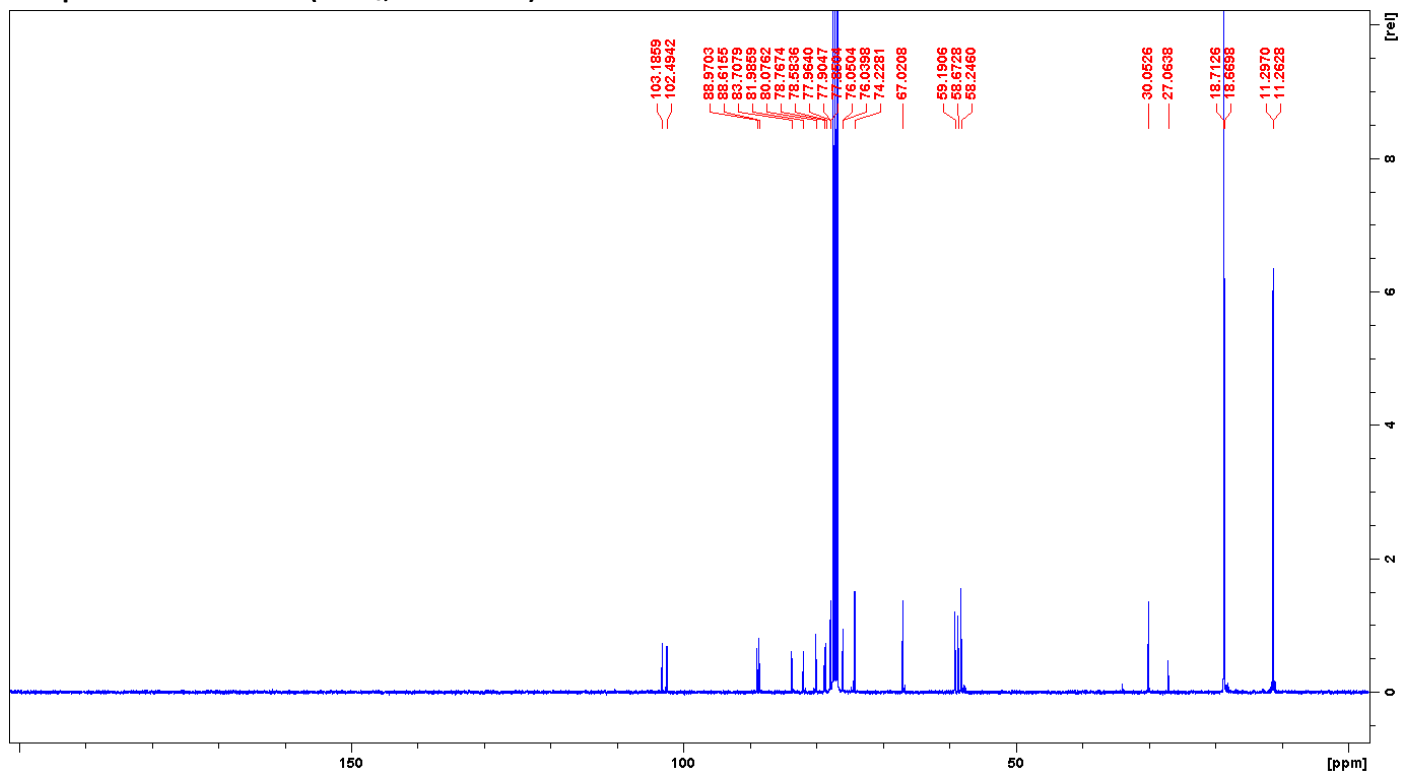
Compound 13 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



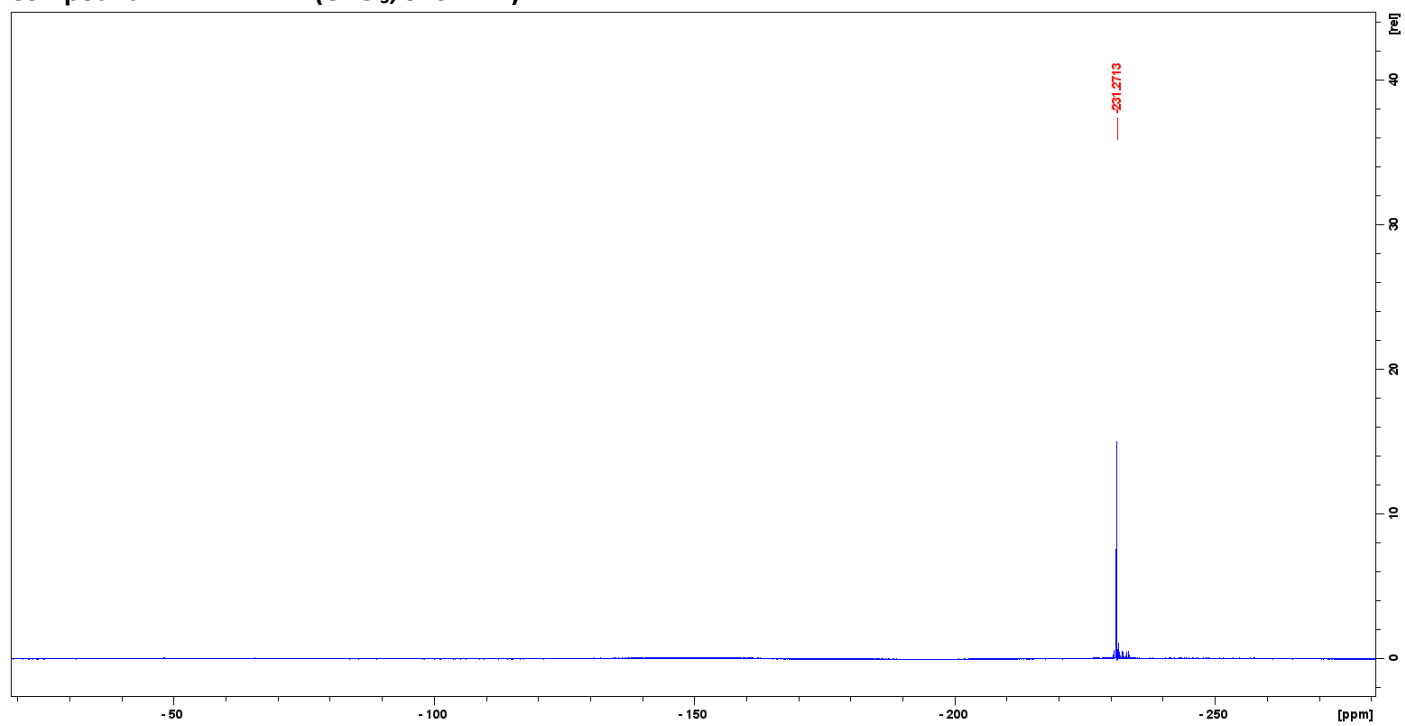
Compound 14 - ^1H NMR (CDCl_3 , 400 MHz)



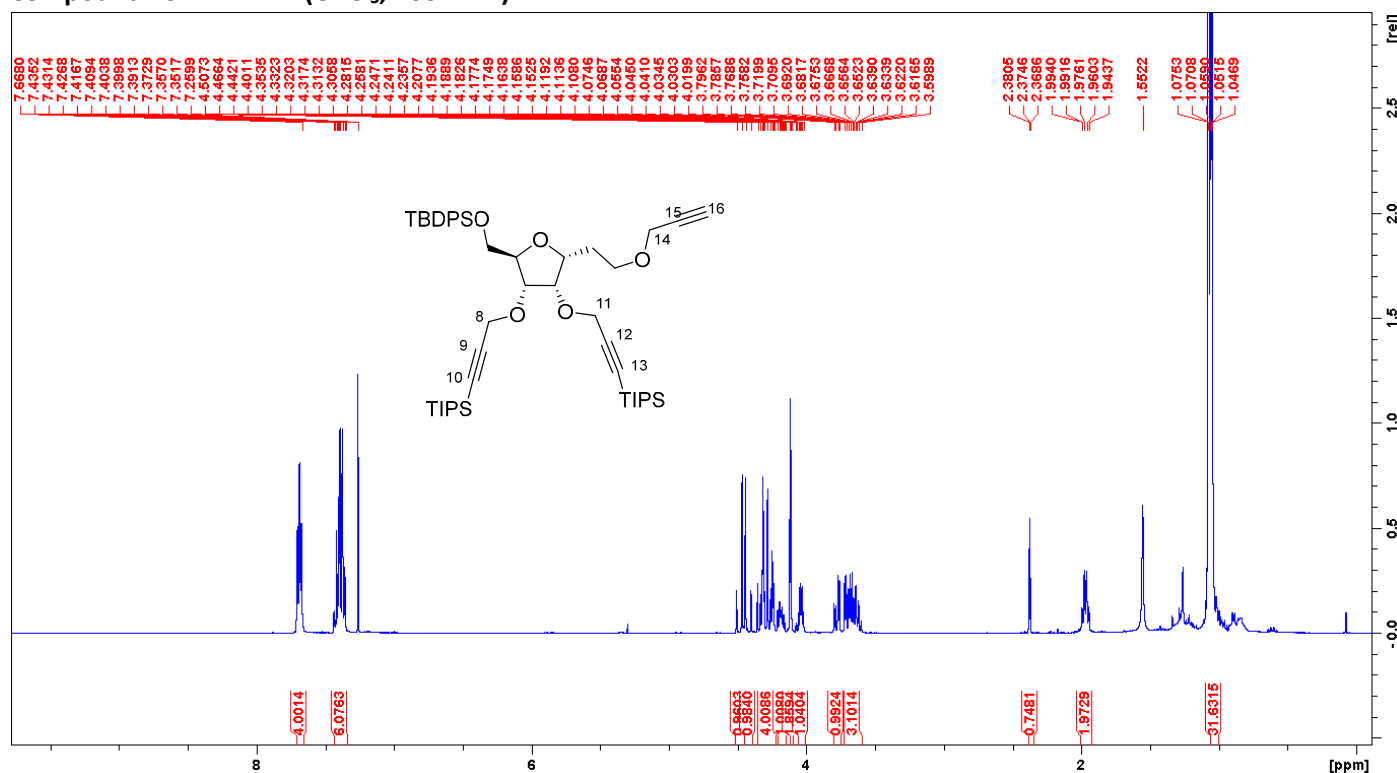
Compound 14 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



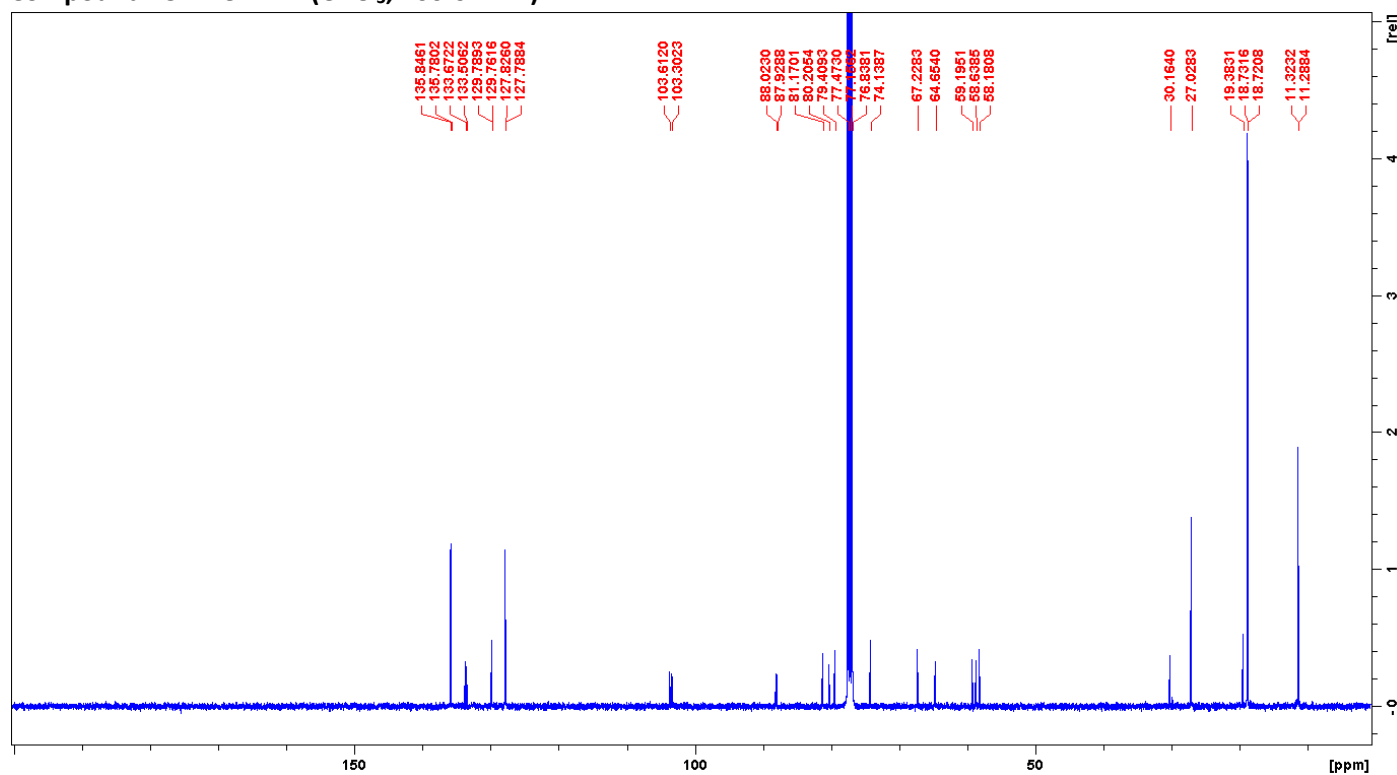
Compound 14 – ^{19}F NMR (CDCl_3 , 376 MHz)



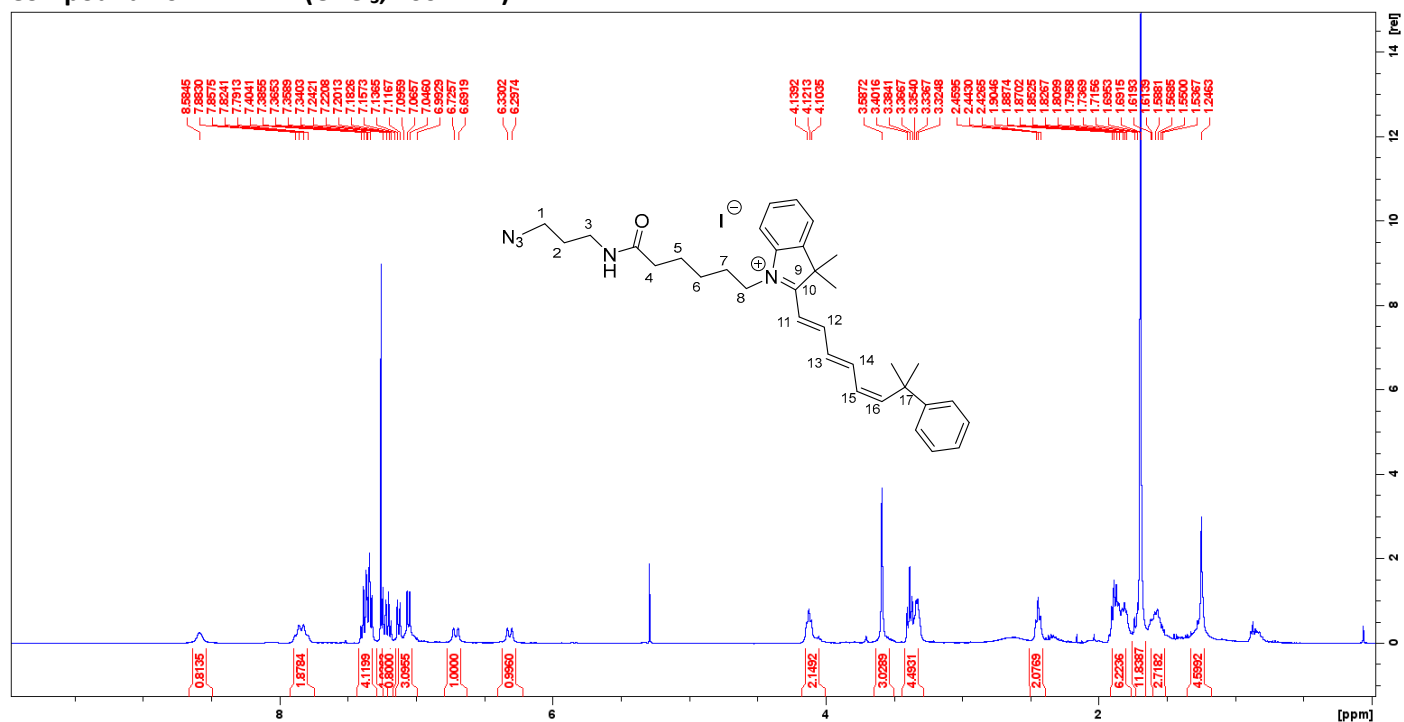
Compound 15 - ^1H NMR (CDCl_3 , 400 MHz)



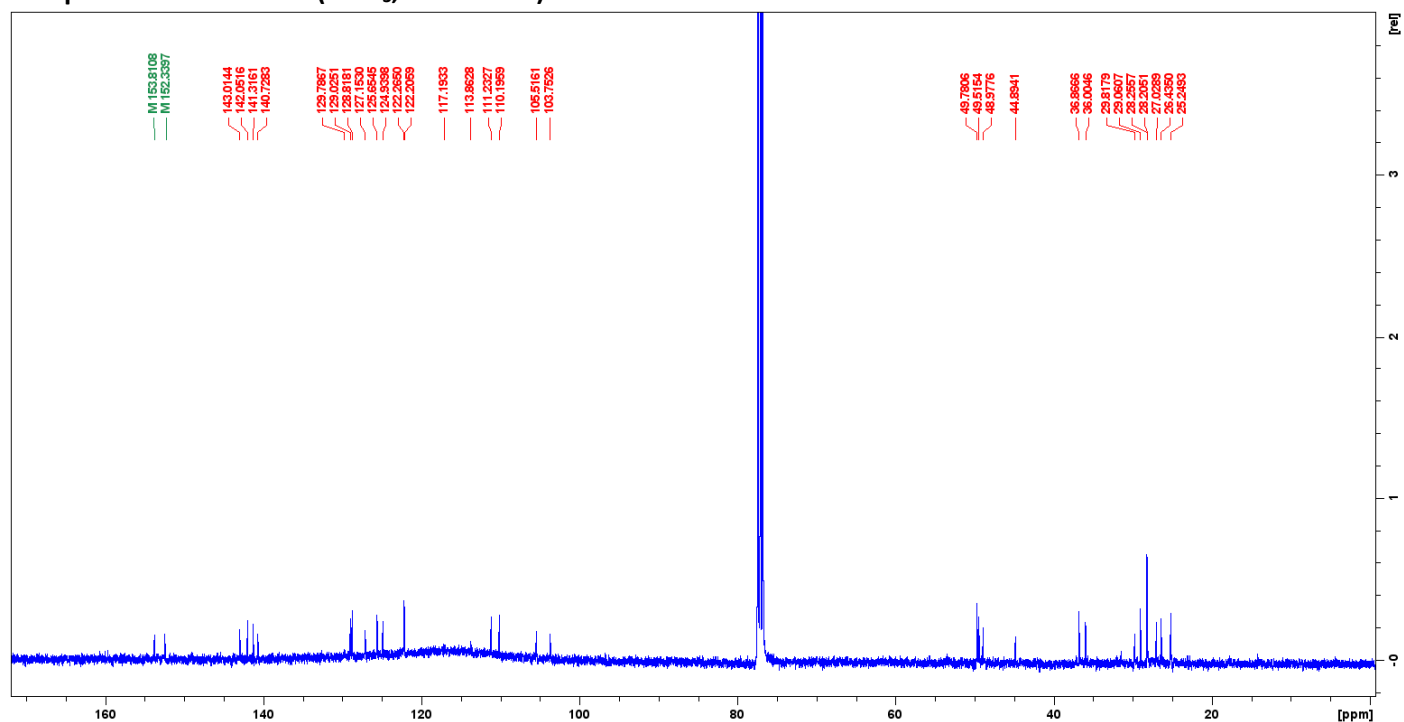
Compound 15 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



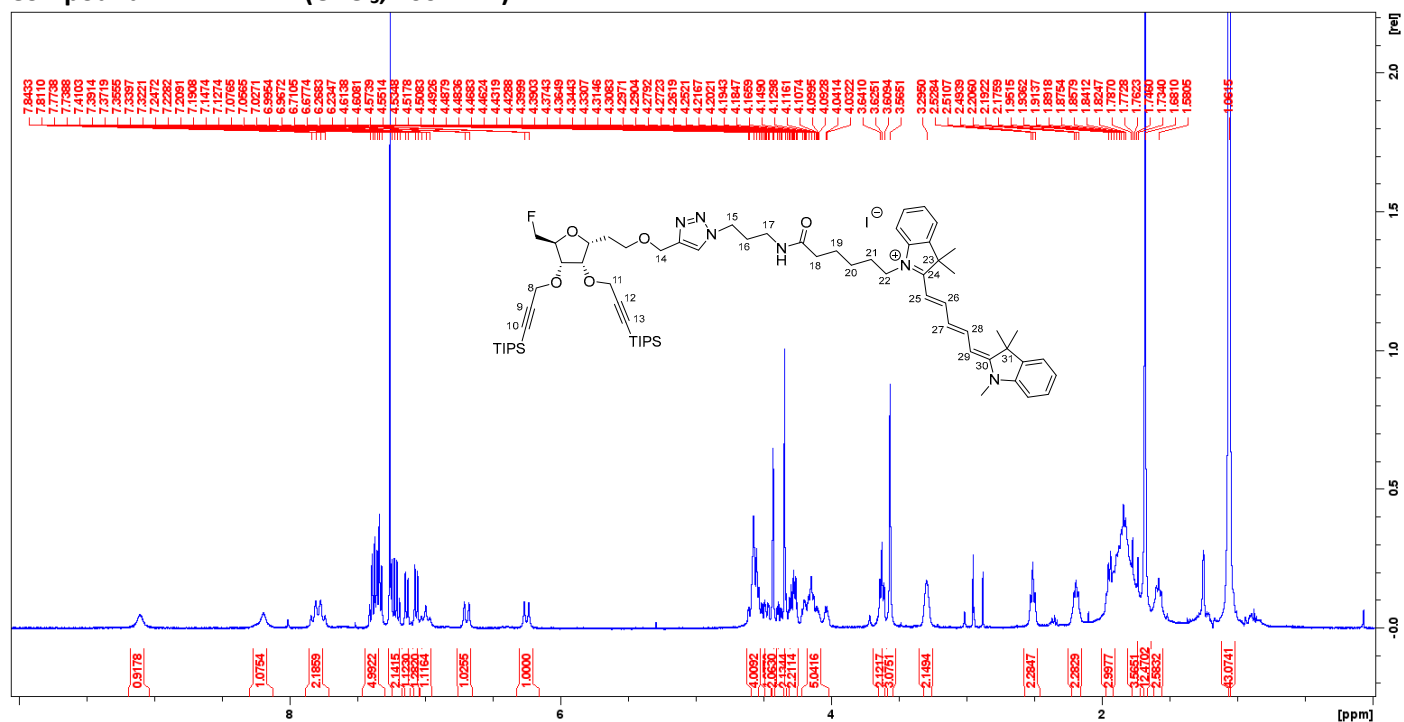
Compound 16 - ^1H NMR (CDCl_3 , 400 MHz)



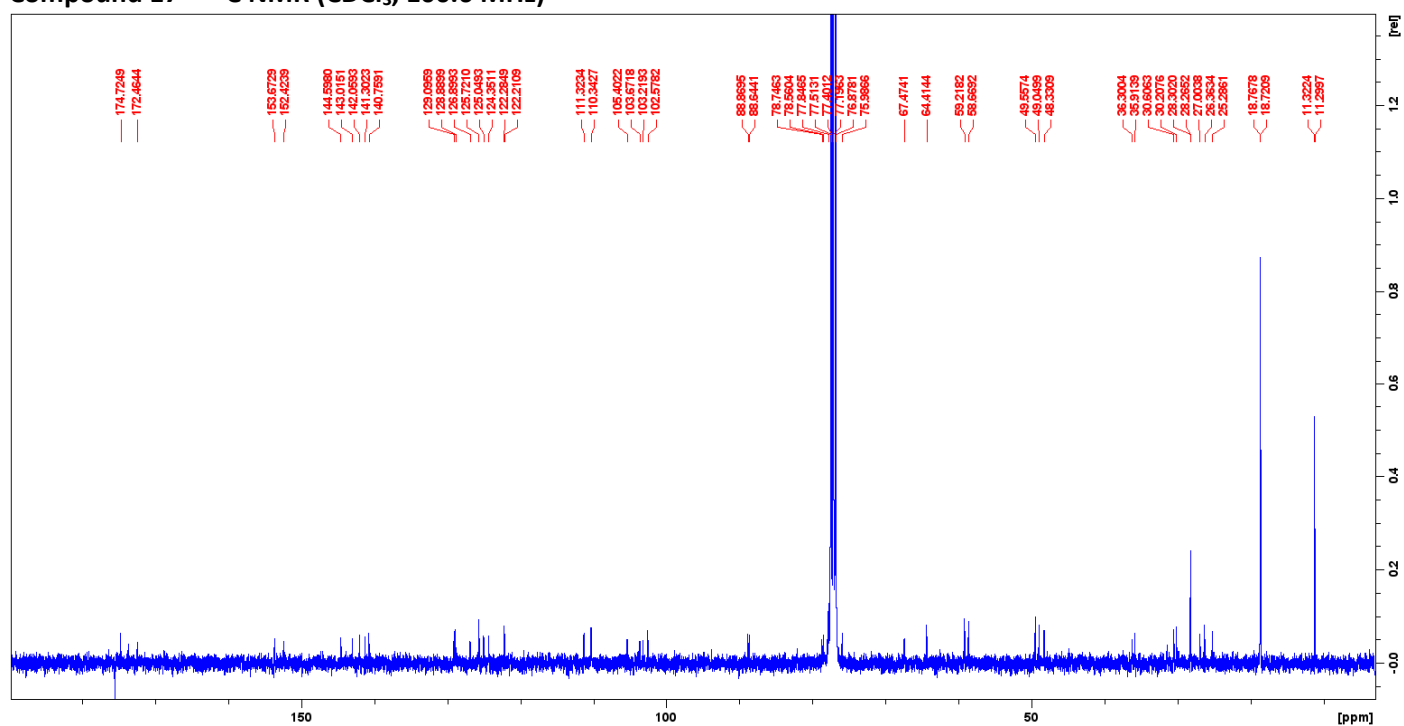
Compound 16 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



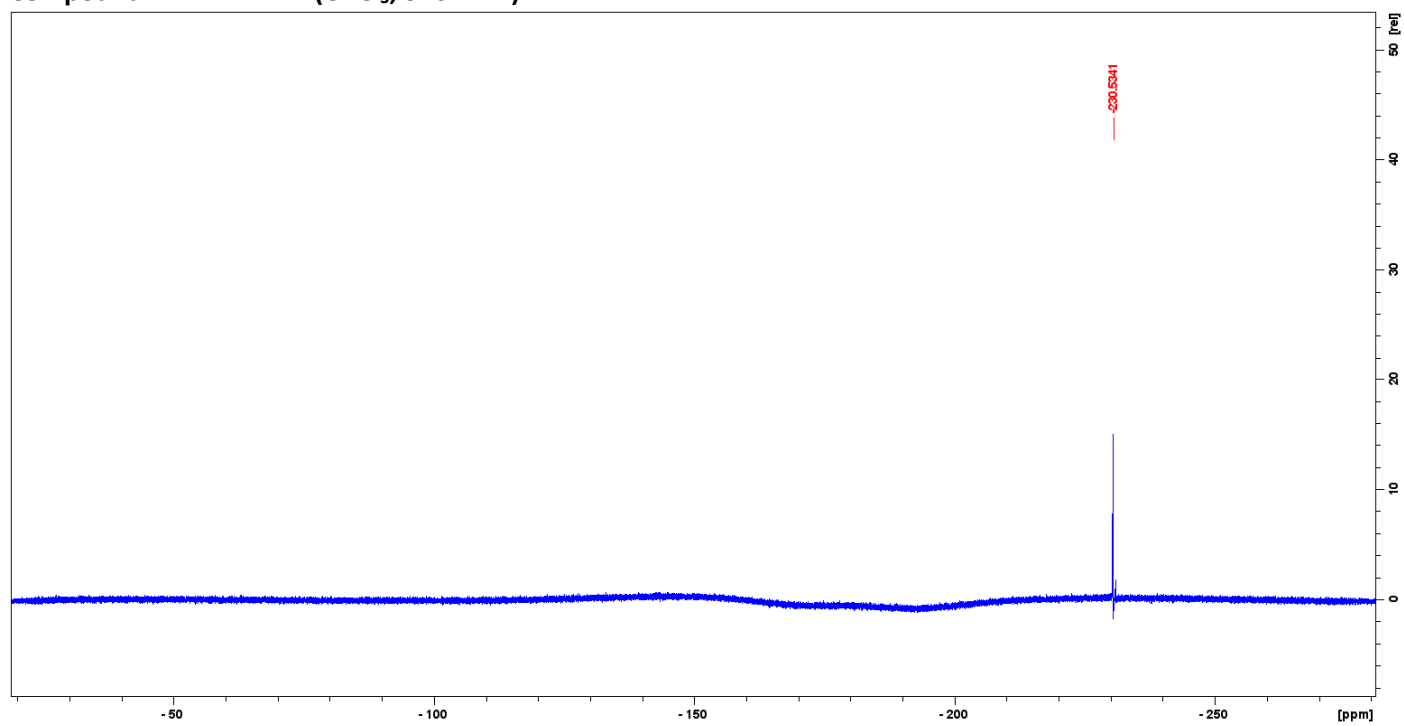
Compound 17 - ^1H NMR (CDCl_3 , 400 MHz)



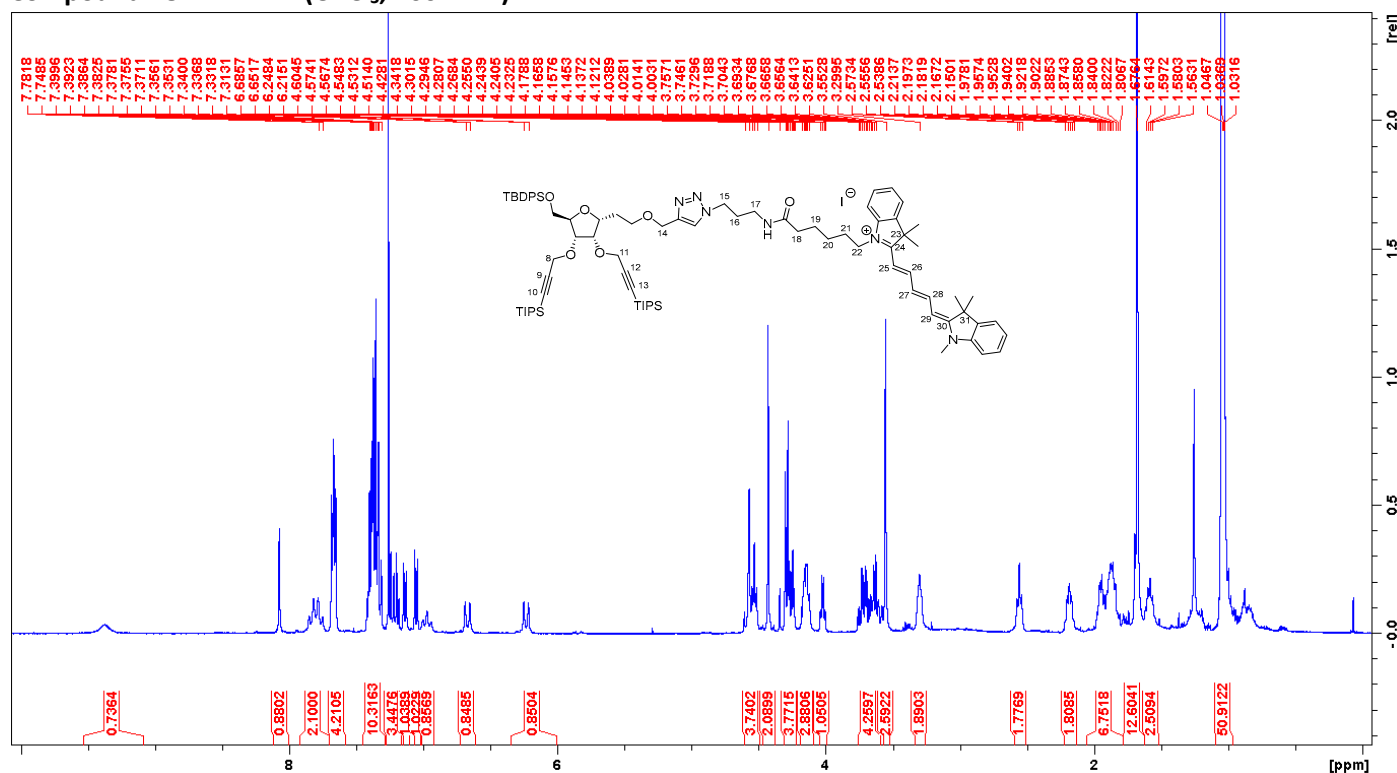
Compound 17 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



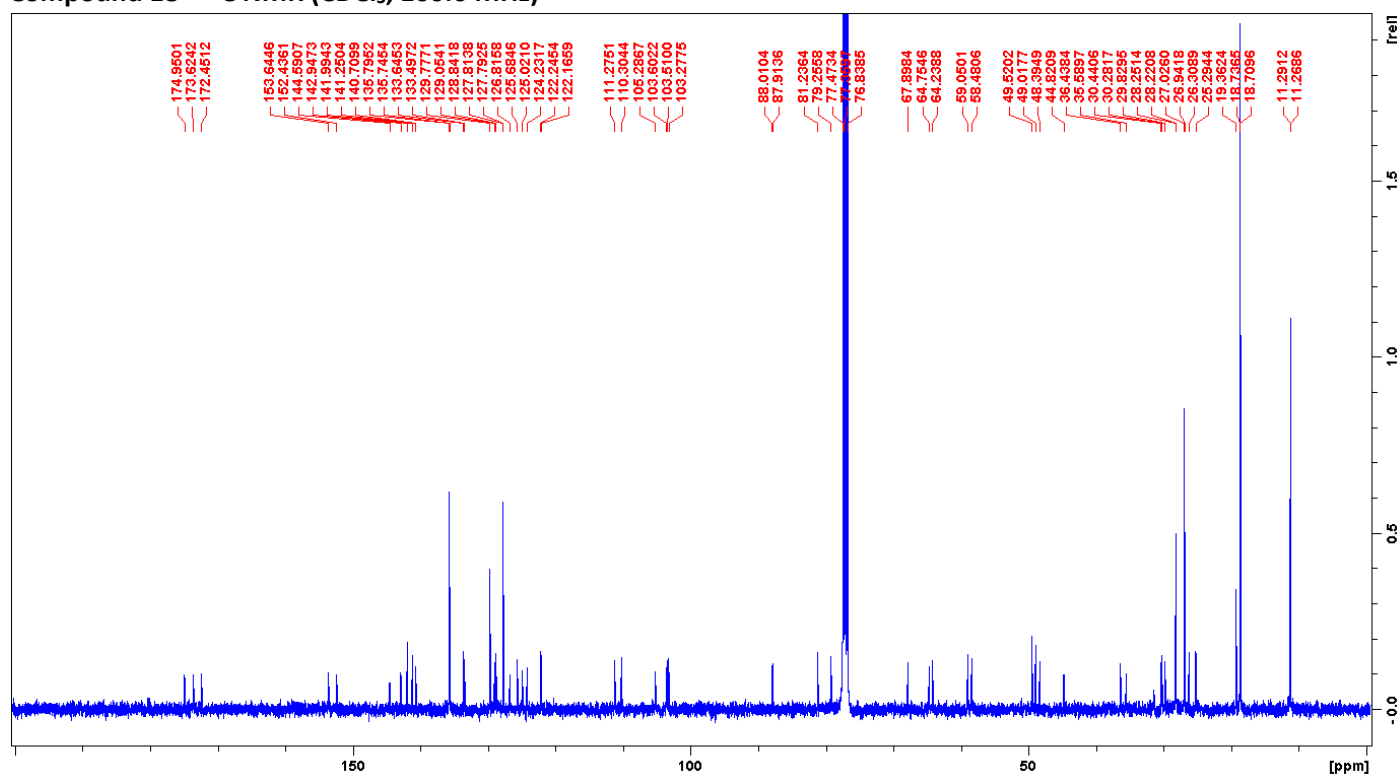
Compound 17 – ^{19}F NMR (CDCl_3 , 376 MHz)



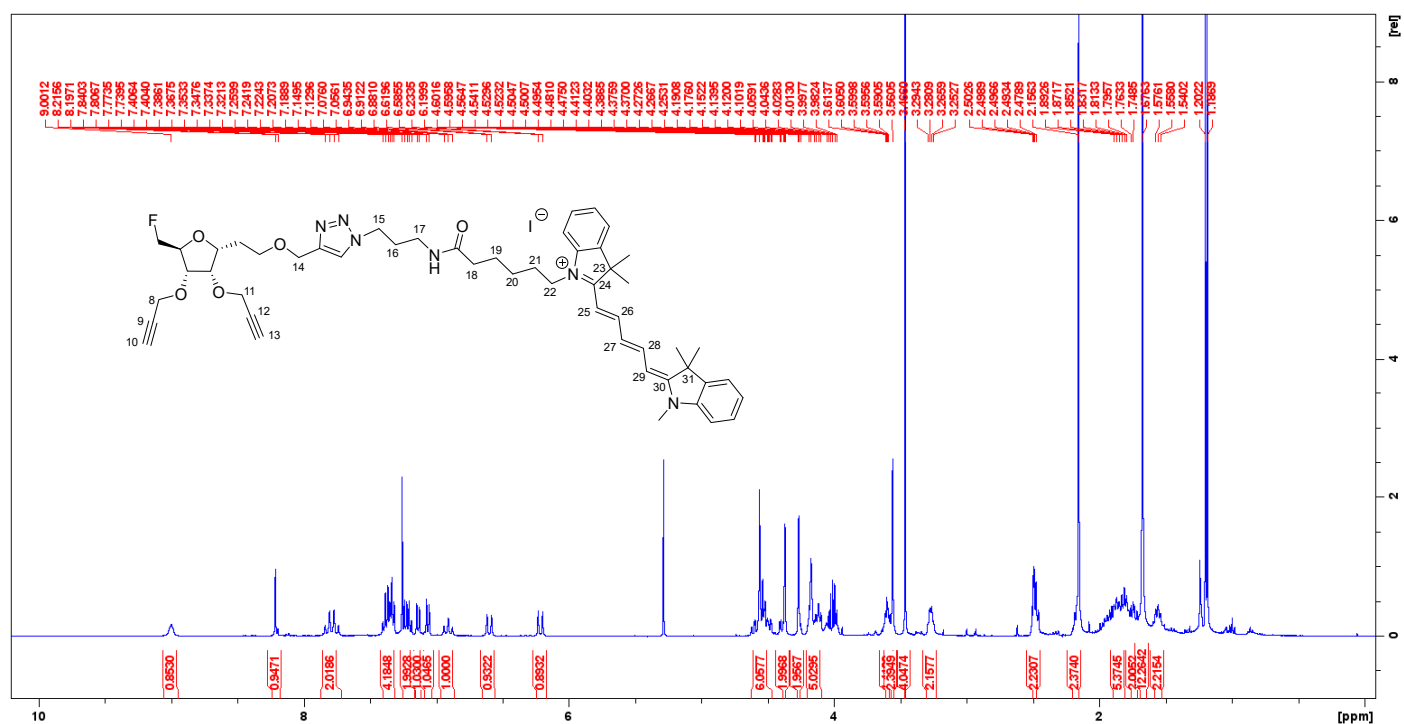
Compound 18 - ^1H NMR (CDCl_3 , 400 MHz)



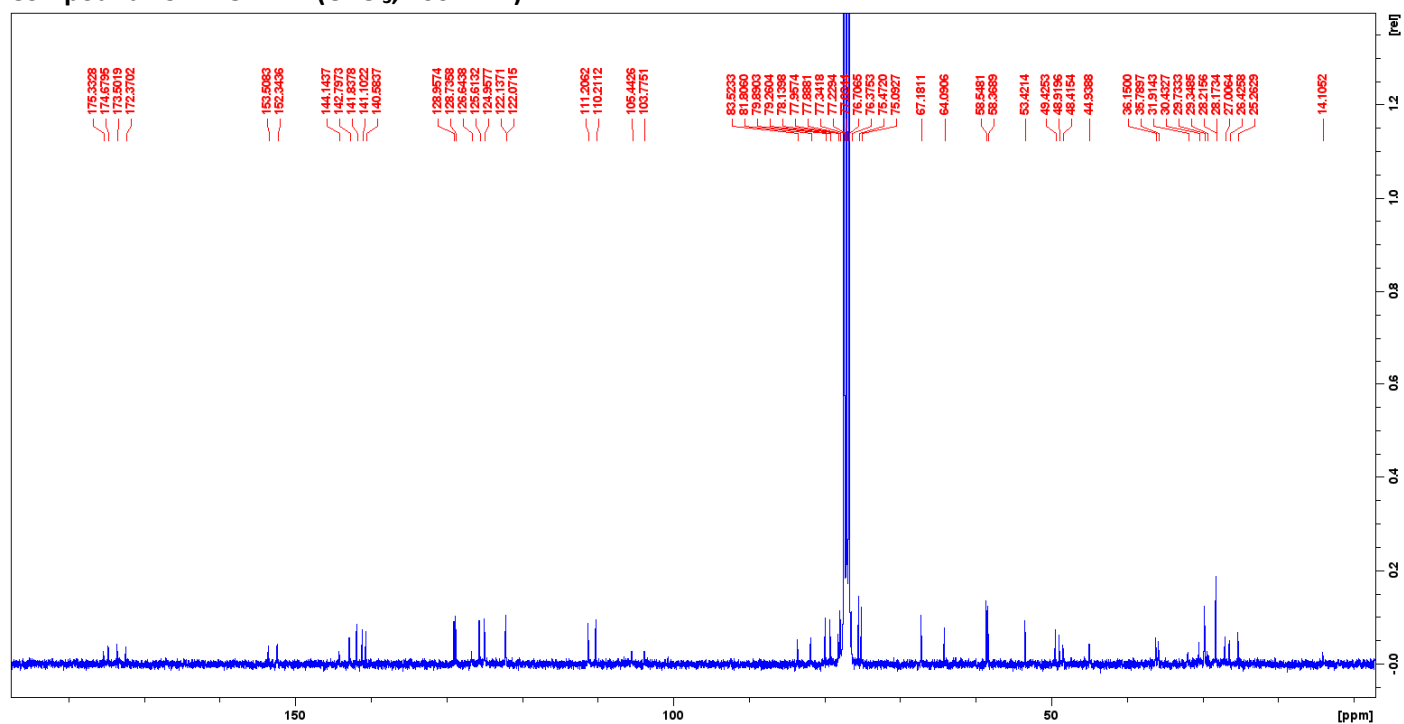
Compound 18 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



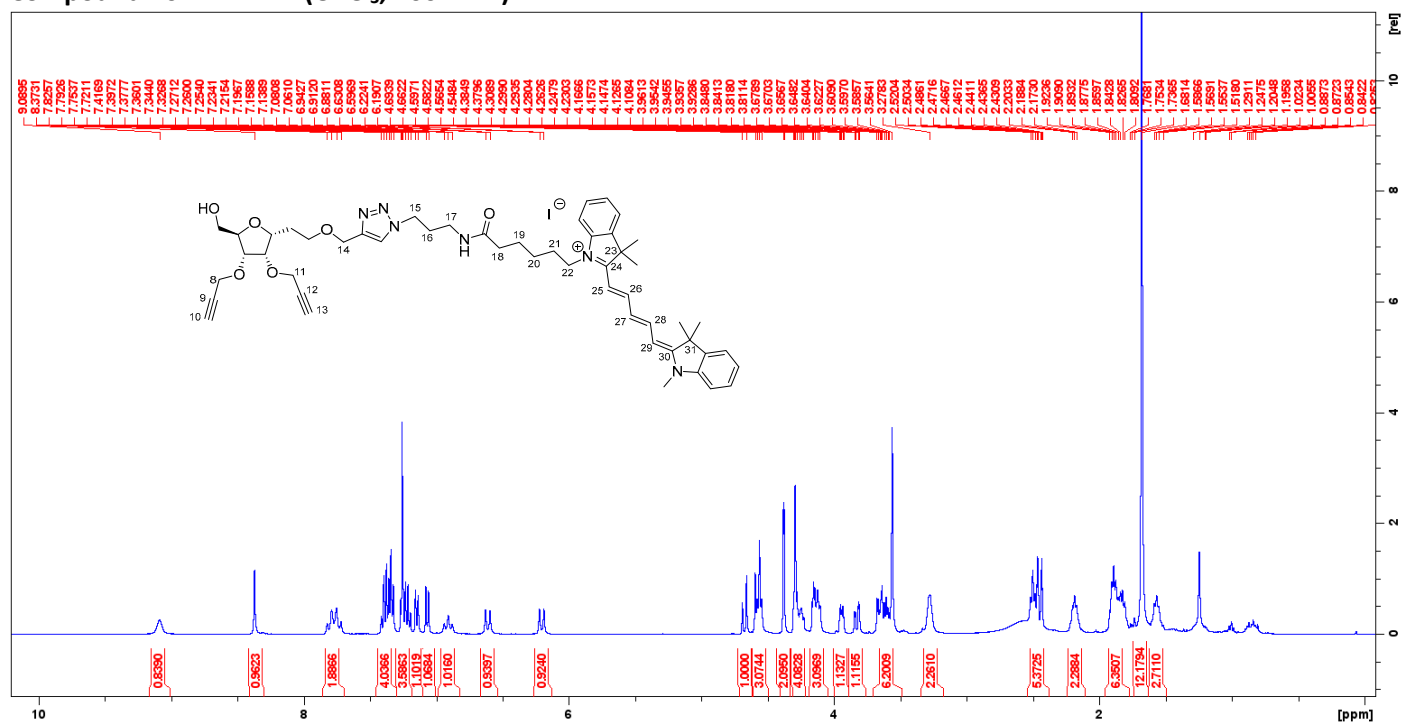
Compound 19 - ^1H NMR (CDCl_3 , 400 MHz)



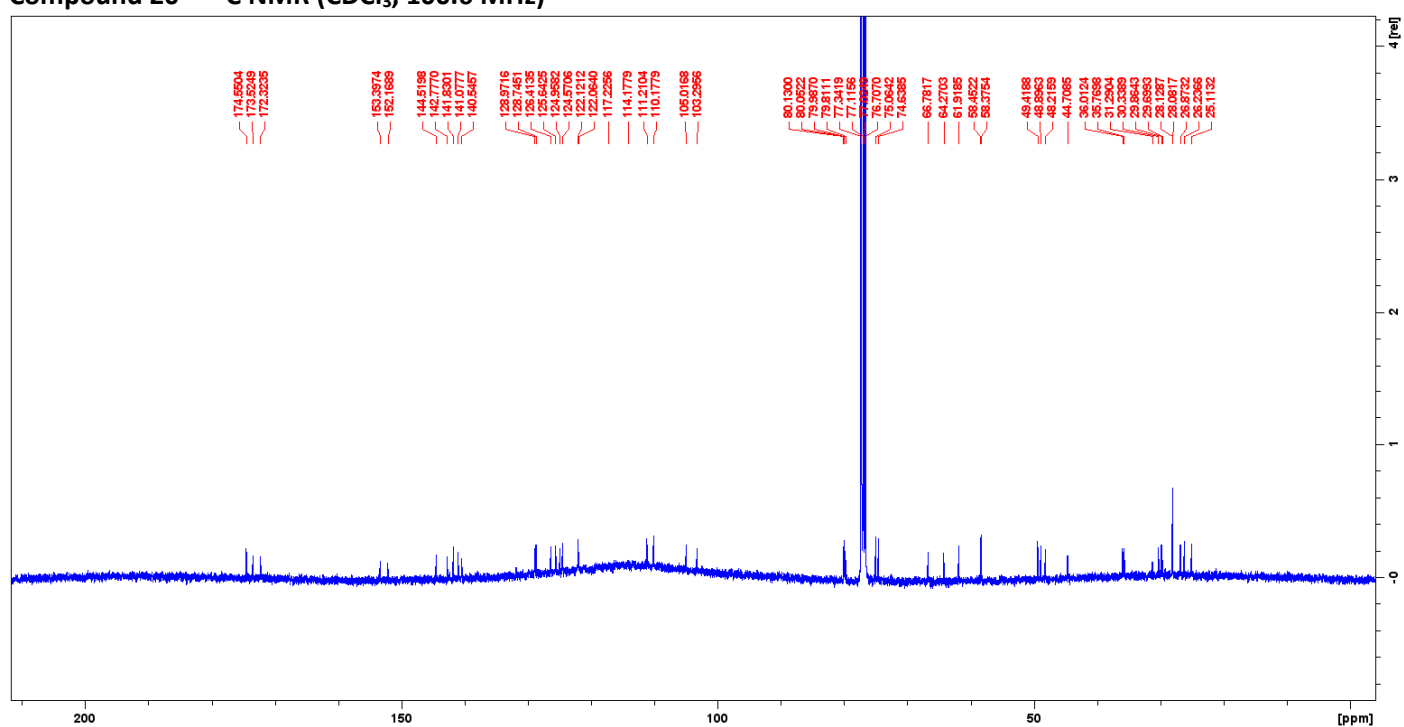
Compound 19 - ^{13}C NMR (CDCl_3 , 400 MHz)



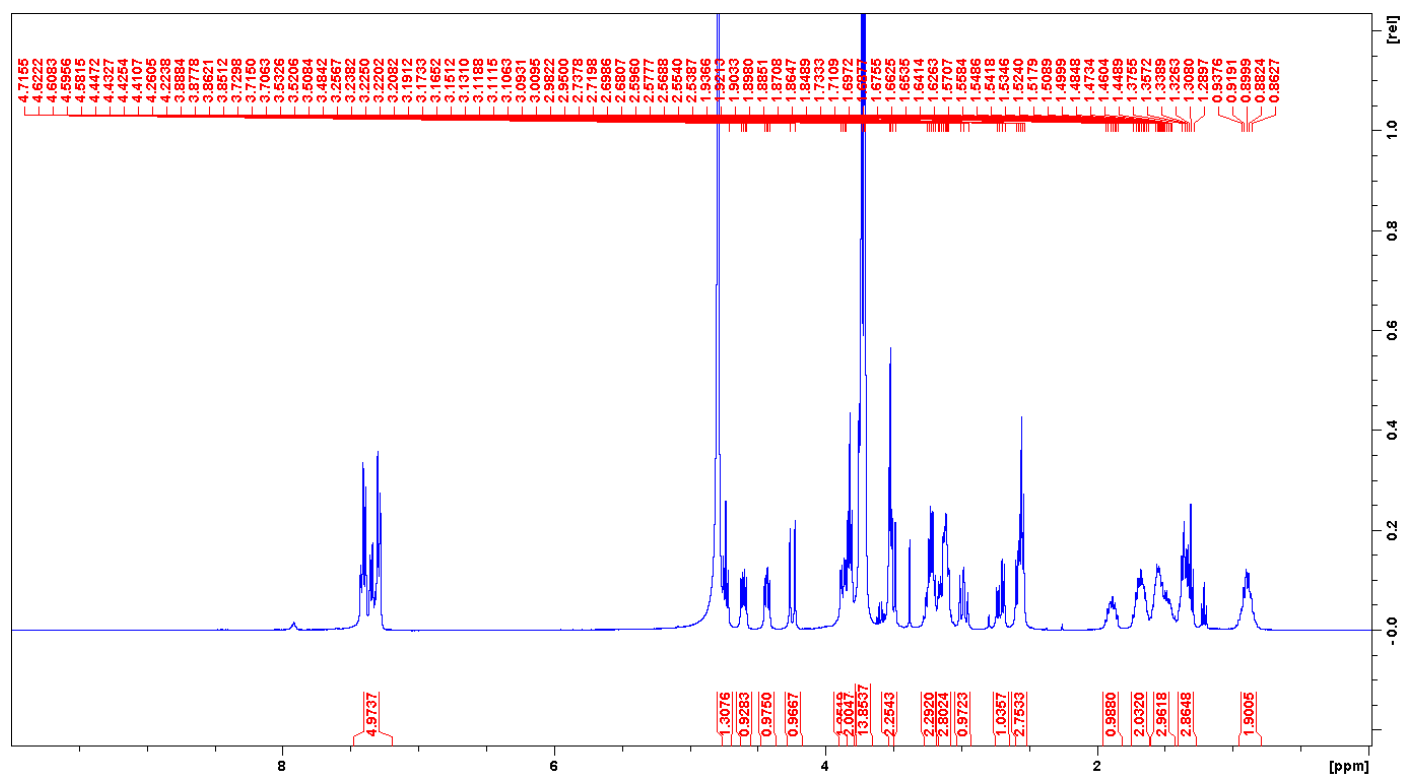
Compound 20 - ^1H NMR (CDCl_3 , 400 MHz)



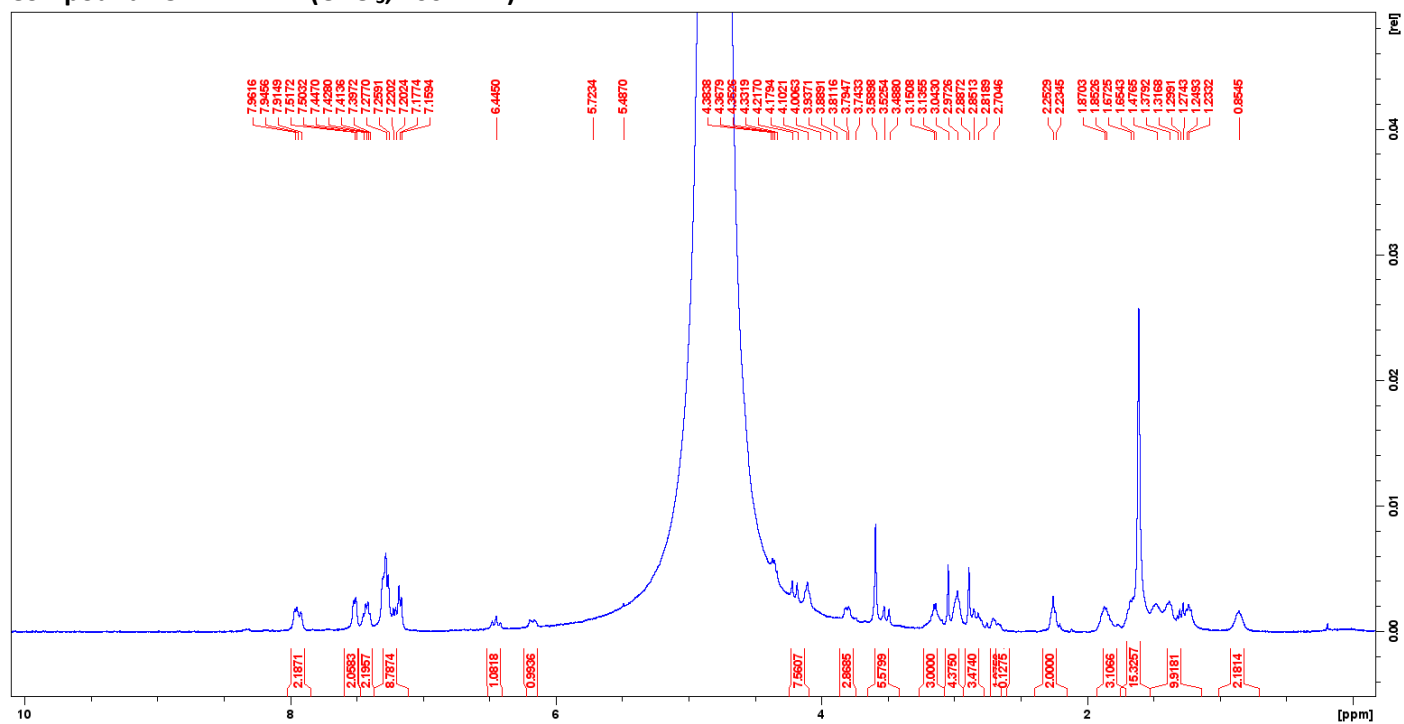
Compound 20 - ^{13}C NMR (CDCl_3 , 100.6 MHz)



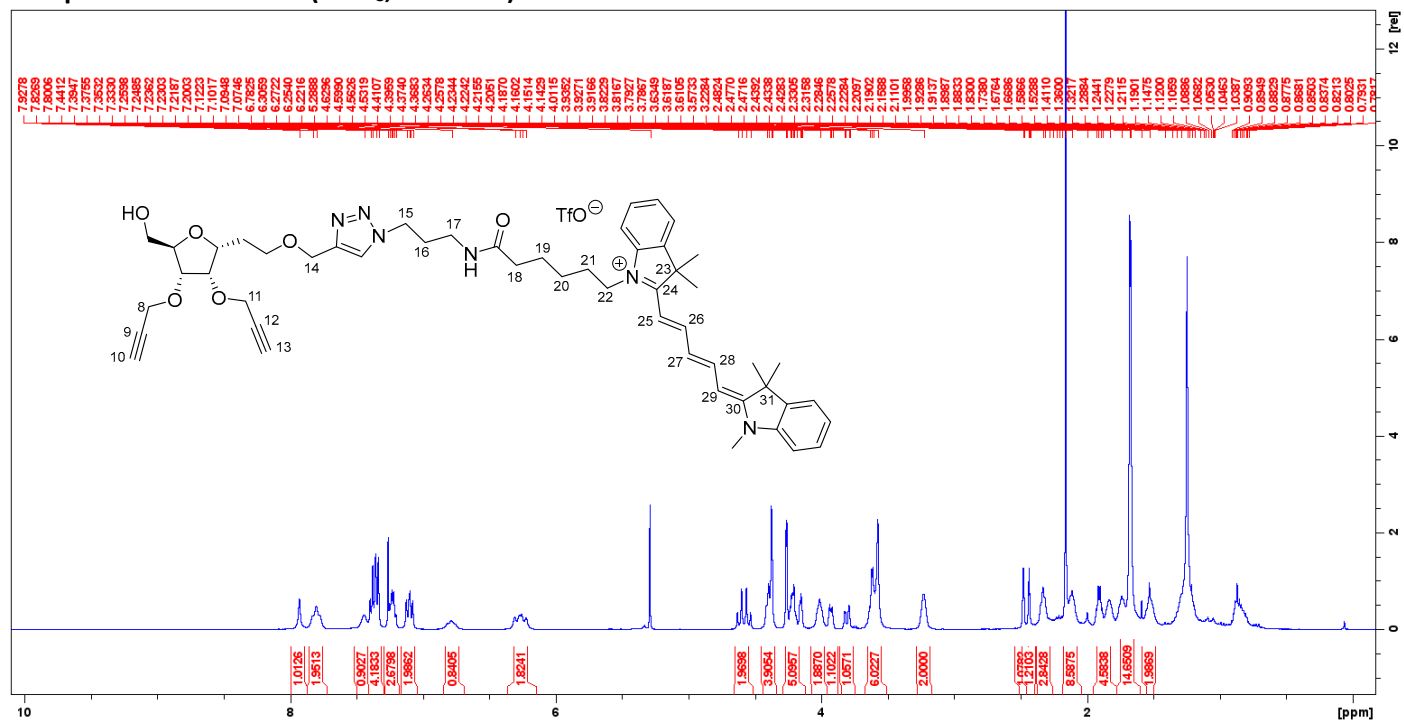
Compound 22 – ^1H NMR (D_2O , 400 MHz)



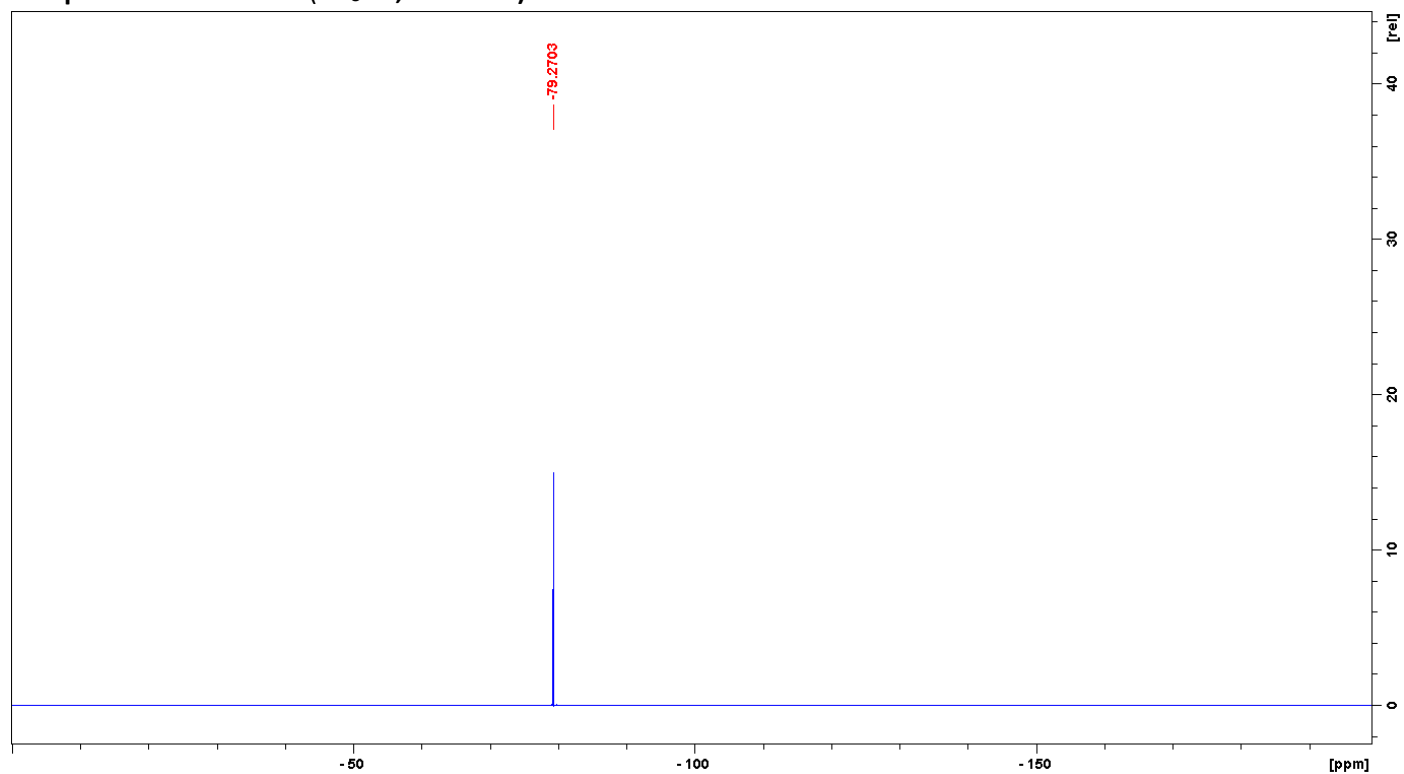
Compound 25 – ^1H NMR (CDCl_3 , 400 MHz)



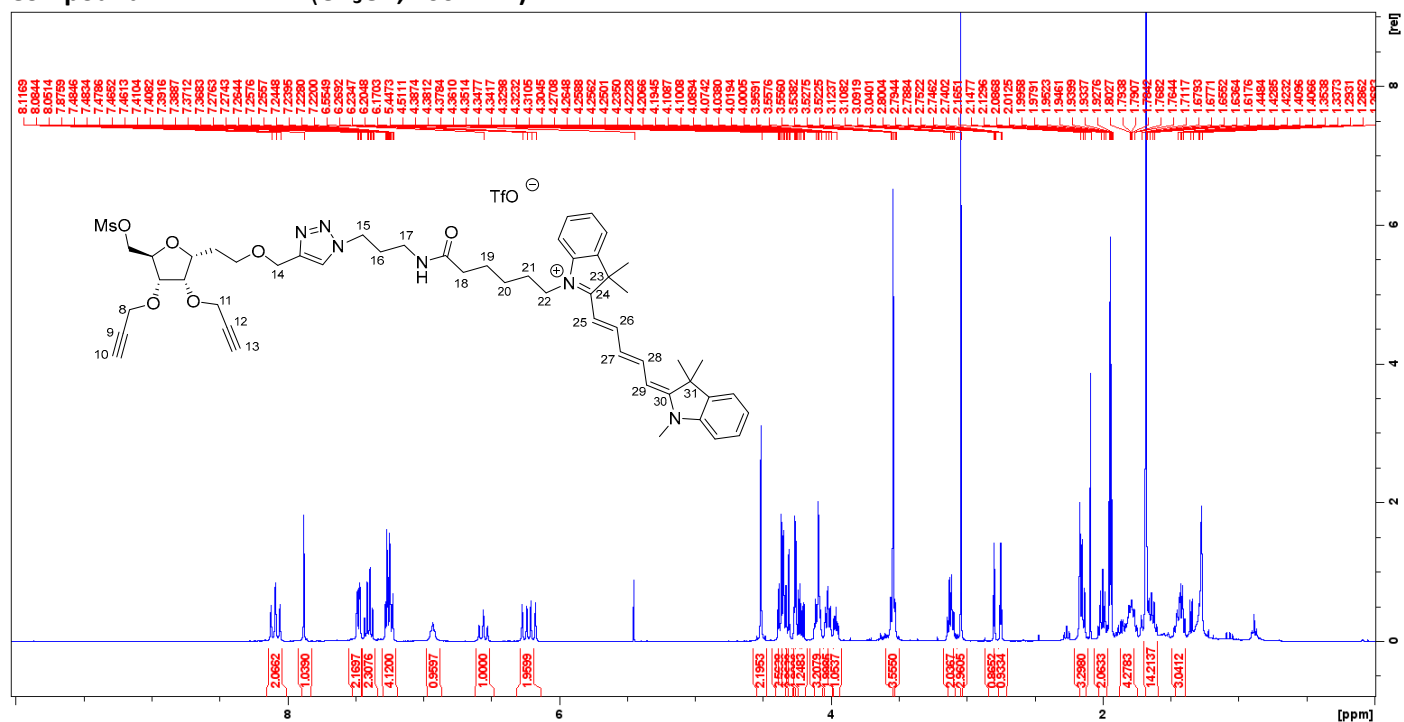
Compound 26 – ^1H NMR (CDCl_3 , 400 MHz)



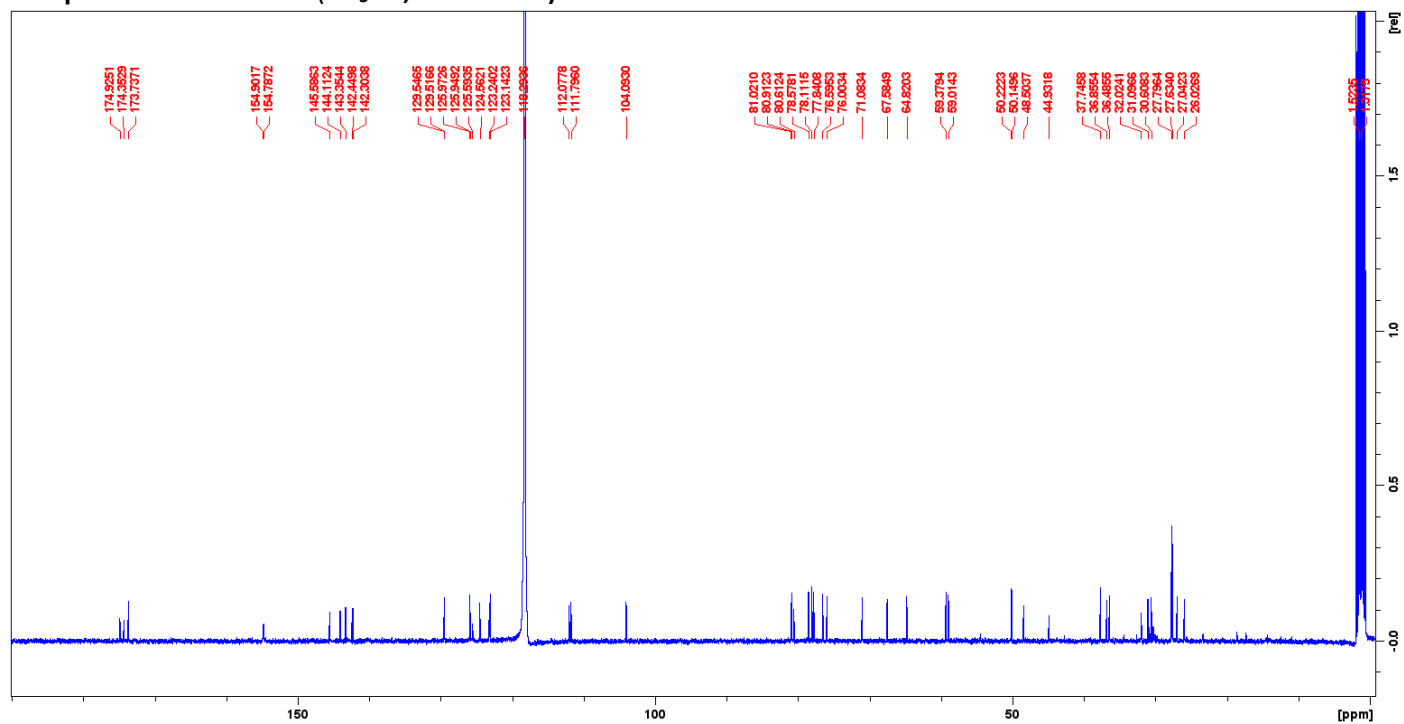
Compound 26 - ^{19}F NMR (CD_3CN , 376 MHz)



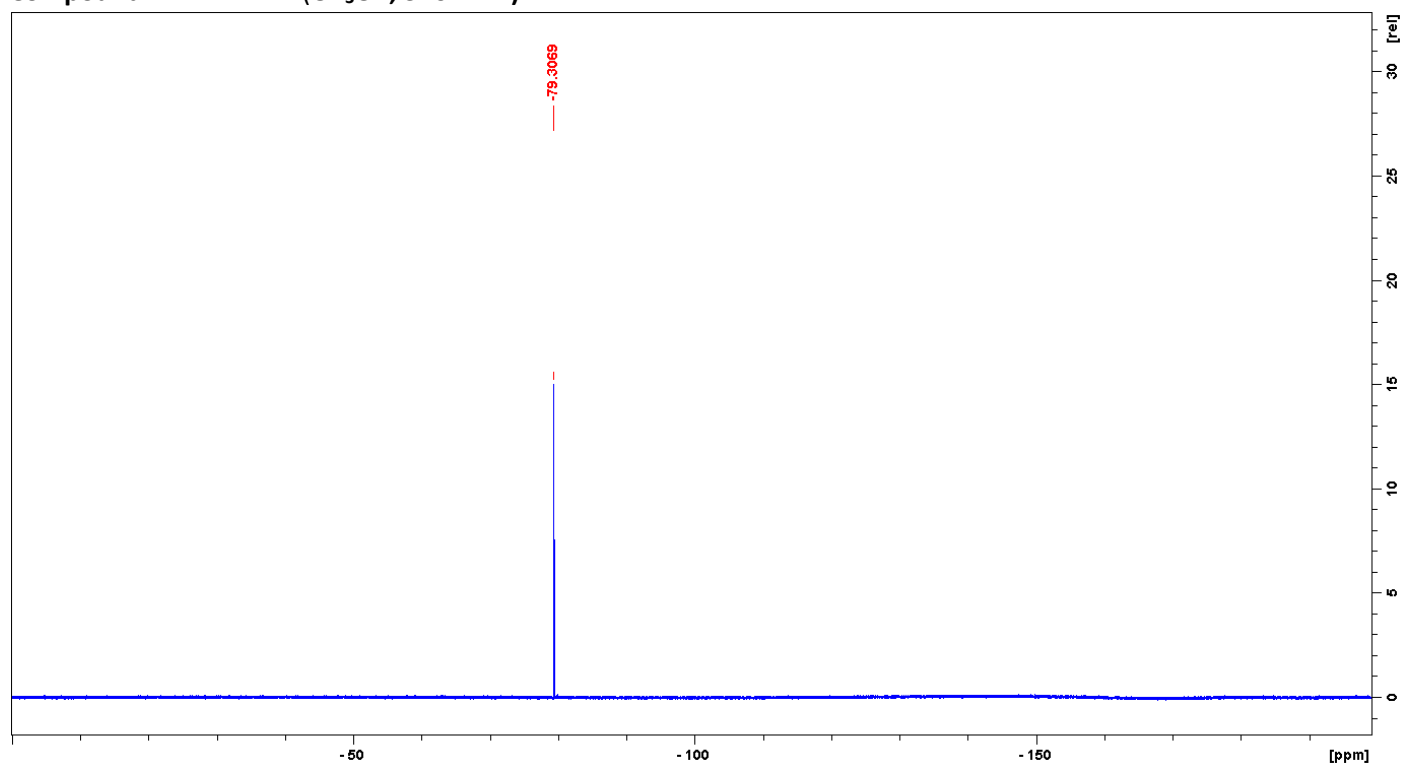
Compound 27 – ^1H NMR (CD_3CN , 400 MHz)



Compound 27 – ^{13}C NMR (CD_3CN , 100.6 MHz)

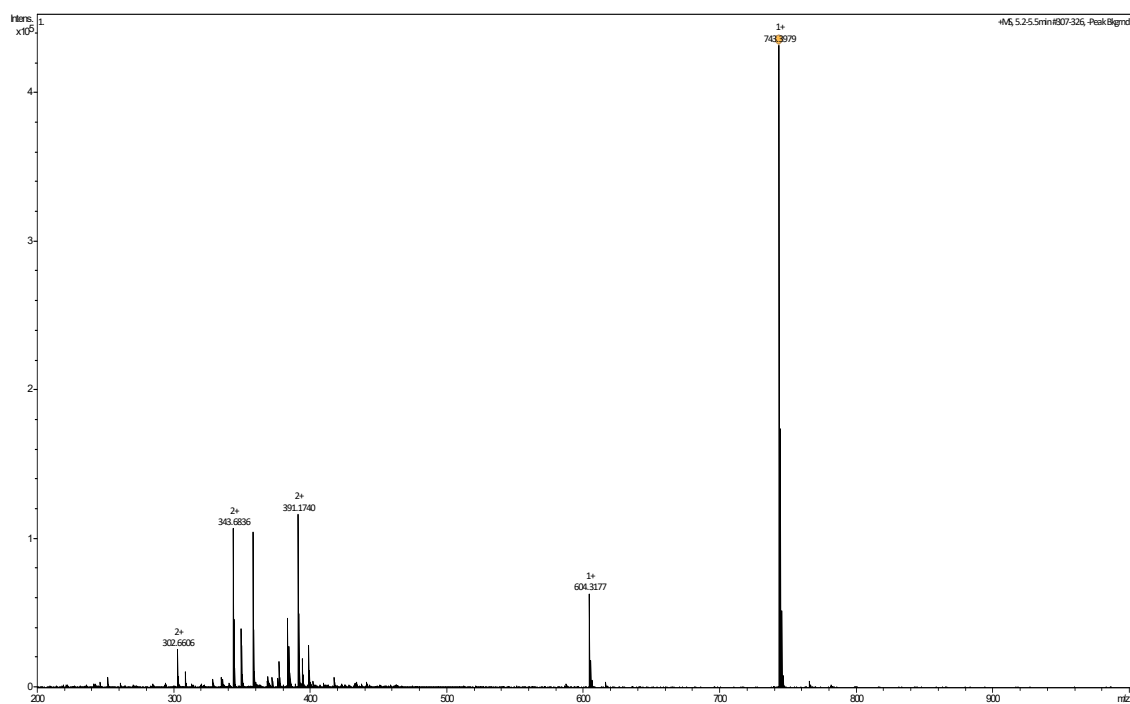


Compound 27- ^{19}F NMR (CD_3CN , 376 MHz)



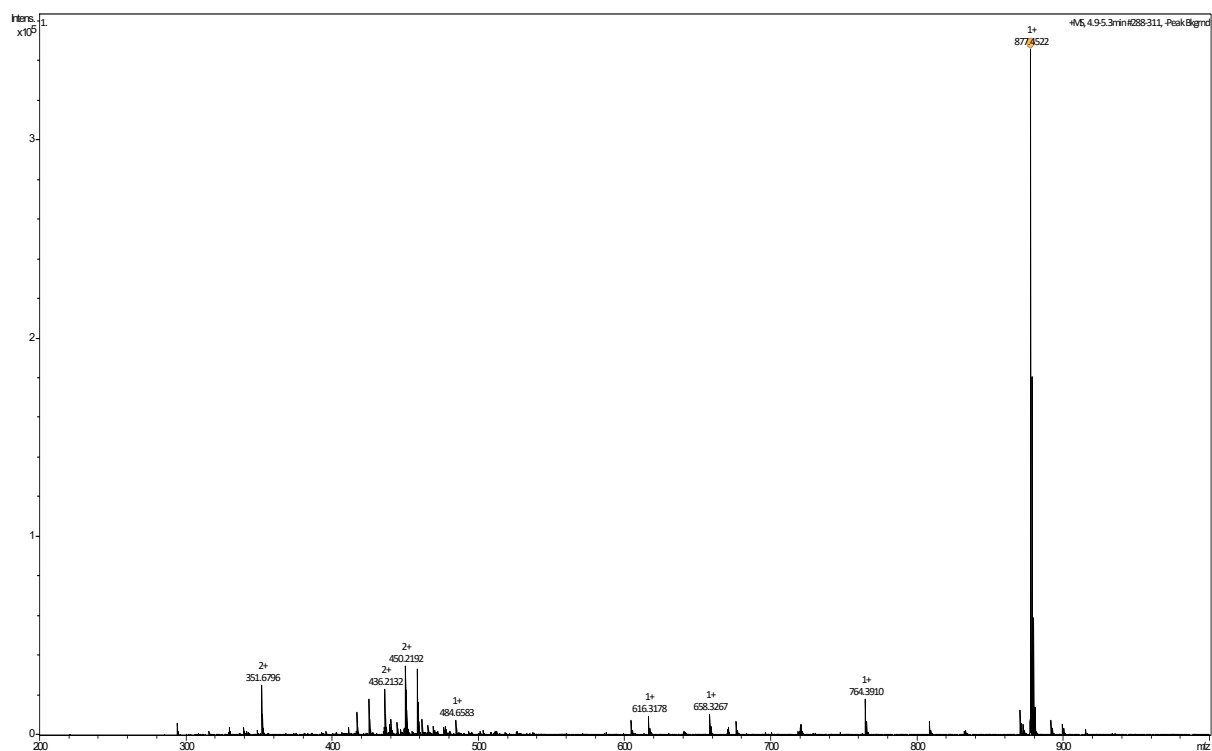
Copy of HRMS for compounds **21-22** and **23-24**

Compound **21**



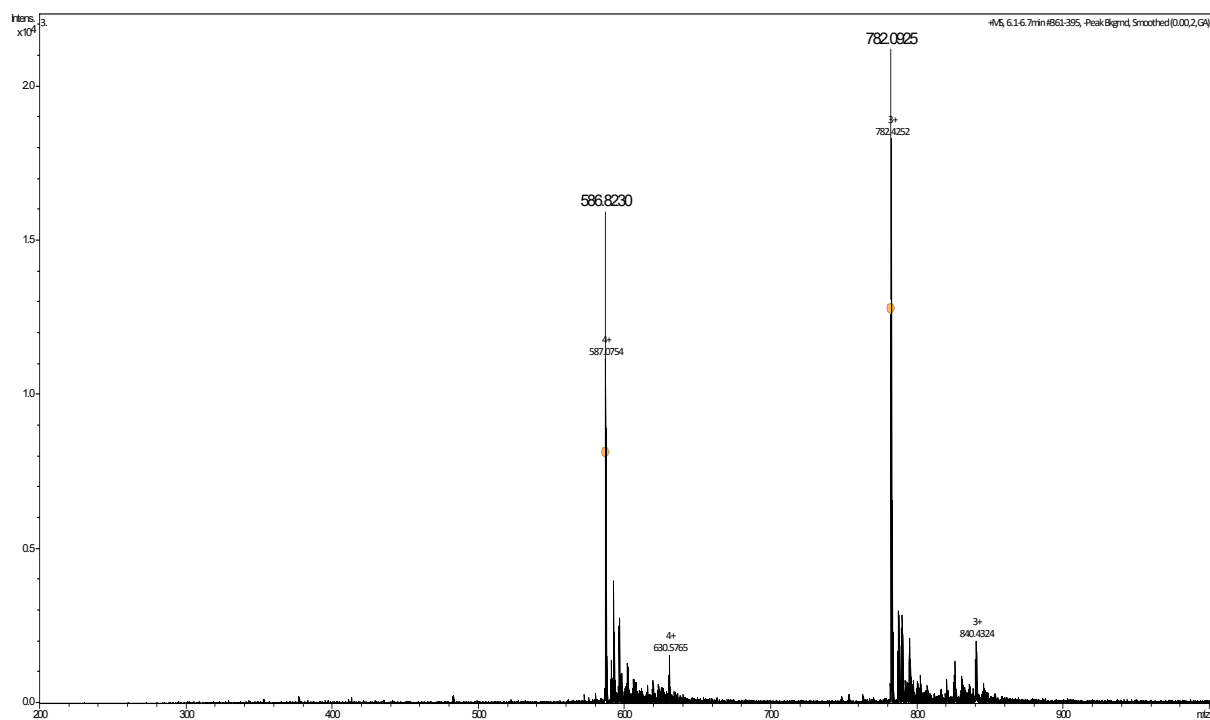
HRMS $[M]^+$ m/z = 743.3979 (calculated for $C_{33}H_{51}N_{12}O_8^+$: 743.3947). 3.2 mDa and 4.3 ppm

Compound 22



HRMS $[M]^+$ m/z = 877.4522 (calculated for $C_{38}H_{61}N_{12}O_{12}$: 877.4526).

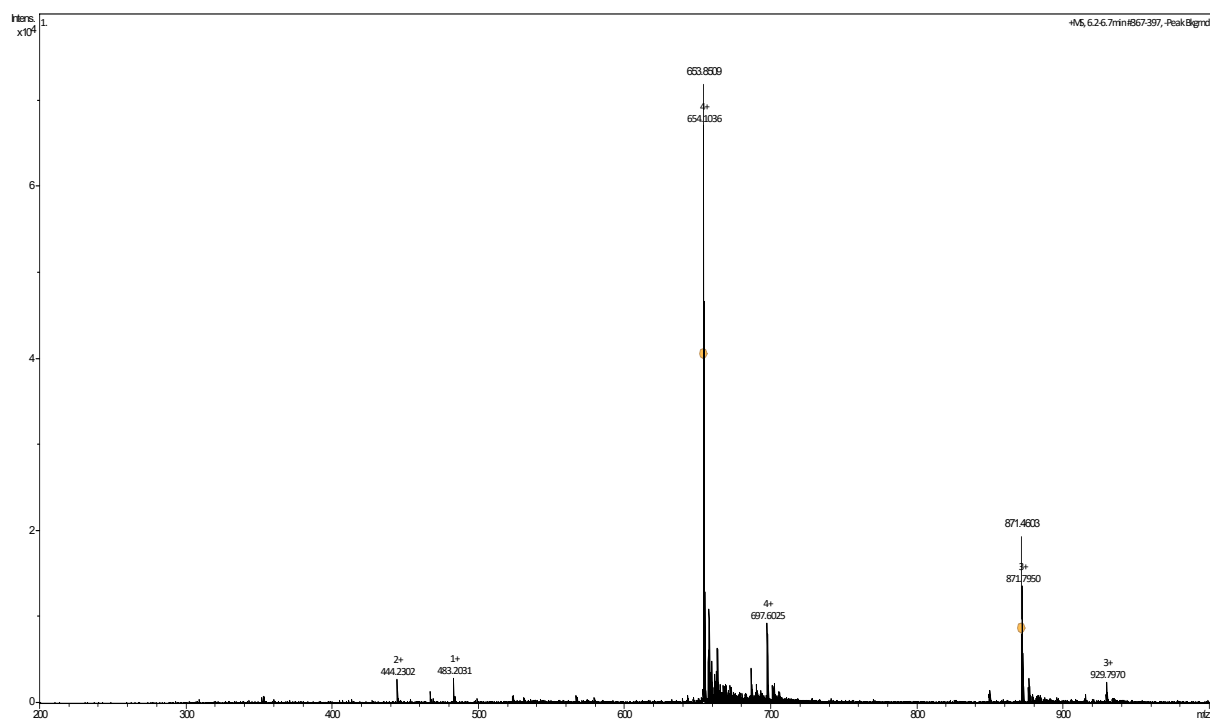
Compound 23



$[M+H]^{4+}$ m/z = 586.8230 (calculated for $C_{117}H_{167}FN_{30}O_{21}$: 586.8221). 1.2 mDa and 1.6 ppm

$[M]^{3+}$ m/z = 782.0925 (calculated for $C_{117}H_{166}FN_{30}O_{21}$: 782.0937). 0.69 mDa and 1.5 ppm

Compound **24**



$[\text{M}+\text{H}]^{4+}$ m/z = 653.8509 (calculated for $\text{C}_{127}\text{H}_{187}\text{FN}_{30}\text{O}_{29}$: 653.8511). 5.3 mDa and 6.1 ppm

$[\text{M}]^{3+}$ m/z = 871.4603 (calculated for $\text{C}_{127}\text{H}_{186}\text{FN}_{30}\text{O}_{29}$: 871.4657). 0.2 mDa and 0.3 ppm

Analytical HPLC profile of compound **19** (Figures S1 and S2)

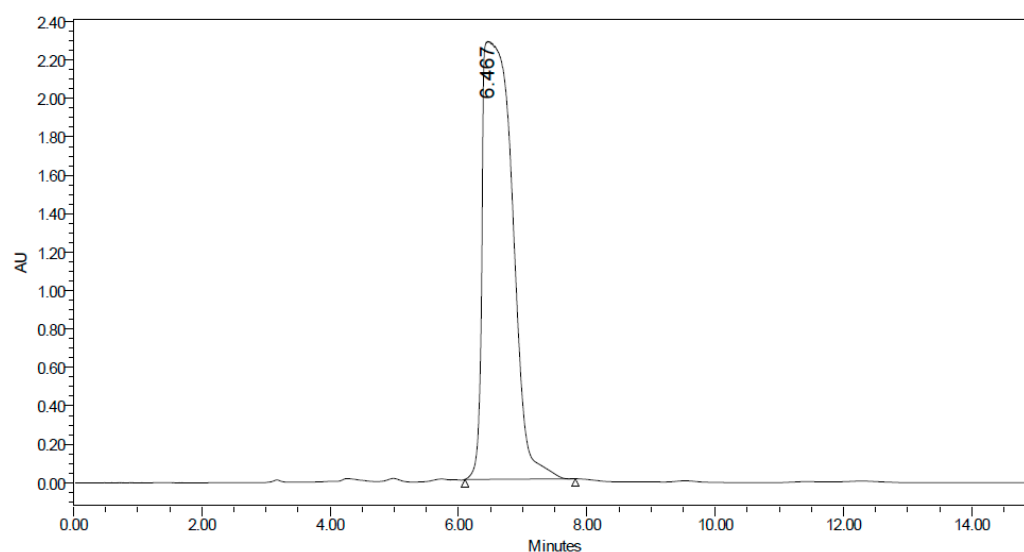


Figure S1. Analytical HPLC profile of **19** on Waters analytical HPLC. Retention time of: 6.5 min; Conditions: Luna PFP column eluted with ACN/H₂O 60/40 (v/v) with 0.1% of TFA in isocratic conditions with a flow rate of 1.0 mL/min; UV detection (650 nm).

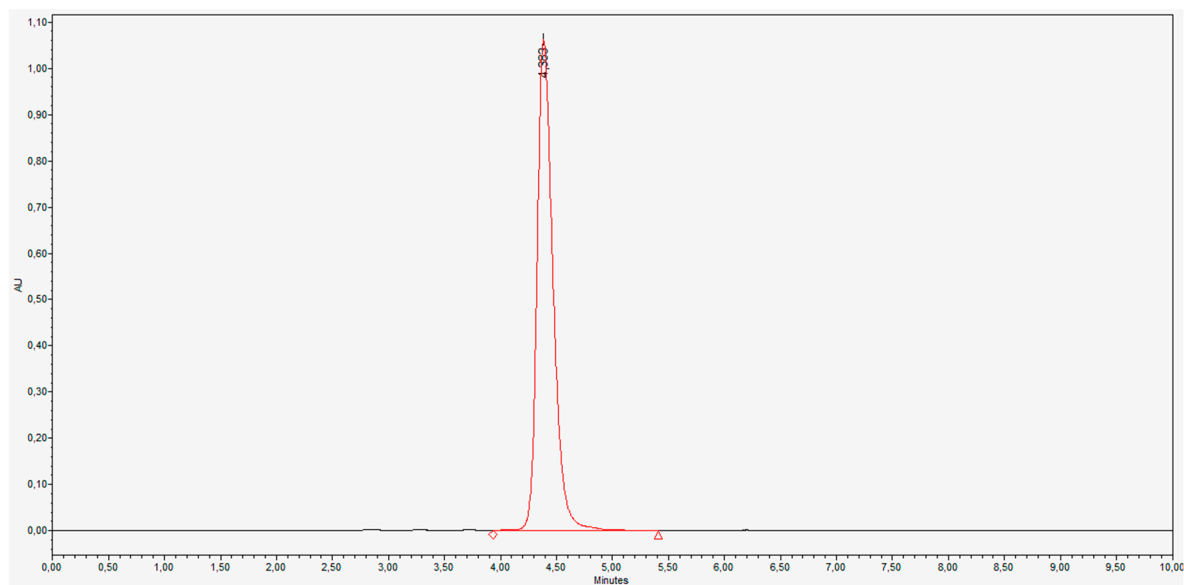


Figure S2. Analytical HPLC profile of **19** on Waters Alliance 2690. Retention time of: 4.4 min; Conditions: Luna PFP column eluted with ACN/H₂O 60/40 (v/v) with 0.1% of TFA in isocratic conditions with a flow rate of 1.5 mL/min; UV detection (650 nm).

References

- 1) M. Richard, J. Ariztia, S. Lamandé-Langle, N. Pellegrini Moïse, *Chemistry Select*, **2018**, 3(31), 9121-9126
- 2) Y. Li, Y. Liu, L. Zhang, Y. Xu, *J. Label Compd. Radiopharm*, **2012**, 55, 229–234.