Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
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Electronic Supplementary Information

for

Glyco-functionalized dinuclear rhenium(I) complexes for cell imaging.

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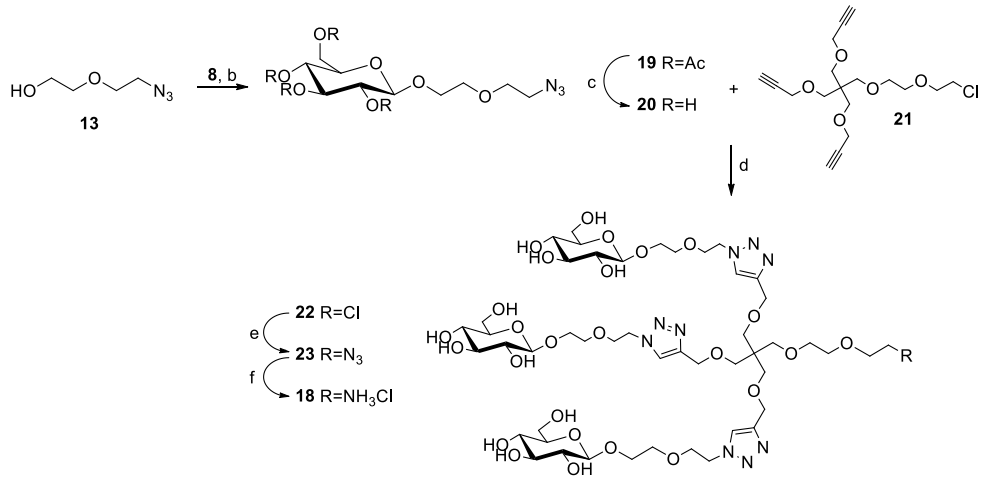

Scheme S1 Synthetic pathway for the preparation of the multivalent glycoconjugate **18**

Compound 19. penta-*O*-acetyl-D-glucose (**8**) (1.25 g, 3.2 mmol, 1 eq) were added to the solution of (**13**) (4 mmol, 1.25 eq) in DCM (20 mL) and treated with BF_3-Et_2O (608 µL, 4.8 mmol, 1.5 eq) at 0°C under nitrogen atmosphere. Then the mixture was slowly allowed to warm, stirred at room temperature and followed by TLC (Hex:AcOEt 6:4 Rf prod.= 0.24). After 5h the reaction was quenched by addition of Et_3N (1 mL) stirred for additional 15 min and concentrated under reduced pressure. The crude was purified by flash chromatography (Hex:AcOEt gradient elution) affording pure compound (**19**) (1.03 g, 70%, β-anomer). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.21 (t, $J_{2,3}$ = 9.6 Hz, 1H, H3), 5.12 – 5.05 (m, 1H, H4), 5.00 (dd, $J_{2,3}$ = 9.6, J1,2 = 8.0 Hz, 1H, H2), 4.61 (d, $J_{1,2}$ = 8.0 Hz, 1H, H1), 4.26 (dd, $J_{7a,7b}$ = 12.3, $J_{7a,8}$ = 4.7 Hz, 1H, H7a), 4.14 (dd, $J_{7a,7b}$ = 12.3, $J_{7a,8}$ = 2.4 Hz, 1H, H7b), 3.95 (dt, $J_{6a,6b}$ = 11.1, $J_{6a,5}$ = 4.0 Hz, 1H, H6a), 3.79 – 3.67 (m, 2H, H6b, H5), 3.67 – 3.63 (m, 4H, H8, H9), 3.42 – 3.32 (m, 2H, H10), 2.09 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.00 (s, 3H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.82, 170.40, 169.57, 169.53 (CO), 100.97 (C1), 72.95 (C3), 71.94 (C5), 71.42 (C2), 70.56, 70.35 (C8, C9), 69.20 (C6), 68.53 (C4), 62.07 (C7), 50.91 (C10), 20.88, 20.82, 20.76, 20.75 (OAc); MS (ESI) m/z calculated for $[C_{18}H_{27}N_3O_{11}Na]^+$: 484.15 found: 484.3 $[M+Na]^+$; $[\alpha]^D_{25}$: -15.4 (C = 0.865, $CHCl_3$).

Compound 20. A solution of compound (**19**) (1 g, 2.21 mmol) in freshly distilled MeOH (90 mL) was treated with a solution of NaOMe (10 mL, 1 M in MeOH). The reaction was stirred at room temperature under nitrogen atmosphere. After 1 h the reaction was complete. The mixture was diluted with MeOH and neutralized by addition of Amberlite IR 120-H+ resin, then the beads was filtered off and washed with MeOH. Finally the solvent was removed under reduced pressure and the crude was purified by flash chromatography (DCM: MeOH 9:1) affording pure compound (**20**) (590 mg, 97 %). ^{1}H NMR (400 MHz, MeOD) δ 4.32 (d, $J_{1,2}$ = 7.8 Hz, 1H, H1), 4.02 (m, 1H, H7a), 3.87 (dd, $J_{6a,6b}$ = 11.9, $J_{6,5}$ 1.7 Hz, 1H, H6a), 3.79 – 3.62 (m, 6H, H8, H9, H6b, H7b), 3.43 – 3.37 (m, 2H, H10), 3.35 (m, 1H, H3), 3.29 – 3.26 (m, 2H, H4, H5), 3.20 (dd, $J_{2,3}$ = 9.1, $J_{1,2}$ = 7.8 Hz, 1H, H2); ^{13}C NMR (100 MHz, MeOD) δ 104.48 (C1), 77.95 (C3, C5), 75.08 (C2), 71.60 (C4), 71.44, 71.00 (C8, C9), 69.76 (C7), 62.74 (C6), 51.76 (C10); MS (ESI) m/z calculated for $[C_{10}H_{19}N_3O_7Na]^+$: 316.11, found: 316.2 $[M+Na]^+$; $[\alpha]^D_{25}$: -25.3 (C = 0.61, MeOH).

Compound 21. Prepared as reported in literature[1]

Compound 22. To a reaction vessel containing compound (**21**) (55 mg, 155 µmol, 1 eq), TBTA (16.3 mg, 30 µmol, 0.2 eq), $CuSO_4$ (3.85 mg, 15 µmol, 0.1 eq), sodium ascorbate (12 mg, 61 µmol, 0.4 eq), compound (**20**) (150 mg, 511 µmol , 3.3 eq) were added and dissolved in a mixture of H_2O:THF 1:1 (8 mL), then the reaction was stirred under nitrogen at room temperature for 16 hours. The crude mixture was directly loaded in a C-18 column and purified by RP-18 chromatography (H_2O:MeOH gradient elution) obtaining 183 mg of pure compound (**22)** (yield 96%). ^{1}H NMR (400 MHz, MeOD) δ 8.03 (s, 3H, H11), 4.59 (t, $J_{10,9}$ = 5.0 Hz, 6H, H10), 4.54 (s, 6H, H13), 4.29 (d, $J_{1,2}$ = 7.8 Hz, 3H, H1), 4.01 – 3.94 (m, 3H, H7a), 3.94 – 3.89 (m, 6H, H9), 3.87 (dd, $J_{6a,6b}$ = 11.8, $J_{6a,5}$ = 1.7 Hz, 3H, H6a), 3.74 – 3.62 (m, 16H, H20, H8, H7b, H6b, H19), 3.62 – 3.58 (m, 2H, H18), 3.53 – 3.50 (m, 2H, H17), 3.47 (s, 6H, H14), 3.42 (s, 2H, H16), 3.39 – 3.33 (m, 3H, H3), 3.28 (m, 6H, H4, H5), 3.20 (dd, $J_{2,3}$ = 9.1, J1,2 = 7.8 Hz, 3H, H2); ^{13}C NMR (100 MHz, MeOD) δ 146.07 (C12), 125.96 (C11), 104.47 (C1), 78.04, 78.00 (C3, C5), 75.11 (C2), 72.48 (C19), 72.11 (C17), 71.64 (C4), 71.38 (C8), 71.36 (C18), 70.78 (C16), 70.35 (C9), 70.04 (C14), 69.81 (C7), 65.31 (C13), 62.77 (C6), 51.35 (C10), 46.54 (C15), 44.06 (C20); MS (ESI) m/z calculated for $[C_{48}H_{82}ClN_9O_{26}Na]^+$: 1258.50, found: 1259.0 $[M+Na]^+$; $[\alpha]^D_{25}$: -16.1 (C = 0.88, MeOH).

Compound 23. To a solution of compound (**22**) (178 mg, 143 µmol, 1 eq) in anhydrous DMF, NaN_3 (75 mg, 1.15 mmol, 8 eq) and NaI (2.14 mg, 14.3 µmol, 0.1 eq) were added. The reaction mixture was stirred at 60°C under nitrogen overnight. The crude mixture was directly loaded in a C-18 column and purified by RP-18 chromatography (H_2O:MeOH gradient elution) obtaining 170 mg of pure compound (**23**) (yield 96.5%). ^{1}H NMR (400 MHz, MeOD) δ 8.02 (s, 3H, H11), 4.61 – 4.56 (m, 6H, H10), 4.54 (s, 6H, H13), 4.29 (d, J1,2 = 7.8 Hz, 3H, H1), 4.01 – 3.94 (m, 3H, H7a), 3.94 – 3.90 (m, 6H, H9), 3.89 – 3.83 (m, 3H, H6a), 3.74 – 3.62 (m, 14H, H6b, H7b, H8, H19), 3.61 – 3.56 (m, 2H, H18), 3.54 – 3.49 (m, 2H, H17), 3.47 (s, 6H, H14), 3.43 (s, 2H, H16), 3.39 – 3.33 (m, 5H, H3, H20), 3.29 – 3.25 (m, 6H, H4, H5), 3.20 (dd, J2,3 = 9.0, $J_{1,2}$ = 7.8 Hz, 3H, H2); ^{13}C NMR (100 MHz, MeOD) δ 146.10 (C12), 125.93 (C11), 104.49 (C1), 78.05, 78.01 (C3, C5), 75.11 (C2), 72.18 (C17), 71.65 (C4), 71.40 (C8, C18), 71.16 (C19), 70.84 (C16), 70.36 (C9), 70.08 (C14), 69.81 (C7), 65.33 (C13), 62.79 (C6), 51.83 (C20), 51.36 (C10), 46.56 (C15); MS (ESI) m/z calculated for $[C_{48}H_{82}N_{12}O_{26}Na]^+$:1265.54, found: 1265.8 $[M+Na]^+$; $[\alpha]^D_{25}$: -18.5 (C = 0.52, MeOH).

Compound 18. To a solution of compound (**23**) (26.2 mg, 21 µmol, 1 eq) in freshly distilled MeOH (1 mL), HCl (21 µL, 1.25 M in MeOH) was slowly added under nitrogen atmosphere. After 10 min a catalytic amount of Pd/C was added, then the mixture was stirred under H_2 atmosphere at r.t. for 1 hour. The crude was diluted with MeOH and the cataylst was filtered off through a celite pad. Remotion of the solvent under reduced pressur afforded pure compound (**18**) (22.1 mg, 84% yield) as chlorohydrate salt. ^{1}H NMR (400 MHz, MeOD) δ 8.05 (s, 3H, H11), 4.63 – 4.56 (m, 6H, H10), 4.53 (s, 6H, H13), 4.30 (d, $J_{1,2}$ = 7.8 Hz, 3H, H1), 4.01 – 3.95 (m, 3H, H7a), 3.93 – 3.90 (m, 6H, H9), 3.89 – 3.83 (m, 3H, H6a), 3.76 – 3.63 (m, 14H, H6b, H7b, H8, H19), 3.62 – 3.57 (m, 2H, H18), 3.56 – 3.51 (m, 2H, H17), 3.46 (s, 6H, H14), 3.42 (s, 2H, H16), 3.40 – 3.35 (m, 3H, H3), 3.30 – 3.26 (m, 6H, H4, H5), 3.20 (dd, $J_{2,3}$ = 9.0, $J_{1,2}$ = 7.8 Hz, 3H, H2), 3.12 – 3.07 (m, 2H, H20); ^{13}C NMR (100 MHz, MeOD) δ 145.92 (C12), 126.05 (C11), 104.44 (C1), 78.03, 77.99 (C3, C5), 75.09 (C2), 72.20 (C17), 71.62 (C4), 71.37, 71.34 (C8, C18), 70.88 (C16), 70.31 (C9), 70.03 (C14), 69.78 (C7), 68.49 (C19), 65.22 (C13), 62.72 (C6), 51.34 (C10), 46.49 (C15), 40.95 (C20); MS (ESI)

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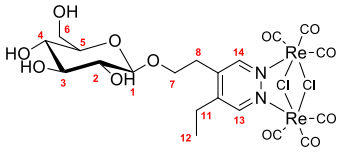
[1] N. Varga, I. Sutkeviciute, R. Ribeiro-Viana, A. Berzi, R. Ramdasi,
A. Daghetti, G. Vettoretti, A. Amara, M. Clerici, J. Rojo, F.Fieschi, A. Bernardi *Biomaterials* **2014**, *35*, 4175–4184

m/z calculated for $[C_{48}H_{84}N_{10}O_{26}Na]^+$: 1239.55, found: 1240.0 $[M+Na]^+$ and 620.7 $[M+H+Na]^{2+}$; $[\alpha]^{D}_{25}$: -17.25 (C = 1.10, MeOH).

Compounds Numbering for spectral assignment



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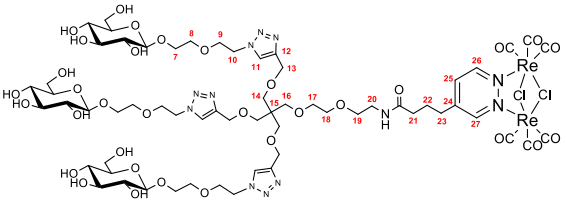
Molecular Weight: 925,7149



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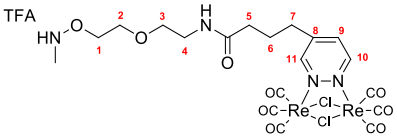

Compound 17b
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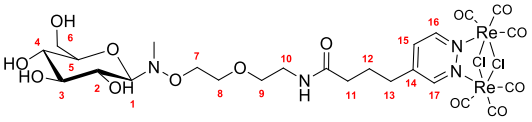

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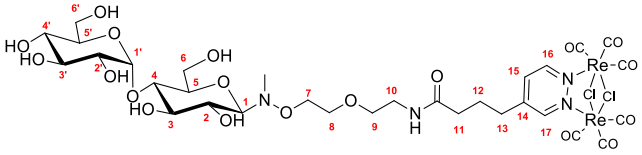
Molecular Weight: 1055,8600

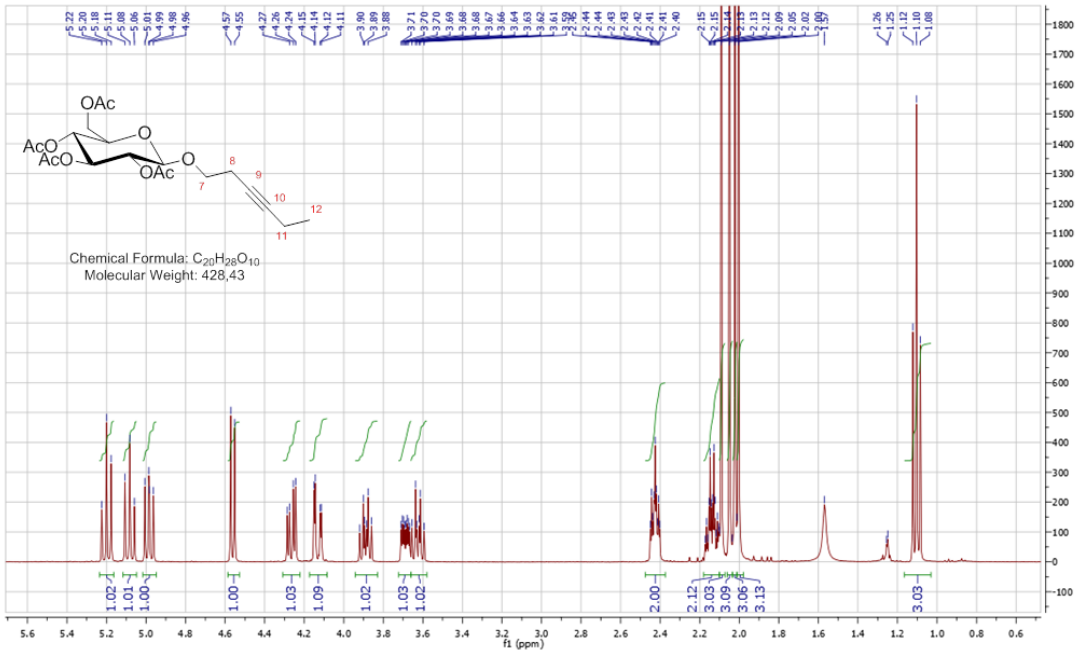


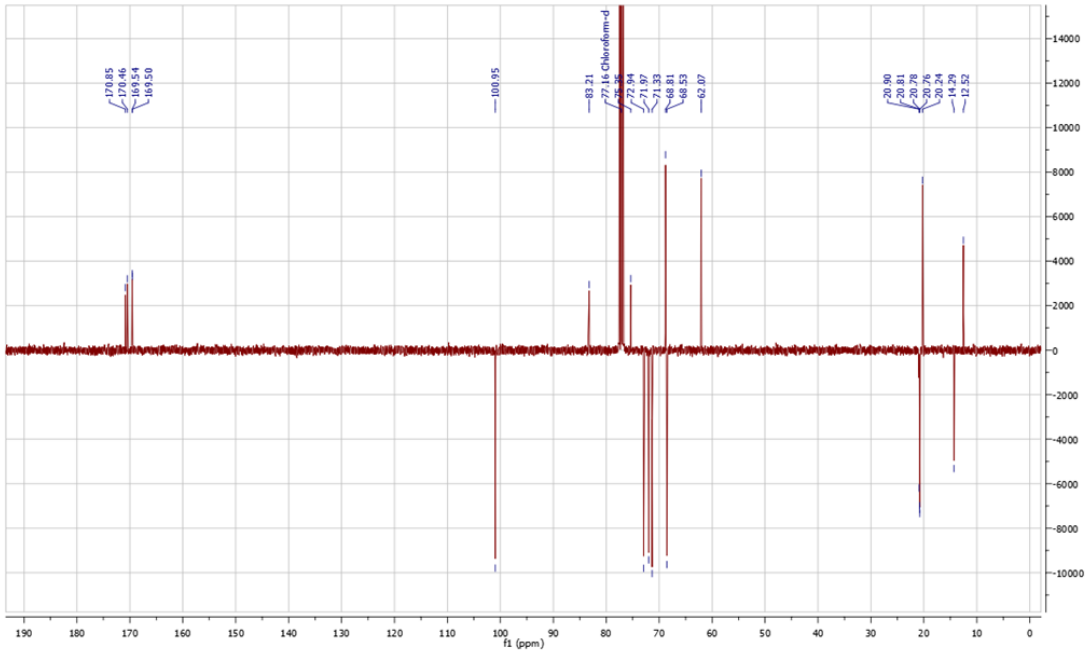
Compound 5
Chemical Formula: $C_{31}H_{42}Cl_2N_4O_{19}Re_2$
Exact Mass: 1218,0935
Molecular Weight: 1218,0006

Figure S2: numbering of compounds (**3**-**6**) and precursor (**17b**).

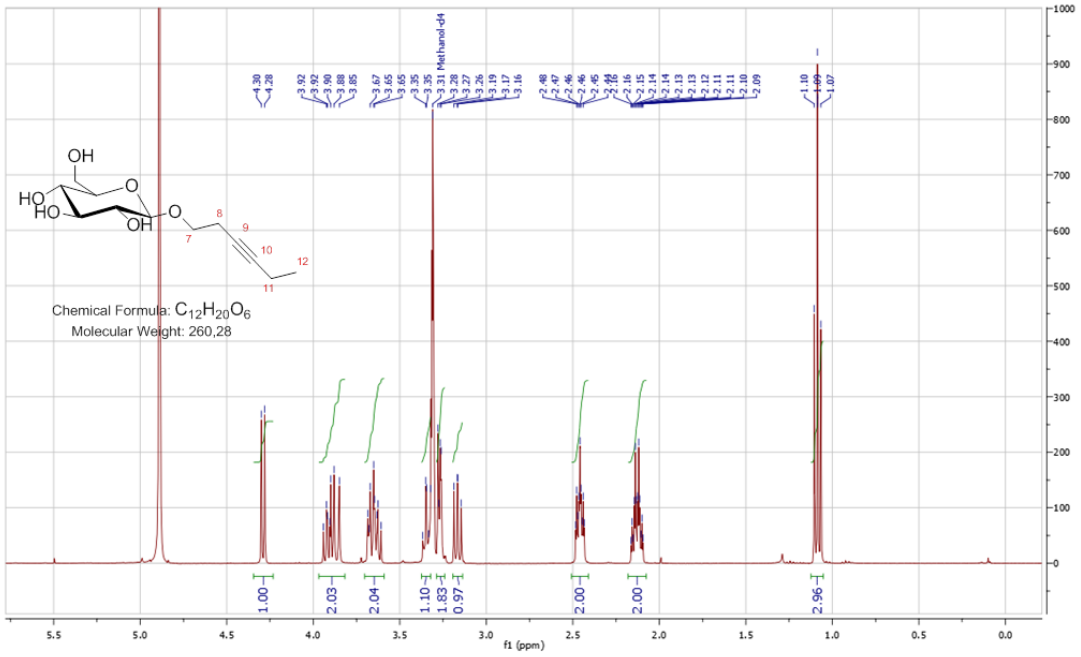
^{1}H and ^{13}C spectra

Compound 9





Compound 10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HO
HO
OH
O
O
7
8
9
10
11
12
Chemical Formula: C12H20O6
Molecular Weight: 260,28
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2.00
2.96
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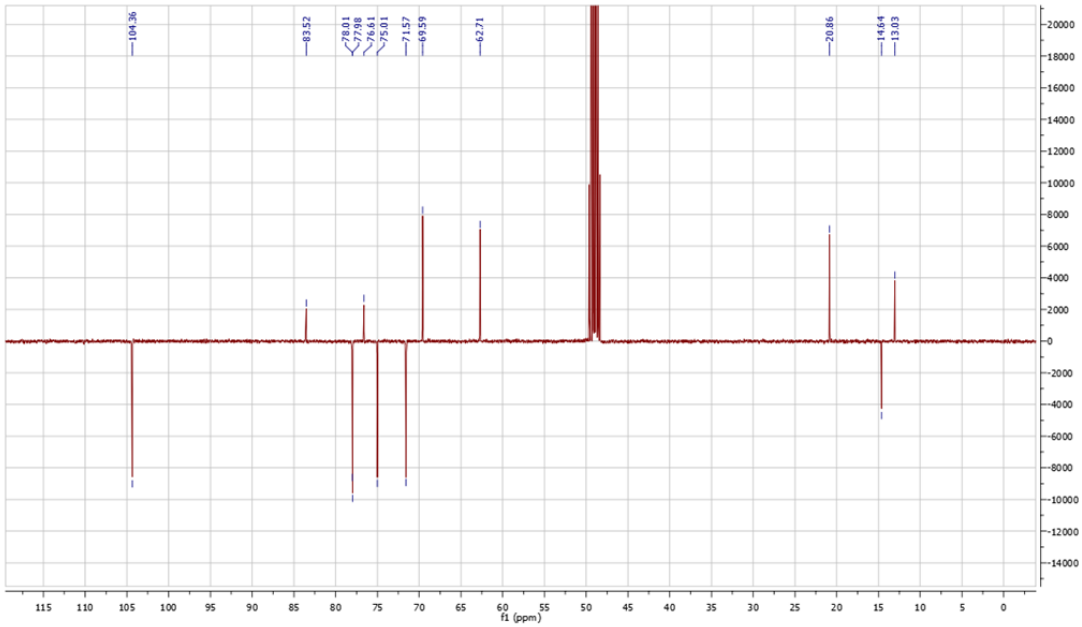
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83.52
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77.98
76.61
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71.57
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20.86
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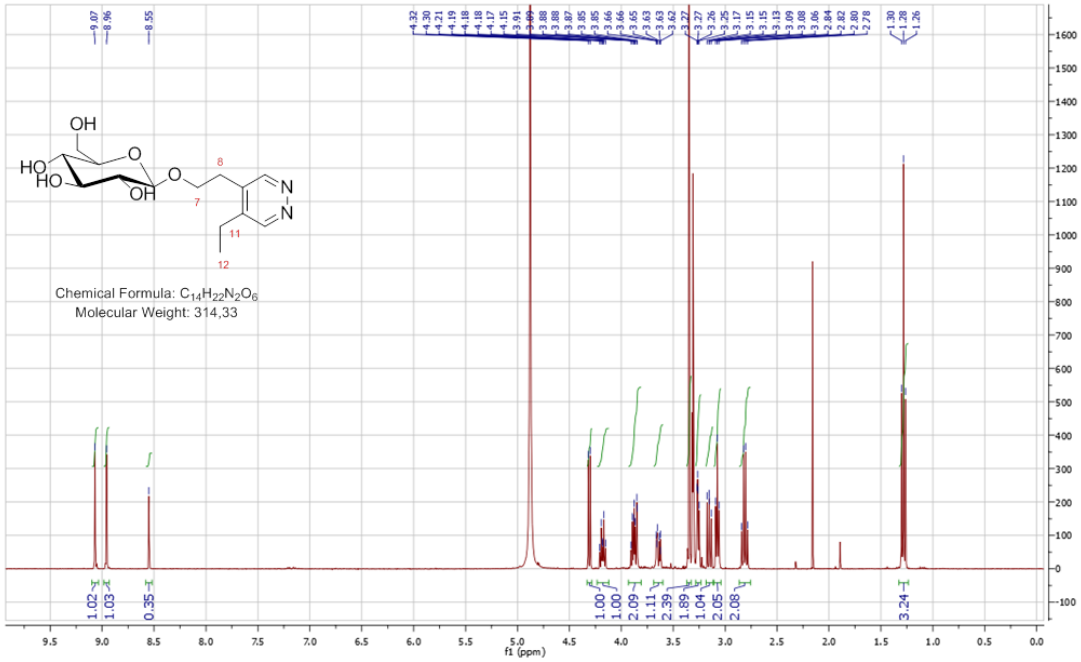
Compound 11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OH
O
O
8
7
N
N
11
12
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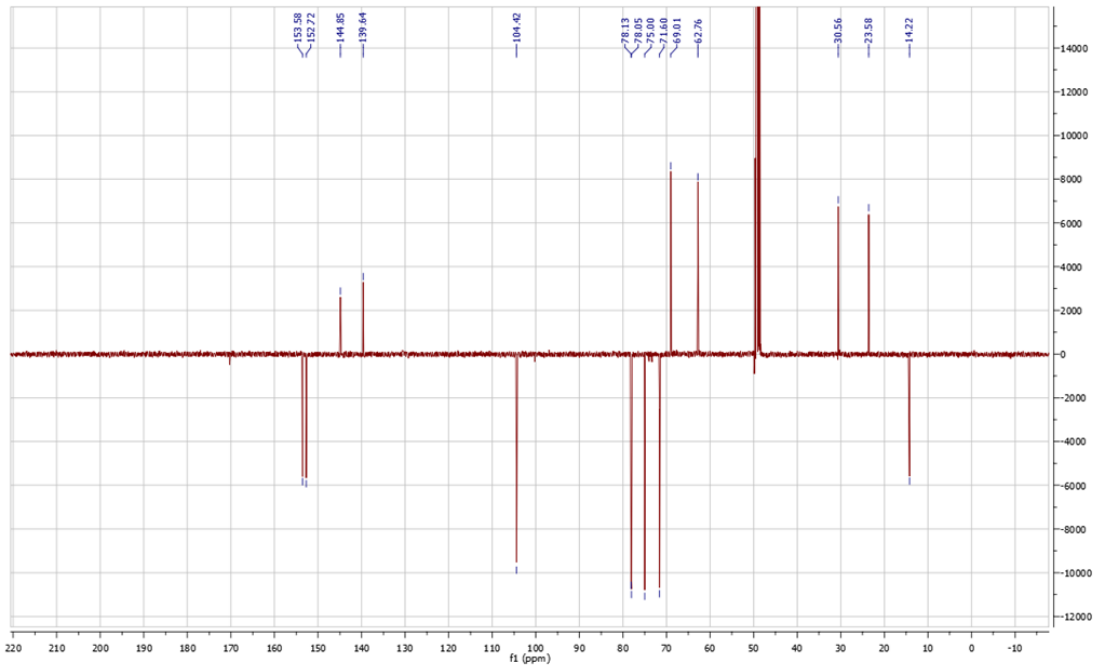
Molecular Weight: 314,33
f1 (ppm)


153.58
152.72
144.85
139.64
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78.13
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69.01
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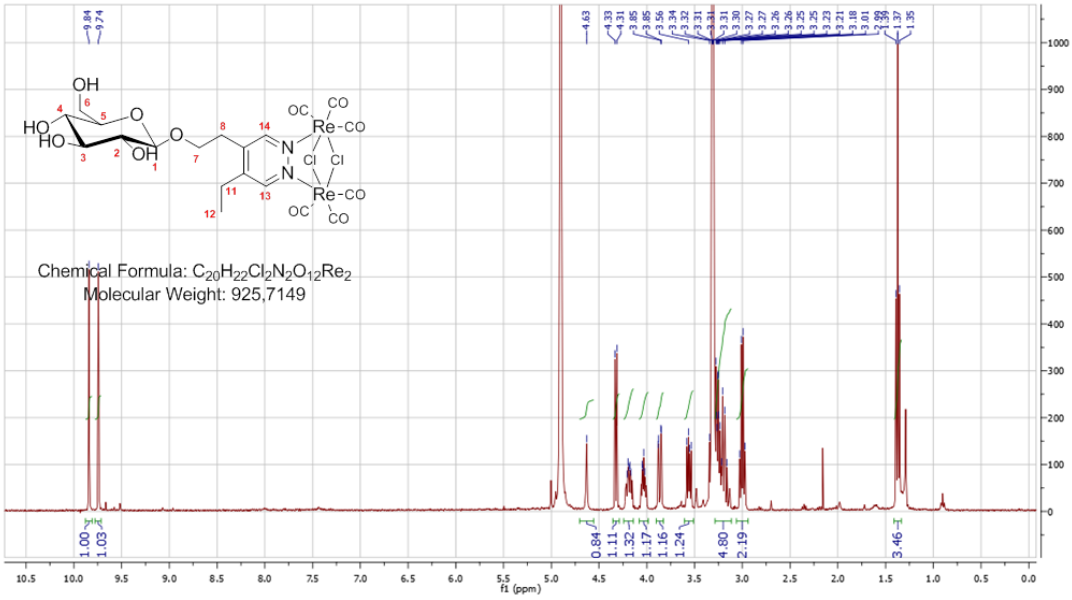
23.58
14.22
f1 (ppm)

Compound 3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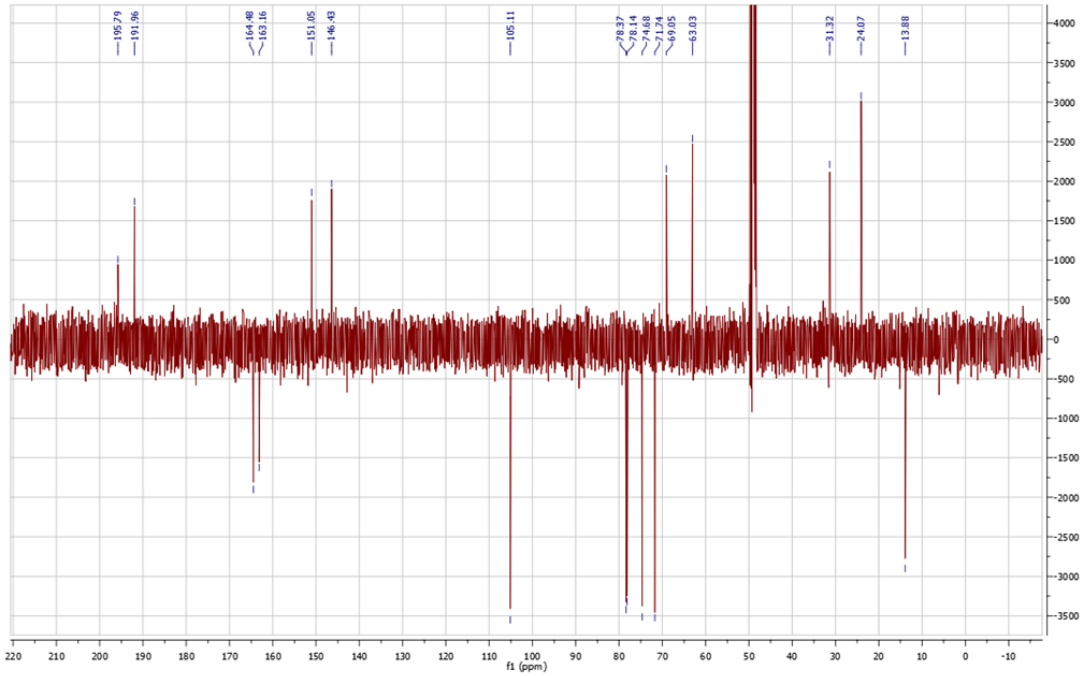
Molecular Weight: 925,7149
f1 (ppm)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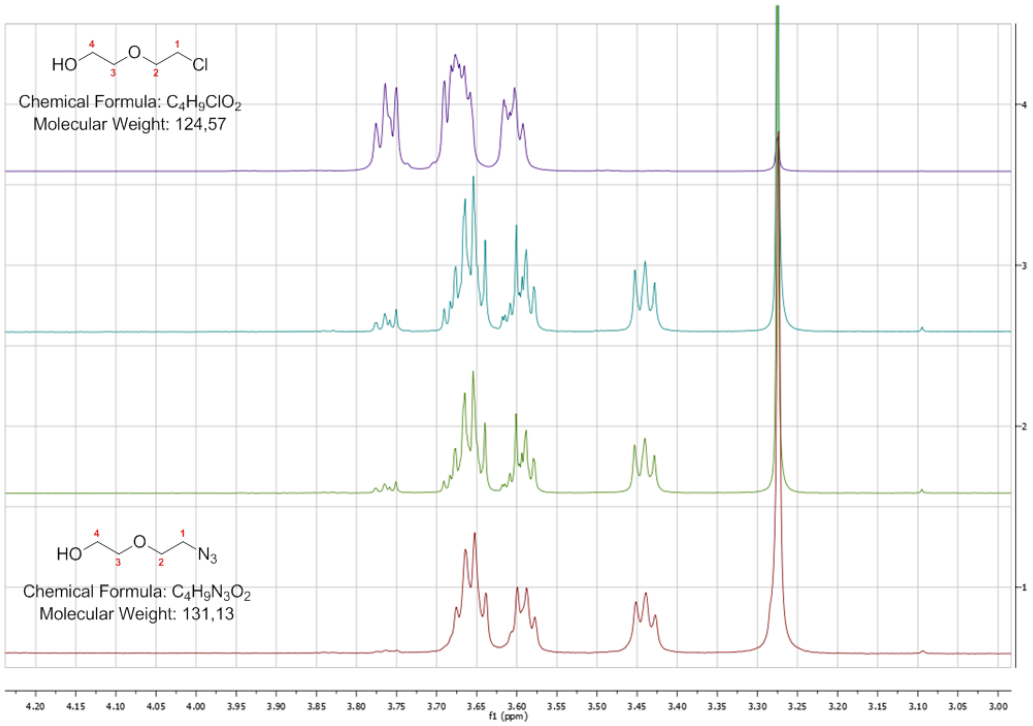
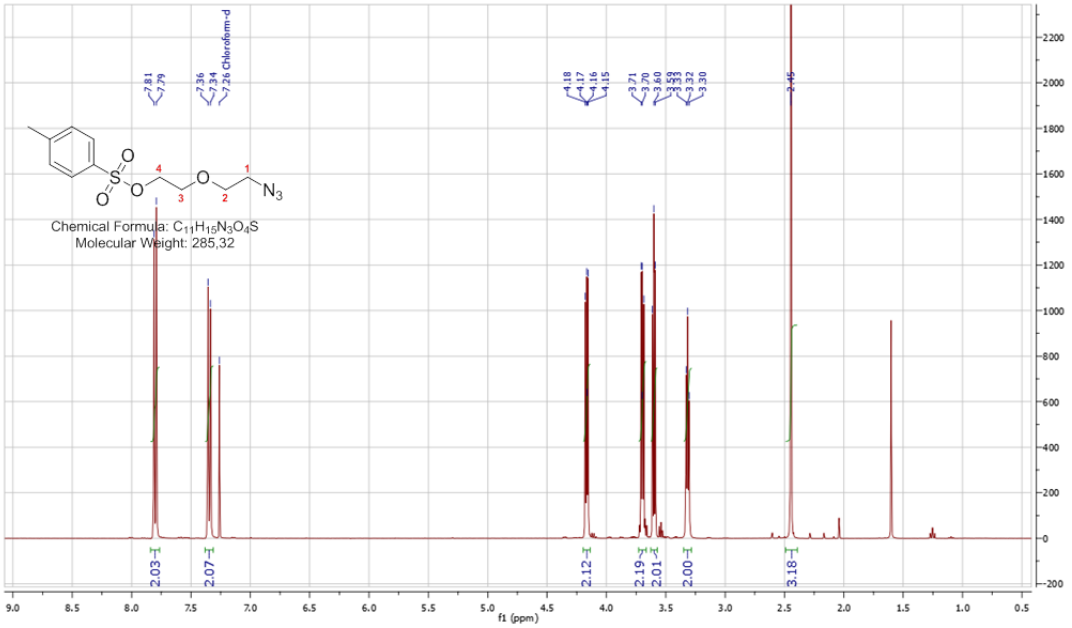
Conversion of compound 12 in compound 13



Compound 14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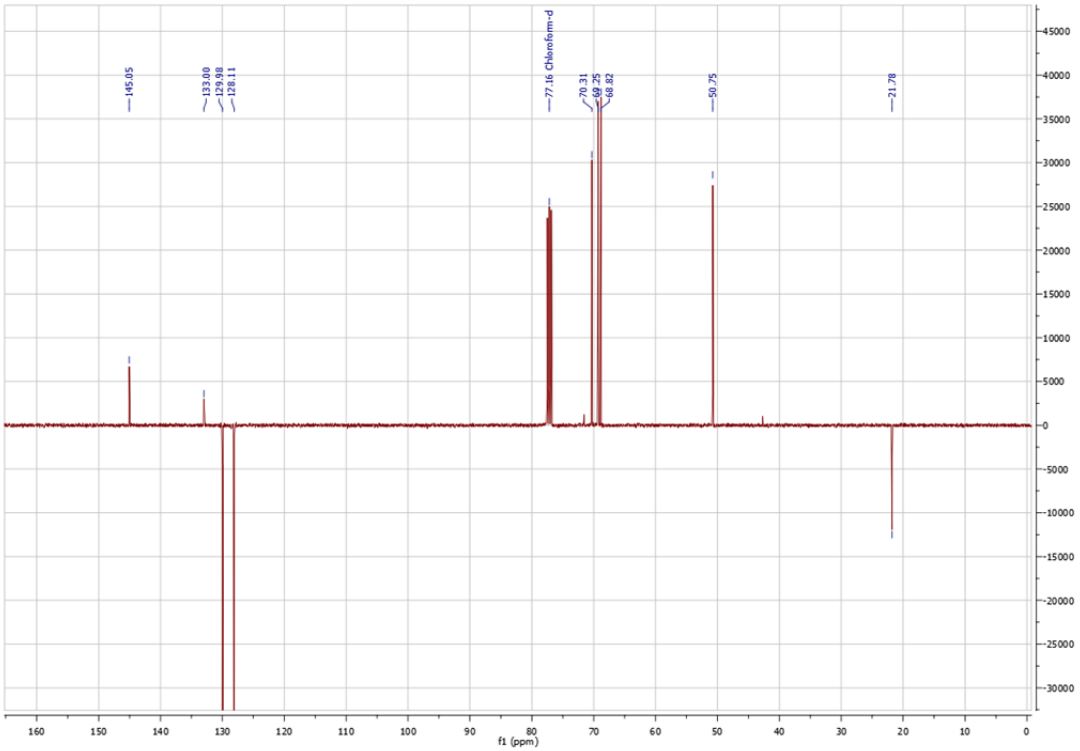
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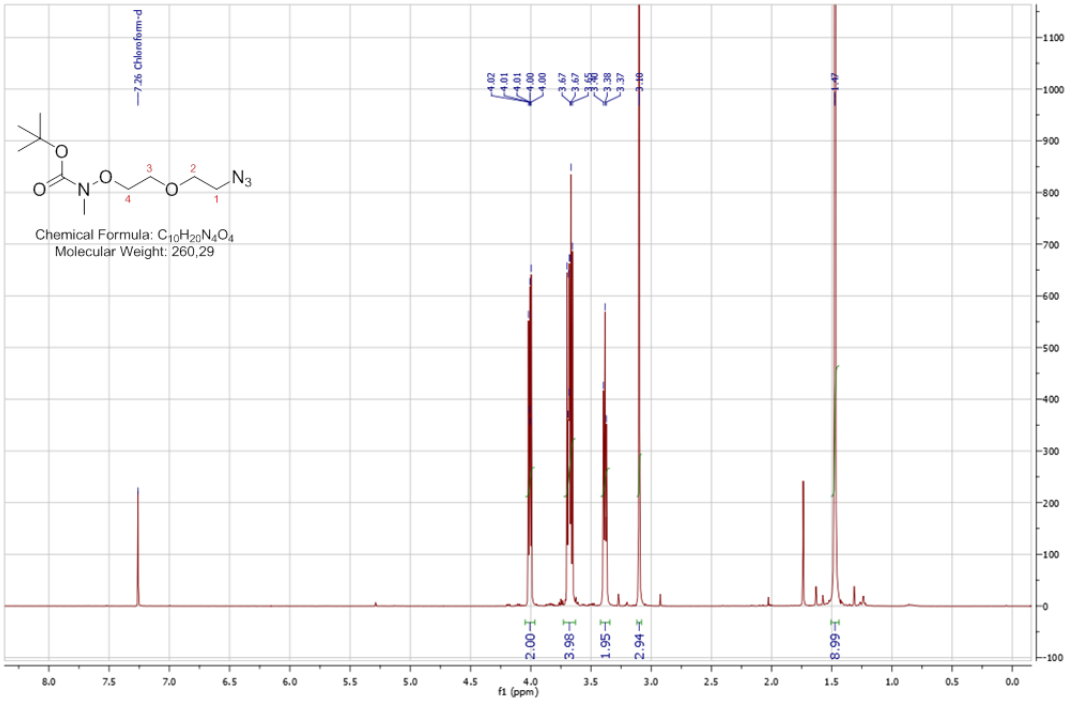
Compound 15


7.26 Chloroform-d
4.02
4.01
4.01
4.00
4.00
3.67
3.67
3.66
3.66
3.38
3.37
3.18
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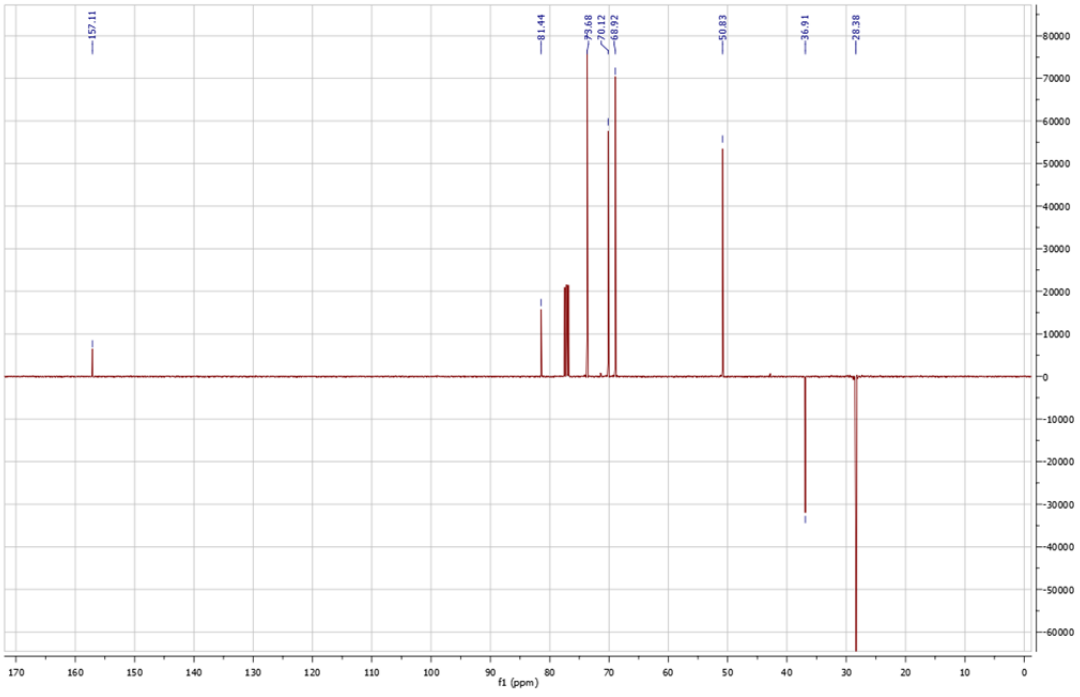
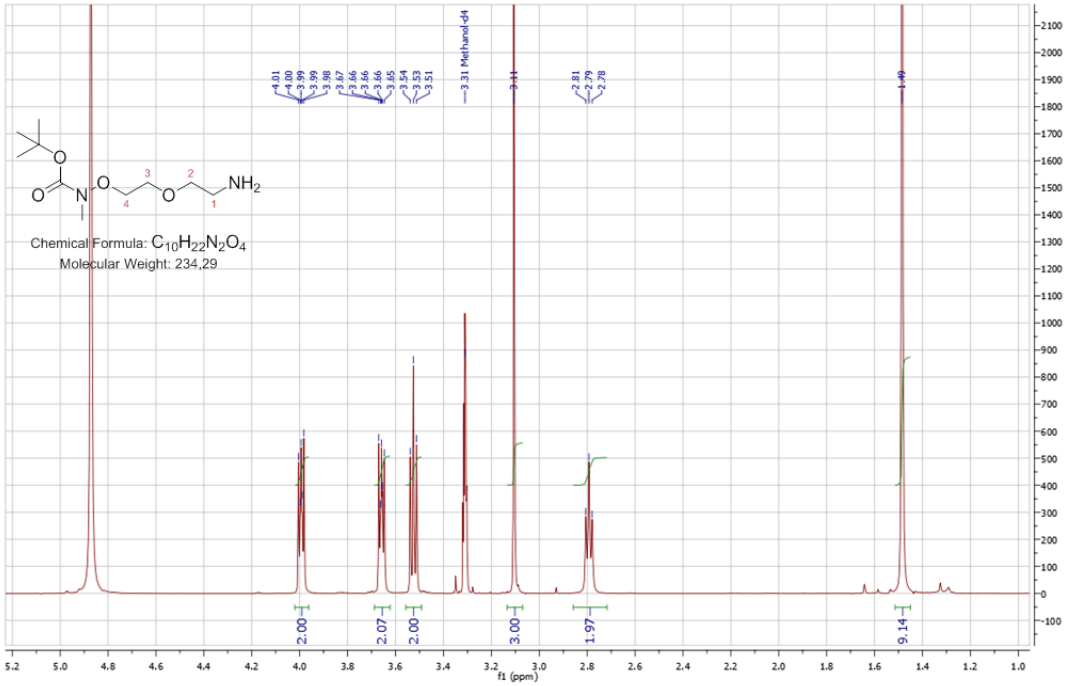
8.99
f1 (ppm)
Chemical Formula: C10H20N4O4
Molecular Weight: 260,29


157.11
81.44
79.68
70.12
68.92
50.83
36.91
28.38
f1 (ppm)

Compound 16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Molecular Weight: 234,29
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4.00
3.99
3.99
3.98
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3.51
3.31 Methanol-d4
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2.00
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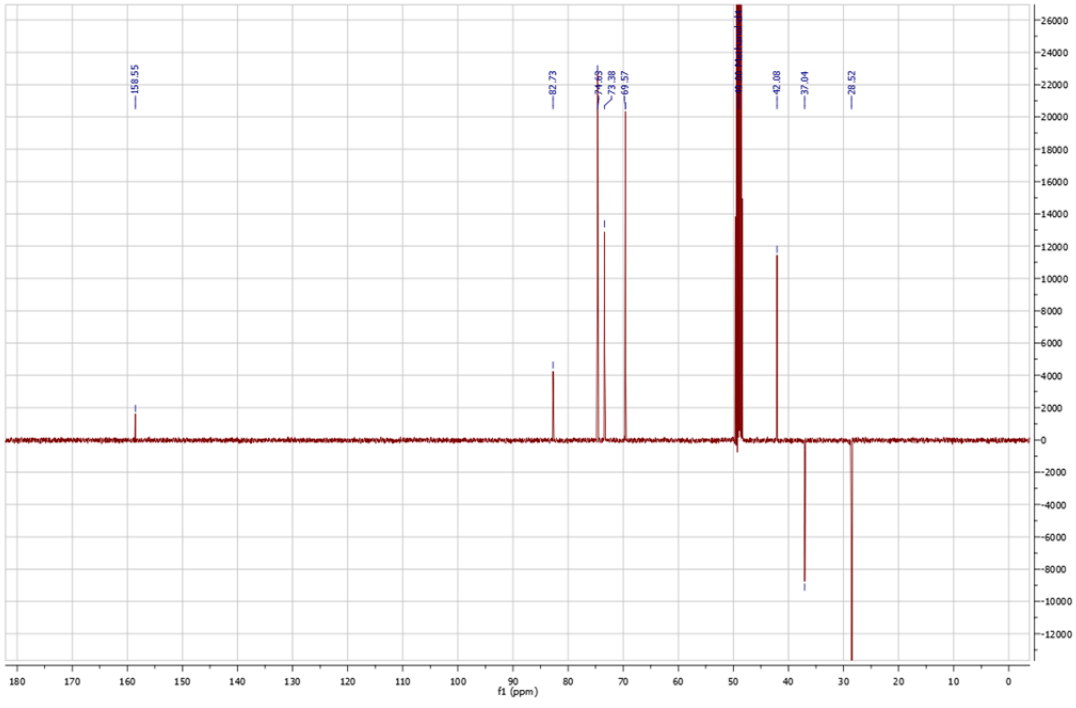
1.97
9.14
f1 (ppm)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73.38
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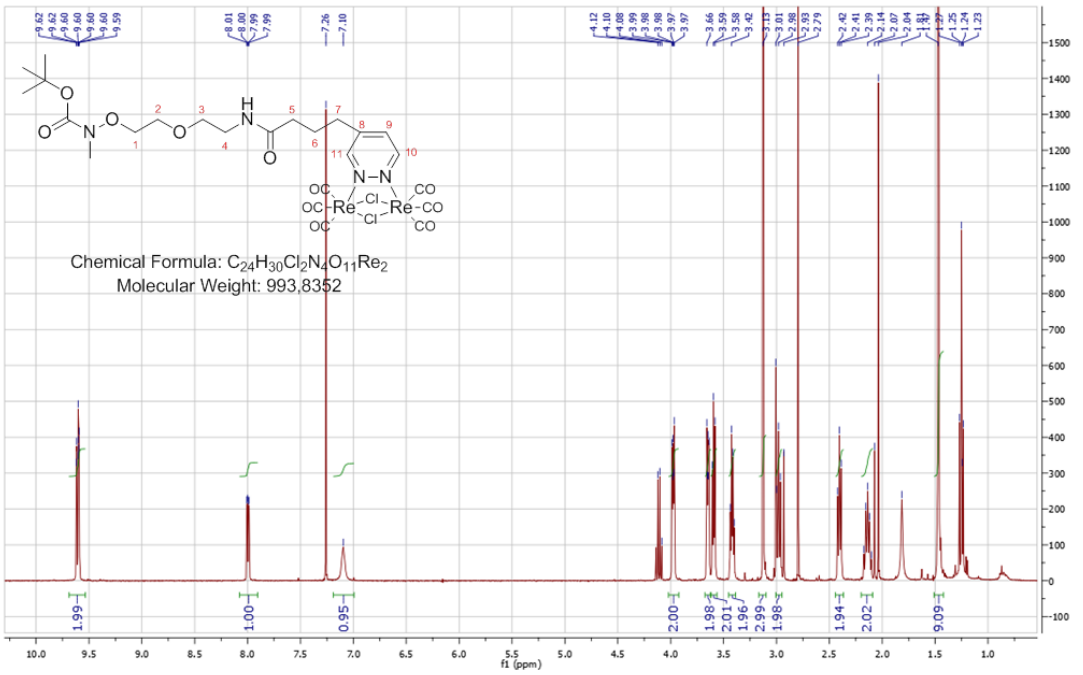
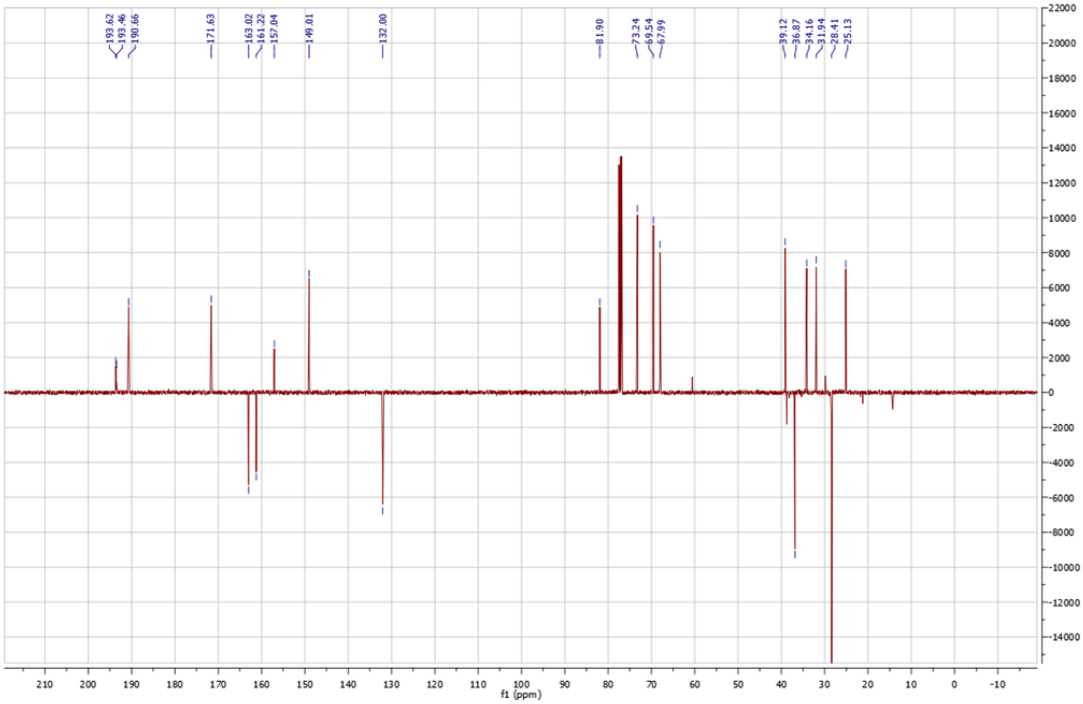
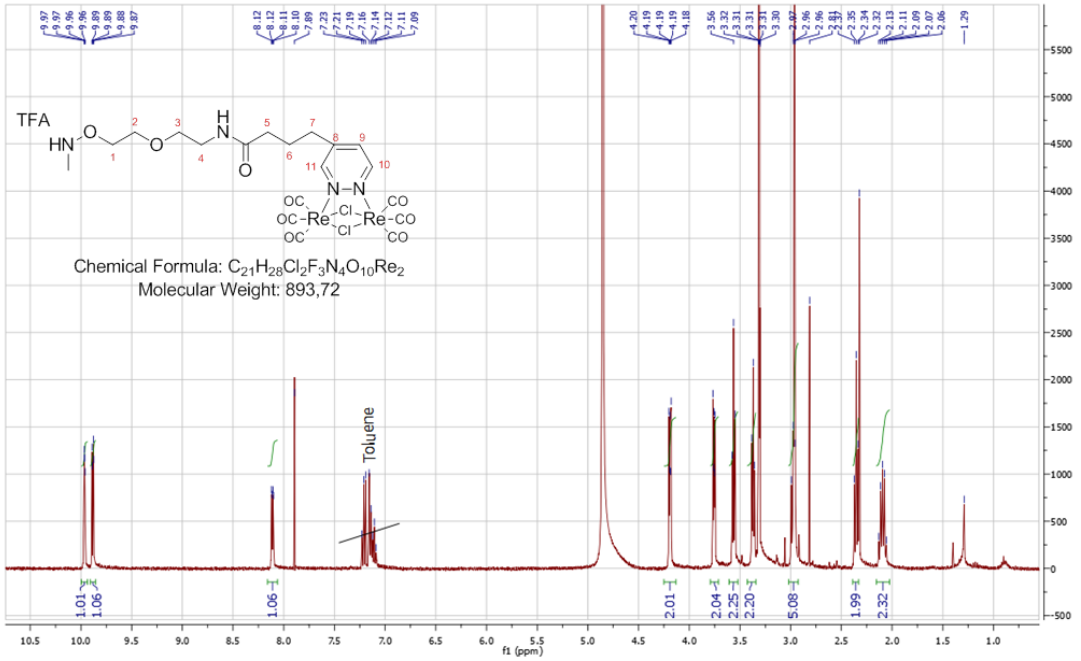
42.08
37.04
28.52
f1 (ppm)

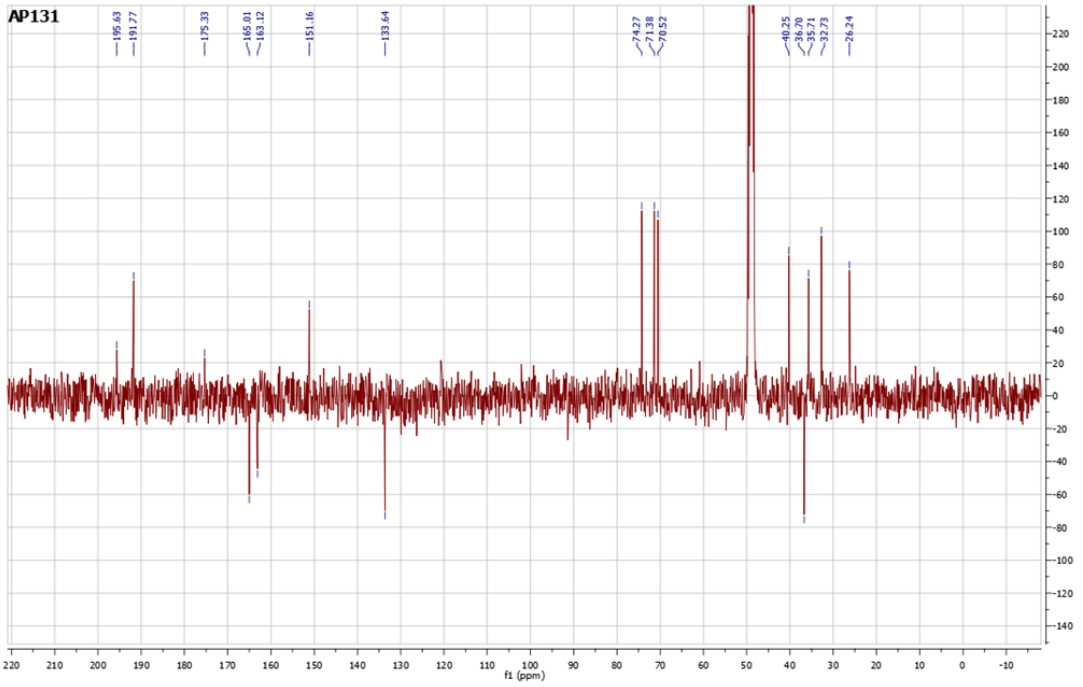
Compound 17a



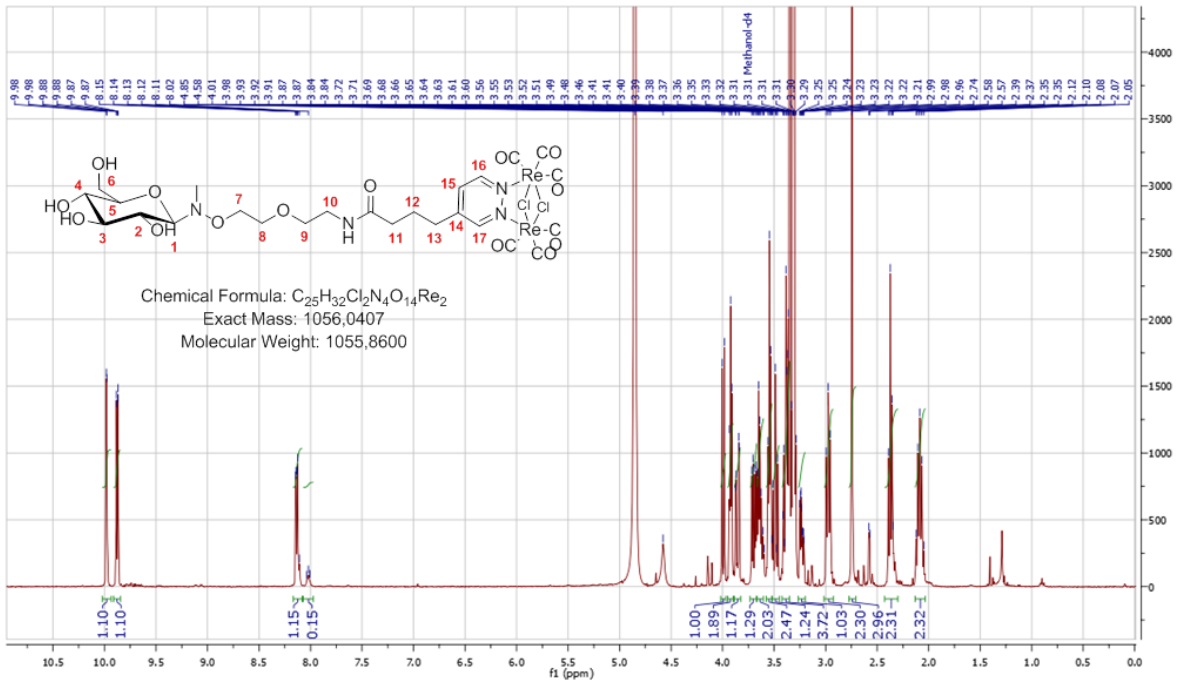


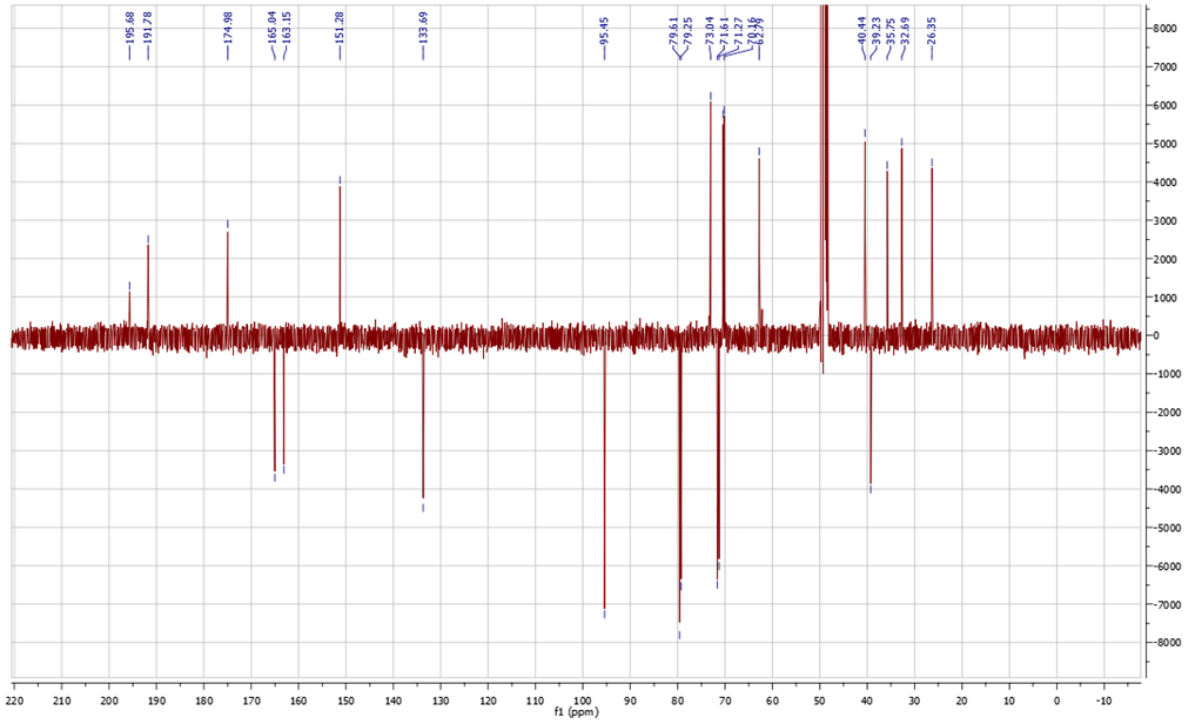
Compound 17b



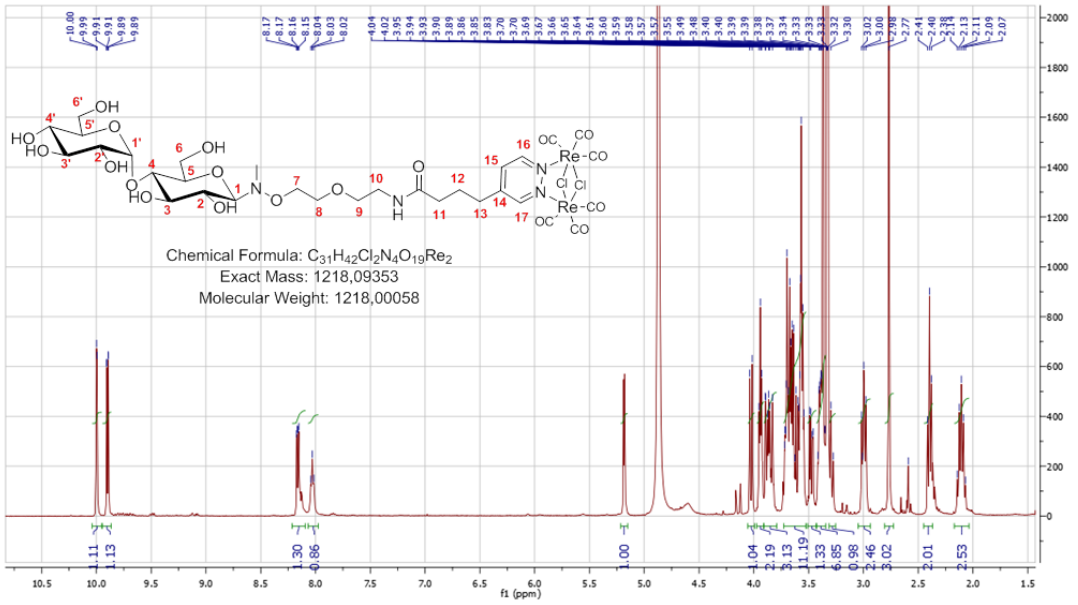


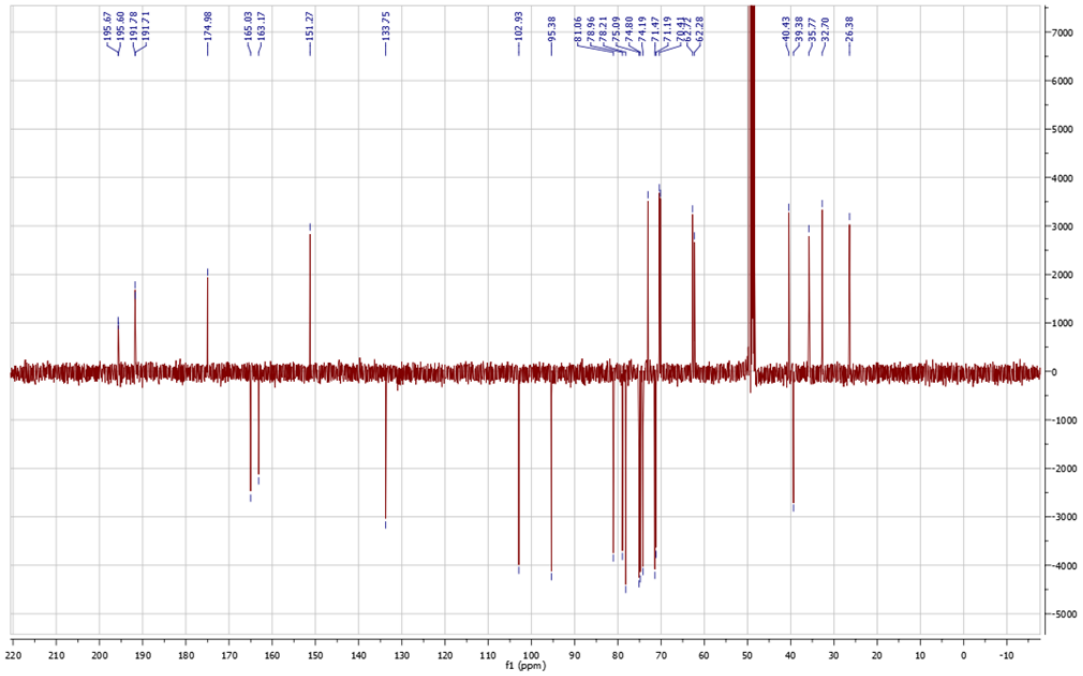
Compound 4


Chemical Formula: $C_{25}H_{32}Cl_2N_4O_{14}Re_2$
Exact Mass: 1056,0407
Molecular Weight: 1055,8600
Methanol-d4
f1 (ppm)

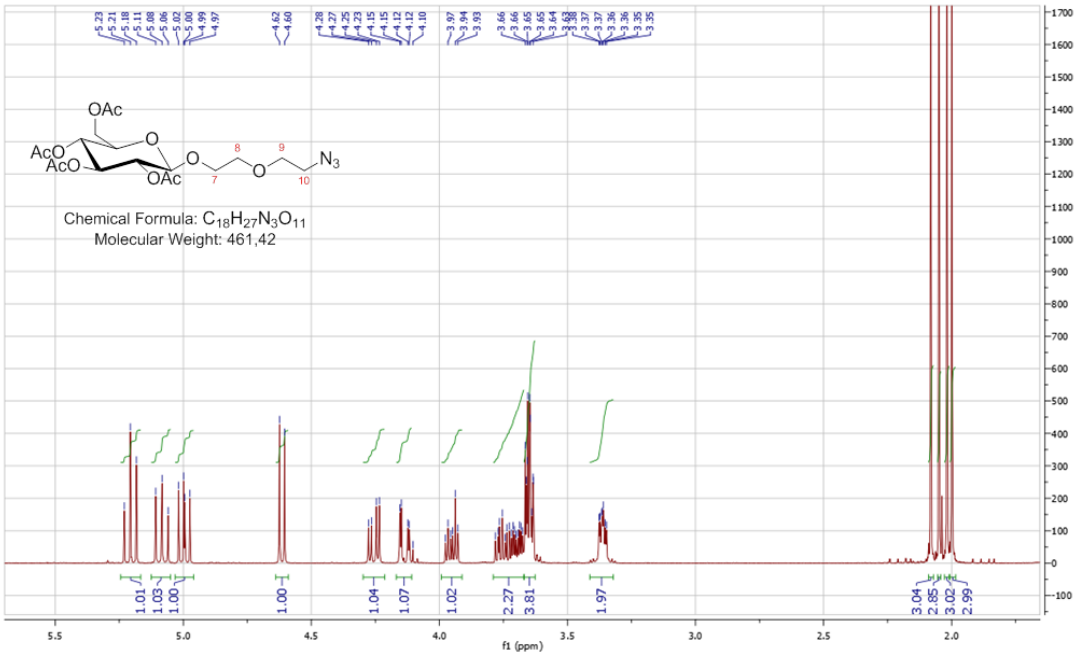

195.68
191.78
174.98
165.04
163.15
151.28
133.69
95.45
79.61
79.25
73.04
71.61
71.27
69.38
40.44
39.23
35.75
32.69
26.35
f1 (ppm)

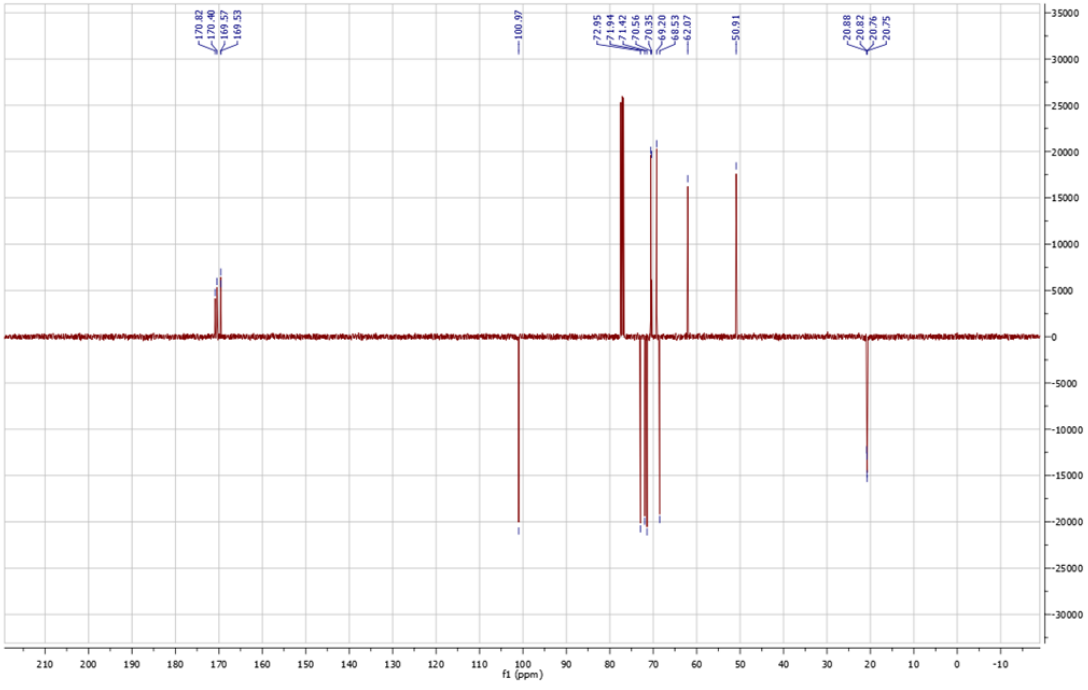
Compound 5


Chemical Formula: $C_{31}H_{42}Cl_2N_4O_{19}Re_2$
Exact Mass: 1218,09353
Molecular Weight: 1218,00058
f1 (ppm)

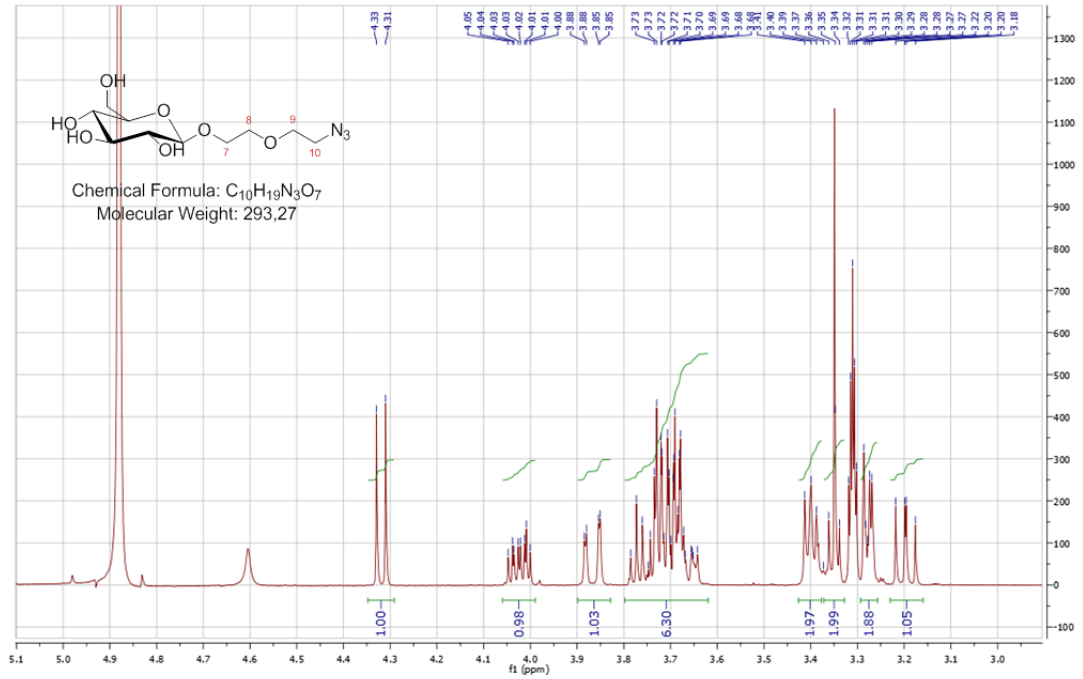

f1 (ppm)

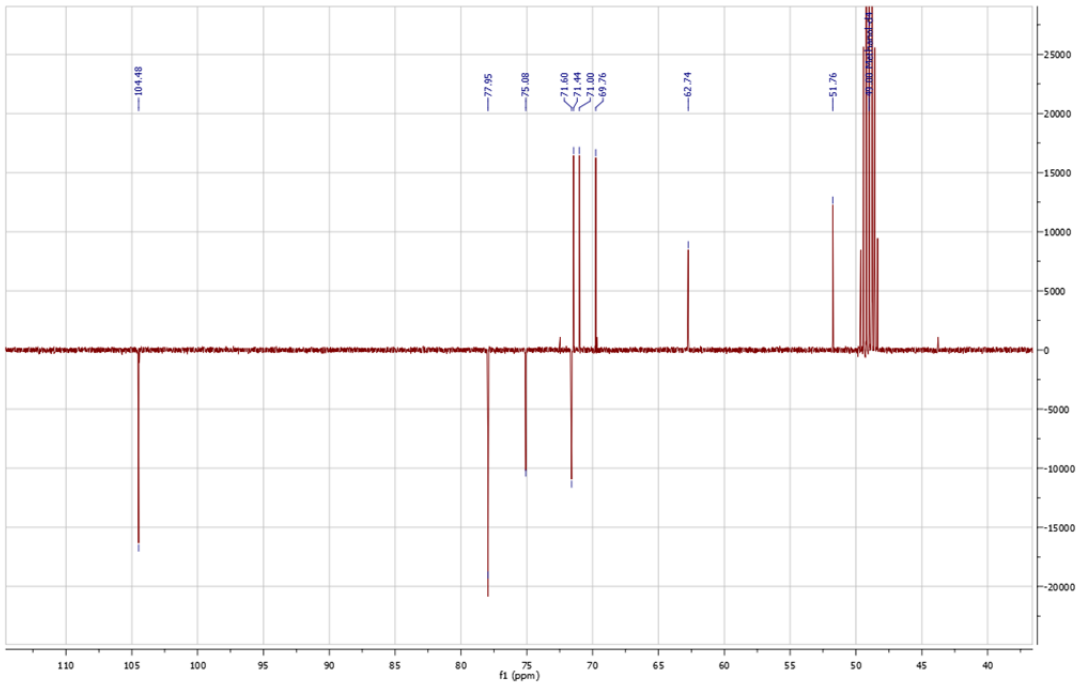
Compound 19


OAc
AcO
AcO
OAc
O
O
8
9
7
O
10
N3
Chemical Formula: C18H27N3O11
Molecular Weight: 461,42
f1 (ppm)

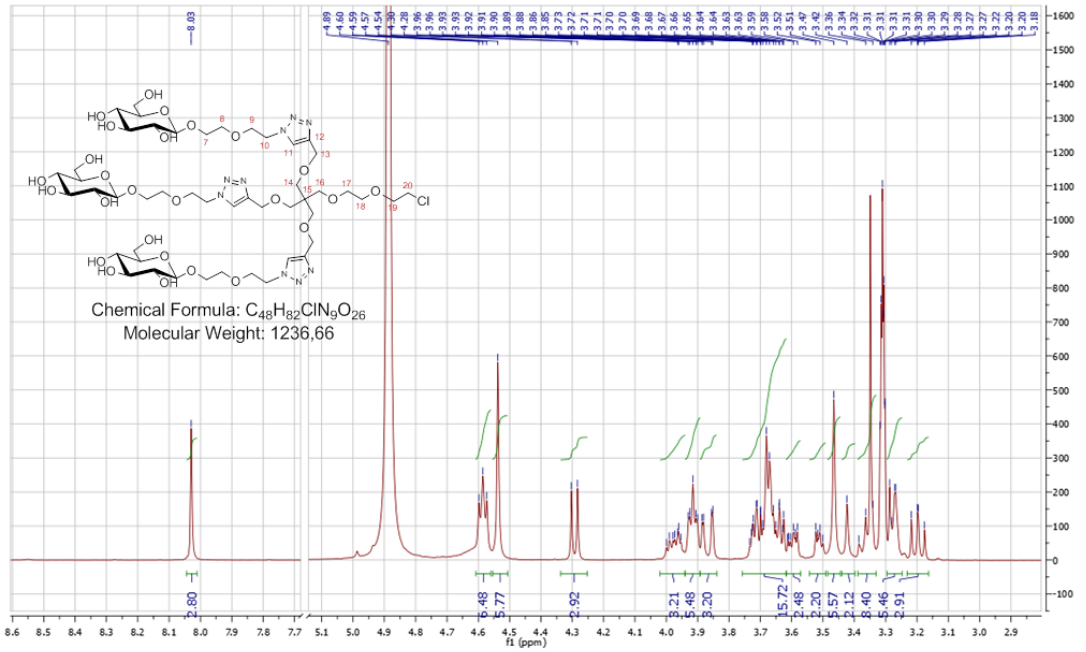

f1 (ppm)

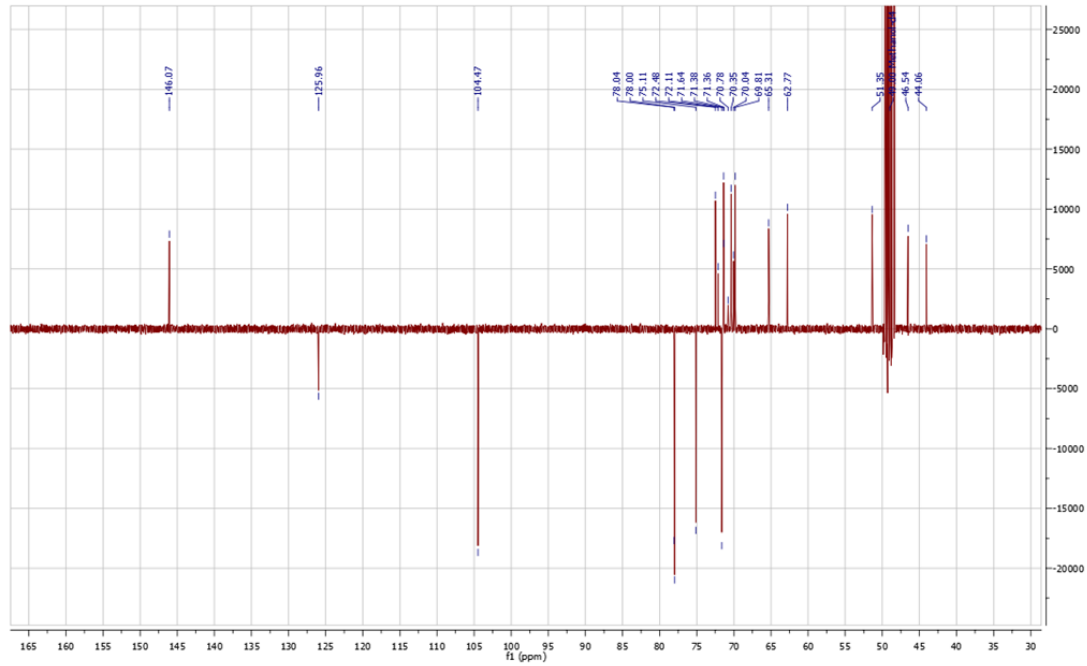
Compound 20


Chemical Formula: $C_{10}H_{19}N_3O_7$
Molecular Weight: 293,27
f1 (ppm)

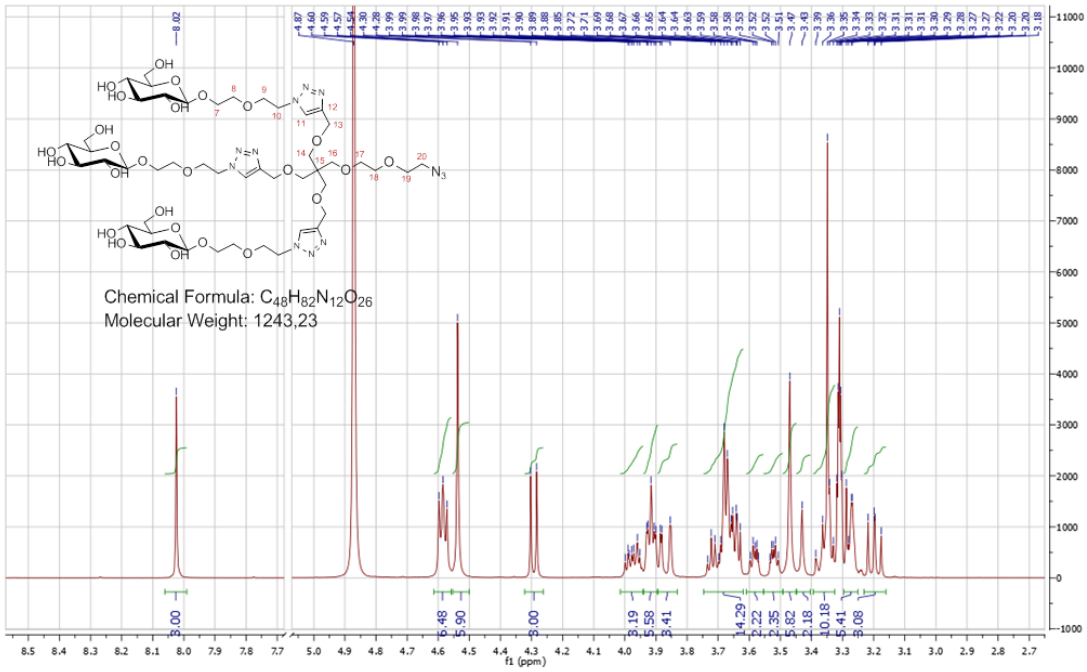

104.48
77.95
75.08
71.60
71.44
71.00
69.76
62.74
51.76
f1 (ppm)

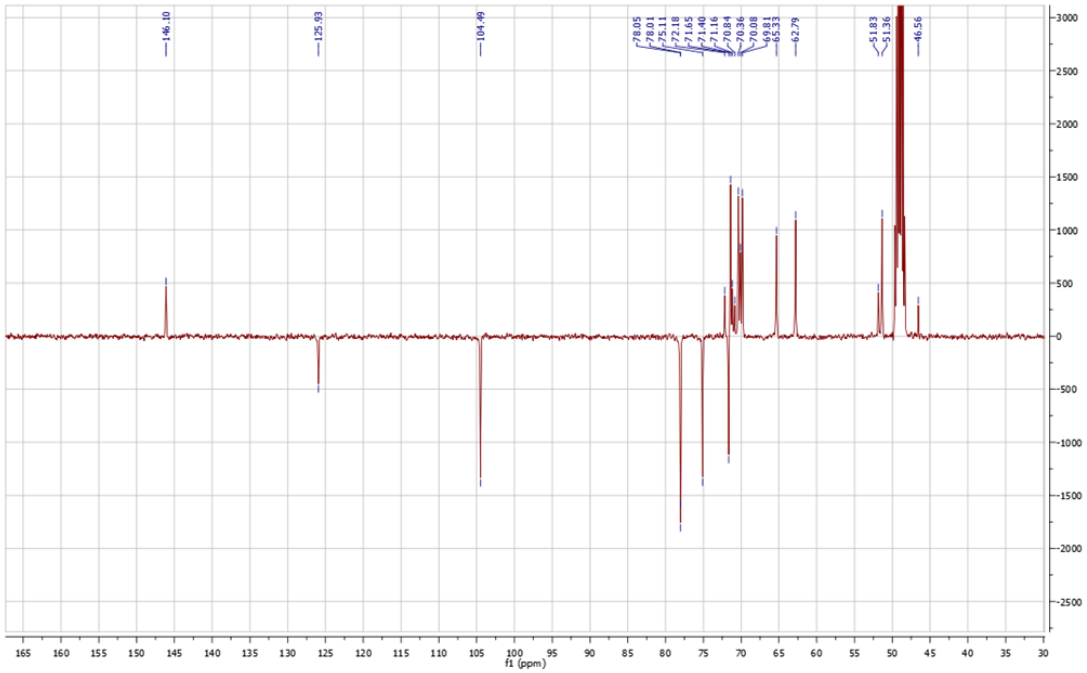
Compound 22


Chemical Formula: $C_{48}H_{82}ClN_9O_{26}$
Molecular Weight: 1236,66
f1 (ppm)

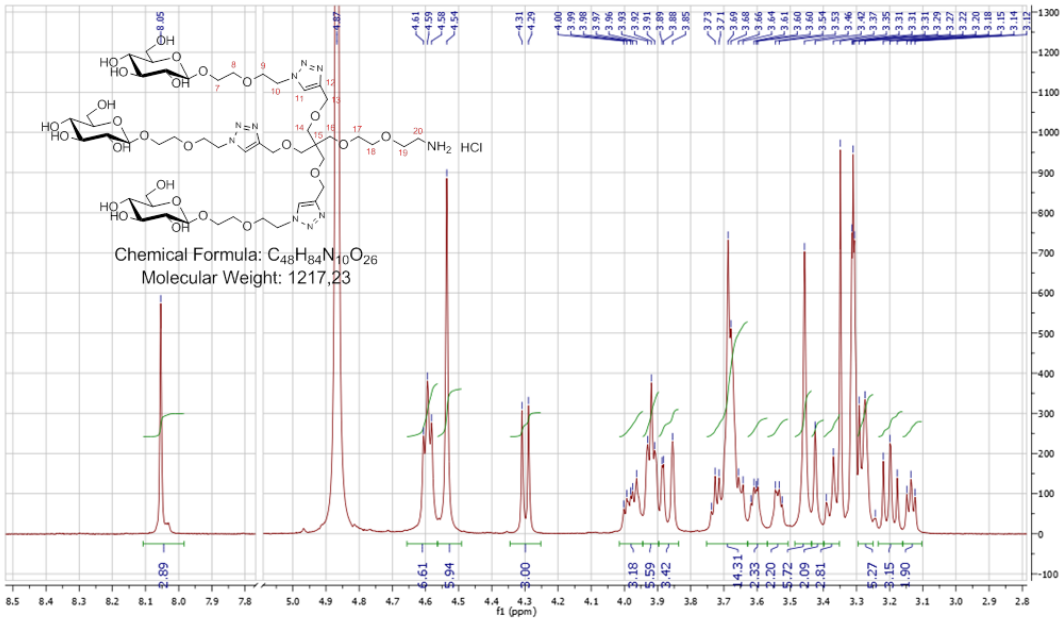

f1 (ppm)

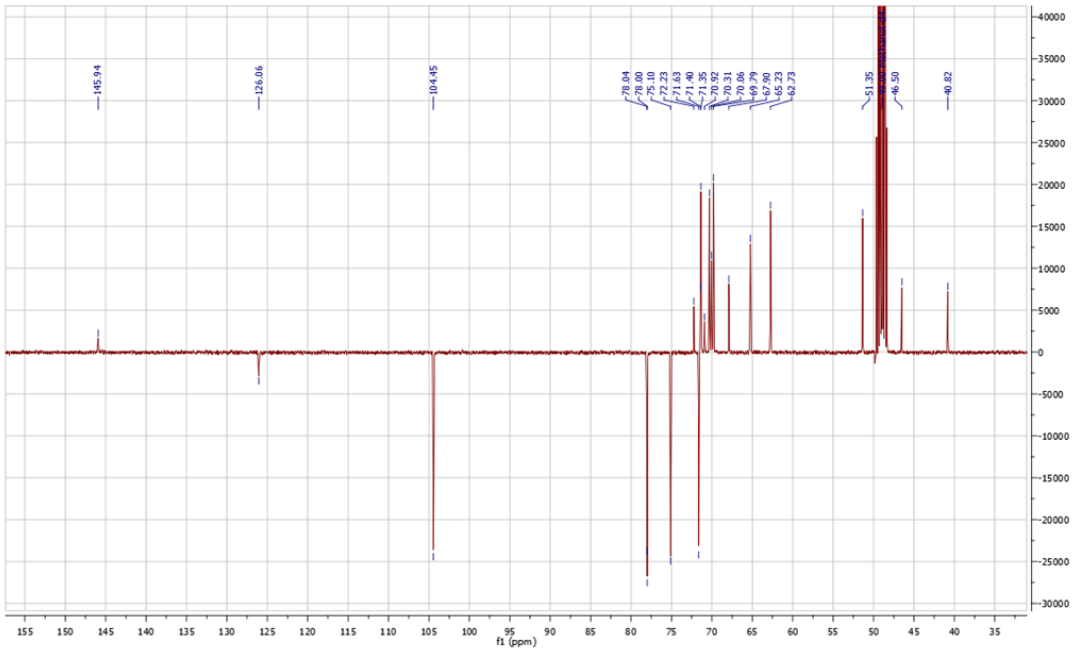
Compound 23


Chemical Formula: $C_{48}H_{82}N_{12}O_{26}$
Molecular Weight: 1243,23
f1 (ppm)

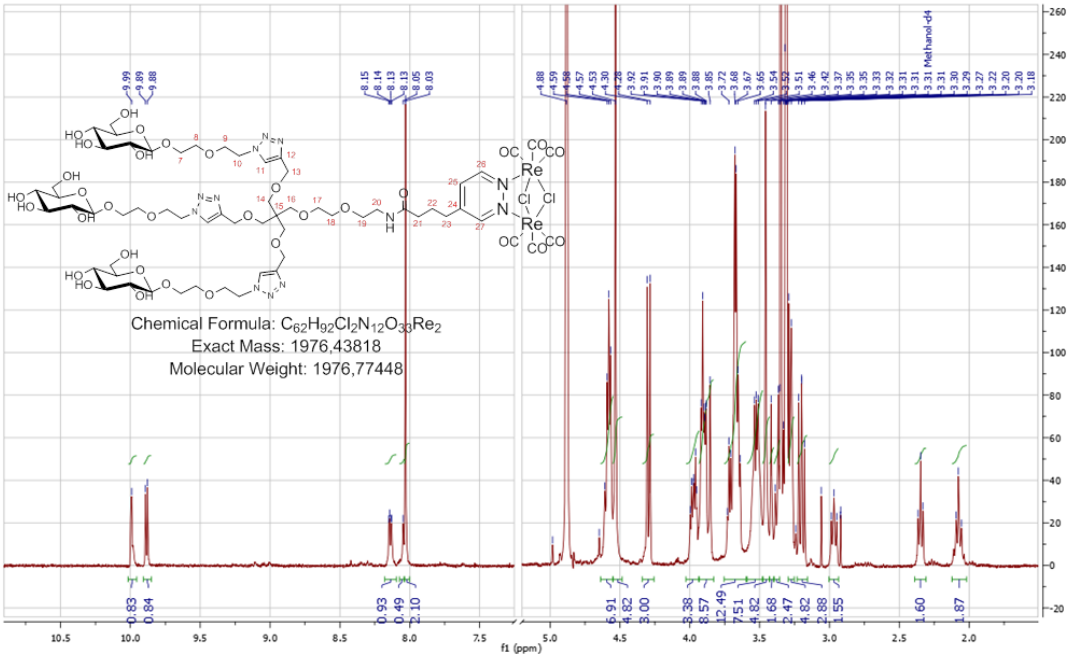

f1 (ppm)

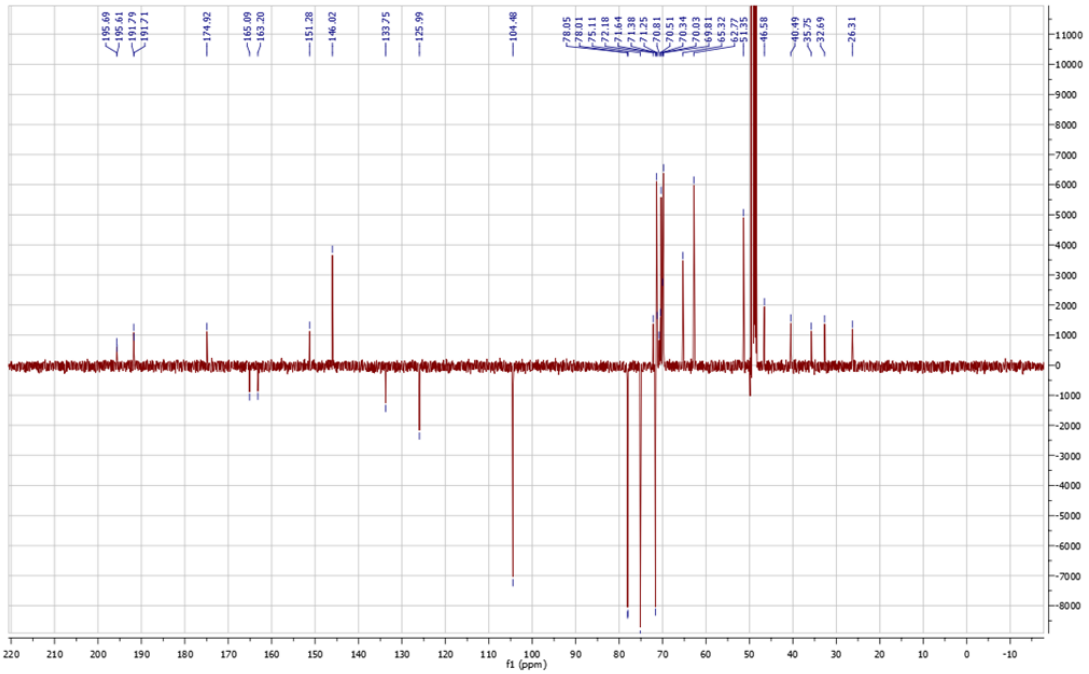
Compound 18


Chemical Formula: C48H84N10O26
Molecular Weight: 1217,23
NH2 HCl
f1 (ppm)


145.94
126.06
104.45
78.04
78.00
75.10
72.23
71.63
71.40
71.35
70.92
70.31
70.06
69.79
67.90
65.23
62.73
51.35
46.50
40.82
f1 (ppm)

Compound 6


Chemical Formula: $C_{62}H_{92}Cl_2N_{12}O_{33}Re_2$
Exact Mass: 1976,43818
Molecular Weight: 1976,77448
Methanol-d4
f1 (ppm)


f1 (ppm)

Fluorescence confocal microscopy

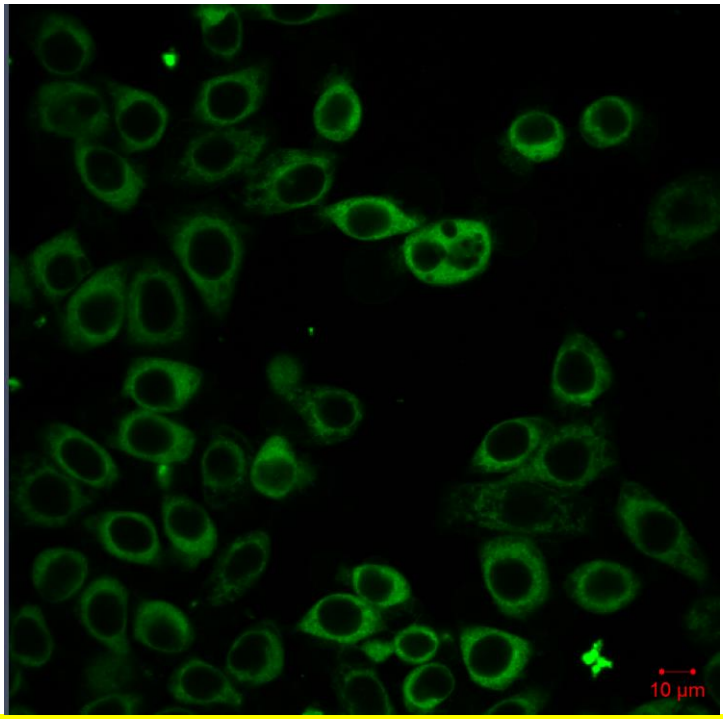


Figure S3: Zoom-out fluorescence confocal image of HeLa cells after the incubation of the compound **3** (50 µM in less than 1% DMSO containing PBS). The samples were excited at λ_{exc} = 405 nm.

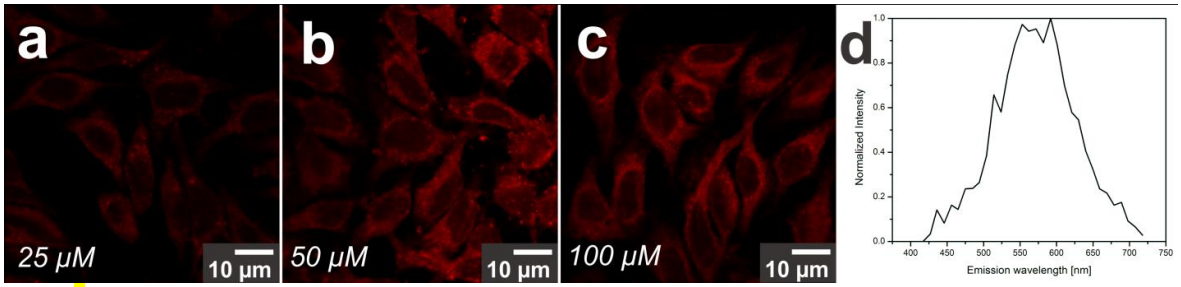


Figure S4 Fluorescence confocal images of HeLa cell after the incubation of compound **5** at three different concentration (a) 25, (b) 50, and (c) 100 µM (in less than 1% DMSO containing PBS). d. The emission spectrum recorded from the cytoplasm of the cell. The samples were excited at λ_{exc} = 405 nm.

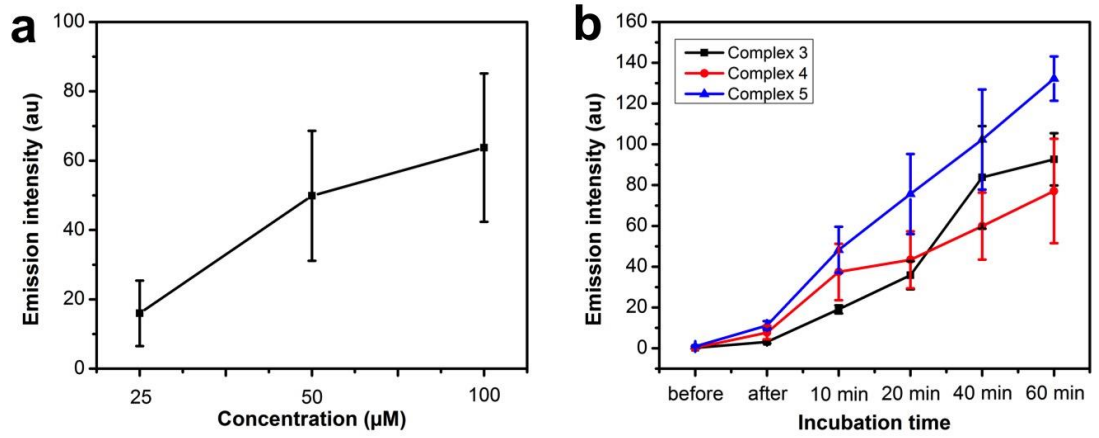


Figure S5 a.) Recorded emission intensity of stained HeLa cell after the incubation of compound **4** at three different concentrations: 25, 50, and 100 µM (in less than 1% DMSO containing PBS). b.) The emission intensity from three different complexes recorded from cytoplasmic region of the cells at different incubation times. The samples were excited at λ_{exc} = 405 nm, respectively.

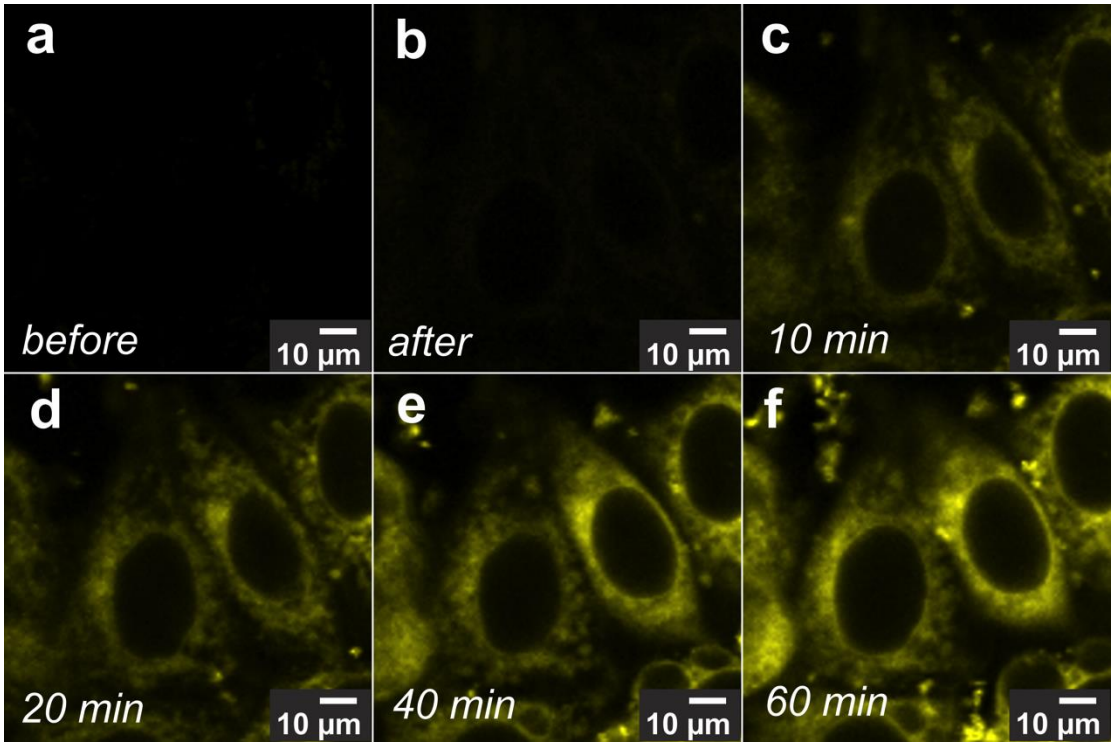


Fig S6 Confocal images of HeLa cells line (a) before and (b-f) after addition of the compound **4** at concentration 100 µM in less than 1% v/v DMSO containing PBS. Kinetic experiment shows fast internalization of compound at different times (b) seconds, (c) 10 minutes, (d) 20 minutes, (e) 40 minutes, and (f) 1 hour after incubation. The samples were excited at λ_{exc} = 405 nm.

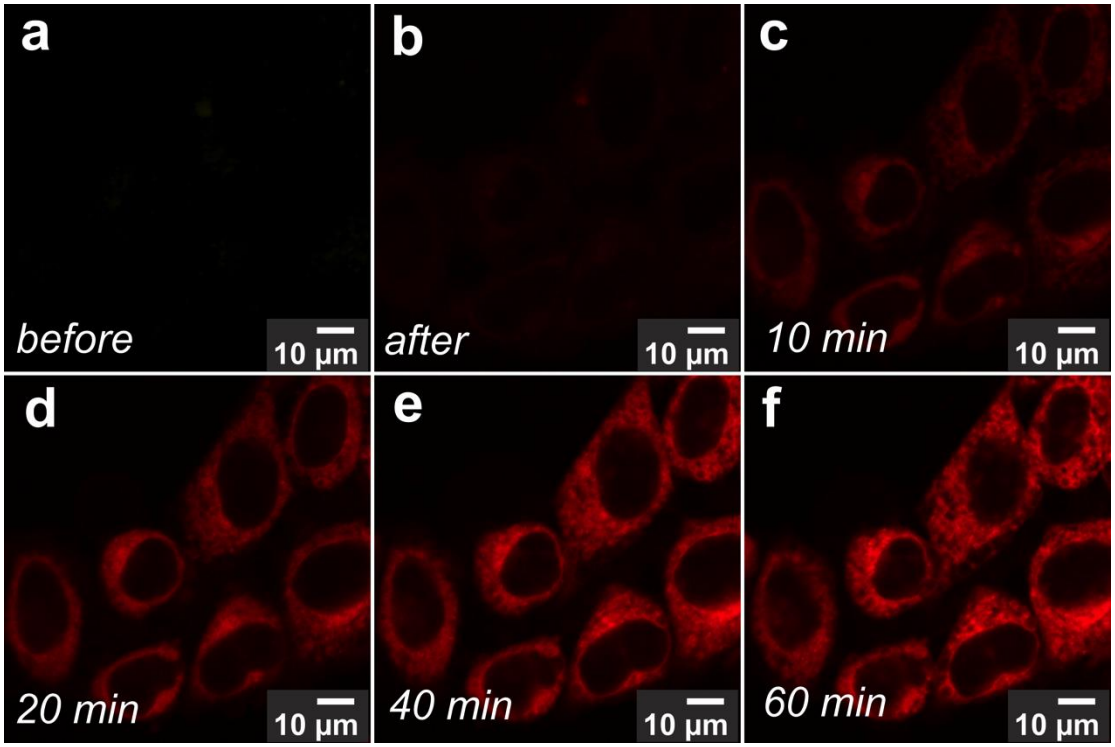


Fig S7 Confocal images of HeLa cells line (a) before and (b-f) after addition of the compound **5** at concentration 100 µM in less than 1% v/v DMSO containing PBS. Kinetic experiment shows fast internalization of compound at different time (b) seconds, (c) 10 minutes, (d) 20 minutes, (e) 40 minutes, and (f) 1 hour after incubation. The samples were excited at λ_{exc} = 405 nm.

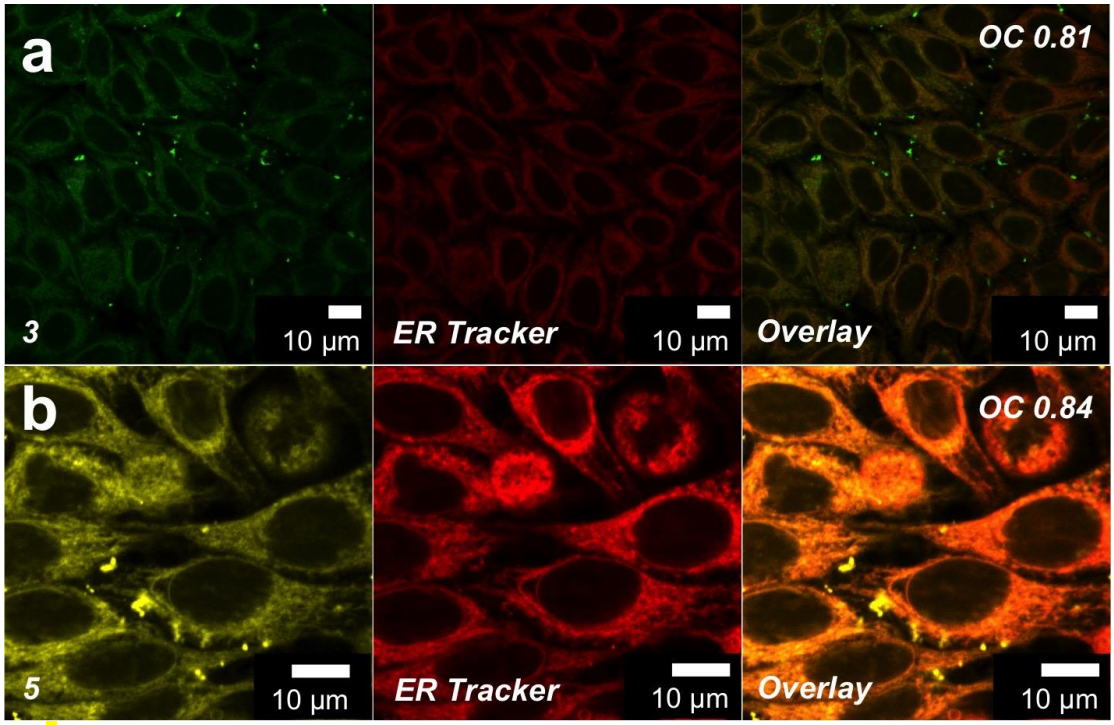


Fig S8 Fluorescence confocal micrograph showing the distribution of compound a. **3** and b. **5** inside endoplasmic reticulums of HeLa cells at concentration of 100 µM in less than 1% DMSO containing PBS as the incubation media. The excitation wavelength for compound **3** and **5** was 405 nm while ER-Tracker™ Red was excited at 594 nm.

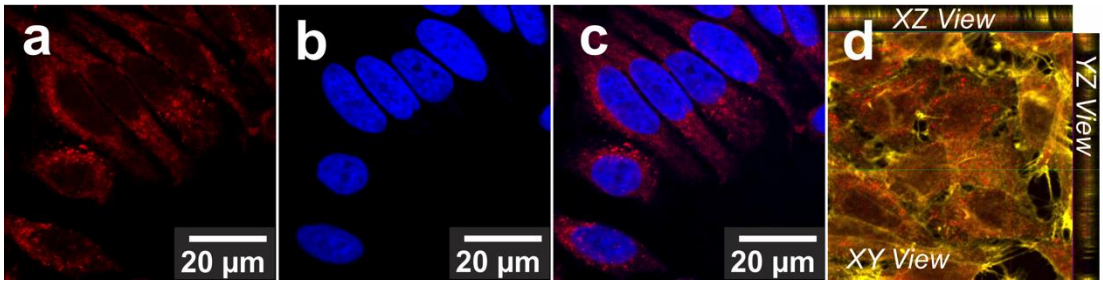


Fig S9 Fluorescence confocal images of collocalization studies neglecting the presence of **5** (100 µM in less than 1% DMSO containing PBS) inside cell nucleus of HeLa cell. (a) **5**, (b) DAPI stains nucleus, and (c) overlap image of a and b. (d). The orthogonal view of the presence of **5** inside the cytoplasmic region of the cell. The excitation wavelength for DAPI and **5** was 405 nm, while Phalloidin Alexa Fluor® 647 was excited at 633 nm.

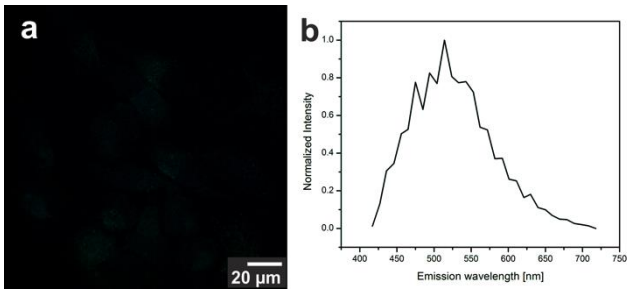


Fig S10 Fluorescence confocal image of HeLa cell after the incubation of compound **6** at concentration 50 M in less than 1% DMSO containing PBS b.) Emission spectrum recorded from the cytoplasm of the cell which shown typical broad-band autofluorescence signal coming from NADH and FAD molecules. The samples were excited at λ_{exc} = 405 nm, respectively.