Supporting Information

Bioorthogonal activation of prodrugs, for the potential treatment of breast cancer, using the Staudinger reaction

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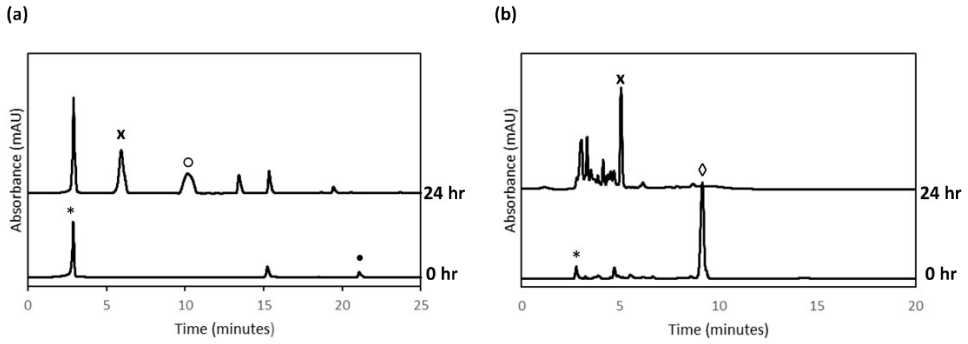


Figure *S1*: a) HPLC chromatograph of doxorubicin **11** release from prodrug **12** by 9-azido sialic acid **4** at 37 C° in aqueous MeCN (1:1) as a function of time. b) HPLC chromatogram of *N*-Mustard release from prodrug **10** by 9-azido sialic acid **4** at 37 C° in aqueous MeCN (1:1) as a function of time. Legend: *: azido-sialic acid **4**; •: Dox prodrug **12**; ○: Dox **11**; **x**: phosphine-oxide ligation product; ◊: *N*-Mustard prodrug **10**.

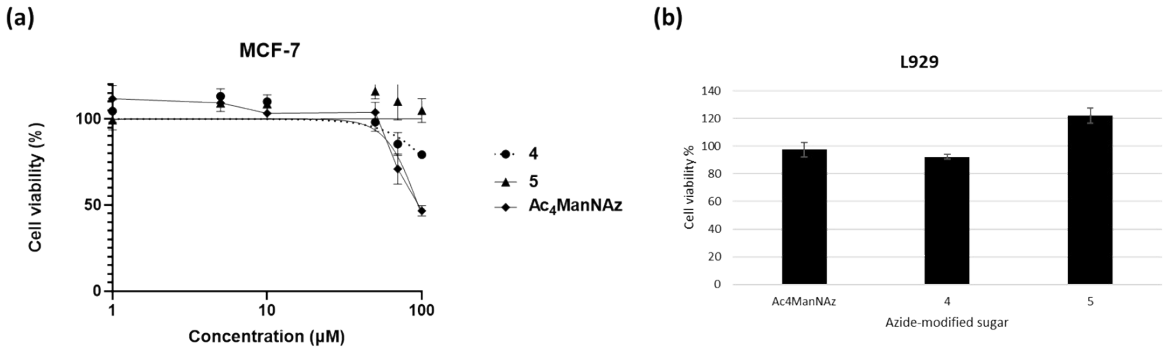


Figure *S2*: a) Viability of MCF-7 cells after incubation with Ac_4ManNAz, **4** or **5** at various concentrations (1-100 µM) determined by the MTT assay. b) Viability of L929 cells after incubation with 50 µM Ac_4ManNAz, **4** or **5** determined by the MTT assay. Data are presented as mean ± SEM (n = 3).

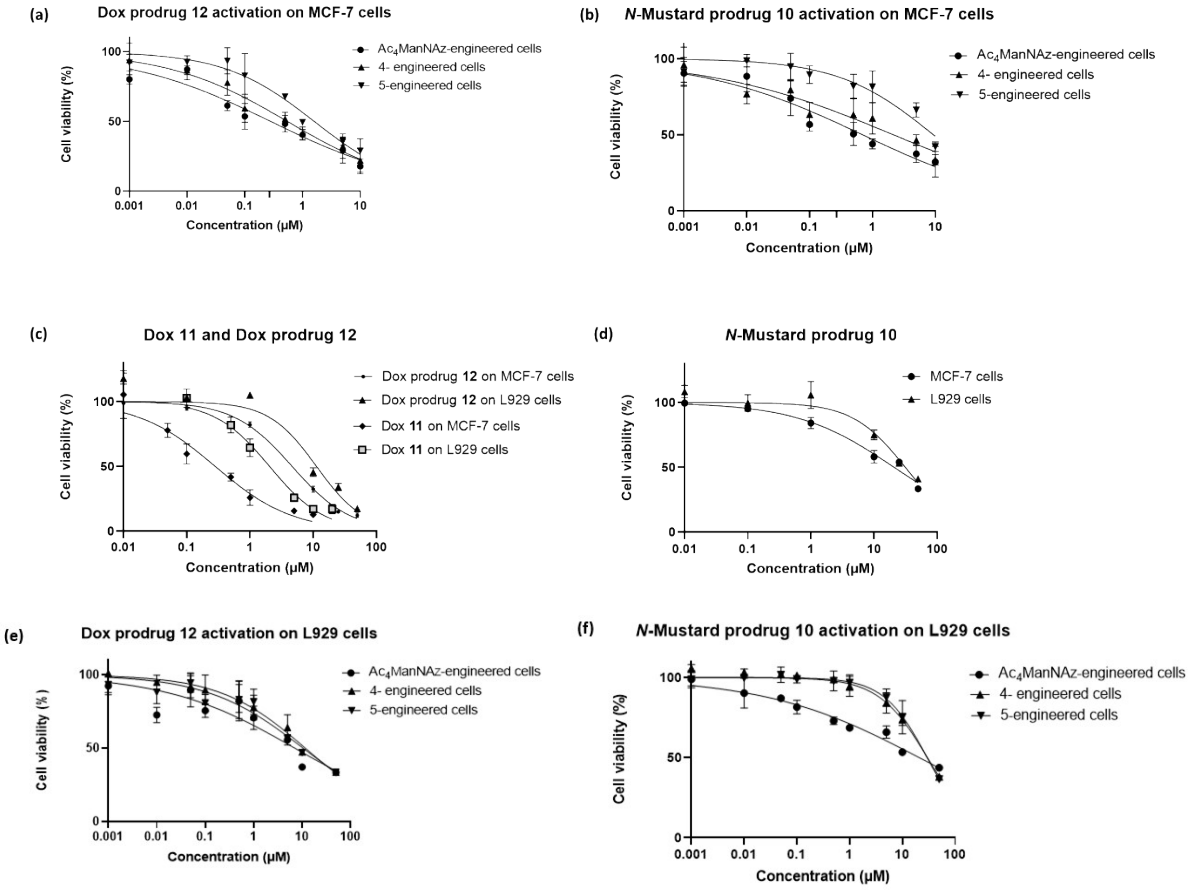


Figure *S*3: a) Determined IC_{50} for Dox prodrug **12** activation against **4**, **5** and Ac_4ManNAz-engineered MCF-7 cells. b) Determined IC_{50} for *N*-Mustard prodrug **10** activation against **4**, **5** and Ac_4ManNAz-engineered MCF-7 cells. c) Determined IC_{50} of Dox **11** and Dox prodrug **12** against MCF-7 cells and L929 cells. d) Determined IC_{50} of *N*-Mustard prodrug **10** against MCF-7 cells and L929 cells. e) Determined IC_{50} for Dox prodrug **12** activation against **4**, **5** and Ac_4ManNAz-engineered L929 cells. f) Determined IC_{50} for *N*-Mustard prodrug **10** activation against **4**, **5** and Ac_4ManNAz-engineered L929 cells. Data are presented as mean ± SEM (n=3).

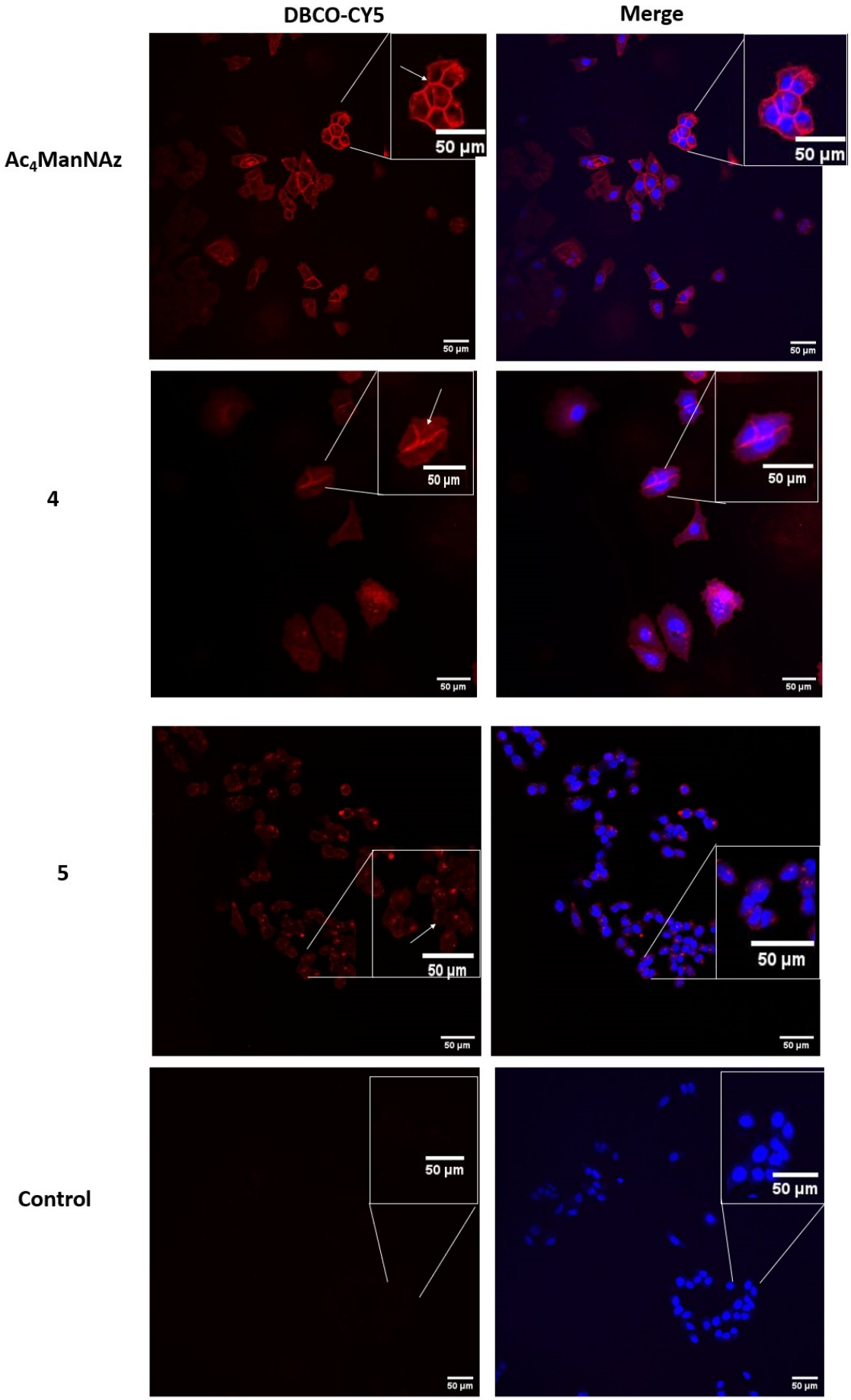
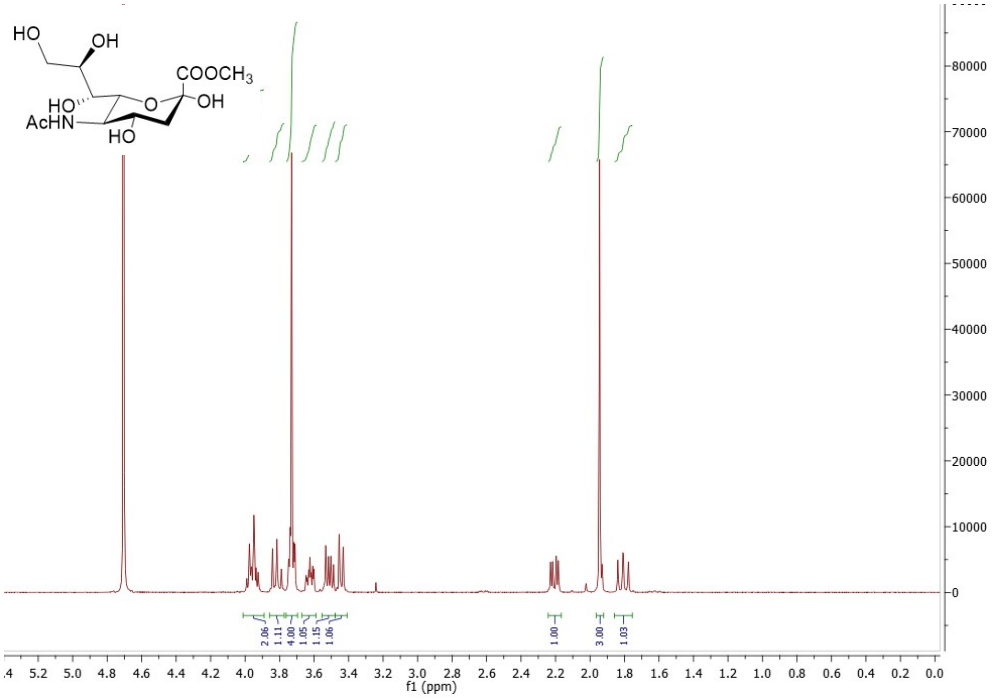
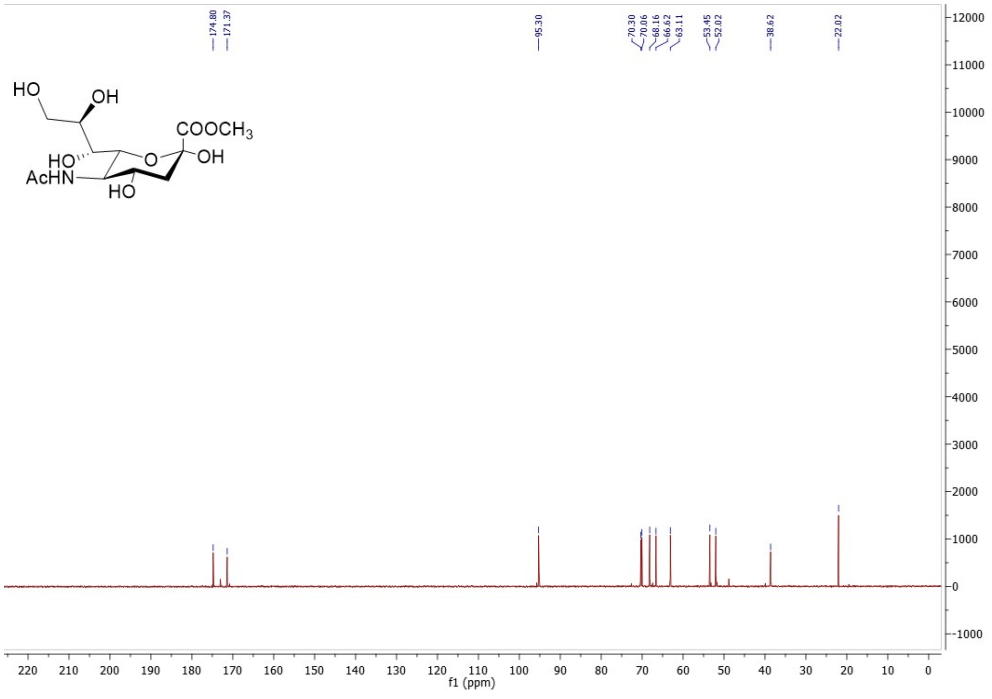

DBCO-CY5
Merge
Ac4ManNAz
4
5
Control
50 µm

Figure *S*4: Confocal fluorescence microscopy images of Ac_4ManNAz, **4**, **5**-treated and untreated control breast cancer cells (MCF-7) (50 µM for 72 h). Arrows indicate higher fluorescence at cell membranes and cell membrane junctions between cells.

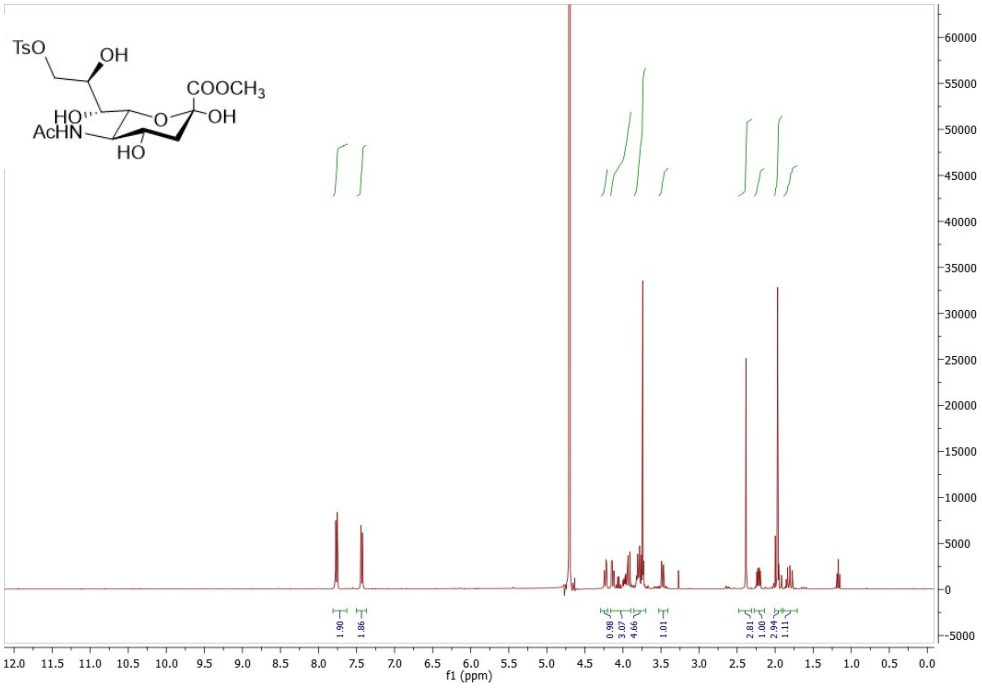
^{1}H and ^{13}C NMR Spectra



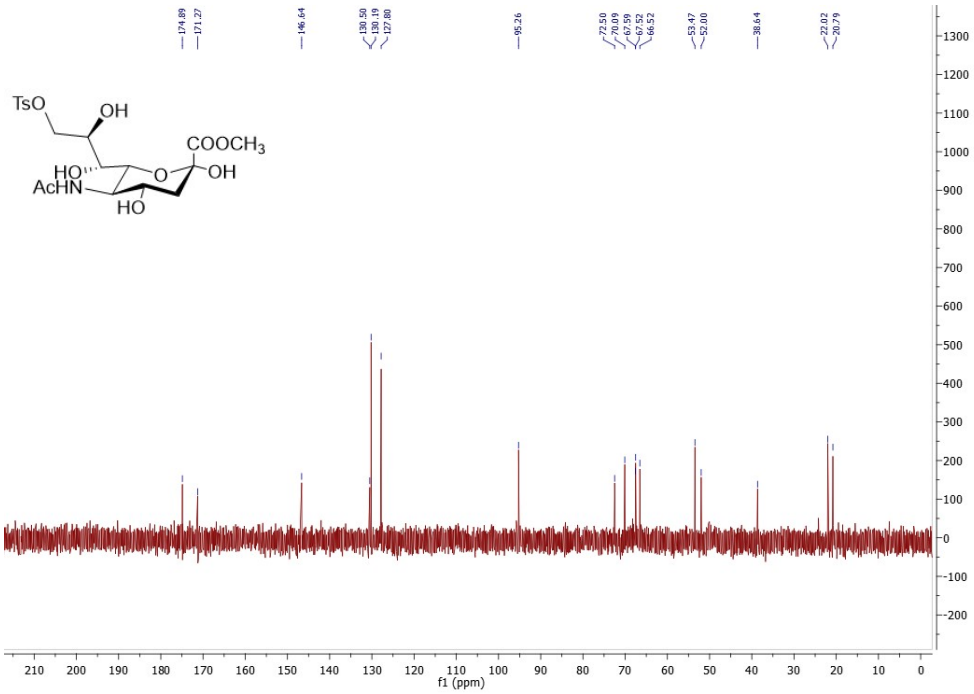
^{1}H NMR spectrum (400 MHz, D_2O) of compound **2**



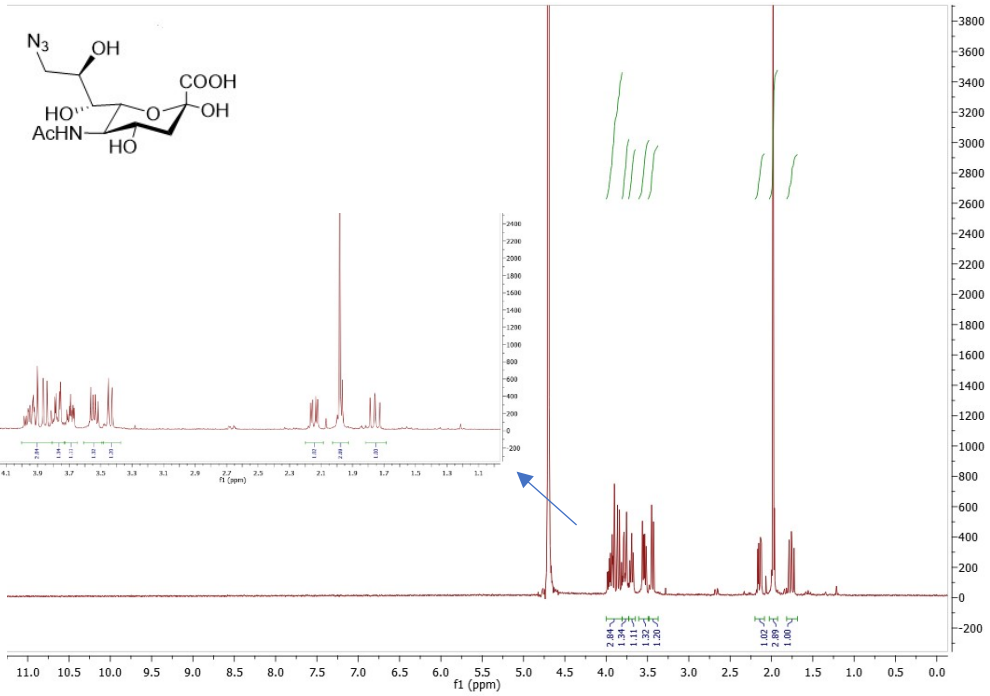
^{13}C NMR spectrum (100 MHz, D_2O) of compound **2**



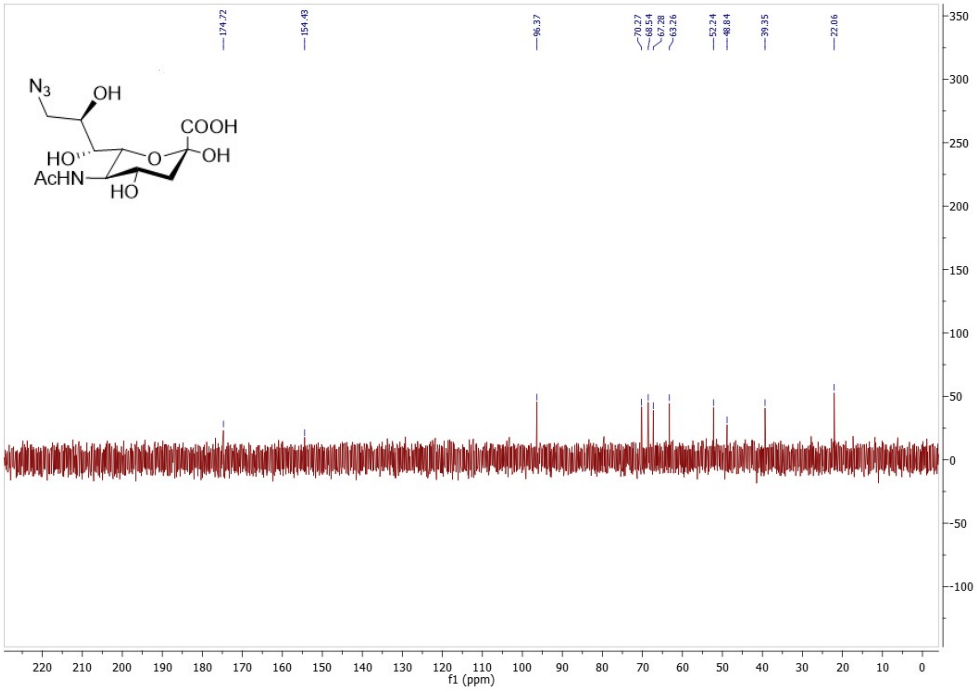
^{1}H NMR spectrum (400 MHz, D_2O) of compound **3**



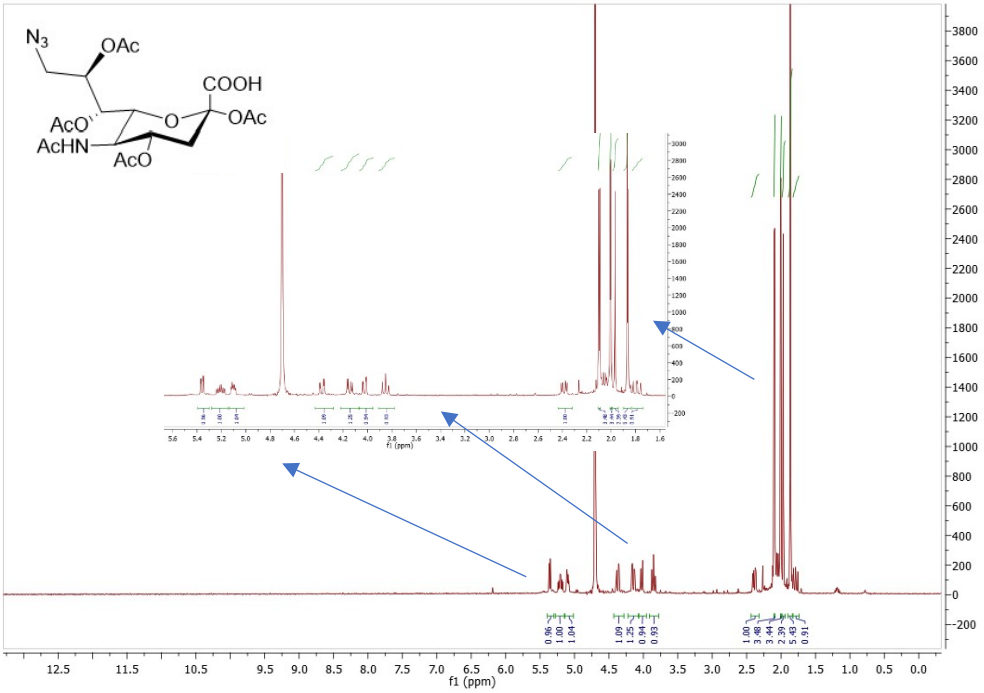
^{13}C NMR spectrum (100 MHz, D_2O) of compound **3**



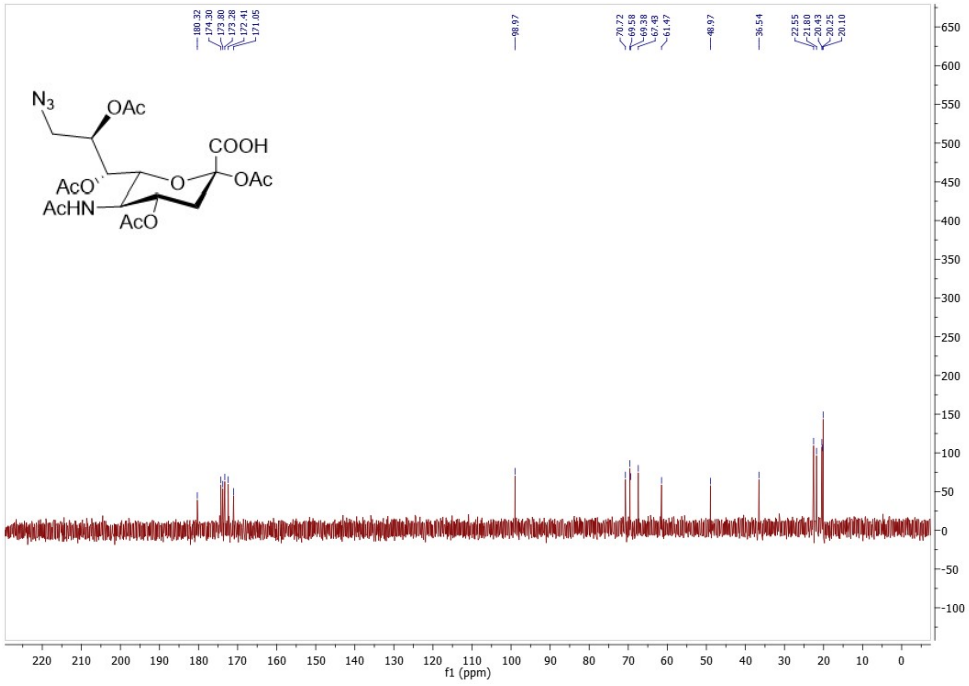
^{1}H NMR spectrum (400 MHz, D_2O) of compound **4**



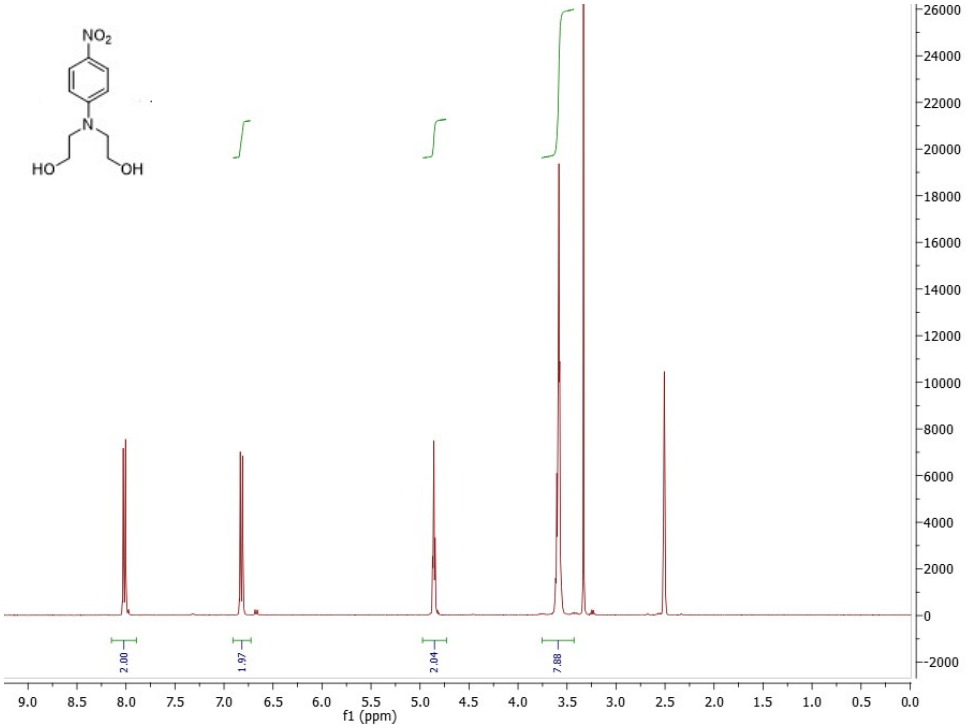
^{13}C NMR spectrum (100 MHz, D_2O) of compound **4**



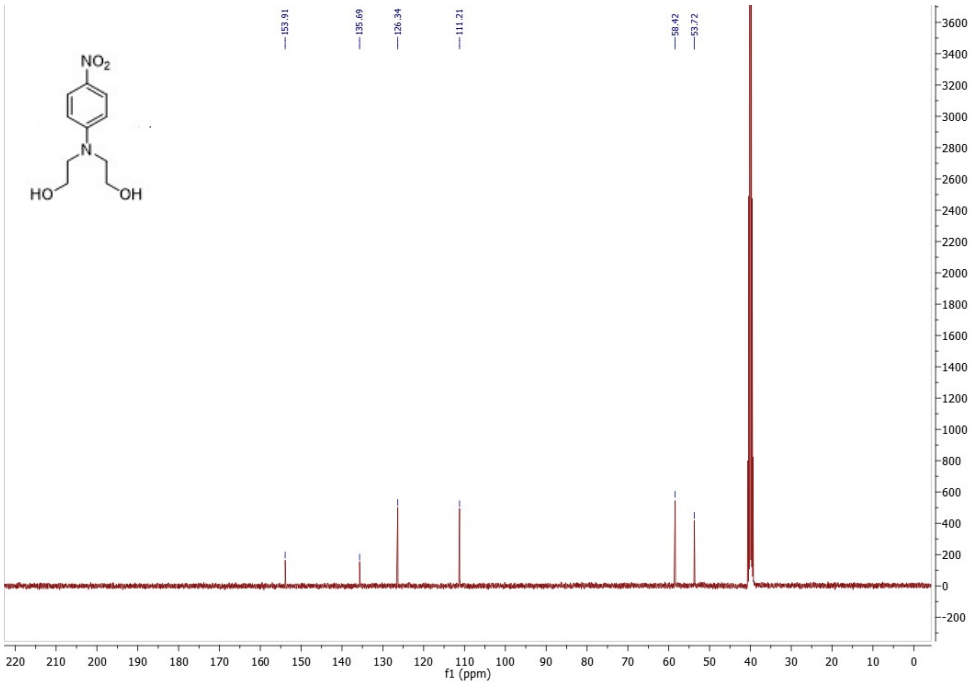
^{1}H NMR spectrum (400 MHz, D_2O) of compound **5**



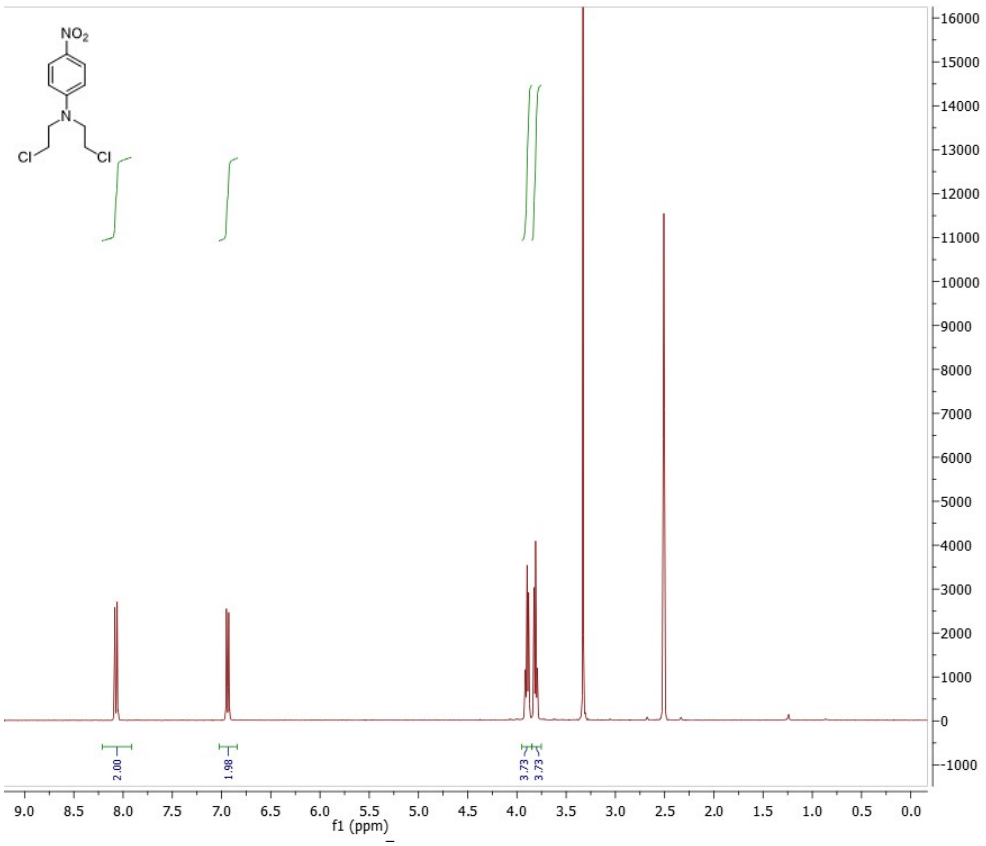
^{13}C NMR spectrum (100 MHz, D_2O) of compound **5**



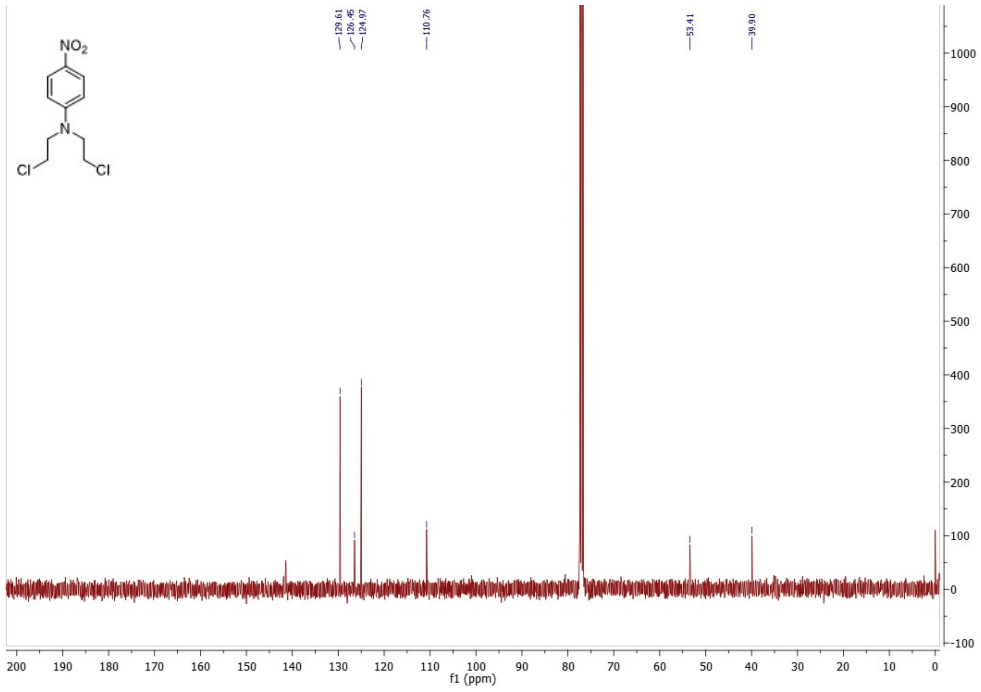
^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **7**



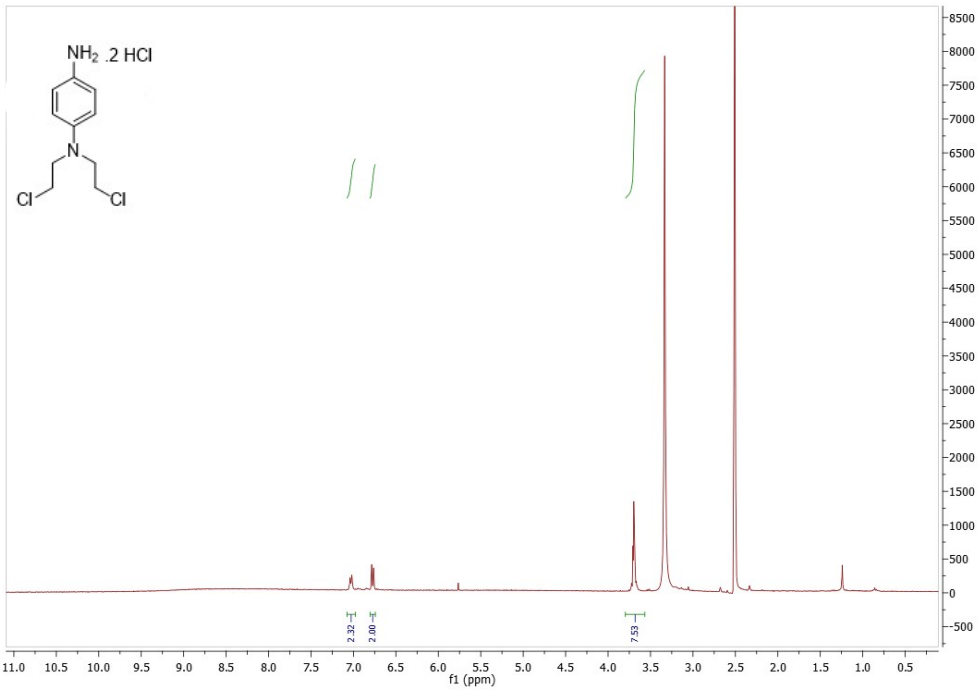
^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **7**



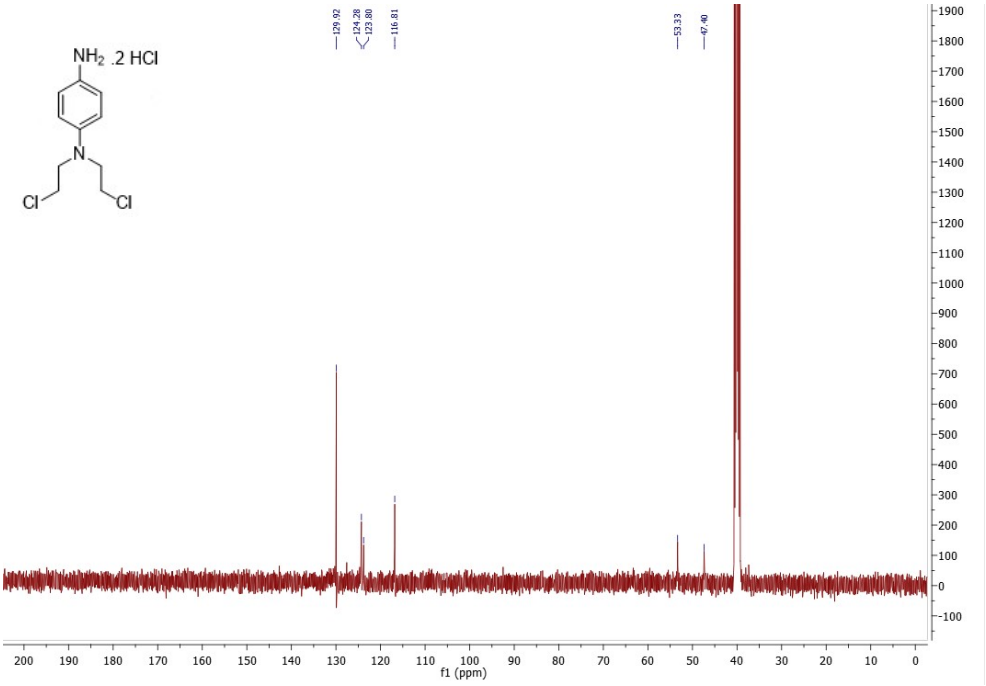
^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **8**



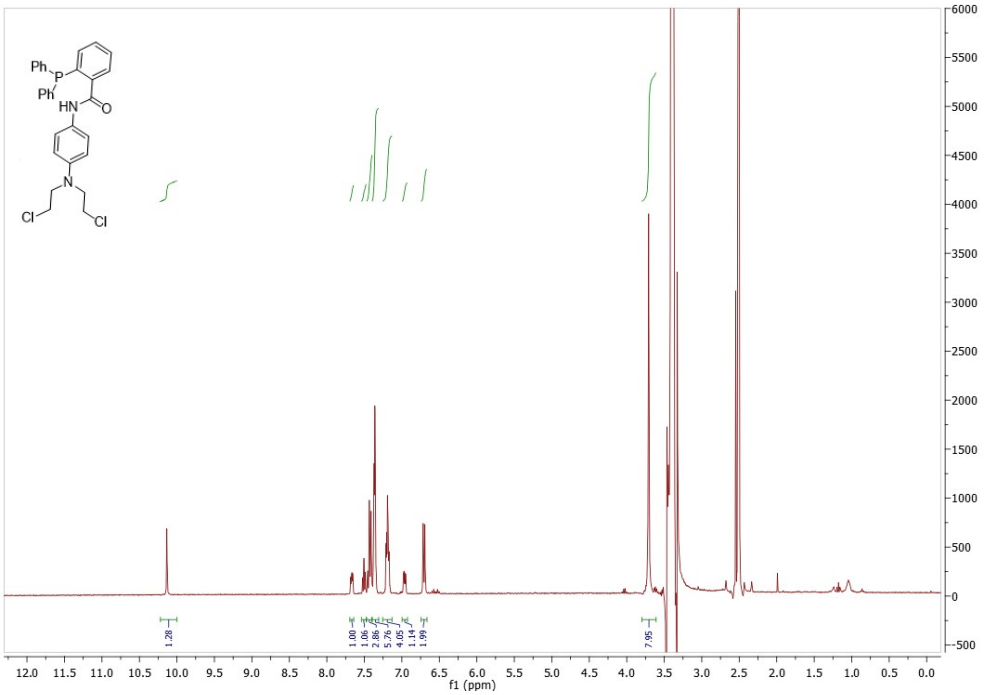
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **8**



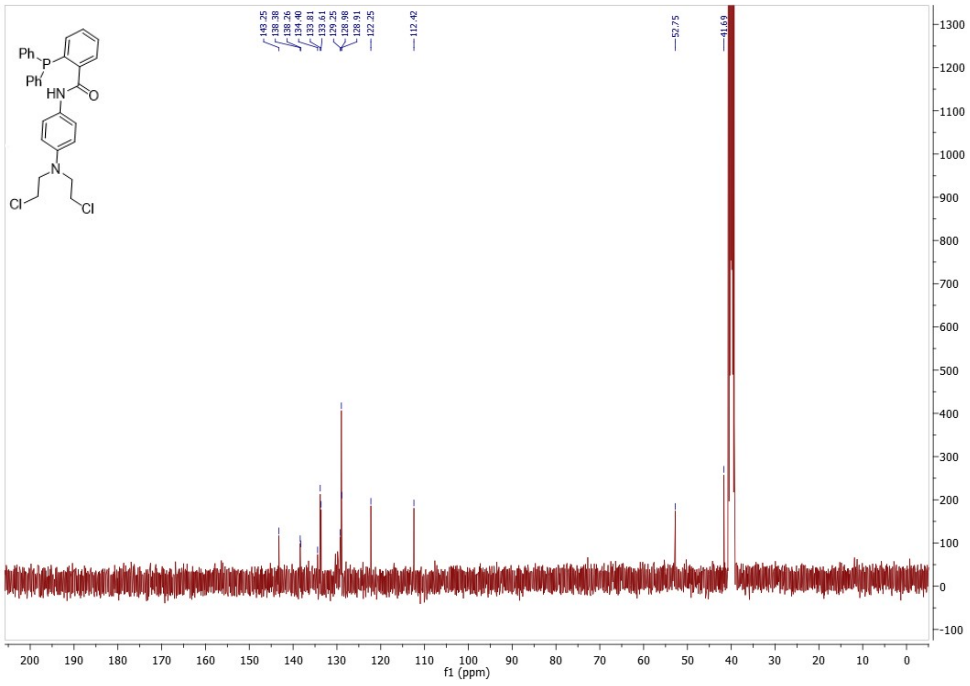
^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **9**



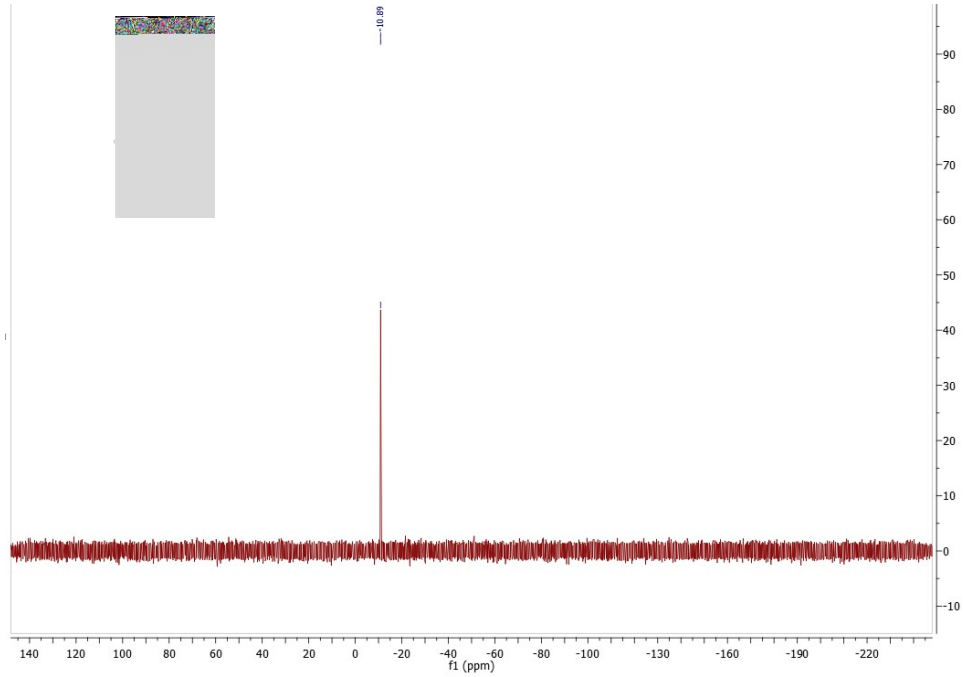
^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **9**



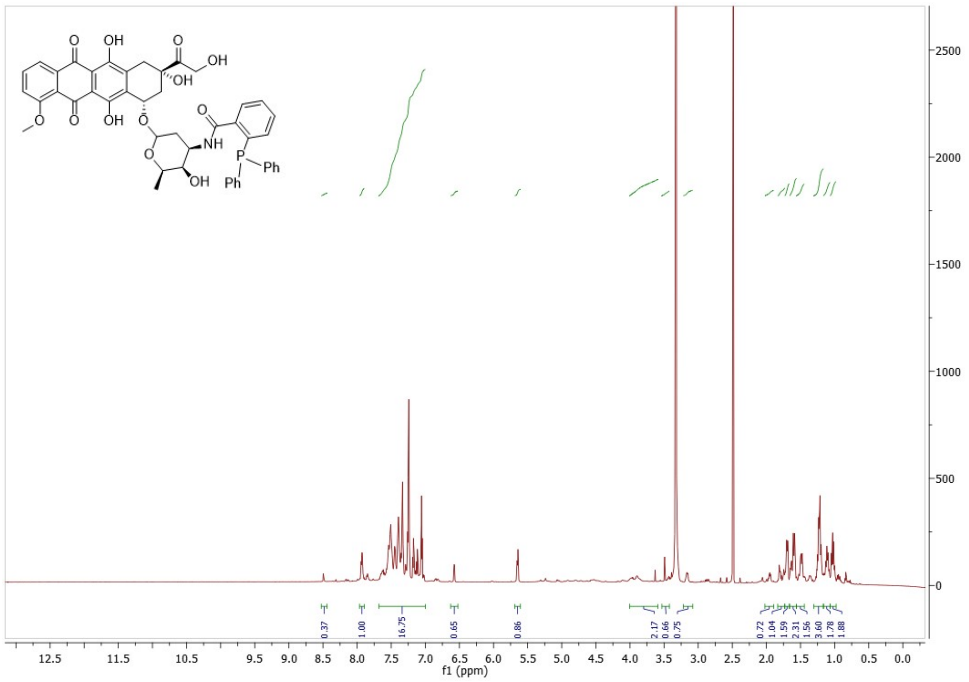
^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **10**



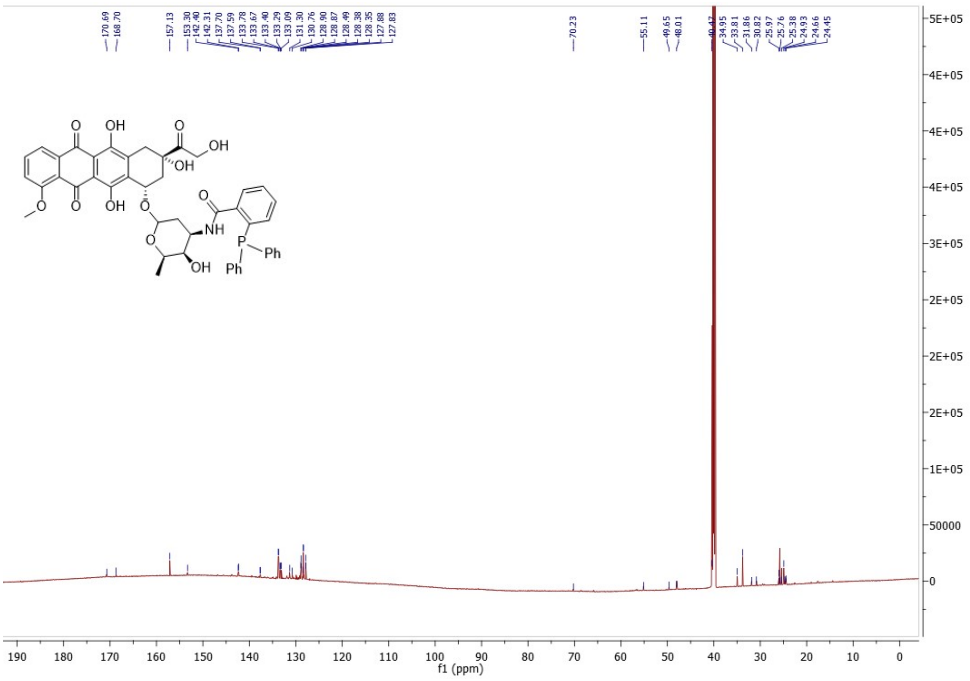
^{13}C NMR spectrum (100 MHz, DMSO-d_6) of compound **10**



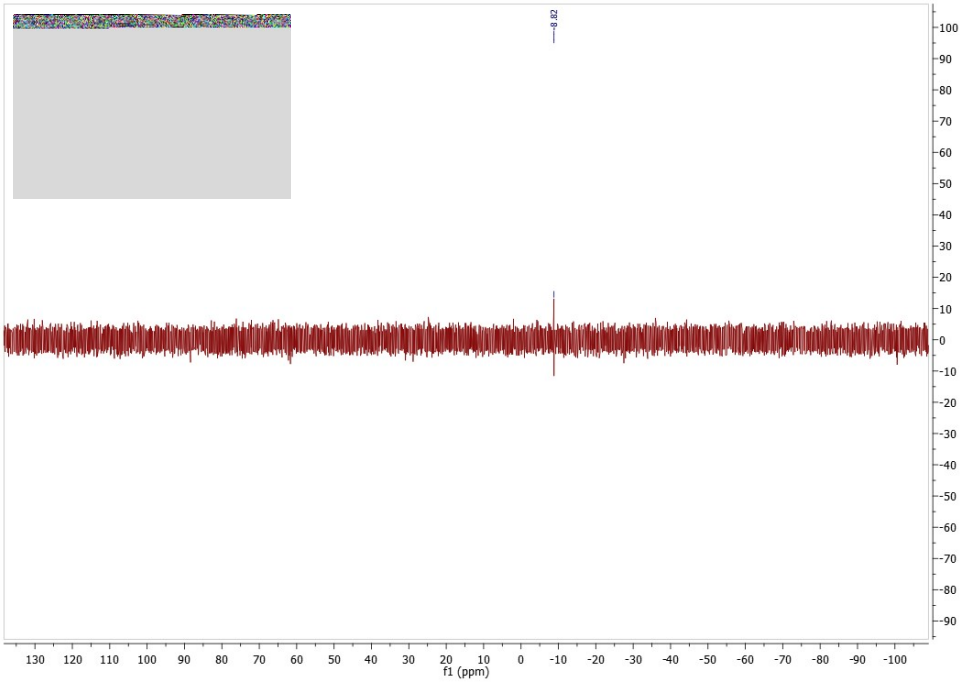
^{31}P NMR spectrum (162 MHz, DMSO-d_6) of compound **10**



^{1}H NMR spectrum (700 MHz, DMSO-d_6) of compound **12**

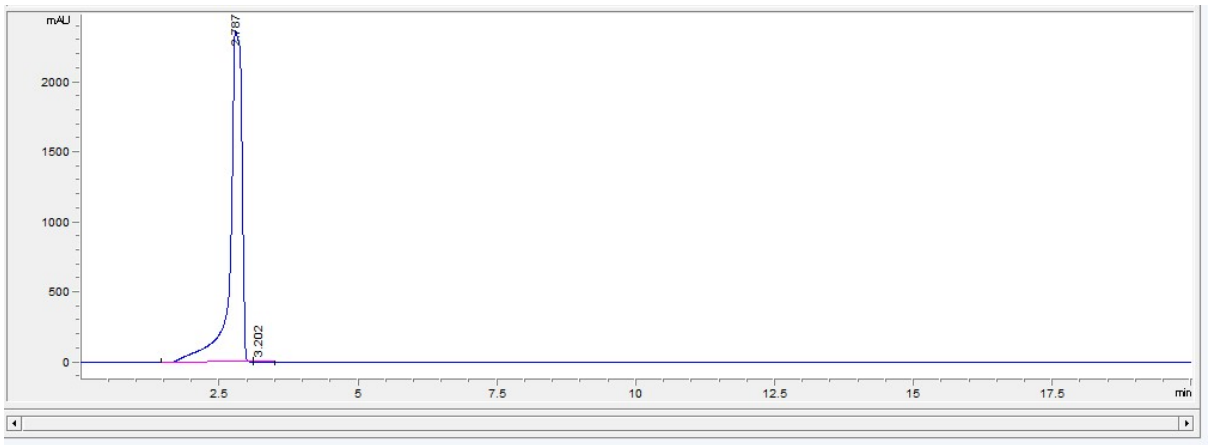


^{13}C NMR spectrum (176 MHz, DMSO-d_6) of compound **12**



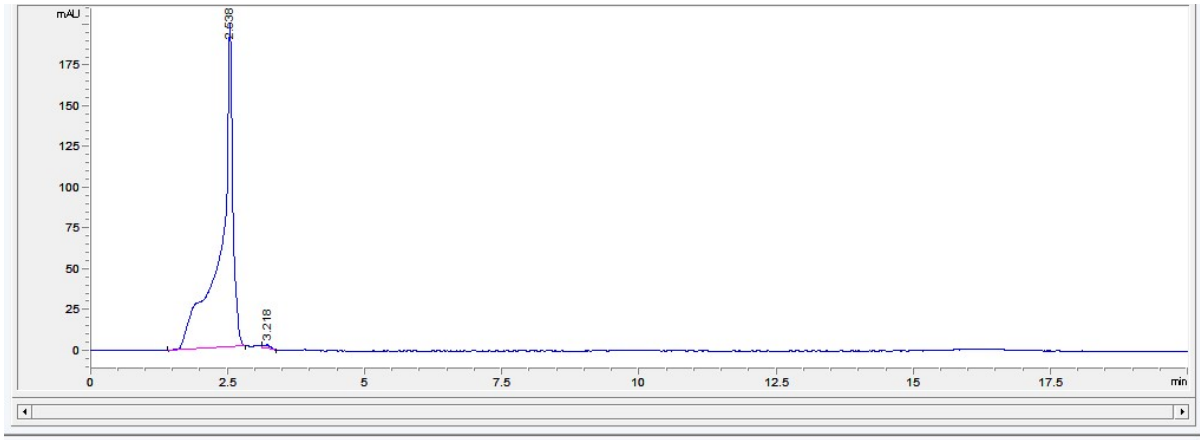
^{31}P NMR spectrum (162 MHz, DMSO-d_6) of compound **12**

HPLC traces of final compounds **4, 5, 10 and 12**



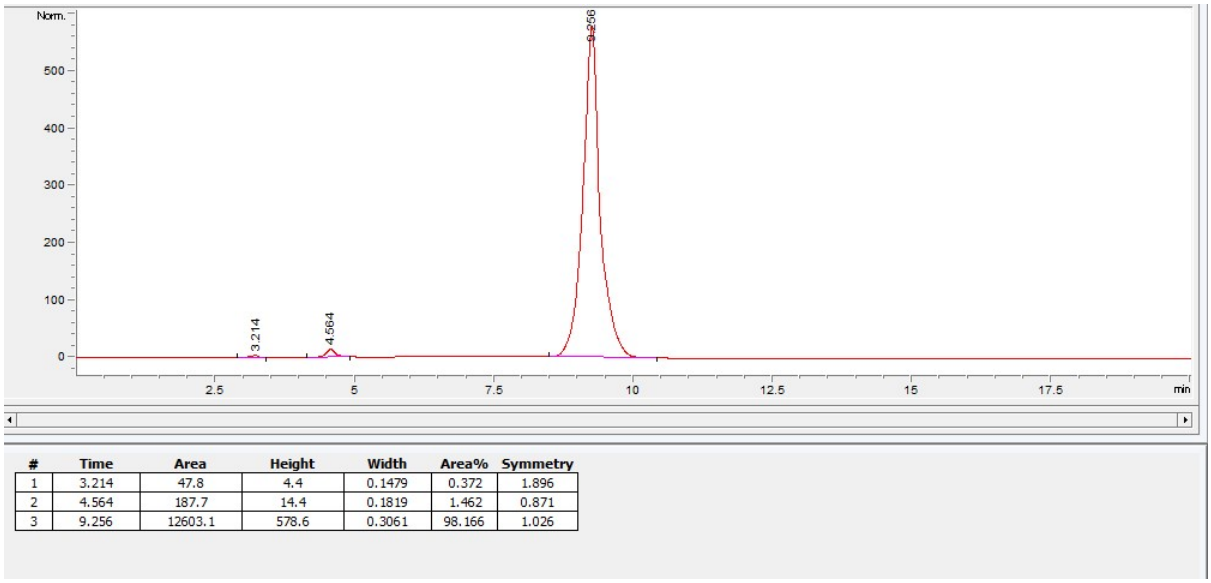
#	Time	Area	Height	Width	Area%	Symmetry
1	2.787	35096.9	2368.6	0.1969	99.897	1.062
2	3.202	36.2	3.2	0.1481	0.103	0.52

The HPLC trace of compound **4**



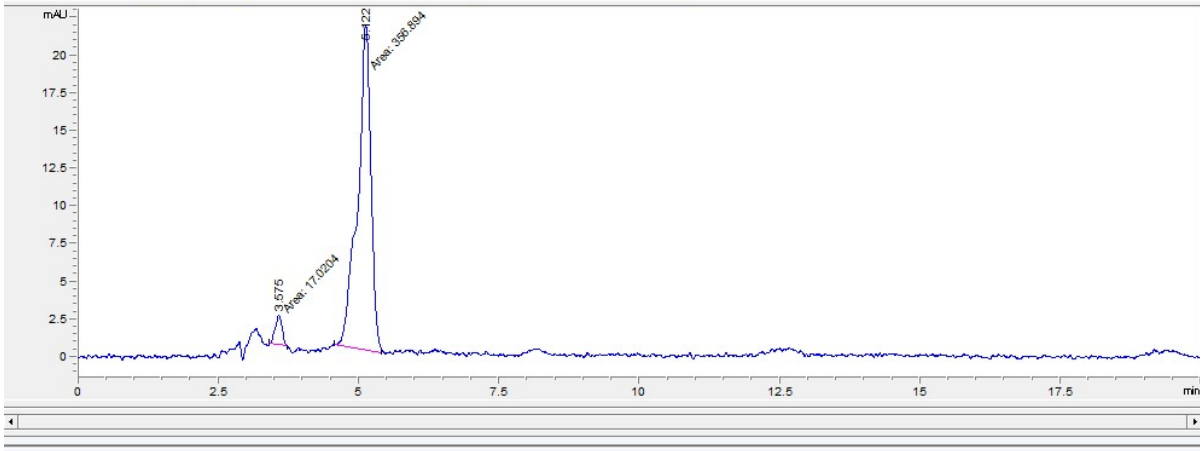
#	Time	Area	Height	Width	Area%	Symmetry
1	2.538	3046.9	198.5	0.197	99.345	3.215
2	3.218	20.1	2.3	0.1138	0.655	0.954

The HPLC trace of compound **5**



#	Time	Area	Height	Width	Area%	Symmetry
1	3.214	47.8	4.4	0.1479	0.372	1.896
2	4.564	187.7	14.4	0.1819	1.462	0.871
3	9.256	12603.1	578.6	0.3061	98.166	1.026

The HPLC trace of compound **10**



#	Time	Area	Height	Width	Area%	Symmetry
1	3.575	17	2	0.1432	4.552	1.111
2	5.122	356.9	21.6	0.2752	95.448	1.506

The HPLC trace of compound **12**