SUPPORTING INFORMATION

Multifunctional Neomycin-Triazine-Based Cationic Lipids for Gene Delivery with Antibacterial Properties

Chiara Pennetta[†,#], Nina Bono[†,#], Federica Ponti[†,°], Maria Cristina Bellucci[§], Fiorenza Viani[¥], Gabriele Candiani[†,*], Alessandro Volonterio[¥,†,*]

[†]Department of Chemistry, Materials, and Chemical Engineer "G. Natta", Politecnico di Milano, Via Mancinelli 7, 20131 Milan, Italy;

° Laboratory for Biomaterials and Bioengineering, Canada Research Chair I in Biomaterials and Bioengineering for the Innovation in Surgery, Department of Min-Met-Materials Engineering & Research Center of CHU de Quebec, Division of Regenerative Medicine, Laval University, Quebec City, QC G1V 0A6, Canada

[§]Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Via Celoria 2, 20133 Milan, Italy;

[¥]Consiglio Nazionale delle Ricerche, Istituto di Scienze e Tecnologie Chimiche "G. Natta" (SCITEC), Via Mario Bianco 9, 20131 Milan, Italy

AUTHOR INFORMATION

Corresponding Authors

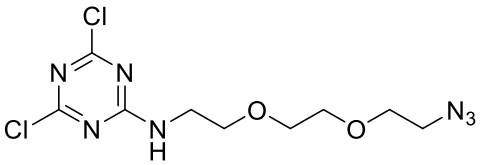
*E-mail: gabriele.candiani@polimi.it (G.C.); alessandro.volonterio@polimi.it (A.V.). Phone: +39-02-23993139. Fax: +39-02-23993180.

Author contribution

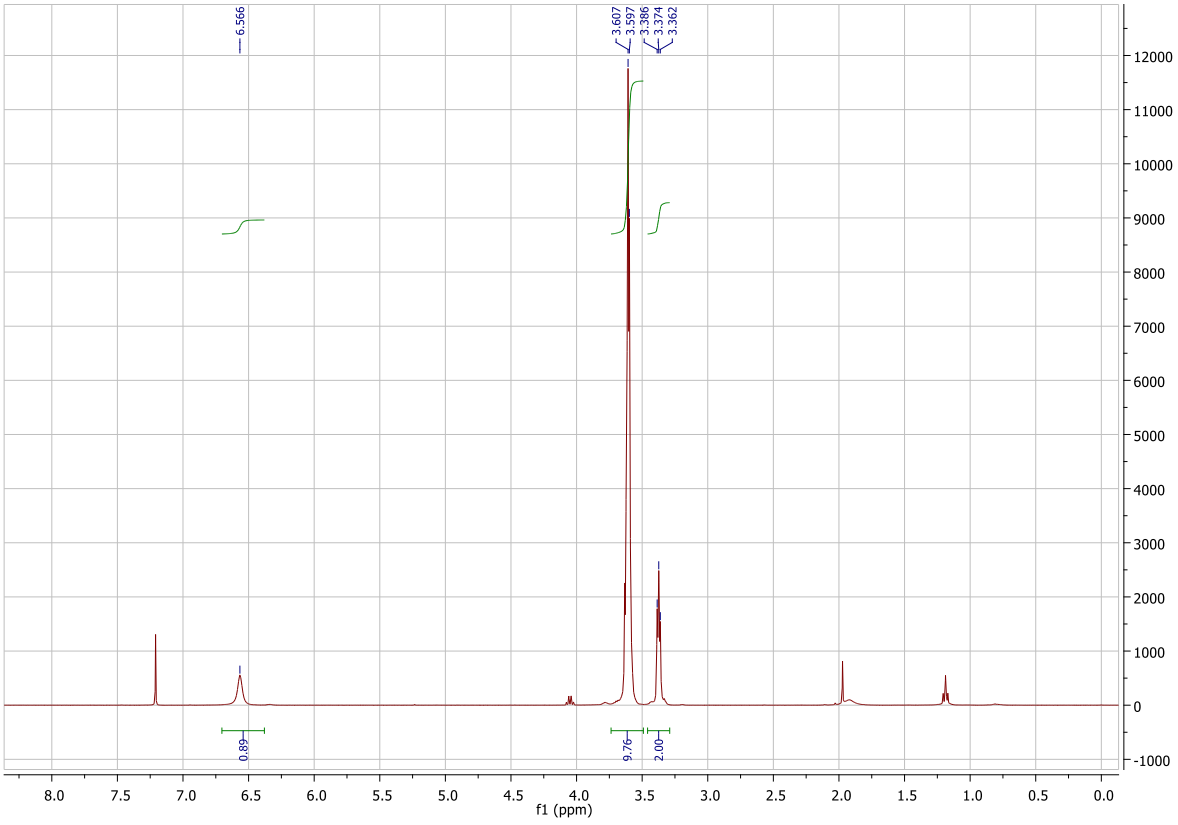
#These authors equally contributed to this work.

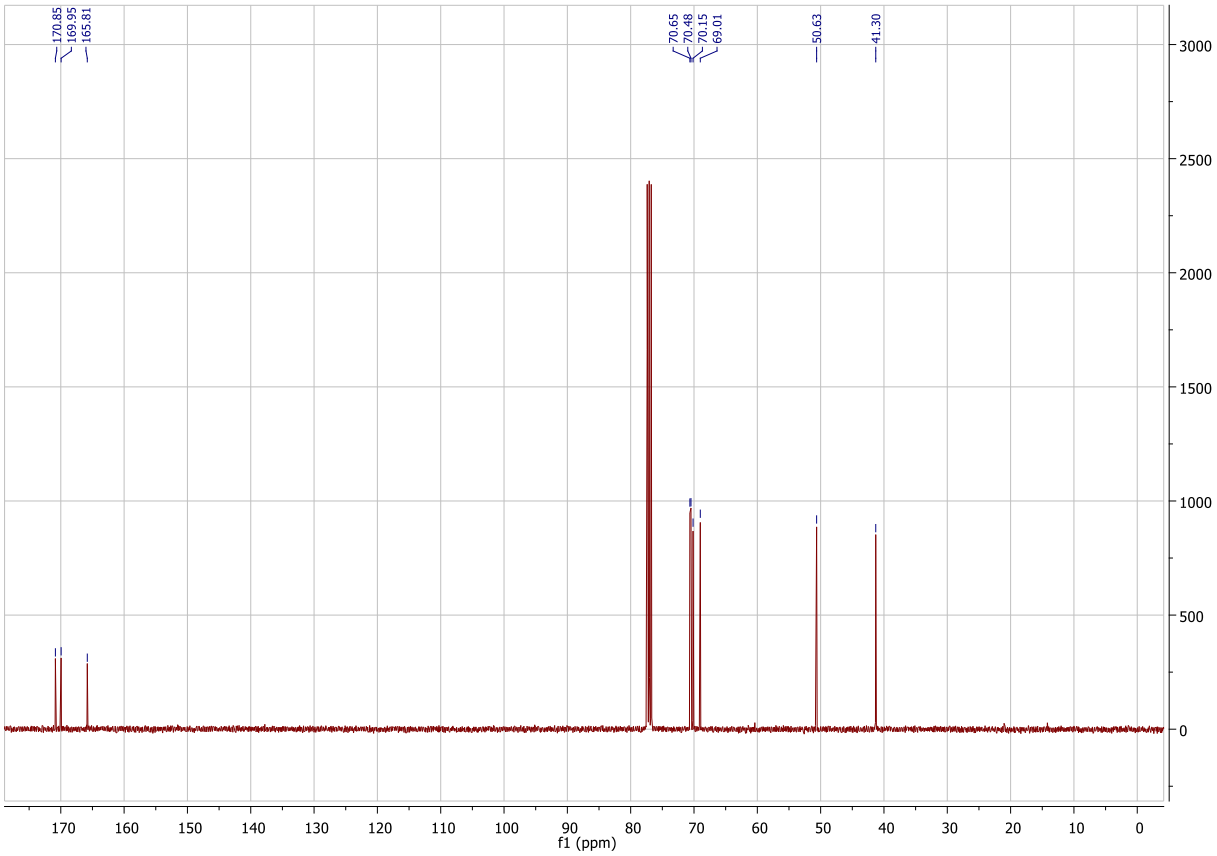
Table of contents

Figure S1. ^{1}H NMR, ^{13}C NMR and mass spectra of N-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-dichloro-1,3,5-triazin-2-amine 3



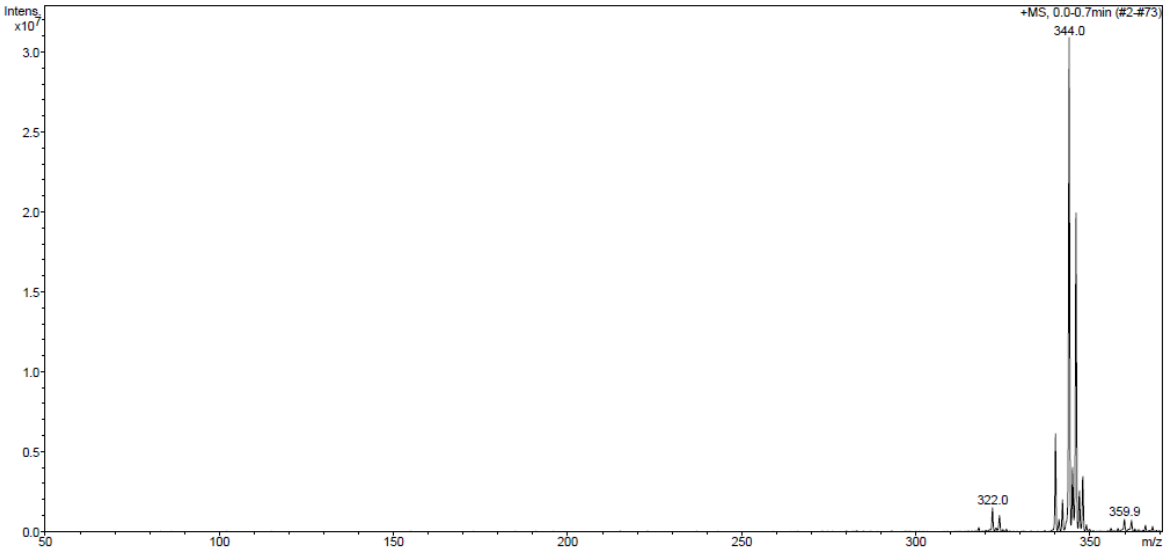
N-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-dichloro-1,3,5-triazin-2-amine 3: R_f = 0.22 (Hexane/AcOEt = 30:70); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.55 (br s, 1H), 3.61-3.58 (m, 10H), 3.37 (t, J = 4.8 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 169.9, 165.8, 70.6, 70.5, 70.1, 69.0, 50.6, 41.3. ESI m/z 344.0 $[M+Na, (100)]^{+}$; Anal. calcd. for $C_9H_{13}Cl_2N_7O_2$: C 33.56, H 4.07, N 30.44; found: C 33.55, H 4.06, N 30.44.





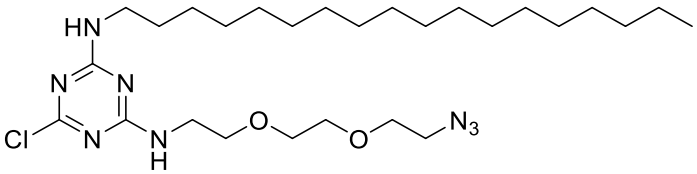
-L.G.S. - Laboratorio Grandi Strumenti - Display Report

Analysis Name	av cpv66.d	Acquisition Date	11/04/16 16:45:15	Operator	Walter Panzeri
Sample Name		Method	Copy of _01tmix_posneg lm.MS	Instrument	esquire3000plus
Comment	1 mg/ml dil 1:100 MeOH Richiedente: Pennetta				

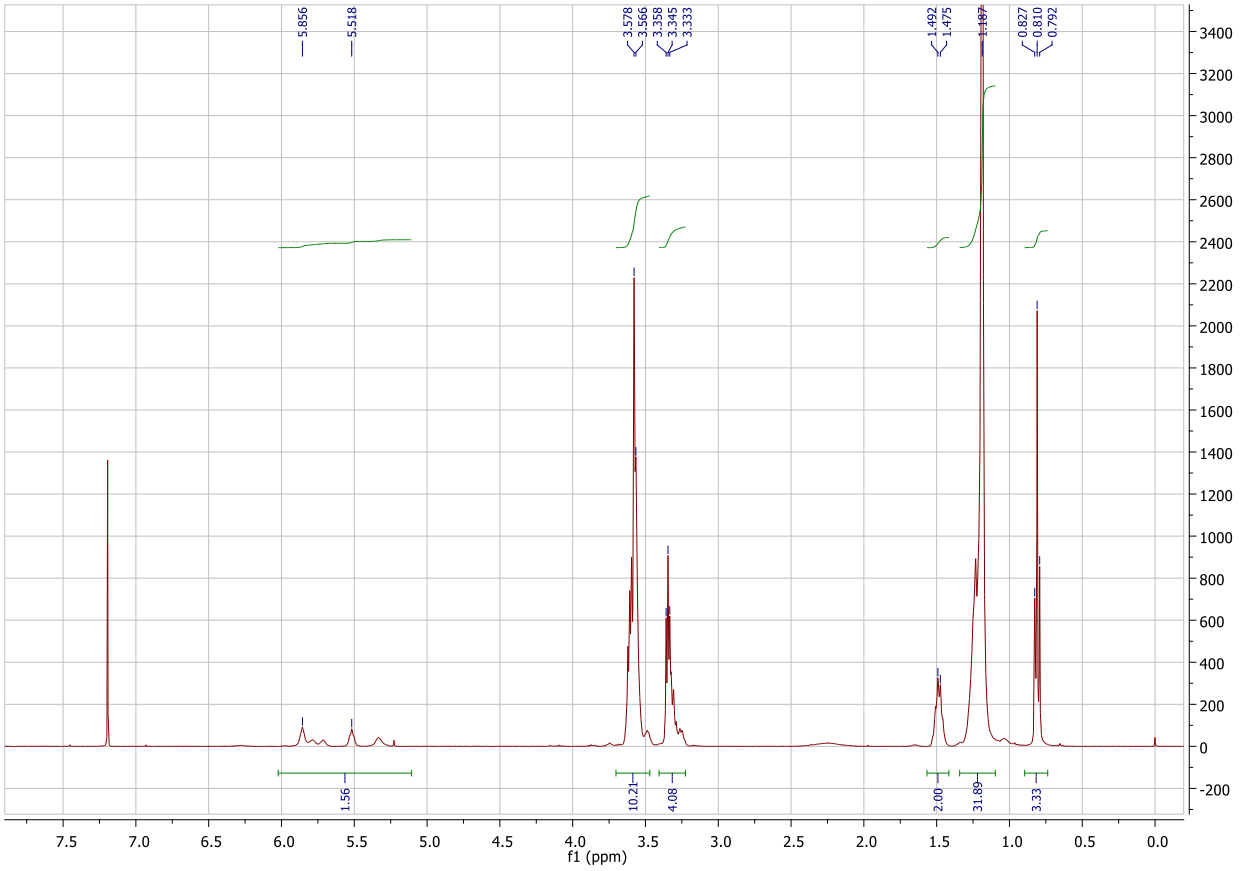


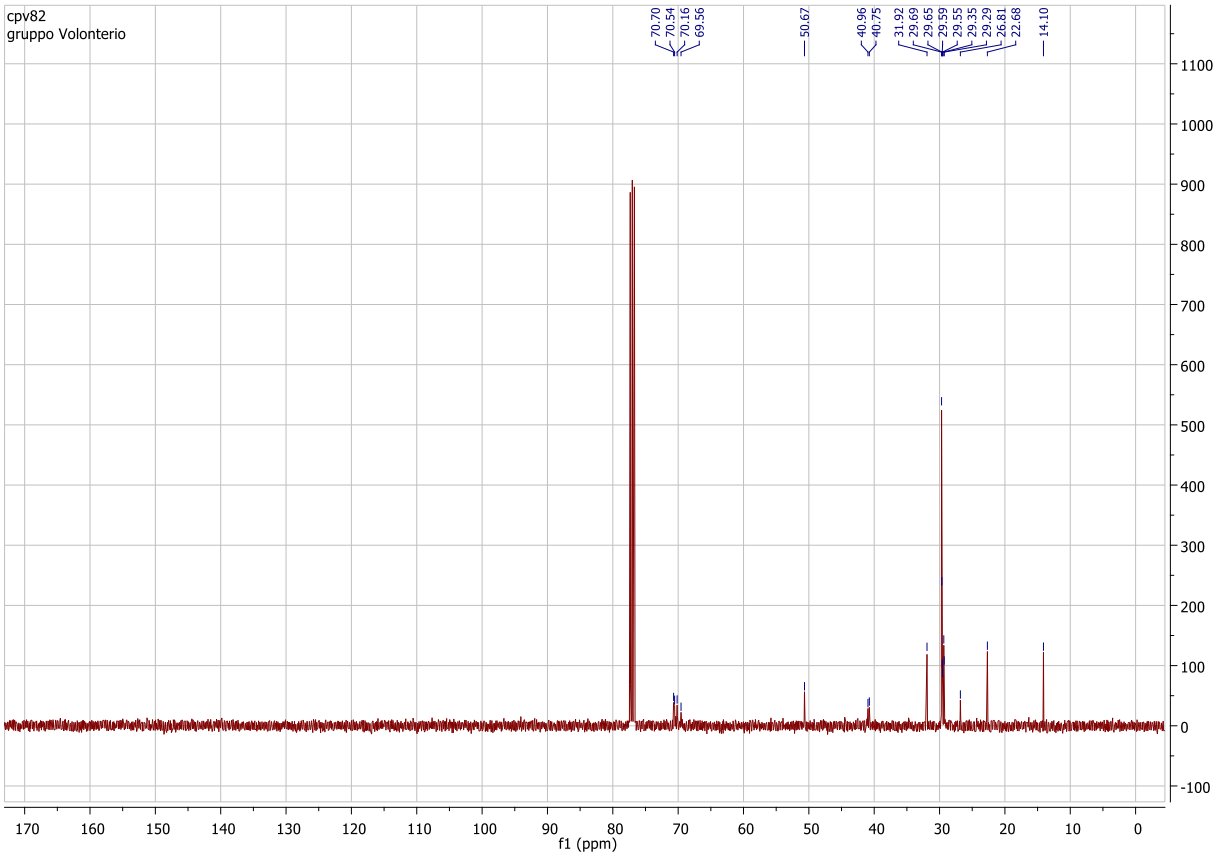
Bruker Daltonics DataAnalysis 3.1 printed: 11/04/16 16:48:53 Page 1 of 1

Figure S2. ^{1}H NMR, ^{13}C NMR and mass spectra of N2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-6-chloro-N4-octadecyl-1,3,5-triazine-2,4-diamine 5a



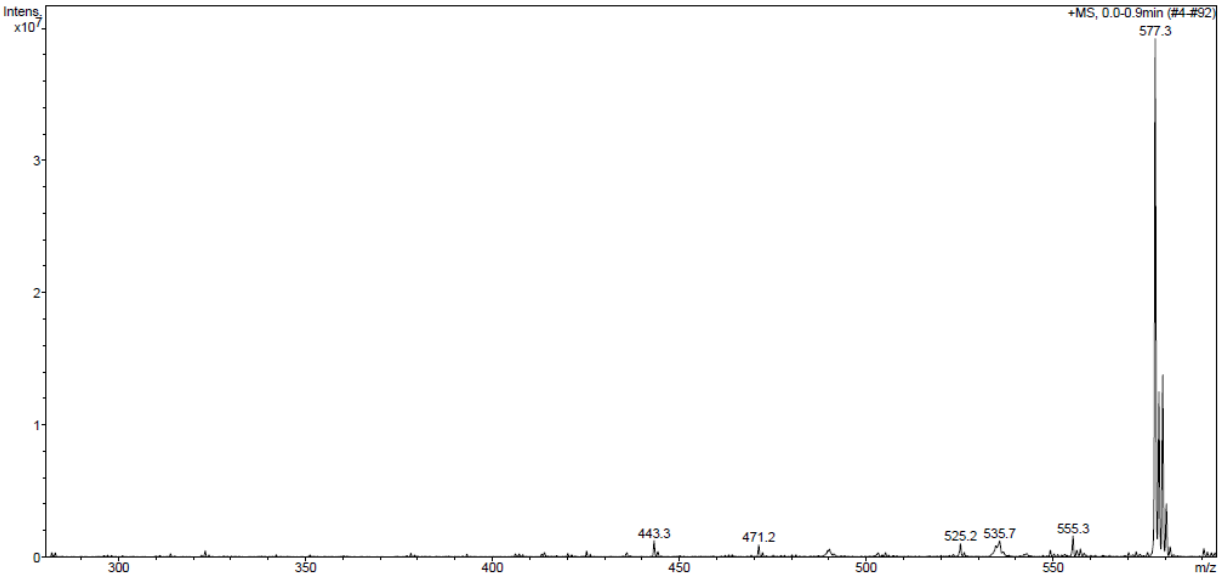
N2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-6-chloro-N4-octadecyl-1,3,5-triazine-2,4-diamine 5a: white solid, 70% yield. R_f = 0.31 (Hexane/AcOEt = 60:40); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (br s, 1H), 5.52 (br s, 1H), 3.67-3.62 (m, 10H), 3.45-3.39 (m, 4H), 1.55-1.53 (m, 2H), 1.28-1.25 (m, 30H), 0.88 (t, J = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 70.8, 70.7, 70.3, 50.8, 41.1, 40.9, 32.1, 29.8, 29.8, 29.7, 29.5, 29.4, 26.9, 22.8, 14.2, the aromatic carbons did not appear due to low intensity; ESI (m/z) 577,3 [M+Na, (100)]$^{+}$; Anal. calcd. for $C_{27}H_{51}ClN_8O_2$: C 58.41, H 9.26, N 20.18; found: C 58.42, H 9.27, N 20.20.





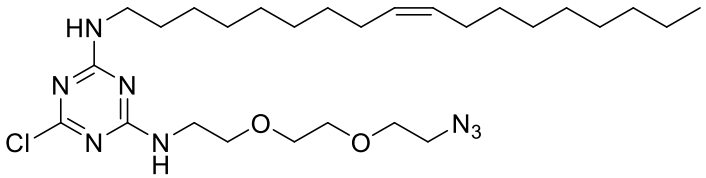
-L.G.S. - Laboratorio Grandi Strumenti - Display Report

Analysis Name	av cpv82.d	Acquisition Date	02/21/17 08:11:56	Operator	Walter Panzeri
Sample Name		Method	Copy of _01tmix_posneg lm.MS	Instrument	esquire3000plus
Comment	1 mg/mL dil 1:100 MeOH Richiedente: Volonterio				

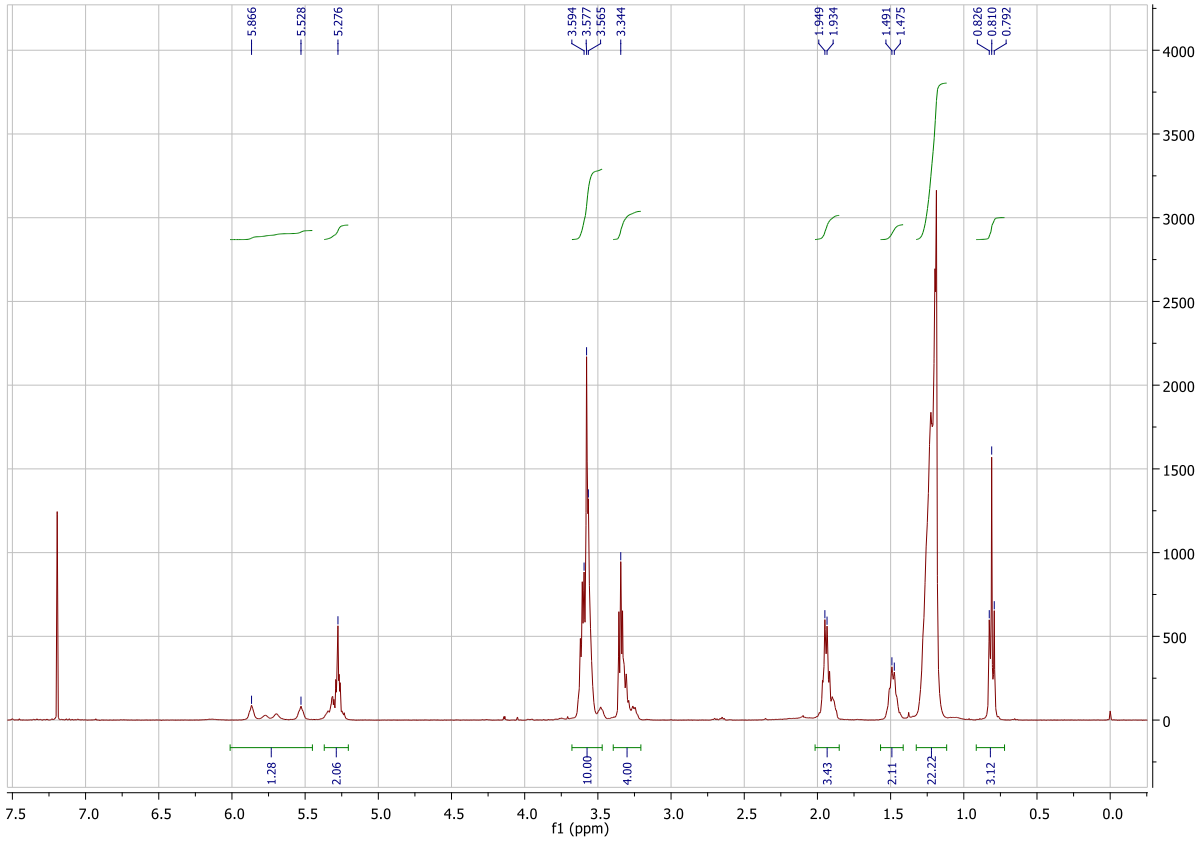


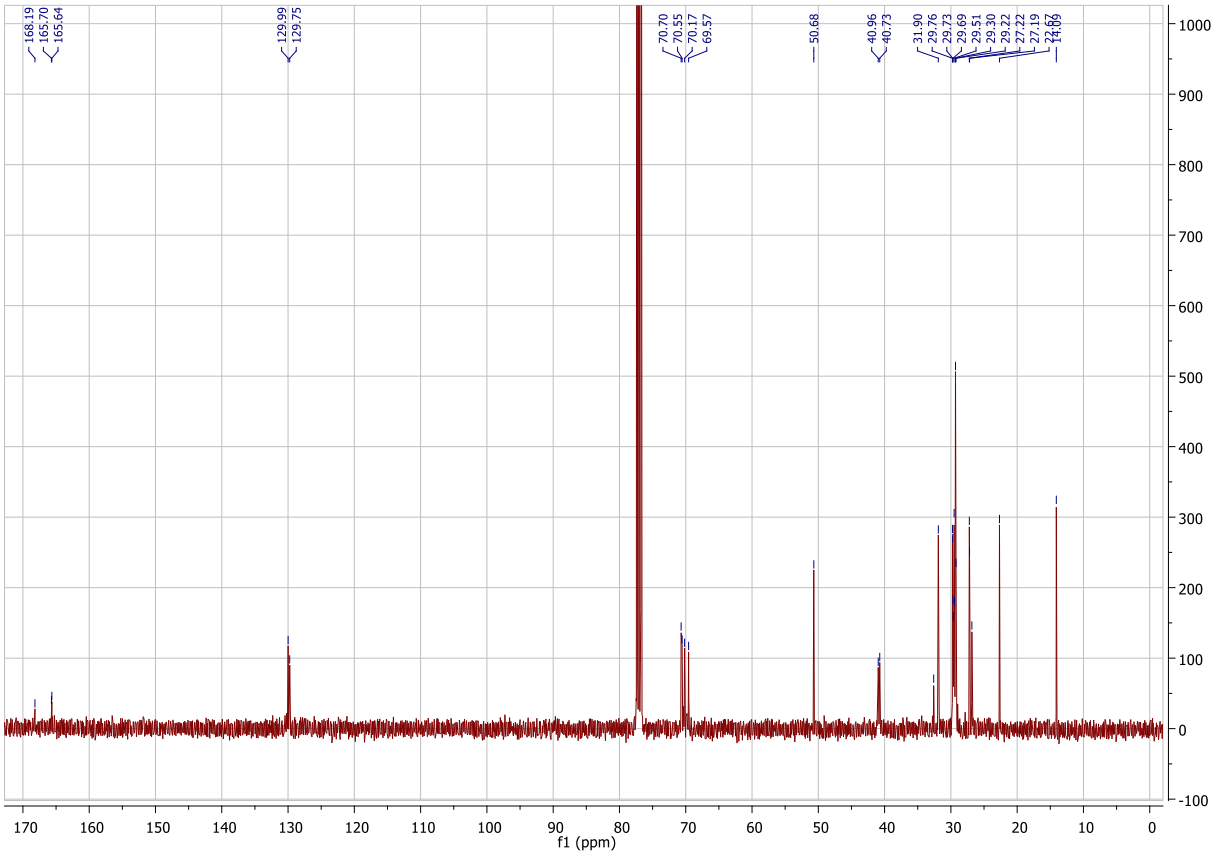
Bruker Daltonics DataAnalysis 3.1 printed: 02/21/17 08:13:14 Page 1 of 1

Figure S3. ^{1}H NMR, ^{13}C NMR and mass spectra of (Z)-N2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-6-chloro-N4-(octadec-9-en-1-yl)-1,3,5-triazine-2,4-diamine, 5b



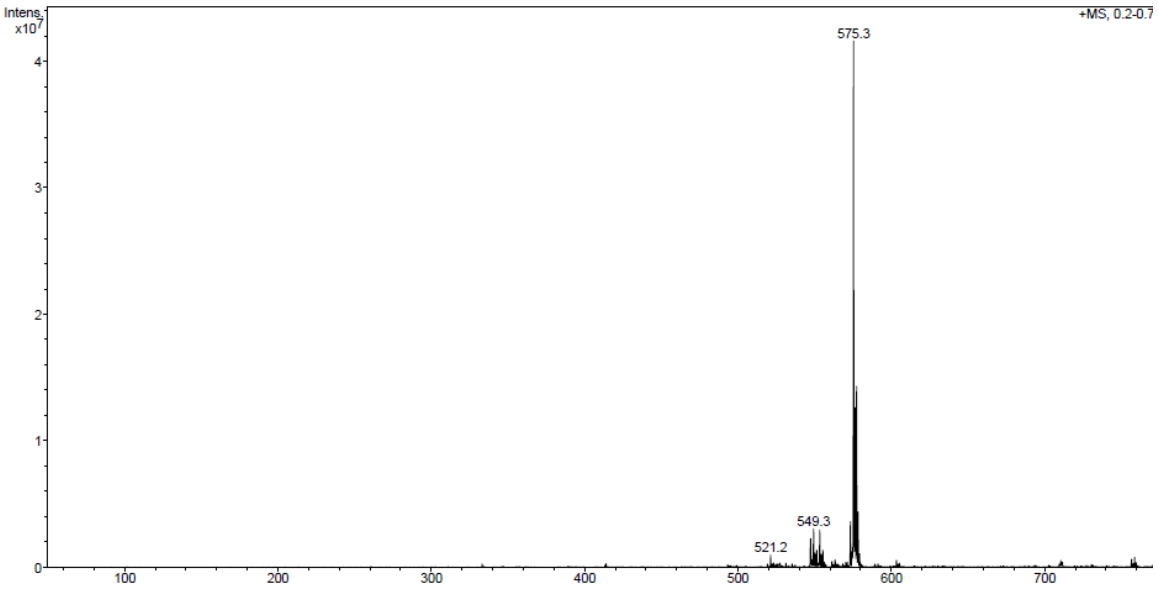
(Z)-N2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-6-chloro-N4-(octadec-9-en-1-yl)-1,3,5-triazine-2,4-diamine, 5b: white solid in 70% yield. R_f = 0.58 (Hexane/AcOEt = 60:40); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.89 (br s, 1H), 5.53 (br s, 1H), 5.35-5.33 (m, 2H), 3.69-3.63 (m, 10H), 3.43-3.41 (m, 4H), 2.05-1.98 (m, 4H), 1.58-1.53 (m, 2H), 1.35-1.26 (m, 22H), 0.88 (t, J = 7,4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 165.7, 165.6, 130.1, 129.9, 70.8, 70.7, 70.3, 69.7, 50.8, 41.1, 40.8, 32.7, 32.0, 29.8, 29.6, 29.4, 27.3, 26.9, 22.8, 14.2. ESI (m/z) 575,3 $[M+Na, (100)]^{+}$; Anal. calcd. for $C_{27}H_{49}ClN_8O_2$: C 58.62, H 8.93, N 20.26; found: C 58.60, H 8.93, N 20.27.





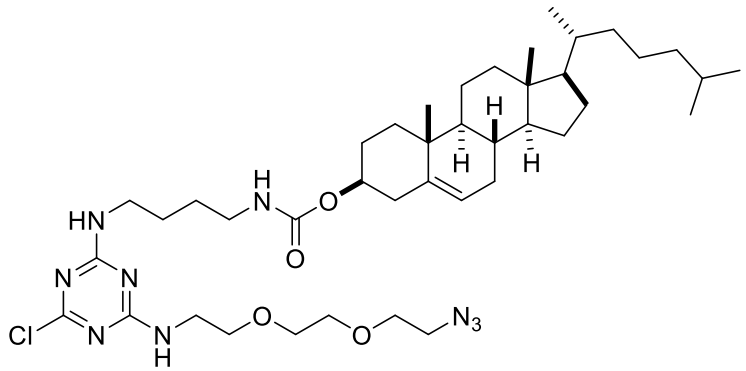
-L.G.S. - Laboratorio Grandi Strumenti - Display Report

Analysis Name	av cpv67.d	Acquisition Date	11/10/16 11:34:14	Operator	Walter Panzeri
Sample Name		Method	Copy of _01tmix_posneg lm.MS	Instrument	esquire3000plus
Comment	1 mg/Ml dil 1:100 MeOH Richiedente: Pennetta				

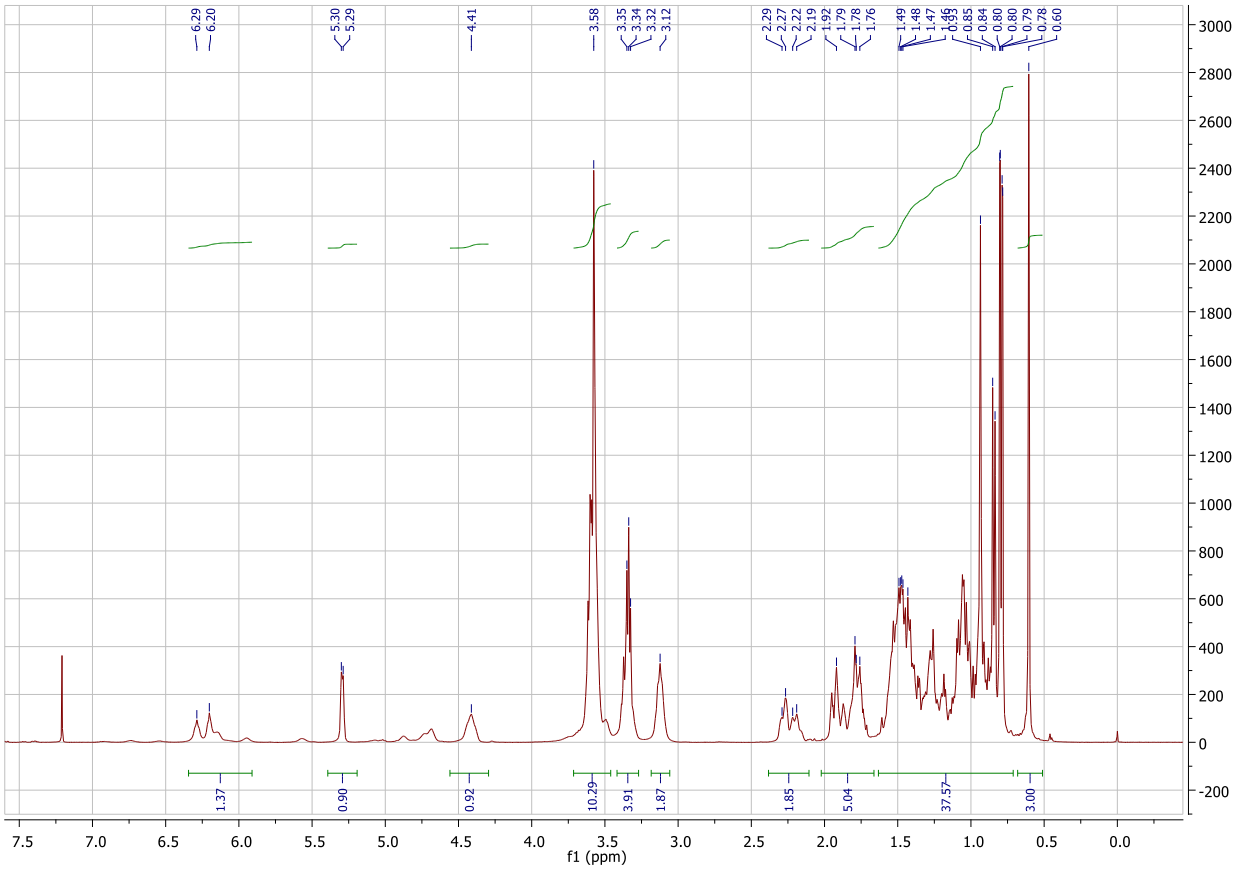


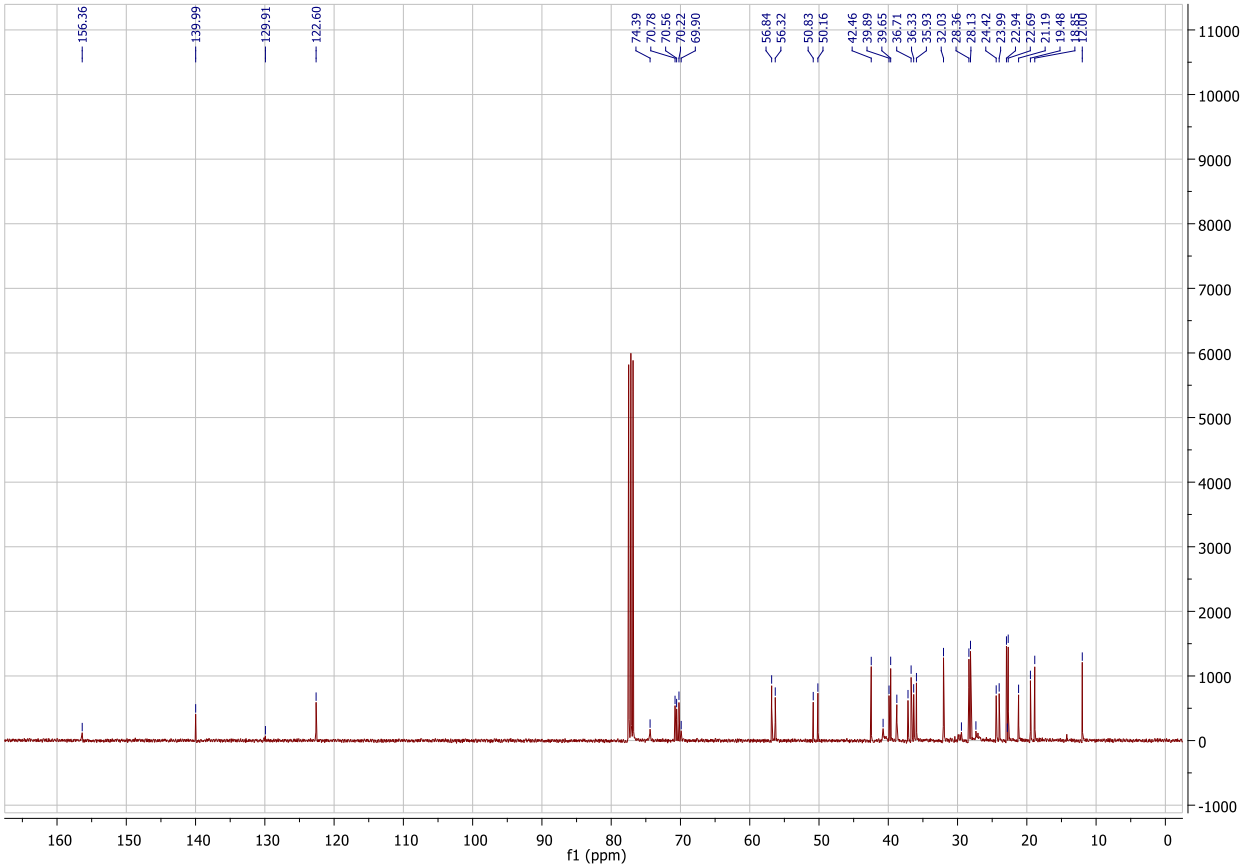
Bruker Daltonics DataAnalysis 3.1 printed: 11/10/16 11:35:31 Page

Figure S4. ^{1}H NMR, ^{13}C NMR, and mass spectra of (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl (4-((4-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-6-chloro-1,3,5-triazin-2-yl)amino)butyl)carbamate 5c



(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl (4-((4-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-6-chloro-1,3,5-triazin-2-yl)amino)butyl)carbamate 5c: white solid, 70% yield. R_f = 0.34 (Hexane/AcOEt = 30:70); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (br s, 1H), 6.20 (br s, 1H), 5.30 (d, *J* = 4.8 Hz, 1H), 4.41 (br s, 1H), 3.60-3.56 (m, 10H), 3.37-3.34 (m, 4H), 3.12(br s, 2H) , 2.32-2.28 (m, 2H), 1.94-1.92 (m, 2H), 1.80-1.77 (m, 3H), 1.66-0.83 (m, 38H), 0.66 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.4, 140.0, 129.9, 122.6, 74.4, 70.8, 70.6, 70.2, 69.9, 56.8, 56.3, 50.8, 50.2, 42.5, 39.9, 39.6, 36.7, 36.3, 35.9, 32.0, 28.4, 28.1, 24.4, 24.0, 22.9, 22.7, 21.2, 19.5, 18.8, 12.0, two aromatic carbons did not appear due to low intensity; ESI *m/z* 808,7 $[M+Na, (100)]^{+}$; Anal. calcd. for $C_{41}H_{68}ClN_9O_4$: C 62.61, H 8.72, N 16.03; found: C 62.60, H 8.74, N 16.01.


6.29
6.20
5.30
5.29
4.41
3.58
3.35
3.34
3.32
3.12
2.29
2.27
2.22
2.19
1.92
1.79
1.78
1.76
1.49
1.48
1.47
1.46
0.93
0.85
0.84
0.80
0.80
0.79
0.78
0.60
1.37
0.90
0.92
10.29
3.91
1.87
1.85
5.04
37.57
3.00
f1 (ppm)


156.36
139.99
129.91
122.60
74.39
70.78
70.56
70.22
69.90
56.84
56.32
50.83
50.16
42.46
39.89
39.65
36.71
36.33
35.93
32.03
28.36
28.13
24.42
23.99
22.94
22.69
21.19
19.48
f1 (ppm)

-L.G.S. - Laboratorio Grandi Strumenti - Display Report

Analysis Name	av cp127d.d	Acquisition Date	09/19/18 13:56:23	Operator	Walter Panzeri
Sample Name		Method	Copy of _01tmix_posneg lm.MS	Instrument	esquire3000plus
Comment	1 mg/ml dil 1:100 MeOH Richiedente: Pennetta				

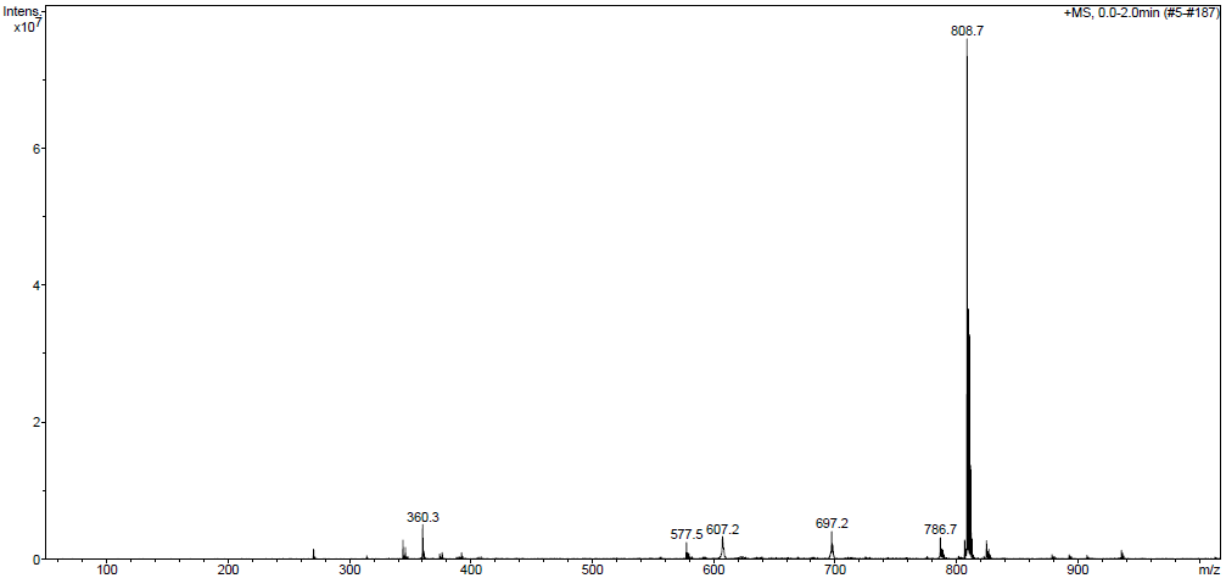
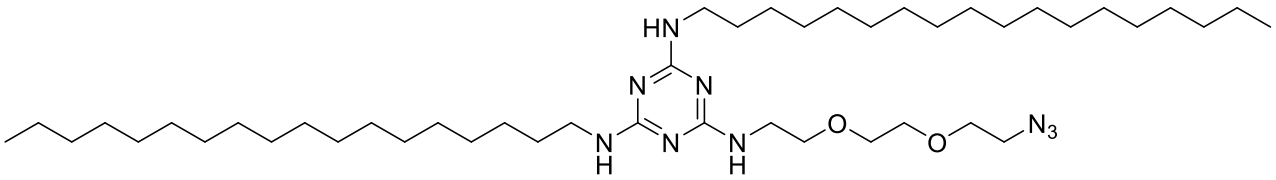
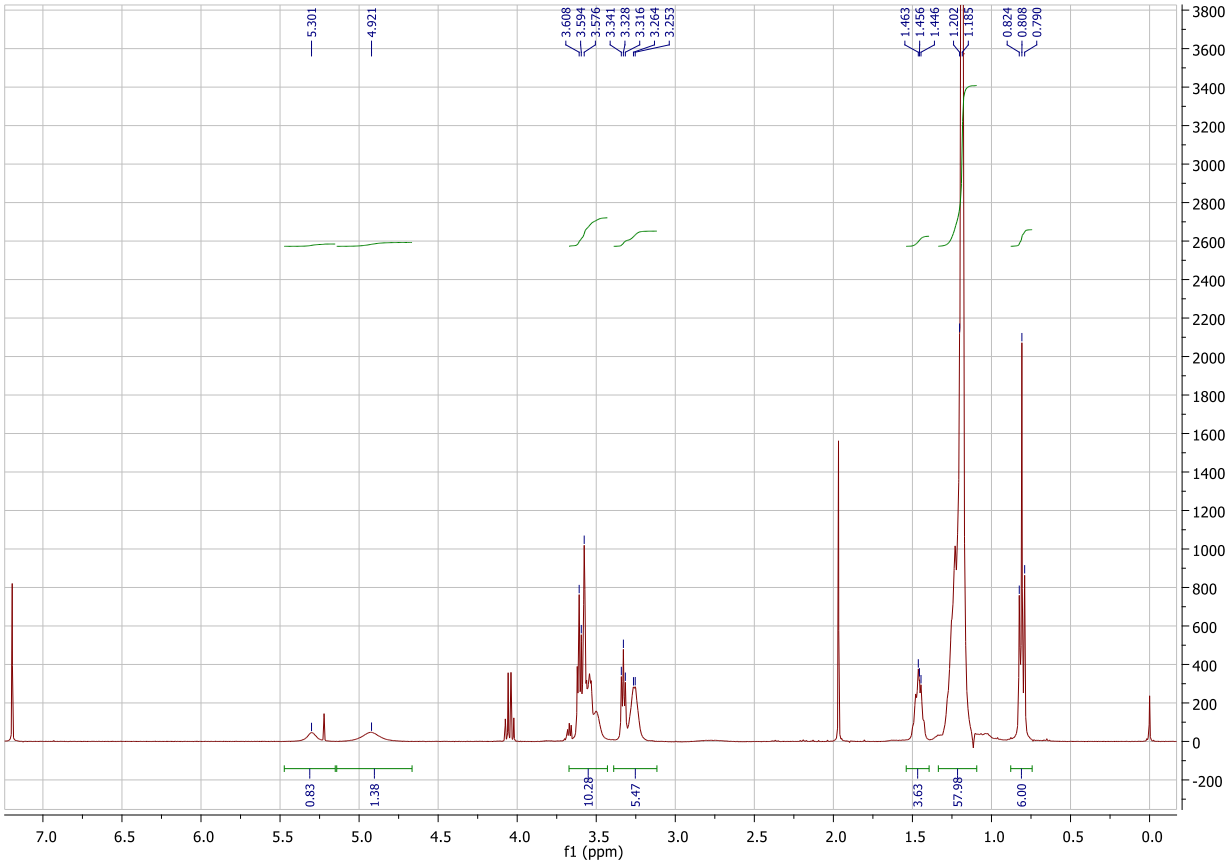
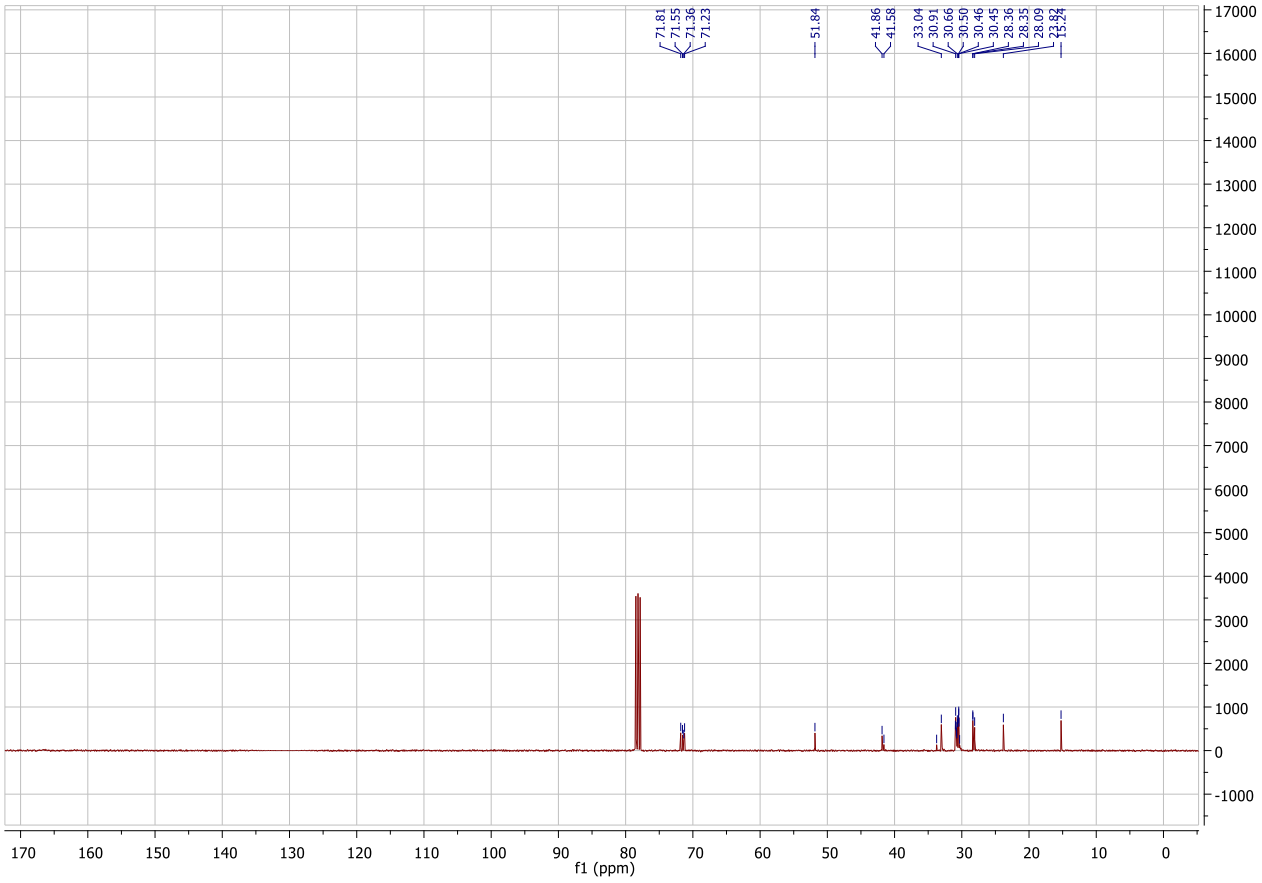


Figure S5. ^{1}H NMR, ^{13}C NMR, and mass spectra of N2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-N4,N6-dioctadecyl-1,3,5-triazine-2,4,6-triamine, 6aa



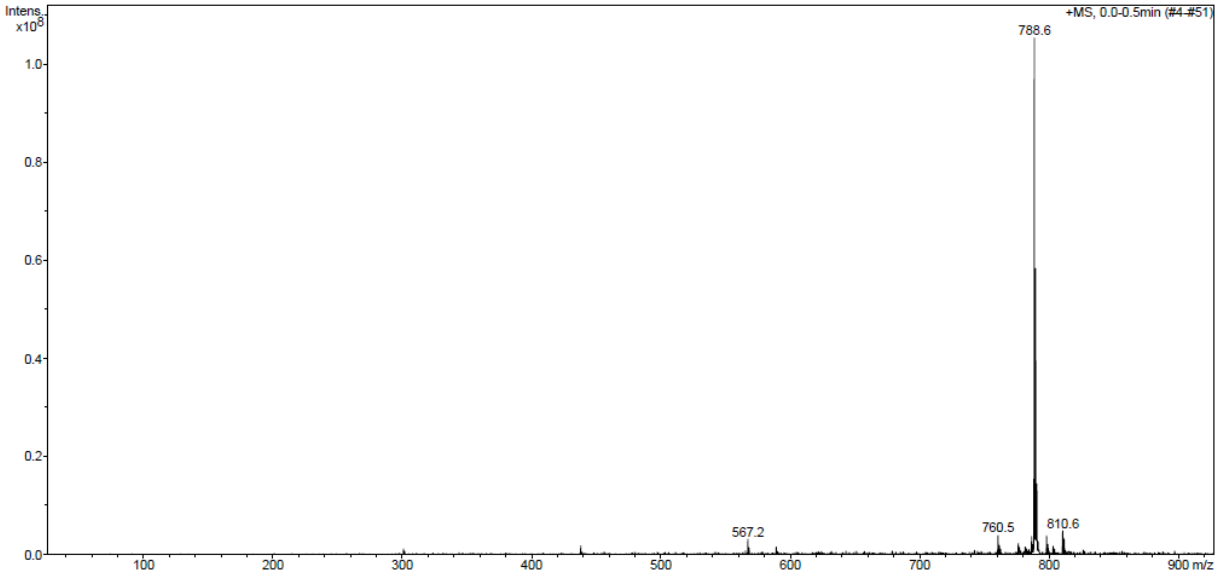
N2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-N4,N6-dioctadecyl-1,3,5-triazine-2,4,6-triamine, 6aa: yellowish solid, 60% yield. R_f = 0.47 (DCM/MeOH = 90:10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (br s, 1H), 4.92 (br s, 2H), 3.62-3.55 (m, 10H), 3.33 (t, *J* = 5.2 Hz, 2H), 3.33-3.30 (m, 4H), 1.48-1.42 (m, 4H), 1.22-1.17 (m, 60H), 0.81 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 71.2, 71.6, 71.4, 71.2, 51.8, 41.9, 41.6, 33.7, 33.0, 30.9, 30.5, 30.4, 28.4, 28.3, 28.1, 23.8, 15.2, the aromatic carbons did not appear due to low intensity; ESI (m/z) 788,6 $[M+H, (100)]^{+}$; Anal. calcd. for $C_{45}H_{89}N_9O_2$: C 68.57, H 11.38, N 15.99; found: C 68.57, H 11.37, N 16.00.





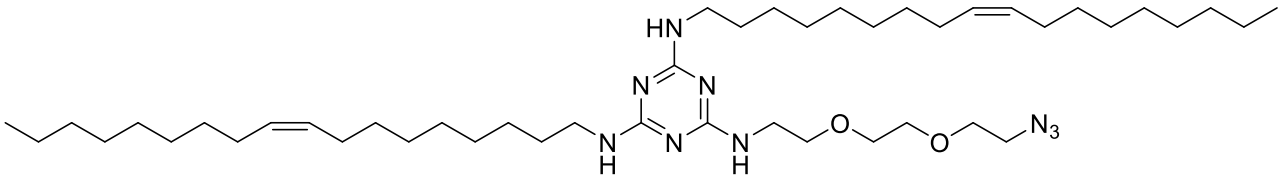
-L.G.S. - Laboratorio Grandi Strumenti - Display Report

Analysis Name	av cpv112b.d	Acquisition Date	09/08/17 15:39:18	Operator	Walter Panzeri
Sample Name		Method	Copy of _01tmix_posneg lm.MS	Instrument	esquire3000plus
Comment	1 mg/ml dil 1:100 MeOH Richiedente: Pennetta				

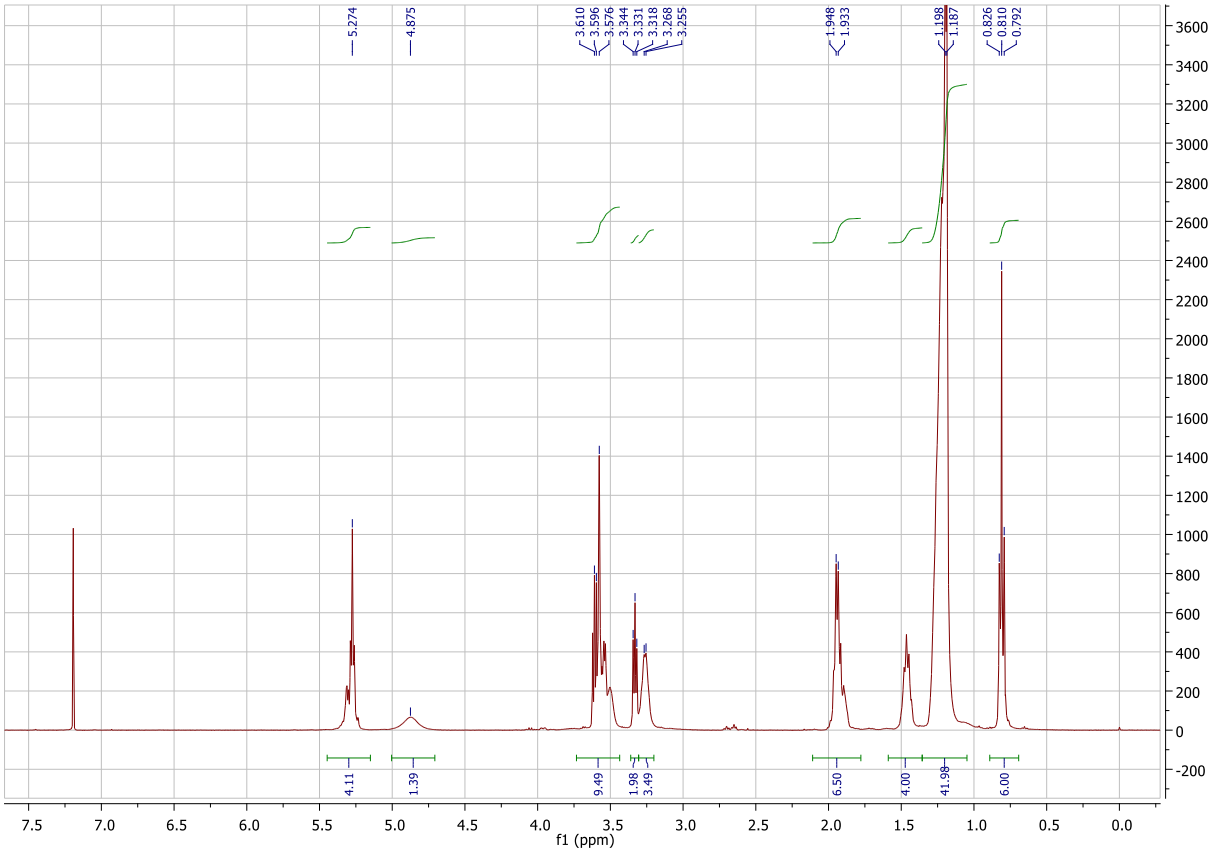


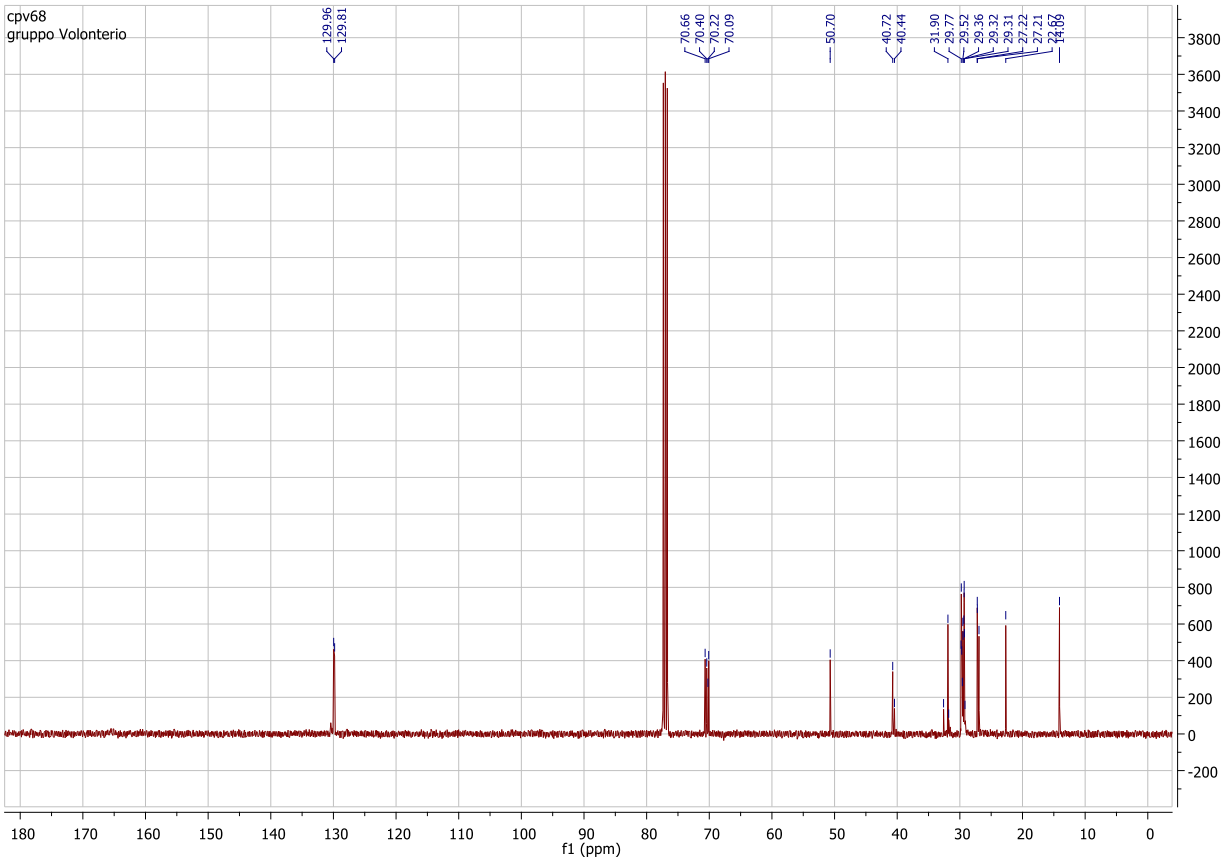
Bruker Daltonics DataAnalysis 3.1 printed: 09/08/17 15:40:18 Page 1 of 1

Figure S6. ^{1}H NMR, ^{13}C NMR, and mass spectra of N2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-N4,N6-di((Z)-octadec-9-en-1-yl)-1,3,5-triazine-2,4,6-triamine, 6bb



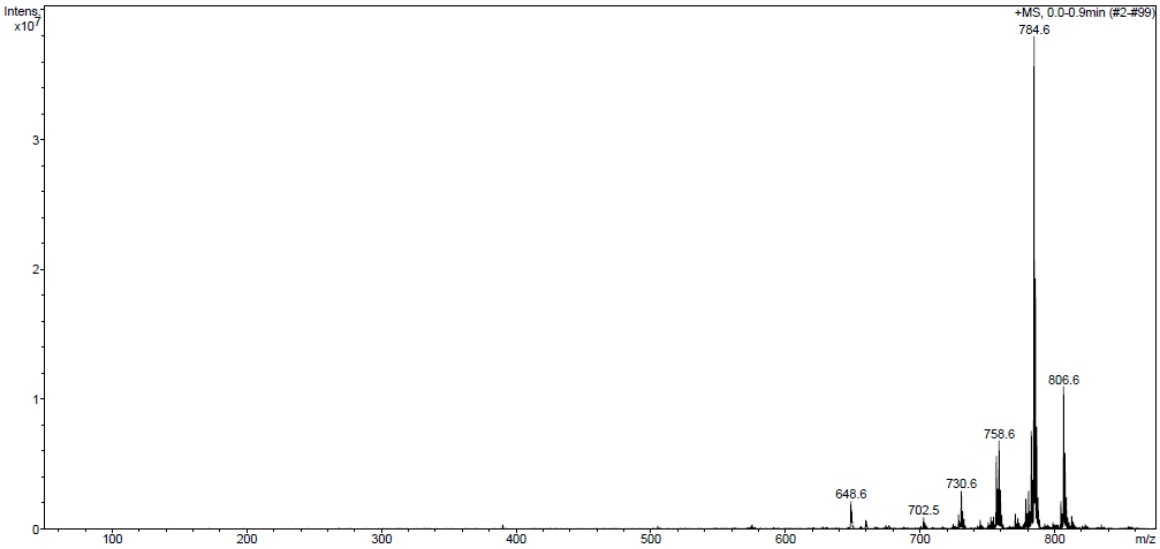
N2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-N4,N6-di((Z)-octadec-9-en-1-yl)-1,3,5-triazine-2,4,6-triamine, 6bb: white solid. 60% yield. R_f = 0.42 (DCM/MeOH = 90:10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36-5.33 (m, 4H), 4.87 (br s, 2H), 3.68-3.56 (m, 10H), 3.39 (t, J = 4.96 Hz, 2H), 3.35-3.32 (m, 4H), 2.02-1.96 (m, 8H), 1.54-1.52 (m, 4H), 1.28-1.25 (m, 44H), 0.87 (t, J = 6.6 Hz, 6H)**;** ^{13}C NMR (101 MHz, $CDCl_3$) δ 129.9, 129.8, 70.7, 70.4, 70.2, 70.1, 50.7, 40.7, 40.4, 31.9, 29.8, 29.5, 29.4, 29.36, 29.32, 29.31, 27.22, 27.21, 26.9, 22.7, 14.1, the aromatic carbons did not appear due to low intensity; ESI (m/z) 806.6 $[M+Na, (26)]^+$, 784,6 $[M+H, (100)]^+$; Anal. calcd. for $C_{45}H_{85}N_9O_2$: C 68.92, H 10.93, N 16.07; found: C 68.93, H 10.95, N 16.06.





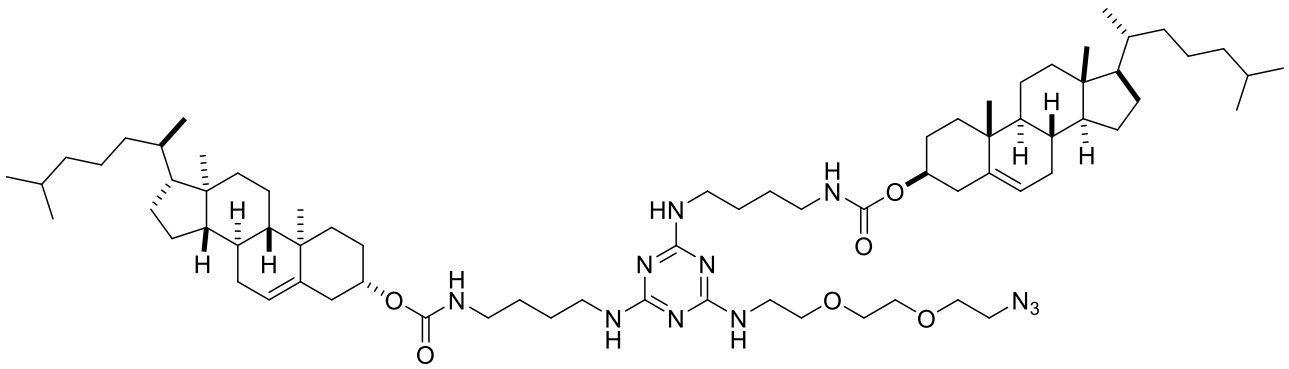
-L.G.S. - Laboratorio Grandi Strumenti - Display Report

Analysis Name	av cpv68.d	Acquisition Date	11/17/16 13:54:22	Operator	Administrator
Sample Name		Method	Copy of _01tmix_posneg lm.MS	Instrument	esquire3000plus
Comment	1 mg/ml dil 1:100 MeOH Richiedente: Pennetta				

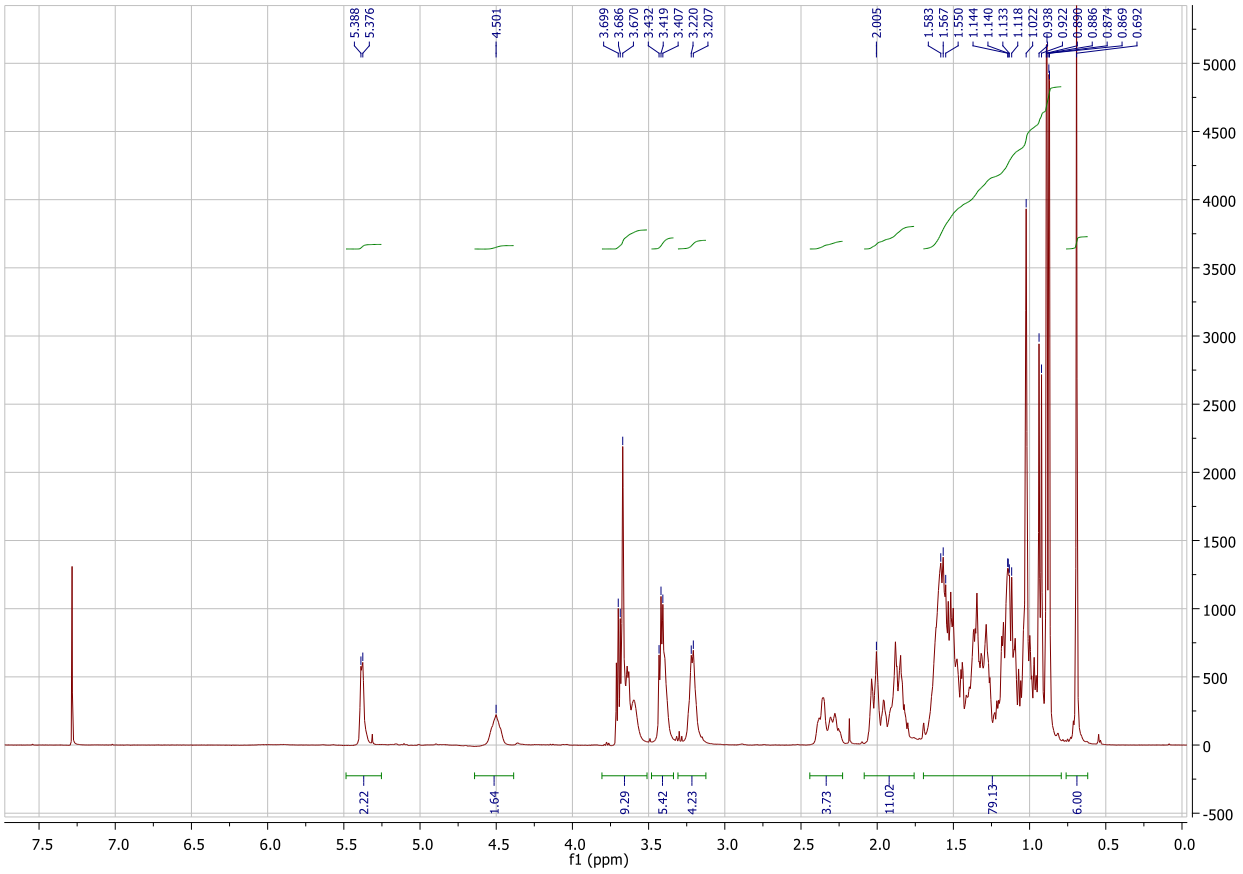


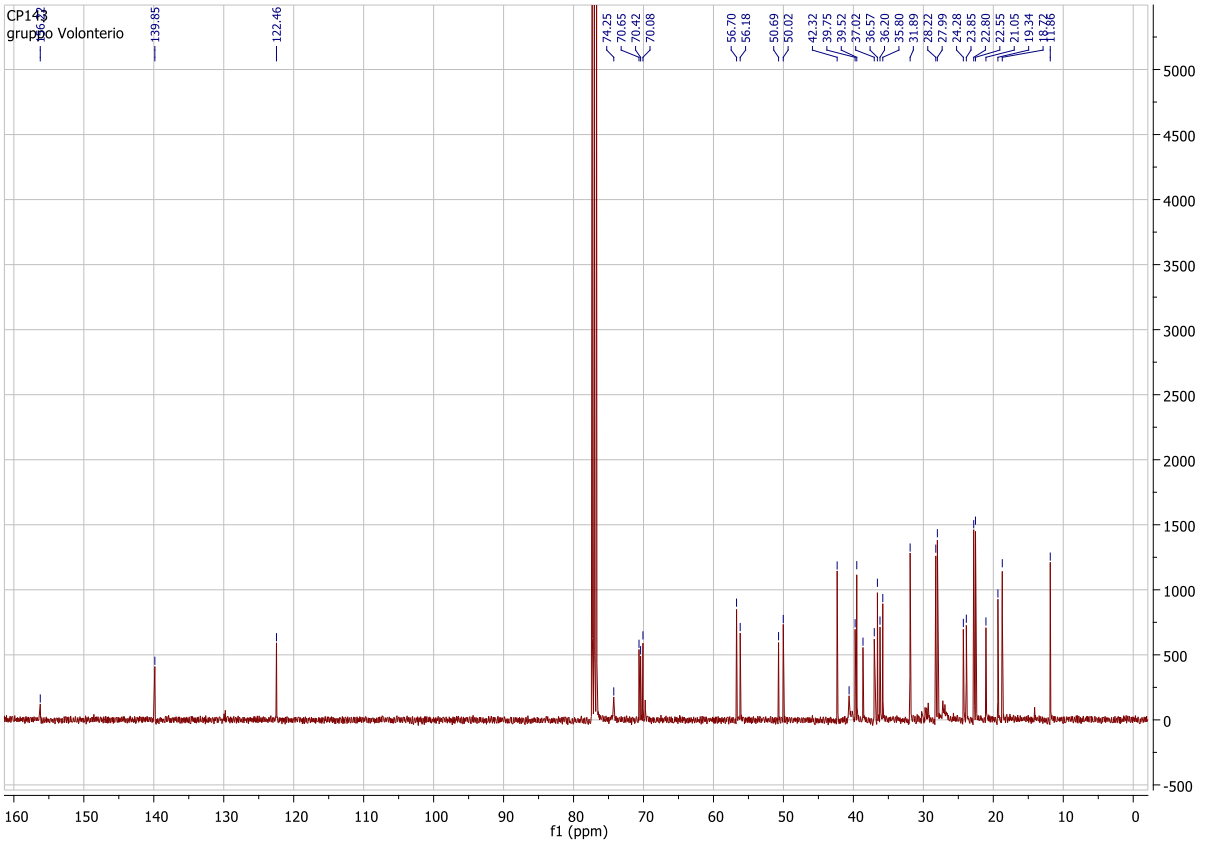
Bruker Daltonics DataAnalysis 3.1 printed: 11/17/16 13:59:18 Page 1 of 1

Figure S7. ^{1}H NMR, ^{13}C NMR and mass spectra of bis((3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl) (((6-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-1,3,5-triazine-2,4-diyl)bis(azanediyl))bis(butane-4,1-diyl))dicarbamate, 6cc



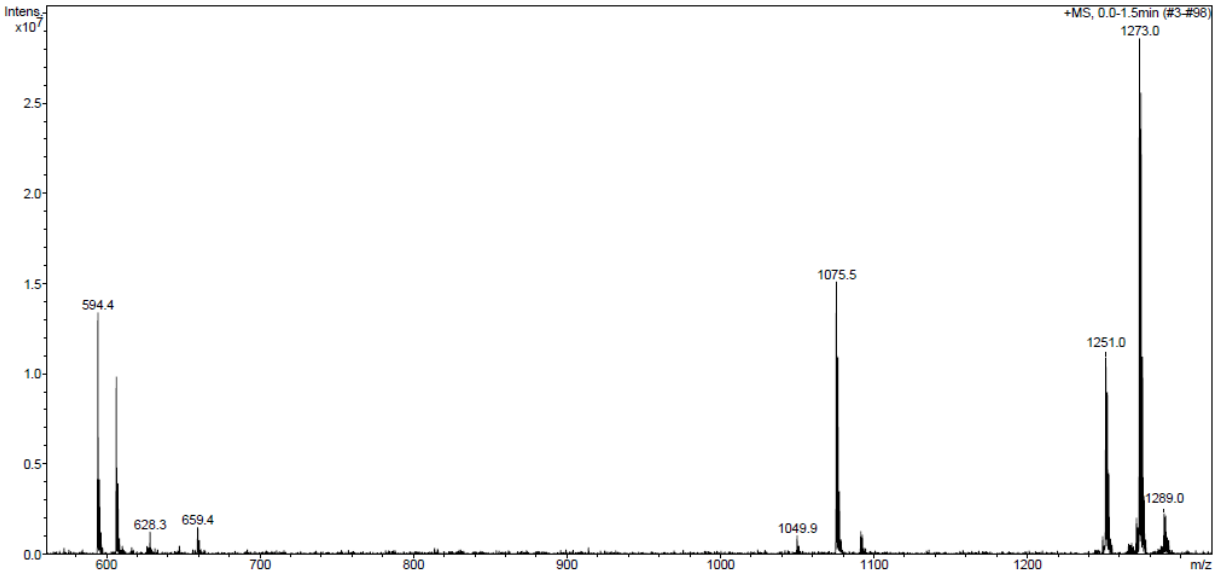
bis((3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl) (((6-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-1,3,5-triazine-2,4-diyl)bis(azanediyl))bis(butane-4,1-diyl))dicarbamate, 6cc: white solid, 45% yield. R_f = 0.39 (DCM/MeOH = 90:10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40-5.37 (m, 2H), 4.50 (br s, 2H), 3.71-3.67 (m, 10H), 3.43-3.40 (m, 6H), 3.23-3.20 (m, 4H), 2.38-2.27 (m 4H), 2.10-1.74 (m, 10H), 1.60-0.87 (m, 76H), 0.69 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.2, 139.8, 122.5, 74.4, 70.8, 70.6, 70.2, 69.9, 56.8, 56.3, 50.8, 50.2, 42.5, 40.7, 39.9, 39.6, 38.8, 37.1, 36.1, 36.3, 35.9, 32.0, 29.9, 29.6, 29.4, 28.4, 28.1, 27.3, 24.4, 24.00, 22.9, 22.8, 22.7, 21.2, 19.5, 18.8, 14.2, 12.0, the aromatic carbons did not appear due to low intensity; ESI (m/z) 1273.0 $[M+Na, (100)]^+$, 1251.0 $[M+H, (36)]^+$; Anal. calcd. for $C_{73}H_{123}N_{11}O_6$: C 70.10, H 9.91, N 12.32; found: C 70.09, H 9.93, N 12.31.


5.388
5.376
4.501
3.699
3.686
3.670
3.432
3.419
3.407
3.220
3.207
2.005
1.583
1.567
1.550
1.144
1.140
1.133
1.118
1.022
0.938
0.922
0.890
0.886
0.874
0.869
0.692
2.22
1.64
9.29
5.42
4.23
3.73
11.02
79.13
6.00
f1 (ppm)


CP143
gruppo Volonterio
139.85
122.46
74.25
70.65
70.42
70.08
56.70
56.18
50.69
50.02
42.32
39.75
39.52
37.02
36.57
36.20
35.80
31.89
28.22
27.99
24.28
23.85
22.80
22.55
21.05
19.34
18.72
11.86
f1 (ppm)

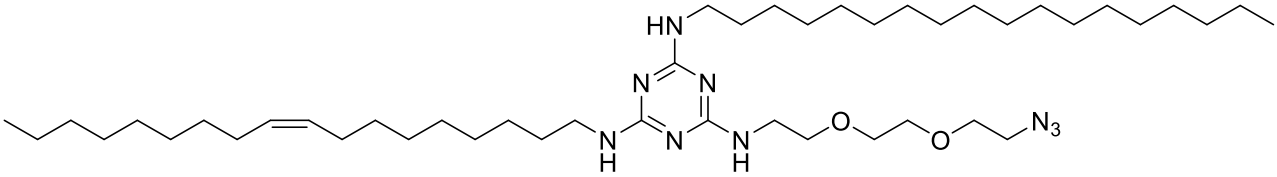
-L.G.S. - Laboratorio Grandi Strumenti - Display Report

Analysis Name	av cp143a.d	Acquisition Date	11/30/18 09:56:16	Operator	Walter Panzeri
Sample Name		Method	Copy of _giov 1620 24713.MS	Instrument	esquire3000plus
Comment	1 mg/ml dil 1:100 MeOH Richiedente: Pennetta				

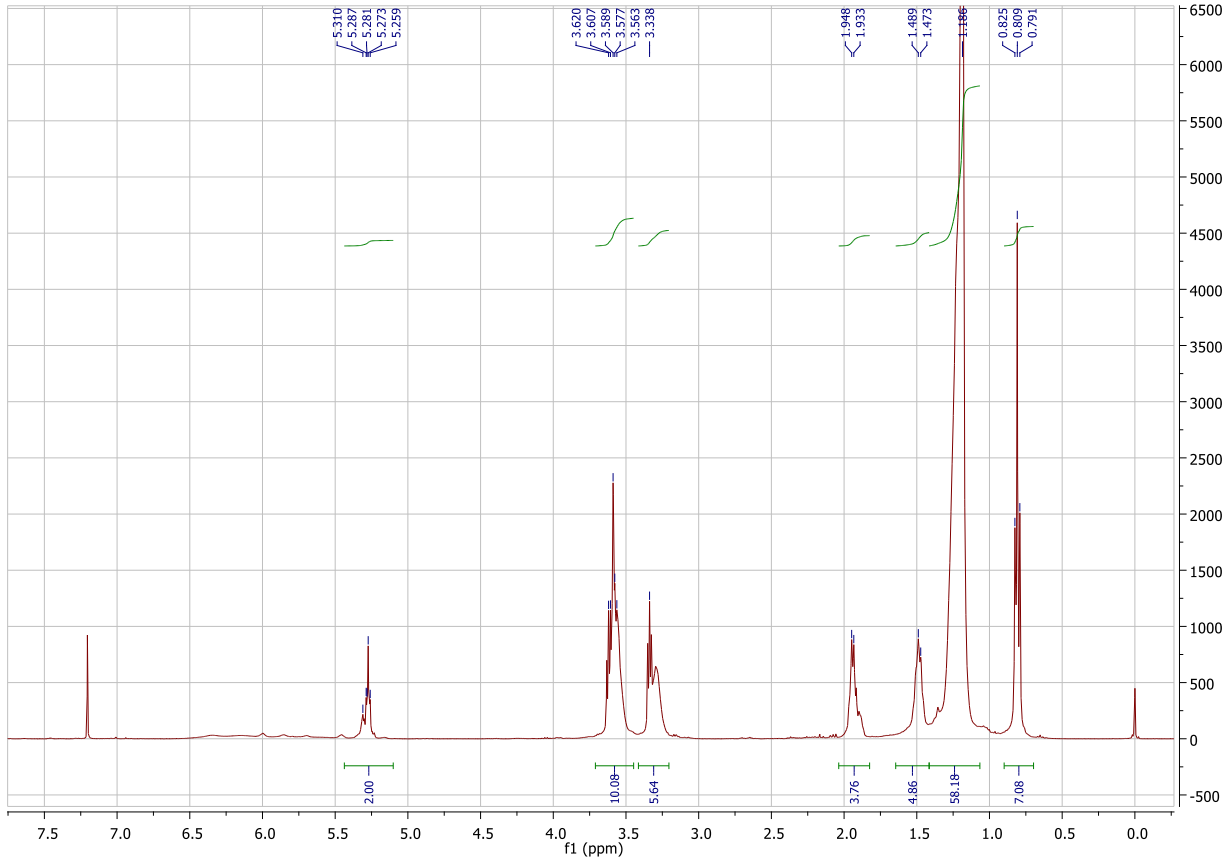


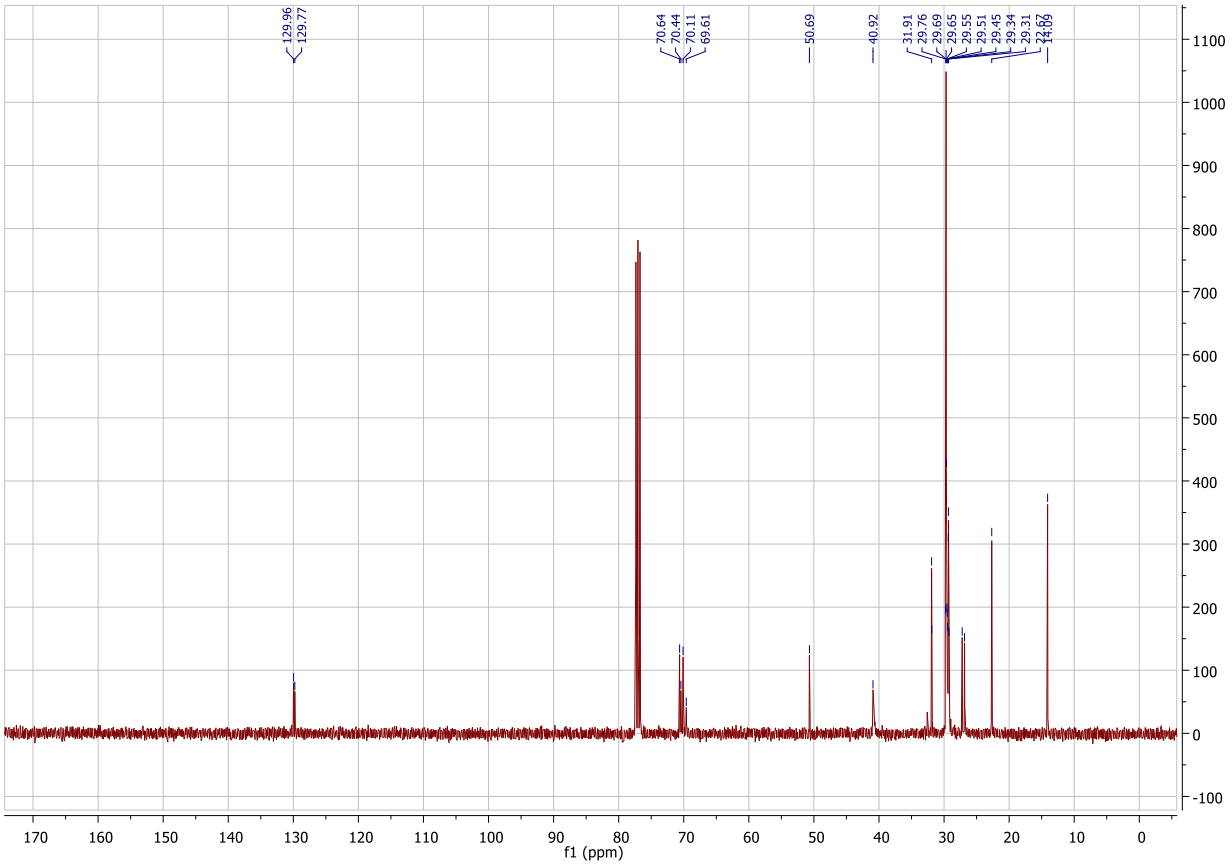
Bruker Daltonics DataAnalysis 3.1 printed: 11/30/18 10:26:14 Page 1 of 1

Figure S8. ^{1}H NMR, ^{13}C NMR, and mass spectra of (Z)-N2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-N4-(octadec-9-en-1-yl)-N6-octadecyl-1,3,5-triazine-2,4,6-triamine, 6ab



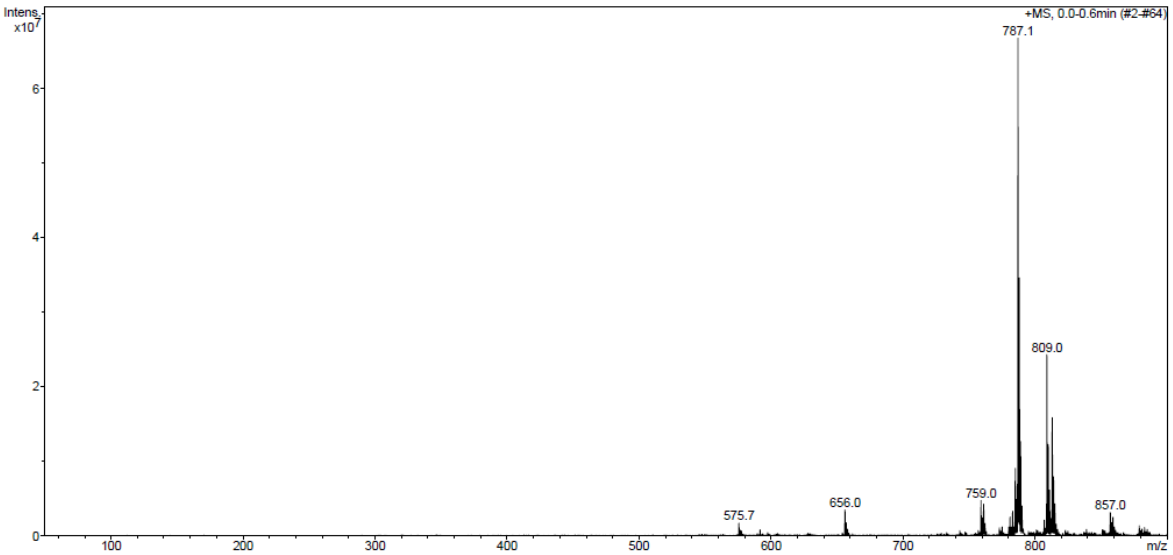
(Z)-N2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-N4-(octadec-9-en-1-yl)-N6-octadecyl-1,3,5-triazine-2,4,6-triamine, 6ab: white solid, 60% yield. R_f = 0.51 (DCM/MeOH = 90:10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31-5.26 (m, 2H), 3.64-3.54 (m, 10H), 3.39-3.31 (m. 6H), 1.96-1.92 (m, 4H), 1.50-1.43 (m, 4H), 1.28-1.14 (m, 52H), 0.81 (t, J = 7.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 130.0, 129.8, 70.8, 70.6, 70.2, 69.7, 50.8, 41.0, 32.7, 32.0, 29.8, 29.6, 29.6, 29.4, 27.3, 27.0, 22.8, the aromatic carbons did not appear due to low intensity; ESI (m/z) 809,0 $[M+Na, (31)]^+$, 787,1 $[M+H, (100)]^+$; Anal. calcd. for $C_{45}H_{87}N_9O_2$: C 68.74, H 11.15, N 16.03; found: C 68.72, H 11.15, N 16.02.





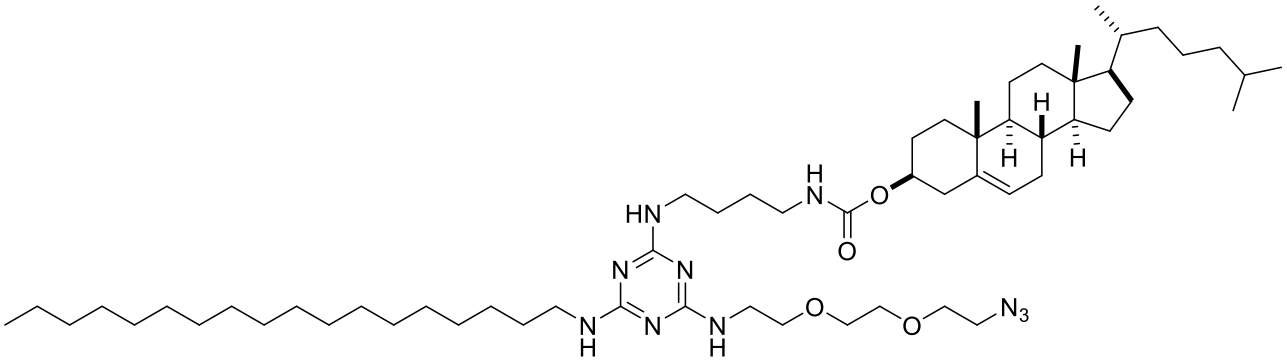
-L.G.S. - Laboratorio Grandi Strumenti - Display Report

Analysis Name	av cpv126.d	Acquisition Date	01/11/18 15:03:22	Operator	Walter Panzeri
Sample Name		Method	Copy of _01tmix_posneg lm.MS	Instrument	esquire3000plus
Comment	1 mg/ml dil 1:100 MeOH Richiedente: Pennetta				

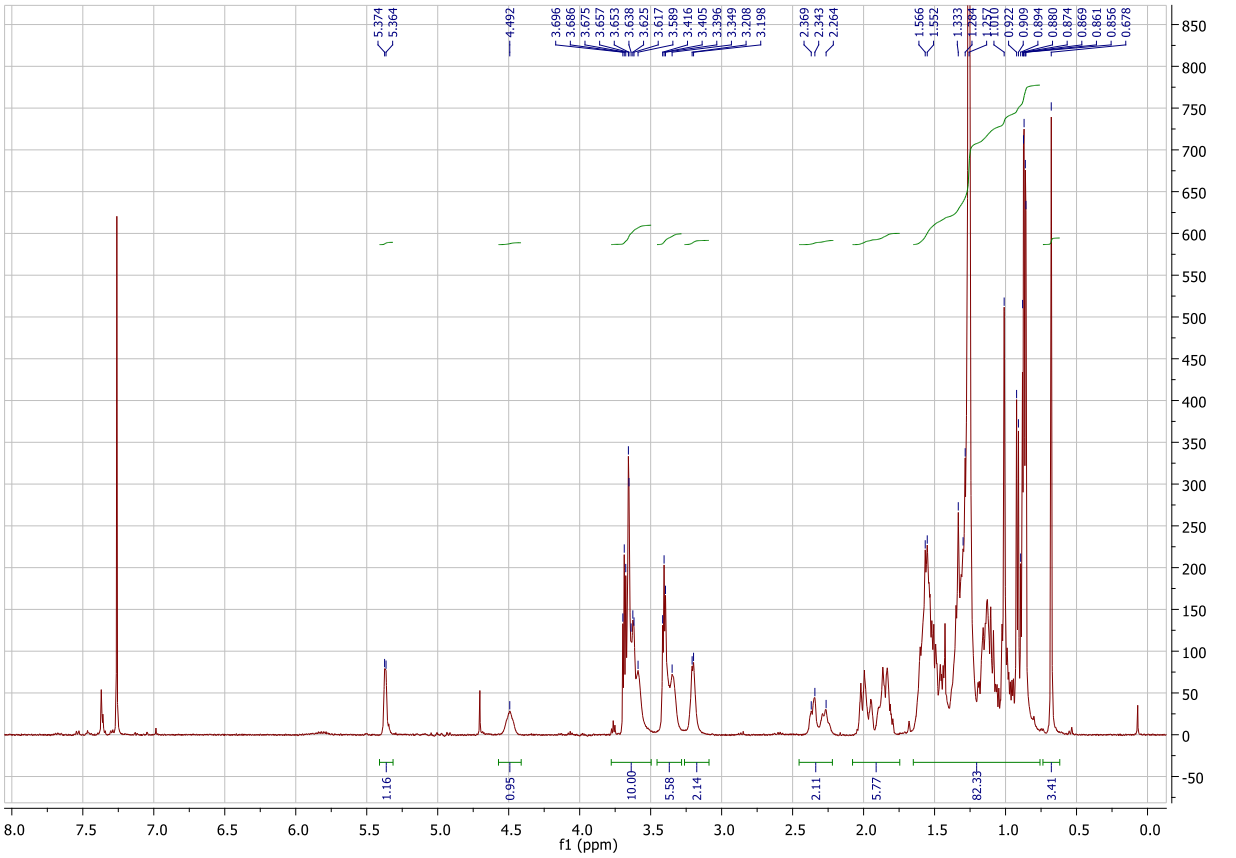


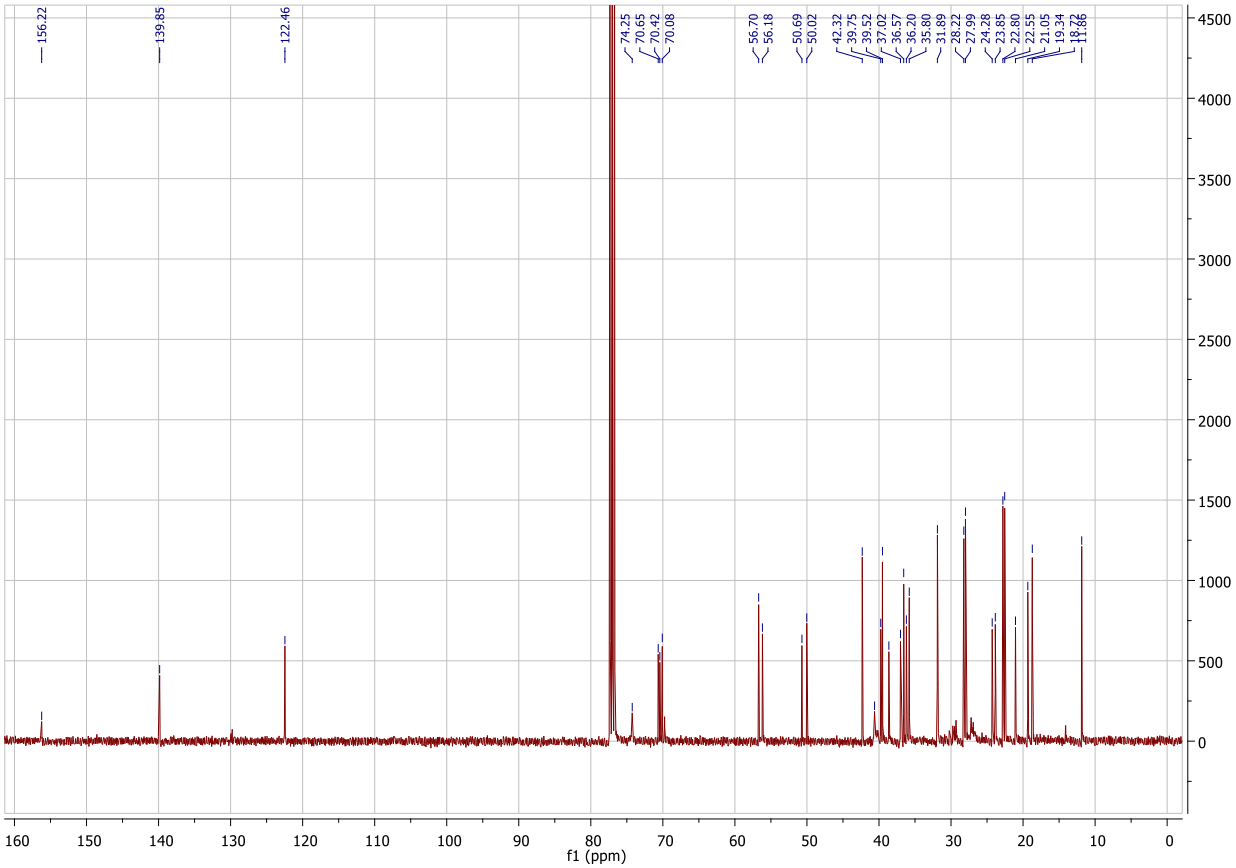
Bruker Daltonics DataAnalysis 3.1 printed: 01/11/18 15:04:16 Page 1 of 1

Figure S9. ^{1}H NMR, ^{13}C NMR, and mass spectra of (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl (4-((4-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-6-(octadecylamino)-1,3,5-triazin-2-yl)amino)butyl)carbamate, 6ac



(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl (4-((4-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-6-(octadecylamino)-1,3,5-triazin-2-yl)amino)butyl)carbamate, 6ac: white solid, 50% yield. R_f = 0.41 (DCM/MeOH = 90:10); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (d, J = 4.0 Hz, 1H), 4.49 (br s, 1H), 3.72-3.55 (m, 10H), 3.44-3.29 (m, 6H), 3.23-3.21 (m, 2H), 2.40-2.21 (m, 2H), 2.05-1.92 (m, 2H), 1.91-1.78 (m, 3H), 1.65-0.83 (m, 72H), 0.68 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.2, 139.8, 122.5, 74.2, 70.6, 70.4, 70.1, 56.7, 56.2, 50.7, 50.0, 42.3, 39.7, 39.5, 37.0, 36.6, 36.2, 35.8, 31.9, 28.2, 28.0, 24.3, 23.8, 22.8, 22.5, 21.0, 19.3, 18.7, 11.9, the aromatic carbons did not appear due to low intensity; ESI (m/z) 1020.0 [M+H, (100)]$^{+}$; Anal. calcd. for $C_{59}H_{106}N_{10}O_4$: C 69.51, H 10.48, N 13.74; found: C 69.50, H 10.50, N 13.73.


5.374
5.364
4.492
3.696
3.686
3.675
3.657
3.653
3.638
3.625
3.617
3.589
3.416
3.405
3.396
3.349
3.208
3.198
2.369
2.343
2.264
1.566
1.552
1.333
1.284
1.257
1.010
0.922
0.909
0.894
0.880
0.874
0.869
0.861
0.856
0.678
1.16
0.95
10.00
5.58
2.14
2.11
5.77
82.33
3.41
f1 (ppm)


156.22
139.85
122.46
74.25
70.65
70.42
70.08
56.70
56.18
50.69
50.02
42.32
39.75
39.52
37.02
36.57
36.20
35.80
31.89
28.22
27.99
24.28
23.85
22.80
22.55
21.05
19.34
11.86
f1 (ppm)

-L.G.S. - Laboratorio Grandi Strumenti - Display Report

Analysis Name	av cp141.d	Acquisition Date	09/28/18 11:48:51	Operator	Walter Panzeri
Sample Name		Method	Copy of _giov 1620 24713.MS	Instrument	esquire3000plus
Comment	1 mg/ml dil 1:100 MeOh Richiedente Pennetta				

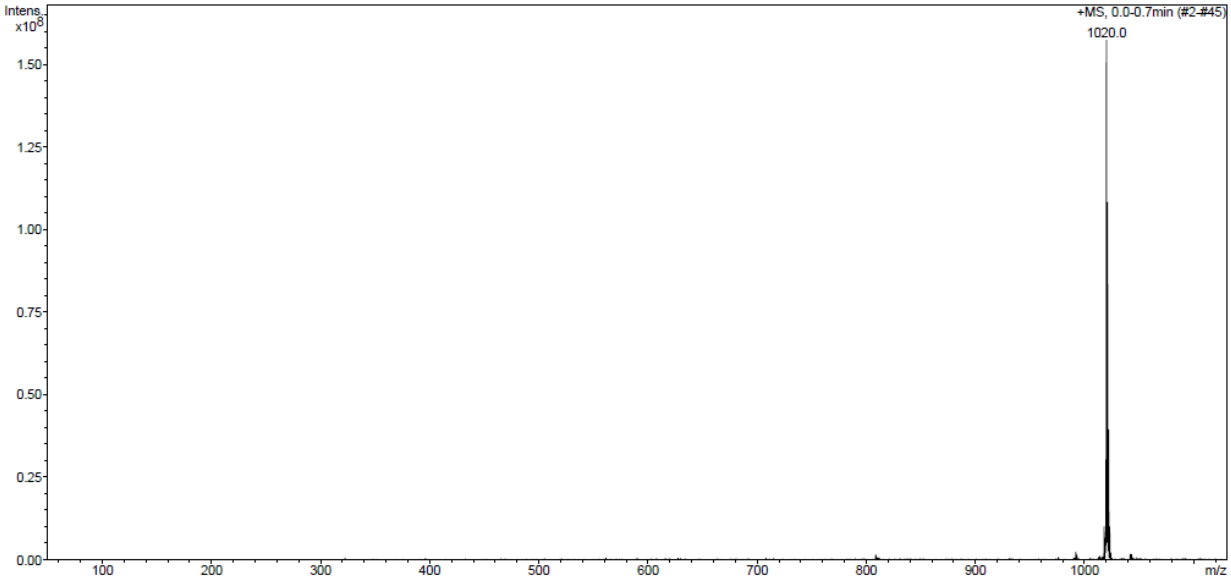
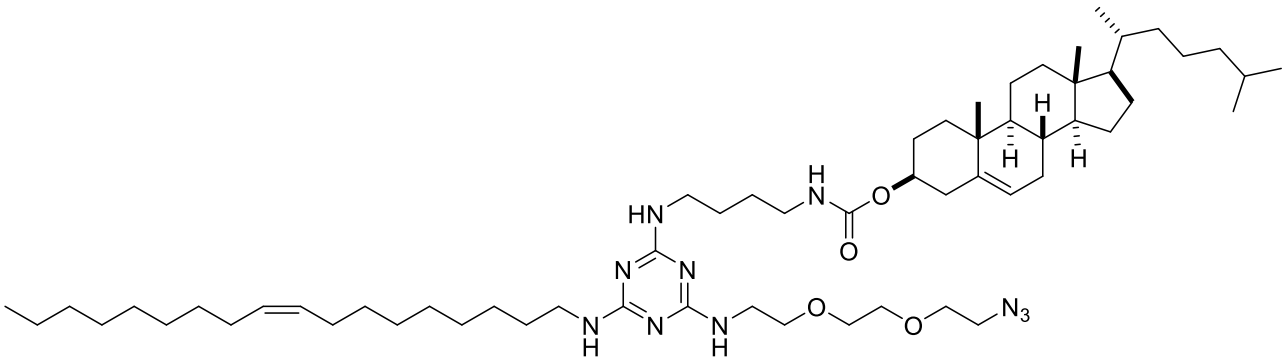
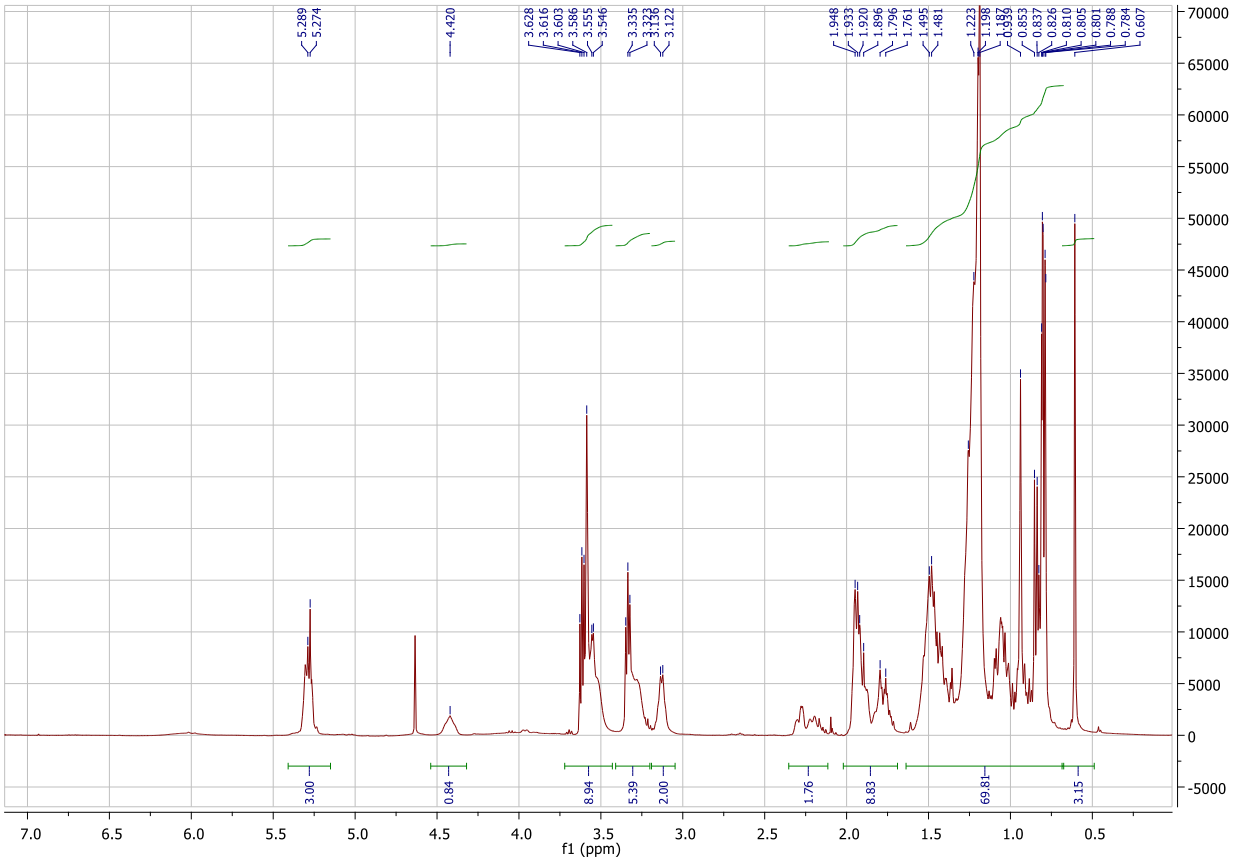
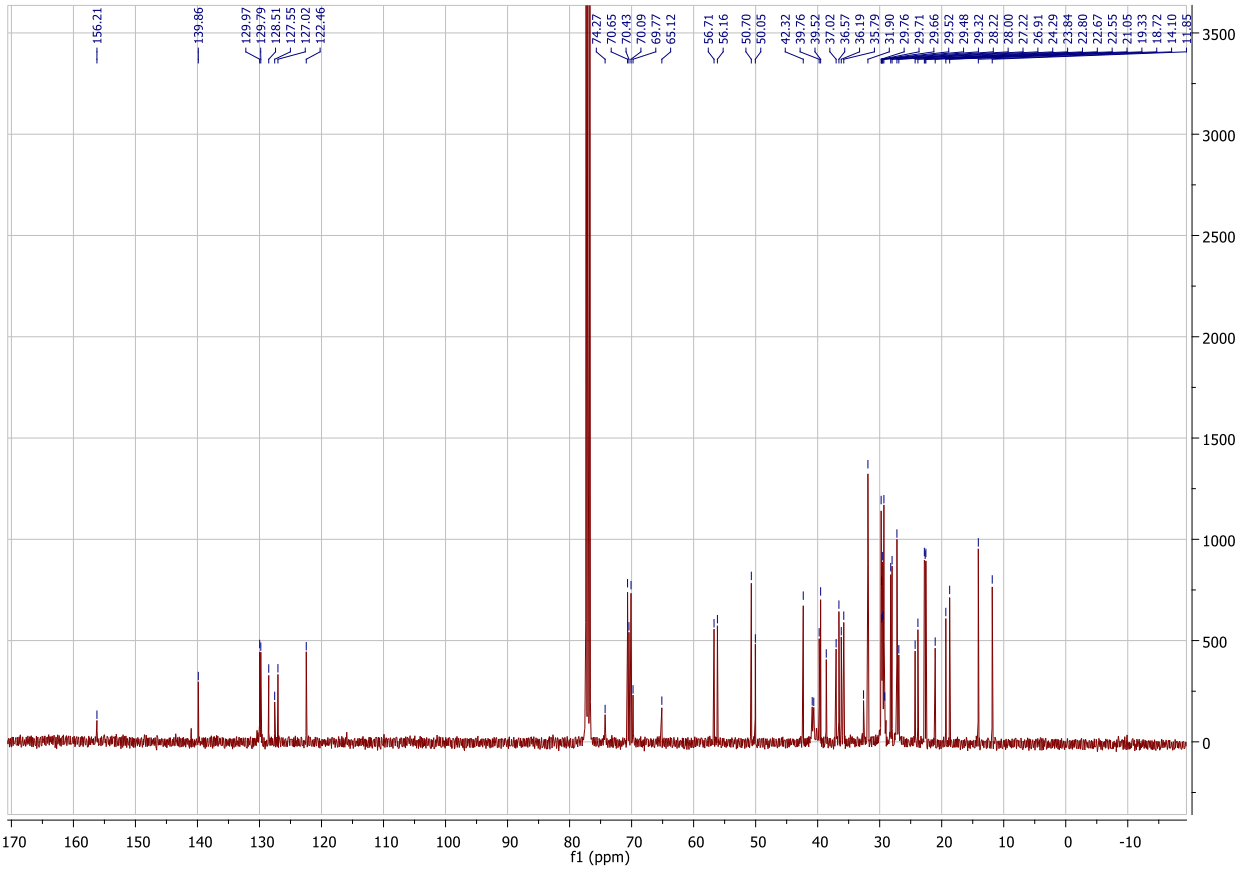


Figure S10. ^{1}H NMR, ^{13}C NMR, and mass spectra of (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl (4-((4-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-6-(((Z)-octadec-9-en-1-yl)amino)-1,3,5-triazin-2-yl)amino)butyl)carbamate, 6bc



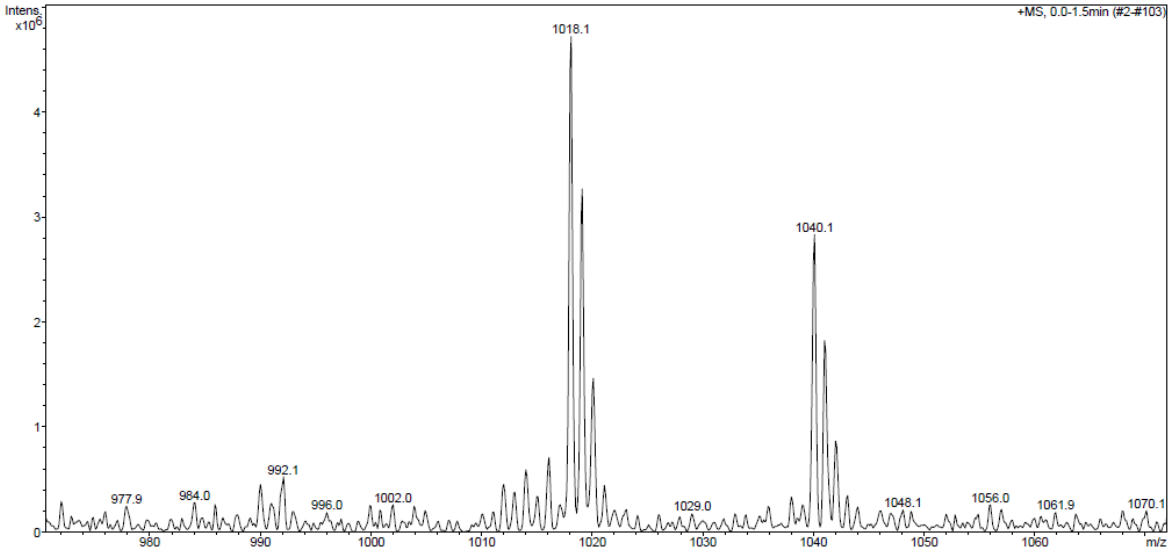
(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl (4-((4-((2-(2-(2-azidoethoxy)ethoxy)ethyl)amino)-6-(((Z)-octadec-9-en-1-yl)amino)-1,3,5-triazin-2-yl)amino)butyl)carbamate, 6bc: white solid, 55% yield. R_f = 0.46 (DCM/MeOH = 90:10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30-5.26 (m, 3H), 4.42 (br s, 1H), 3.65-3.54 (m, 10H), 3.36-3.31 (m, 6H), 3.16-3.10 (m, 2H), 2.28-2.25 (m, 2H), 1.98-1.77 (m, 6H), 1.35-0.79 (m, 68H), 0.61 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 156.3, 140.00, 130.1, 129.9, 128.6, 127.7, 127.1, 122.6, 74.4, 70.8, 70.5, 70.2, 69.9, 65.2, 56.8, 56.3, 50.8, 50.2, 42.4, 41.0, 40.7, 39.9, 39.6, 38.7, 37.1, 36.7, 36.3, 35.9, 32.7, 32.0, 29.9, 29.8, 29.8, 29.6, 29.6, 29.4, 29.3, 29.1, 28.3, 28.1, 27.3, 27.0, 24.4, 23.9, 22.9, 22.8, 22.7, 21.2, 19.5, 18.8, 14.2, 12.0. ESI (m/z) 1018.1 $[M+H, (100)]^{+}$; Anal. calcd. for $C_{59}H_{104}N_{10}O_4$: C 69.64, H 10.30, N 13.77; found: C 69.65, H 10.32, N 13.76.


5.289
5.274
4.420
3.628
3.616
3.603
3.586
3.555
3.546
3.335
3.323
3.138
3.122
1.948
1.933
1.920
1.896
1.796
1.761
1.495
1.481
1.223
1.198
1.187
0.939
0.853
0.837
0.826
0.810
0.805
0.801
0.788
0.784
0.607
3.00
0.84
8.94
5.39
2.00
1.76
8.83
69.81
3.15
f1 (ppm)


156.21
139.86
129.97
129.79
128.51
127.55
127.02
122.46
74.27
70.65
70.43
70.09
69.77
65.12
56.71
56.16
50.70
50.05
42.32
39.76
39.52
37.02
36.57
36.19
35.79
31.90
29.76
29.71
29.66
29.52
29.48
29.32
28.22
28.00
27.22
26.91
24.29
23.84
22.80
22.67
22.55
21.05
19.33
18.72
14.10
11.85
f1 (ppm)

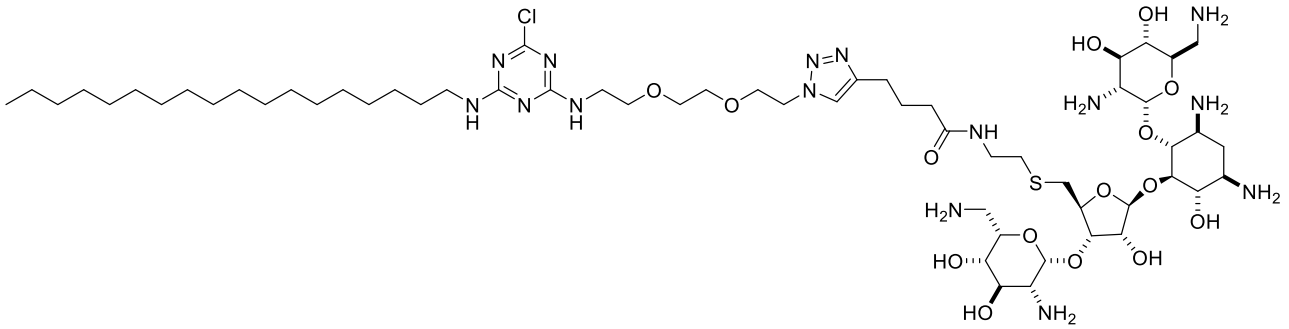
-L.G.S. - Laboratorio Grandi Strumenti - Display Report

Analysis Name	av cp141 28.11.18.d	Acquisition Date	11/28/18 15:12:45	Operator	Walter Panzeri
Sample Name		Method	Copy(2) of _giov 1620 24713.MS	Instrument	esquire3000plus
Comment	1 mg/ml dil 1:100 MeOH Richiedente: Pennetta				

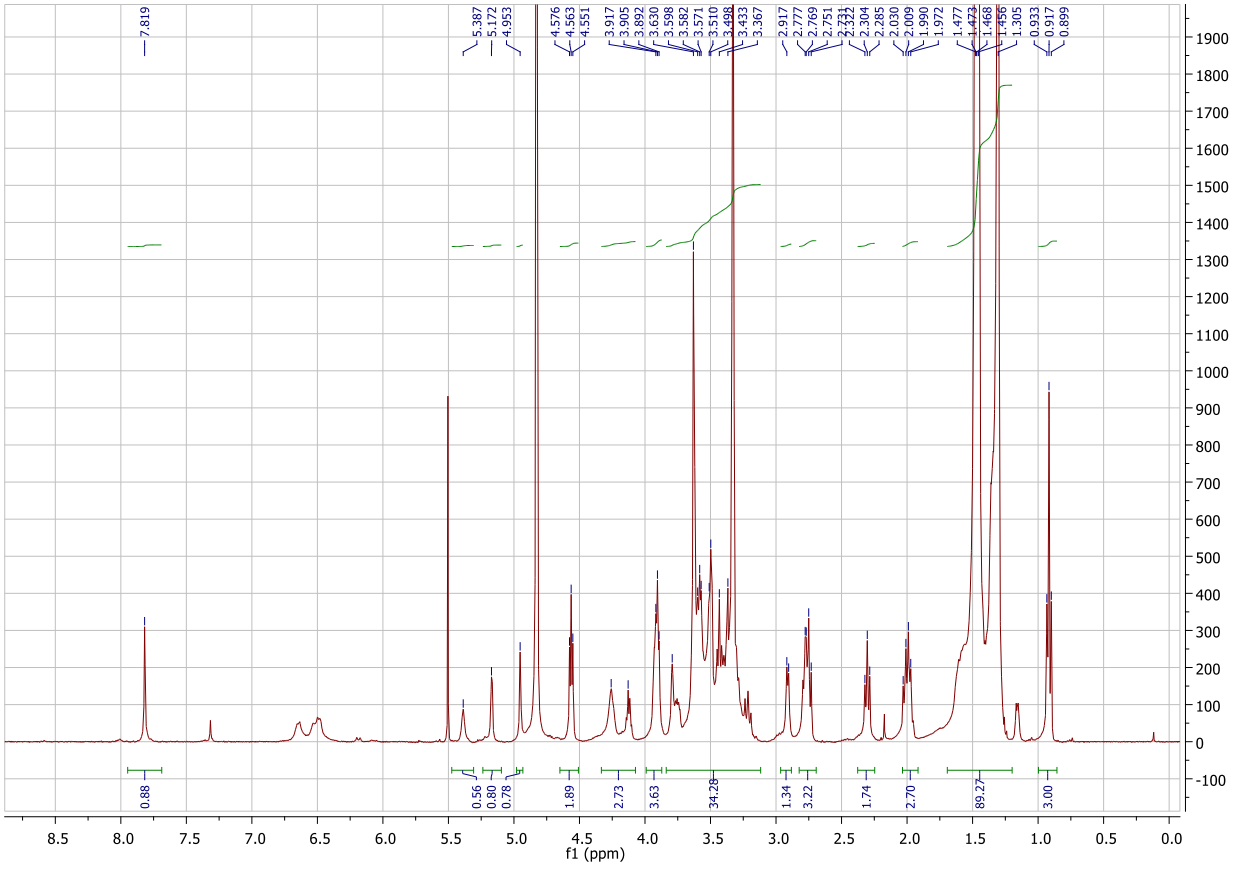


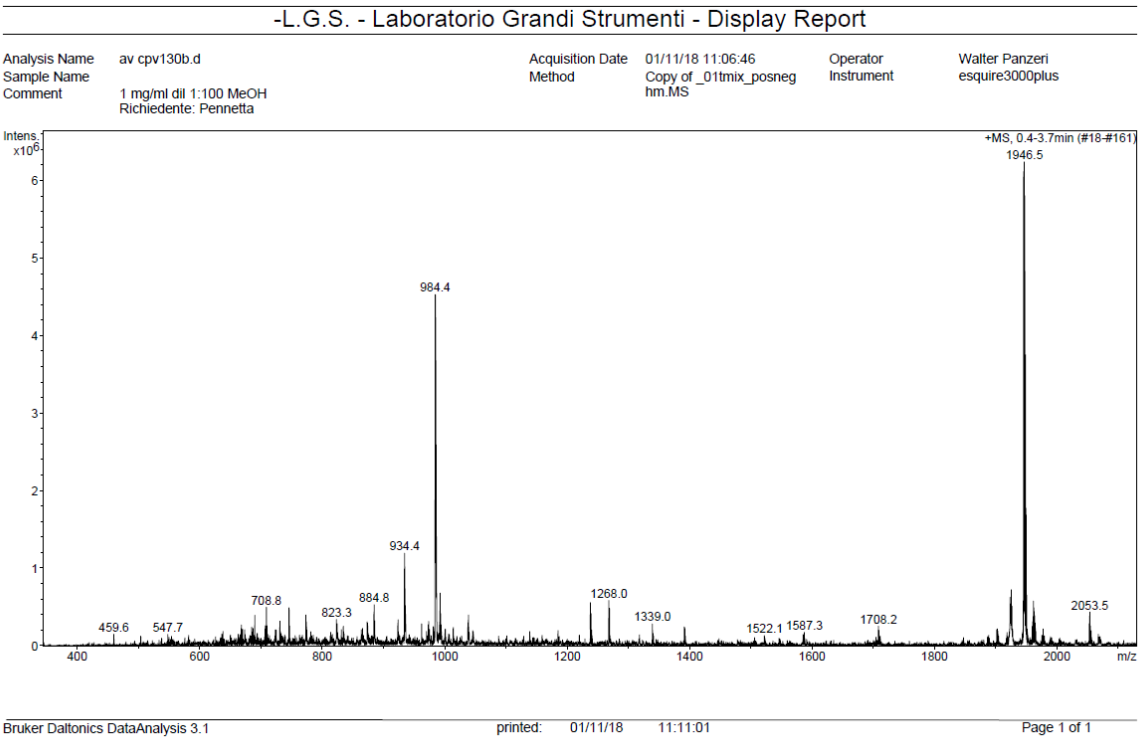
Bruker Daltonics DataAnalysis 3.1 printed: 11/28/18 15:16:56 Page 1 of 1

Figure S11. ^{1}H NMR and mass spectra of compound Boc-8a and ^{1}H NMR and ^{13}C NMR derivative 1_ST (compound 8a)

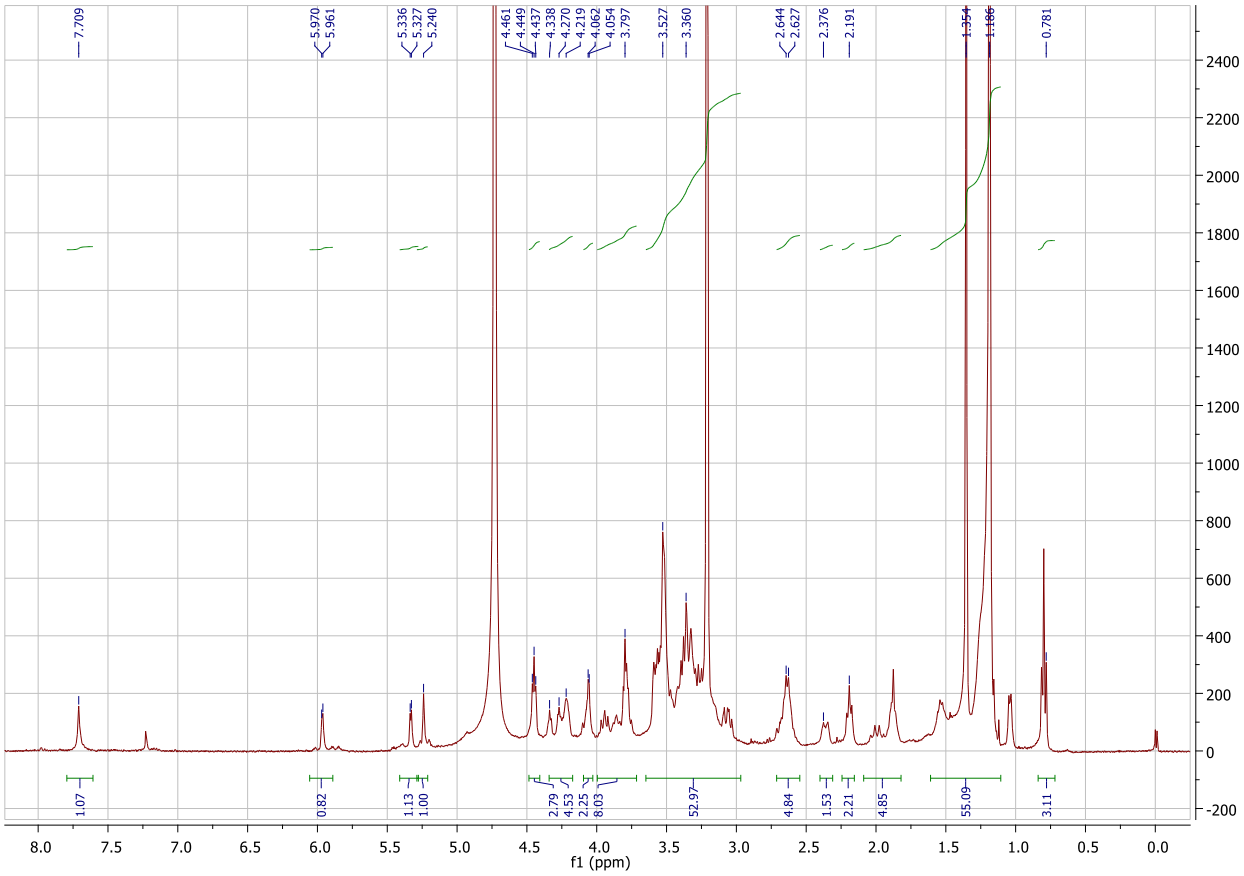


Compound Boc-8a: R_f = 0.32 (DCM/MeOH = 90:10); ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (s, 1H), 5.37 (s, 1H), 5.15 (s, 1H), 4.93 (s, 1H), 4.55 (t, *J* = 5.2 Hz, 2H), 4.26-4.23 (m, 2H), 4.12-4.10 (m, 1H), 3.91-3.88 (m, 4H), 3.78-3.75 (m, 2H), 3.65-3.35 (m, 25H), 2.90 (d, *J* = 5,5 Hz, 2H), 2.78-2.71 (m, 4H), 2.29 (t, *J* = 7.3 Hz, 2H), 1.99-1.96 (m, 3H), 1.64-1.44 (m, 57H), 1.34-1.24 (m, 30H), 0.90 (t, *J* = 6,6 Hz, 3H). ESI (m/z) 1946,5 $[M+Na, (100)]^{+}$





Compound 8a white solid, 50% yield in two steps.^{1}H NMR (400 MHz, CD_3OD) δ 7.83 (s, 1H), 6.06 (d, *J*=3.7 Hz, 1H), 5.43 (d, *J* =3,7 Hz, 1H), 5.34 (s, 1H), 4.55 (t, *J* =4,7 Hz, 2H), 4.44 (t, *J* = 4,6 Hz, 1H), 4.38-4.31 (m, 3H), 4.20-4.03 (m, 4H), 3.92-3.89 (m, 4H), 3.69-3.55 (m, 12H), 3.52-3.35 (m, 12H), 2.75-2.72 (m, 5H), 2.48-2.45 (m, 1H), 2.29 (t, *J* = 6,9 Hz, 2H), 1.99-1.88 (m, 3H), 1.65-1.63 (m, 1H), 1.34-1.27 (m, 30H), 0.89 (t, 7,36 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 174.5, 161.4, 161.1, 156.6, 156.4, 110.6, 95.5, 95.0, 86.1, 74.9, 74.0, 72.7, 71.8, 70.7, 70.0, 69.0, 68.3, 68.0, 67.8, 60.1, 54.1, 51.6, 50.0, 49.8, 48.8, 41.3, 41.1, 40.6, 31.6, 29.3, 29.2, 29.0, 28.9, 28.5, 26.4, 24.3, 22.3, 13.0; Anal. calcd. for $C_{58}H_{108}ClN_{15}O_{15}S$: C 52.65, H 8.23, N 15.88; found: C 52.66, H 8.21, N 15.87.


7.709
5.970
5.961
5.336
5.327
5.240
4.461
4.449
4.437
4.338
4.270
4.219
4.062
4.054
3.797
3.527
3.360
2.644
2.627
2.376
2.191
1.354
1.186
0.781
1.07
0.82
1.13
1.00
2.79
4.53
2.25
8.03
52.97
4.84
1.53
2.21
4.85
55.09
3.11
f1 (ppm)

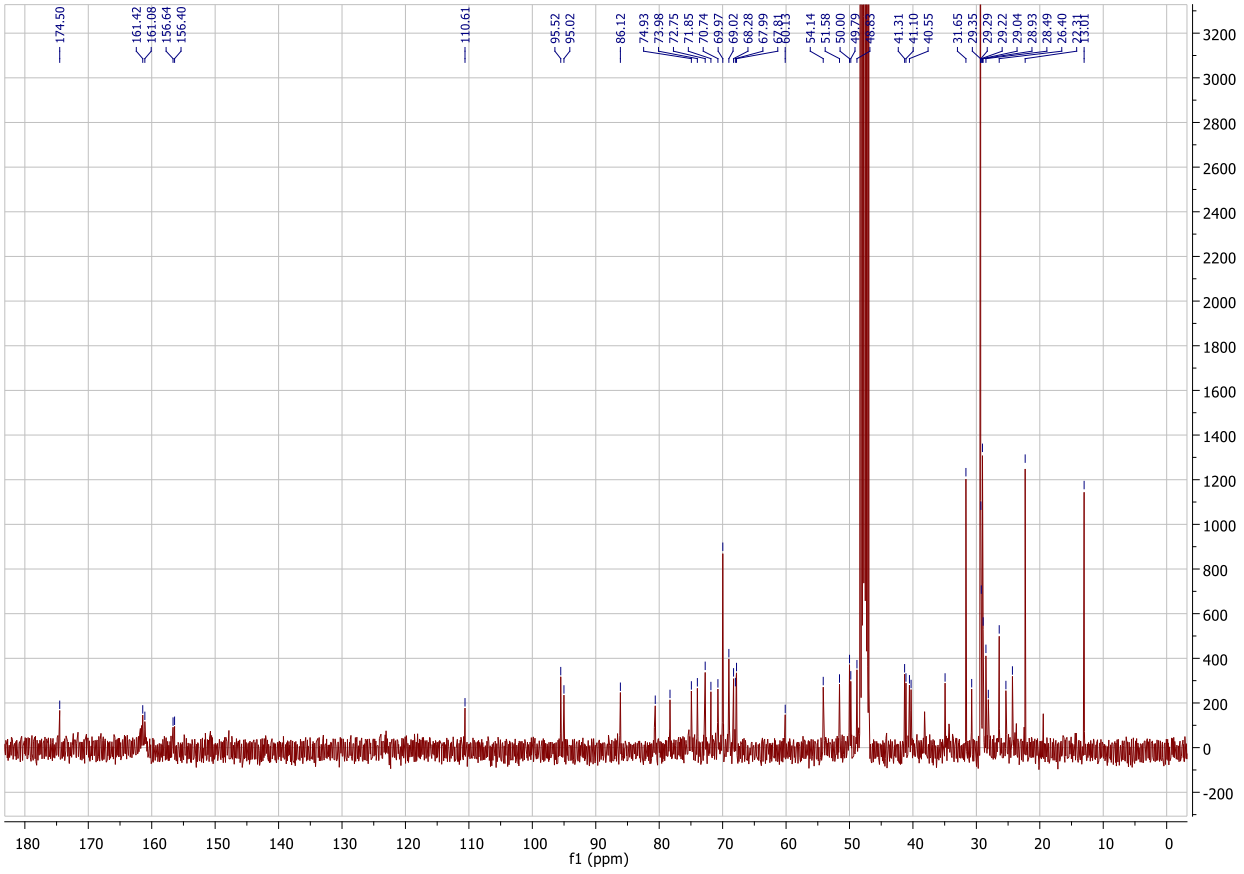
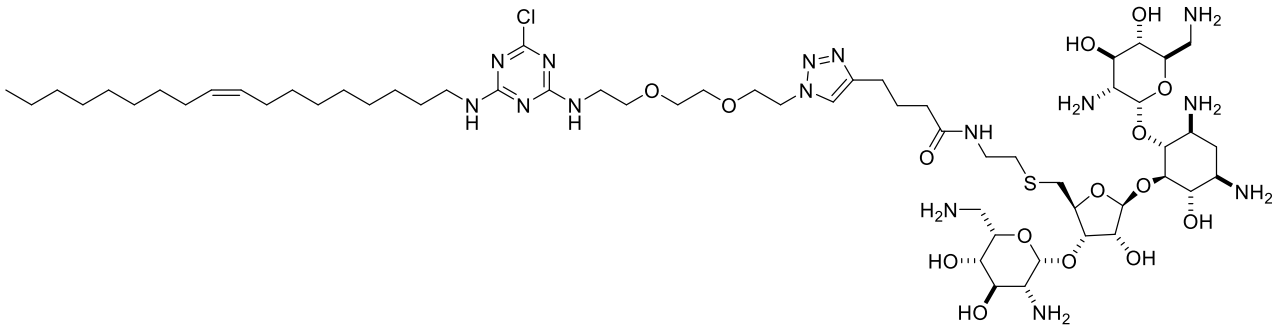
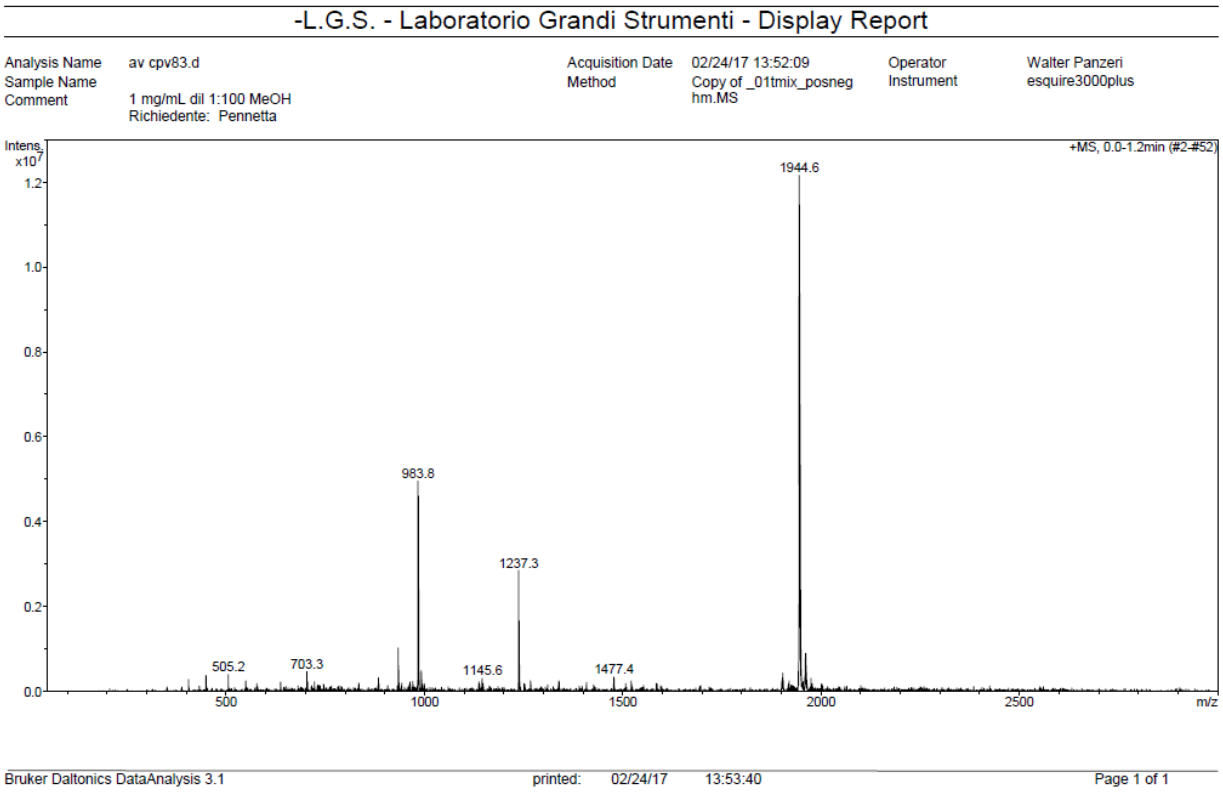

174.50
161.42
161.08
156.64
156.40
110.61
95.52
95.02
86.12
74.93
73.98
72.75
71.85
70.74
69.97
69.02
68.28
67.99
67.81
60.13
54.14
51.58
50.00
49.79
48.83
41.31
41.10
40.55
31.65
29.35
29.29
29.22
29.04
28.93
28.49
26.40
23.11
13.01
f1 (ppm)

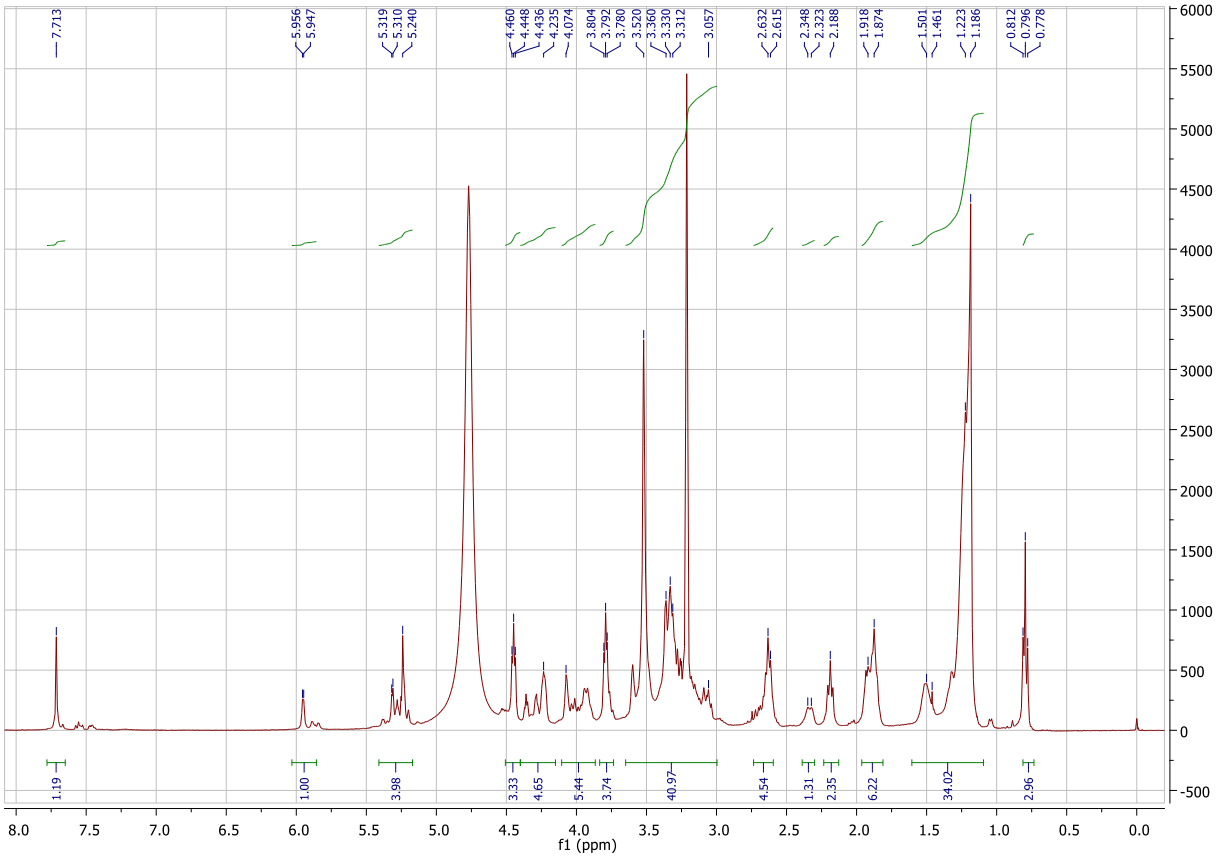
Figure S12. Mass spectra of compound Boc-8b and ^{1}H NMR and ^{13}C NMR derivative 1_OL (compound 8b)



Compound Boc-8b: R_f = 0.35 (DCM/MeOH = 90:10); ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (s, 1H), 5.36-5.33 (m, 3H), 5.15 (d, *J* = 2,3 Hz,1H), 4.93 (s, 1H), 4.54 (t, *J* = 5.1 Hz, 2H), 4.25-4.22 (m, 2H), 4.11-4.08 (m, 1H), 3.95-3.90 (m, 4H), 3.79-3.76 (m, 2H), 3.65-3.35 (m, 25H), 2.90 (d, *J* = 5,5 Hz, 2H), 2.78-2.71 (m, 4H), 2.28 (t, *J* = 7,4 Hz, 2H), 2.04-1.95 (m, 7H), 1.59-1.52 (m, 3H), 1.50-1.40 (m, 54H), 1.32-1.24 (m, 22H), 0.90 (t, *J* = 6,6 Hz, 3H). ESI (m/z) 1944,6 $[M+Na, (100)]^{+}$



Compound 8b: yellowish solid, 35% yield in 2 steps. ^{1}H NMR (400 MHz, CD_3OD) δ 7.71 (s,1H), 5.94 (d, *J* = 6.2 Hz, 1H), 5.32-5.24 (m, 4H), 4.45 (t, *J* = 4.8 Hz, 2H), 4.33 (m, 2H), 4.16 (m, 1H), 3.89 (m, 4H), 3.70 (b, 2H), 3.62-3.35 (m, 25H), 2.75-2.71 (m, 6H) 2.28 (t, *J* = 7.2 Hz, 2H), 2.03-1.95 (m, 7H), 1.61-1.56 (m, 3H), 1.29-1.10 (m, 22H), 0.89 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 148.2, 130.9, 130.8, 124.2, 112.1, 96.8, 96.4, 87.6, 79.7, 76.9, 75.3, 74.2, 73.2, 72.1, 71.3, 71.2, 70.4, 70.0, 69.6, 69.2, 55.6, 52.9, 51.3, 50.3, 49.6, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 42.1, 41.9, 41.7, 38.4, 36.3, 33.6, 33.0, 32.2, 30.8, 30.7, 30.6, 30.4, 30.3, 30.2, 30.1, 28.1, 27.8, 26.7, 25.7, 23.7, 14.4; Anal. calcd. for $C_{58}H_{106}ClN_{15}O_{15}S$: C 52.73, H 8.09, N 15.90; found: C 52.75, H 8.10, N 15.91.



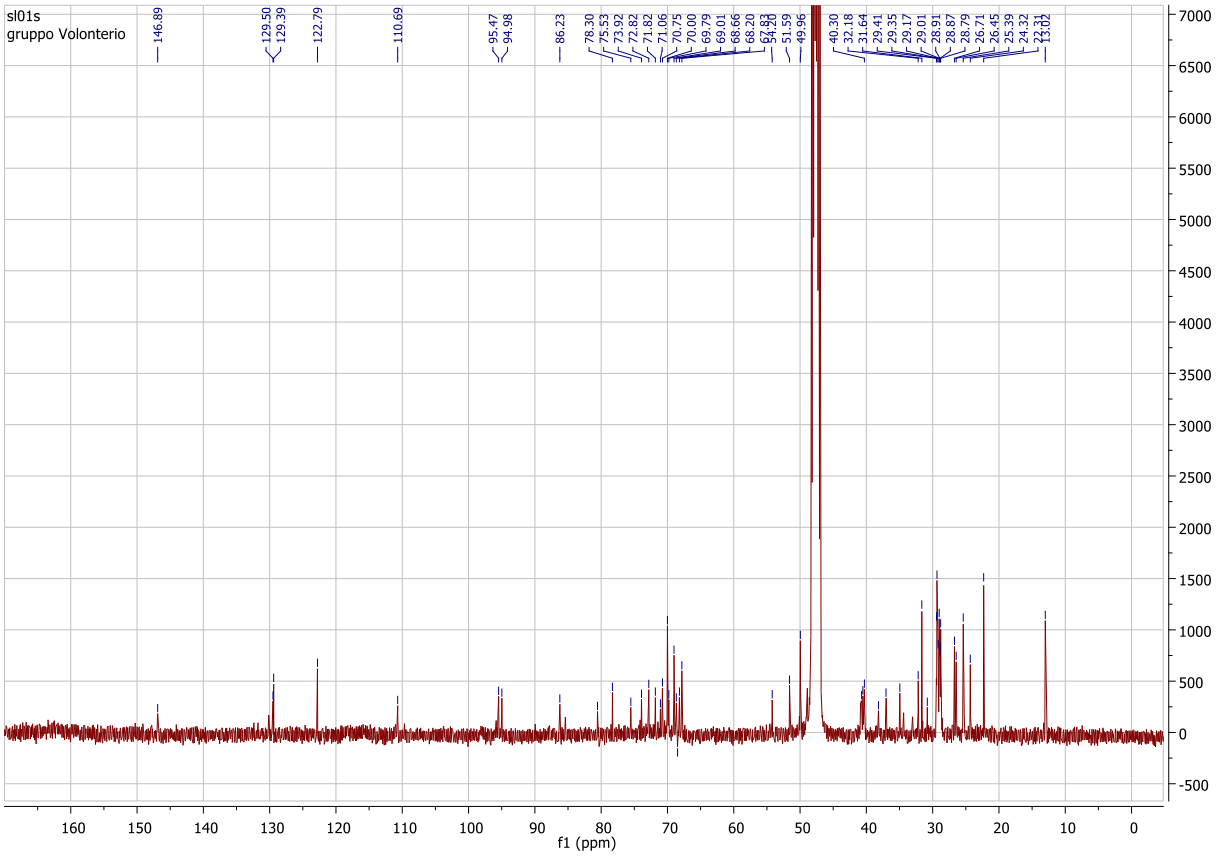
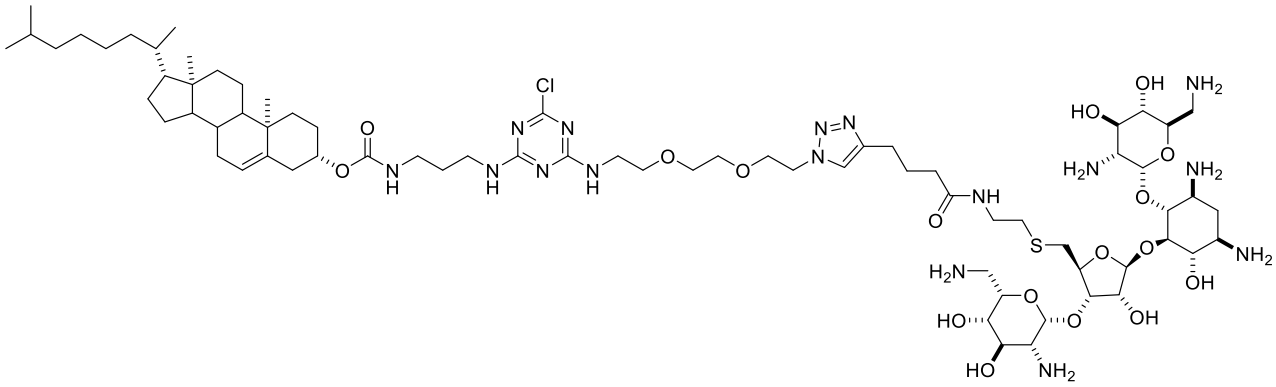
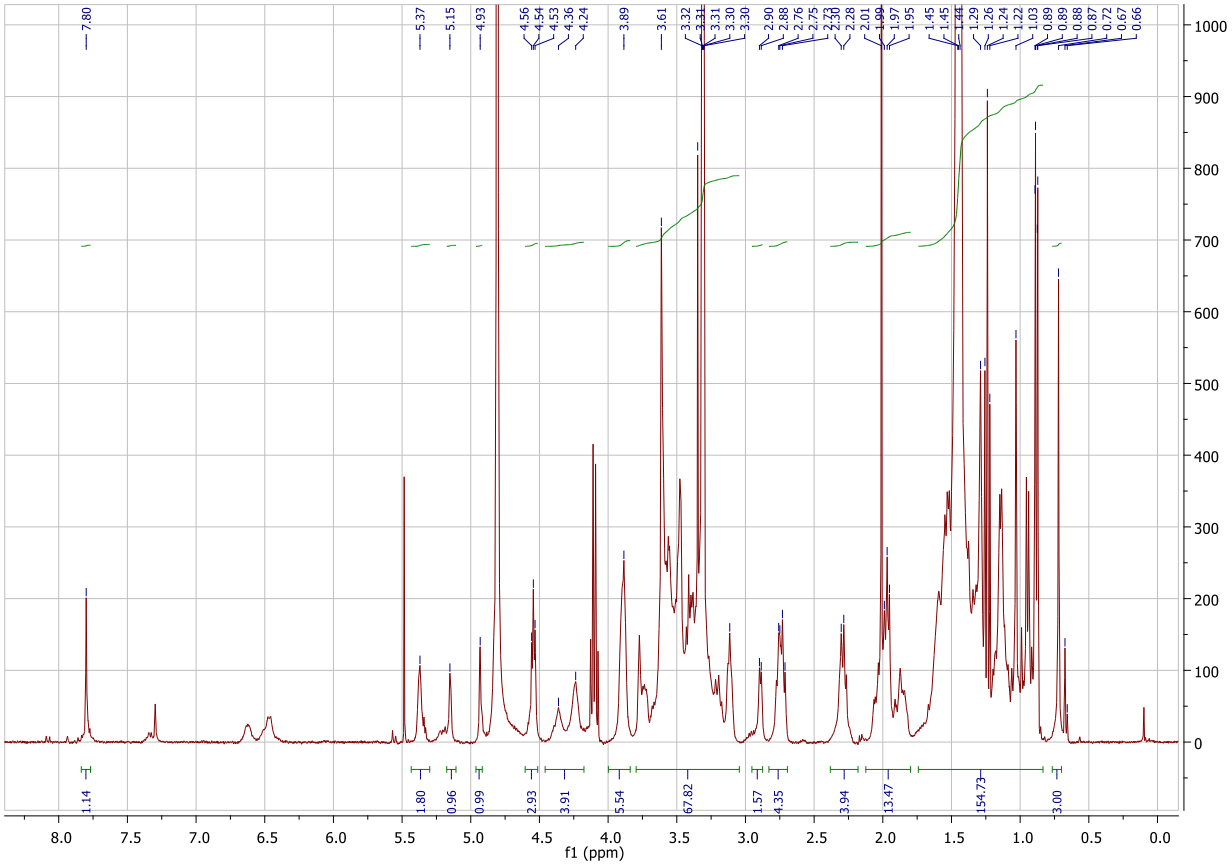
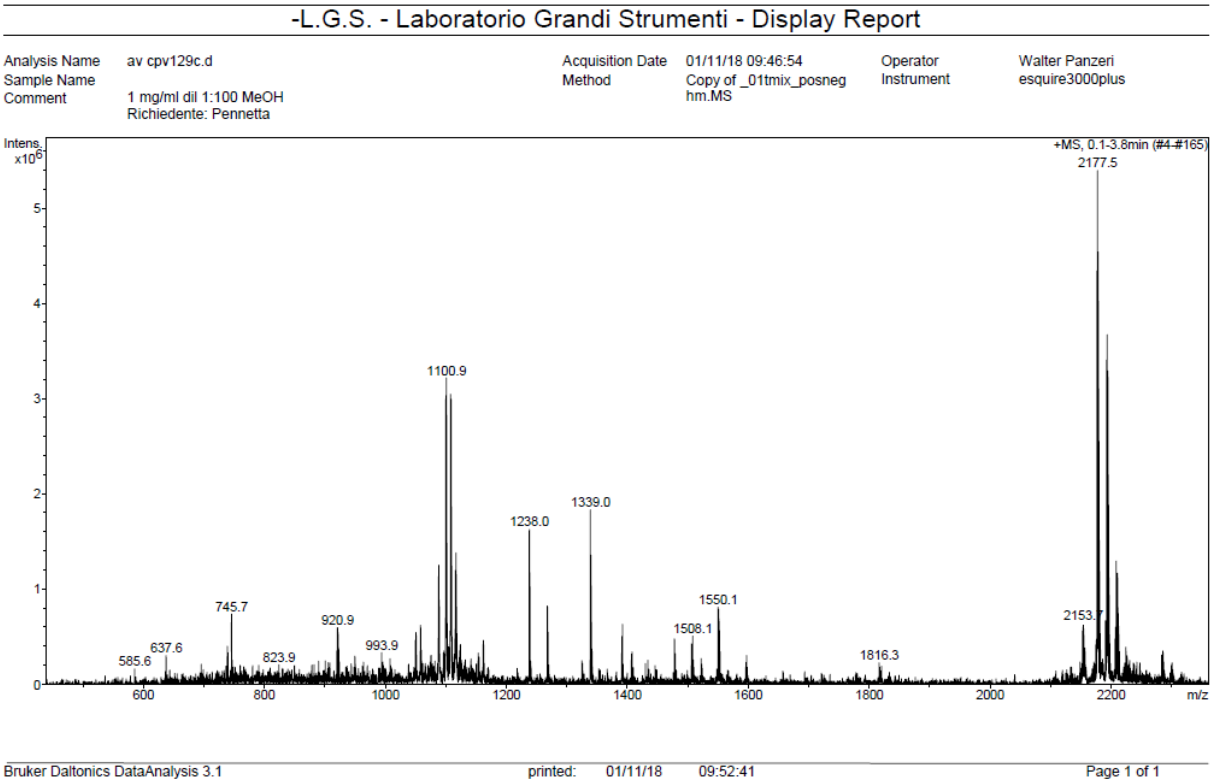

sl01s
gruppo Volonterio
146.89
129.50
129.39
122.79
110.69
95.47
94.98
86.23
78.30
75.53
73.92
72.82
71.82
71.06
70.75
70.00
69.79
69.01
68.66
68.20
67.83
54.20
51.59
49.96
40.30
32.18
31.64
29.41
29.35
29.17
29.01
28.91
28.87
28.79
26.71
26.45
25.39
24.32
23.31
13.02
f1 (ppm)

Figure S13. ^{1}H NMR and mass spectra of compound Boc-8c and ^{1}H NMR and ^{13}C NMR spectra of derivative 1_Chol (compound 8c)

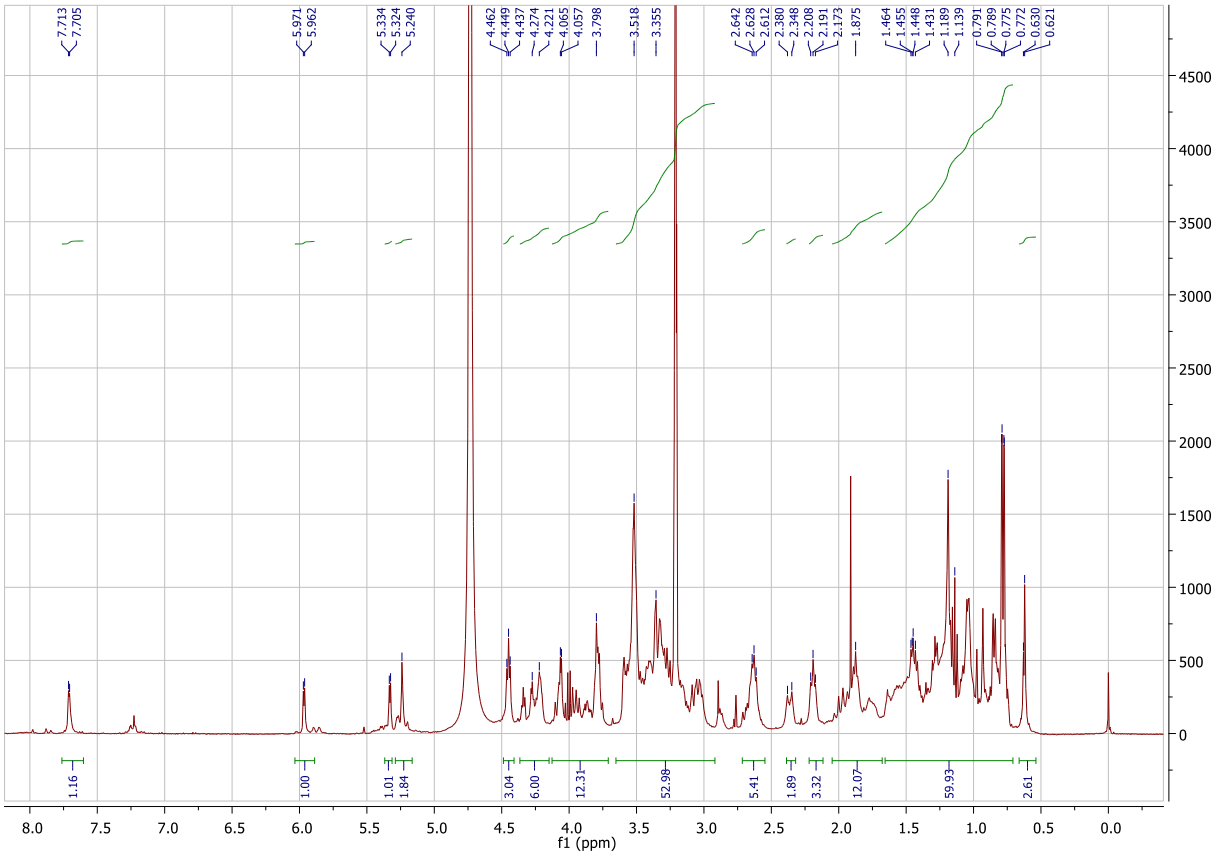


Compound Boc-8c: R_f = 0.27 (DCM/MeOH = 90:10); ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (s, 1H), 5.38-5.36 (m, 2H), 5.15 (s, 1H), 4.93 (s, 1H), 4.55 (t, *J* = 5.1 Hz, 3H), 3.91-3.88 (m, 5H), 3.79-3.76 (m, 3H), 3.61-3.37 (m, 32H), 3.14-3.11 (m, 2H), 2.90 (d, *J* = 5.0 Hz, 1H), 2.77-2.74 (m, 4H), 2.31-2.30 (m, 3H), 1.99-1.95 (m, 5H), 1.87-1.85 (m, 2H), 1.60-0.87 (m, 89H), 0.72 (s, 3H). ESI (m/z) 2177,5 $[M+Na, (100)]^{+}$





Compound 8c: yellowish solid, 60% yield in two steps. ^{1}H NMR (400 MHz, CD_3OD) δ 7.85 (d, *J* = 3.2 Hz, 1H), 6.10 (d, *J* = 3.6 Hz, 1H), 5.47 (d, *J* = 4.1 Hz, 1H), 5.38 (m, 2H), 4.59 (t, *J* = 5.2 Hz, 2H), 4.46 (t, *J* = 4.4 Hz, 1H), 3.93 (m, 2H), 3.69-3.41 (m, 29H), 3.17 (m, 2H), 3.03 (m, 1H), 2.81-2.73 (m, 4H), 2.51 (m, 2H), 2.33 (m, 2H), 2.14 (m, 2H), 1.86 (m, 2H), 1.65-0.86 (m, 46 H), 0.77 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 175.9, 163.3, 163.2, 162.9, 124.1, 112.0, 96.9, 96.4, 87.5, 82.1, 79.7, 76.3, 75.4, 74.1, 73.2, 72.2, 71.3, 70.4, 69.4, 69.3, 61.5, 58.3, 57.6, 55.5, 52.9, 51.4, 51.2, 50.2, 43.5, 43.4, 42.5, 41.9, 41.7, 40.7, 37.8, 37.4, 37.1, 36.3, 32.9, 32.1, 30.7, 29.5, 29.3, 29.1, 26.8, 25.7, 24.9, 23.2, 22.9, 22.1, 20.8, 19.8, 19.2, 14.4, 12.3. Anal. calcd. for $C_{72}H_{125}ClN_{16}O_{17}S$: C 55.64, H 8.11, N 14.42; found: C 55.65, H 8.13, N 14.42.


7.713
7.705
5.971
5.962
5.334
5.324
5.240
4.462
4.449
4.437
4.274
4.221
4.065
4.057
3.798
3.518
3.355
2.642
2.628
2.612
2.380
2.348
2.208
2.191
2.173
1.875
1.464
1.455
1.448
1.431
1.189
1.139
0.791
0.789
0.775
0.772
0.630
0.621
1.16
1.00
1.01
1.84
3.04
6.00
12.31
52.98
5.41
1.89
3.32
12.07
59.93
2.61
f1 (ppm)

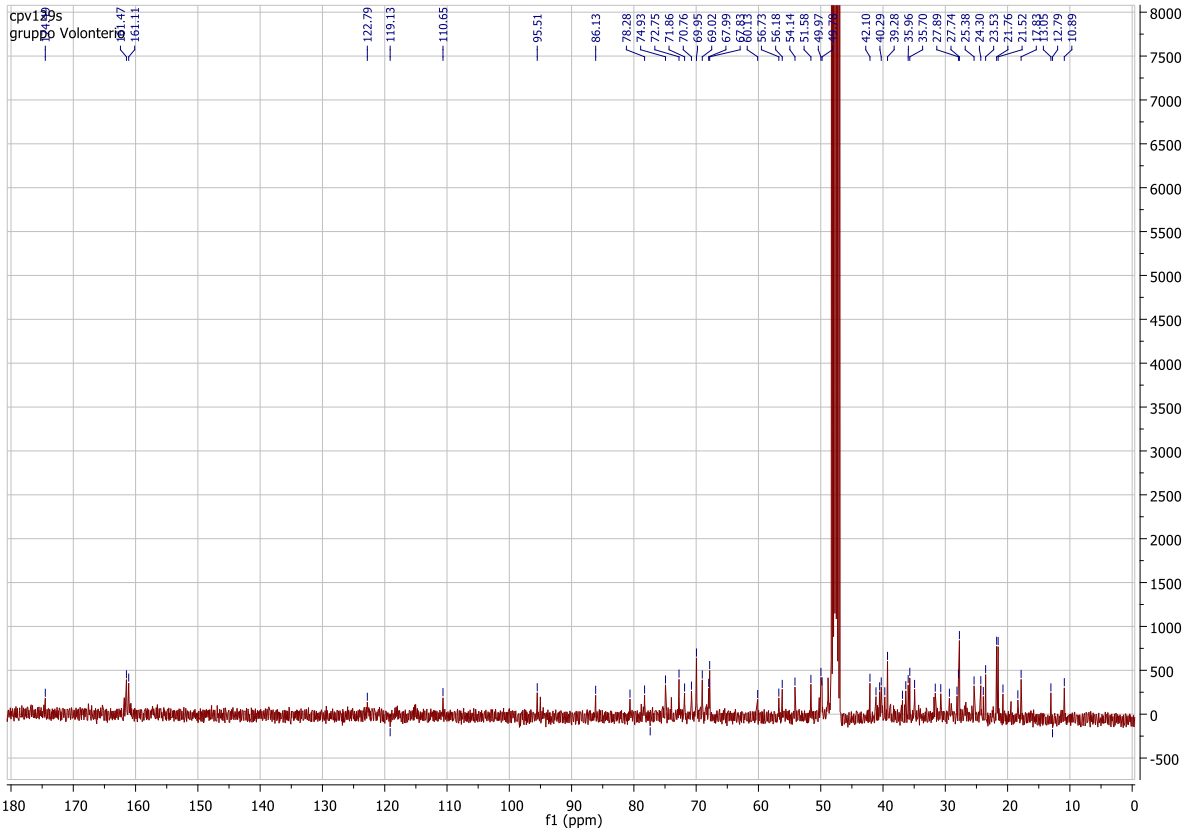
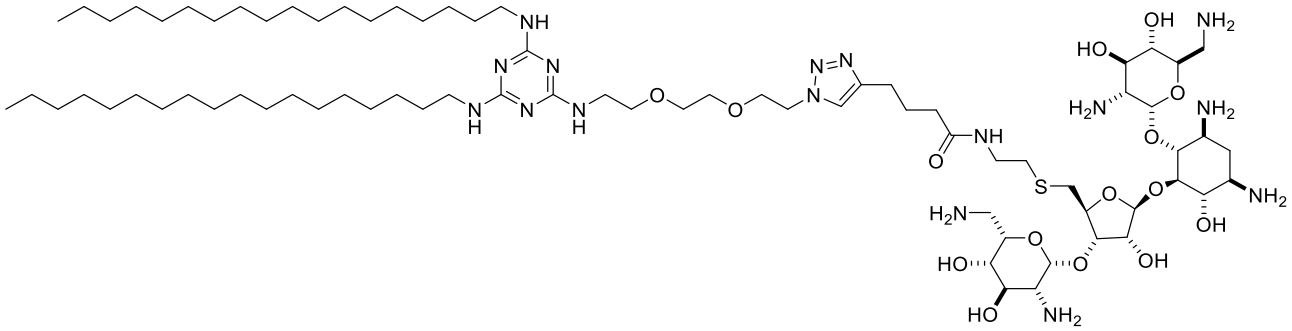
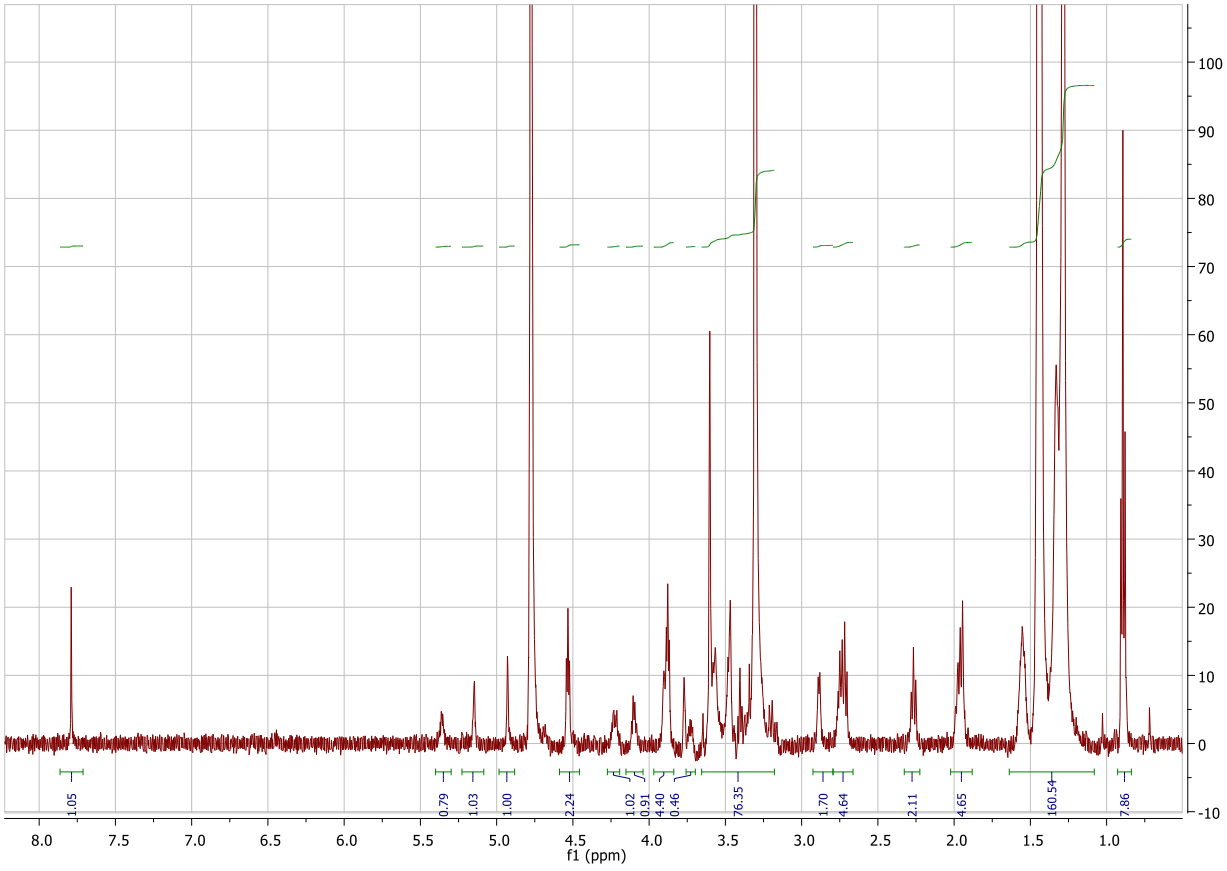
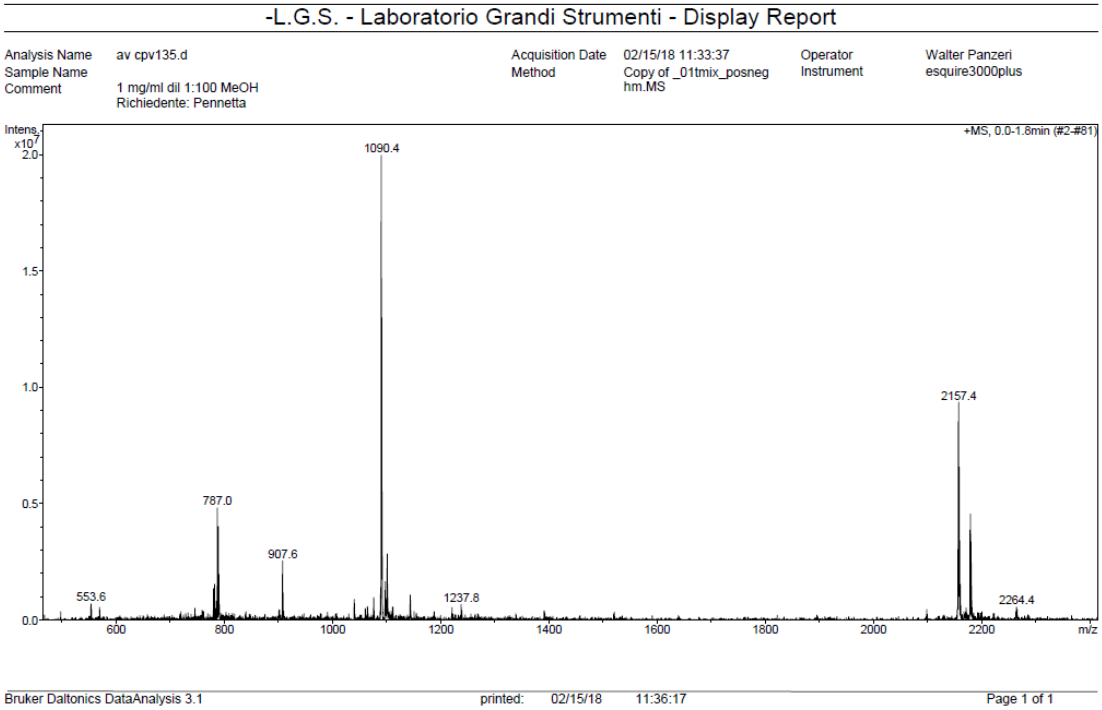

cpv129s
gruppo Volontario
161.47
161.11
122.79
119.13
110.65
95.51
86.13
78.28
74.93
72.75
71.86
70.76
69.95
69.02
67.99
67.83
60.13
56.73
56.18
54.14
51.58
49.97
42.10
40.29
39.28
35.96
35.70
27.89
27.74
25.38
24.30
23.53
21.76
21.52
13.85
13.82
12.79
10.89
f1 (ppm)

Figure S14. ^{1}H NMR and mass spectra of compound Boc-9aa and ^{1}H NMR and ^{13}C NMR spectra of derivative 2_ST (compound 9aa)

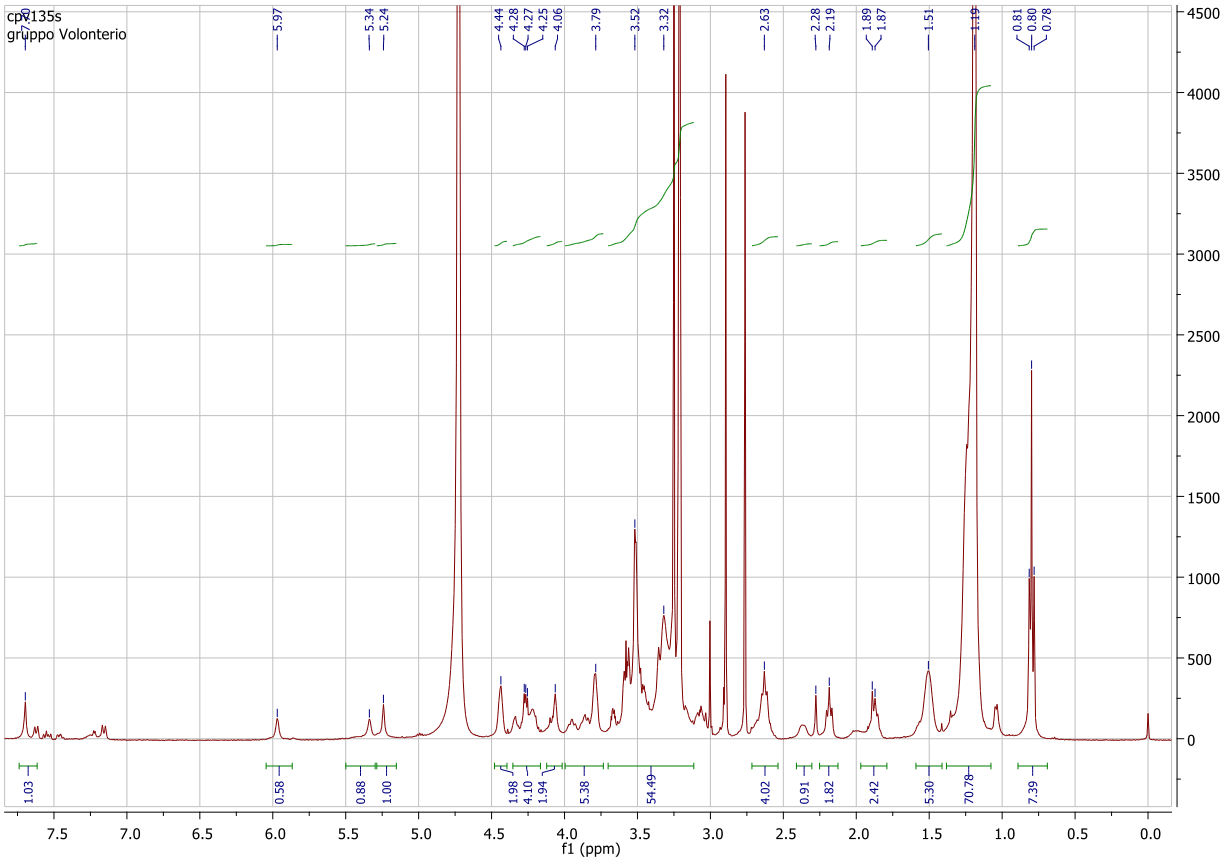


Compound Boc-9aa: R_f = 0.29 (DCM/MeOH = 90:10); ^{1}H NMR (500 MHz, CD_3OD) δ 7.79 (s, 1H), 5.36 (s, 1H), 5.15 (s, 1H), 4.81 (s, 1H), 4.54 (t, 2H), 4.23 (m, 2H), 4.10 (m, 1H), 3.92-3.87 (m, 4H), 3.78-3.72 (m, 2H), 3.68-3.37 (m, 27H), 2.89 (d, 2H), 2.79-2.69 (m, 4H), 2.27 (t, 2H), 1.97 (m, 3H), 1.56 (b, 5H), 1.45(m, 54H), 1.33-1.29 (m, 60H), 0.90 (t, J=5.7Hz, 6H). ESI (m/z) 2157,4 $[M+H, (48)]^{+}$





Compound 9aa: white solid, 55% yield in two steps. ^{1}H NMR (400 MHz, CD_3OD) δ 7.79 (s, 1H), 6.06 (d, *J* = 4.2 Hz, 1H), 5.43 (d, *J* = 3.6 Hz, 1H), 5.34 (s, 1H), 4.54 (m, 2H), 4.39-4.28 (m, 2H), 4.16 (t, *J* = 3.2 Hz, 1H), 3.89 (m, 4H), 3.69-3.54 (m, 13H), 3.48-3.33 (m, 16H), 3.31 (m, 2H), 2.73 (m, 4H), 2.29 (t, *J* = 7.6 Hz, 2H), 1.97 (m, 3H), 1.60 (m, 5H), 1.29 (m, 60H), 0.90 (t, *J* = 6.8Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 109.6, 94.5, 94.0, 85.1, 79.6, 77.3, 73.0, 71.7, 70.8, 69.7, 69.0, 68.9, 68.0, 66.8, 53.1, 50.6, 48.9, 48.8, 47.8, 39.5, 39.2, 37.1, 33.9, 30.6, 29.7, 28.3, 28.0, 25.7, 24.3, 23.3, 21.3, 12.0, the aromatic carbons did not appear due to low intensity; Anal. calcd. for $C_{76}H_{146}N_{16}O_{15}S$: C 58.66, H 9.46, N 14.40; found: C 58.67, H 9.45, N 14.43.


cpx135s
gruppo Volonterio
5.97
5.34
5.24
4.44
4.28
4.27
4.25
4.06
3.79
3.52
3.32
2.63
2.28
2.19
1.89
1.87
1.51
1.19
0.81
0.80
0.78
1.03
0.58
0.88
1.00
1.98
4.10
1.94
5.38
54.49
4.02
0.91
1.82
2.42
5.30
70.78
7.39
f1 (ppm)

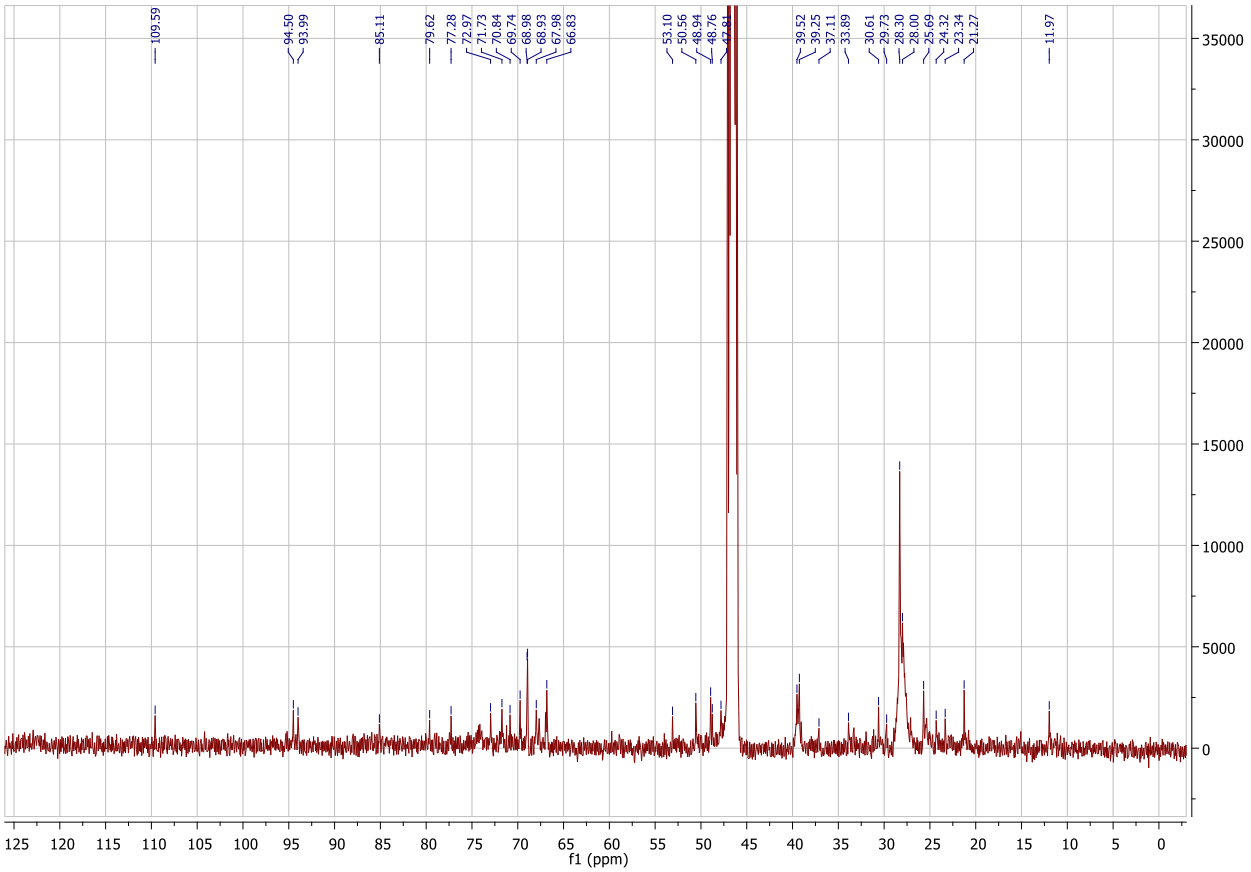
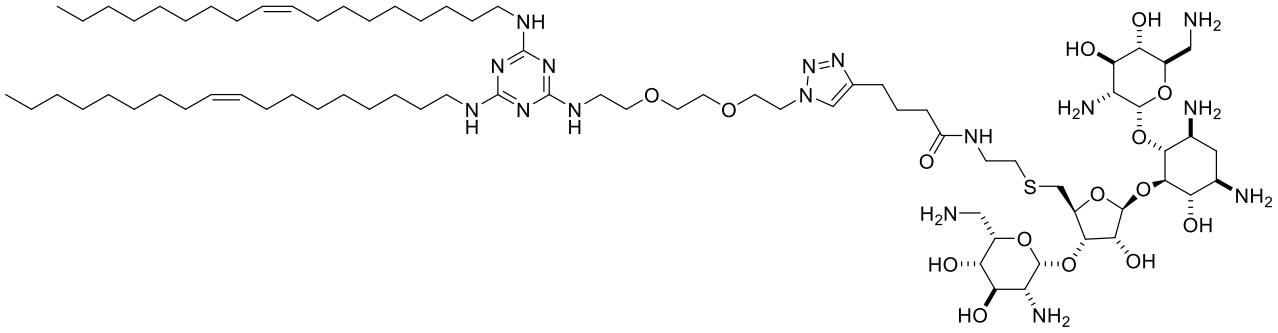
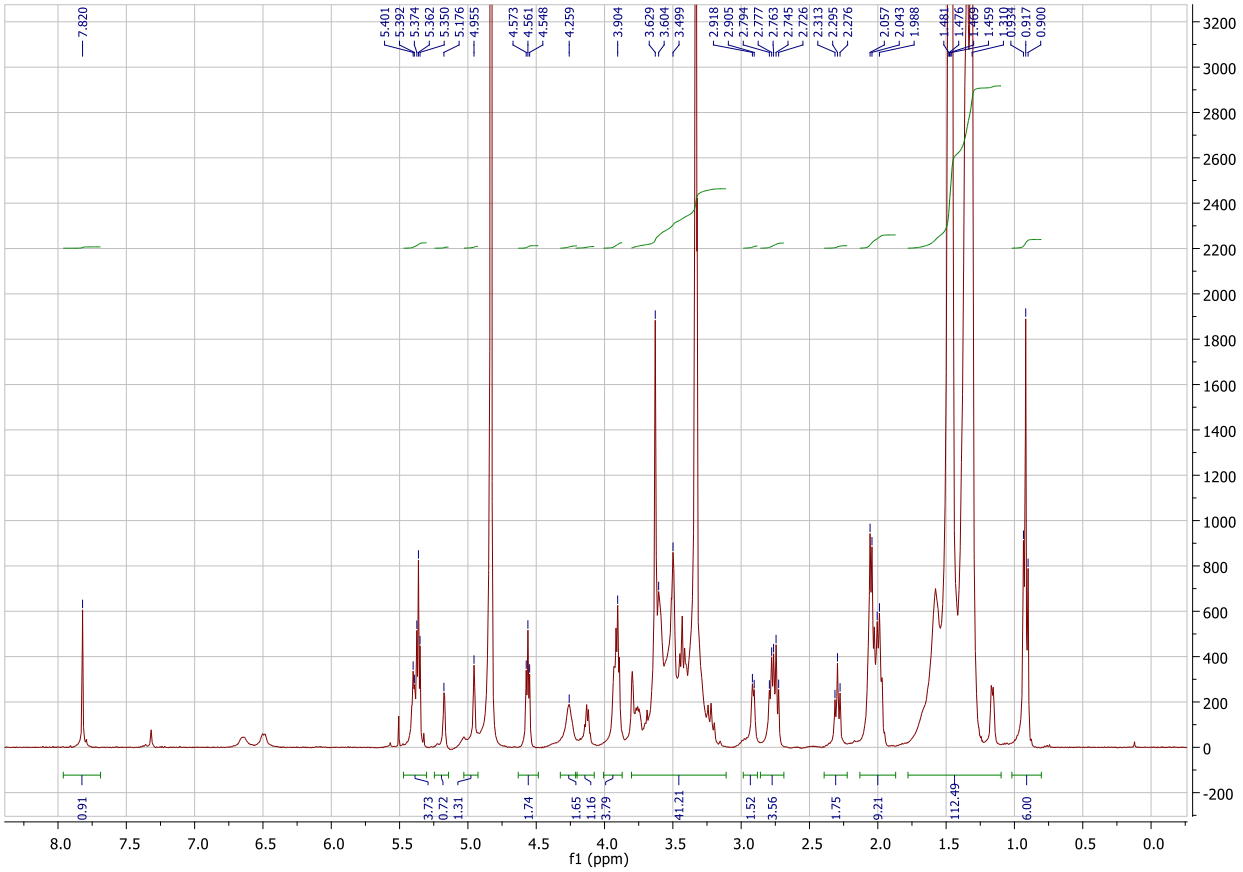
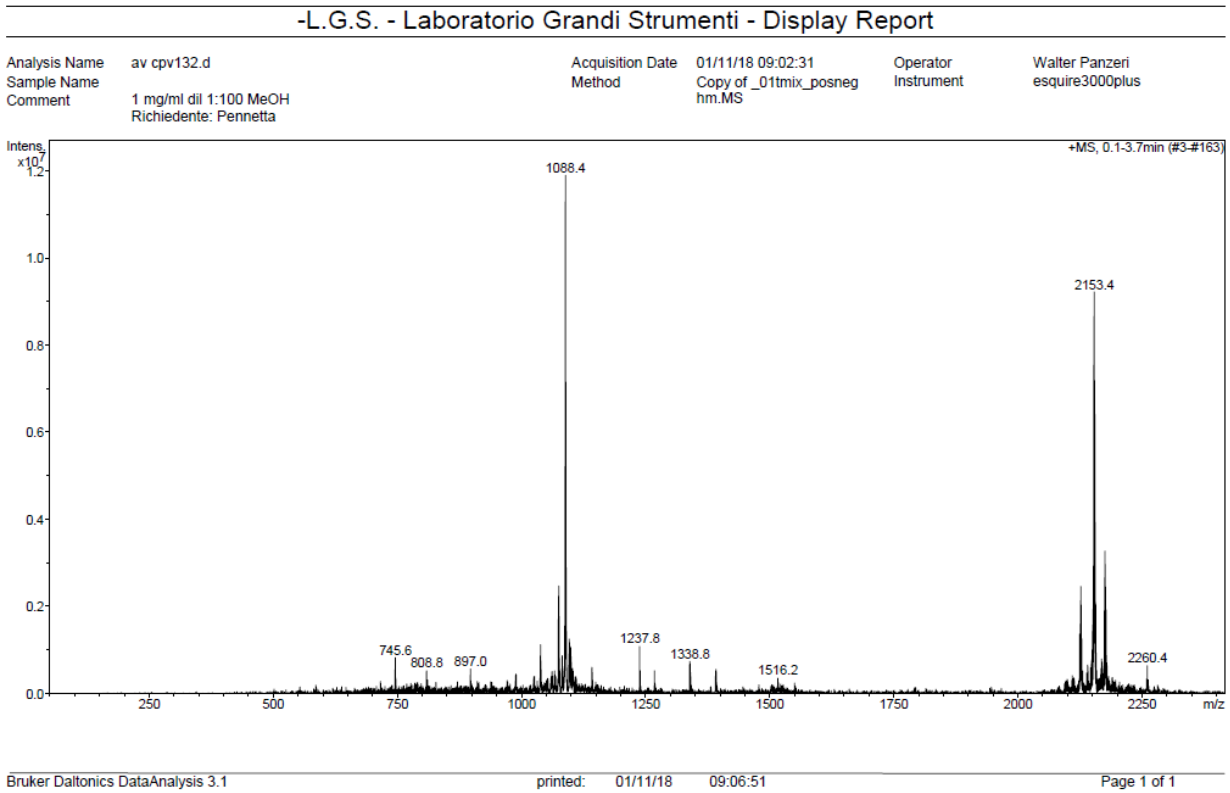

109.59
94.50
93.99
85.11
79.62
77.28
72.97
71.73
70.84
69.74
68.98
68.93
67.98
66.83
53.10
50.56
48.94
48.76
42.84
39.52
39.25
37.11
33.89
30.61
29.73
28.30
28.00
25.69
24.32
23.34
21.27
11.97
f1 (ppm)

Figure S15. ^{1}H NMR and mass spectra of compound Boc-9bb and ^{1}H NMR and ^{13}C NMR spectra of derivative 2_OL (compound 9bb)

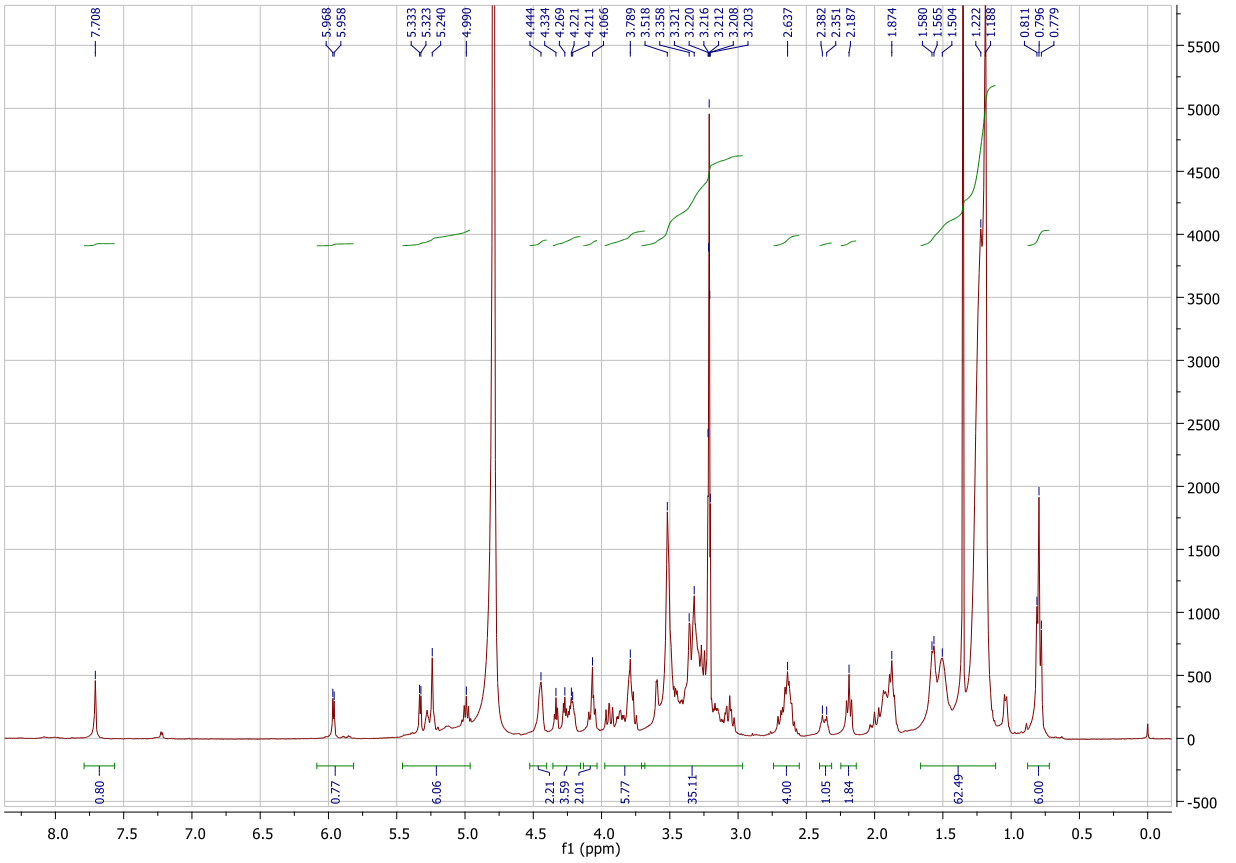


Compound Boc-9bb: R_f = 0.31 (DCM/MeOH = 90:10); ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (s, 1H), 5.41-5.32 (m, 5H), 5.15 (s, 1H), 4.93 (s, 1H), 4.54 (t, 2H), 4.24 (b, 2H), 4.11 (m, 1H), 3.91-3.87 (m, 4H), 3.78-3.71 (m, 2H), 3.65-3.36 (m, 27H), 2.88 (d, 2H), 2.77-2.70 (m, 4H), 2.27 (t, 2H), 2.04-1.95 (m, 11H), 1.56 (m, 5H), 1.46 (m, 54H), 1.32(m, 44H) 0.90 (t, 6H); ESI (m/z) 2153,4 $[M+H, (100)]^{+}$





Compound 9bb: white solid, 20% yield in two steps. ^{1}H NMR (400 MHz, CD_3OD) δ 7.79 (s, 1H), 6.07 (d, *J* = 4.2 Hz, 1H), 5.42 (d, *J* = 3.6 Hz, 1H), 5.34 (m, 4H), 5.09 (q, *J* = 6.4Hz, 1H), 4.54 (b, 2H), 4.38 (m, 2H), 4.16 (m, 1H), 3.89-3.87 (m, 4H), 3.67-3.34 (m, 29H), 2.73 (m, 5H), 2.47 (m, 1H), 2.28 (t, *J* = 7.6Hz, 2H), 2.15-1.92 (m, 8H), 1.68 (m, 4H), 1.61 (m, 4H), 1.32 (m, 44H), 0.90 (t, *J* = 6.4Hz, 6H). ^{13}C NMR (101 MHz, CD_3OD) δ 175.8, 163.3, 162.9, 148.3, 130.8, 130.6, 124.1, 112.0, 96.9, 96.4, 87.5, 81.9, 81.3, 79.7, 76.4, 75.3, 74.2, 73.3, 72.2, 71.3, 70.3, 70.1, 69.4, 69.2, 55.5, 52.9, 51.3, 50.2, 41.7, 39.6, 36.3, 35.7, 34.7, 33.1, 32.9, 32.1, 30.7, 30.4, 30.3, 27.9, 26.8, 26.1, 25.7, 23.7, 14.4; Anal. calcd. for $C_{76}H_{142}N_{16}O_{15}S$: C 58.81, H 9.22, N 14.44; found: C 58.80, H 9.24, N 14.45.


7.708
5.968
5.958
5.333
5.323
5.240
4.990
4.444
4.334
4.269
4.221
4.211
4.066
3.789
3.518
3.358
3.321
3.220
3.216
3.212
3.208
3.203
2.637
2.382
2.351
2.187
1.874
1.580
1.565
1.504
1.222
1.188
0.811
0.796
0.779
0.80
0.77
6.06
2.21
3.59
2.01
5.77
35.11
4.00
1.05
1.84
62.49
6.00
f1 (ppm)

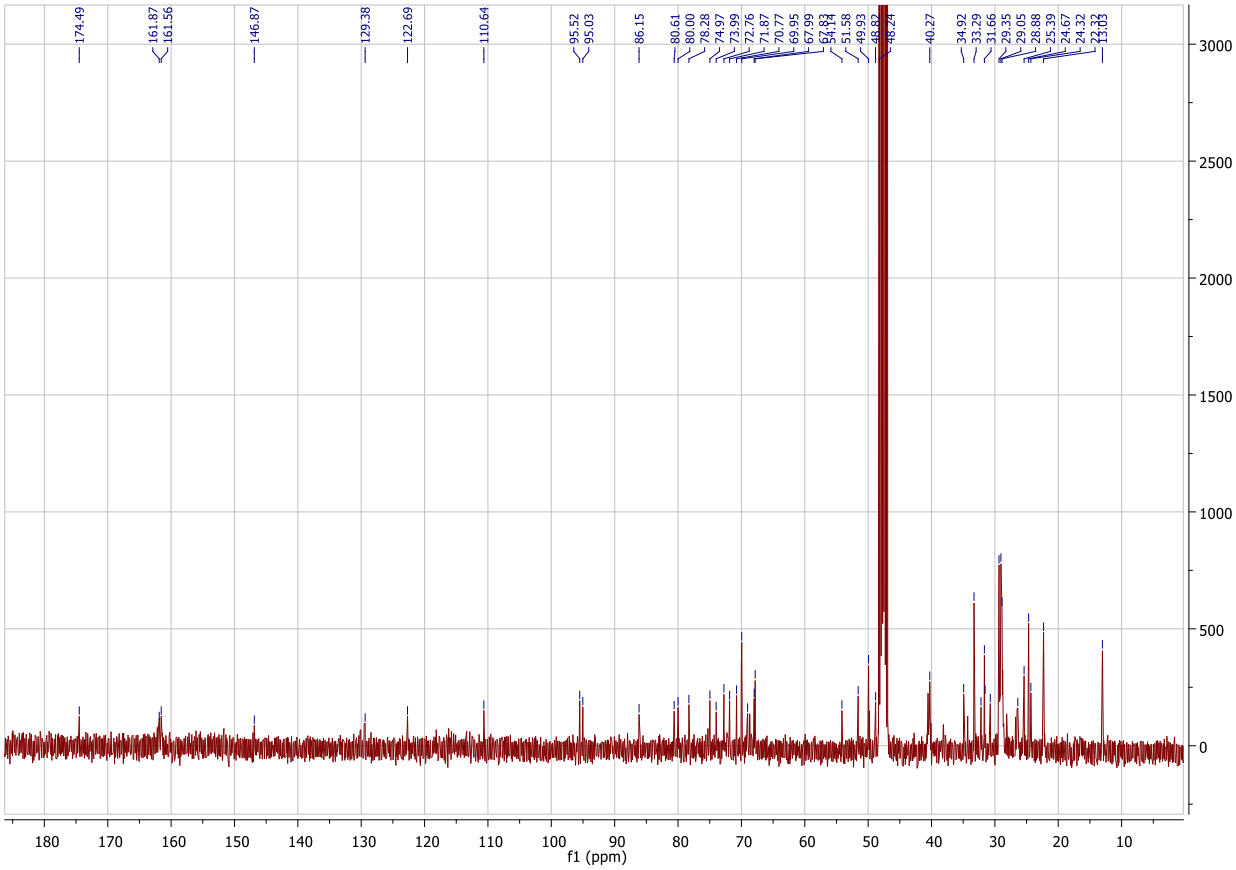
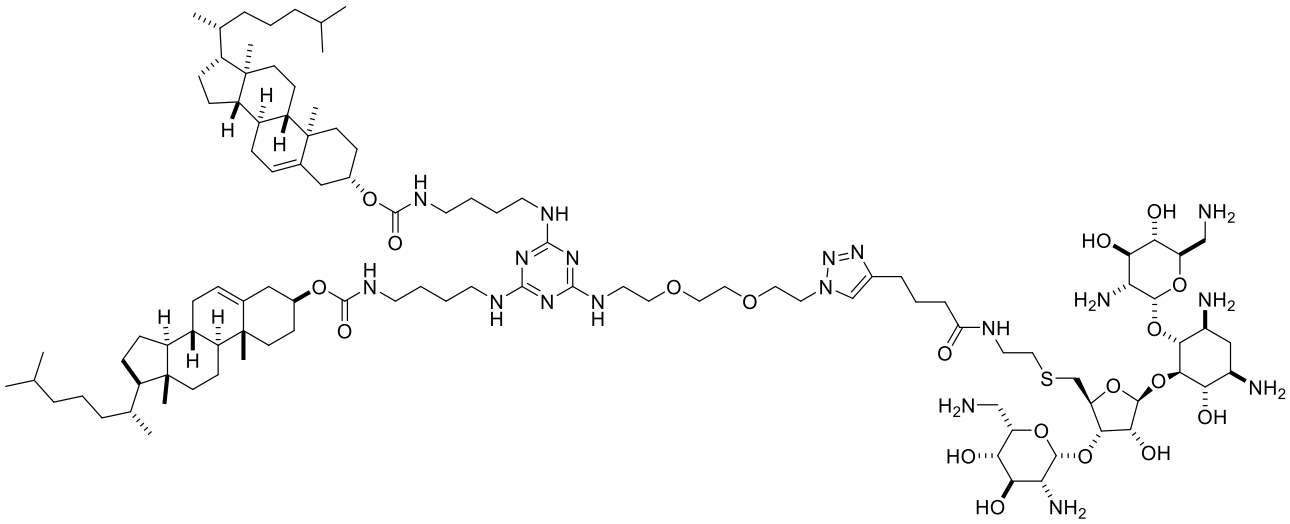
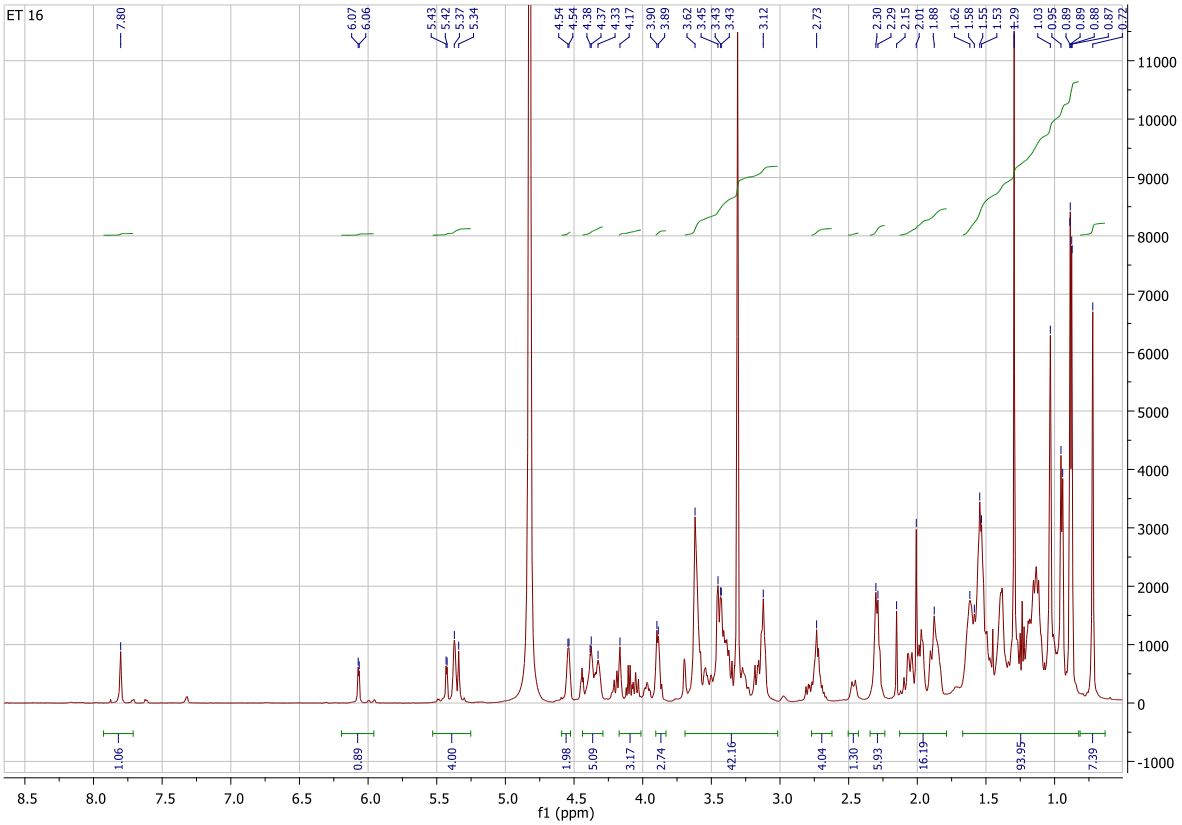

174.49
161.87
161.56
146.87
129.38
122.69
110.64
95.52
95.03
86.15
80.61
80.00
78.28
74.97
73.99
72.76
71.87
70.77
69.95
67.99
51.58
49.93
48.82
48.24
40.27
34.92
33.29
31.66
29.35
29.05
28.88
25.39
24.67
24.32
f1 (ppm)

Figure S16. ^{1}H NMR and ^{13}C NMR spectra of derivative 2_Chol (compound 9cc)



Compound 9cc: yellowish solid, 55% yield in two steps. ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (s, 1H), 6.09 (d, *J* = 3.5 Hz, 1H), 5.42 (d, *J* = 3.5 Hz, 1H), 5.38-5.36 (m, 2H), 5.34 (s, 1H), 4.55-4.53 (m, 2H), 4.44 (t, *J* = 4.5 Hz, 1H), 4.38-4.34 (m, 5H), 3.90-3.87 (m, 4H), 3.69 (s, 1H), 3.62-3.35 (m, 36H), 3.15-3.10 (m, 5H), 2.75-2.70 (m, 5H), 2.32-2.29 (m, 5H), 1.88-1.85 (m, 4H), 1.61-0.91 (m, 75H), 0.72 (br s, 6H).^{13}C NMR (126 MHz, CD_3OD) δ 175.9, 161.6, 158.5, 141.3, 132.2, 129.2, 123.5, 112.1, 96.9, 96.4, 87.5, 82.0, 79.7, 76.3, 75.4, 74.2, 73.3, 72.2, 71.4, 71.3, 70.4, 69.4, 69.3, 61.5, 58.2, 55.6, 53.0, 51.4, 51.2, 50.3, 43.6, 41.9, 41.7, 41.2, 40.7, 39.7, 37.8, 36.4, 33.3, 33.0, 32.2, 29.3, 26.8, 25.7, 22.2; Anal. calcd. for $C_{104}H_{180}N_{18}O_{19}S$: C 61.88, H 8.99, N 12.49; found: C 61.90, H 9.00, N 12.50.


ET 16
f1 (ppm)

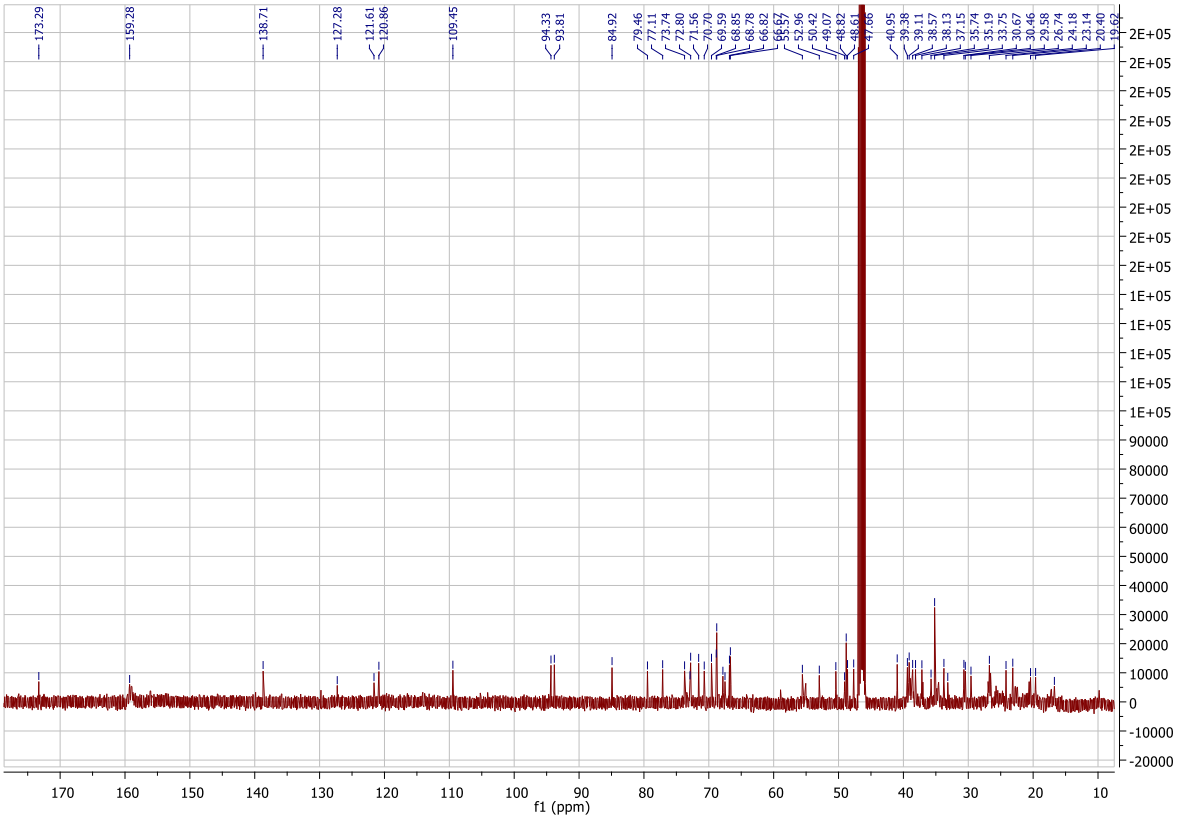
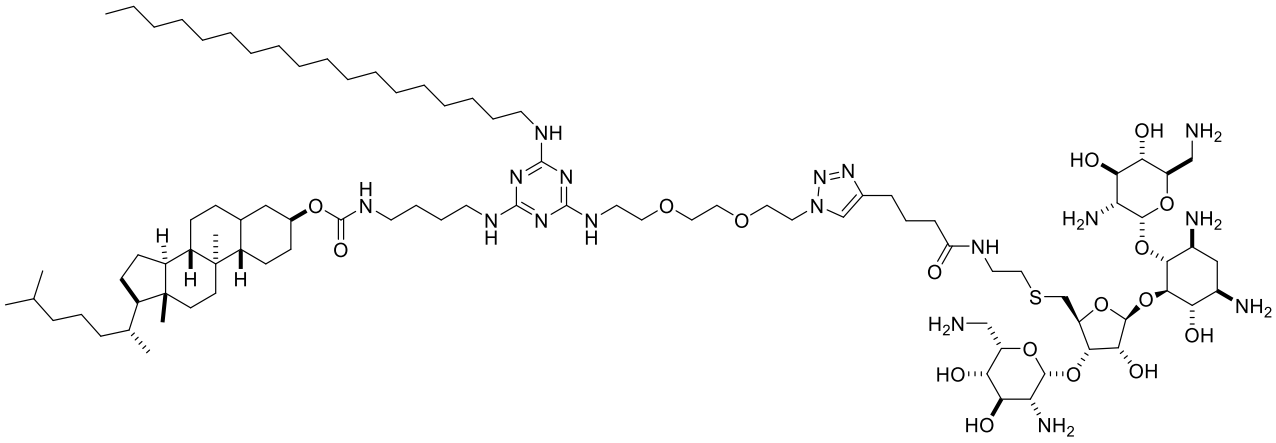
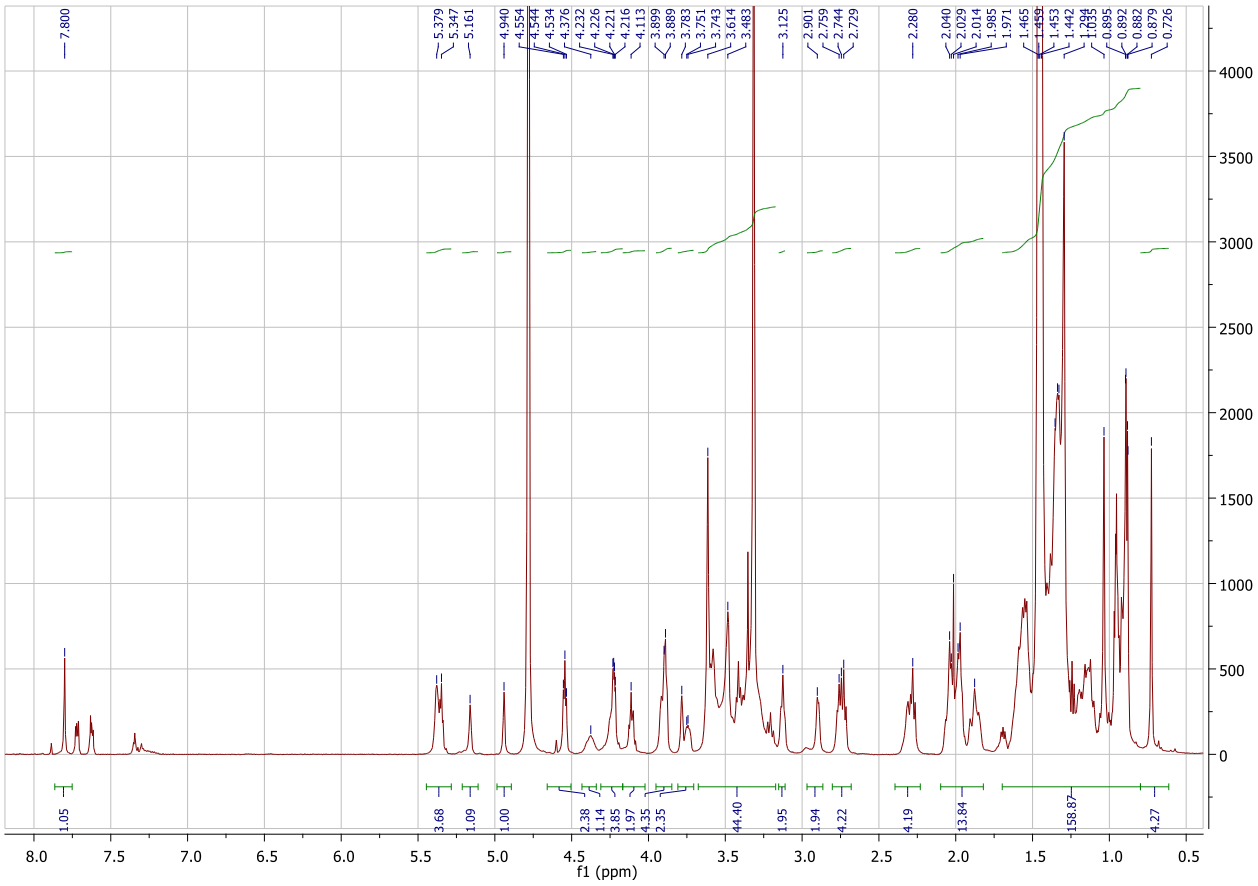
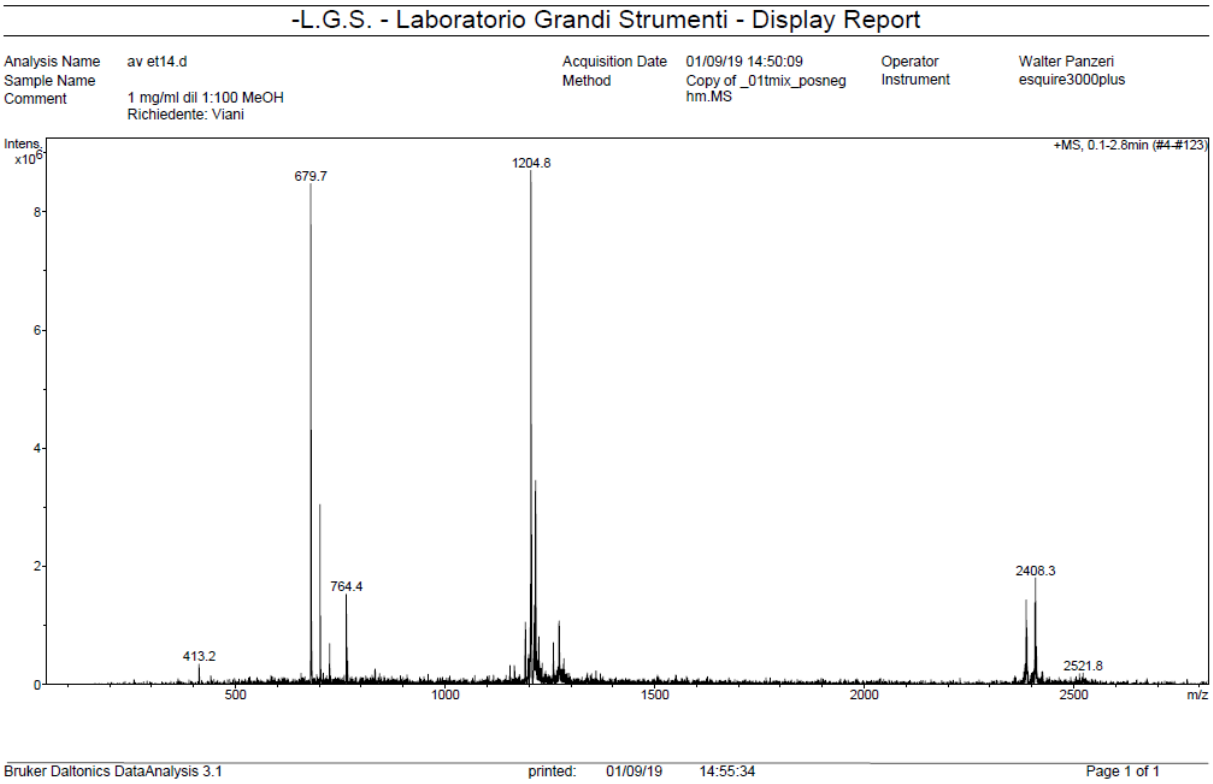

f1 (ppm)

Figure S17. ^{1}H NMR and mass spectra of compound Boc-9ab and ^{1}H NMR and ^{13}C NMR spectra of derivative 1_ST-1_OL (compound 9ab)

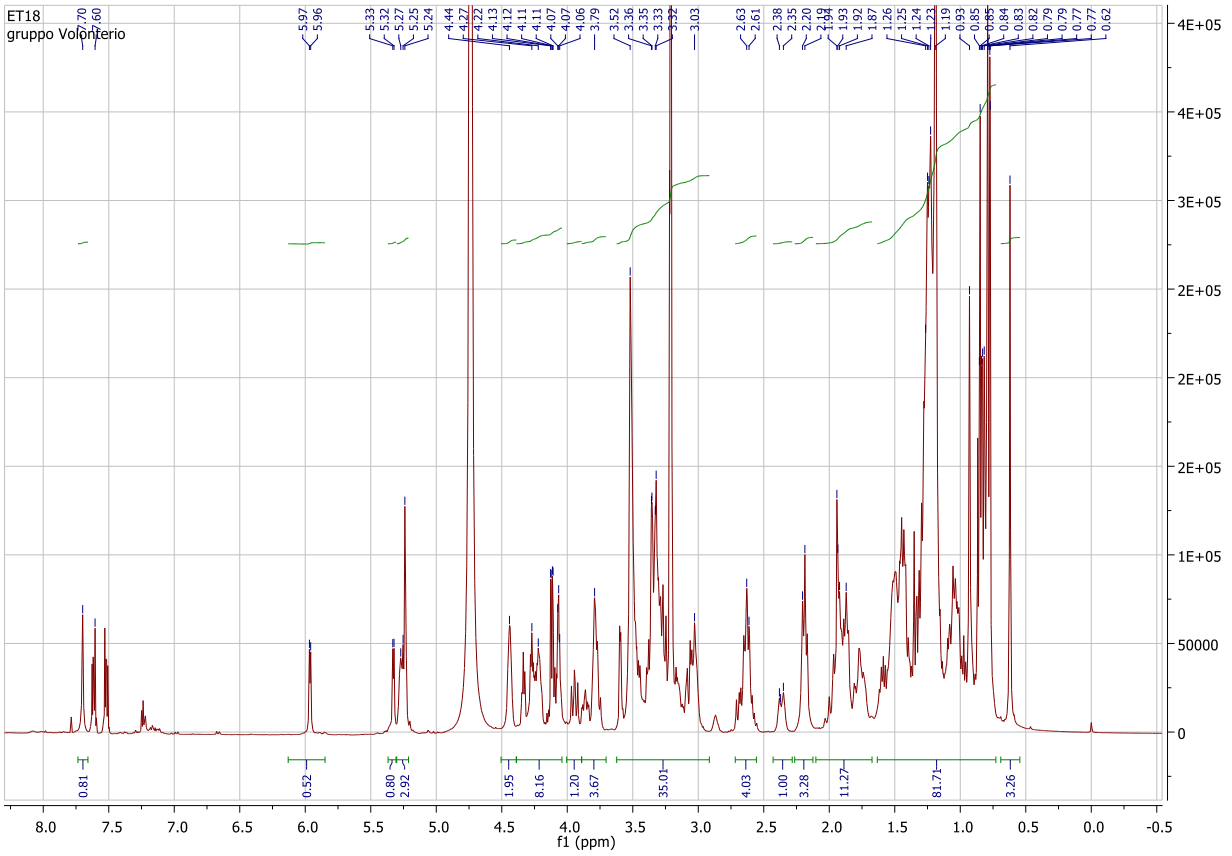


Compound Boc-9ab: R_f = 0.33 (DCM/MeOH = 90:10); ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (s, 1H), 5.38 (d, 1H), 5.34 (t, 2H), 5.15 (d, J=2.4 Hz. 1H), 4.93 (s, 1H), 4.54 (t, J=4.8Hz. 2H), 4.25 (b, 2H), 4.12 (m, 1H), 3.88 (m, 4H), 3.80-3.70 (m, 2H), 3.67-3.35 (m, 27H), 2.89 (m, 2H), 2.72 (m, 4H), 2.27 (t, J=7.6Hz, 2H), 2.08-1.93 (m, 7H), 1.56 (b, 1H), 1.53-1.41 (m, 54H), 1.33 (m, 56H), 0.90 (t, J=6.4Hz, 6H). ESI (m/z) 2177,8 $[M+Na, (34)]^{+}$, 2155,8 $[M+H, (38)]^{+}$, 1089,5 $[M+Na, (100)]^{2+}$





Compound 9ab: white solid, 40% yield in two steps. ^{1}H NMR (500 MHz, CD_3OD) δ 7.81 (s, 1H), 6.07 (d, *J* = 2.8Hz, 1H), 5.42 (d, *J* = 3.2 Hz, 1H), 5.35-5.32 (m, 3H), 4.54-4.51 (m, 2H), 4.33-4.31 (m, 2H), 4.18-4.16 (m, 1H), 3.90-3.86 (m, 4H), 3.68-3.35 (m, 29H), 3.15 (m, 2H), 2.81-2.67 (m, 4H) , 2.29 (t, *J* = 4.1 Hz, 2H), 2.13-1.94 (m, 5H), 1.61-1-59 (m, 3H), 1.35-1.29 (m, 56H), 0.90 (*t*, J = 4.4Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 159.3, 128.4, 128.2, 109.5, 94.4, 93.9, 79.5, 77.2, 72.8, 71.6, 70.7, 69.6, 68.9, 68.8, 67.9, 66.7, 52.9, 50.4, 48.8, 48.6, 47.7, 46.9, 39.4, 39.1, 30.5, 28.2, 27.9, 25.6, 24.2, 23.2, 21.1, 11.8; Anal. calcd. for $C_{76}H_{144}N_{16}O_{15}S$: C 58.74, H 9.34, N 14.42; found: C 58.76, H 9.33, N 14.41.


ET18
gruppo Volonterio
f1 (ppm)

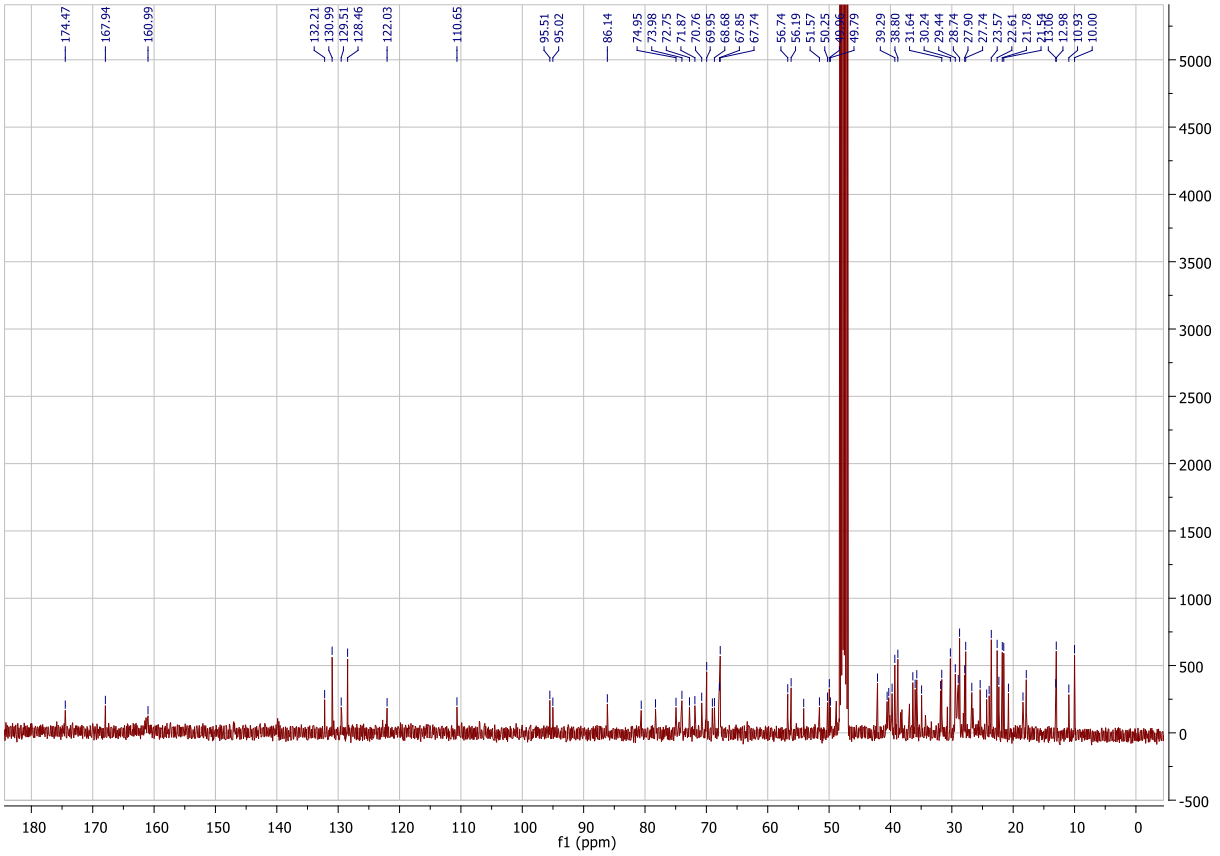
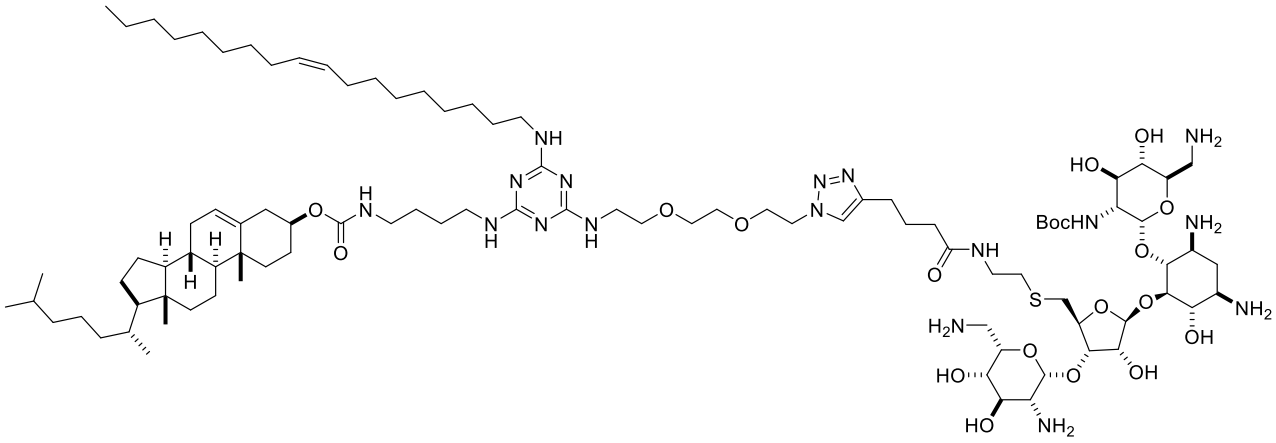
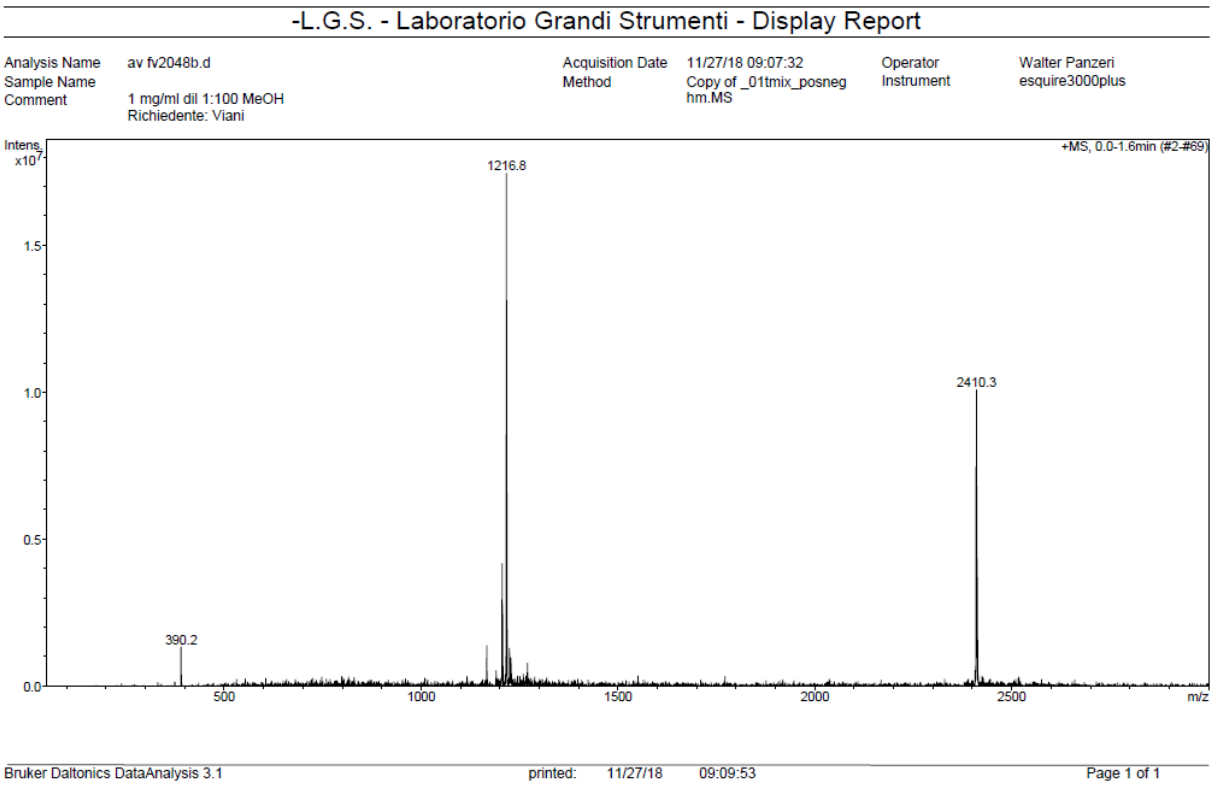

f1 (ppm)

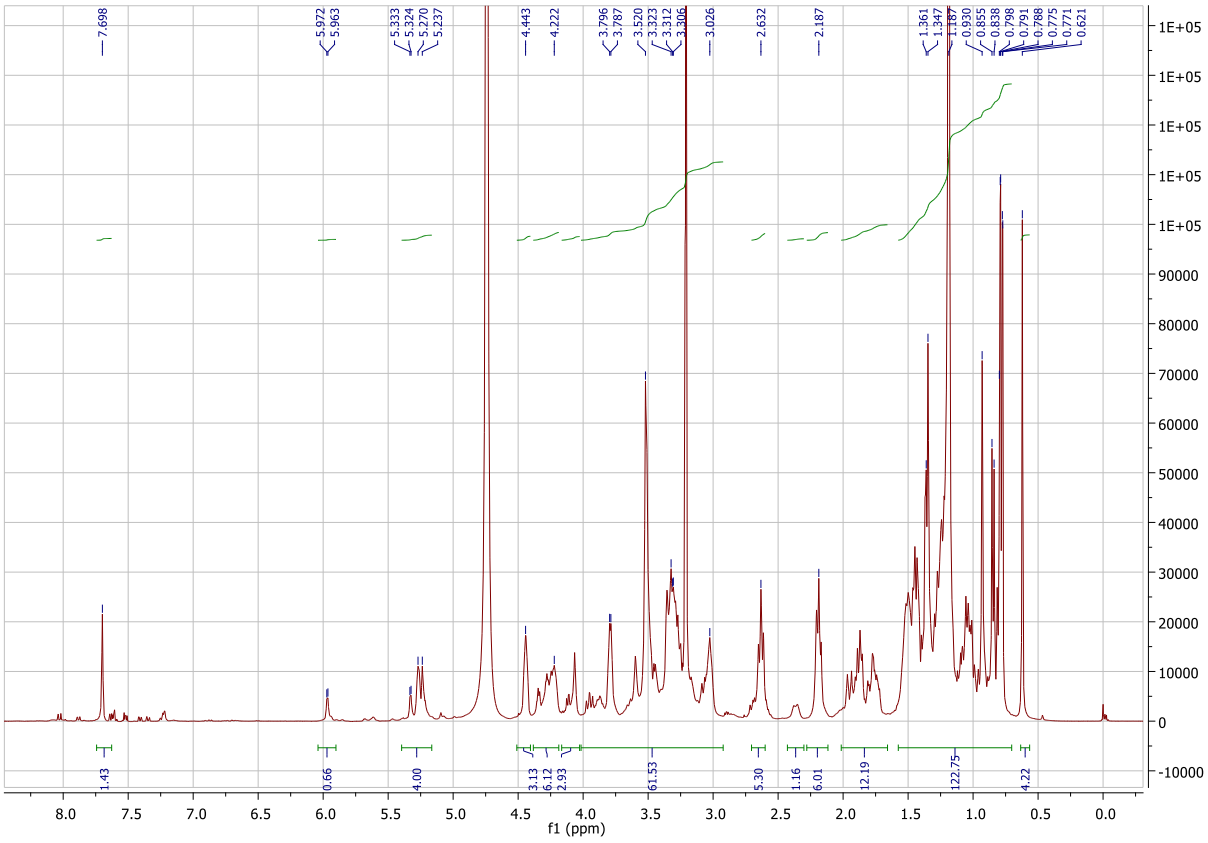
Figure S18. Mass spectra of compound Boc-9bc and ^{1}H NMR and ^{13}C NMR spectra of derivative 1_OL-1_Chol (compound 9bc)



Compound Boc-9bc: R_f = 0.30 (DCM/MeOH = 90:10); ESI (m/z) 2410.3 [M+Na, (100)]$^{+}$, 1216.8 [M+Na, (100)]$^{2+}$



Compound 9bc: white solid, 35% yield in two steps. ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (s, 1H), 6.06 (s, 1H), 5.42 (d, *J* = 3.6 Hz, 1H), 5.34 (s, 4H), 4.54 (bs, 1H), 4.38-4.36 (m, 2H), 3.89 (s, 2H), 3.73-3.25 (m, 35H), 3.16-3.12 (m, 3H), 2.73 (t, *J* = 4.2 Hz, 3H), 2.32-2.30 (m, 4H), 2.10-1.87 (m, 6H) 1.69-0.87 (m, 70H), 0.72 (br s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.0, 162.6, 162.1, 124.2, 119.1, 71.4, 71.3, 69.4, 58.1, 57.6, 51.6, 46.1, 43.5, 41.1, 40.7, 38.3, 37.7, 37.4, 37.1, 36.4, 33.2, 33.1, 30.8, 30.5, 29.3, 29.1, 28.8, 24.9, 23.7, 23.2, 22.9, 22.2, 19.3, 14.4, 12.3; Anal. calcd. for $C_{90}H_{161}N_{17}O_{17}S$: C 60.54, H 9.09, N 13.34; found: C 60.55, H 9.10, N 13.36.



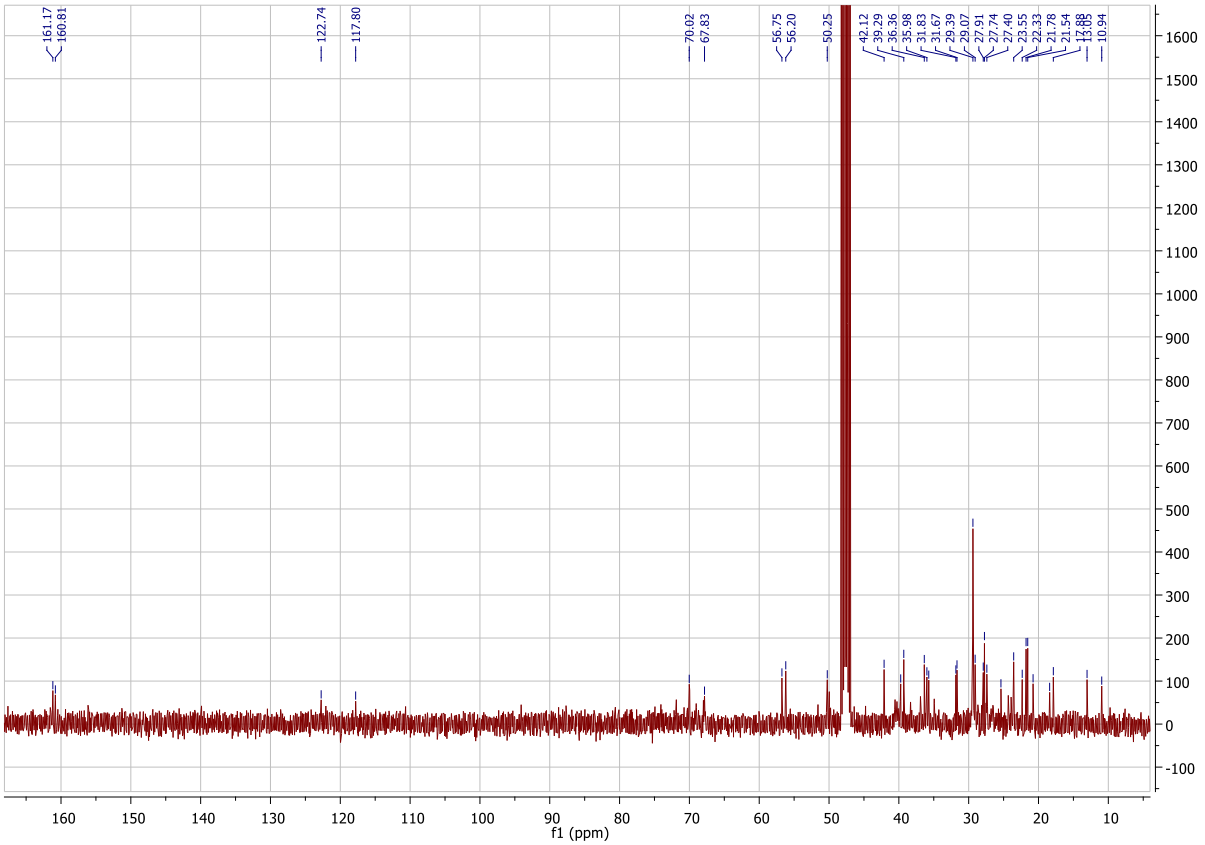
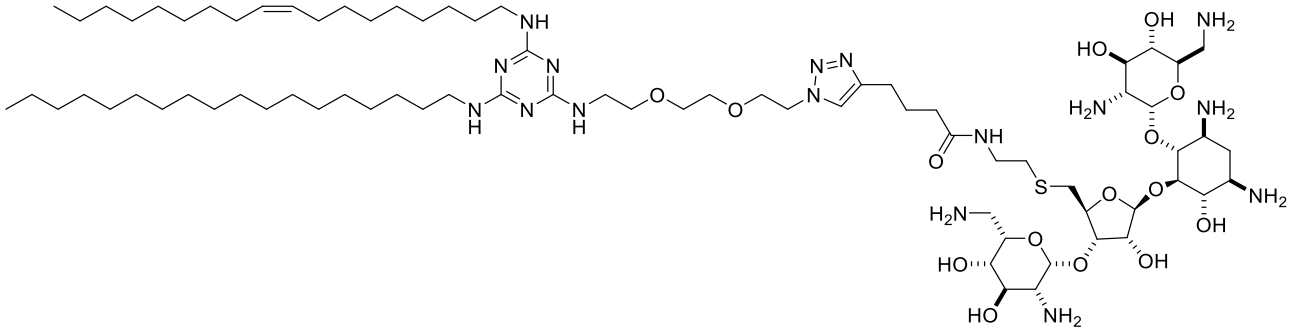
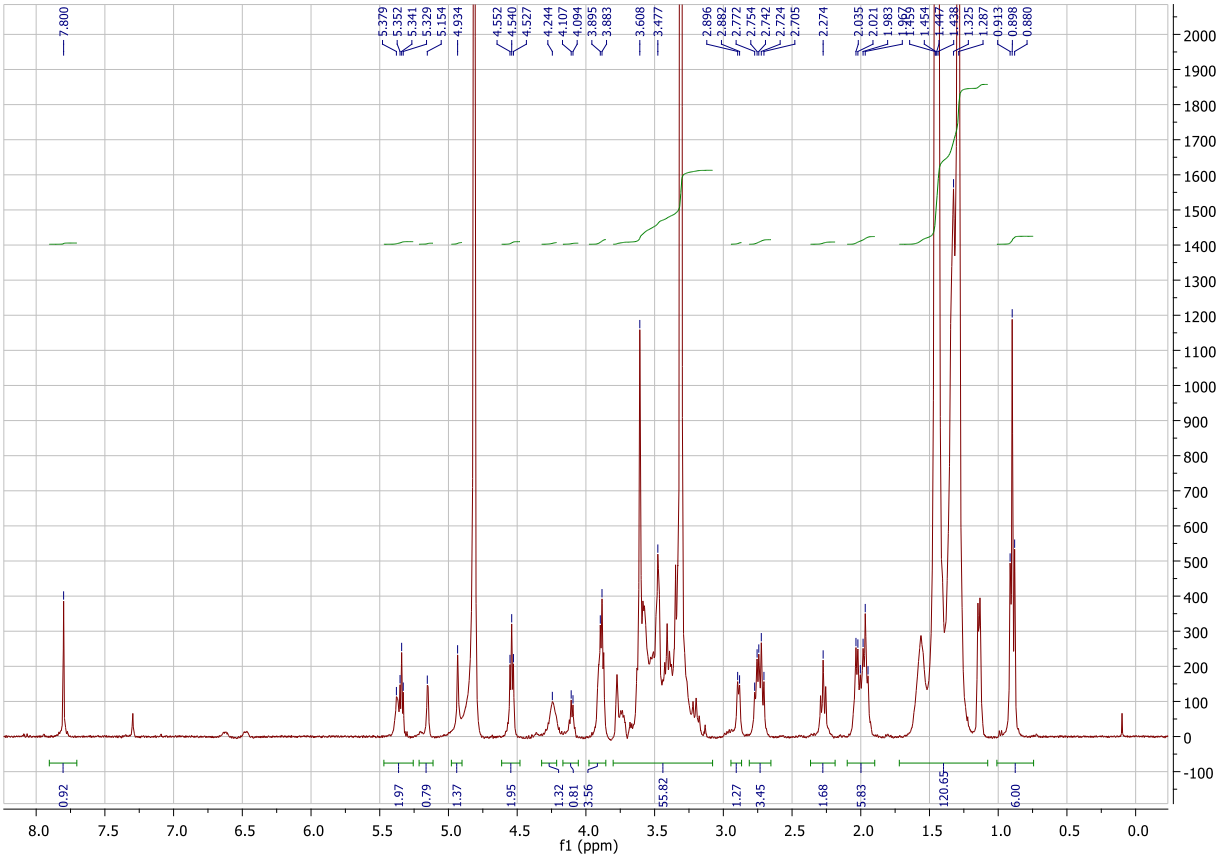
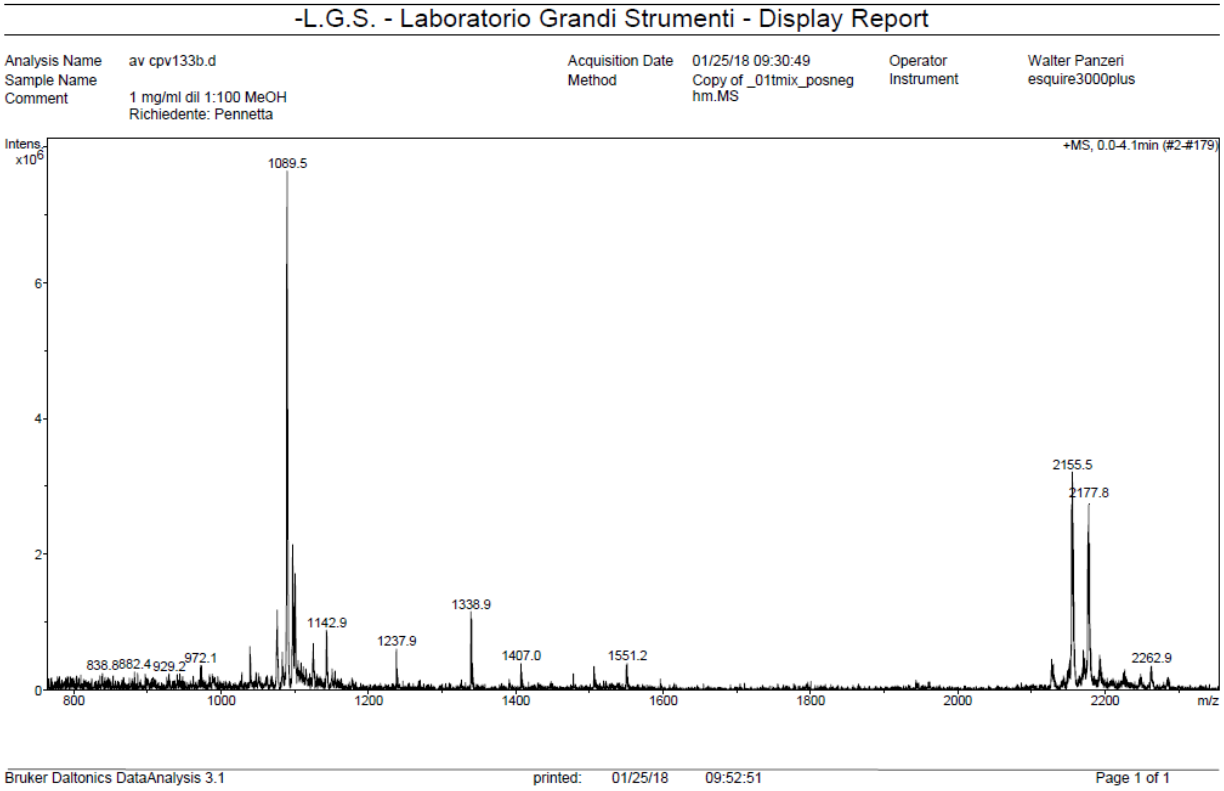

161.17
160.81
122.74
117.80
70.02
67.83
56.75
56.20
50.25
42.12
39.29
36.36
35.98
31.83
31.67
29.39
29.07
27.91
27.74
27.40
23.55
22.33
21.78
21.54
10.94
f1 (ppm)

Figure S19. ^{1}H NMR and mass spectra of Boc-9ac and ^{1}H NMR and ^{13}C NMR spectra of derivative 1_ST-1_Chol (compound 9ac)

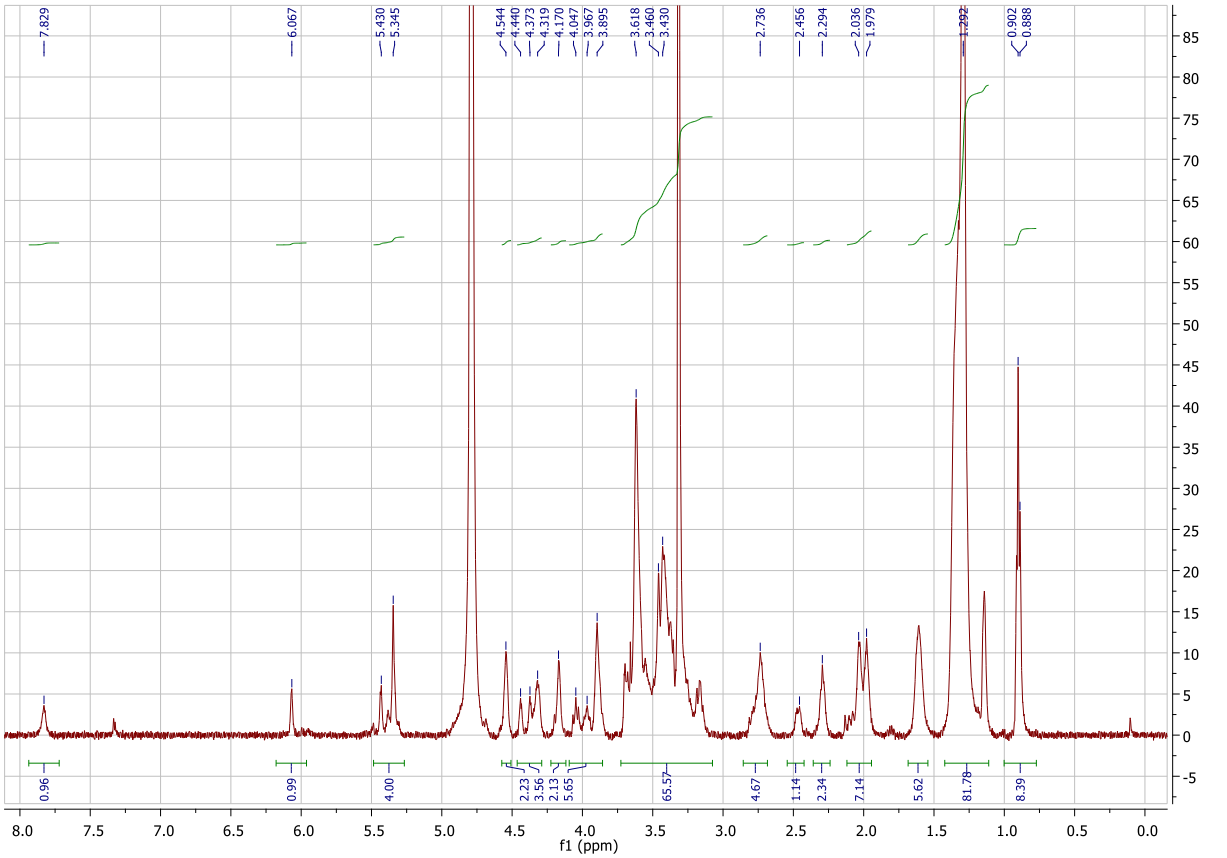


Compound Boc-9ac: R_f = 0.21 (DCM/MeOH = 90:10); ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (s, 1H), 5.37-5.32 (m, 2H), 5.16 (s, 1H), 4.94 (s, 1H), 4.54 (t, 2H), 4.36 (b, 1H), 4.21 (m, 3H), 4.11 (m, 2H), 3.89 (m, 3H), 3.75 (m, 2H), 3.61-3.35 (m, 30H), 3.13 (t, 2H), 2.90 (b, 1H), 2.76 (m, 4H), 2.29 (m, 3H), 2.05-1.95 (m, 5H), 1.86 (m, 2H), 1.60-0.94 (m, 125H), 0.72 (s, 3H). ESI (m/z) 2408.3 $[M+Na, (22)]^{+}$, 1204.8 $[M+Na, (100)]^{2+}$





Compound 9ac: white solid, 44% yield in two steps. ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (s,1H), 6.06 (d, *J* = 3.5 Hz, 1H), 5.43 (s, 1H) , 5.36-5.34 (m, 2H), 4.55-4.53 (m, 2H), 4.42 (s, 1H), 3.89-3,52 (m, 31H), 3.14-3.12 (m, 1H), 2.97-2.81 (m, 4H), 2.66-2.64 (m, 2H), 2.46-2.44 (m, 2H), 2.30-2.28 (m, 2H), 1.61-0.89 (m, 81H), 0.72 (br s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 169.3, 133.6, 132.4, 130.9, 129.9, 123.4, 112.0, 96.9, 96.4, 87.5, 76.3, 75.4, 74.1, 73.3, 72.1, 71.3, 70.4, 70.1, 69.2, 69.1, 58.1, 57.6, 55.5, 52.9, 51.6, 51.3, 51.2, 50.2, 43.5, 41.9, 41.7, 41.1, 40.7, 40.2, 38.3, 37.7, 37.4, 37.1, 36.3, 33.2, 33.0, 32.1, 31.6, 30.8, 30.3, 30.1, 29.3, 29.1, 28.1, 26.8, 25.7, 25.3, 24.9, 24.0, 23.7, 23.2, 22.9, 22.2, 19.8, 19.3, 14.4, 14.3, 12.3, 11.4; Anal. calcd. for $C_{90}H_{163}N_{17}O_{17}S$: C 60.48, H 9.19, N 13.32; found: C 60.50, H 9.17, N 13.31.


7.829
6.067
5.430
5.345
4.544
4.440
4.373
4.319
4.170
4.047
3.967
3.895
3.618
3.460
3.430
2.736
2.456
2.294
2.036
1.979
1.292
0.902
0.888
0.96
0.99
4.00
2.23
3.56
2.13
5.65
65.57
4.67
1.14
2.34
7.14
5.62
81.78
8.39
f1 (ppm)

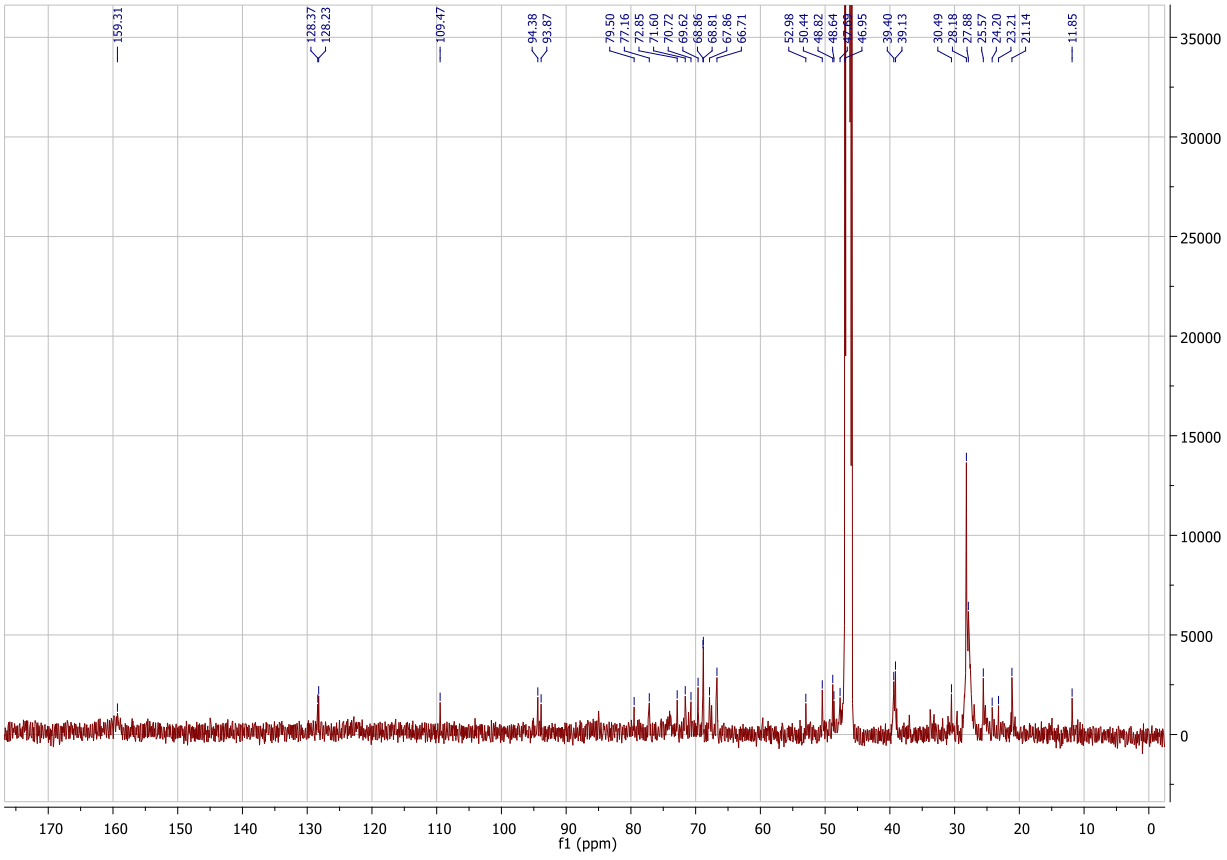
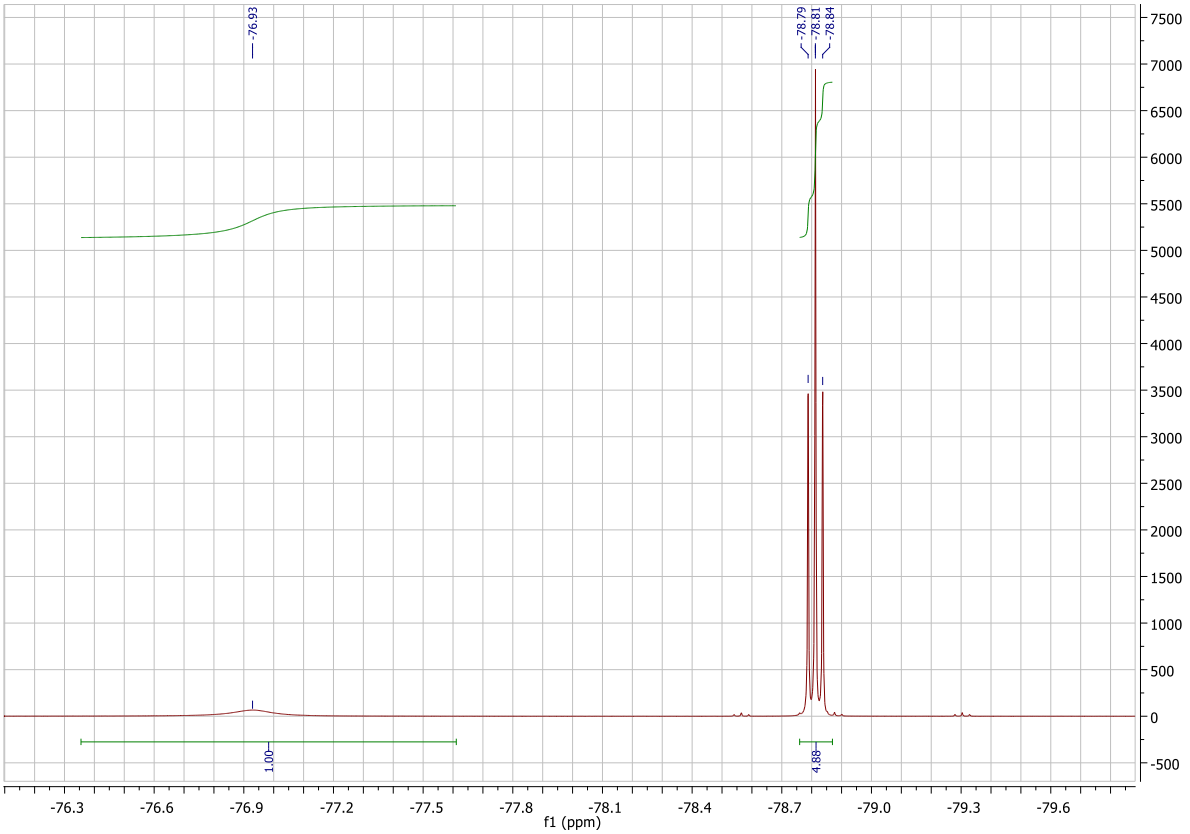
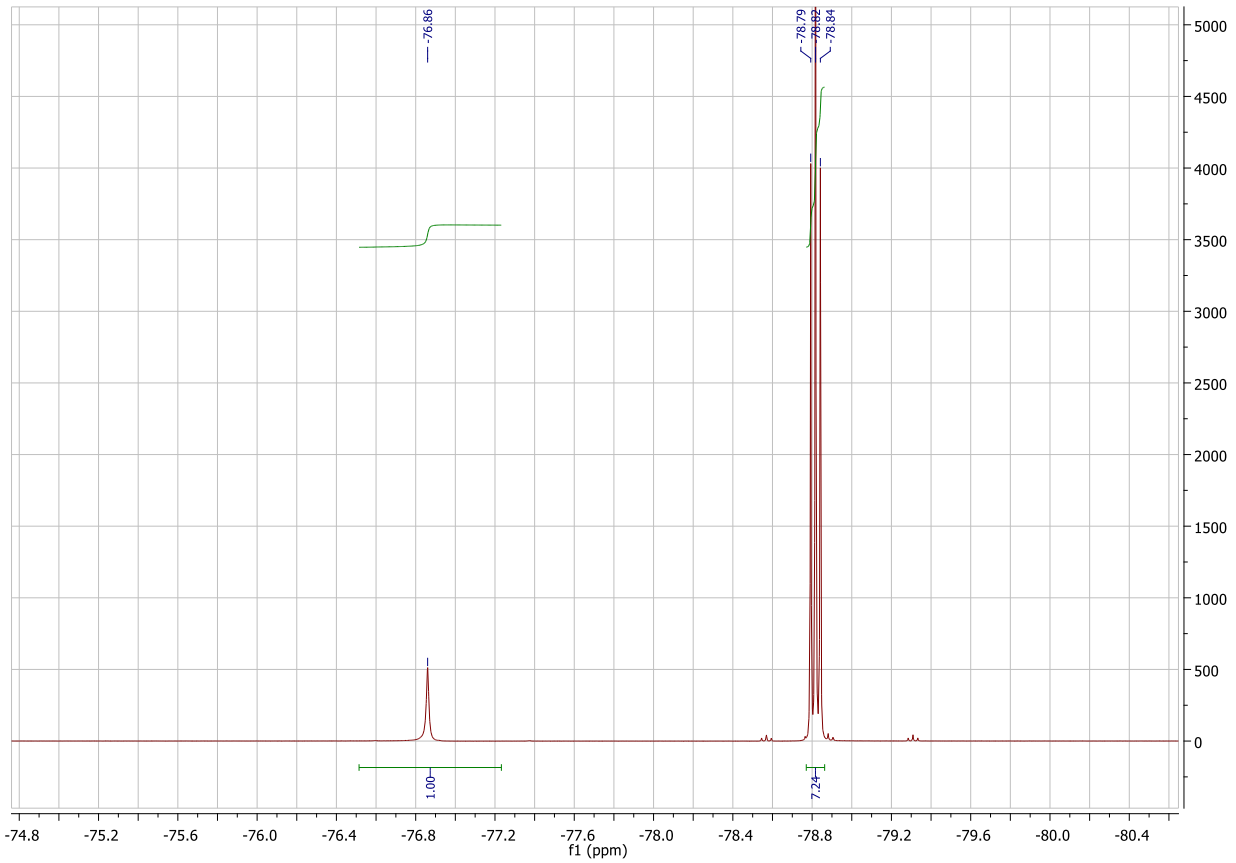

159.31
128.37
128.23
109.47
94.38
93.87
79.50
77.16
72.85
71.60
70.72
69.62
68.86
68.81
67.86
66.71
52.98
50.44
48.82
48.64
46.95
39.40
39.13
30.49
28.18
27.88
25.57
24.20
23.21
21.14
11.85
f1 (ppm)

Figure S20. ^{19}F spectra of derivative 1_OL (compound 8b)



To a solution of 8.1 mg of triazine **8b** (MW = 1319.74 + xTFA) in 750 µL of CD_3OD trifluoroethanol (10 µL, 0.133 mmol) was added. The ^{19}F NMR was recorded showing a 4.88:1 molar ratio between the trifluoromethyl group of trifluoroethanol and the trifluoromethyl groups of the trifluoroacetic acid molecules belonging to the triazine **8b**. Thus in 8.1 mg of triazine there are 0.133 mmol / 4.88 = 0.027 mmol of trifluoroacetic acid which weight 3.1 mg. 8.1 mg – 3.1 mg = 5.0 mg of desalted triazine which account for 0.0038 mmol. The molar ratio between triazine and TFA is 0.027/0.0038 = 7.1

Figure S21. ^{19}F titration spectra of derivative 2_OL (compound 9bb)



To a solution of 6.8 mg of triazine **9bb** (MW = 1551.06 + xTFA) in 750 µL of CD_3OD trifluoroethanol (10 µL, 0.133 mmol) was added. ^{19}F NMR was recorded showing a 7.24:1 molar ratio between the trifluoromethyl group of trifluoroethanol and the trifluoromethyl groups of the trifluoroacetic acid molecules belonging to the triazine **9bb**. Thus in 6.8 mg of triazine there are 0.133 mmol / 7.24 = 0.018 mmol of trifluoroacetic acid which weight 2.09 mg. 6.8 mg – 2.9 mg = 3.9 mg of desalted triazine which account for 0.0025 mmol. The molar ratio between triazine and TFA is 0.018/0.0025 = 7.2

Figure S22. Cytotoxicity and transfection efficiency of Neo-triazine derivatives evaluated on HeLa cells.

Complexes were prepared by mixing pGL3 with Neo-triazine derivatives at different charge ratios (CRs, +/−), 25 kDa *b*PEI (C/R 10) and Lipofectamine 2000 (Lipo). Transfection efficiency is expressed as luminescence signal (RLU) normalized to the total protein content in each cell lysate. A) mono-tailed Neo-triazine conjugates **1_ST**, **1_OL** and **1_Chol**; B) symmetric two-tailed Neo-triazine conjugates **2_ST**, **2_OL** and **2_Chol**; C) asymmetric two-tailed Neo-triazine conjugates **1_ST-1_OL**, **1_ST-1_Chol** and **1_OL-1_Chol**. Results are expressed as mean ± SD (n ≥ 3) (*p < 0.05 *vs.* other lipids for any given CR).

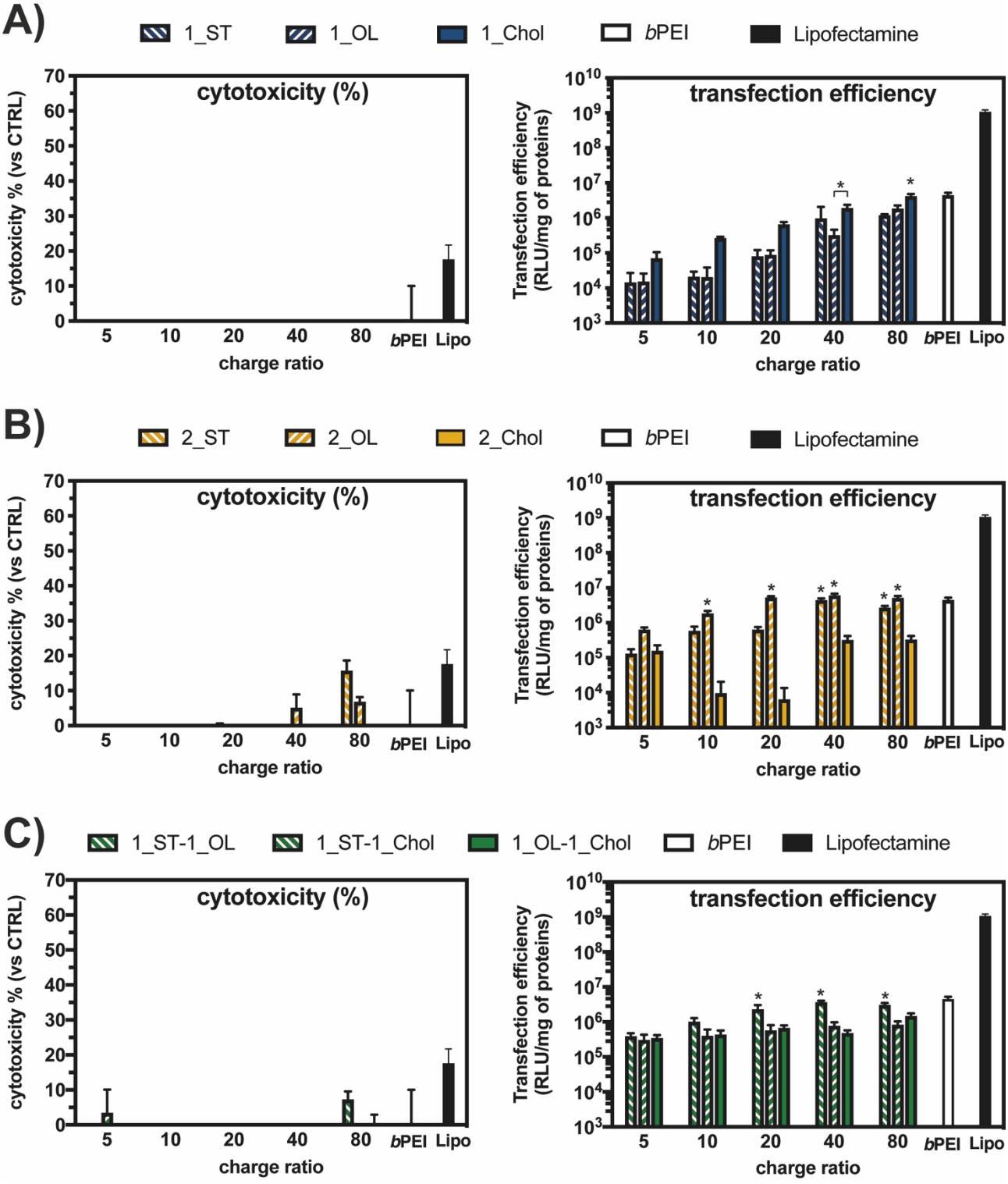


Figure S23. MIC of Neomycin.

Evaluation of the MIC of Neo against *E. coli*. Results are expressed as mean ± SD (n ≥ 3)