# Influence of linker length in Shape Recognition of B* DNA by Dimeric Aminoglycosides (Supporting Information)

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## Procedure for the synthesis of neomycin dimers

### Synthesis of linkers (diisothiocyanate from diamine)

To a solution of diamine (0.20 mmol) in dichloromethane (anhydrous, 5 mL), 1,10-Thiocarbonyldi-2(1H)-pyridone (0.22 mmol, 2.2 equiv.) was added and the reaction started with constant stirring in inert atmosphere. The progress of the reaction was monitored by TLC. Flash chromatography (in dichloromethane) afforded the desired product as colorless oil (see Table S1 for % yields).

### Synthesis of *N-Boc* protected neomycin dimers

To a solution of neomycin-boc-5”-amine (1 or 2) (0.05 mmol) in dry pyridine (5 mL), DMAP (cat. amount) was added followed by diisothiocyanate/diisocynate linker (0.025 mmol, 0.50 eq) and the reaction started under inert atm. The reaction mixture stirred at r.t. for 15 h in the atmosphere of argon. The progress of the reaction was monitored by TLC. The volatiles were removed on rotovap. Purification by flash column chromatography [0 to 10 % ethanol in $CH_2Cl_2$ (v/v)] afforded the desired product as a white solid (% yields are reported for individual compounds in table). [$R_f$ 0.38-0.46, 10 % ethanol in $CH_2Cl_2$ (v/v)].

### Deprotection of *N-Boc* protected neomycin dimers

To a solution of neomycin dimer (0.012 mmol) in dioxane (3 mL), 4 M HCl/dioxane (1 mL) was added and the reaction started at room temperature. There is a formation of white precipitate after 15 min. The reaction mixture was centrifuged and the solid was collected. The solid was washed with a solution of diethyl ether/hexane [3 × 5 mL, 1:1 (v/v)]. The solid was dissolved in water and lyophilized to afford the desired product as a white powder (% yields are reported for individual compounds in table).

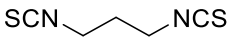
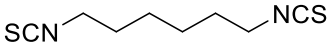
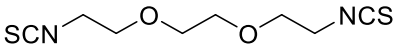
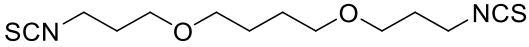
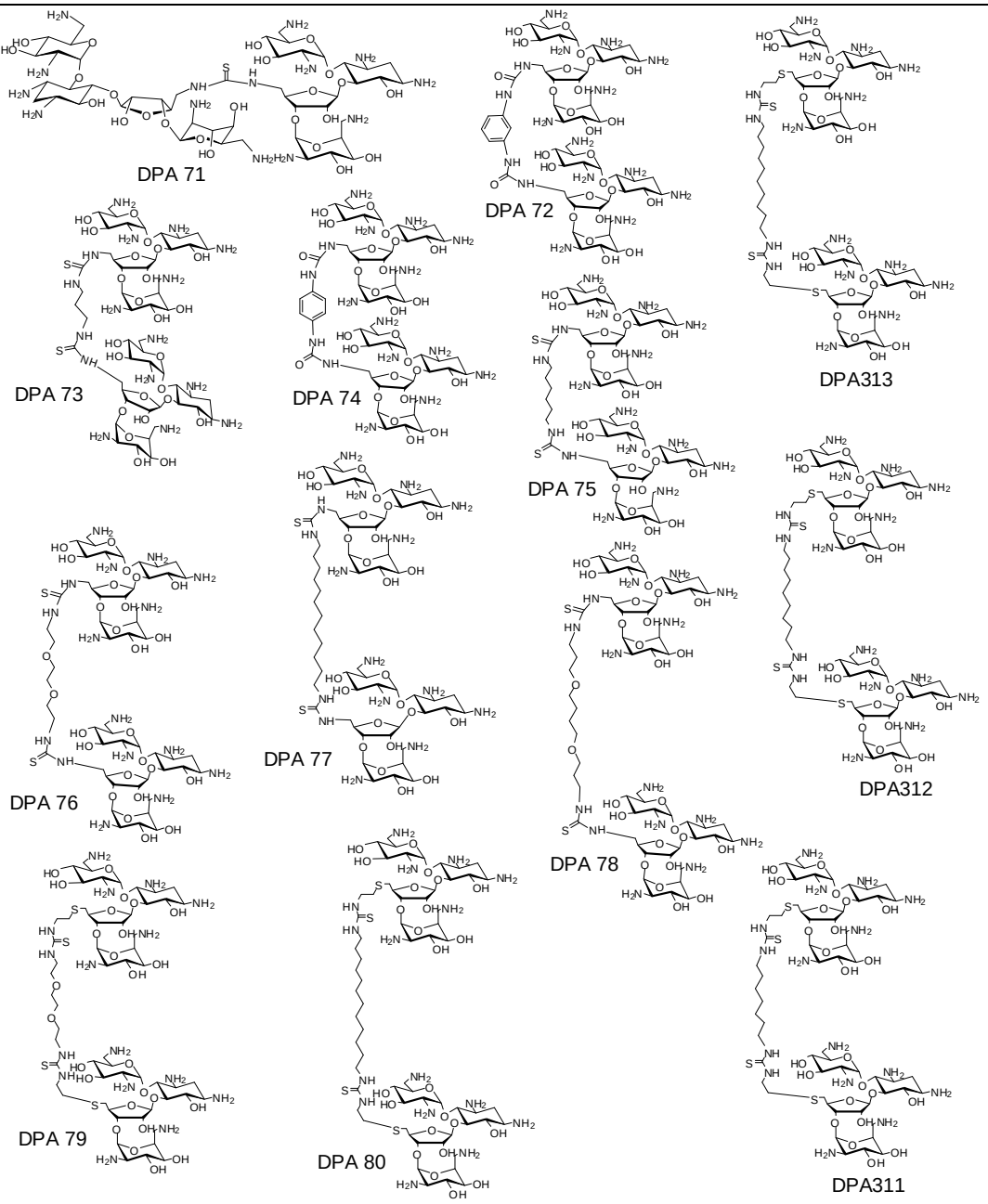
| Linker | % yield |
|---|---|
|  | 96.2 |
|  | 97.4 |
|  | 95.8 |
|  | 95.5 |

**Table S1.** % yields of the diisothicyanate linkers synthesized from diammines.

| Neomycin Dimer | Linker length | % yield |
|---|---|---|
| DPA71 | 1 | 66.2 |
| DPA72 | 7 | 61.7 |
| DPA73 | 7 | 65.0 |
| DPA74 | 8 | 61.2 |
| DPA75 | 10 | 61.5 |
| DPA76 | 14 | 60.4 |
| DPA77 | 16 | 69.2 |
| DPA78 | 16 | 68.0 |
| DPA79 | 18 | 66.3 |
| DPA80 | 22 | 67.2 |
| DPA311 | 17 | 70.1 |
| DPA312 | 19 | 70.7 |
| DPA313 | 20 | 69.8 |

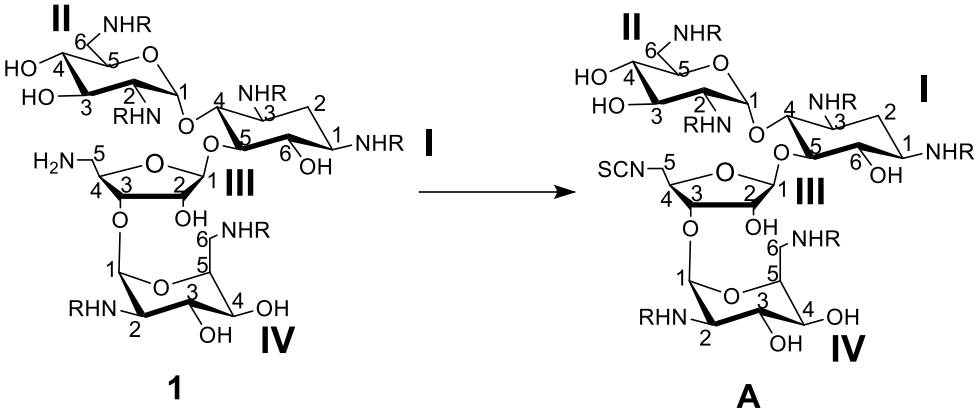
**Table S2.** Overall % yields of the neomycin dimers from two steps (N-boc protected and deprotected).




**Scheme 1.** Chemical structure of neomycin dimers.

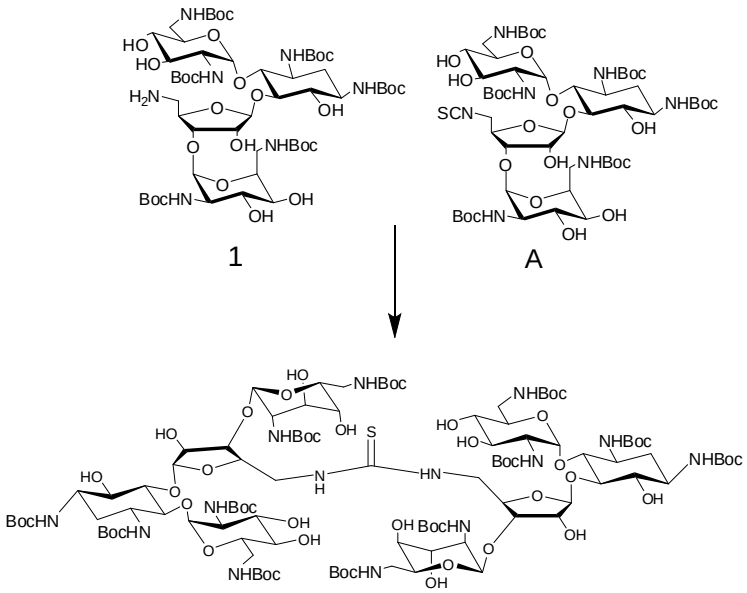
**Characterization of neomycin dimers**

*Synthesis of Hexa-N-Boc deoxy-neomycin-5''-isothiocyanate*



To a solution of Hexa-*N*-Boc deoxy-neomycin-5''-amine (1) (30.0 mg, 24.0 µmol) in dry DCM (3.0 mL), DMAP (cat. amount) was added followed by the addition of TCDP (8.4 mg, 36.0 µmol) and the reaction started with constant stirring at r.t. in the atmosphere of argon gas. The reaction ran for 12 h. The progress of the reaction was monitored by TLC. The solvents were removed on evaporater. Flash column chromatography [0 to 10 % EtOH in DCM (v/v)] yields an off white powder (24.55 mg, 80 %). [$R_f$ 0.62, 10 % EtOH in DCM (v/v)]; IR (KBr, $cm^{-1}$) 2980, 2910, 2105 (NCS), 1701, 1510; $^1$H NMR (500 MHz, $CD_3COCD_3$) δ 6.41 (t, *J* = 5.36 Hz, 1 H, $NH_{6IV}$), 6.25-6.33 (m, 1 H, $NH_{1I}$ or $NH_{6II}$), 6.12 (d, *J* = 7.72 Hz, 1 H, $NH_{1I}$ or $NH_{6II}$), 5.94-6.12 (m, 3 H, $NH_{3I}$, $NH_{2IV}$, $NH_{2II}$), 5.23-5.25 (m, 2 H), 5.27-5.31 (m, 2 H), 4.85-4.95 (m, 1 H), 4.48 (m, 1 H), 4.42 (m, 1 H), 4.31 (t, *J* = 5.05 Hz, 1 H), 4.22-4.27 (m, 1 H), 4.12 (q, *J* = 4.73 Hz, 1 H), 3.99-4.08 (m, 2 H), 3.89-3.97 (m, 1 H), 3.74-3.88 (m, 4 H), 3.52-3.74 (m, 9 H), 3.40-3.52 (m, 4 H), 3.16-3.35 (m, 4 H), 1.35-1.65 (m, 56 H, $H_{2Iax}$, 6 × Boc). MS MALDI-TOF calcd. for $C_{54}H_{93}N_7O_{24}$ ($M+Na^+$), 1277.60, obsd: 1276.55.

*Synthesis of N-Boc DPA71.*

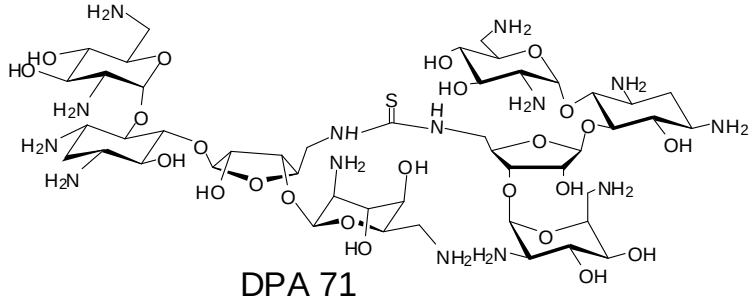



*N*-Boc DPA71

To a solution of Hexa-*N*-Boc deoxy-neomycin-5’’-amine (1) (15.0 mg, 12.0 µmol) in dry pyridine (3.0 mL), DMAP (cat. amount) was added followed by the addition of a solution of *Hexa-N-Boc deoxy-neomycin-5’’*-isothiocyanate, A (14.8 mg, 12.0 µmol) in dry pyridine (1.0 mL) and the reaction started with constant stirring at r.t. in the atmosphere of argon gas. The reaction ran for 12 h. The progress of the reaction was monitored by TLC. The solvents were removed on evaporater. Flash column chromatography [0 to 10 % EtOH in DCM (v/v)] yields an off white powder (25.95 mg, 82 %). [$R_f$ 0.44, 10 % EtOH in DCM (v/v)]; IR (KBr, $cm^{-1}$) 3300-3500 (br), 2975, 2918, 2105 (br, -C=S), 1705, 1619; $^1$H NMR (500 MHz, $CD_3COCD_3$) δ 6.42 (t, $J$ = 5.84 Hz, 2 H, $NH_{6IV}$), 6.36 (d, $J$ = 9.15 Hz, 2 H, $NH_{1I}$ or $NH_{6II}$), 6.23-6.30 (m, 2 H, $NH_{1I}$ or $NH_{6II}$), 6.18 (d, $J$ = 6.13 Hz, 2 H, $NH_{2IV}$ or $NH_{3I}$), 5.99-6.06 (m, 2 H, $NH_{2IV}$ or $NH_{3I}$), 5.88-5.99 (br, s, 2 H, $NH_{2II}$), 5.14-5.22 (m, 4 H), 4.94-5.01 (m, 4 H), 4.77-4.82 (m, 2 H), 4.39-4.45 (m, 4 H), 4.37 (d, $J$ = 6.47 Hz, 2 H), 4.31-4.35 (m, 4 H), 4.15-4.21 (m, 4 H), 4.02-4.10 (m, 4 H), 3.82-3.95 (m, 8 H), 3.74-3.82 (m, 6 H), 3.58-3.70 (m, 10 H), 3.54-3.58 (m, 4 H), 3.50-3.54 (m, 2 H),

3.40-3.50 (m, 6 H), 3.15-3.26 (m, 6 H), 1.50-1.60 (m, 2 H, $H_{2Iax}$), 1.35-1.50 (m, 108 H, 12 × Boc). MS MALDI-TOF calcd. for $C_{107}H_{188}N_{14}O_{48}S$ ($M+H_2O^+$), 2486.24, obsd: 2485.70.

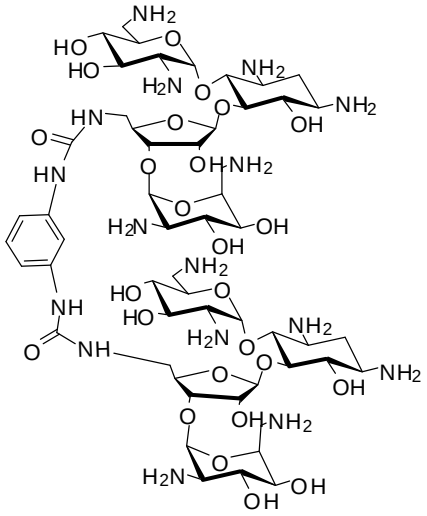
**DPA71.**



DPA 71

IR (KBr, $cm^{-1}$) 3401 (br, OH), 2093, 1642; $^1H$ NMR (500 MHz, $D_2O$): 5.80 (m, 2 H, $H_{1II}$), 5.28 (d, $J$ = 3.16 Hz, 2 H, $H_{1III}$), 5.06 (br, 2 H, $H_{1IV}$), 4.41 (m, 2 H, $H_{4III}$), 4.26 (d, $J$ = 5.83 Hz, 2 H, $H_{2III}$), 4.15-4.20 (m, 2 H, $H_{4IV}$), 4.05-4.11 (m, 2 H, $H_{4I}$), 3.96-4.03 (m, 4 H), 3.85 (t, $J$ = 9.78 Hz, 2 H, $H_{6II}$), 3.78 (t, $J$ = 10.25 Hz, 2 H, $H_{5I}$), 3.64-3.74 (m, 8 H, $H_{2IV}$, $H_{4II}$, $H_{5IV}$, and $H_{3III}$), 3.60 (s, 2 H, $H_{6I}$), 3.40-3.53 (m, 6 H, $H_{3II}$, $H_{3I}$ and $H_{5II}$), 3.27-3.38 (m, 4 H), 3.17-3.27 (m, 8 H, $H_{3I}$, $H_{5II}$, $H_{3II}$), 3.08-3.17 (m, 4 H), 2.23-2.31 (m, 2 H, $H_{2Ieq.}$), 1.69 (q, $J$ = 12.45 Hz, 2 H, $H_{2Iax.}$); MS MALDI-TOF m/z for $C_{47}H_{92}N_{14}O_{24}S$ $[M+H_2O]^+$, calcd 1287.38, found 1287.67.

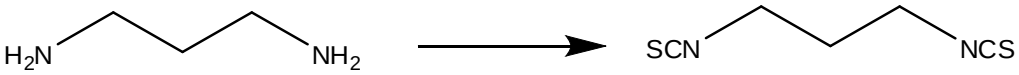
**DPA72.**



DPA 72

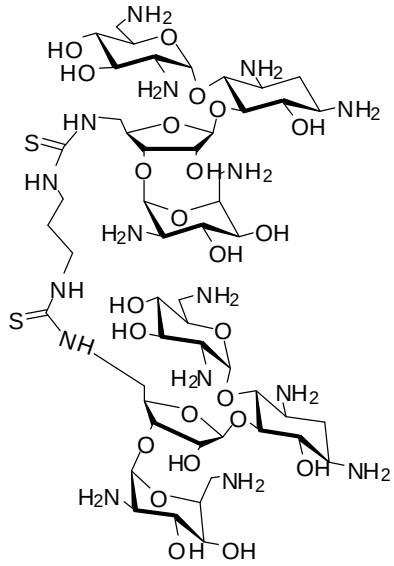
IR (KBr, $cm^{-1}$) 3434 (br, OH), 2093, 1638, 1366; $^1H$ NMR (500 MHz, $D_2O$) δ 7.32 (m, 1 H, Ar), 7.24 (m, 1 H, Ar), 7.05 (m, 2 H, Ar), 5.85-5.91 (m, 2H, $H_{1II}$), 5.28-5.32 (m, 2H, $H_{1III}$), 5.14-5.20 (m, 2H, $H_{1IV}$), 4.35-4.43 (m, 2 H), 4.25-4.34 (m, 2 H), 4.14-4.24 (m, 6 H), 4.12 (t, *J* = 2.84 Hz, 4 H), 4.05 (t, *J* = 9.46 Hz, 2 H, $H_{6II}$), 3.85-3.94 (m, 4 H), 3.82 (t, *J* = 8.86 Hz, 2 H, $H_{5I}$), 3.71-3.78 (m, 4 H), 3.53-3.70 (m, 8 H), 3.47-3.51 (m, 4 H), 3.39-3.47 (m, 4 H), 3.30-3.38 (m, 8 H), 3.18-3.30 (m, 8 H), 3.00-3.17 (m, 8 H), 2.32-2.41 (m, 2 H, $H_{2Ieq.}$), 1.80-1.91 (m, 2 H, $H_{2Iax.}$); MS MALDI-TOF m/z for $C_{54}H_{98}N_{16}O_{26}$ $[M+Na]^+$, calcd 1410.45, found 1410.33; UV (water) $\lambda_{max}$ = 234 nm.

**DPA73 (Linker = 1,3-diisothiocyanatopropane).**




[$R_f$ = 0.45 in $CH_2Cl_2$]; IR (KBr, $cm^{-1}$); 3400, 2967, 2912, 2050 (br, NCS), 1728, 1602, 1472; $^1H$ NMR (300 MHz, $CDCl_3$): ∂ 3.75 (t, *J* = 4.25 Hz, 4 H), 2.07 (p, *J* = 3.21 Hz, 2 H), $^{13}C$ NMR (125 MHz, $CDCl_3$): ∂ 42.09, 30.17.

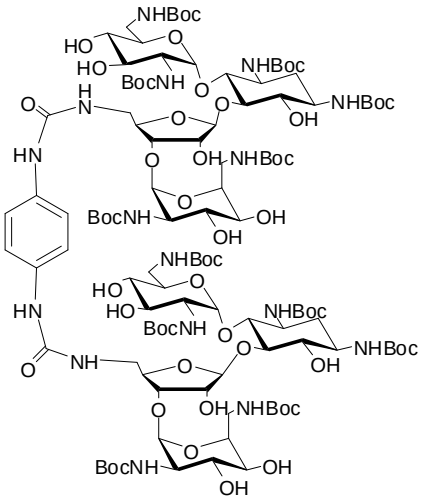
**DPA73**



DPA 73

IR (KBr, $cm^{-1}$) 3431 (br, OH), 2091 (weak), 1654, 1524, 1366; $^{1}H$ NMR (500 MHz, $D_2O$): 5.98-6.04 (m, 2 H, $H_{1II}$), 5.30-5.34 (m, 2 H, $H_{1III}$), 5.18-5.23 (br, 2 H, $H_{1IV}$), 4.33-4.42 (m, 4 H, $H_{4III}$, $H_{2III}$), 4.26-4.32 (m, 2 H), 4.19-4.25 (m, 2 H, $H_{4IV}$), 4.13 (m, 2 H, $H_{4I}$ ), 4.10 (t, *J* =10.09 Hz, 2 H, $H_{6II}$), 3.95 (t, *J* = 10.56 Hz, 2 H, $H_{5I}$), 3.74-3.90 (m, 8 H), 3.60-3.75 (m, 8 H, $H_{2IV}$, $H_{4II}$, $H_{5IV}$, and $H_{3III}$), 3.51-3.60 (m, 6 H), 3.42-3.52 (m, 8 H), 3.32-3.41 (m, 6 H), 3.20-3.32 (m, 8 H, $H_{3I}$, $H_{5II}$, $H_{3II}$), 3.00-3.20 (m, 6 H), 2.67-2.87 (m, 4 H, linker protons), 2.37 (dt, *J1* = 3.62 Hz, *J2* = 4.25 Hz, 2 H, $H_{2Ieq.}$), 1.89 (q, *J* = 13.25 Hz, 2 H, $H_{2Iax.}$), 1.16 (m, 2 H, linker protons); MS MALDI-TOF m/z for $C_{51}H_{100}N_{16}O_{24}S_2$ $[M+H_2O]^+$, calcd 1403.44, found 1402.91.

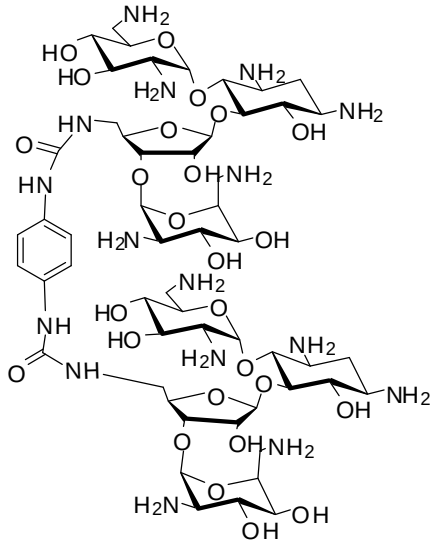
**N-Boc DPA74.**




*N*-Boc DPA74

[$R_f$ 0.37 in 10% EtOH in DCM (v/v)]; IR (KBr, $cm^{-1}$) 3375 (OH), 2930, 2870, 1691, 1600-1500 [-C=C- (aromatic region)]; $^1$H-NMR (500 MHz, $CD_3COCD_3$) δ 7.42 (s, 4 H, aromatic region), 6.86 (m, 4 H, $NH_{6IV}$), 6.57-6.77 (m, 4 H, $NH_{1I}$), 6.09-6.33 (m, 4 H, $NH_{6II}$, $NH_{3I}$), 5.85-6.09 (m, 4 H, $NH_{2IV}$, and $NH_{2II}$), 5.02-5.16 (m, 8 H, $H_{1II}$, $H_{1III}$, $OH_{4IV}$, $H_{1IV}$), 4.92-4.98 (m, 4 H), 4.09-4.38 (m, 10 H), 3.95-4.07 (m, 8 H), 3.77-3.91 (m, 8 H), 3.67-3.77 (m, 4 H), 3.52-3.65 (m, 16 H), 3.40-3.52 (m, 10 H), 3.25-3.39 (m, 4 H), 3.07-3.23 (m, 6 H), 2.11-2.16 (m, 2 H), 1.52-1.70 (m, 2 H, $H_{2Iax}$), 1.10-1.53 (m, 110 H, , $H_{2Iax}$, 12 × Boc); MALDI-TOF : calcd for $C_{114}H_{194}N_{16}O_{50}$ $[M+H_2O]^+$ 2605.31, found 2604.10.

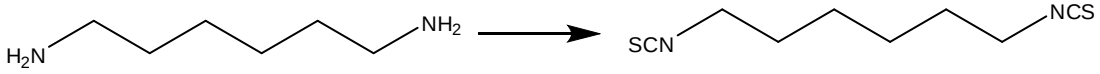
**DPA74.**



DPA 74

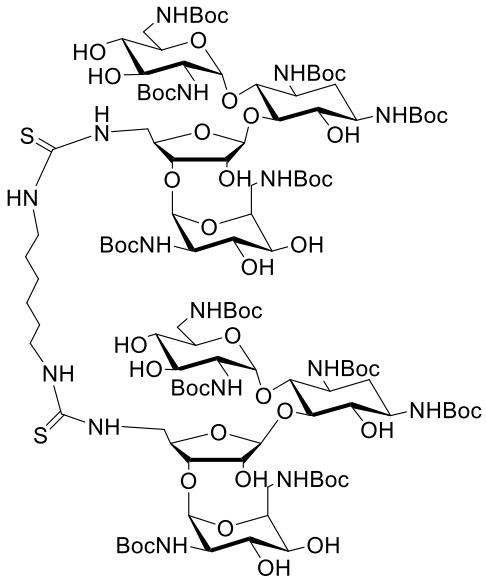
IR (KBr, $cm^{-1}$) 3434 (br, OH), 2089, 1642; $^1$H NMR (500 MHz, $D_2O$) δ 7.27 (s, 2H, Ar), 5.91-5.94 (m, 2H, $H_{1II}$), 5.34 (d, *J* = 3.47 Hz, 2H, $H_{1III}$), 5.20 (d, *J* = 3.47 Hz, 2H, $H_{1IV}$), 4.42 (t, 2H, *J* = 4.73 Hz, 2 H $H_{4III}$), 4.33-4.35 (m, 2H, $H_{2III}$), 4.24 (d, *J* = 5.83 Hz, 2H, $H_{5IV}$ or $H_{3IV}$ ), 4.17-4.22 (m, 4H, $H_{5IV}$ or $H_{3IV}$, $H_{3III}$), 4.14 (t, *J* = 2.99 Hz, 2 H), 3.85-3.95 (m, 8H), 3.65-3.70 (m, 8 H), 3.48-3.55 (m, 4 H), 3.22-3.38 (m, 12 H), 3.12-3.20 (m, 4 H), 3.03 (t, *J* = 9.30 Hz, 2 H), 2.35-2.43 (m, 2 H, $H_{2Ieq}$), 1.89-2.00 (m, 2 H, $H_{2Iax}$); MS MALDI-TOF m/z for $C_{54}H_{98}N_{16}O_{26}$ $[M+Na]^+$, calcd 1410.44, found 1410.79; Anal. Calcd for $C_{54}H_{110}N_{16}O_{26}Cl_{12}$: C, 35.54; H, 6.08; Cl, 23.31; N, 12.28; O, 22.79. Found: C, 35.18; H, 5.92; N, 11.98; UV (water) $\lambda_{max}$ = 253 nm.

**DPA75 (Linker = 1,6-diisothiocyanatohexane).**



$R_f$ = 0.60, (silica gel, $CH_2Cl_2$); IR (KBr, $cm^{-1}$); 3000-3400 (br), 2105 (NCS), 1670 (br), 1420; $^1$H NMR (300 MHz, $CDCl_3$): ∂ 3.31-3.38 (m, 4 H), 1.59-1.62 (m, 4 H), 1.44 (t, *J* = 3.62 Hz, 4H).$^{13}$C NMR (125 MHz, $CDCl_3$, 25 ˚C): ∂ 122 (NCS), 42.82, 31.05, 25.93.

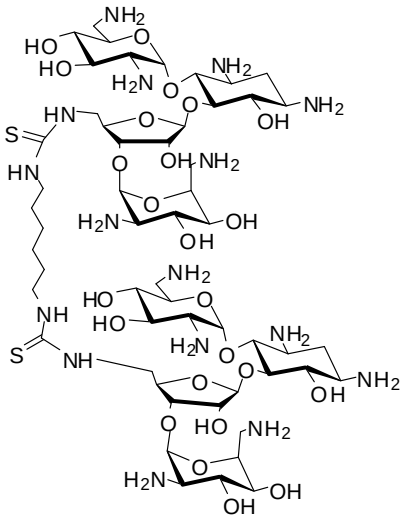
**N-Boc DPA75.**




*N*-Boc DPA75

[$R_f$ = 0.36, 10% $CH_3OH$ in $CH_2Cl_2$, (v/v)]; $^1$H-NMR (500 MHz, $CD_3COCD_3$) δ 6.55-5.92 (m, 12 H, $NH_{6IV}$, $NH_{1I}$, $NH_{6II}$, $NH_{3I}$, $NH_{2IV}$, and $NH_{2II}$), 5.11-5.31 (s, br, 4H, $H_{1II}$, $H_{1III}$), 4.96-5.09 (m, 4 H, $OH_{4IV}$, $H_{1IV}$), 4.85 (s, 2H, OH), 4.72 (s, 2H, OH), 4.40-4.65 (m, 4 H, OH), 4.20-4.31 (m, 4 H, $H_{5IV}$ or $H_{3IV}$), 4.13-4.30 (m, 4 H, $H_{3III}$ and $H_{5IV}$ or $H_{3IV}$), 4.02-4.12 (m, 4 H), 3.91-3.98 (t, 2H, *J* = 7.25 Hz, $H_{2IV}$), 3.81-3.90 (m, 8 H), 3.62 (m, 16 H, -O-$CH_2$-$CH_2$-O-, linker protons), 3.42-3.54 (m, 6 H), 3.17-3.38 (m, 10 H), 1.32-1.63 (m, 110 H, $H_{2Iax}$, 12 × Boc); MALDI-TOF : calcd for $C_{114}H_{202}N_{16}O_{48}S_2$ $[M]^+$ 2627.32, found 2627.14.

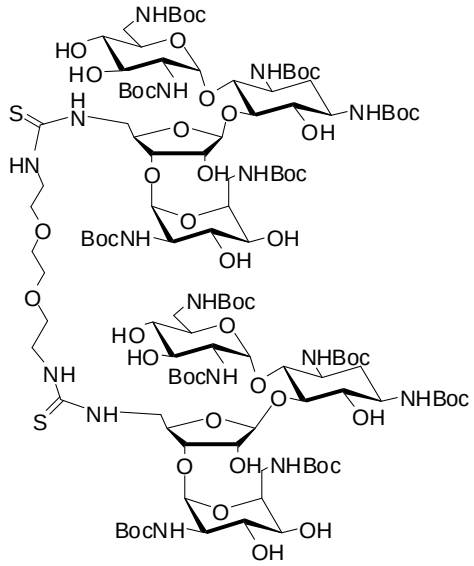
**DPA75.**



DPA 75

IR (KBr, $cm^{-1}$) 3432 (br, OH), 2089, 1644; $^1H$ NMR (500 MHz, $D_2O$) δ 5.91-5.95 (m, 2H, $H_{1II}$), 5.32 (d, *J* = 3.94 Hz, 2H, $H_{1III}$), 5.17 (m, 2H, $H_{1IV}$), 4.30 (t, *J* = 4.73 Hz, 2 H, $H_{4III}$), 4.23 (t, *J* = 4.73 Hz, 2 H), 4.19 (t, *J* = 4.89 Hz, 2 H), 4.13-4.17 (m, 2 H), 4.11 (t, *J* = 2.99 Hz, 2 H), 4.05 (t, *J* = 9.93 Hz, 2 H, $H_{6II}$), 4.92 (t, *J* = 9.14 Hz, 2 H, $H_{5I}$), 3.80-3.88 (m, 4 H), 3.69-3.74 (m, 2 H), 3.63 (t, *J* = 9.93 Hz, 2 H, $H_{3II}$), 3.40-3.50 (m, 4 H), 3.28-3.40 (m, 8 H), 3.21-3.28 (m, 6 H), 3.12-3.17 (m, 2 H), 2.91-3.02 (m, 2 H), 2.34-2.42 (m, 2 H, $H_{2Ieq.}$), 1.77 (q, *J* = 11.51, 2 H, $H_{2Iax.}$); MS MALDI-TOF m/z for $C_{54}H_{106}N_{16}O_{24}S_2$ $[M]^+$, calcd 1427.64, found 1427.57.

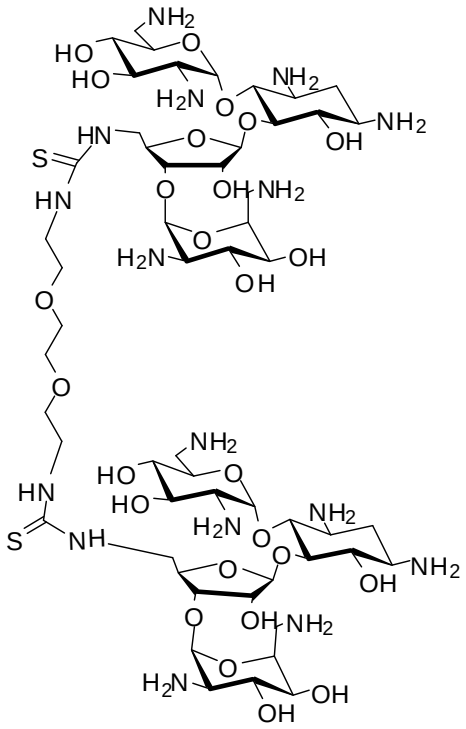
**N-Boc DPA76.**




*N*-Boc DPA76

[$R_f$ = 0.38, 10% $CH_3OH$ in $CH_2Cl_2$, (v/v)]; IR ($cm^{-1}$): 3375 (OH), 2930, 2870, 2150 (-C=S), 1691, 1510; $^1$H-NMR (500 MHz, $CD_3COCD_3$) δ 7.42 (s, 2 H, (S=C-N**H**-$CH_2$-$CH_2$-O-), 7.32 (s, 2 H, $C_{5III}$-$CH_2$-$CH_2$-**NH**-C=S), 6.59 (t, 2H, *J* = 5 Hz, $NH_{6IV}$), 6.47 (m, 2H, $NH_{1I}$), 6.34-5.90 (m, 8 H, $NH_{6II}$, $NH_{3I}$, $NH_{2IV}$, and $NH_{2II}$), 5.29 (s, 2H, $H_{1II}$), 5.13 (s, 2H, $H_{1III}$), 4.95-5.05 (m, 4 H, $OH_{4IV}$, $H_{1IV}$), 4.77 (s, 2H, OH), 4.63 (s, 2H, OH), 4.38-4.49 (m, 4 H, OH), 4.21-4.32 (m, 4 H, $H_{5IV}$ or $H_{3IV}$), 4.10-4.21 (m, 4 H, $H_{3III}$ and $H_{5IV}$ or $H_{3IV}$), 4.05 (s, 2H, $H_{4IV}$), 3.94 (t, 2H, *J* = 7.25 Hz, $H_{2IV}$), 3.80-3.92 (m, 8 H), 3.70-3.81 (m, 8 H), 3.66 (m, 12 H, -O-$CH_2$-$CH_2$-O-, linker protons), 3.39-3.58 (m, 12 H), 3.17-3.37 (m, 10 H), 1.35-1.60 (m, 110 H, $H_{2Iax}$, 12 × Boc); MALDI-TOF : calcd for $C_{118}H_{210}N_{16}O_{50}S_2$ $[M+Na]^+$ 2650.33, found 2650.44.

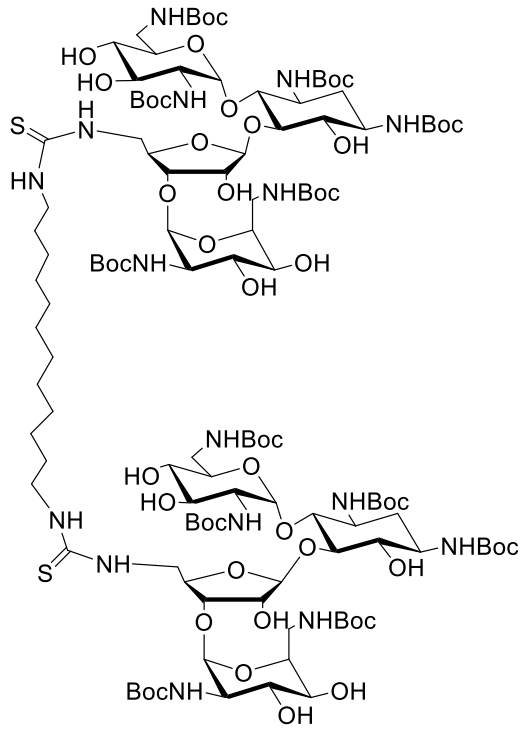
**DPA76.**



DPA 76

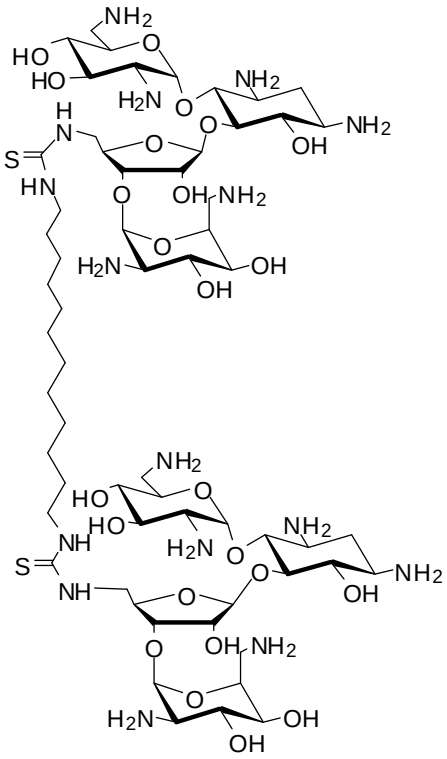
IR (KBr, $cm^{-1}$) 3433 (br, OH), 2926, 1648, 1524; $^1H$ NMR (500 MHz, $D_2O$) δ 5.96 (m, 2H, $H_{1II}$), 5.32 (s, 2H, $H_{1III}$), 5.18 (s, 2H, $H_{1IV}$), 4.38 (m, 2H, $H_{4III}$), 4.23-4.35 (m, 4H, $H_{2III}$), 4.07-4.16 (m, 2H, $H_{5IV}$ or $H_{3IV}$ ), 4.02 (t, *J* = 9.78 Hz, 2 H, $H_{6II}$), 3.92 (t, *J* = 9.93 Hz, 2 H), 3.78-3.87 (m, 8 H), 3.72 (m, 4 H), 3.54-3.68 (m, 12 H), 3.39-3.53 (m, 10 H), 3.14-3.38 (m, 16 H), 2.30-2.43 (m, 2 H, $H_{2Ieq}$), 1.74-1.88 (m, 2 H, $H_{2Iax}$); MS MALDI-TOF m/z for $C_{54}H_{106}N_{16}O_{26}S_2$ $[M+H_2O]^+$, calcd 1482.64, found 1481.89; Anal. Calcd for $C_{54}H_{118}N_{16}O_{26}Cl_{12}$ $S_2$: C, 34.19; H, 6.27; Cl, 22.42; N, 11.81; O, 21.93; S, 3.38. Found: C, 33.95; H,6.16; N, 11.68.

**N-Boc DPA77.**




[$R_f$ = 0.40, 10% $CH_3OH$ in $CH_2Cl_2$, (v/v)]; $^1$H-NMR (500 MHz, $CD_3COCD_3$) δ 6.54-6.63 (t, 2H, *J* = 5 Hz, $NH_{6IV}$), 6.40-6.53 (m, 2H, $NH_{1I}$), 6.35-5.87 (m, 10 H, $NH_{6II}$, $NH_{3I}$, $NH_{2IV}$, and $NH_{2II}$), 5.28 (s, 2H, $H_{1II}$), 5.15 (s, 2H, $H_{1III}$), 4.94-5.08 (m, 4 H, $OH_{4IV}$, $H_{1IV}$), 4.75 (s, 2H, OH), 4.62 (s, 2H, OH), 4.40-4.51 (m, 4 H, OH), 4.20-4.31 (m, 4 H, $H_{5IV}$ or $H_{3IV}$), 4.11-4.20 (m, 4 H, $H_{3III}$ and $H_{5IV}$ or $H_{3IV}$), 4.01-4.11 (s, 4H), 3.91-3.98 (t, 2H, *J* = 7.25 Hz, $H_{2IV}$), 3.81-3.90 (m, 8 H), 3.71-3.81 (m, 8 H), 3.66 (m, 8 H, -O-$CH_2$-$CH_2$-O-, linker protons), 3.39-3.58 (m, 10 H), 3.20-3.38 (m, 10 H), 1.32-1.63 (m, 110 H, $H_{2Iax}$, 12 × Boc); MALDI-TOF : calcd for $C_{120}H_{214}N_{16}O_{48}S_2$ $[M]^+$ 2711.40, found 2710.96.

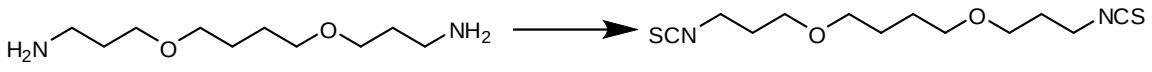
**DPA77.**



DPA 77

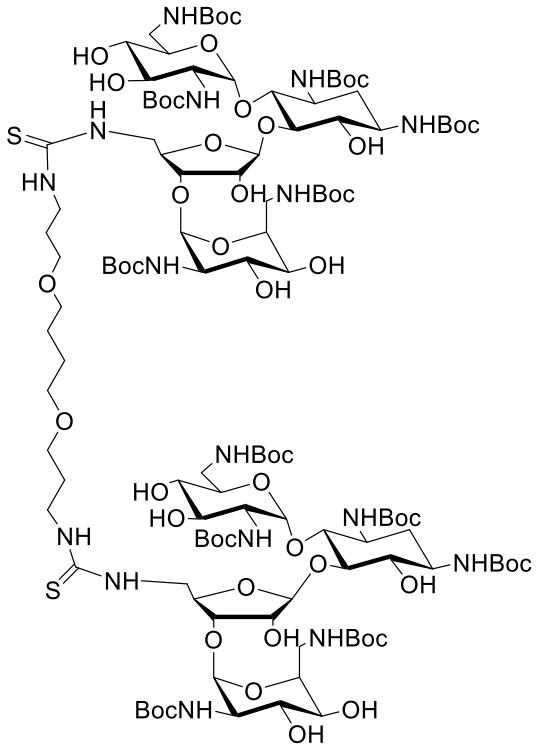
IR (KBr, $cm^{-1}$) 3412 (br, OH), 2928, 1684, 1521, 1366; $^{1}H$ NMR (500 MHz, $D_2O$): 5.94 (d, *J* = 4.09 Hz, 2 H, $H_{1II}$), 5.30 (d, *J* = 2.68 Hz, 2 H, $H_{1III}$), 5.19 (d, *J* = 1.58 Hz, 2 H, $H_{1IV}$), 4.29-4.34 (m, 4 H), 4.16-4.23 (m, 4 H), 4.11 (t, *J* = 2.99 Hz, 2 H), 4.02-4.08 (m, 2 H), 3.98 (t, *J* = 10.25 Hz, 2 H, $H_{6II}$), 3.76-3.84 (m, 4 H), 3.90 (t, *J* = 9.14 Hz, 2 H, $H_{5I}$), 3.76-3.84 (m, 4 H), 3.69-3.73 (m, 2 H), 3.59 (t, *J* = 9.15 Hz, 2 H), 3.44-3.51 (m, 4 H), 3.42 (d, *J* = 3.94 Hz, 2 H), 3.40 (d, *J* = 4.10 Hz, 2 H), 3.30-3.80 (m, 6 H), 3.20-3.30 (m, 8 H), 3.09-3.18 (m, 6 H), 3.01 (dd, *J1* = 3.47 Hz, *J2* = 3.62 Hz, 2 H), 2.76-2.84 (m, 4 H, linker protons), 2.70 (q, *J* = 8.98 Hz, 2 H), 2.37 (dt, *J1* = 3.94 Hz, *J2* = 4.25 Hz, 2 H, $H_{2Ieq.}$), 1.80 (q, *J* = 12.61 Hz, 2 H, $H_{2Iax.}$), 1.19 (s, 2 H, linker protons), 1.05-1.12 (m, 18 H, linker protons); MS MALDI-TOF m/z for $C_{60}H_{118}N_{16}O_{24}S_2$ $[M+Na]^+$, calcd 1534.98, found 1535.81.

**N-Boc DPA78 (Linker = 4, 9-dioxa-1, 12- dodecadiisothiocyanate).**




$R_f$ = 0.50, (silica gel, $CH_2Cl_2$); IR (KBr, $cm^{-1}$) 3350, 2950, 2110 (NCS), 1250, 1050; $^1H$ NMR (300 MHz, $CDCl_3$): $\partial$ 3.65 (t, *J* = 4.22 Hz, 4H), 3.55(t, *J* = 3.84 Hz, 4H), 3.46 (t, *J* = 3.72 Hz, 4H), 1.93 (p, *J* = 3.64 Hz, 4H), 1.61 (p, *J* = 3.58 Hz, 4H).$^{13}C$ NMR (125 MHz, $CDCl_3$) : $\partial$ 131, 70.88, 66.58, 53.5, 42.15, 30.21, 26.36; MS/GC calcd.: 288.07, obsd ($M^+$): 288.

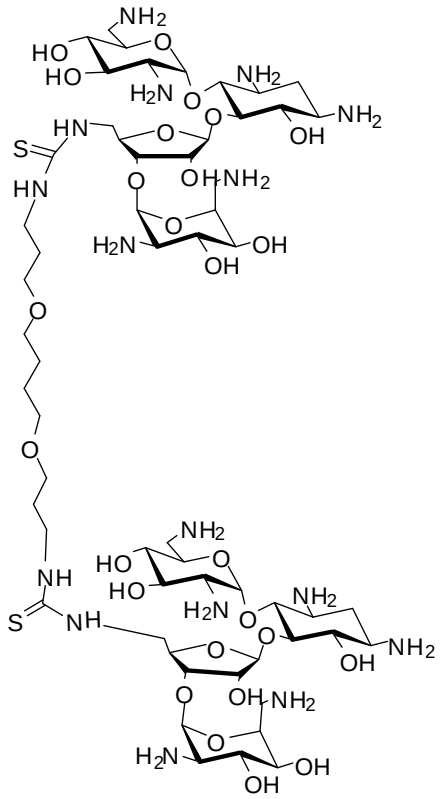
**N-Boc DPA78.**




[$R_f$ 0.40, 10 % MeOH in $CH_2Cl_2$, (v/v)]; $^1H$ NMR (300 MHz, $CDCl_3$, 25 °C); $\partial$ 6.15 (s, 2H), 5.46 (br s, 2 H), 5.13 (br s, 2 H), 4.92 (m, 2 H), 4.23 (m, 2 H), 4.09 (br s, 4 H), 3.88-3.91 (m, 4 H), 3.74-3.77 (m, 4 H), 3.63-3.67 (m, 4 H), 3.53-3.56 (m, 4 H), 3.44-3.47 (m,

4 H), 3.56-3.44 (m, 30 H), 3.00-3.30 (m, 24 H), 1.92-1.95 (m, 2 H), 1.87-1.91 (m, 4 H), 1.60-1.64 (m, 8 H), 1.38-1.58 (m, 110 H); $^{13}$C NMR (500 MHz, $D_2O$) δ 167 (C=S), 155-158 (m, 6 × Boc, C=O), 100, 71, 69, 67, 62, 53, 52, 51, 42.5, 38.5, 33, 32, 31.8, 31.1, 31, 27.5, 26, 25; MALDI-TOF : calcd for $C_{118}H_{210}N_{16}O_{50}S_2$ $[M]^+$ 2716.39, found 2716.90.

**DPA78.**



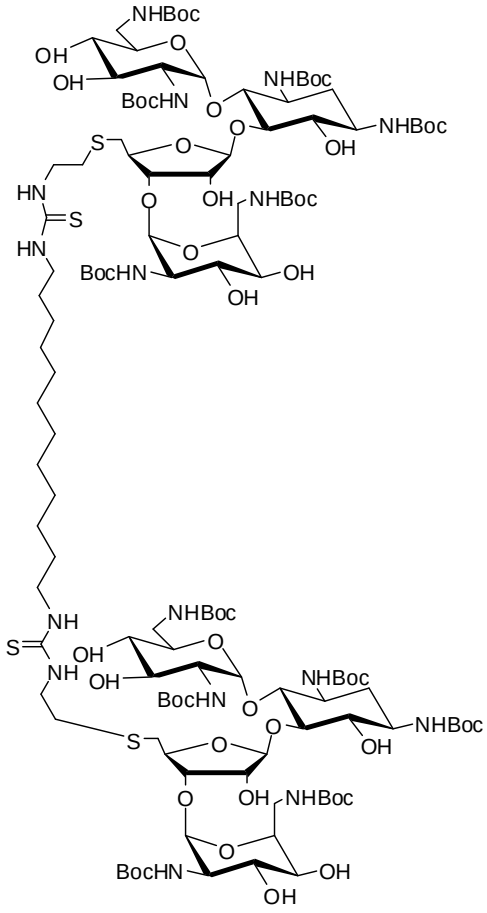
DPA 78

$^1$H NMR (500 MHz, $D_2O$): 5.99 (d, *J* = 3.92 Hz, 2 H, $H_{1II}$), 5.28-5.36 (m, 2 H, $H_{1III}$), 5.16-5.22 (m, 2 H, $H_{1IV}$), 4.36-4.44 (m, 2 H), 4.22-4.32 (m, 4 H), 4.17-4.23 (m, 4 H), 4.08-4.15 (m, 2 H), 4.03 (t, *J* = 9.78 Hz, 2 H, $H_{6II}$), 3.90 (t, *J* = 9.93 Hz, 2 H, $H_{5I}$), 3.78-3.87 (m, 4 H), 3.67-3.77 (m, 4 H), 3.54-3.65 (m, 12 H, linker protons), 3.40-3.54 (m, 10 H), 3.32-3.39 (m, 6 H), 3.30 (t, *J* = 4.58 Hz, 2 H), 3.12-3.29 (m, 8 H), 2.38 (dt, *J1* = 3.78 Hz, *J2* = 4.41 Hz, 2 H, $H_{2Ieq.}$), 1.71-1.91 (m, 4 H, $H_{2Iax.}$, linker protons), 1.05-1.25 (m, 4

H, linker protons), 1.05-1.12 (m, 18 H, linker protons); $^{13}$C NMR (500 MHz, $D_2O$) δ 176, 109, 97, 91, 77, 75, 74, 73, 72, 70.9, 70.6, 70.1, 69.5, 68.1, 68, 62.5, 61, 53, 52, 51, 50, 49, 48, 42.5, 40.5, 40.1, 27.5, 25; MALDI-TOF : calcd for $C_{58}H_{114}N_{16}O_{26}S_2$ $[M+H_2O]^+$ 1533.75, found 1533.43.

**DPA79 (**Synthesis and characterization of DPA79 is reported in S. Kumar, L. Xue and D. P. Arya, *J. Am. Chem. Soc.*, 2011, **133**, 7361-7375).

**N-Boc DPA80.**

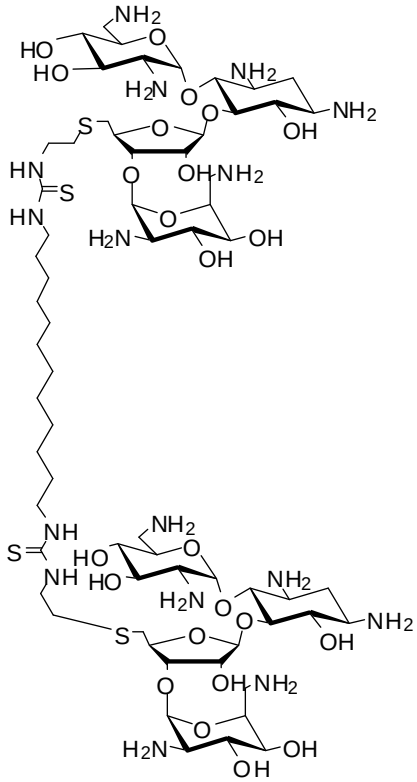



*N*-Boc DPA80

[$R_f$ = 0.42, 10% $CH_3OH$ in $CH_2Cl_2$ (v/v)]; IR (KBr, $cm^{-1}$) 3300 (br, OH), 2950, 2902, 2833, 2107 (br, -C=S), 1727, 1615, 1450; $^1$H-NMR (500 MHz, $CD_3COCD_3$) δ 7.58 (s, 2

H, (S=C-N**H**-$CH_2$-$CH_2$-O-), 6.60 (m, 2 H, $NH_{6IV}$), 6.19-6.35 (m, 4 H, $NH_{1I}$, $NH_{6II}$), 6.14 (s, br, 2 H, $NH_{3I}$), 6.06 (d, *J* = 9.62 Hz, 2 H, $NH_{2IV}$), 5.91 (s, 2 H, $NH_{2II}$), 5.77 (m, 2 H, OH), 4.96-5.14 (m, 8 H, $H_{1II}$, $H_{1III}$, $OH_{4IV}$, $H_{1IV}$), 4.76 (s, 2 H, $OH_{6I}$), 4.56-4.70 (s, 2 H, $OH_{4II}$), 4.18-4.27 (m, 4 H, $H_{3III}$ and $H_{5IV}$ or $H_{3IV}$), 4.10-4.17 (m, 2 H, $H_{3IV}$ or $H_{5IV}$), 3.98-4.10 (m, 8 H, $H_{4IV,}$ $H_{2IV}$), 3.90-3.98 (m, 2 H), 3.78-3.86 (m, 6 H, $H_{5II}$ or $H_{5I}$), 3.74 (t, *J* = 8.82 Hz, 2 H, $H_{5II}$ or $H_{5I}$), 3.51-3.70 (m, 12 H, $H_{6I}$, $H_{4I}$, $H_{3I}$, $H_{5III}$, and $H_{2II}$), 3.38-3.51 (m, 8 H, $H_{3II}$ and $H_{1I}$), 3.22-3.38 (m, 10 H), 3.12-3.22 (m, 4 H, $H_{3I}$ or $H_{1I}$), 2.98-3.12 (m, 4 H, $H_{3I}$ or $H_{1I}$), 1.10-1.65 (m, 130 H, $H_{2Iax}$, 6 × Boc, linker protons); MS MALDI-TOF calcd. for $C_{124}H_{222}N_{16}O_{50}S_2$ ($M+Na^+$), 2817.30, obsd, 2818.45.

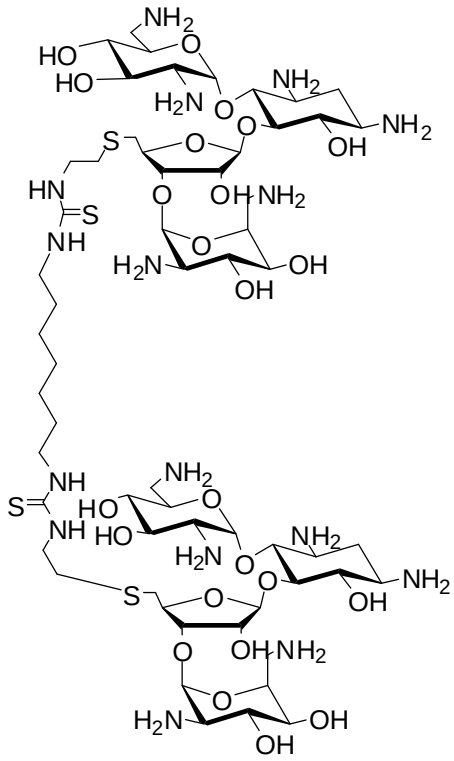
**DPA80.**



DPA 80

IR (KBr, $cm^{-1}$) 3432 (br, OH), 2102, 1646; $^1H$ NMR (500 MHz, $D_2O$): 5.96 (t, *J* = 3.94 Hz, 2 H, $H_{1II}$), 5.32 (m, 2 H, $H_{1III}$), 5.18-5.24 (m, 2 H, $H_{1IV}$), 4.29-4.36 (m, 4 H), 4.17-4.26 (m, 4 H), 4.10-4.15 (m, 2 H), 3.97-4.09 (m, 4 H, $H_{6I}$), 3.92 (t, *J* = 9.30 Hz, 2 H, $H_{5I}$), 3.77-3.87 (m, 6 H), 3.68-3.74 (m, 2 H), 3.60 (t, *J* = 9.30 Hz, 2 H), 3.47-3.52 (m, 2 H), 3.40-3.47 (m, 6 H), 3.31-3.40 (m, 8 H), 3.20-3.32 (m, 8 H), 3.09-3.20 (m, 6 H), 2.98-3.05 (m 2 H), 2.96 (m, 2 H), 2.77-2.89 (m, 4 H), 2.72 (q, *J* = 8.98 Hz, 2 H), 2.37 (dt, *J1* = 3.94 Hz, *J2* = 4.25 Hz, 2 H, $H_{2Ieq.}$), 1.81 (q, *J* = 12.45 Hz, 2 H, $H_{2Iax.}$), 1.25-1.32 (s, 4 H, linker protons), 1.04-1.13 (m, 18 H, linker protons); MS MALDI-TOF m/z for $C_{64}H_{126}N_{16}O_{24}S_4$ $[M+2H_2O]^+$, calcd 1668.04, found 1668.15.

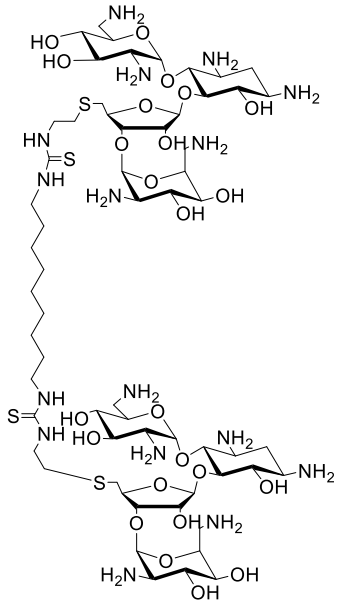
**DPA311.**



DPA311

IR (KBr, $cm^{-1}$) 3433 (br, OH), 2110, 1641; $^1H$ NMR (500 MHz, $D_2O$) δ 5.85-5.60 (m, 2 H, $H_{1II}$), 5.25-5.38 (m, 2 H, $H_{1III}$), 5.10-5.20 (m, 2 H, $H_{1IV}$), 4.15-4.35 (m, 8H), 4.08-4.14 (m, 2 H), 3.97-4.06 (m, 2 H), 3.88-3.96 (m, 2 H, $H_{5I}$), 3.75-3.86 (m, 4 H), 3.68-3.74 (m, 2 H), 3.54-3.64 (m, 4 H), 2.95-3.50 (m, 30 H), 2.50-2.85 (m, 6 H), 2.30-2.40 (m, 2 H, $H_{2Ieq.}$), 1.71-1.90 (m, 2 H, $H_{2Iax.}$), 1.30-1.58 (m, 4 H, linker protons), 1.02-1.25 (m, 12 H, linker protons); MS MALDI-TOF m/z for $C_{59}H_{116}N_{16}O_{24}S_4$ $[M+H_2O]^+$, calcd 1579.91, found 1580.21.

**DPA312.**

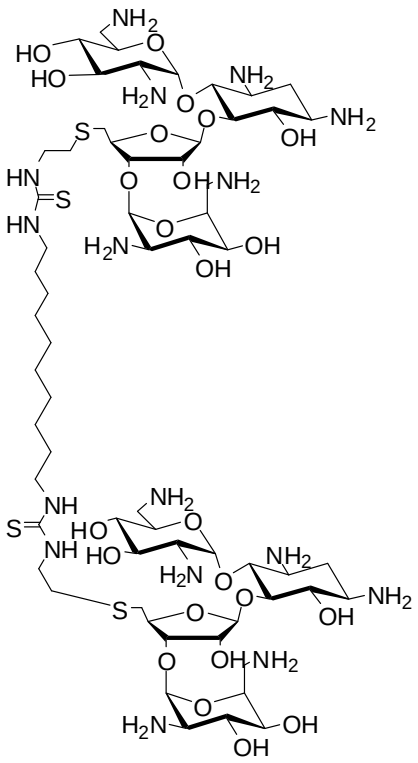


DPA312

IR (KBr, $cm^{-1}$) 3430 (br, OH), 2106, 1642; $^1H$ NMR (500 MHz, $D_2O$) δ 5.85-5.96 (m, 2 H, $H_{1II}$), 5.20-5.28 (m, 2 H, $H_{1III}$), 5.10-5.15 (m, 2 H, $H_{1IV}$), 4.17-4.30 (m, 6 H), 4.12-4.16 (m, 2 H), 4.06 (t, *J* = 2.84 Hz, 2 H), 3.94 (t, *J* = 9.78 Hz, 2 H, $H_{5I}$), 3.86 (t, *J* = 9.30 Hz, 2 H), 3.70-3.80 (m, 6 H), 3.64-3.68 (m, 2 H), 3.48-3.59 (m, 6 H), 3.41-3.45 (m, 2 H), 3.31-3.40 (m, 4 H), 3.26-3.30 (m, 6 H), 3.14-3.25 (m, 8 H), 3.04-3.13 (m, 4 H), 2.94-3.04 (m,

2 H), 2.55-2.80 (m, 6 H), 2.27-2.37 (m, 2 H, $H_{2Ieq.}$), 1.75 (q, $J$ = 12.45 Hz, 2 H, $H_{2Iax.}$), 1.30-1.41 (m, 4 H, linker protons), 1.02-1.20 (m, 12 H, linker protons); MS MALDI-TOF m/z for $C_{61}H_{120}N_{16}O_{24}S_4$ $[M+H_2O]^+$, calcd 1589.96, found 1608.0.

**DPA313.**



DPA313

IR (KBr, $cm^{-1}$) 3431 (br, OH), 2104, 1646; $^1H$ NMR (500 MHz, $D_2O$) δ 5.85-5.97 (m, 2 H, $H_{1II}$), 5.24-5.33 (m, 2 H, $H_{1III}$), 5.13-5.21 (m, 2 H, $H_{1IV}$), 4.20-4.37 (m, 6 H), 4.13-4.19 (m, 2 H), 4.05-4.12 (m, 2 H), 3.96 (t, $J$ = 9.62 Hz, 2 H, $H_{5I}$), 3.88 (t, $J$ = 9.62 Hz, 2 H), 3.72-3.83 (m, 6 H), 3.66-3.72 (m, 2 H), 3.51-3.64 (m, 6 H), 3.29-3.50 (m, 10 H), 3.16-3.30 (m, 6 H), 2.95-3.15 (m, 6 H), 2.60-2.83 (m, 6 H), 2.30-2.40 (m, 2 H, $H_{2Ieq.}$), 1.75 (q, $J$ = 12.13 Hz, 2 H, $H_{2Iax.}$), 1.30-1.57 (m, 4 H, linker protons), 1.02-1.20 (m, 12 H, linker

protons); MS MALDI-TOF m/z for $C_{62}H_{122}N_{16}O_{24}S_4$ $[M+H_2O]^+$, calcd 1603.98, found 1622.18.

*Hexa-N-Boc deoxy-neomycin-5''-isothiocyanate* (A).

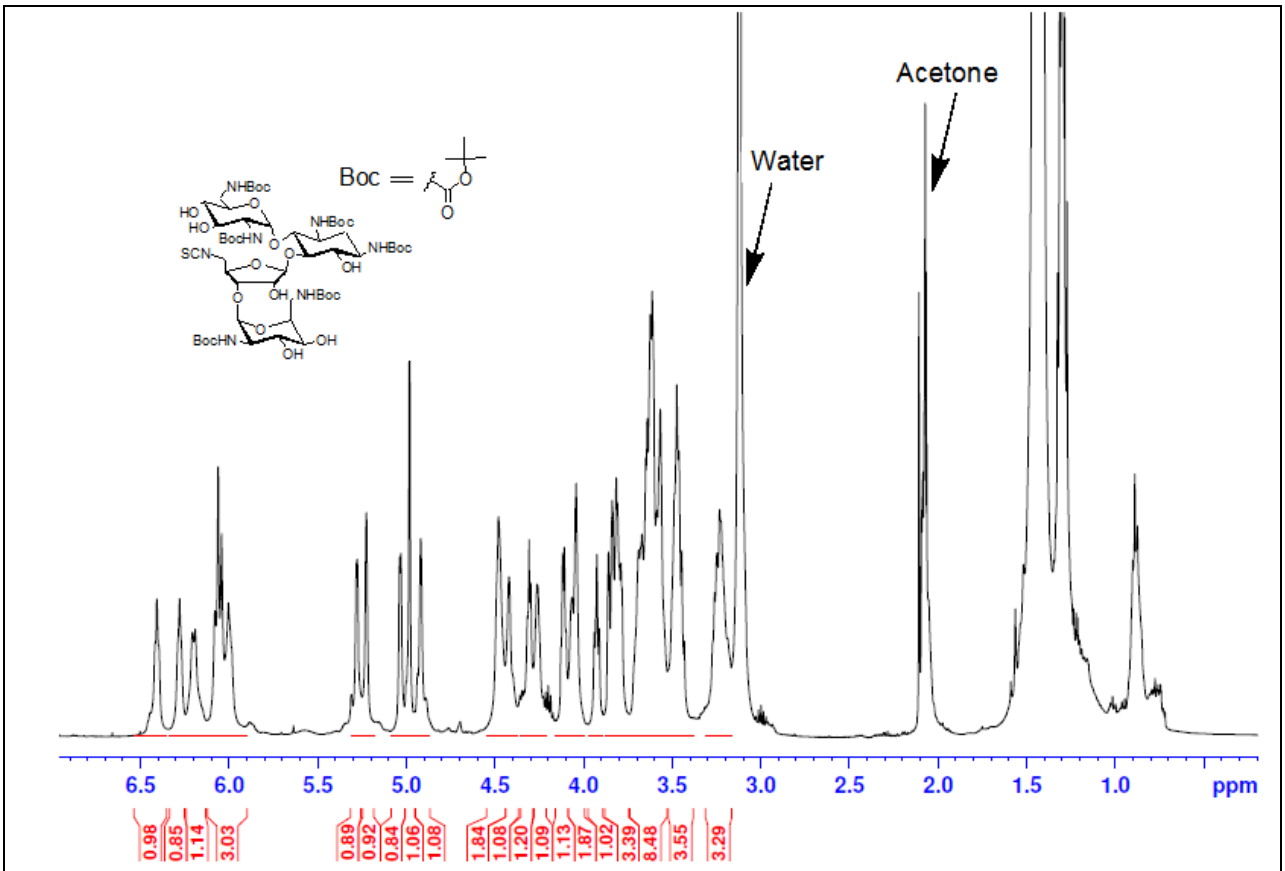



***Figure S1.*** $^{1}$H-NMR of A in $CD_3COCD_3$.

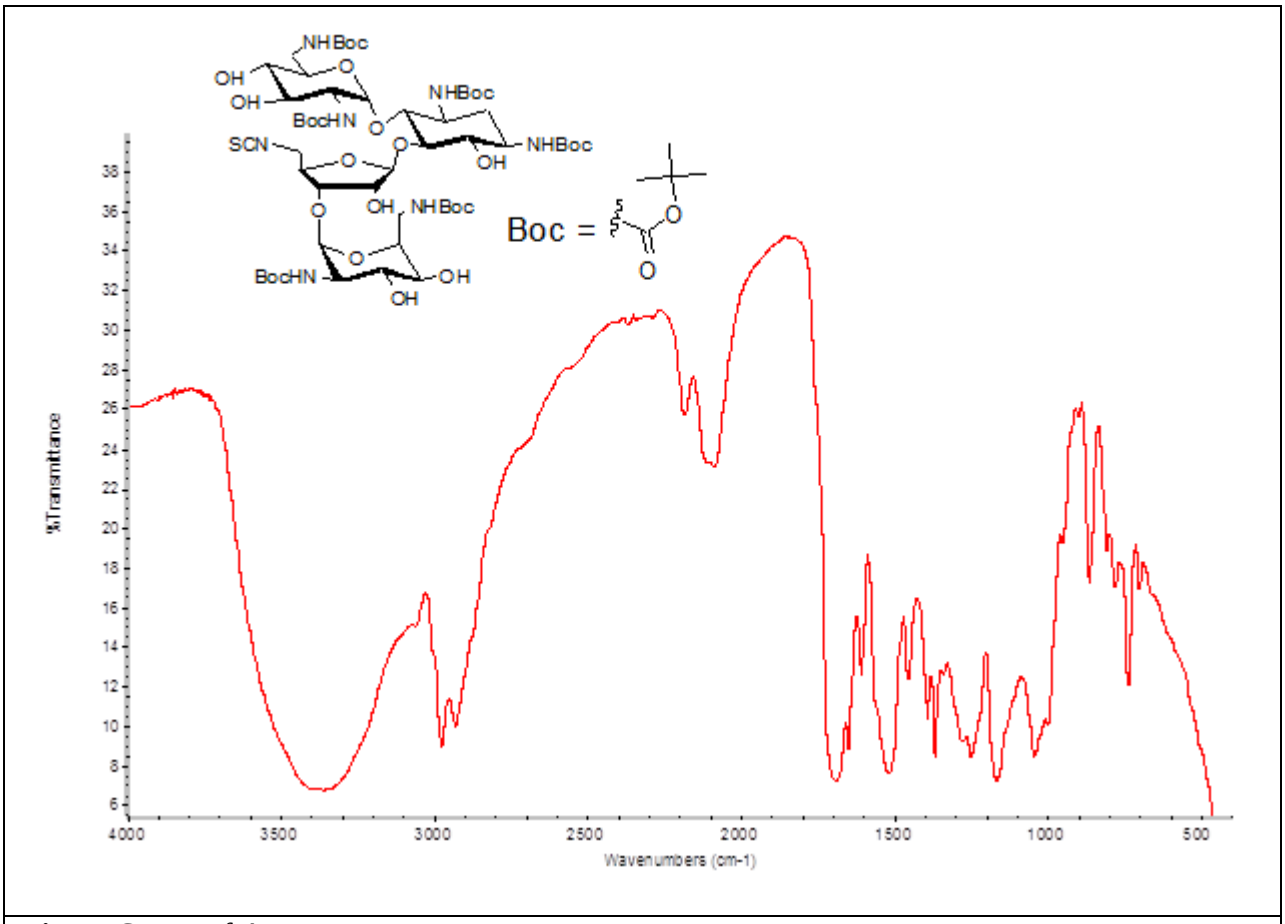



***Figure S2.*** IR of A.

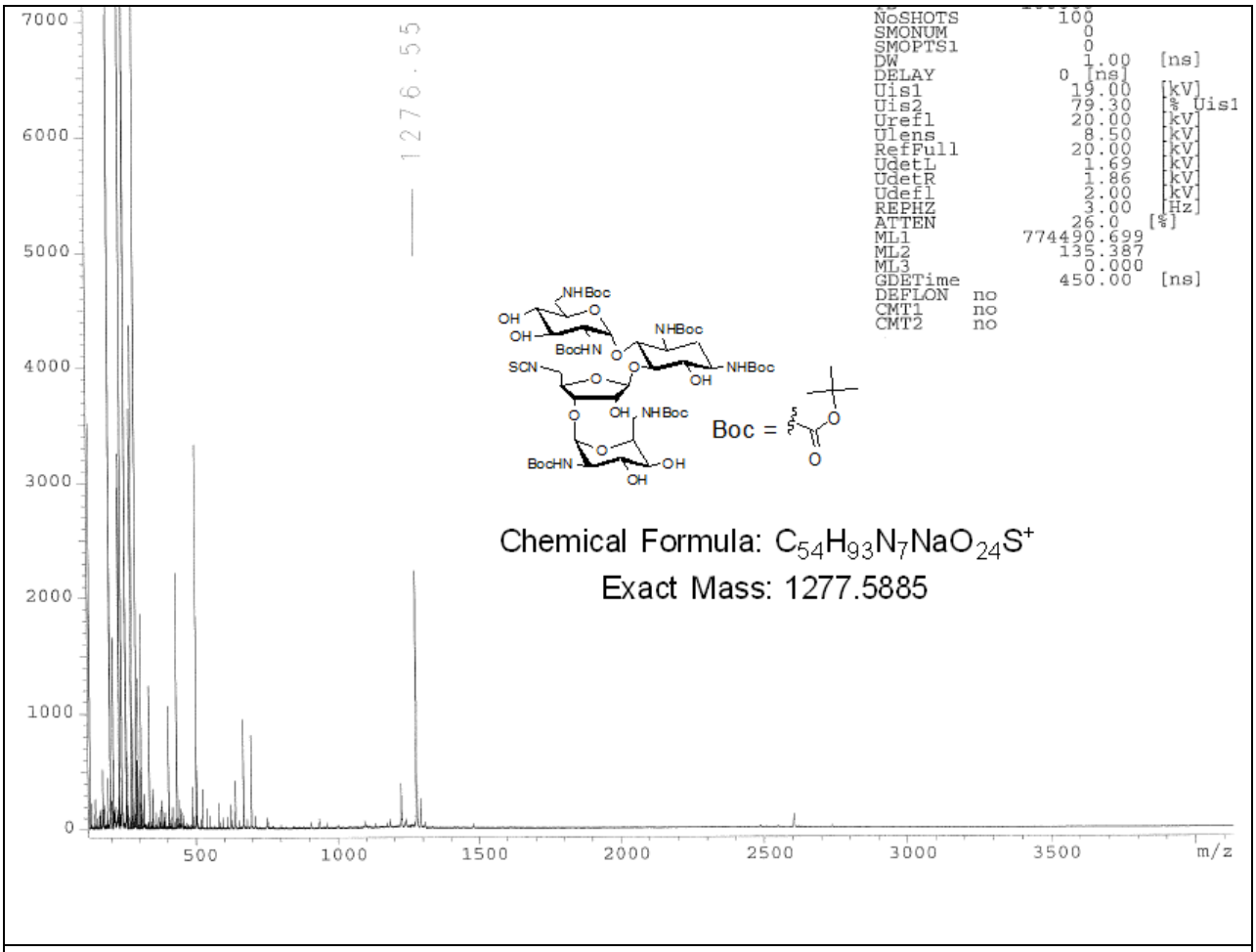



***Figure S3.*** MALDI-TOF of A.

N-Boc DPA 71.

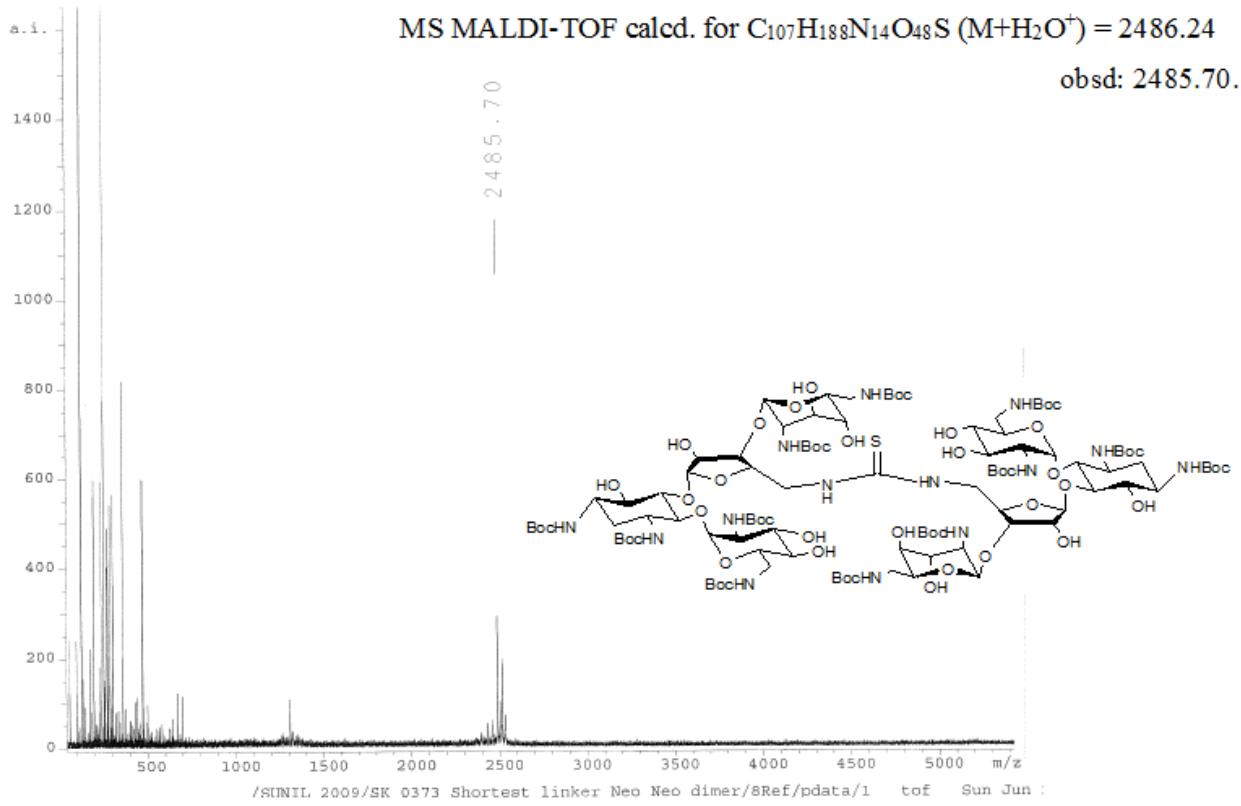



***Figure S4.*** MALDI-TOF of N-Boc DPA71.

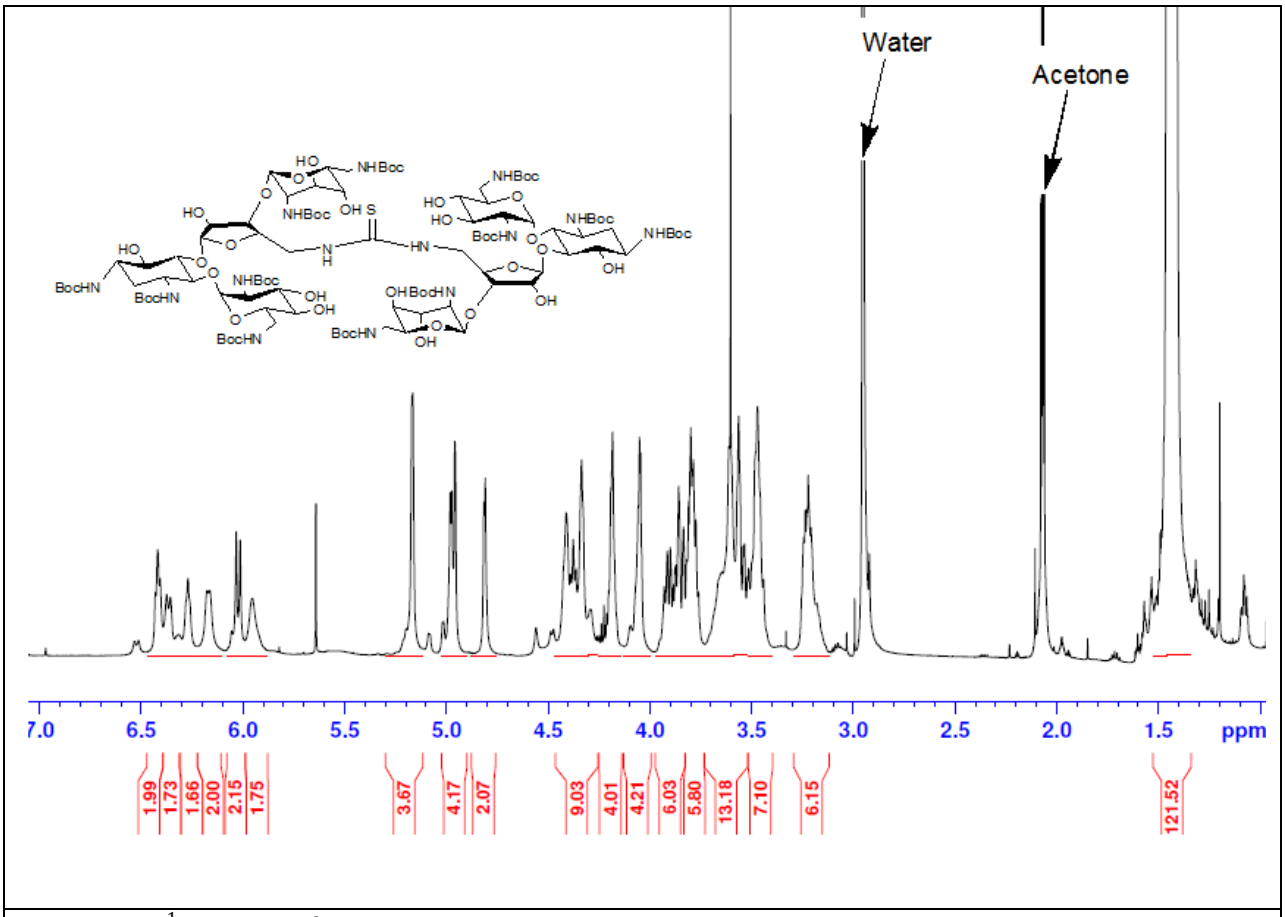



***Figure S5.*** $^{1}$H-NMR of N-Boc DPA71 in $CD_3COCD_3$.

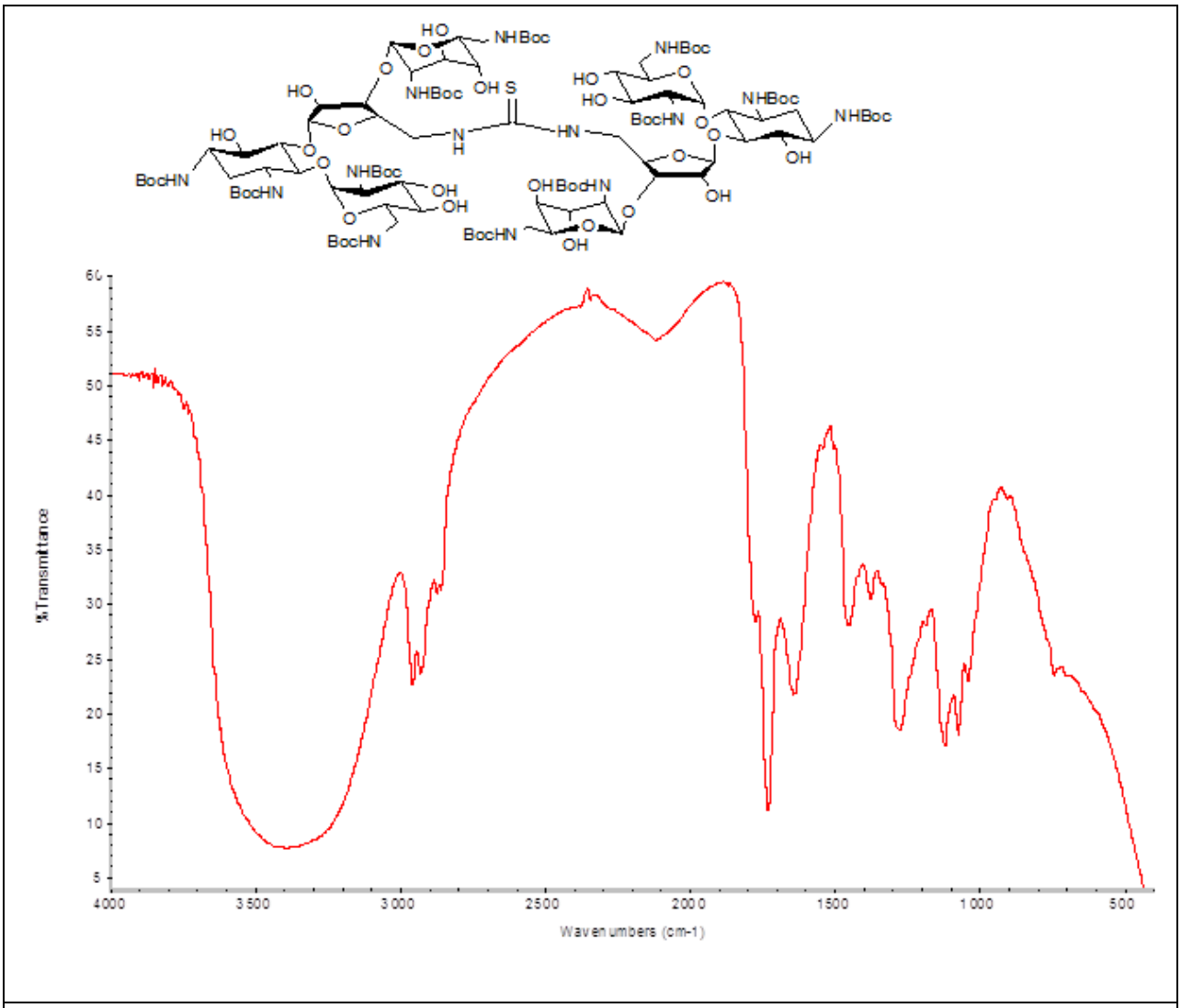



***Figure S6..*** IR of N-Boc DPA71.

DPA71.

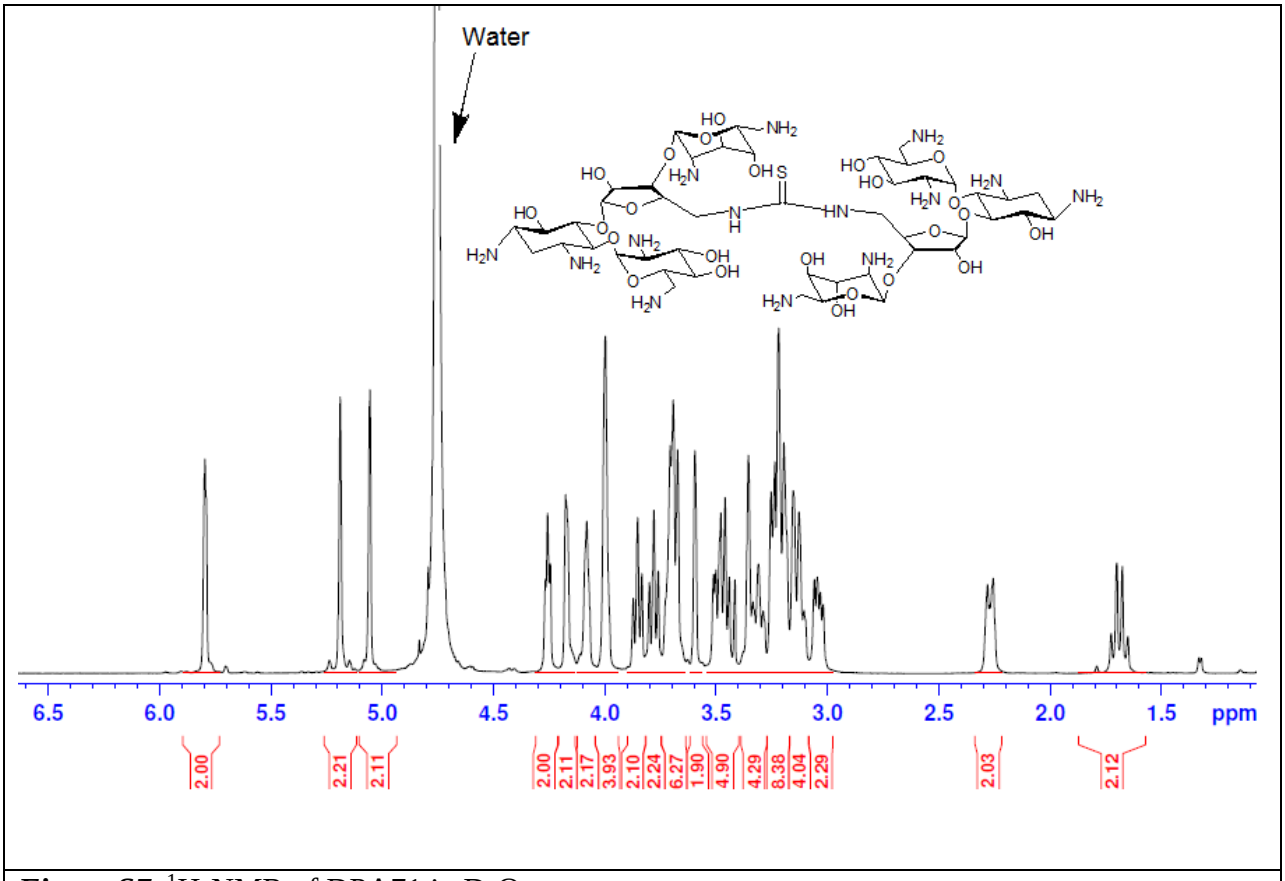



***Figure S7.*** $^{1}$H-NMR of DPA71 in $D_2O$.

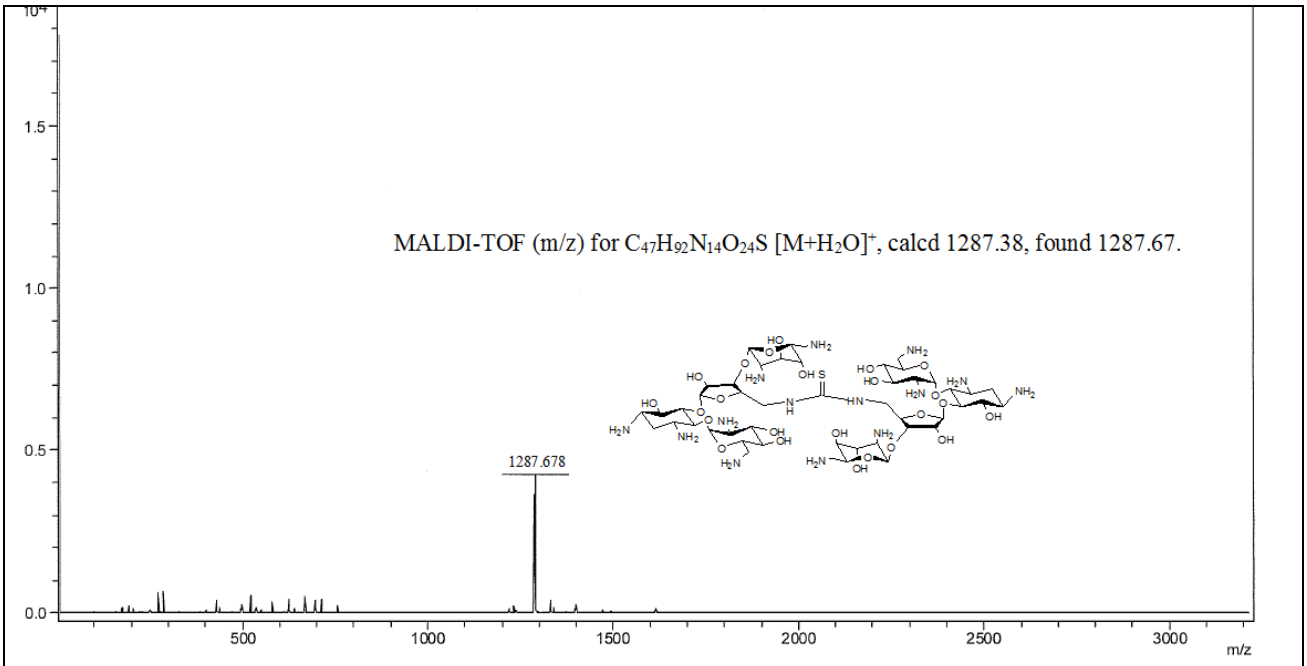



***Figure S8.*** MALDI-TOF of DPA71.

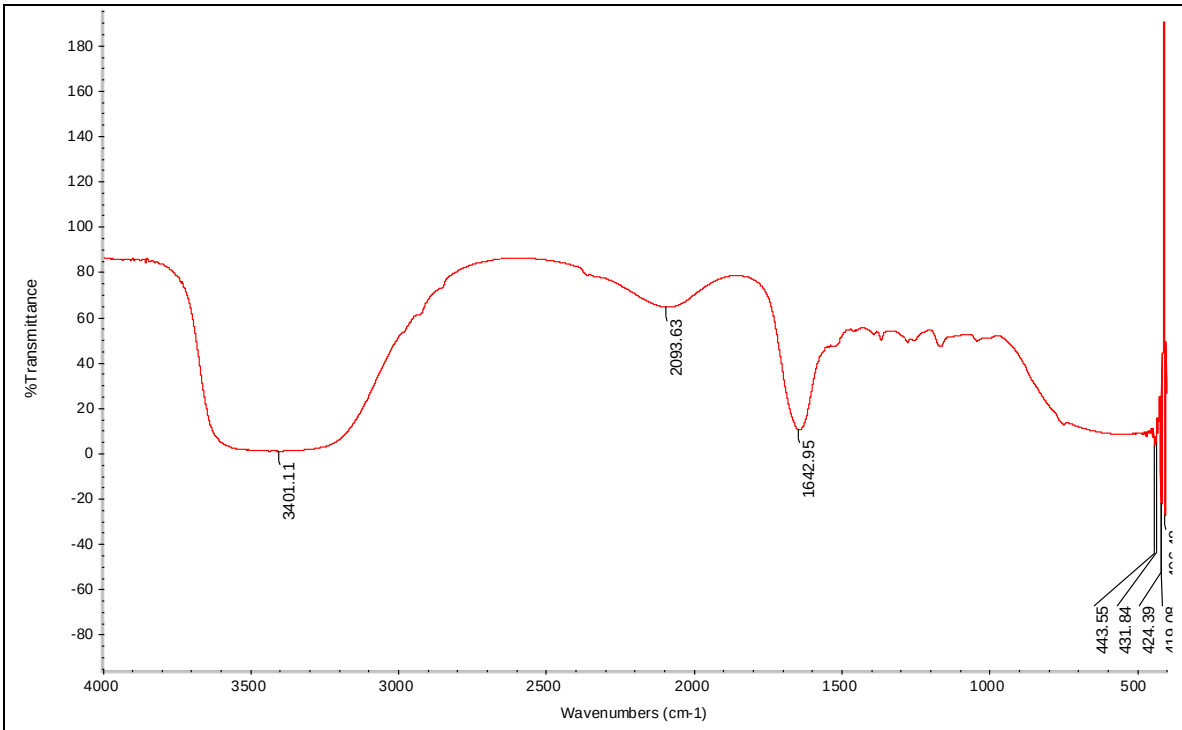



***Figure 9.*** IR of DPA71.

DPA 72.

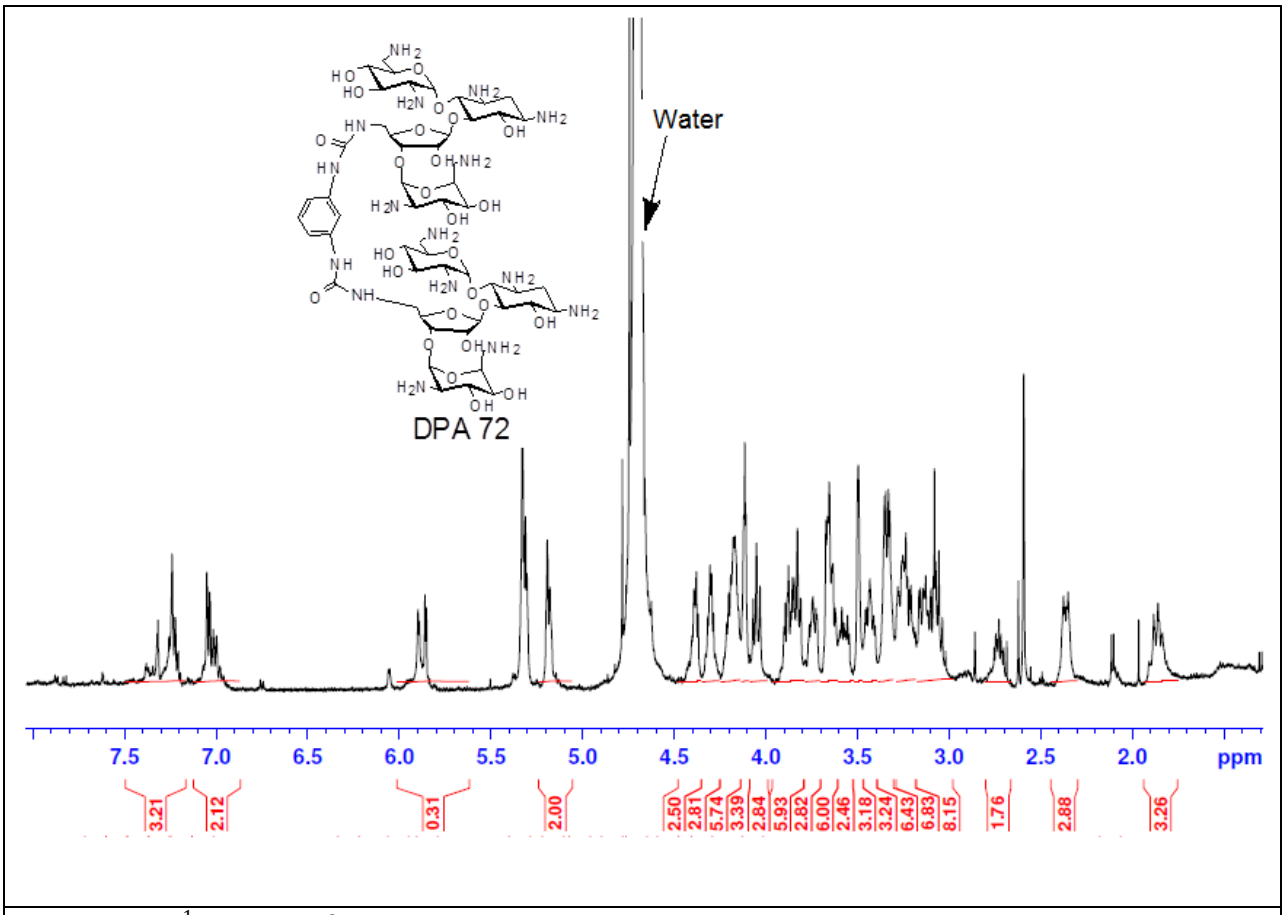



***Figure S10.*** $^{1}$H-NMR of DPA72 in $D_2O$.

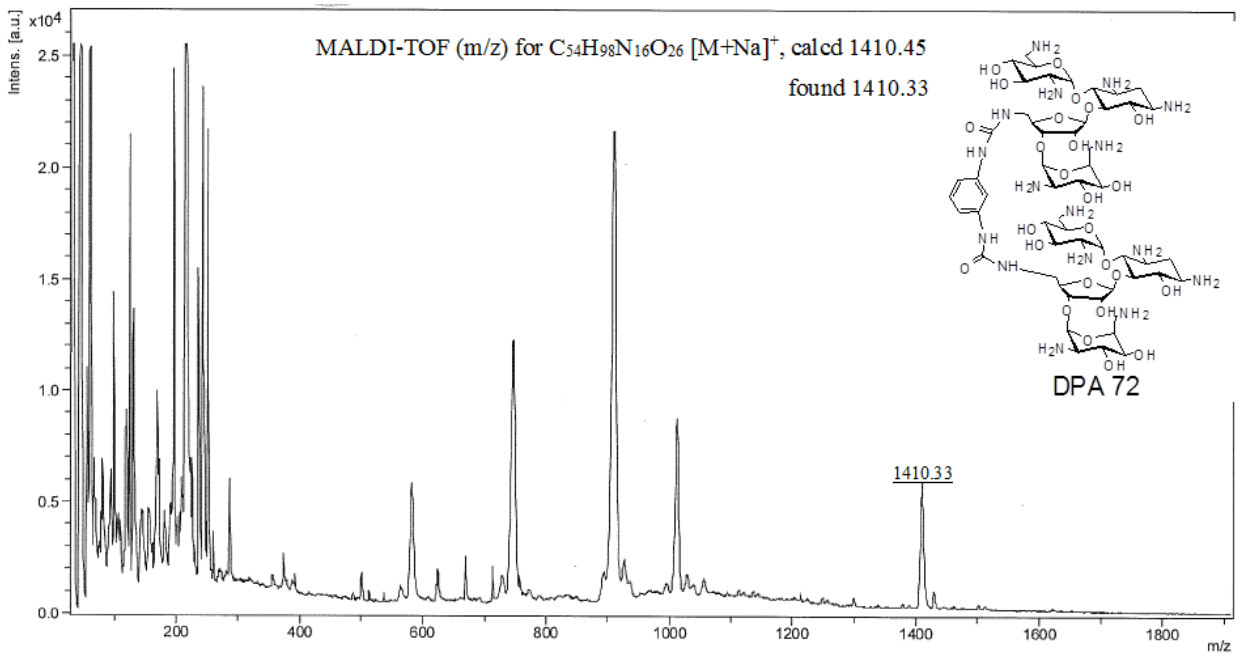



***Figure S11.*** MALDI-TOF of DPA72.

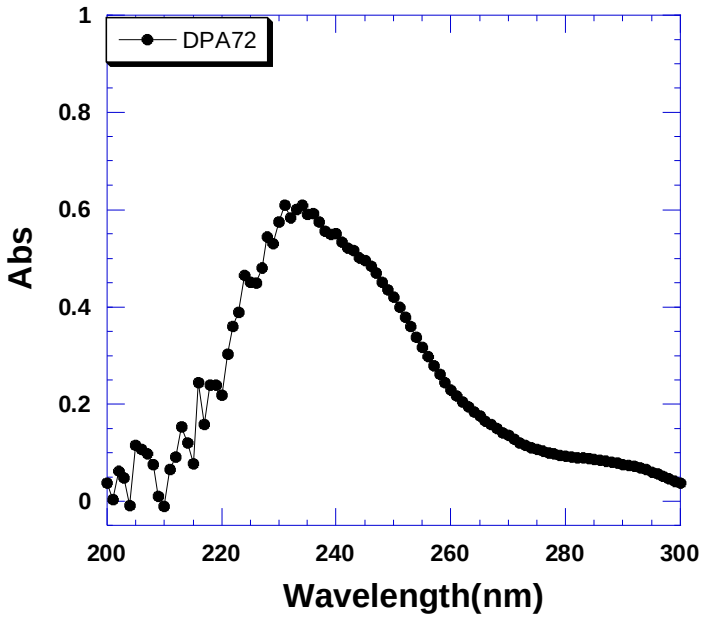



***Figure S12.*** UV absorbance scan of DPA72. Scan was taken in water and the concentration of DPA72 was 25 µM. T = 25 °C.

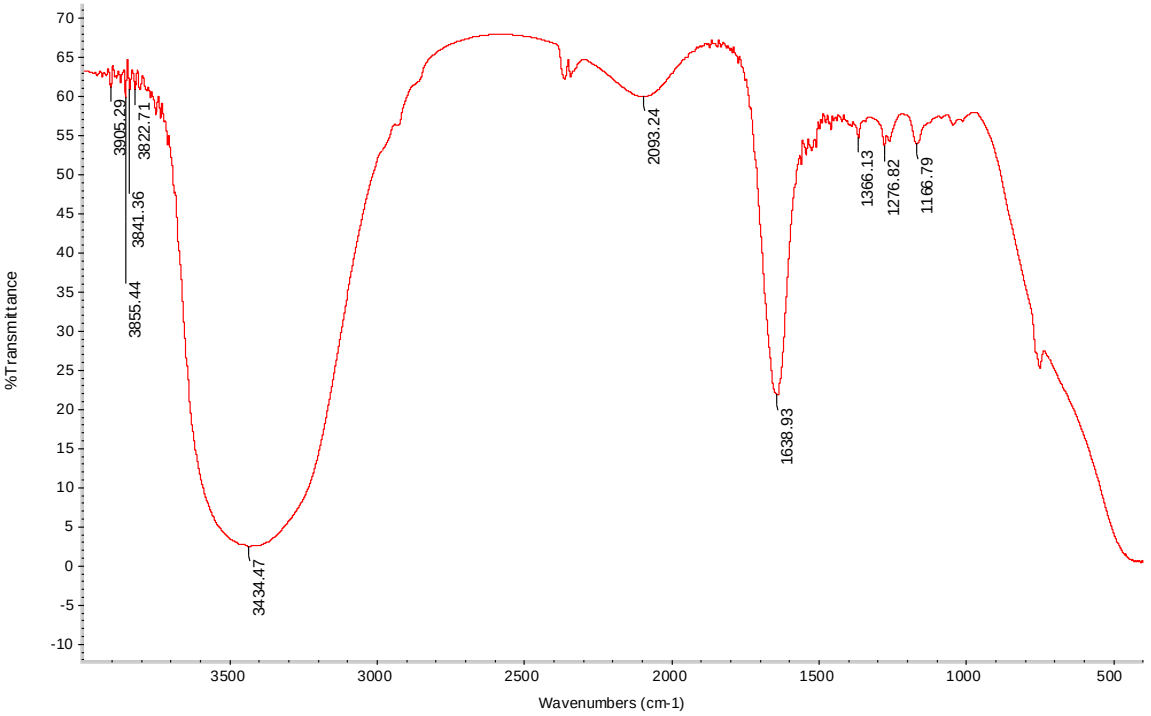



***Figure S13.*** IR of DPA72.

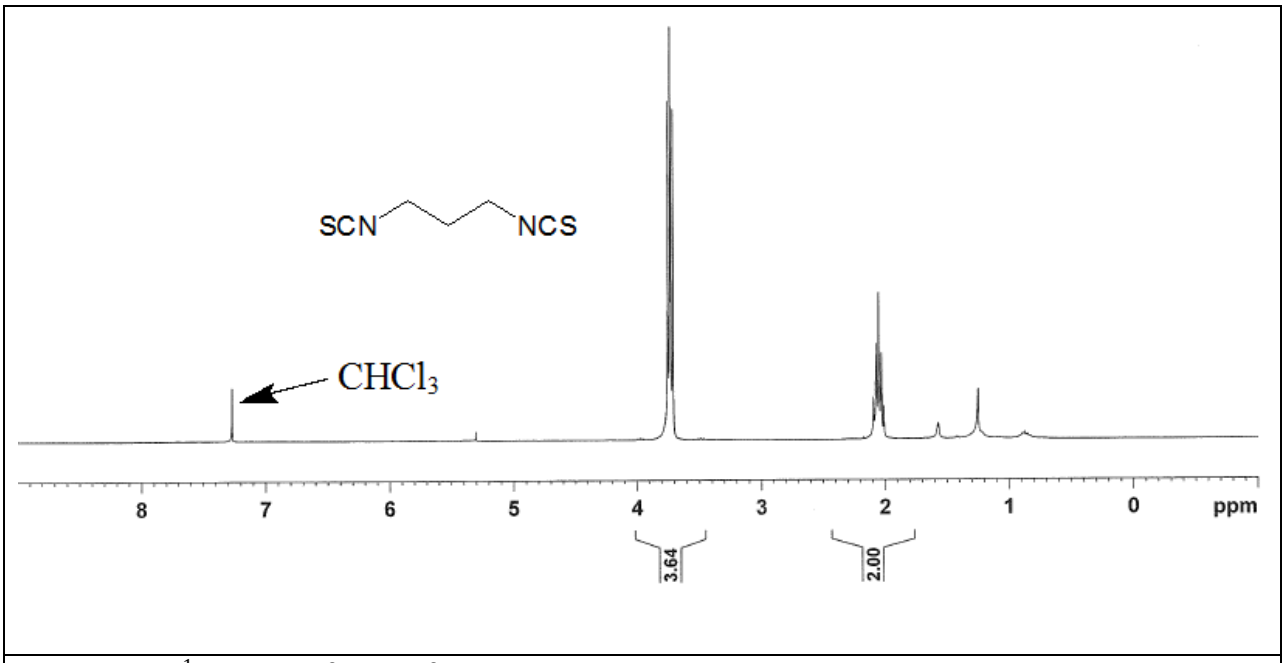



***Figure S14.*** $^{1}$H-NMR of linker for DPA73 in $CDCl_3$.

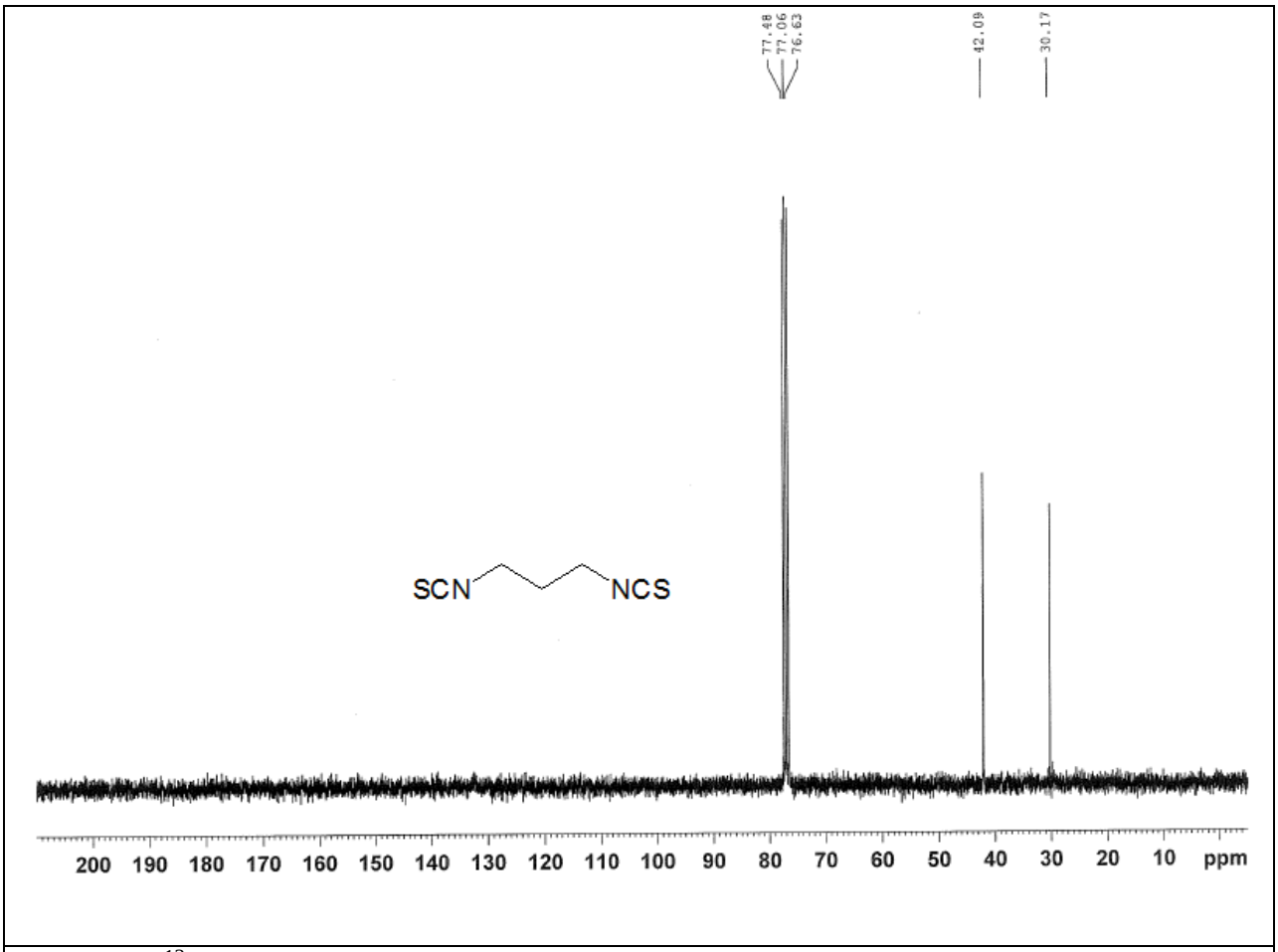



***Figure 15.*** $^{13}$C-NMR of linker for DPA73 in $CDCl_3$.

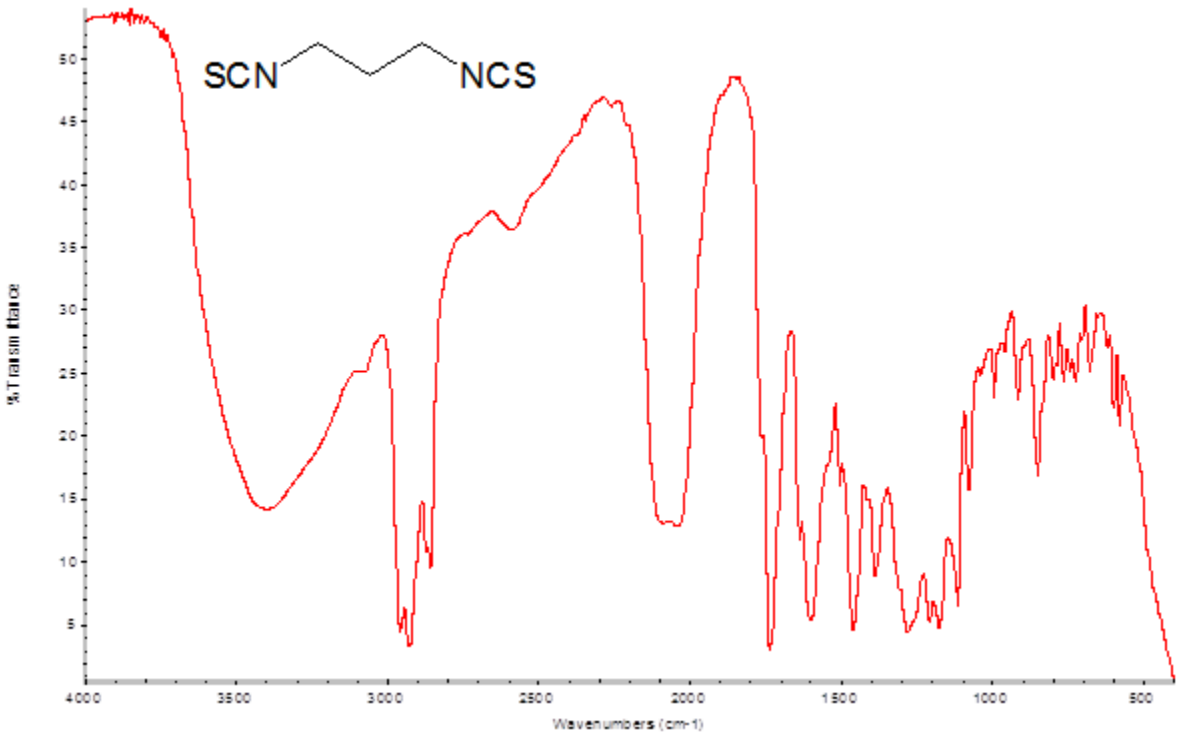



***Figure S16.*** IR of linker for DPA73.

DPA73.

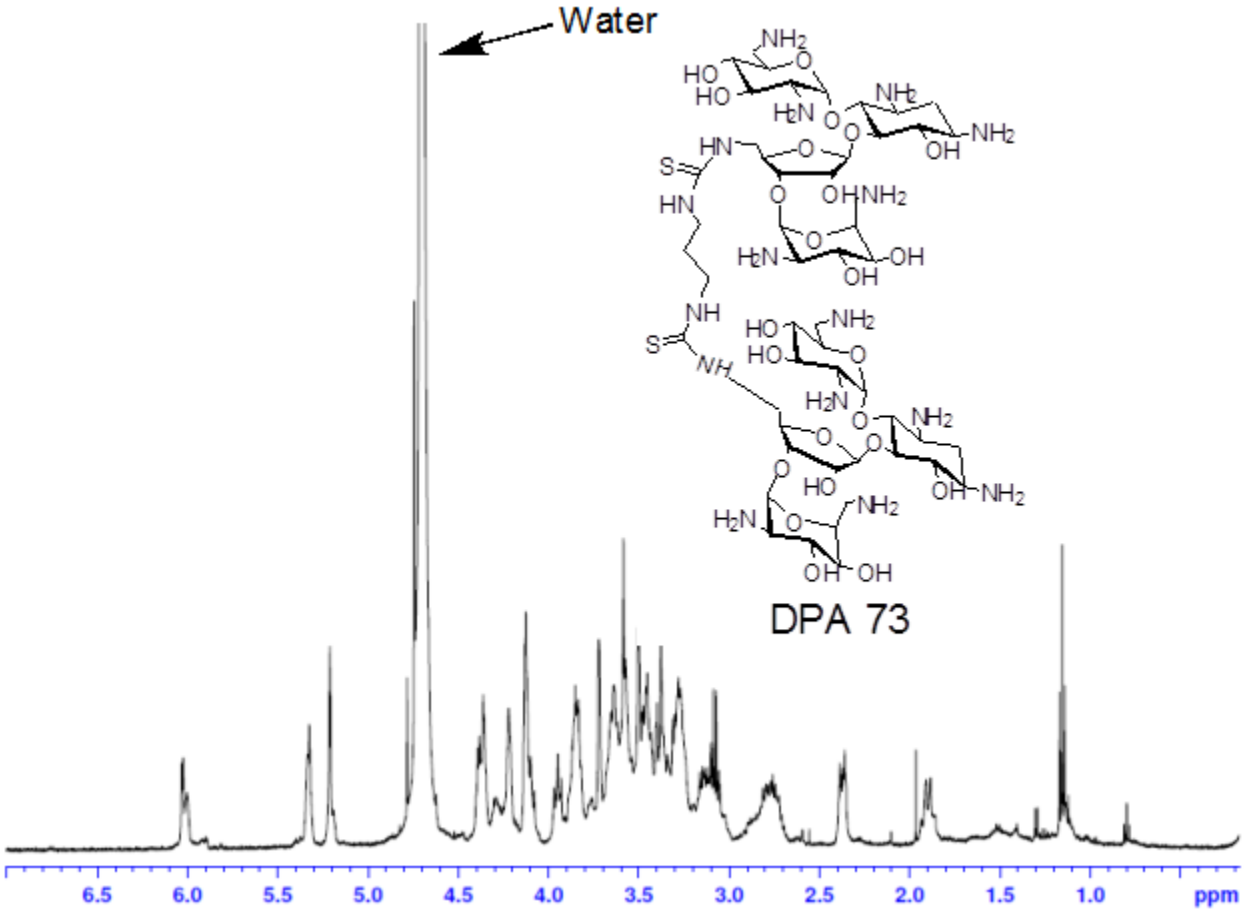



***Figure S17.*** $^{1}$H-NMR of DPA73 in $D_2O$.

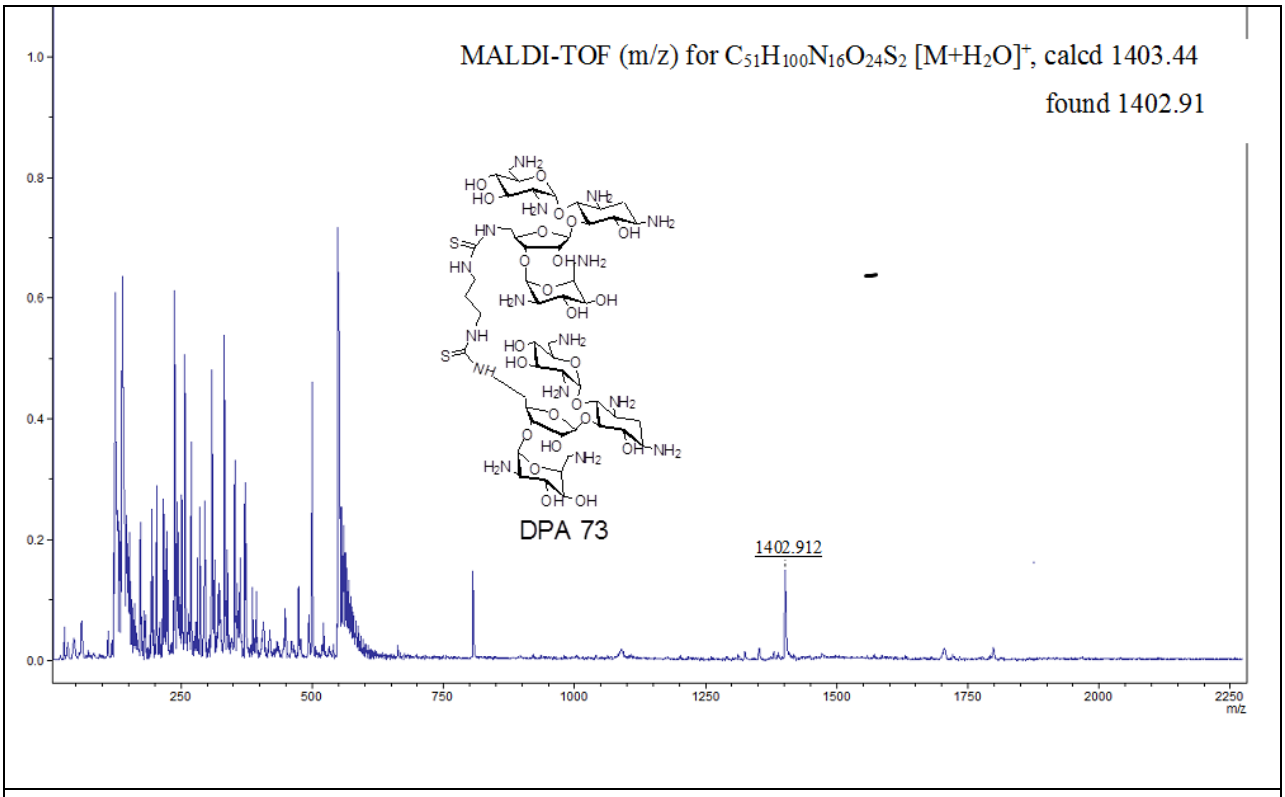



***Figure S18.*** MALDI-TOF of DPA73.

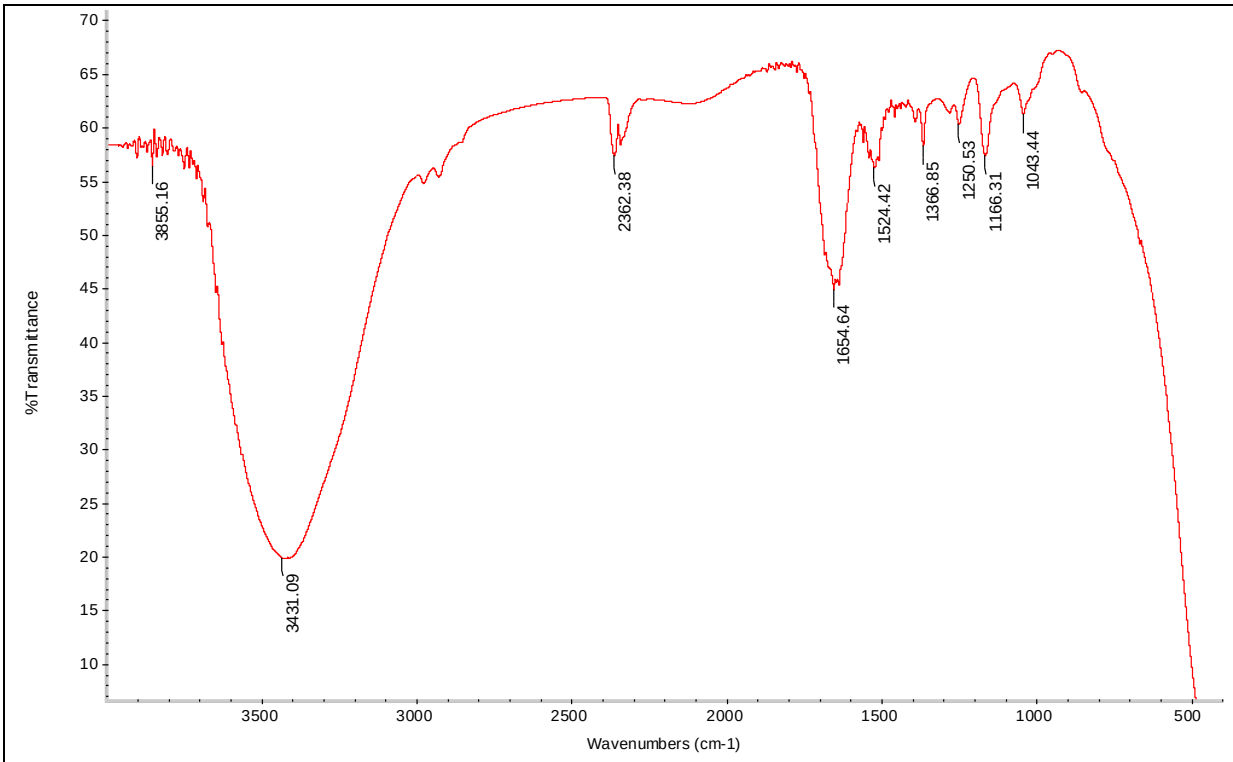



***Figure S19.*** IR of DPA73.

N-Boc DPA 74.

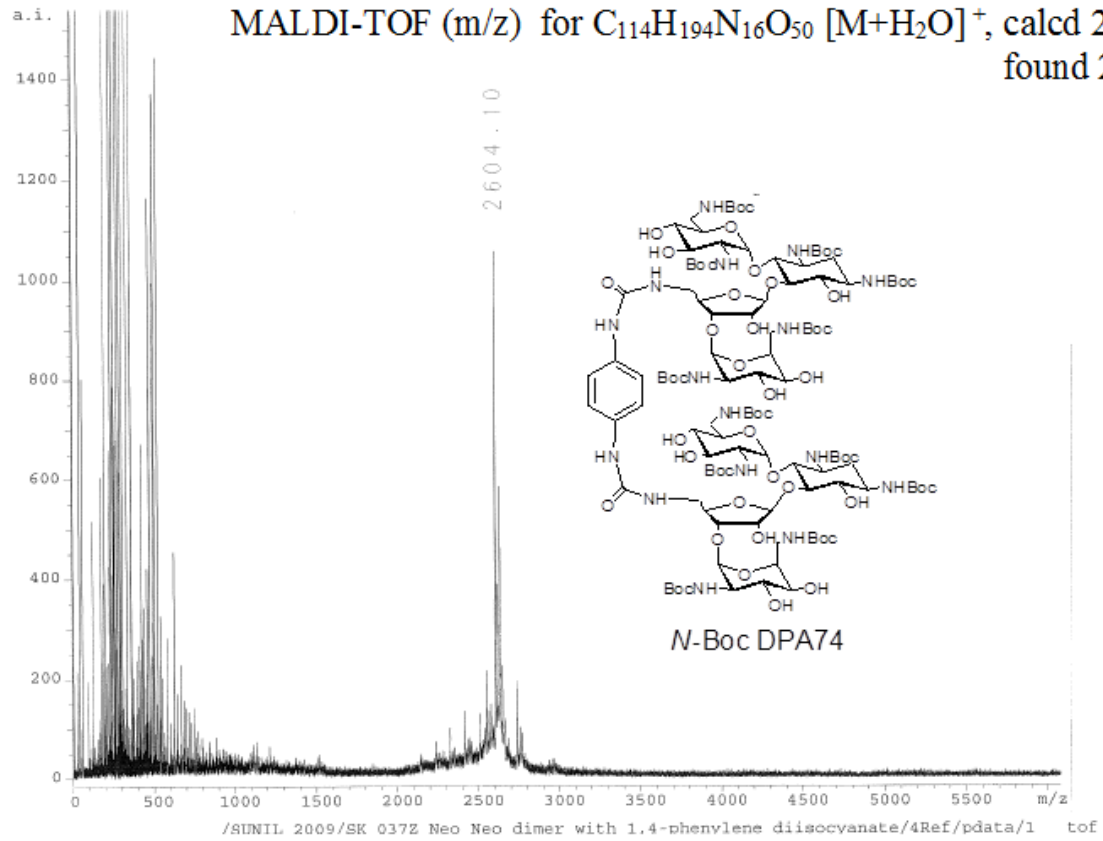



***Figure S20.*** MALDI-TOF of N-Boc DPA74.

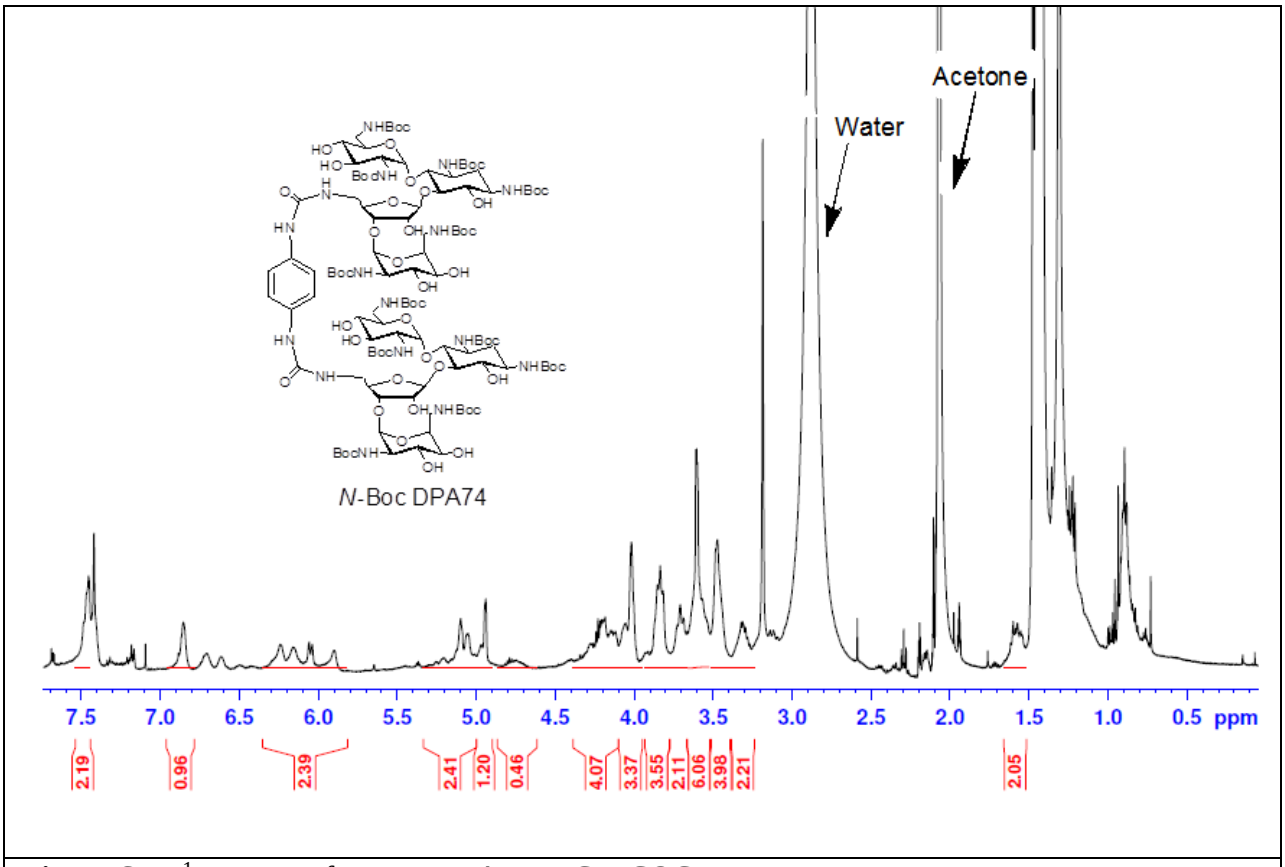



***Figure S21.*** $^{1}$H-NMR of N-Boc DPA74 in $CD_3COCD_3$.

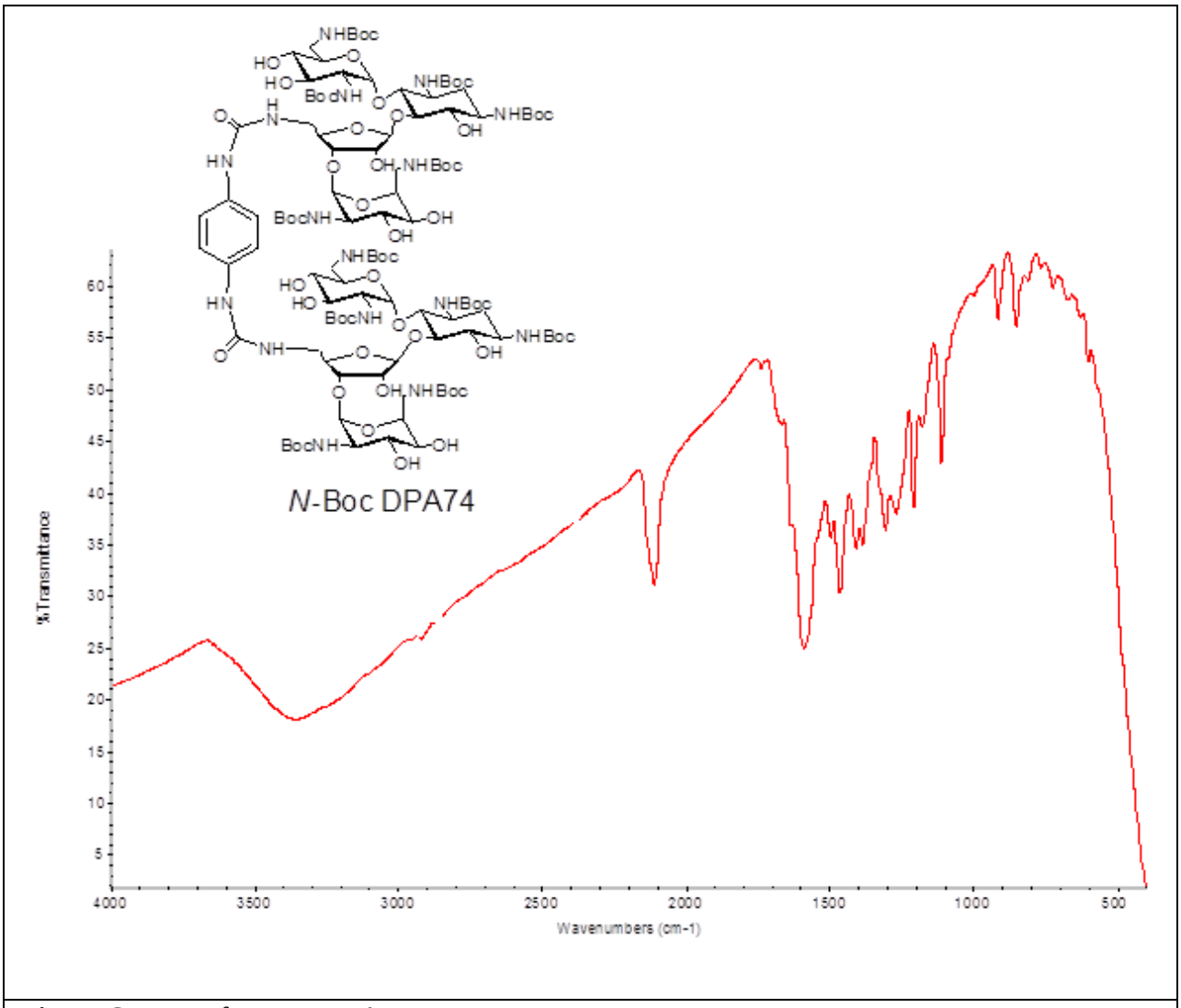



***Figure S22.*** IR of N-Boc DPA74.

DPA74.

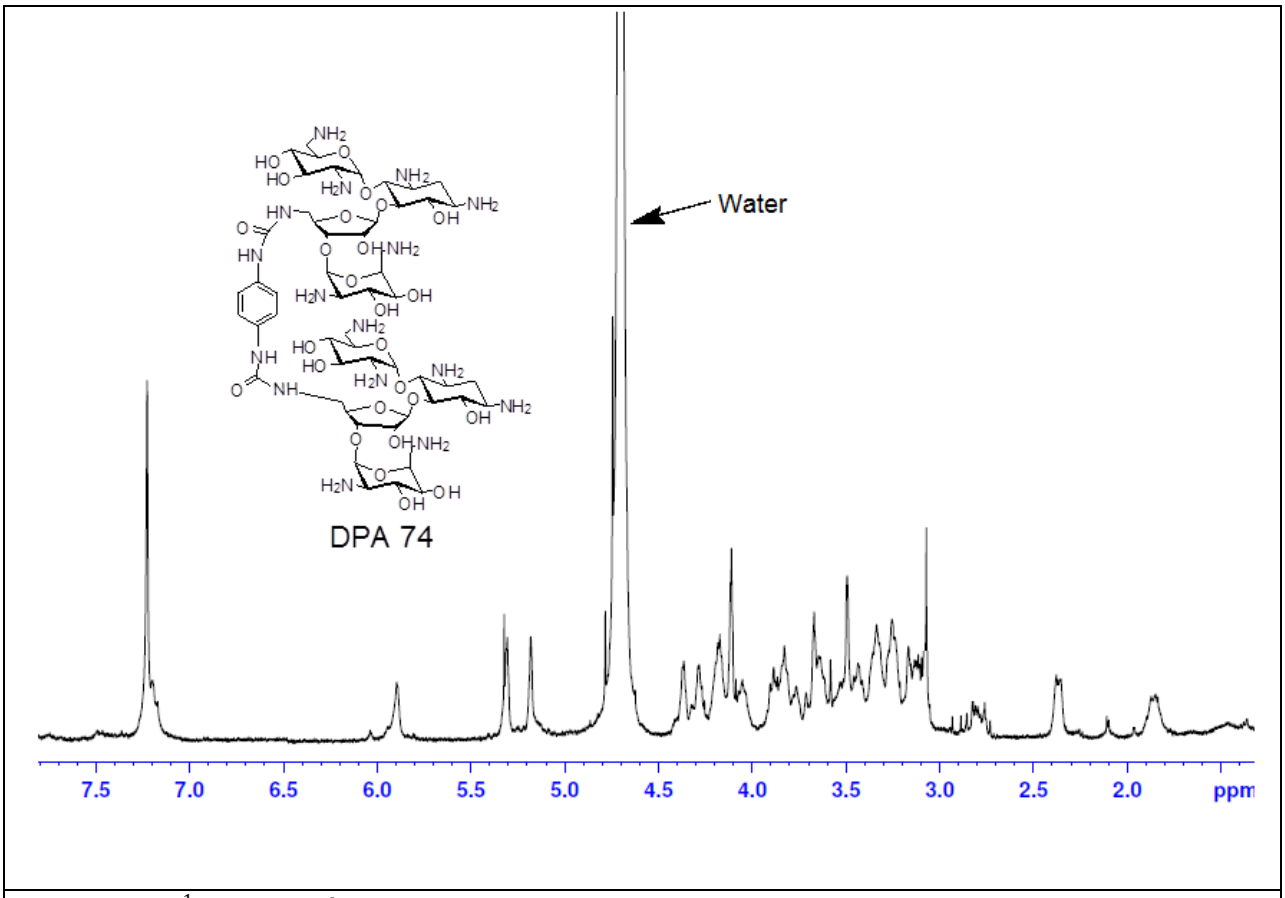



***Figure S23.*** $^{1}$H-NMR of DPA74 in $D_2O$.

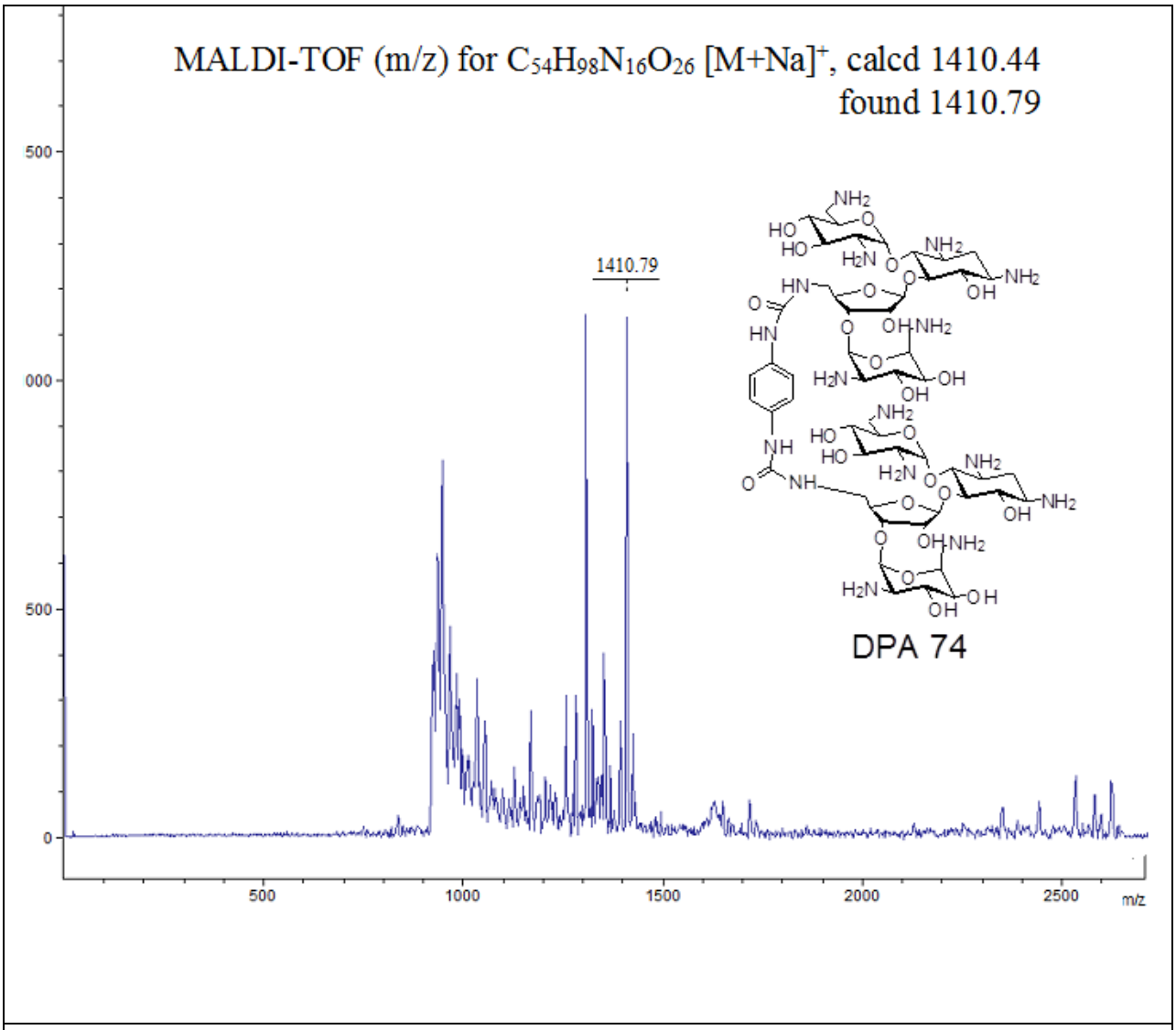



***Figure S24.*** MALDI-TOF of DPA74.

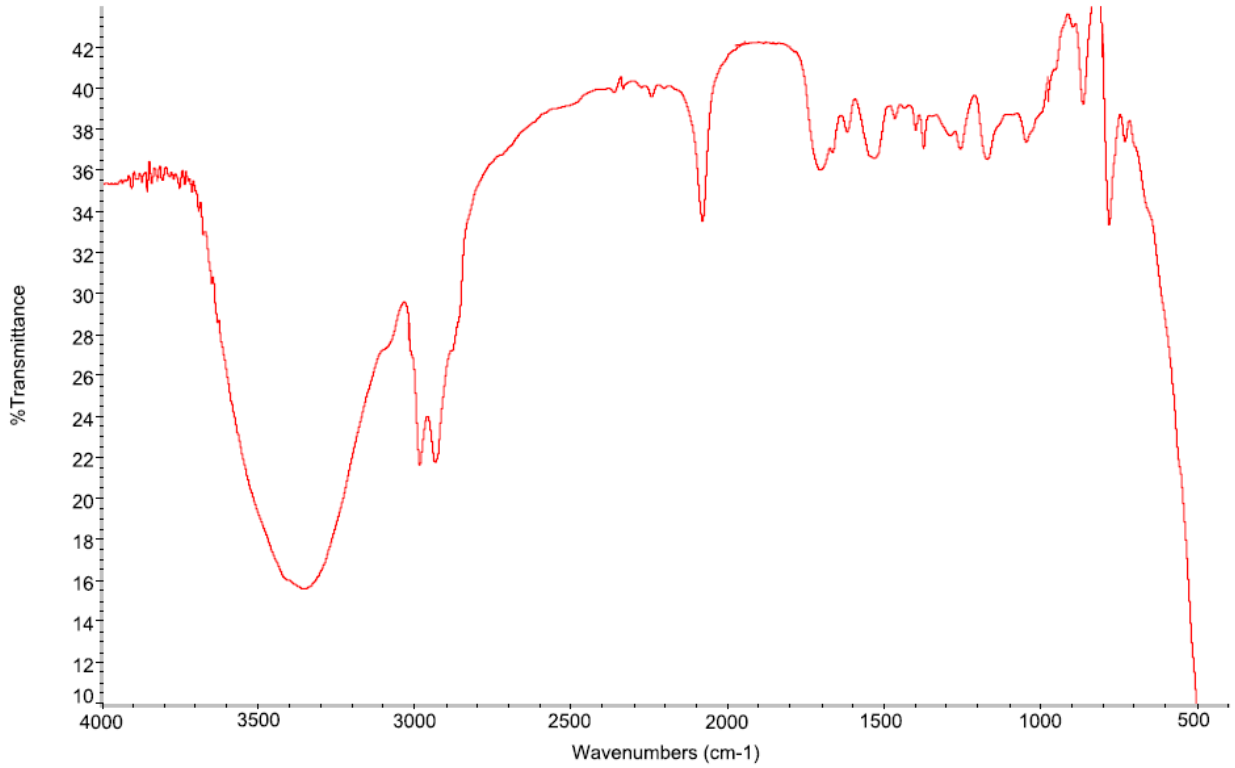



***Figure S25.*** IR of DPA74.

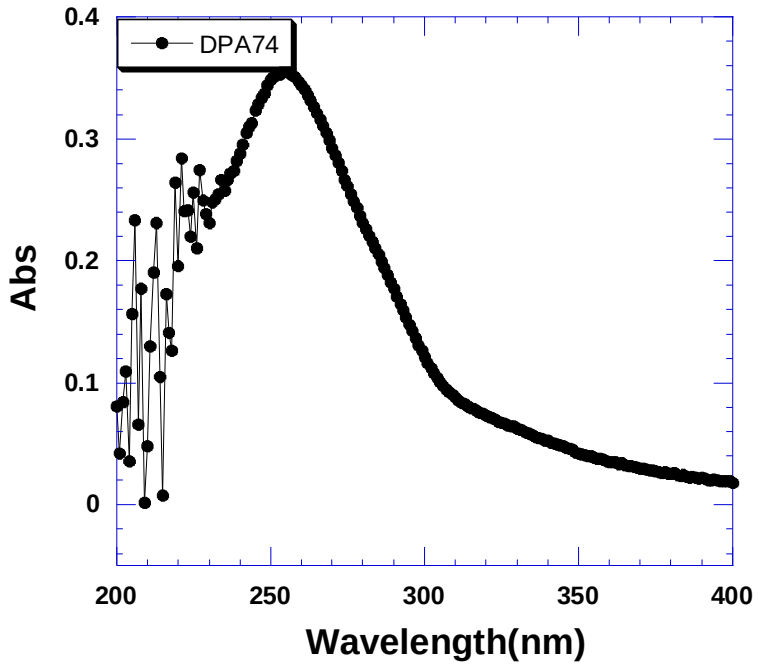



***Figure S26.*** UV absorbance scan of DPA74. Scan was taken in water and the concentration of DPA74 was 25 μM. T = 25 °C.

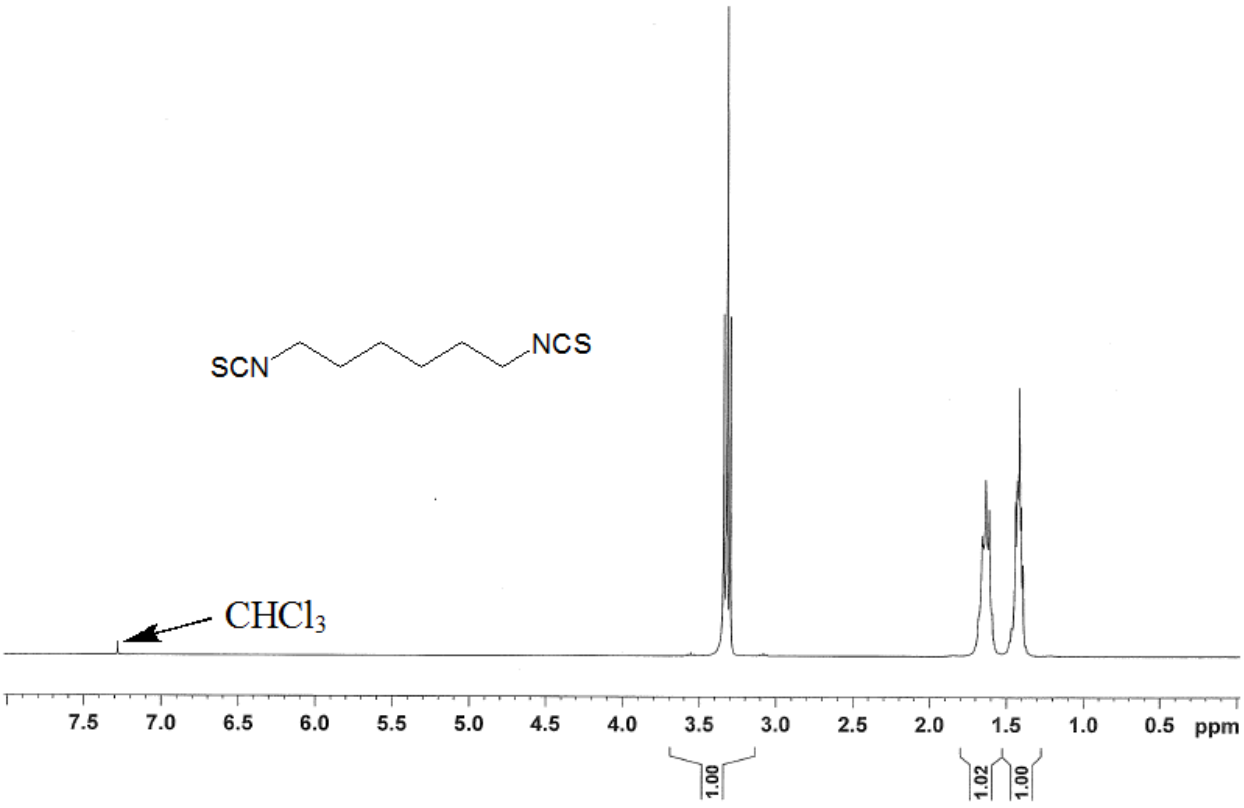



***Figure 27.*** $^{1}$H-NMR of linker for N-Boc DPA75 in $CDCl_3$.

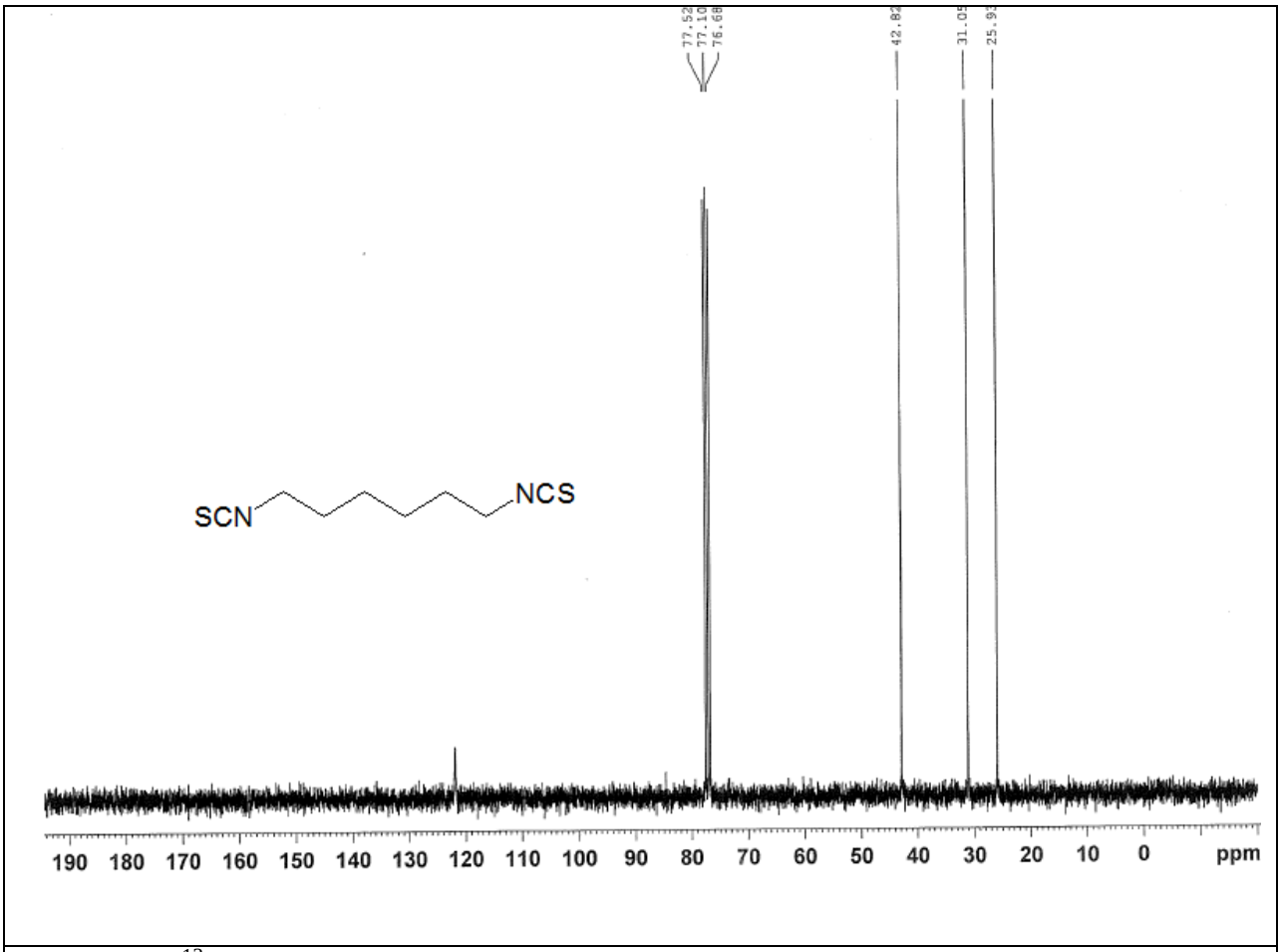



***Figure S28.*** $^{13}$C-NMR of linker for N-Boc DPA75 in $CDCl_3$.

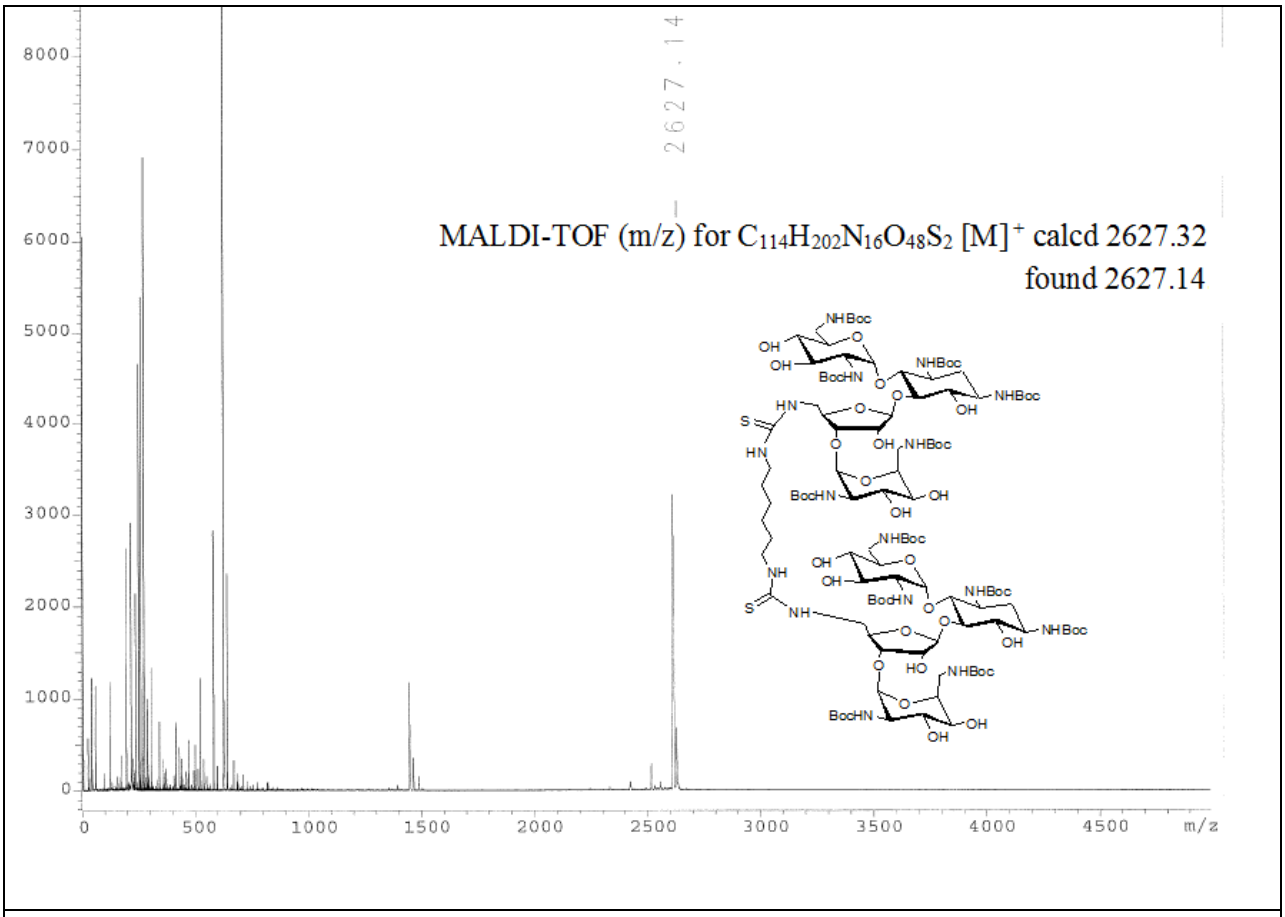



***Figure S29.*** MALDI-TOF of N-Boc DPA75.

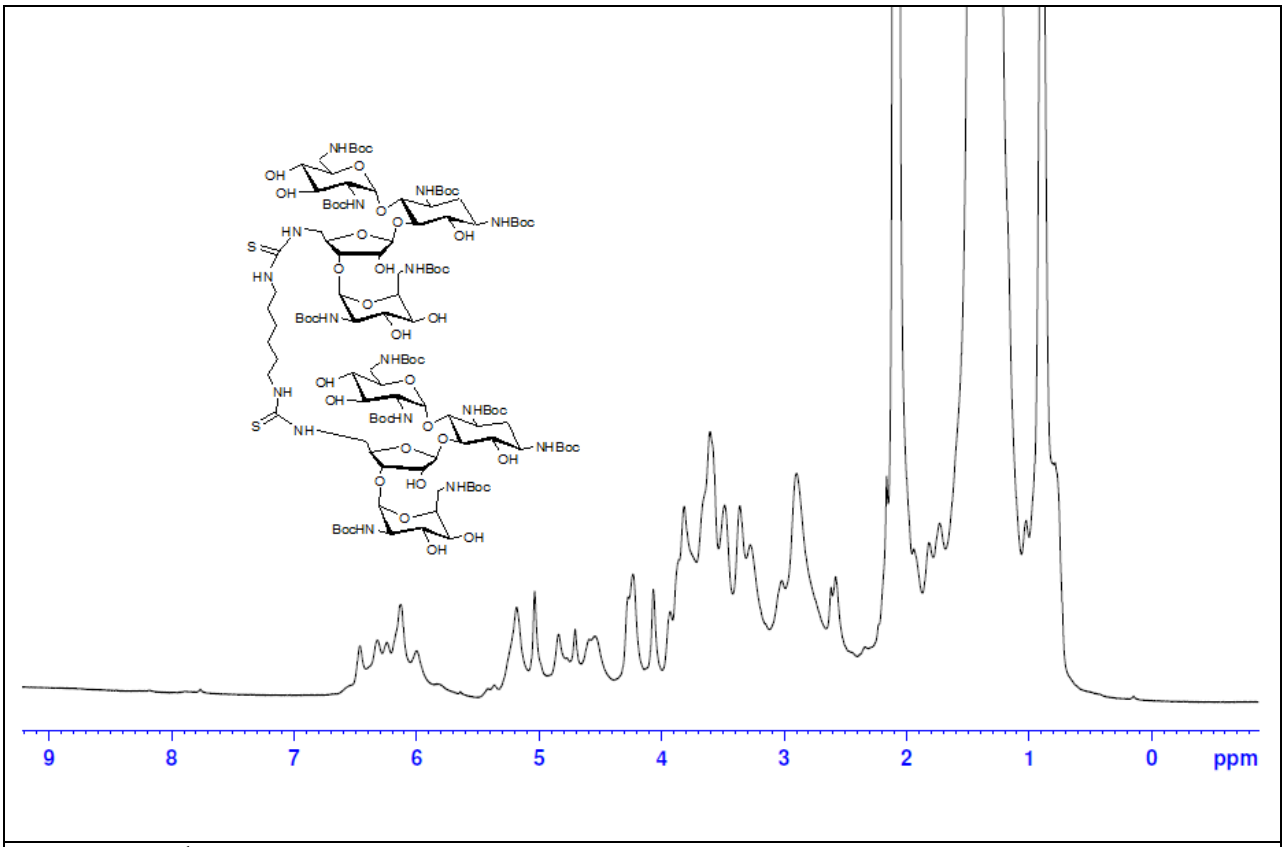



***Figure S30.*** $^{1}$H-NMR of DPA75 in $CD_3COCD_3$.

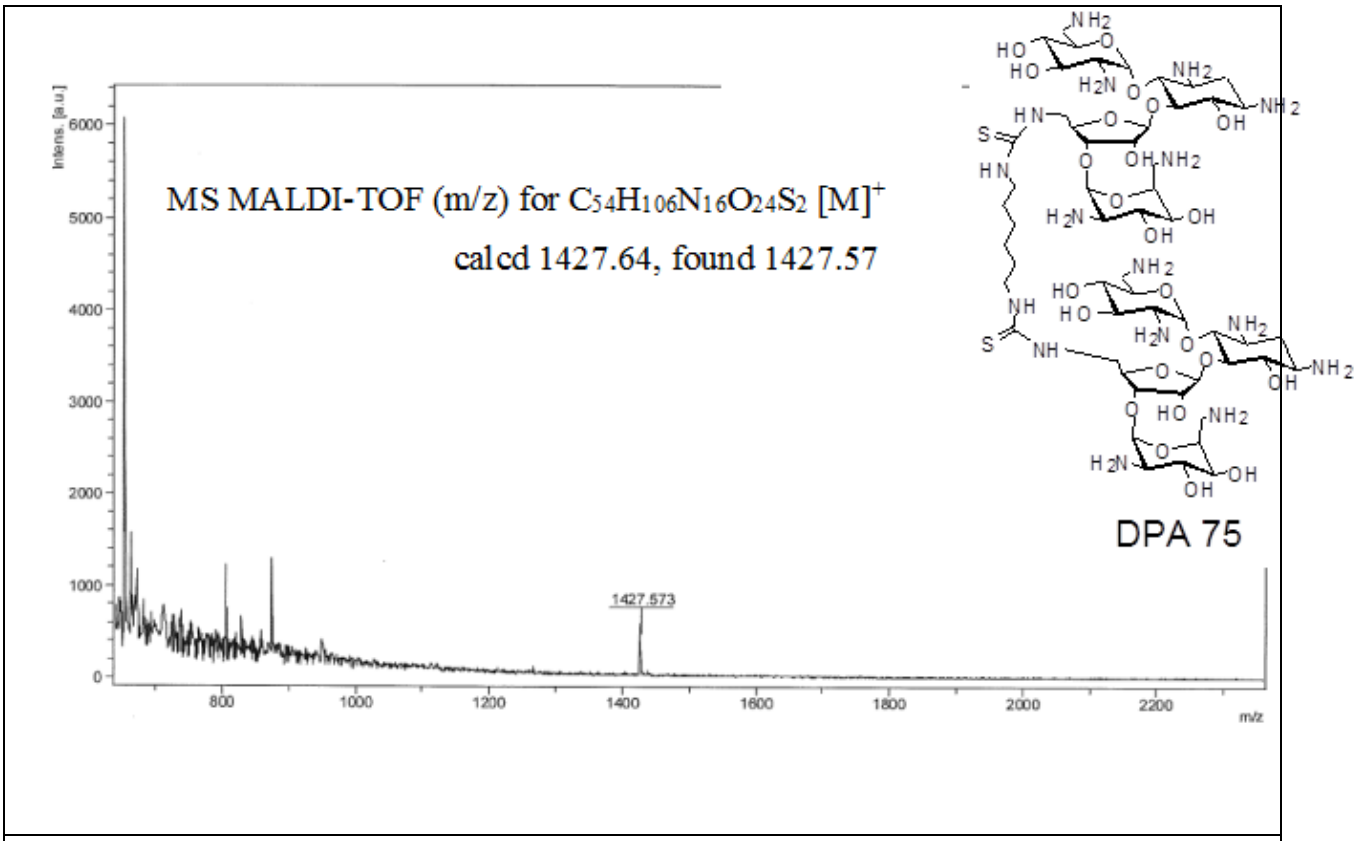



***Figure 31.*** MALDI-TOF of DPA75.

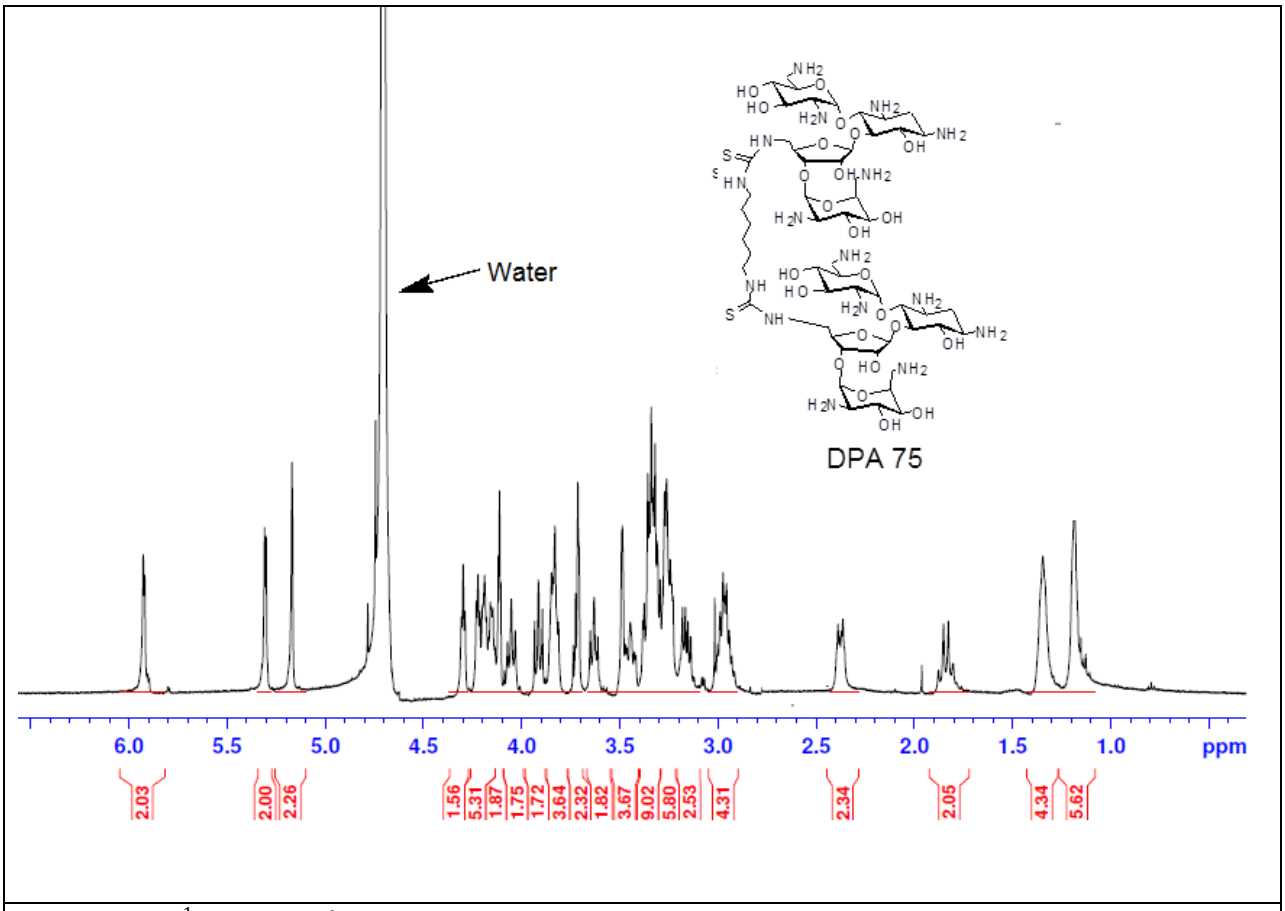



***Figure S32.*** $^{1}$H-NMR of DPA75 in $D_2O$.

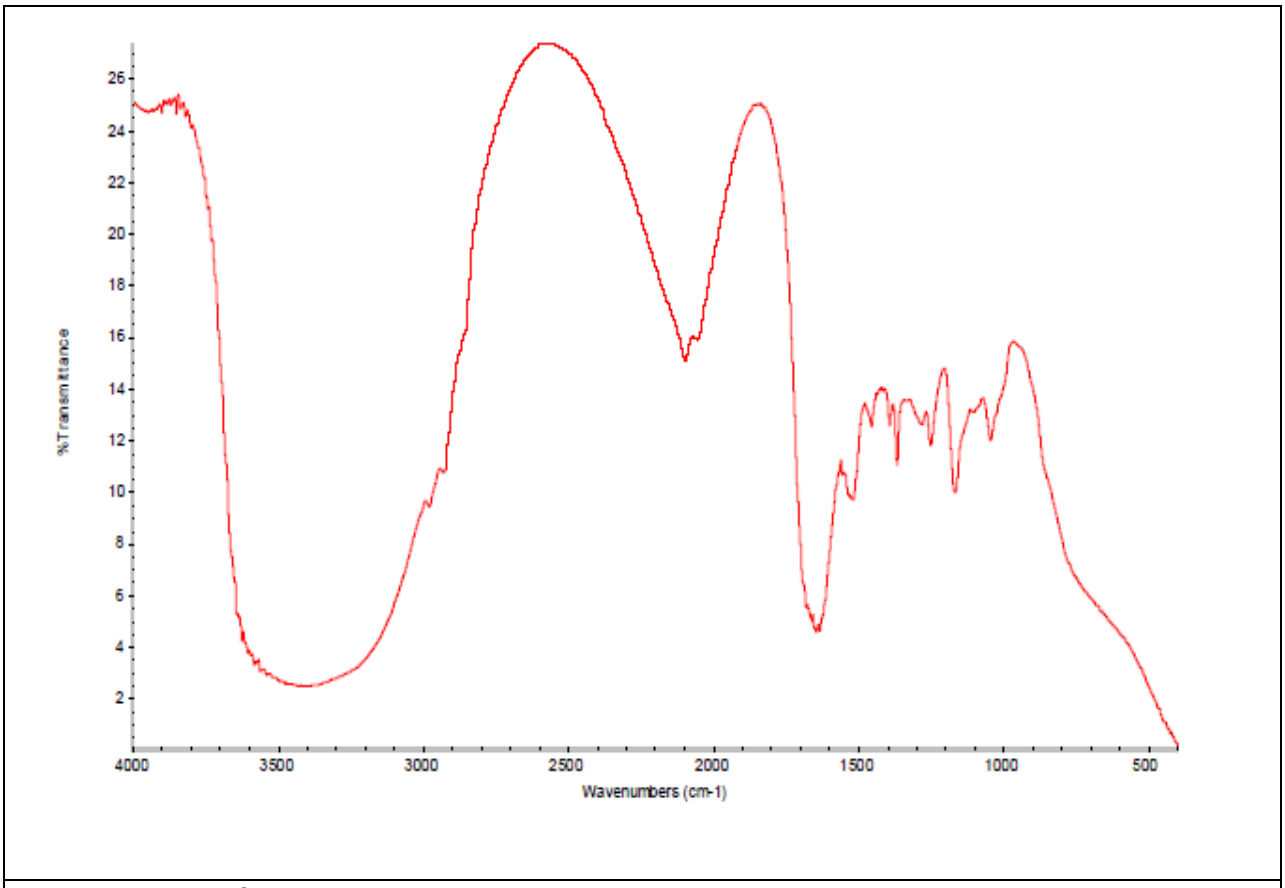



***Figure S33.*** IR of DPA75.

N-Boc DPA 76.

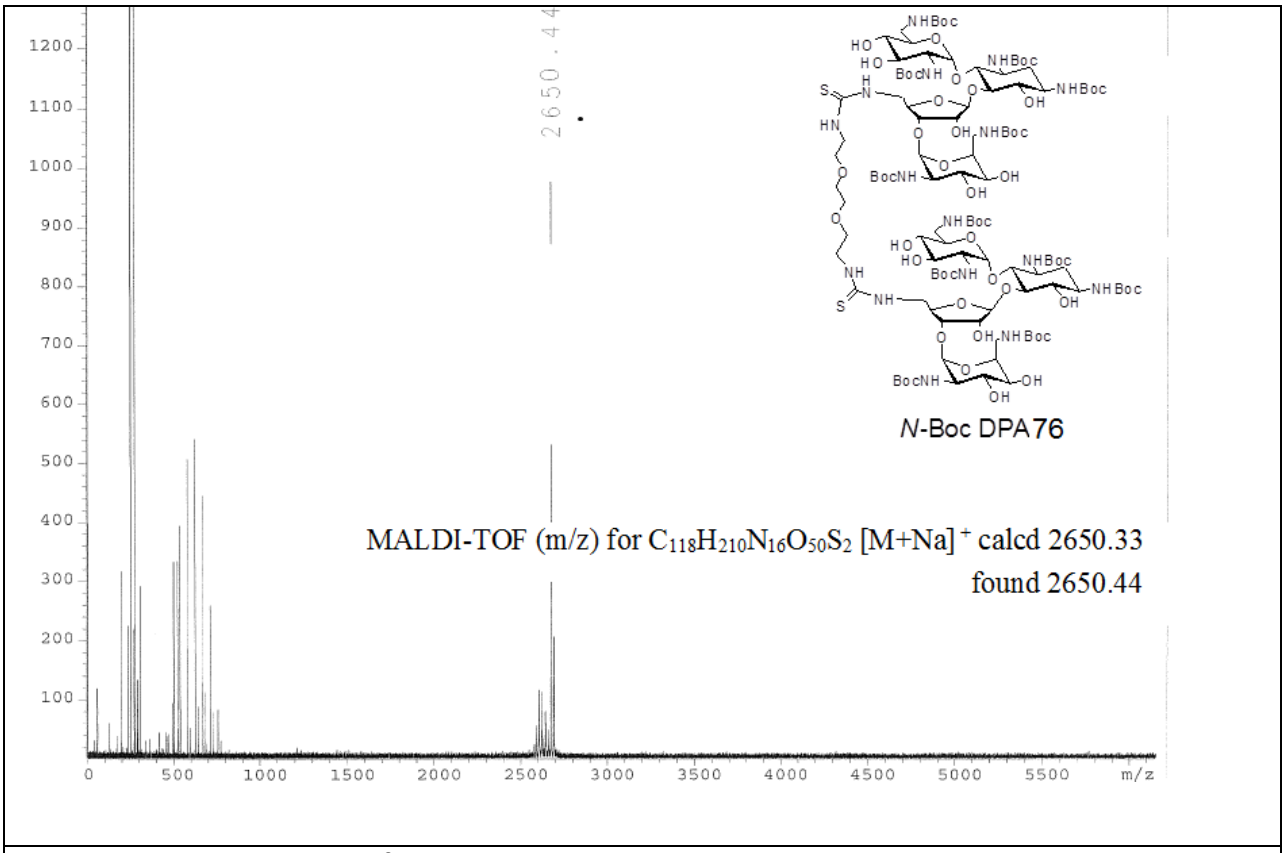



***Figure S34.*** MALDI-TOF of N-Boc DPA76.

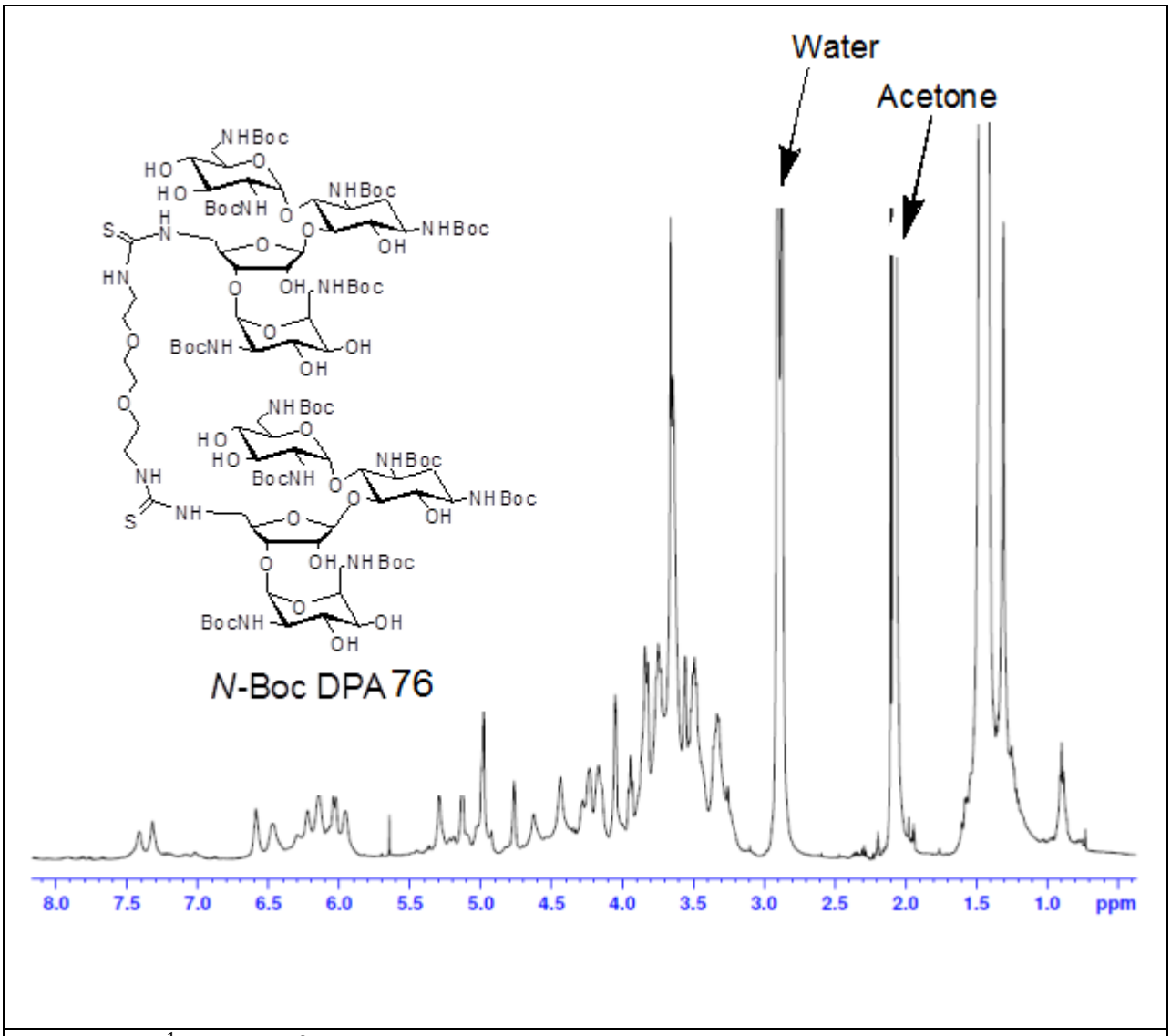



***Figure S35.*** $^{1}$H-NMR of N-Boc DPA76 in $CD_3COCD_3$.

DPA76.

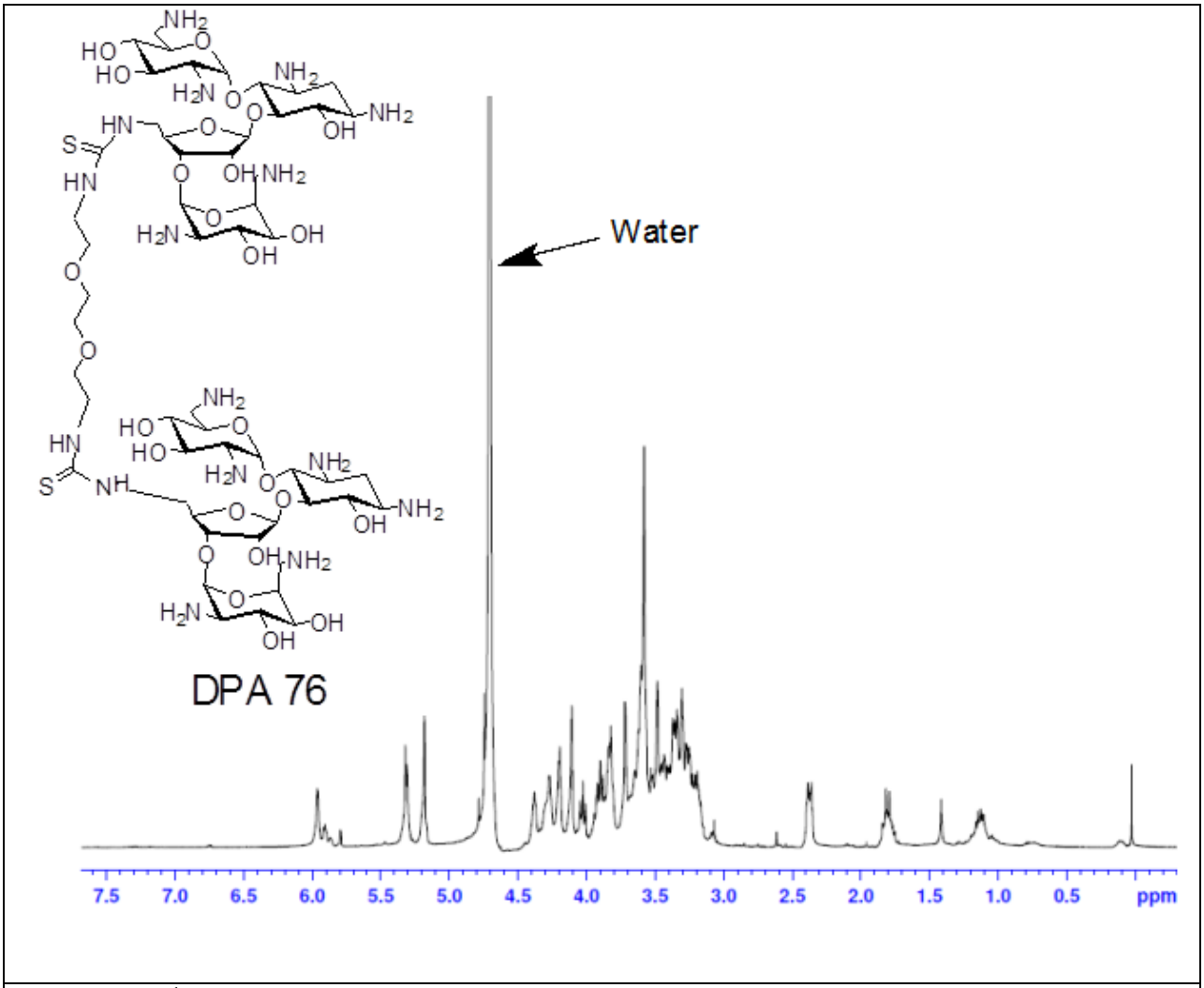



***Figure S36.*** $^{1}$H-NMR of DPA76 in $D_2O$.

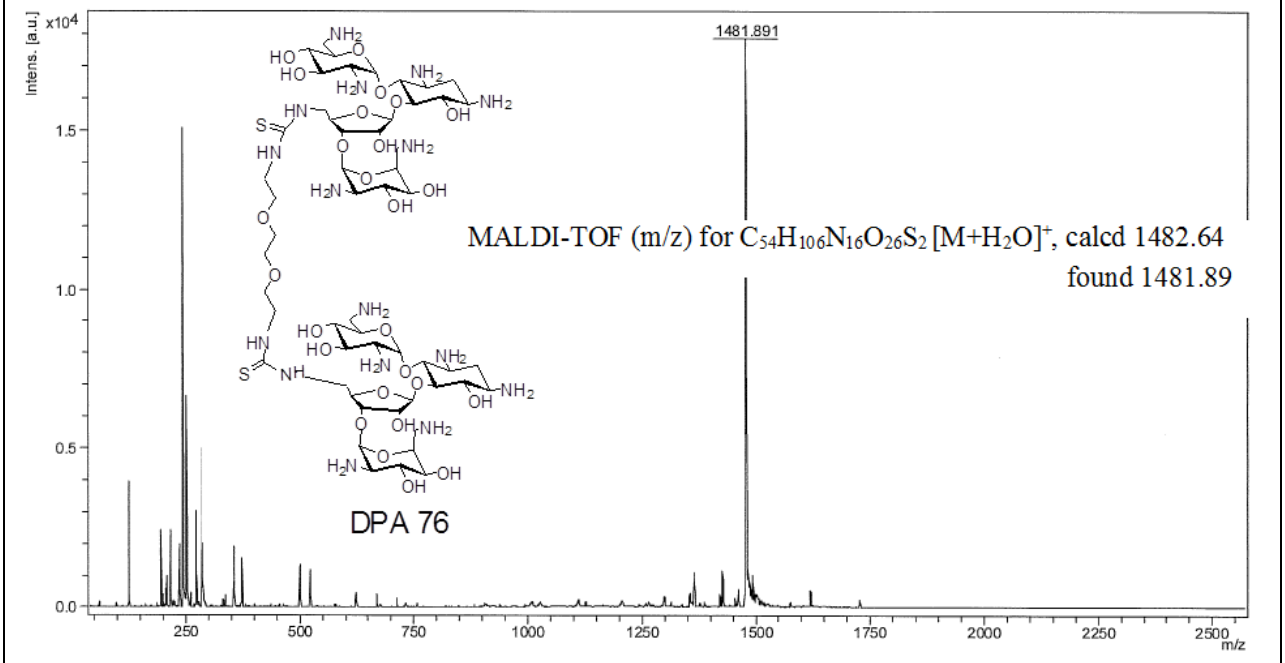



***Figure S37.*** MALDI-TOF of DPA76.

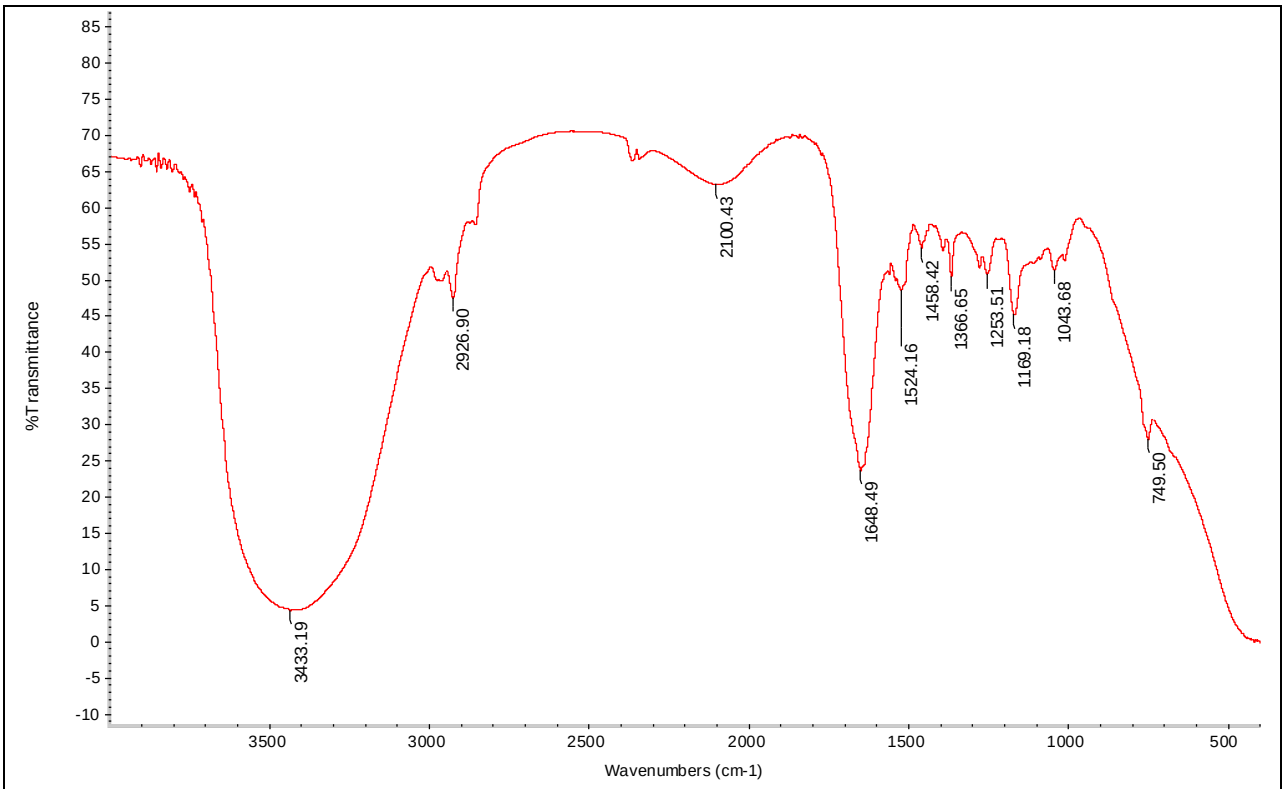



***Figure S38.*** IR of DPA76.

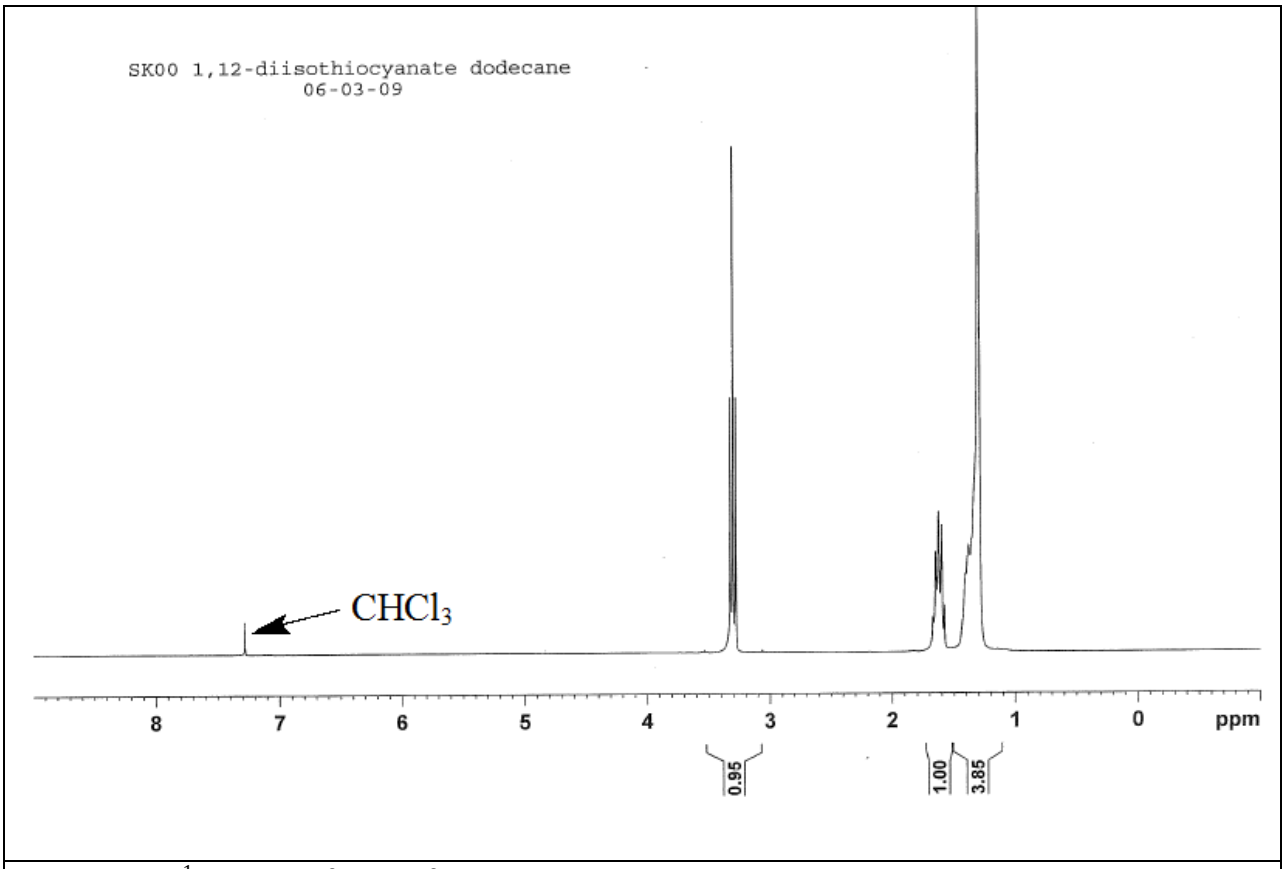



***Figure S39.*** $^{1}$H-NMR of linker for N-Boc DPA77 in $CDCl_3$.

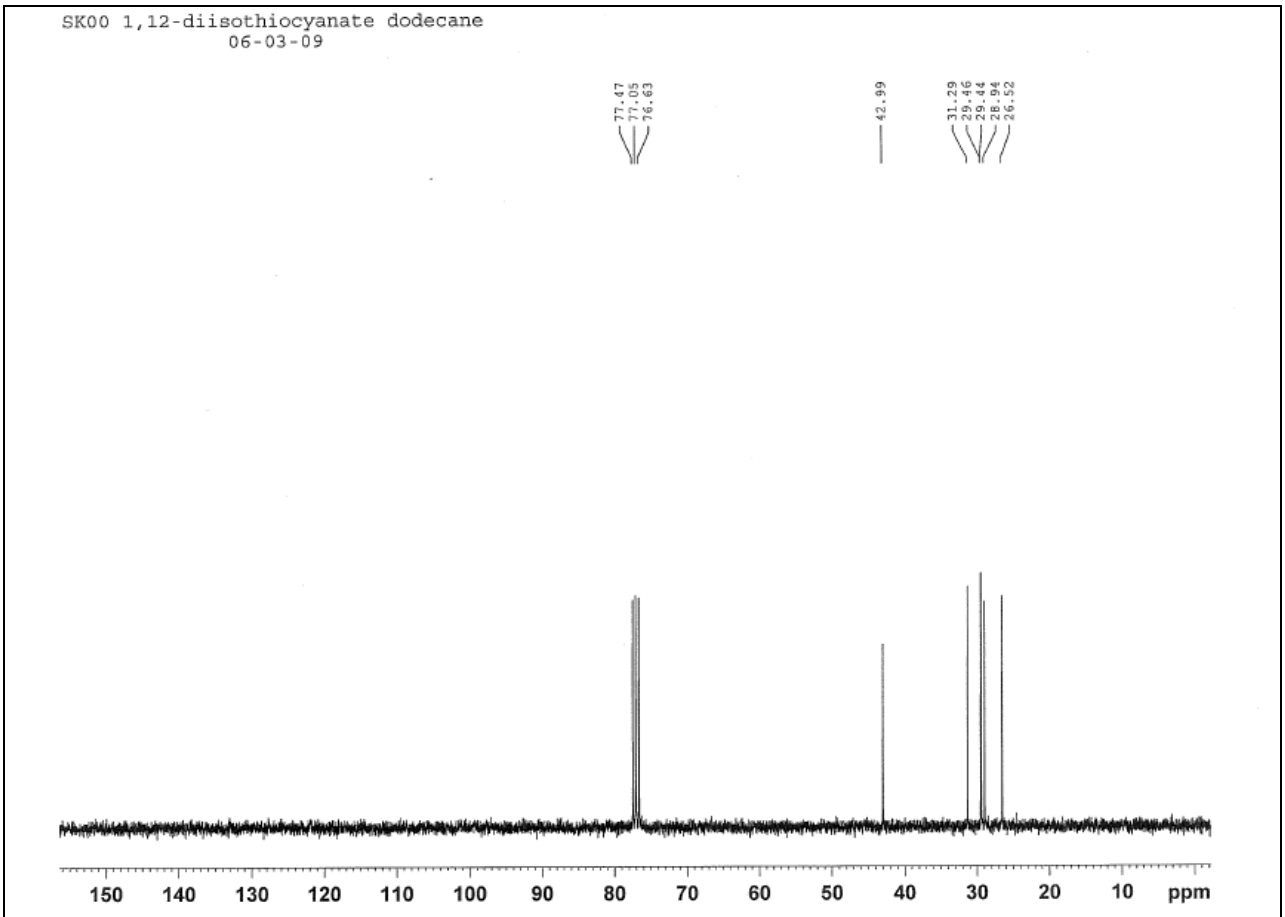



***Figure S40.*** $^{13}$C-NMR of linker for N-Boc DPA77 in $CDCl_3$.

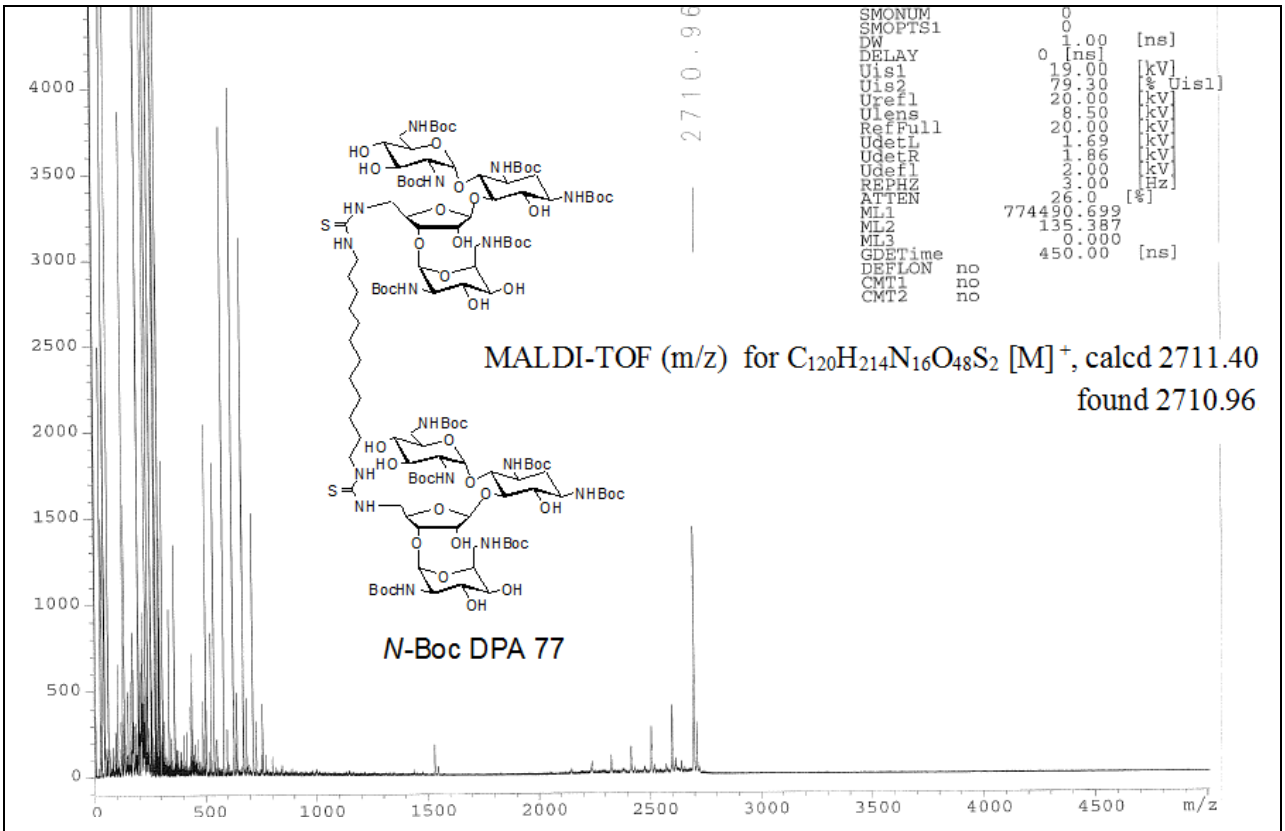



***Figure S41.*** MALDI-TOF of N-Boc DPA77.

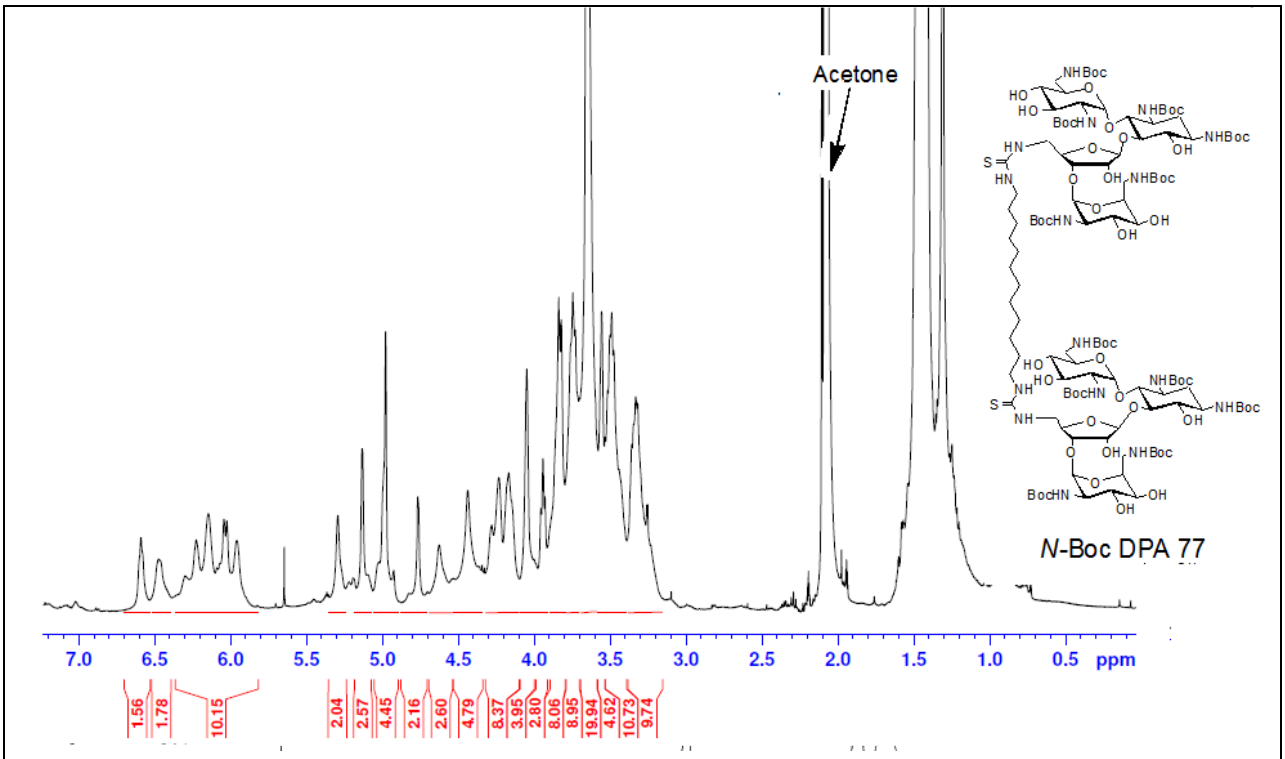



***Figure S42.*** $^{1}$H-NMR of N-Boc DPA77 in $CD_3COCD_3$.

DPA77.

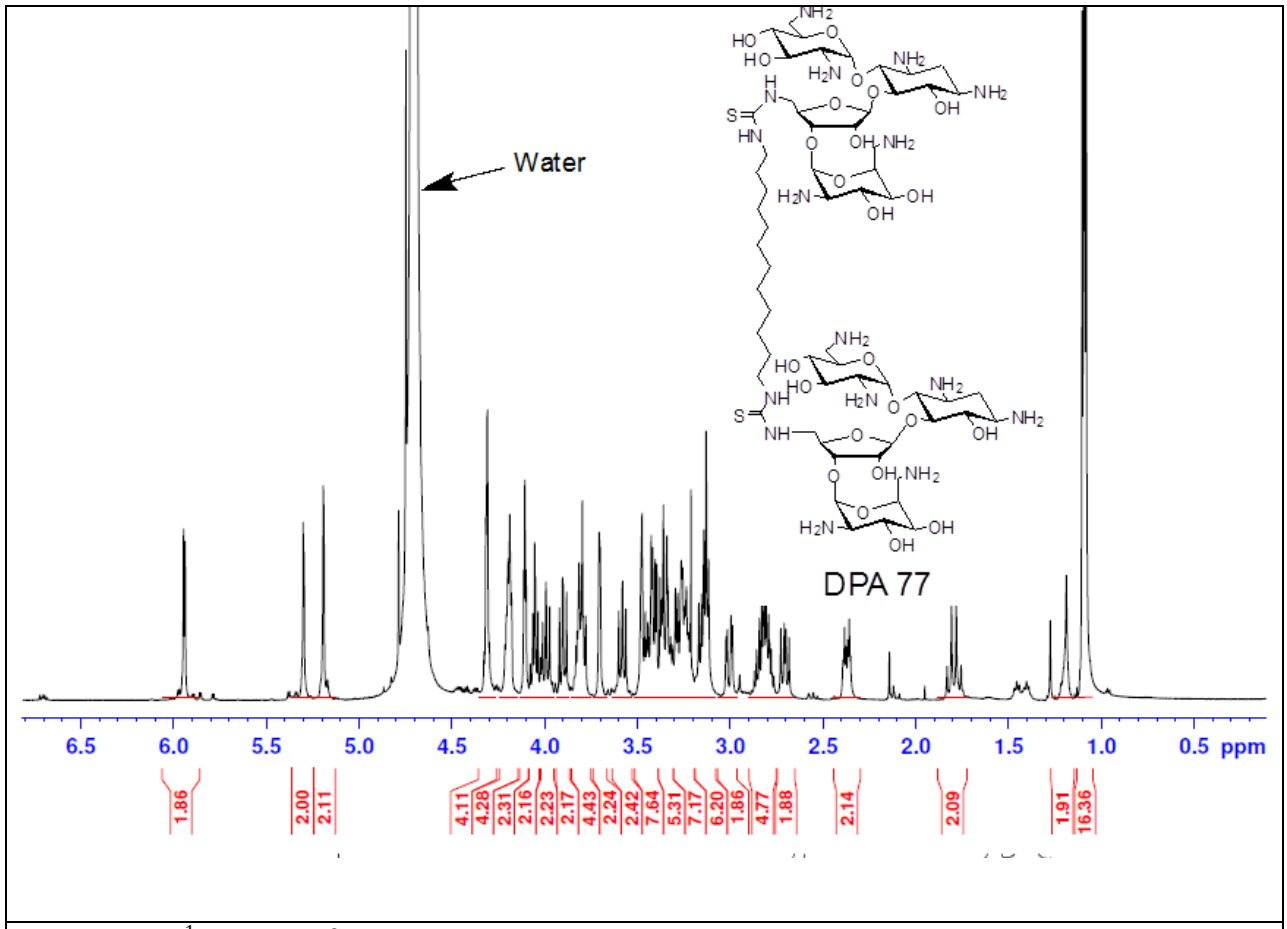



***Figure S43.*** $^{1}$H-NMR of DPA77 in $D_2O$.

DPA77.

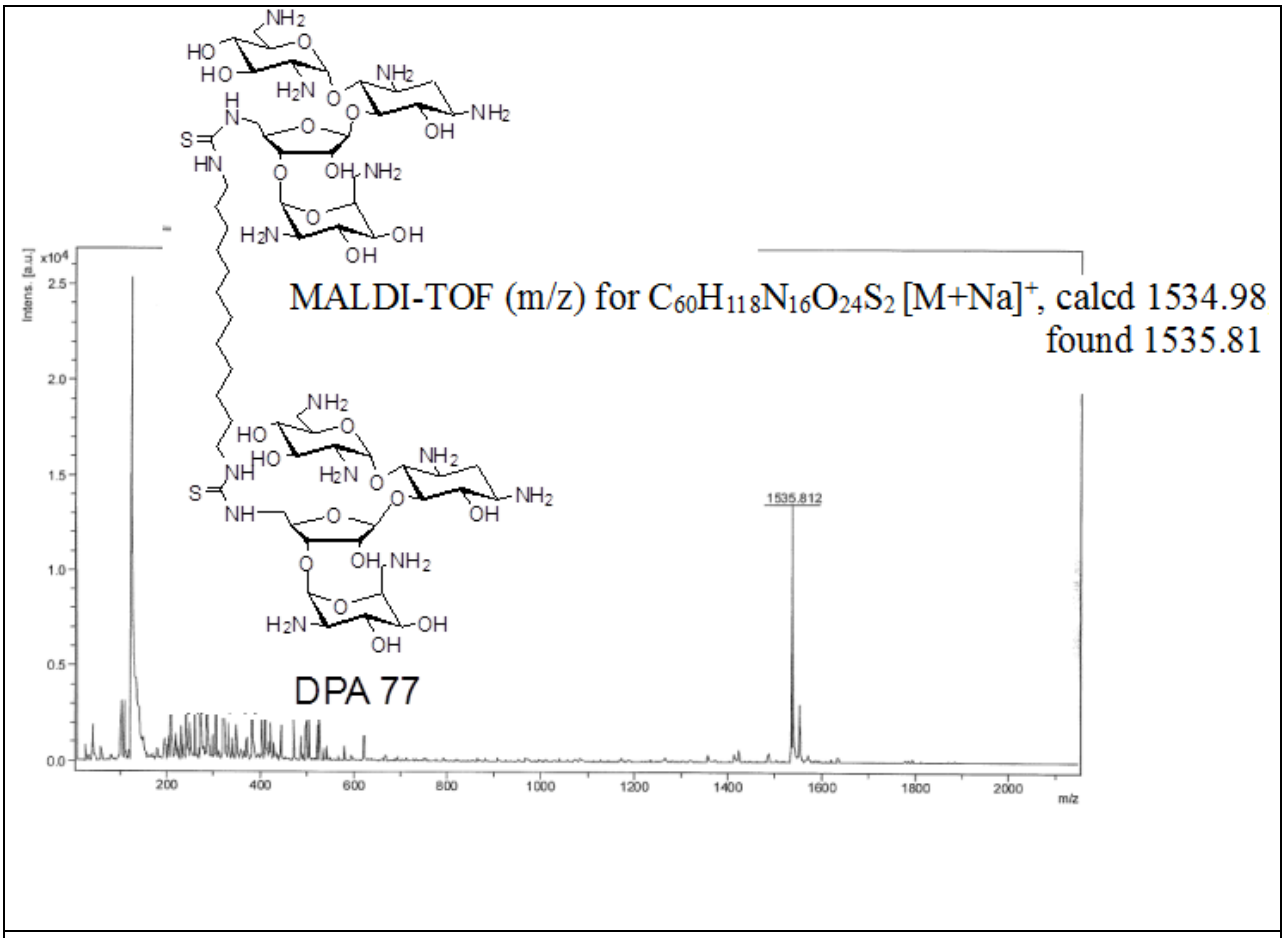



***Figure S44.*** MALDI-TOF of DPA77.

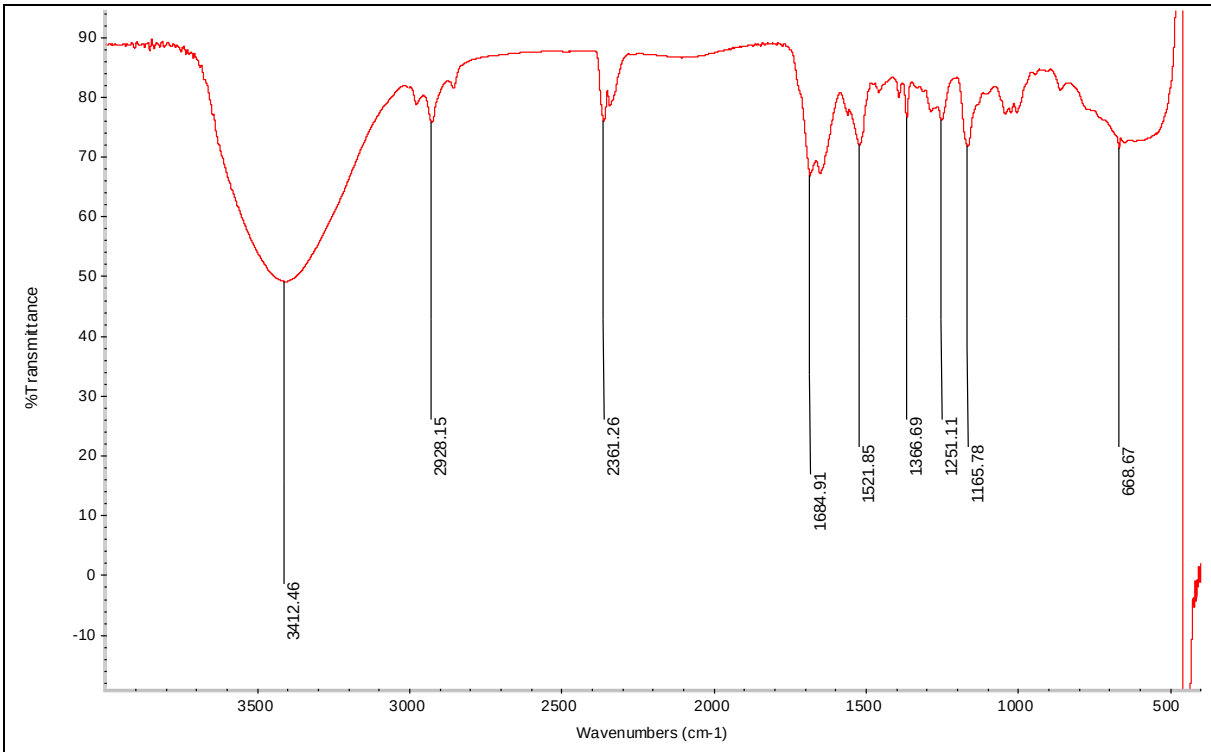



***Figure S45.*** IR of DPA77.

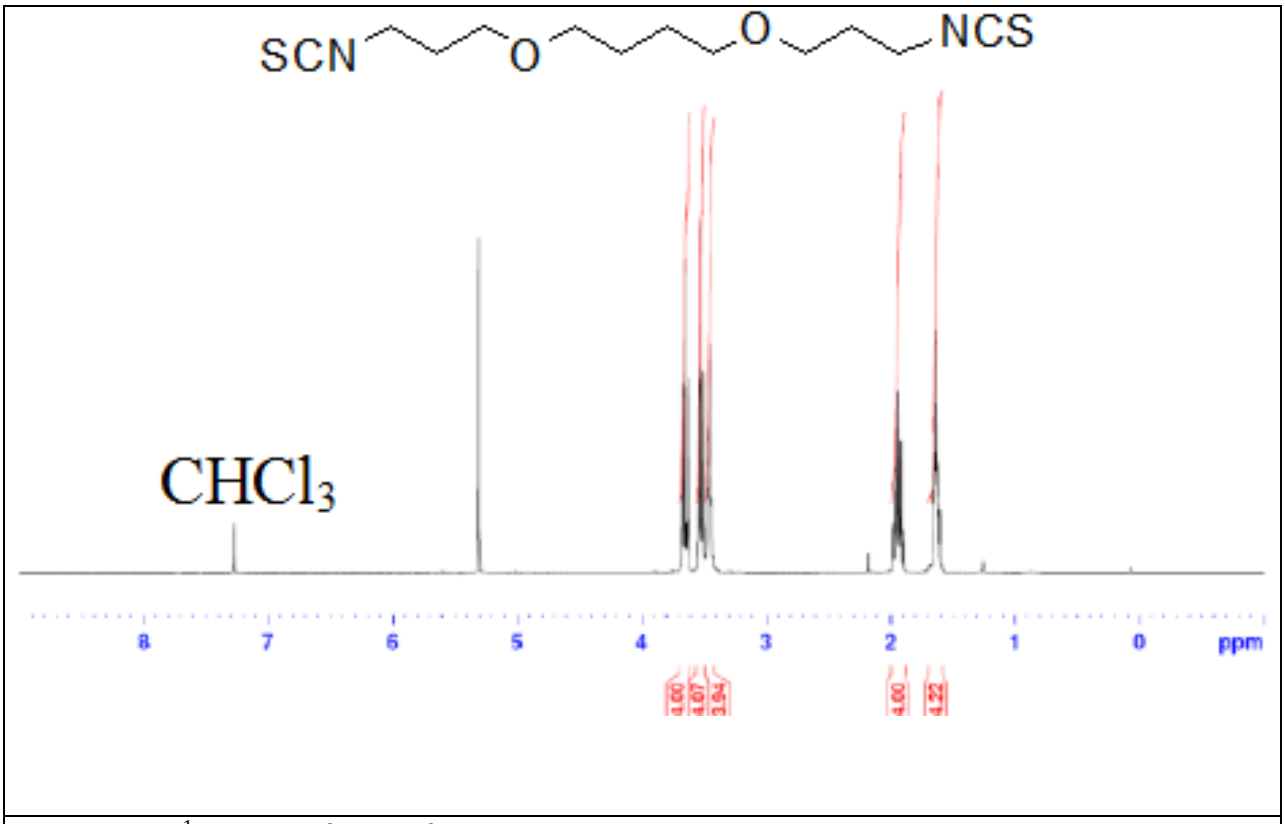



***Figure S46.*** $^{1}$H-NMR of linker for N-Boc DPA78 in $CDCl_3$.

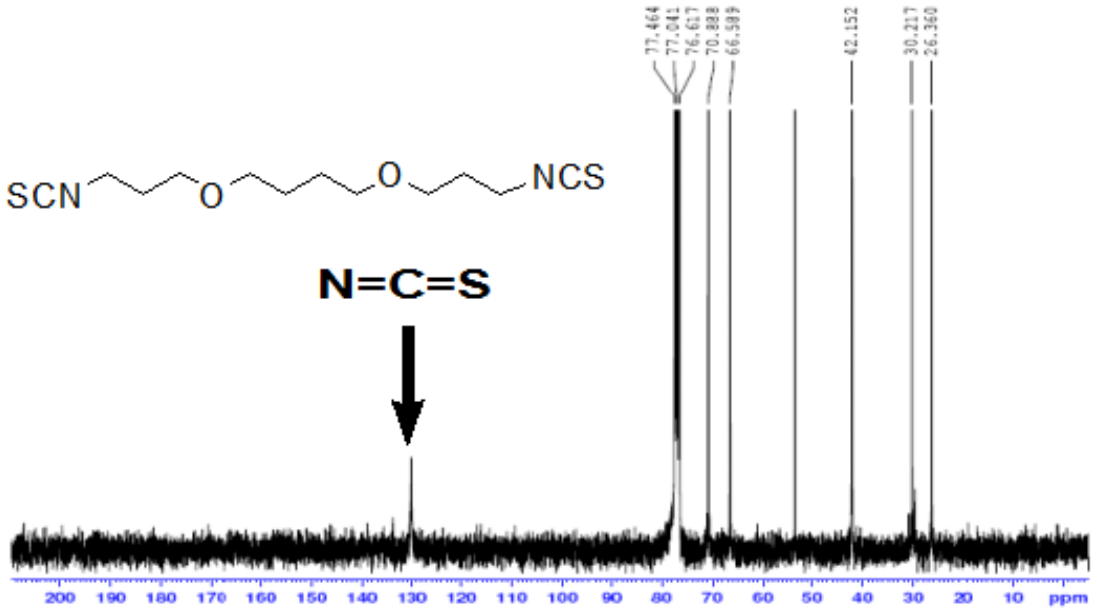



***Figure S47.*** $^{13}$C-NMR of linker for N-Boc DPA78 in $CDCl_3$.

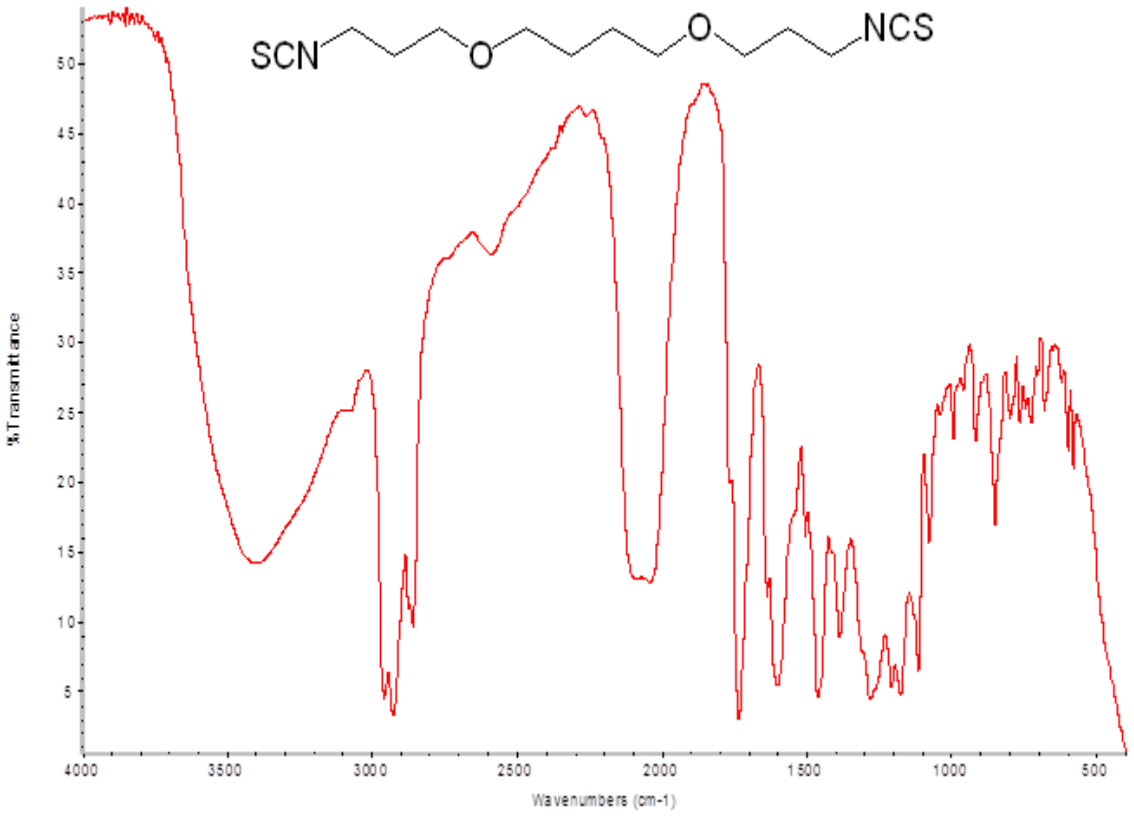



***Figure S48.*** IR of linker for N-Boc DPA78.

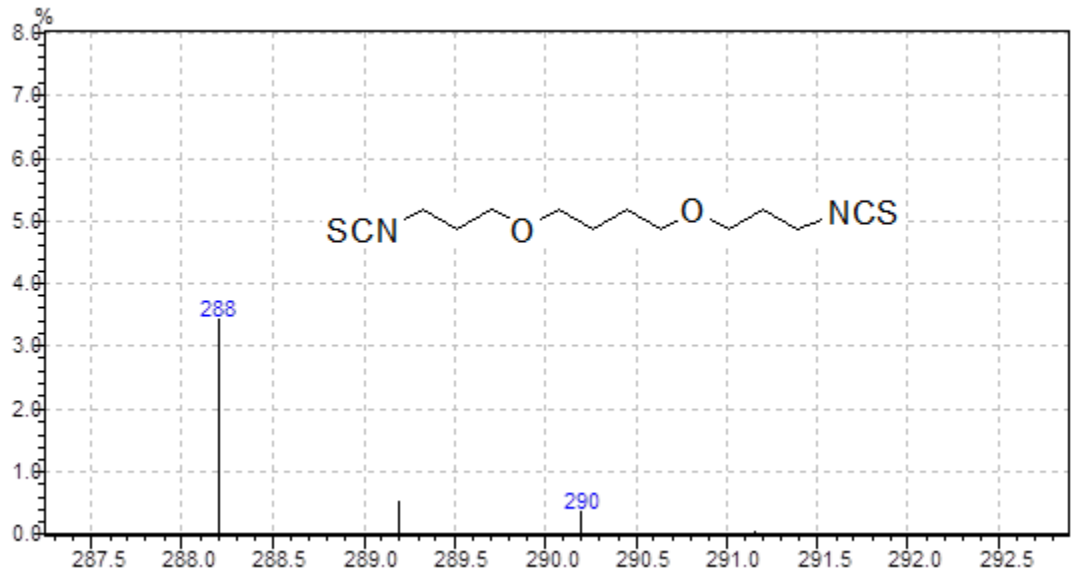



***Figure S49.*** GC/MS of linker for N-Boc DPA78.

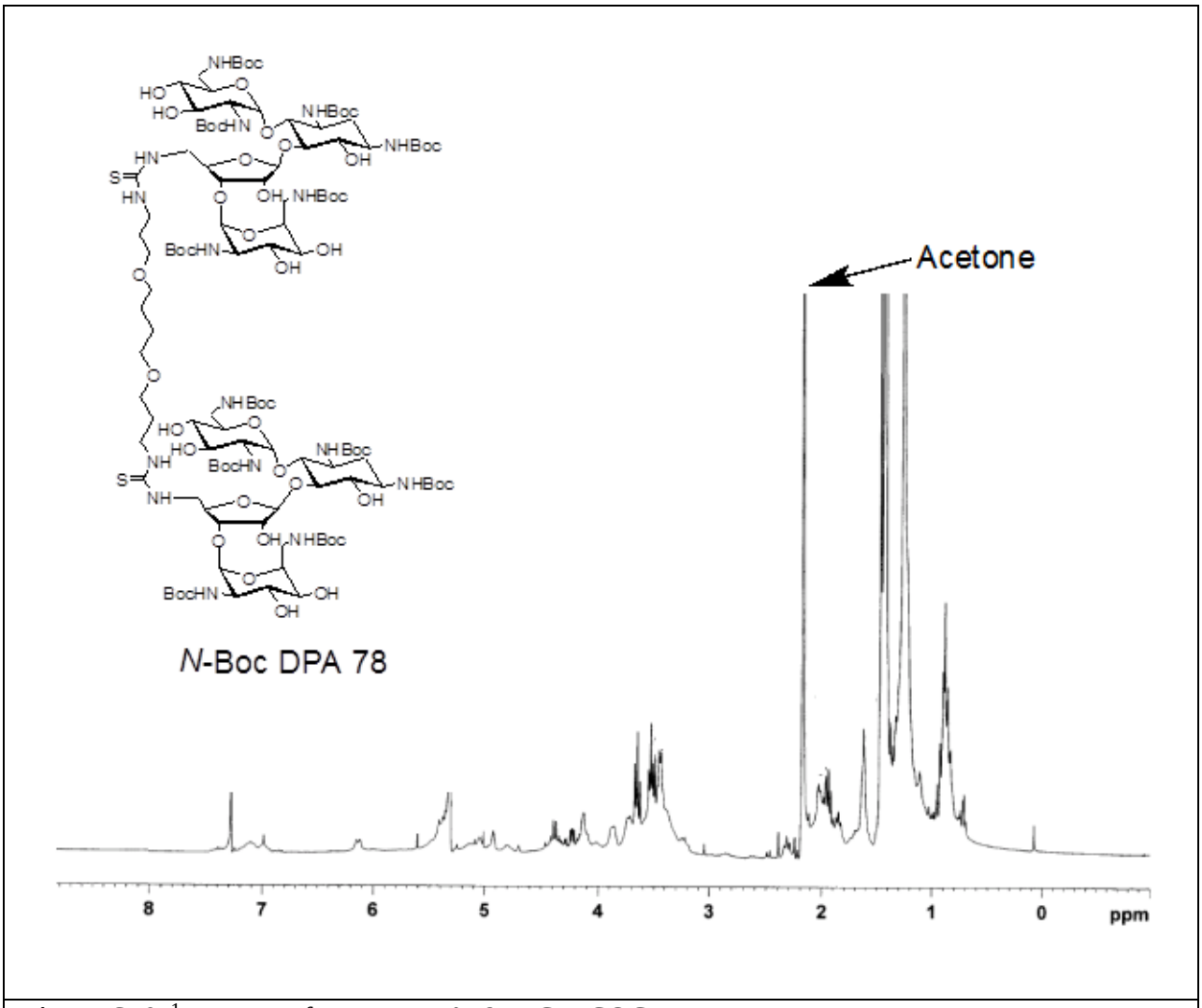



***Figure S50.*** $^{1}$H-NMR of N-Boc DPA78 in $CD_3COCD_3$.

DPA78.

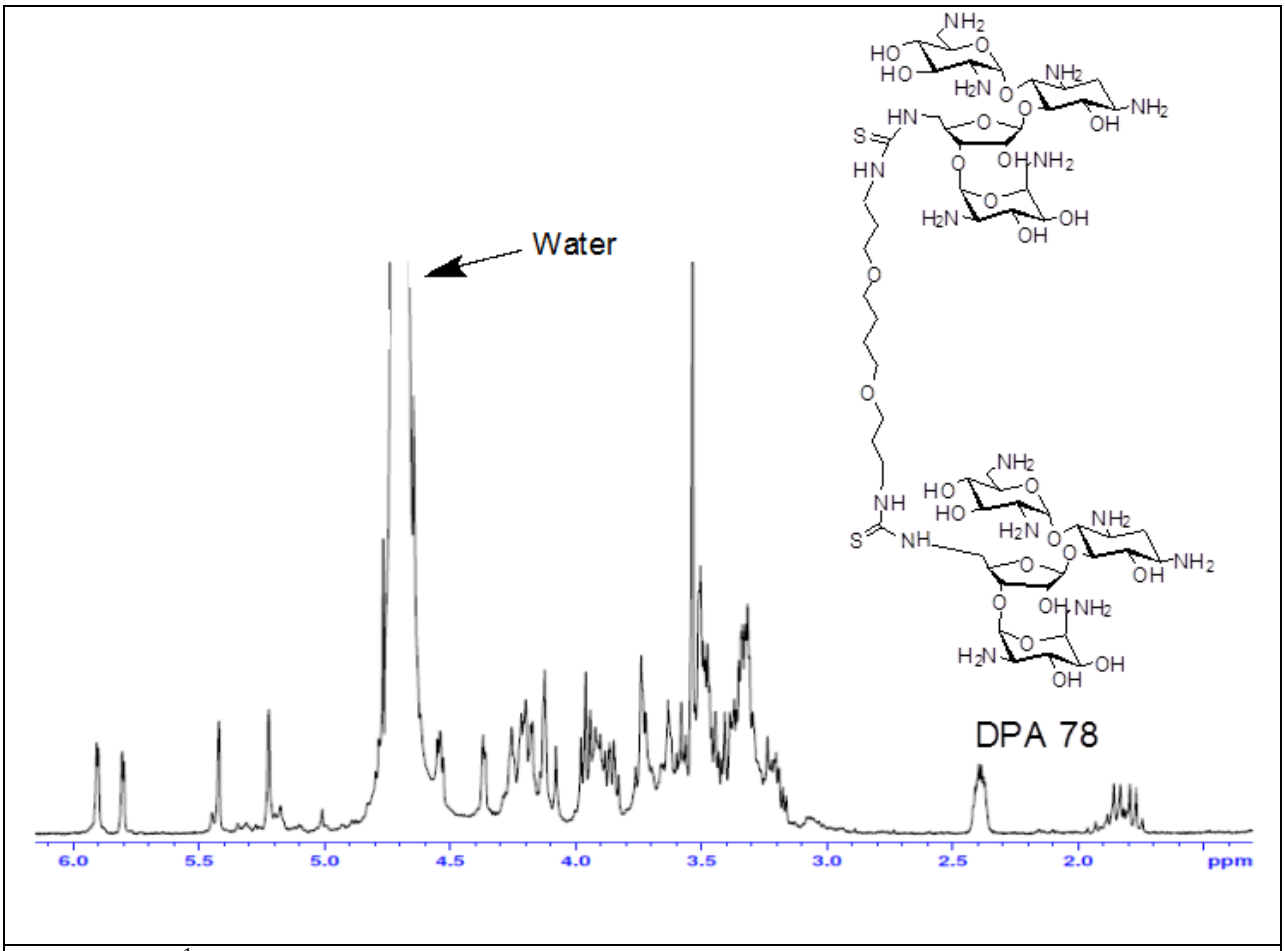
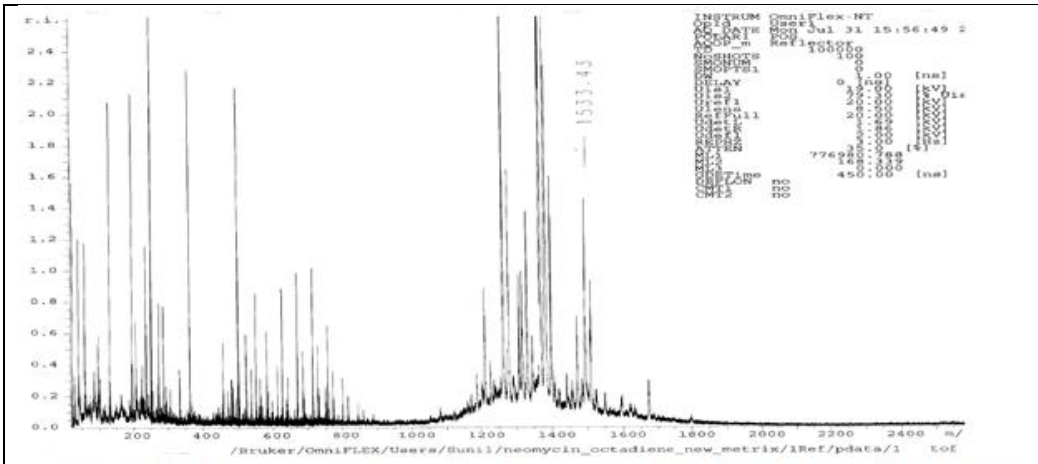



***Figure S51.*** $^{1}$H-NMR of DPA78 in $D_2O$.




MALDI-TOF (m/z) for $C_{58}H_{114}N_{16}O_{26}S_2$ $[M+H_2O]^+$ calcd 1533.75
found 1533.43

***Figure S52.*** MALDI-TOF of DPA78.

N-Boc DPA 80.

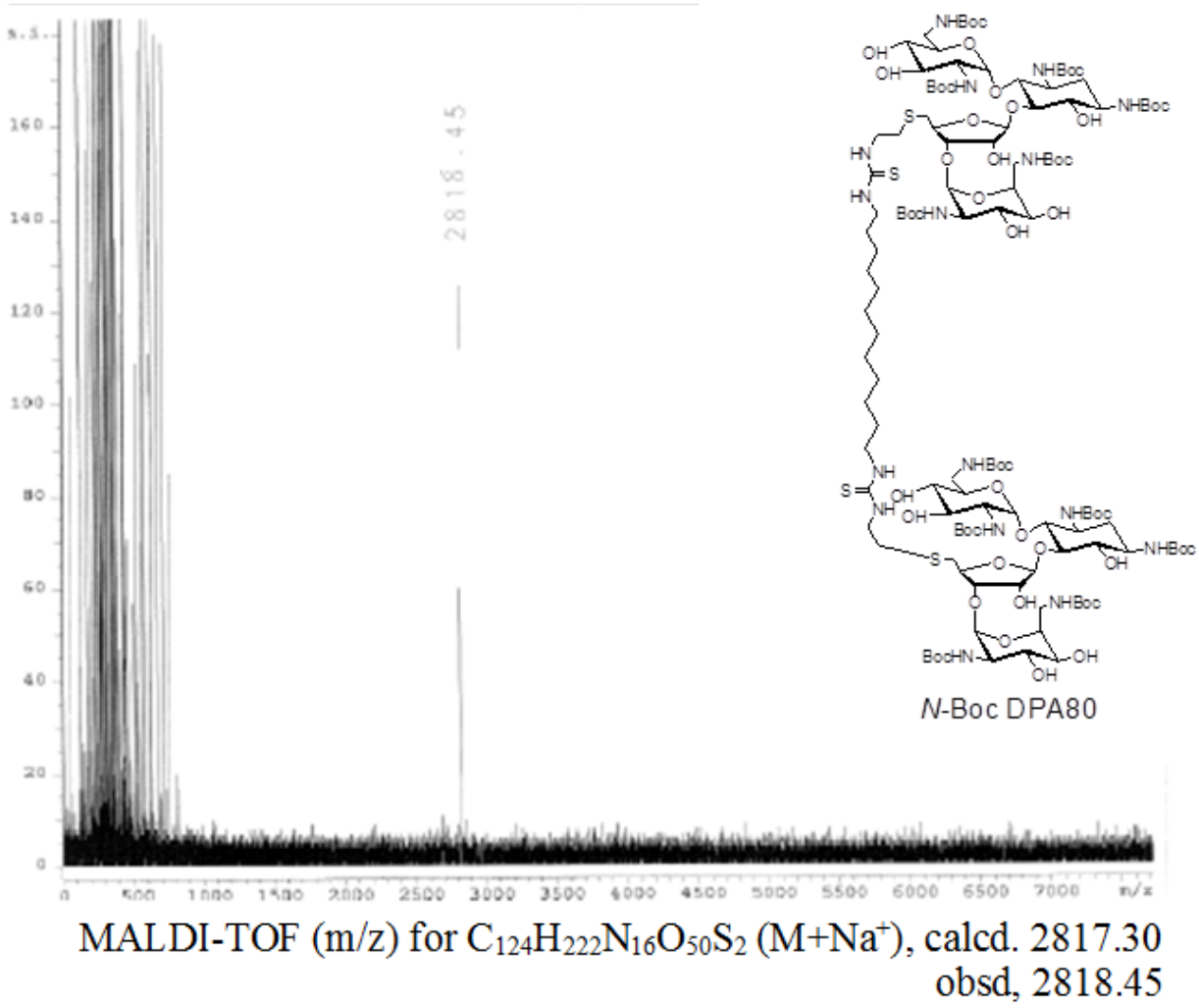



***Figure S53.*** MALDI-TOF of N-Boc DPA80.

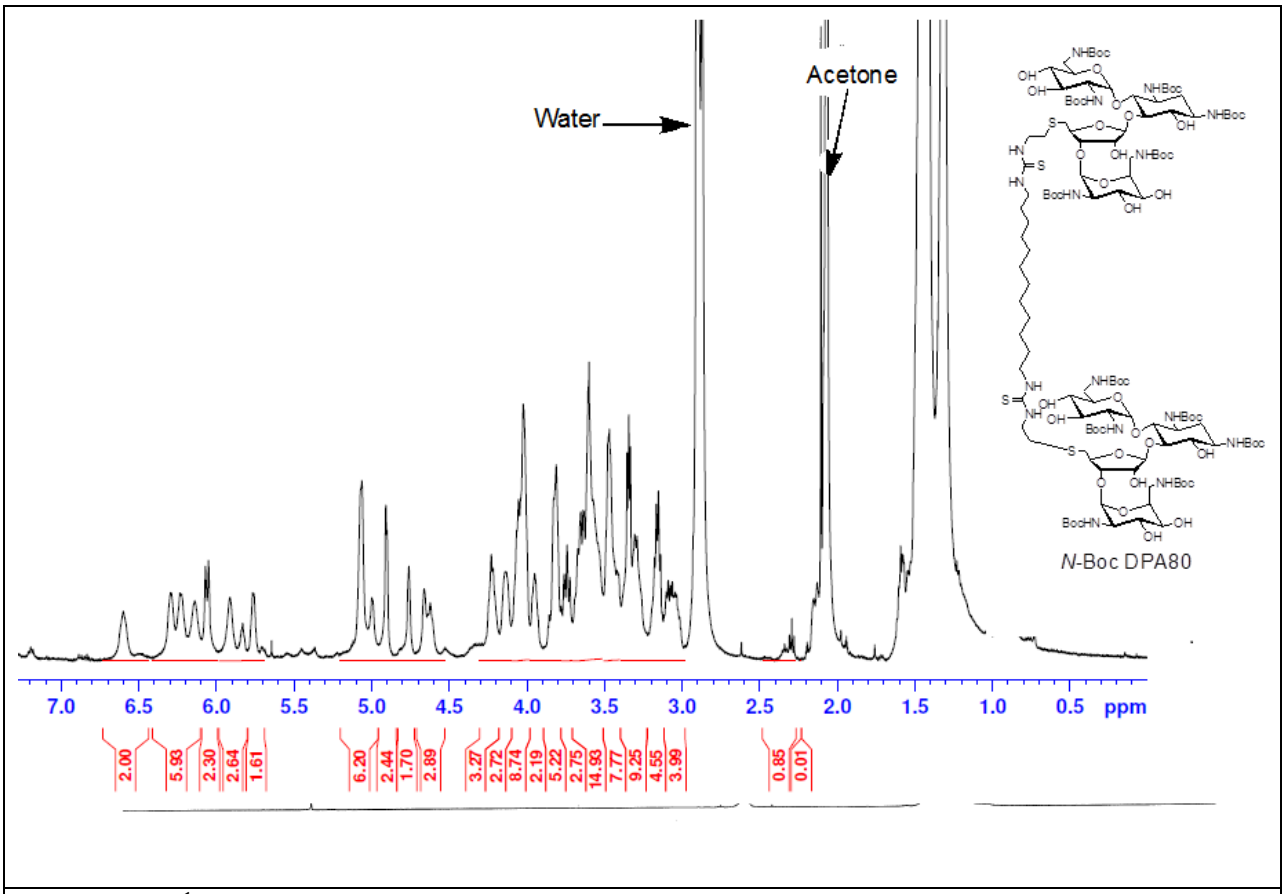



***Figure S54.*** $^{1}$H-NMR of N-Boc DPA80 in $CD_3COCD_3$.

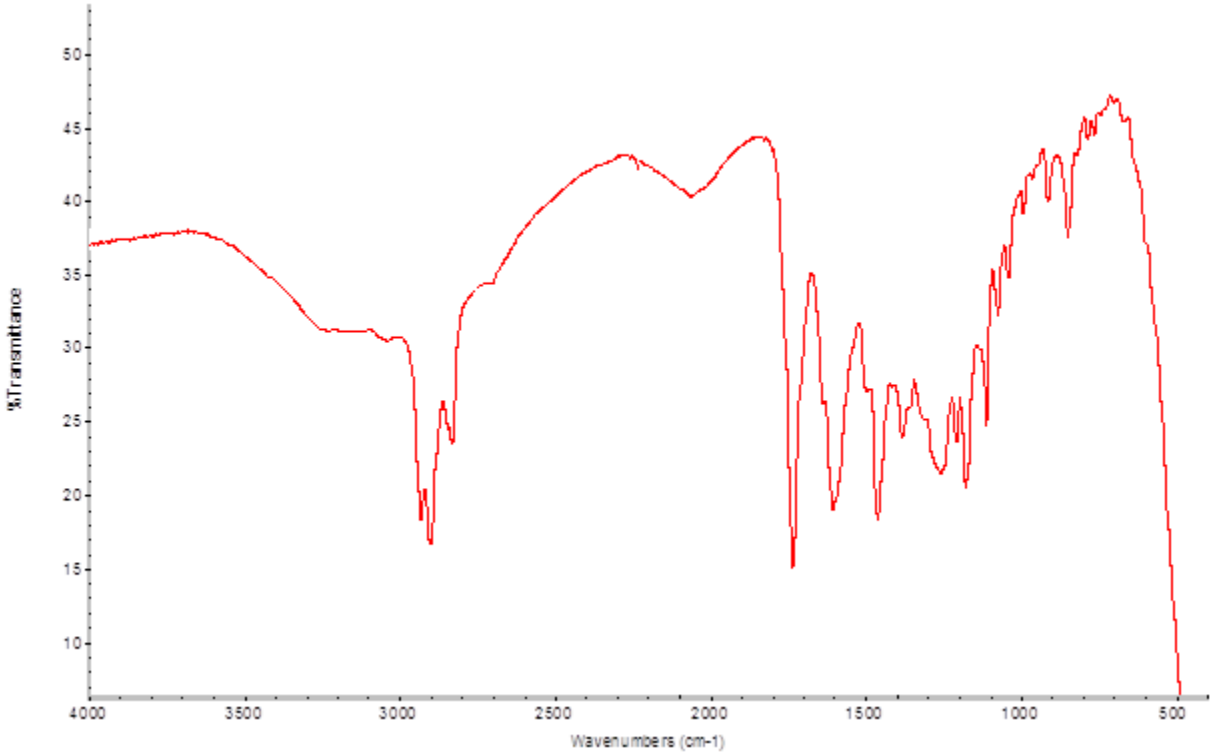



***Figure S55.*** IR of N-Boc DPA80.

DPA80.

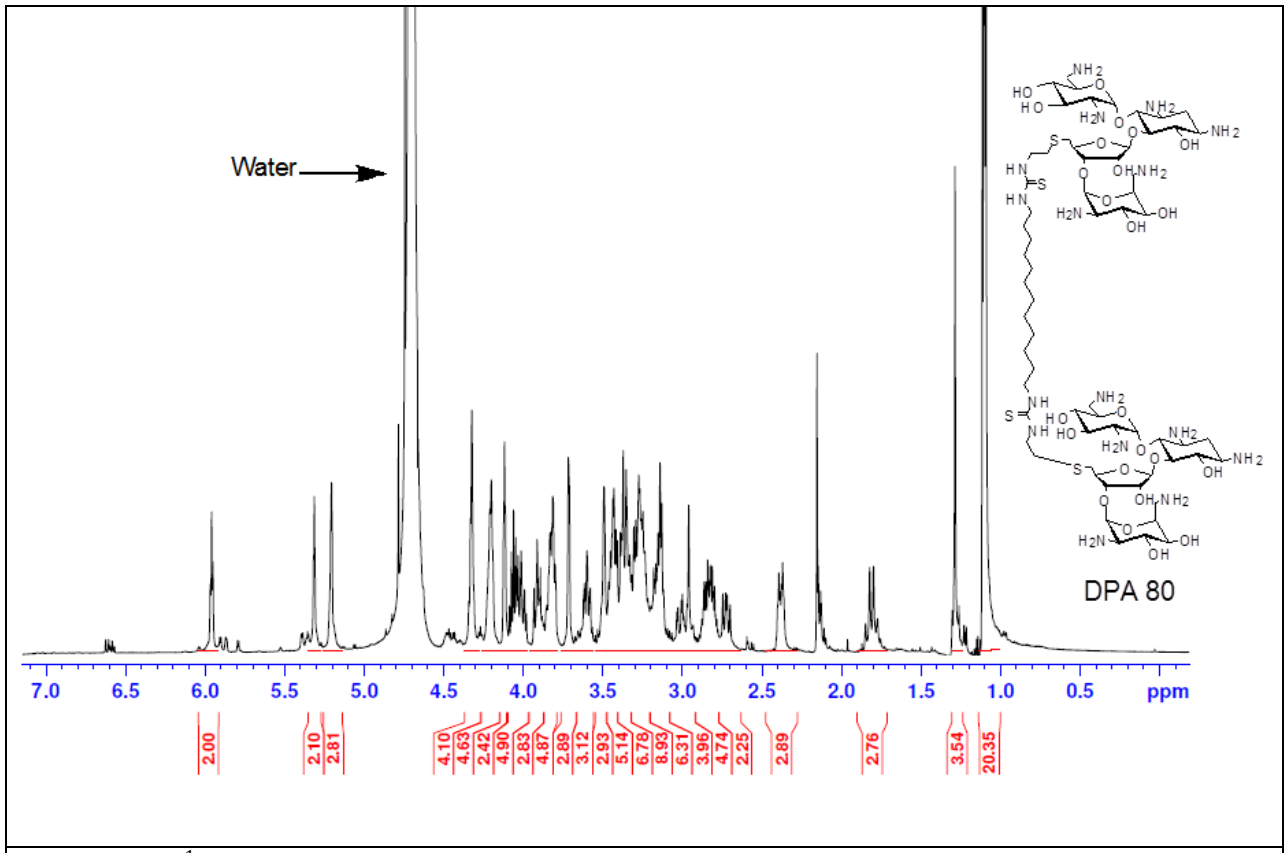



***Figure S56.*** $^{1}$H-NMR of DPA80 in $D_2O$.

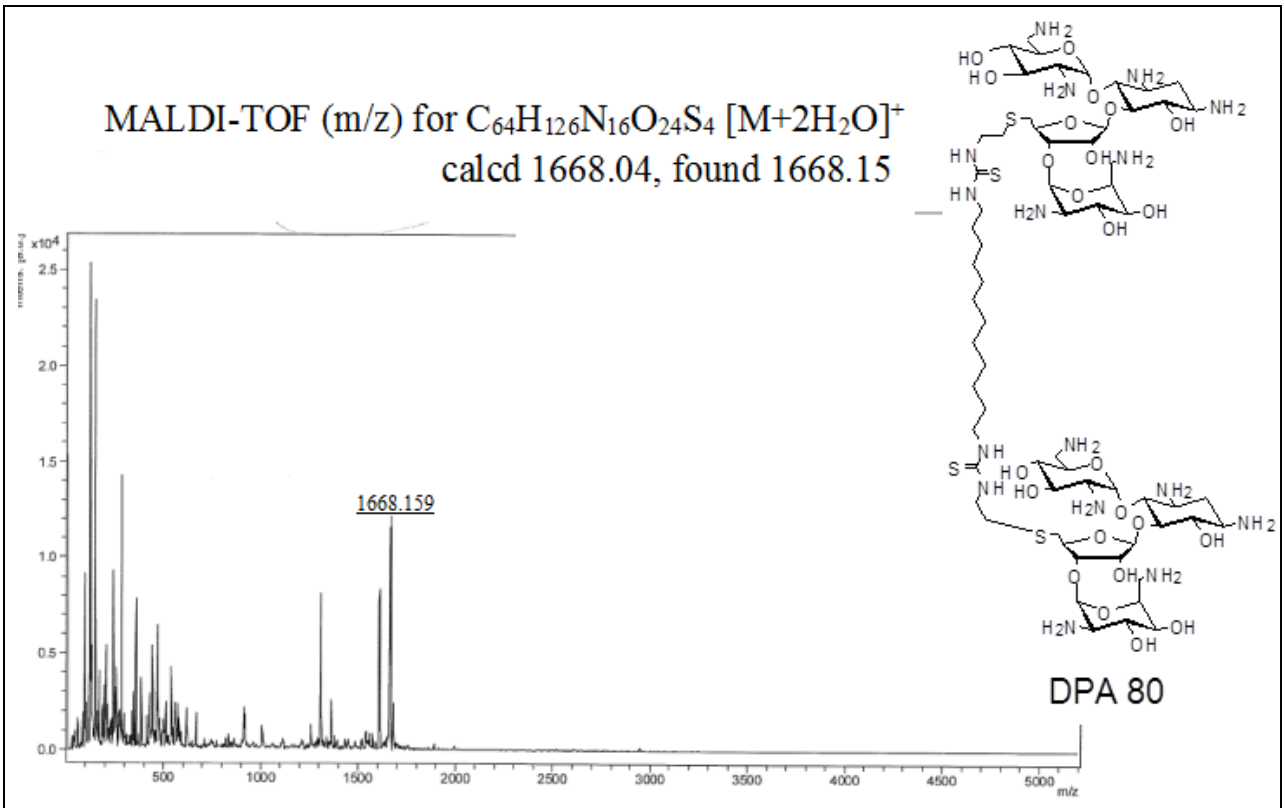



***Figure S57.*** MALDI-TOF of DPA80.

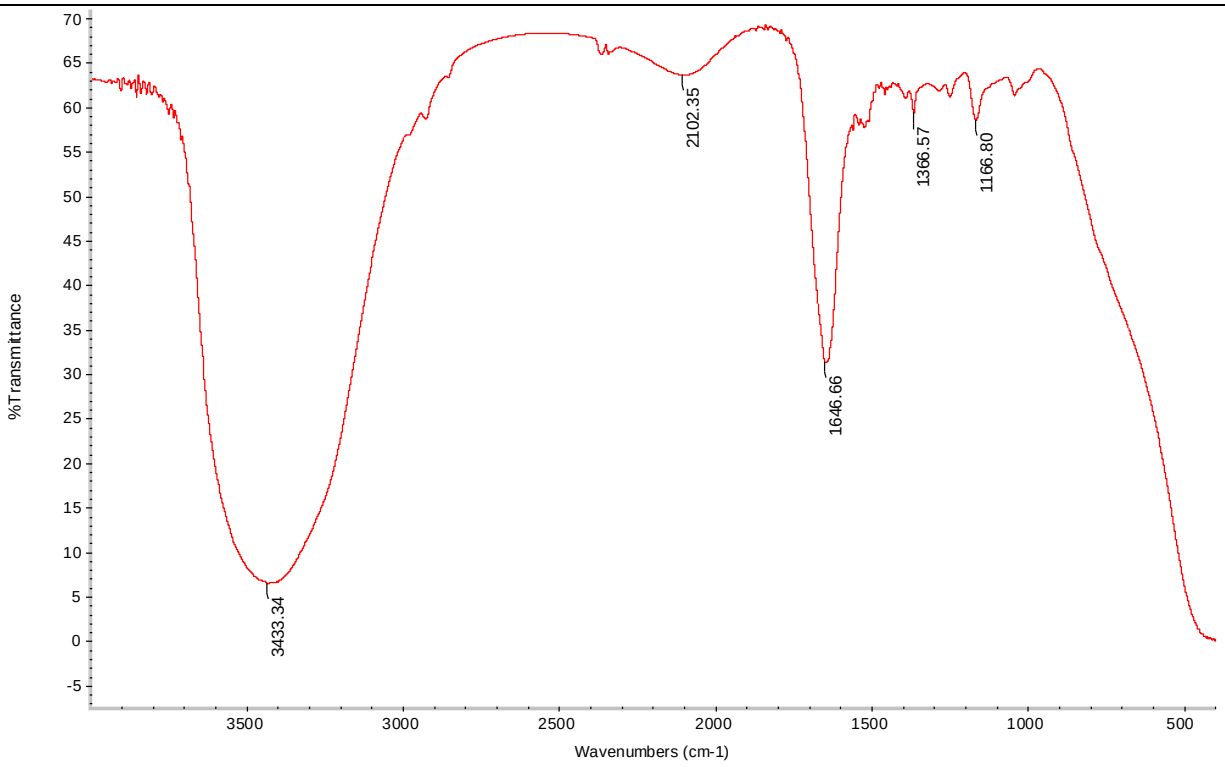



***Figure S58.*** IR of DPA80.

**DPA311.**

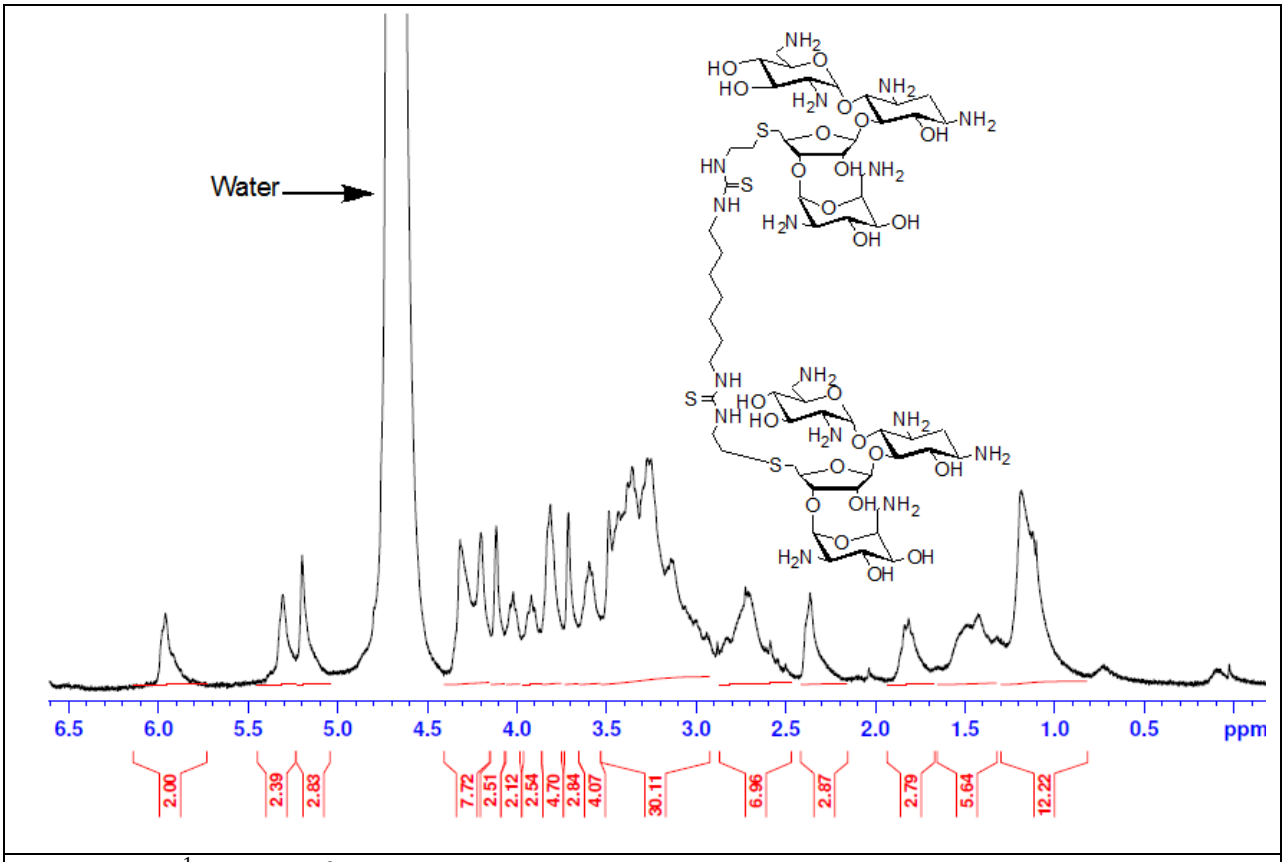



***Figure S59.*** $^{1}$H-NMR of DPA311 in $D_2O$.

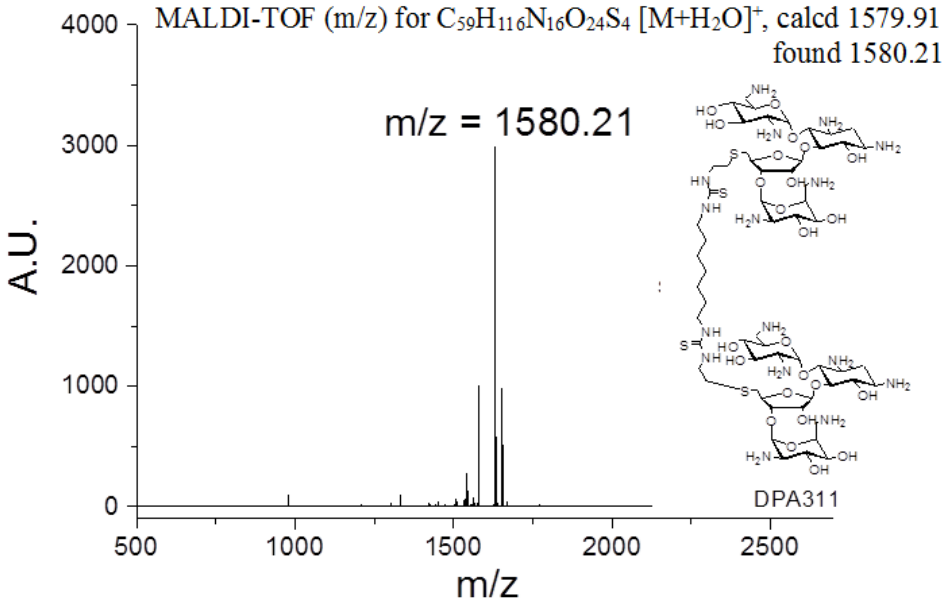



***Figure S60.*** MALDI-TOF of DPA311.

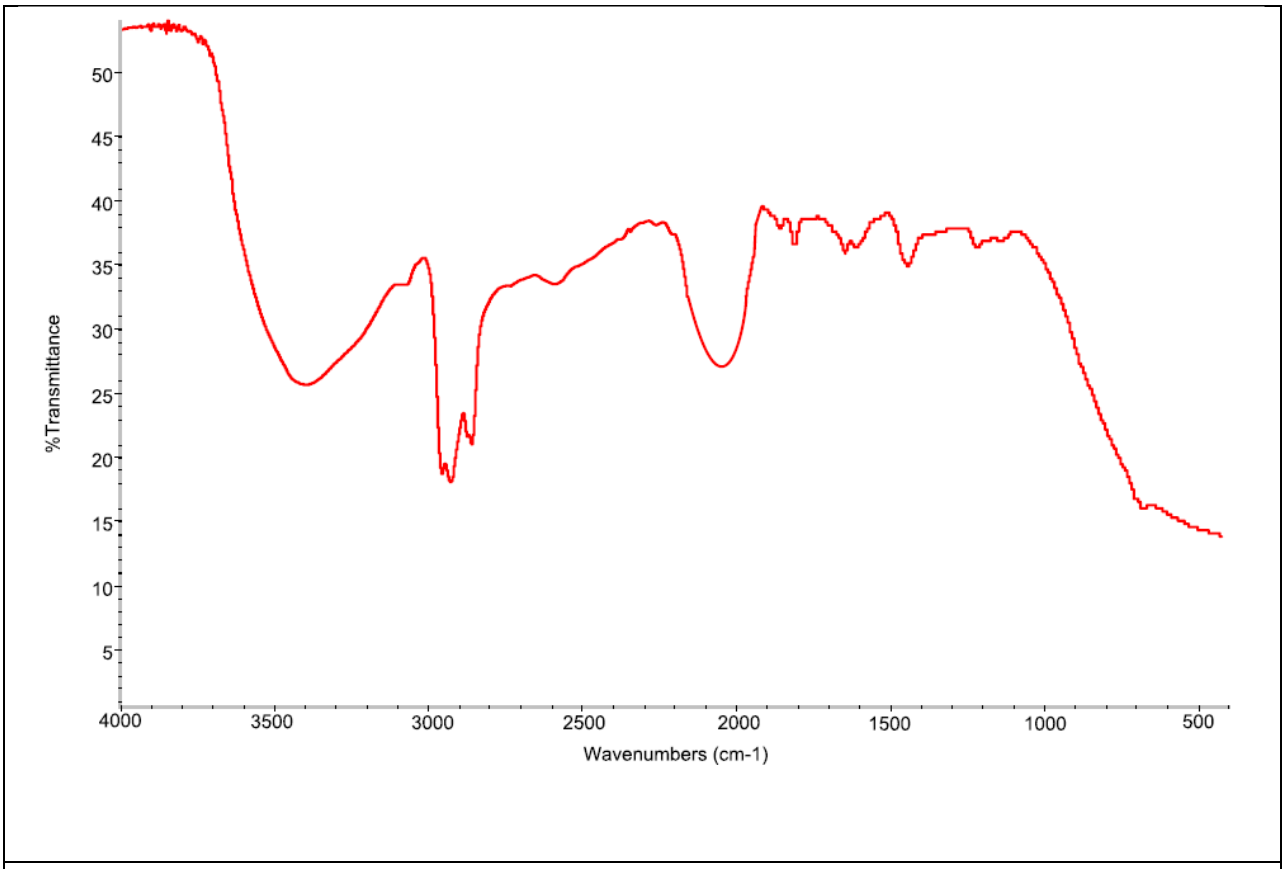



***Figure S61.*** IR of DPA311.

**DPA312.**

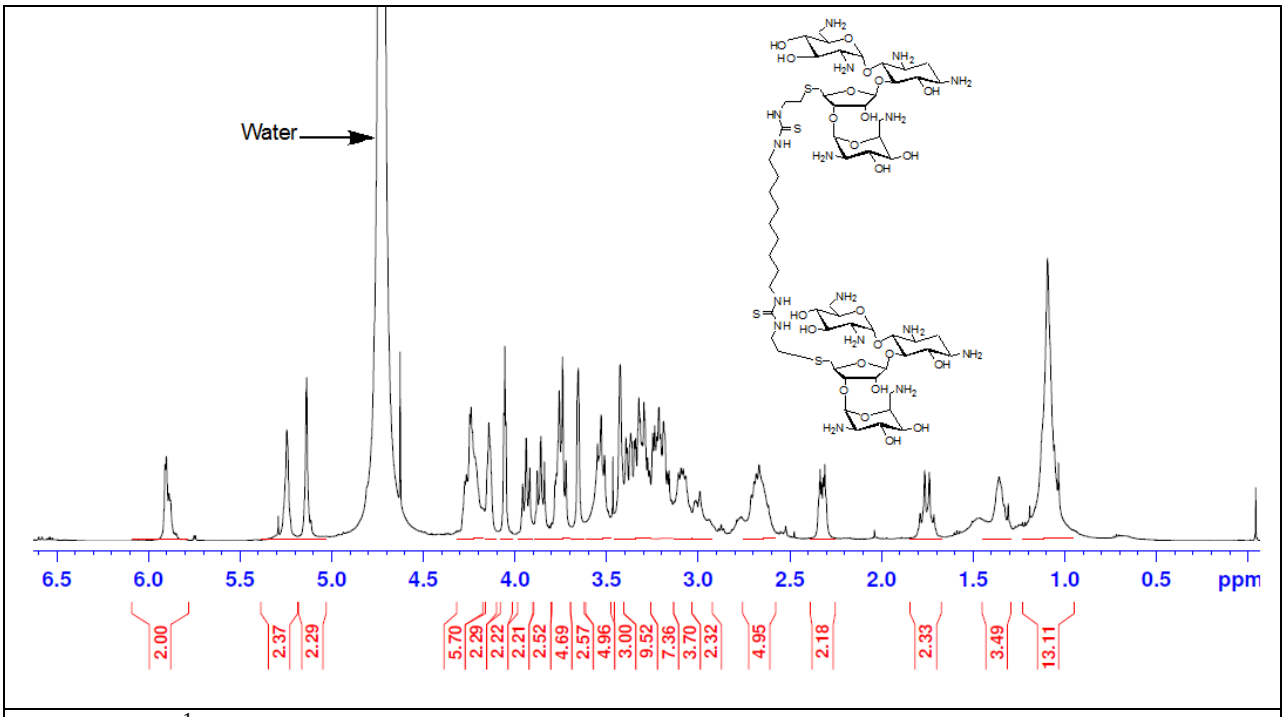



***Figure S62.*** $^{1}$H-NMR of DPA312 in $D_2O$.

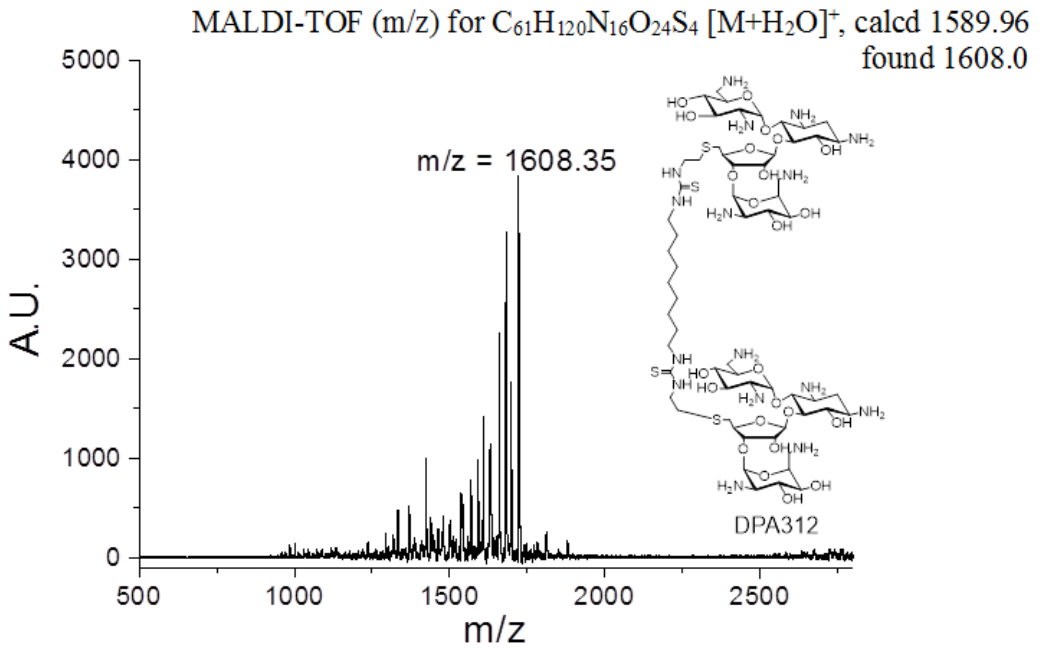



***Figure S63.*** MALDI-TOF of DPA312.

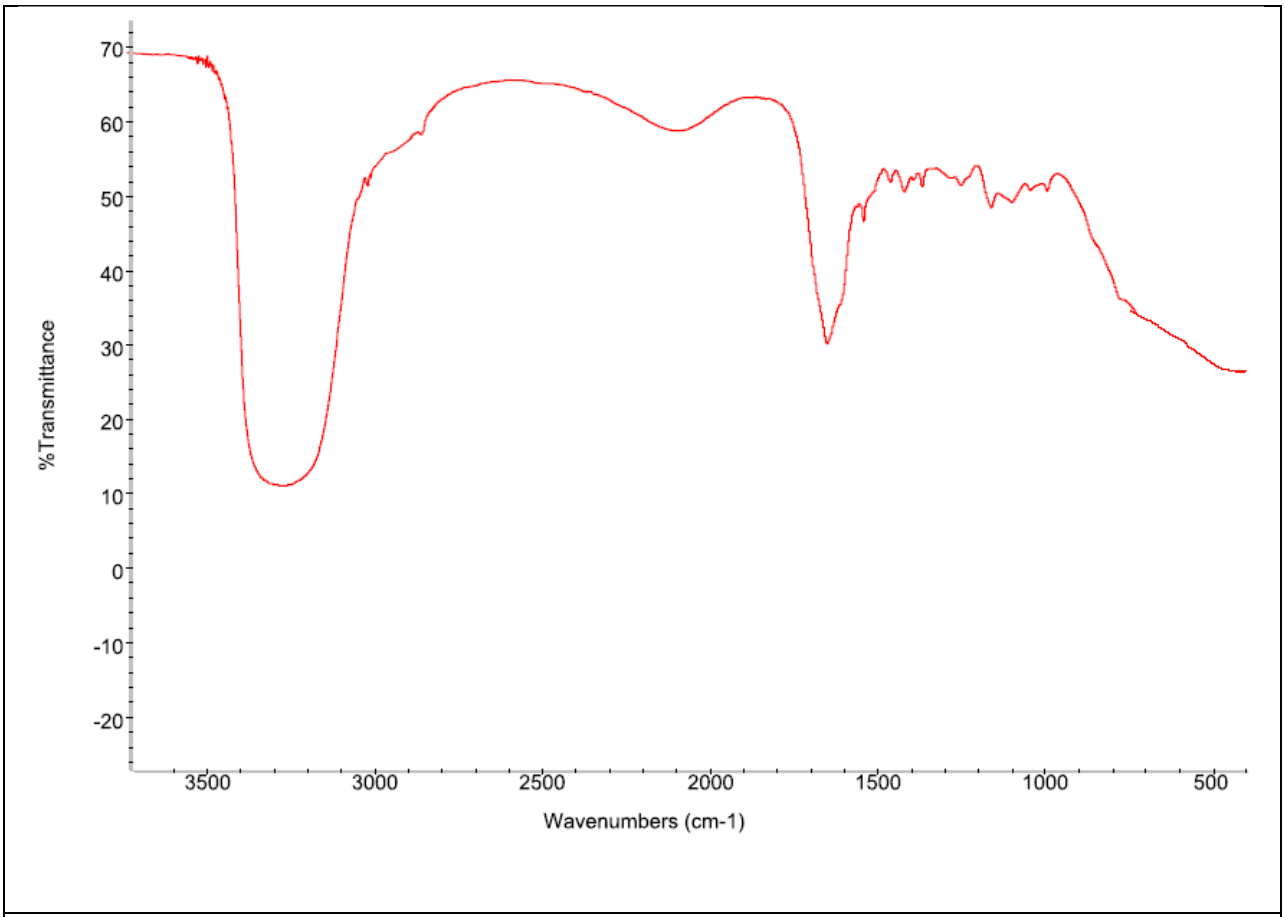



***Figure S64.*** IR of DPA312.

**DPA313.**

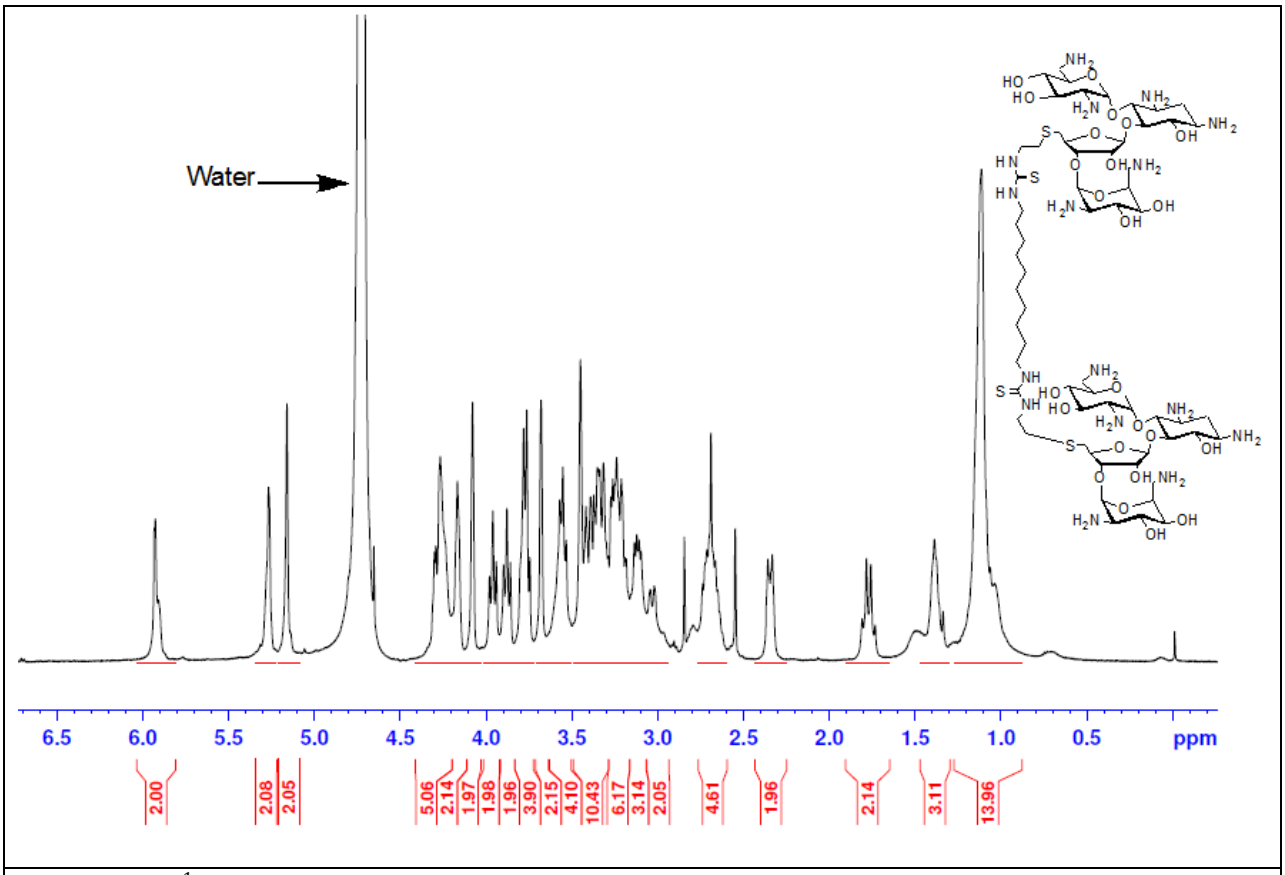



***Figure S65.*** $^{1}$H-NMR of DPA313 in $D_2O$.

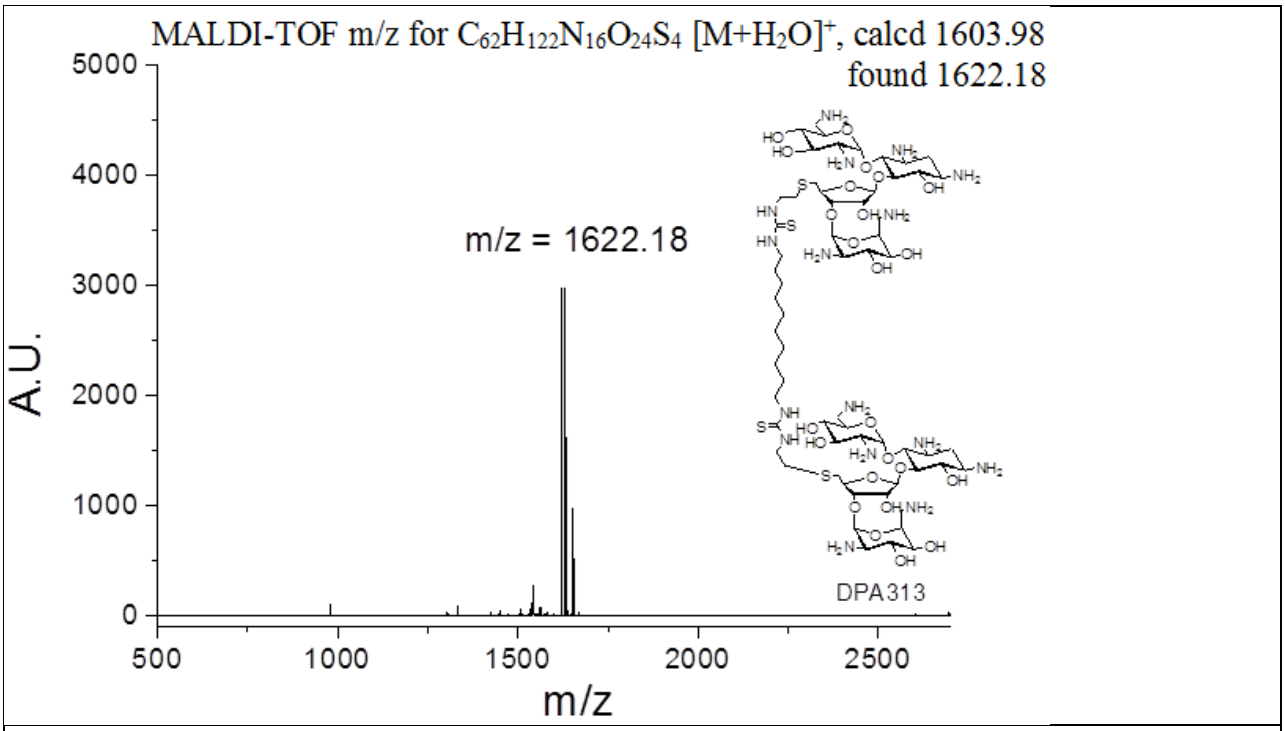



***Figure S66.*** MALDI-TOF of DPA313.

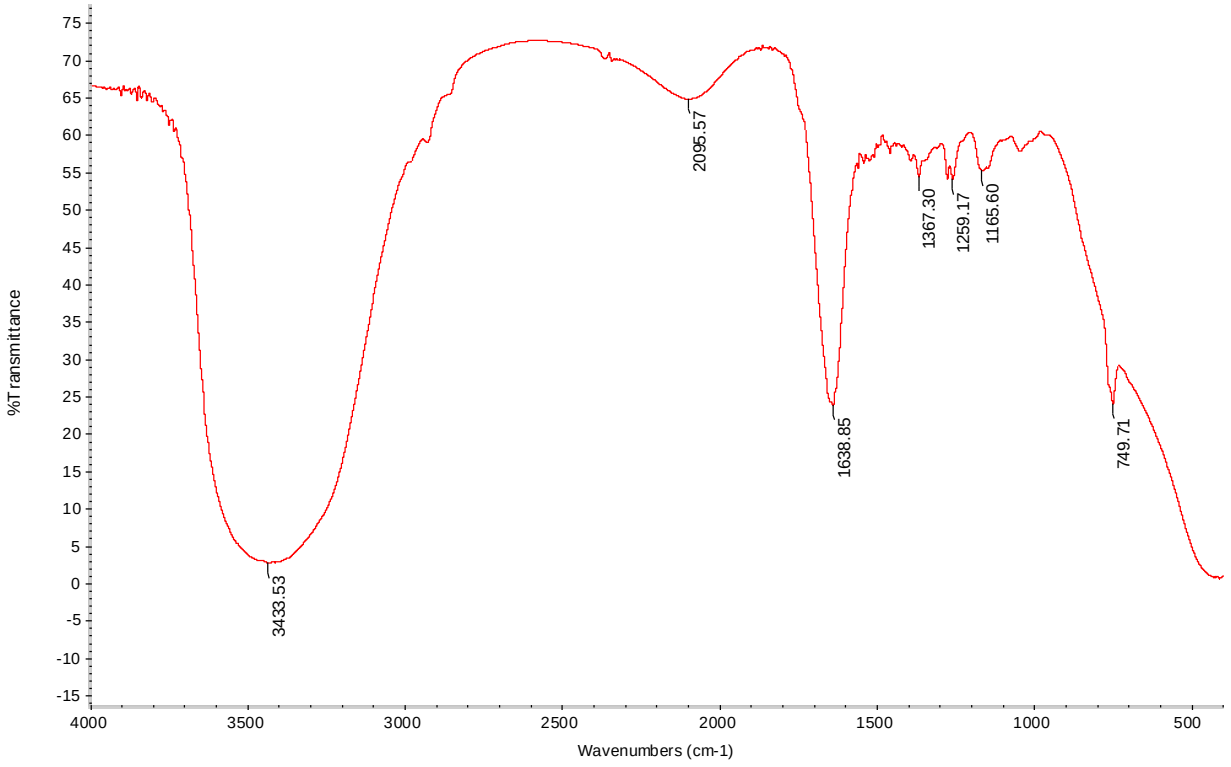
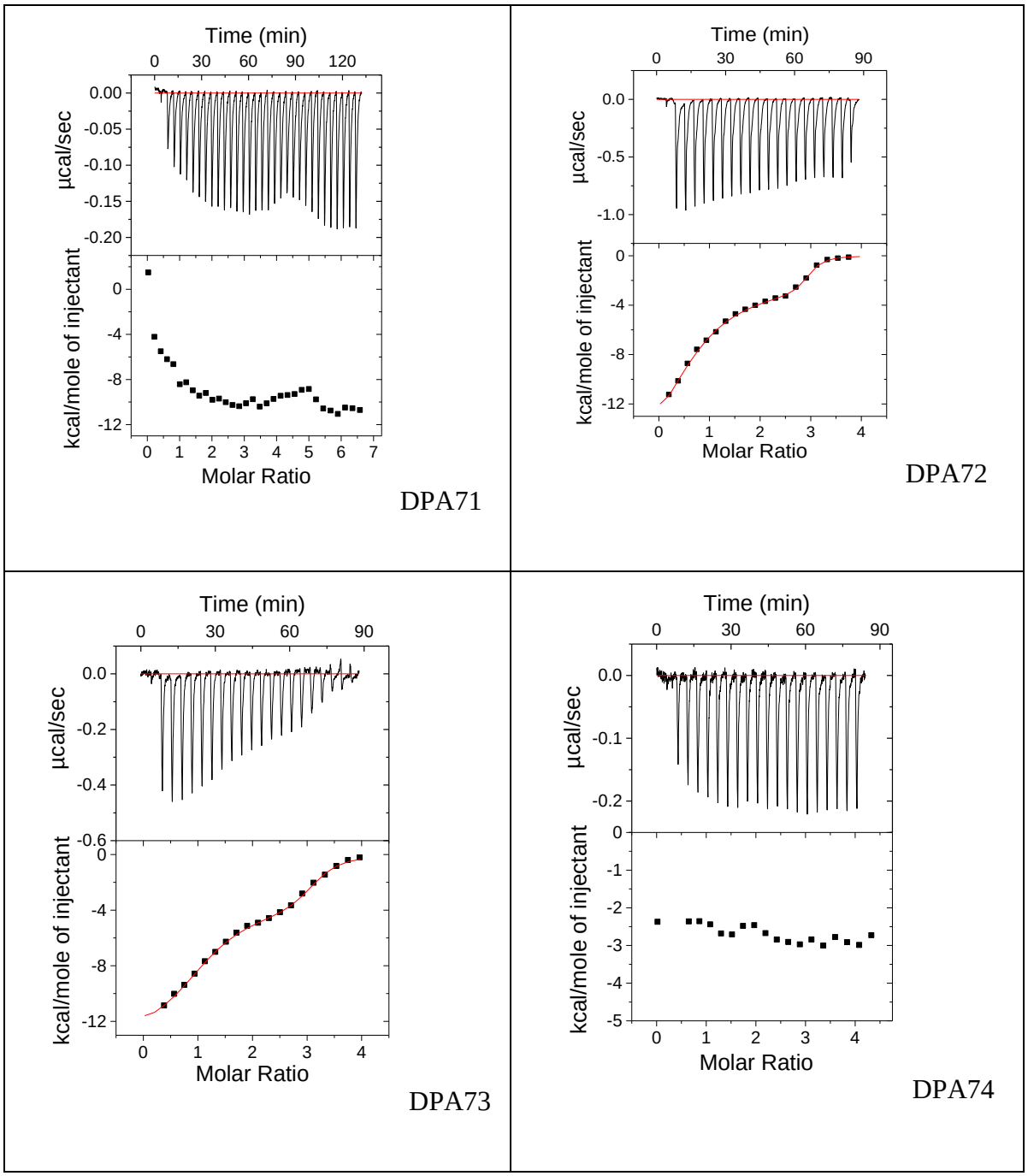
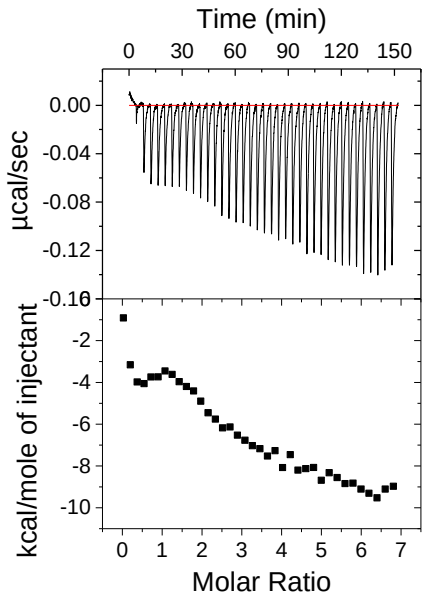


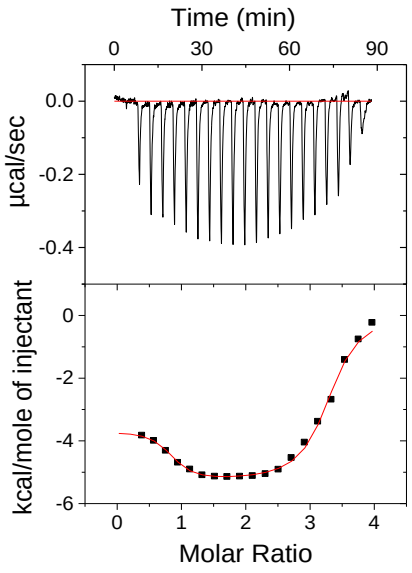

***Figure S67.*** IR of DPA313.

## ITC profile of neomycin and neomycin dimers with AT DNA

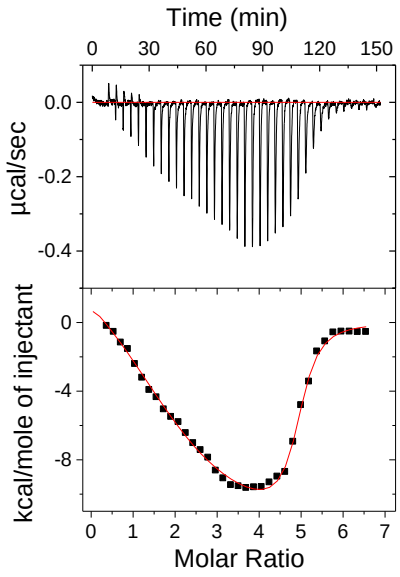




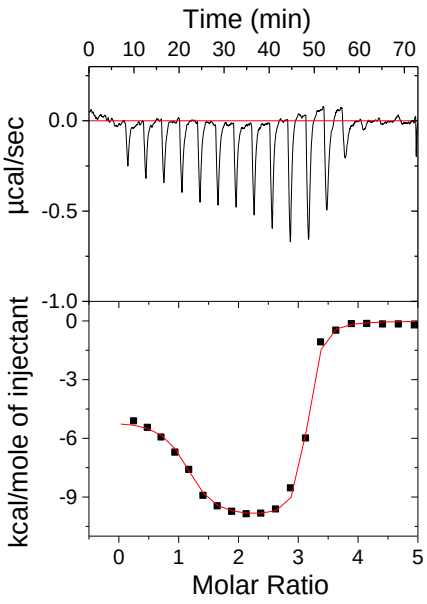

DPA75



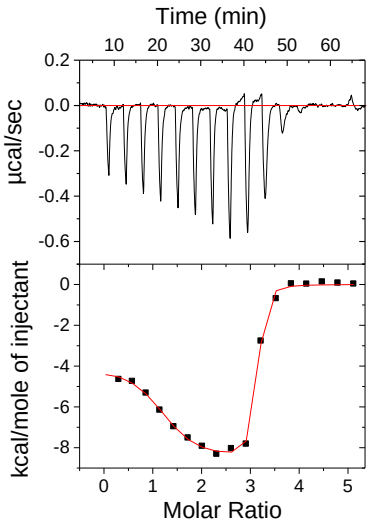

DPA76



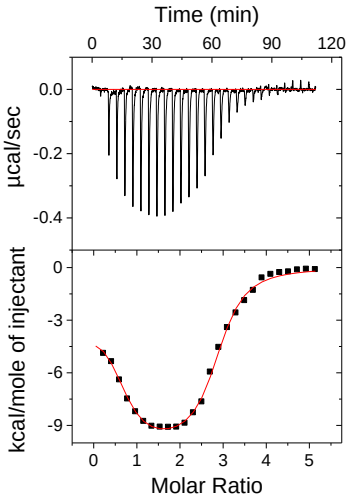

DPA77



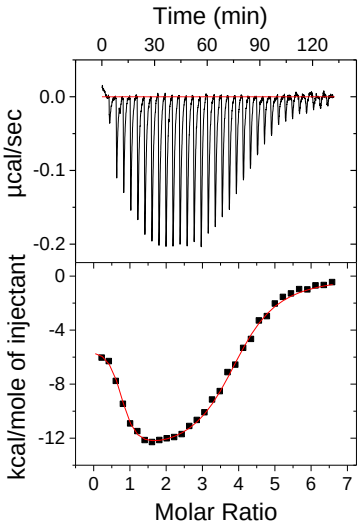

DPA78



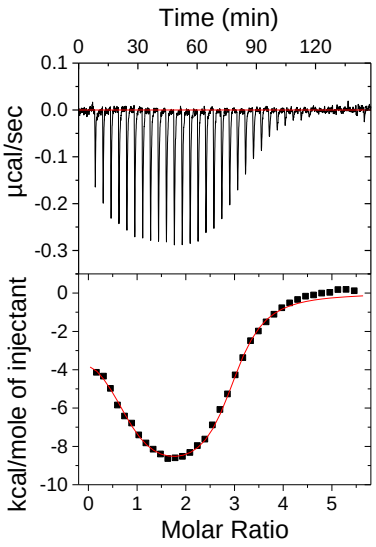

DPA79



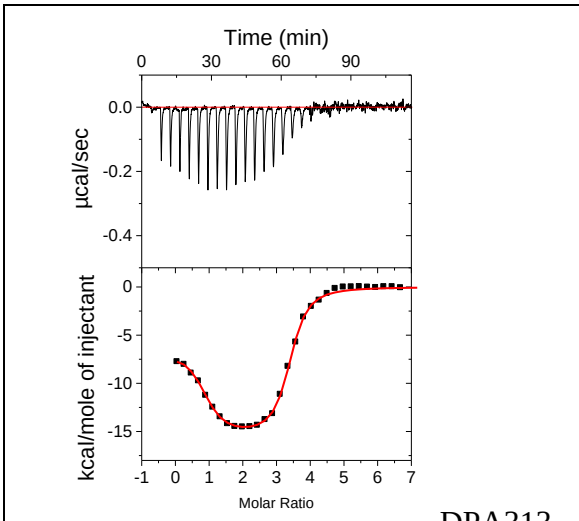

DPA80



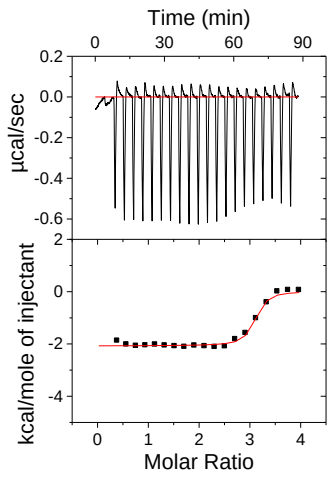

DPA311




DPA312

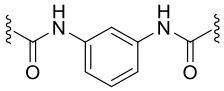
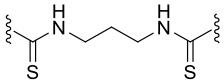
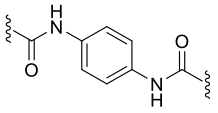
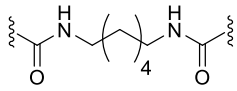
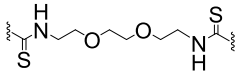
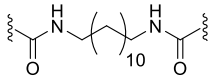
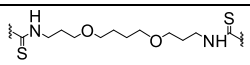
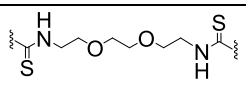
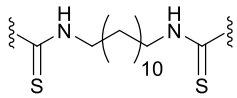



DPA313




Neomycin

***Figure S68.*** ITC profile of indicated ligands including neomycin and neomycin dimers with d[5'-$G_2A_6T_6C_2$-3']. The upper panel in each ITC titration represents the heat burst curves, each of which is the result of a 9 µL injection of 125 µM of **neomycin dimer**, with a DNA concentration of 4 µM/duplex. The area under each heat burst curve was calculated by integration and yielded the associated injection heats that are plotted as a function of molar ratio of drug-to-DNA in the lower panel in each figure. Corrected injection heats plotted as a function of the [drug]/DNA ratio. Buffer conditions: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 5.5. T = 25 $^{o}$C. [DNA] = 4 µM/duplex. [**neomycin dimer**] = 125 µM. [Neomycin] = 250 uM and the [DNA] = 8 µM/duplex.

| Compound name | Structure of the linker | $N_1$ | $K_1$ ($M^{-1}$) | $\Delta H_1$ (kcal/mol) | $\Delta S_1$ (cal/mol.K) |
|---|---|---|---|---|---|
| DPA71 |  | ND | ND | -3.9±0.2 | ND |
| DPA72 |  | 1.0±0.0 | (2.5±0.3)X$10^7$ | -13.5±0.7 | -10.5 |
| DPA73 |  | 0.8±0.1 | (7.6±1.8)X$10^7$ | -19.6±3.0 | -29.6 |
| DPA74 |  | ND | ND | -2.4±0.3 | ND |
| DPA75 |  | ND | ND | -4.2±0.4 | ND |
| DPA76 |  | 0.8±0.1 | (7.2±0.5)X$10^7$ | -3.6±0.3 | 26.4 |
| DPA77 |  | 1.0±0.1 | (6.1±0.4)X$10^7$ | -6.1±0.5 | 15.0 |
| DPA78 |  | 1.1±0.1 | (9.1±1.0)X$10^7$ | -5.1±0.3 | 16.8 |
| DPA79* |  | 1.1±0.0 | (1.6±0.2)X$10^8$ | -5.2±0.1 | 19.9 |
| DPA80* |  | 0.8±0.1 | (1.2±0.2)X$10^8$ | -4.4±0.3 | 22.4 |

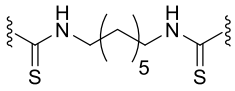
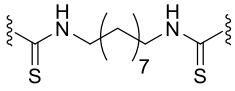
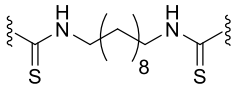
| | | | | | |
|---|---|---|---|---|---|
| DPA311* |  | 0.7±0.0 | (1.3±0.1)X$10^8$ | -5.2±0.3 | 19.8 |
| DPA312* |  | 0.7±0.0 | (1.6±0.2)X$10^8$ | -3.7±0.2 | 21.3 |
| DPA313* |  | 0.8±0.0 | (1.3±0.2)X$10^8$ | -6.7±0.4 | 12.2 |
| Neomycin | NA | 3.1±0.0 | (3.1±0.3)X$10^5$ | -2.0±0.2 | 4.6 |

***Table S3.*** ITC-derived thermodynamic characterization of neomycin dimers and neomycin with DNA duplex d[5'-$G_2A_6T_6C_2$-3'] at 25 ˚C. d[5'-$G_2A_6T_6C_2$-3'] = 8 μM/duplex [neomycin dimer] = 125 μM. Buffer condition: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 5.5.

## $AC_{50}$ values of neomycin dimers and neomycin against AT rich DNA

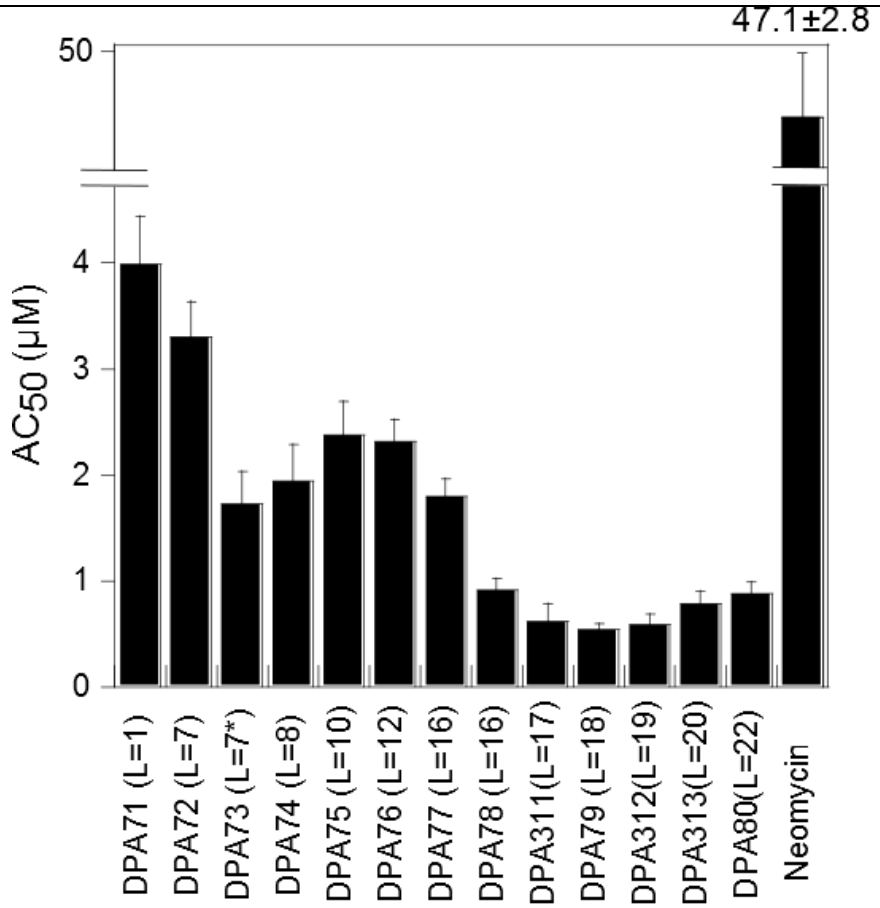



***Figure S69.*** Bar graph represents the comparison of $AC_{50}$ (concentration of aminoglycoside required for 50% change in fluorescence) values derived from FID assay between indicated ligands and d[5'-$A_{12}$-x-$T_{12}$-3'] at 25 °C. d[5'-$A_{12}$-x-$T_{12}$-3'] = 1 μM/duplex. Buffer condition: 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8.