SUPPORTING INFORMATION

Synthesis and evaluation of new spacers for use as dsDNA endcaps

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Table S4 Oligonucleotide MALDI-TOFMS data

Oligonucleotide	$(M+H)^+$ Calculated	$(M+H)^+$ Observed	Comments
GCTA-**8**-TAGC	2693.9	2693.4	
ATGC-**8**-GCAT	2693.9	2693.2	
GCAT-**14**-ATGC	2717.9	2717.3	Confirmed by enzymatic sequencing*
TACG-**14**-CGTA	2717.9	2715.3	Confirmed by enzymatic sequencing
CGAT-**14**-ATCG	2717.9	2717.5	
GCTA-**14**-TAGC	2717.9	2715.9	Confirmed by enzymatic sequencing
ATGC-**14**-GCAT	2717.9	2715.4	Confirmed by

			enzymatic sequencing
GCAT-**18**-ATGC	2716.9	2719.6	
TACG-**18**-CGTA	2716.9	2712,2	
CGAT-**18**-ATCG	2716.9	2714.0	
GCTA-**18**-TAGC	2716.9	2713.9	
ATGC-**18**-GCAT	2716.9	2714.7	
GCTA-**22**-TAGC	2744.9	2743.5	
ATGC-**22**-GCAT	2744.9	2748.0	
GCTA-**29**-TAGC	2748.9	2750.6	
ATGC-**29**-GCAT	2748.9	2750.6	

*Enzymatic sequencing involved exonuclease digestion from the 3'-end in combination with MALDI

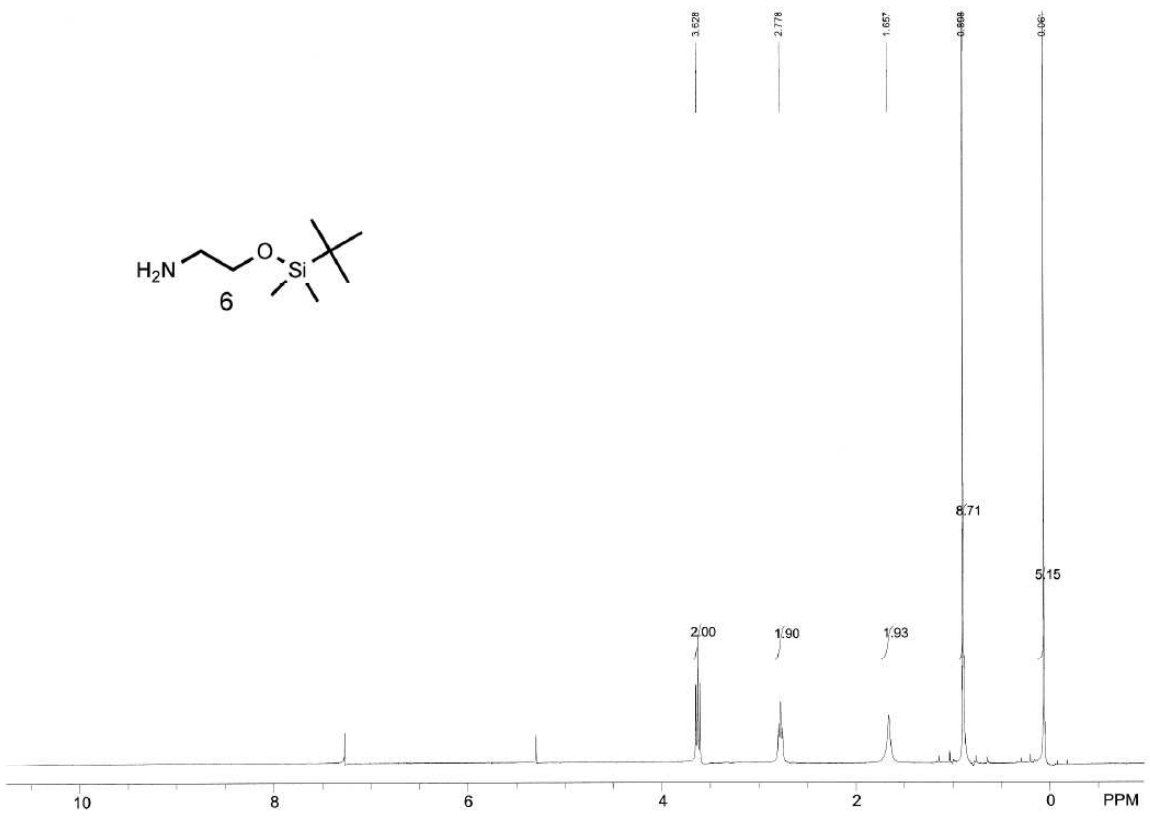

Figure S1: ^{1}H NMR spectrum of 2-(*tert*-Butyldimethylsilyloxy)ethylamine (**6**)

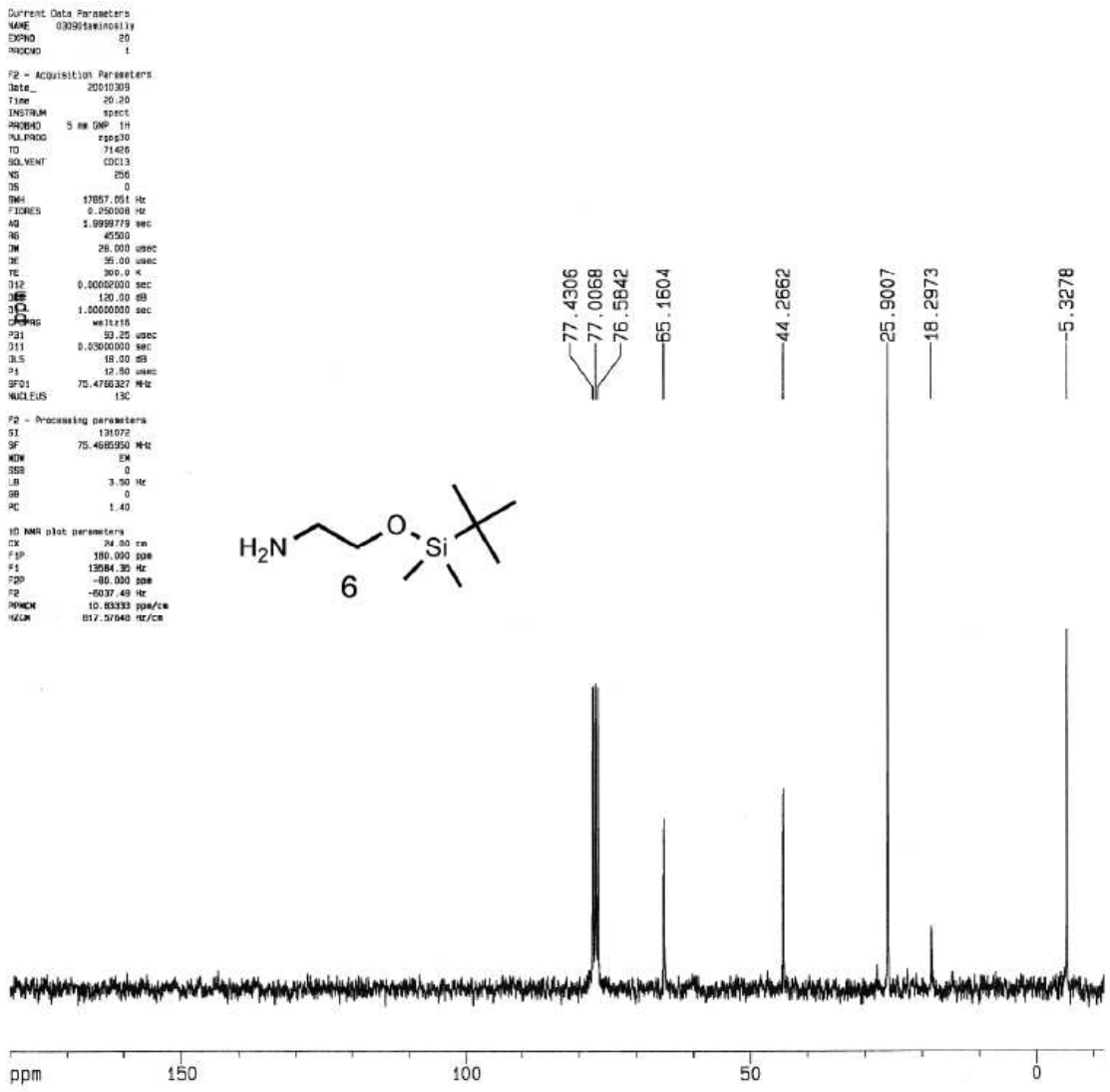

Figure S2: ^{13}C NMR spectrum of 2-(*tert*-Butyldimethylsilyloxy)ethylamine (**6**)

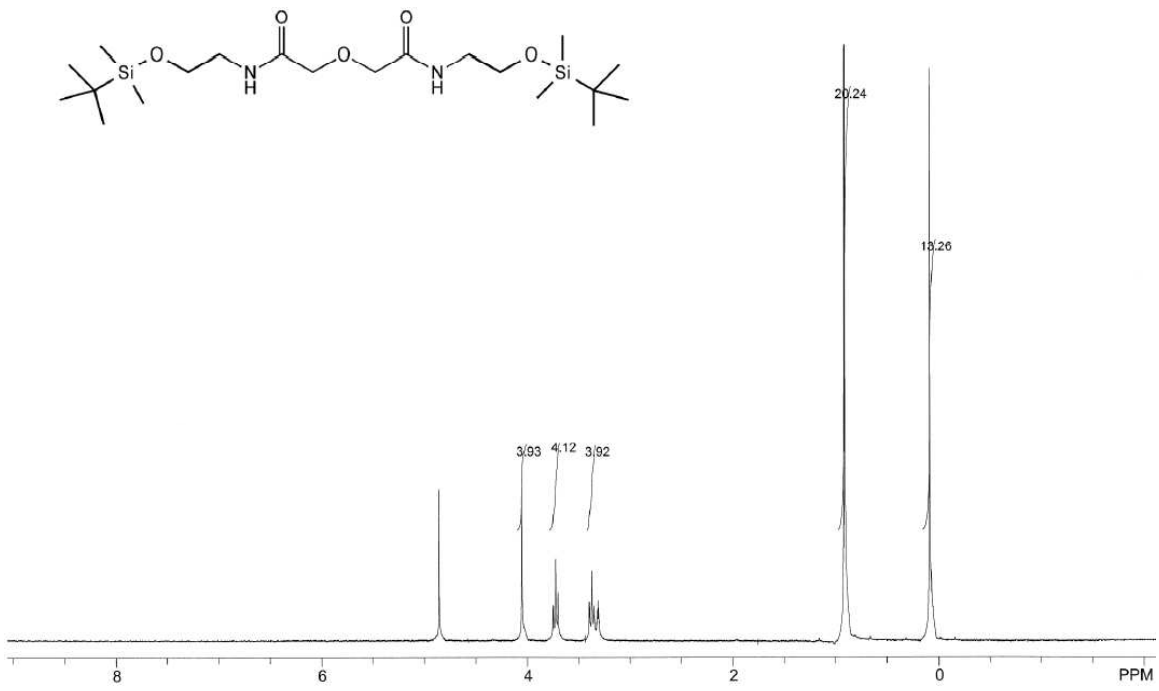

Figure S3: ^{1}H NMR spectrum of *N*,*N*'-Bis[2-(*tert*-butyldimethylsilyloxyethyl]-2,2'-oxydiacetamide (**7**)

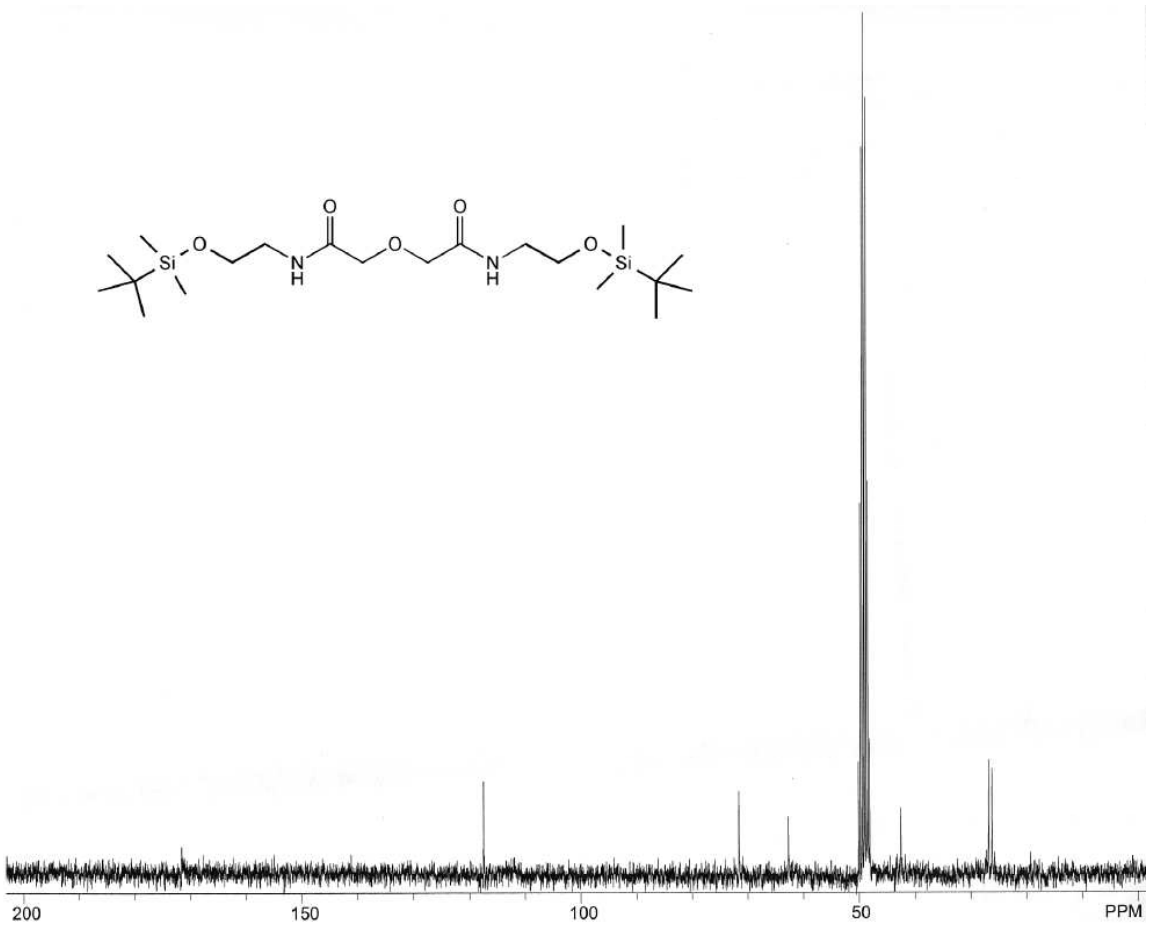

Figure S4: ^{13}C NMR spectrum of *N*,*N*'-Bis[2-(*tert*-butyldimethylsilyloxyethyl]-2,2'-oxydiacetamide (**7**)

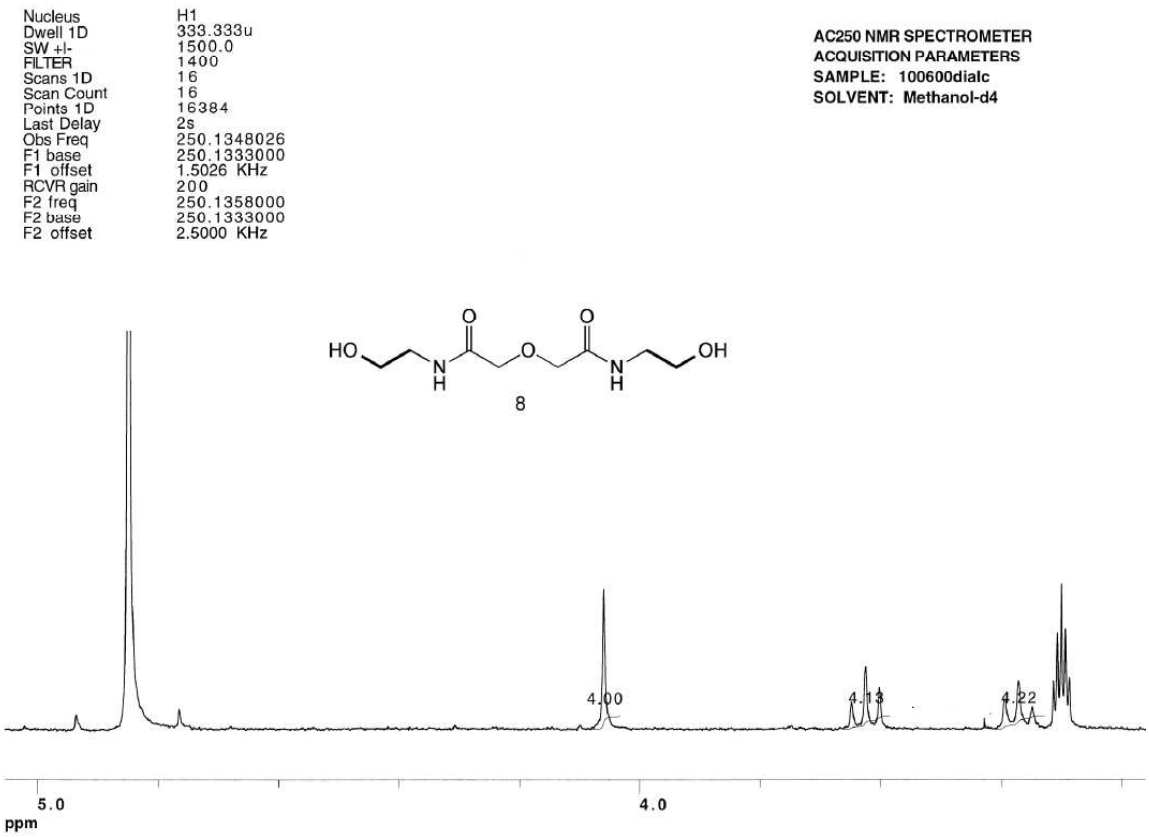

Figure S5: ^{1}H NMR spectrum of *N*,*N*'-Bis(2-hydroxyethyl)-2,2'-oxydiacetamide (**8**)

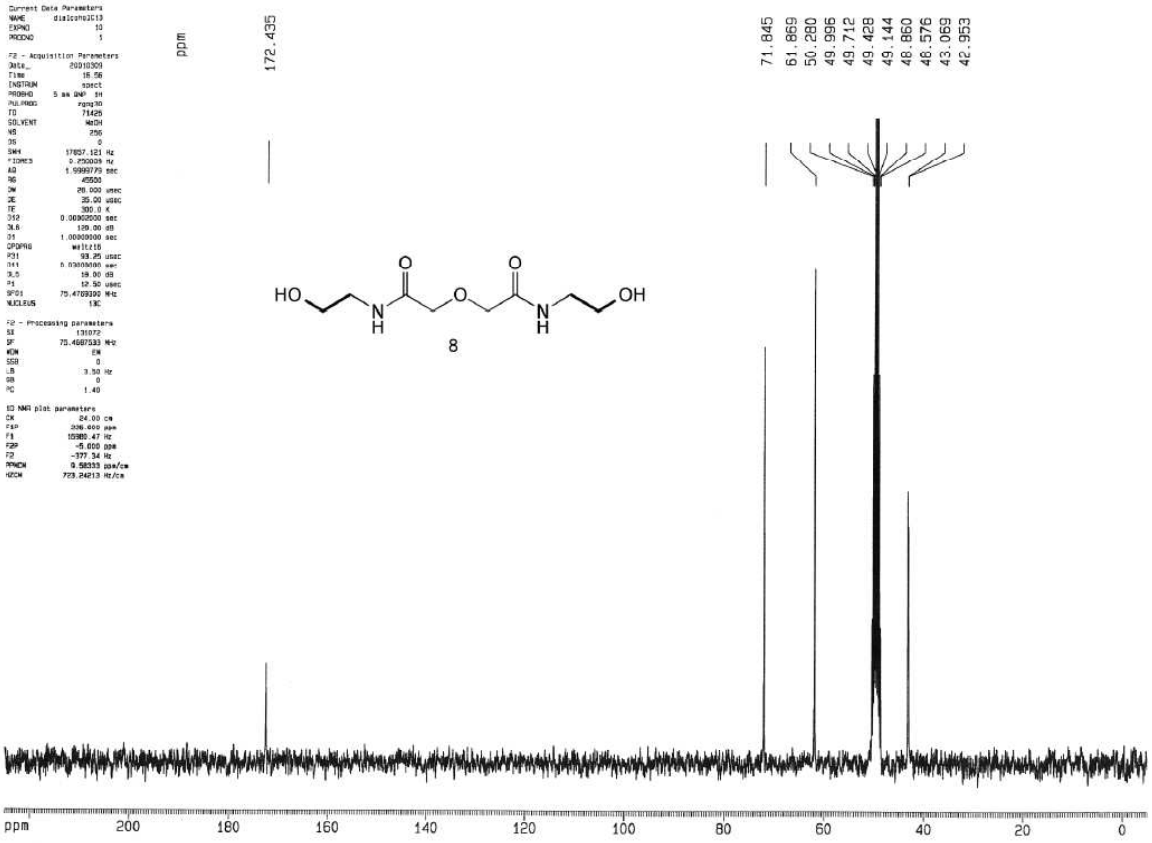

Figure S6: ^{13}C NMR spectrum of *N*,*N*'-Bis(2-hydroxyethyl)-2,2'-oxydiacetamide (**8**)

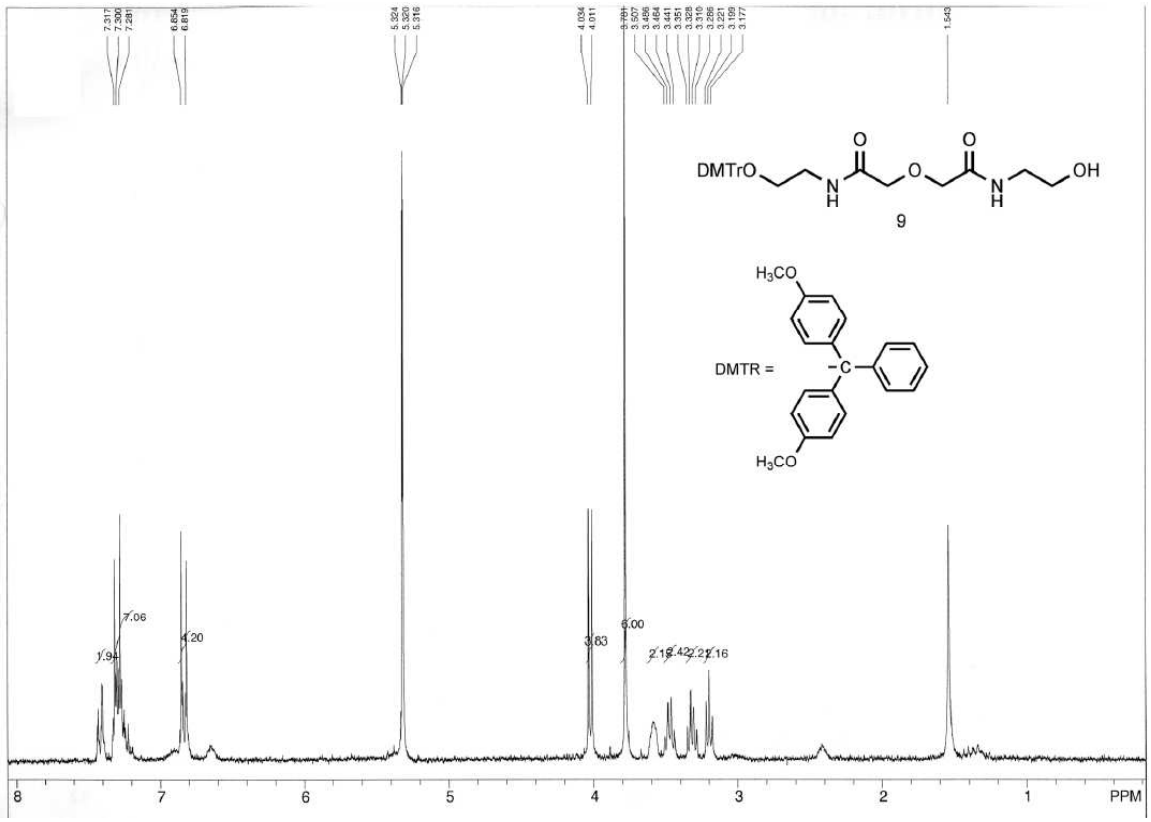

Figure S7: ^{1}H NMR spectrum of *N*-[2-(4, 4'-dimethoxytrityl)oxyethyl]-*N*'-(2-hydroxyethyl)-2,2'-oxydiacetamide (**9**)

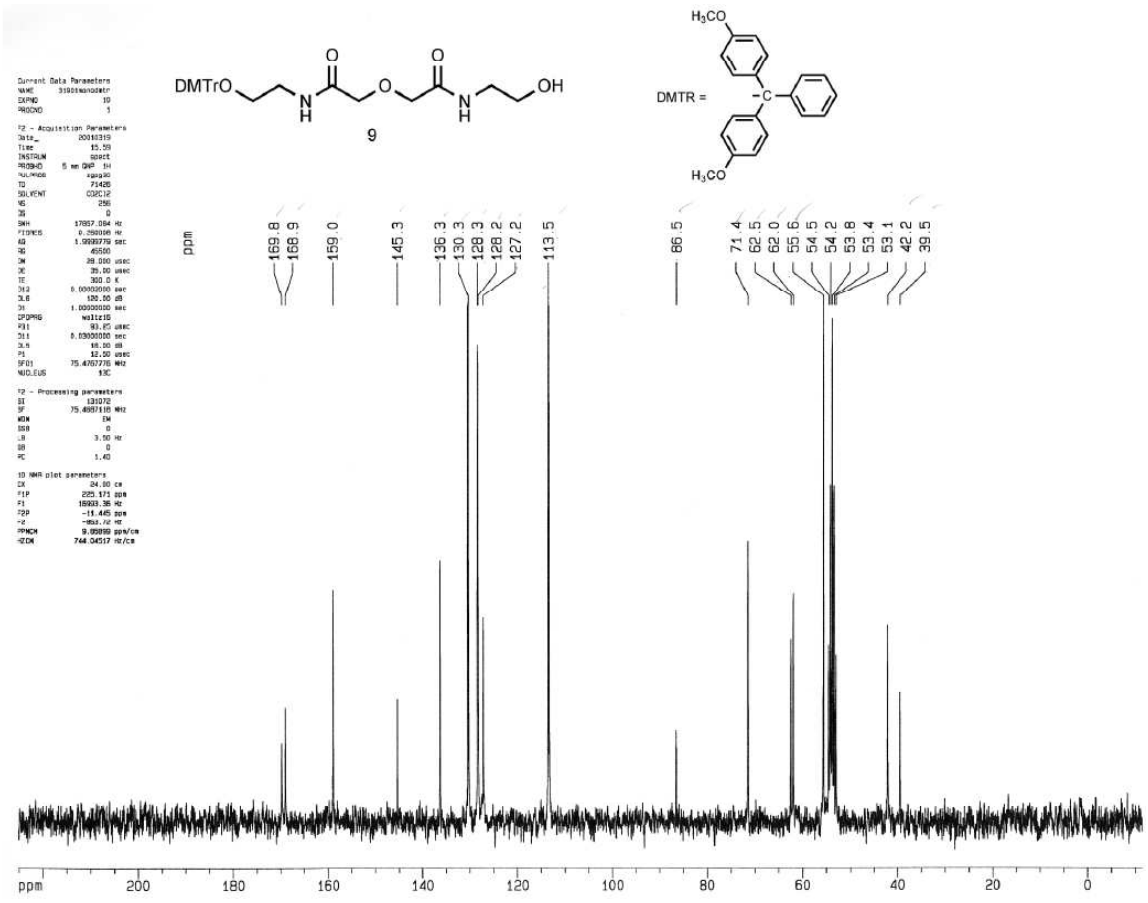

Figure S8: ^{13}C NMR spectrum of *N*-[2-(4, 4'-dimethoxytrityl)oxyethyl]-*N*'-(2-hydroxyethyl)-2,2'-oxydiacetamide (**9**)

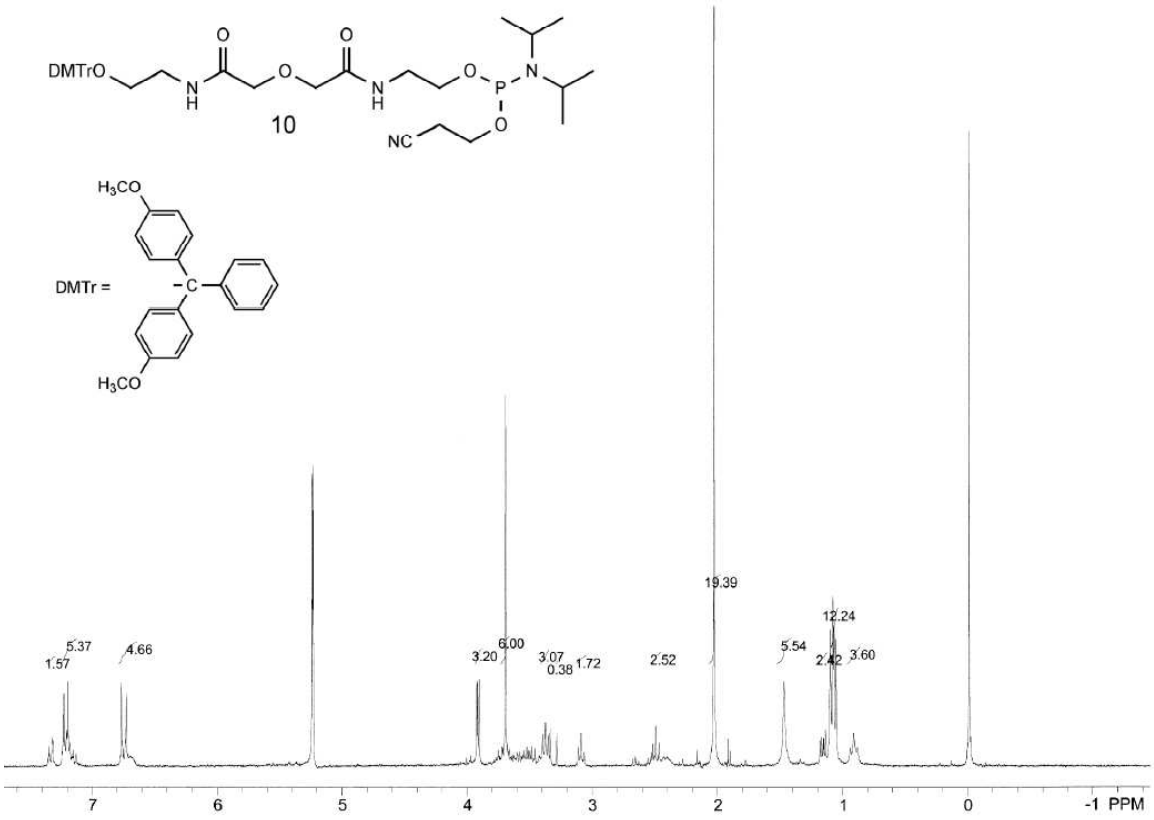

Figure S9: ^{1}H NMR spectrum of *N*-[3-O-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)ethyl]-*N*'-[2-(4,4' –dimethoxytrityl)oxyethyl]-2,2'-oxydiacetamide (**10**)

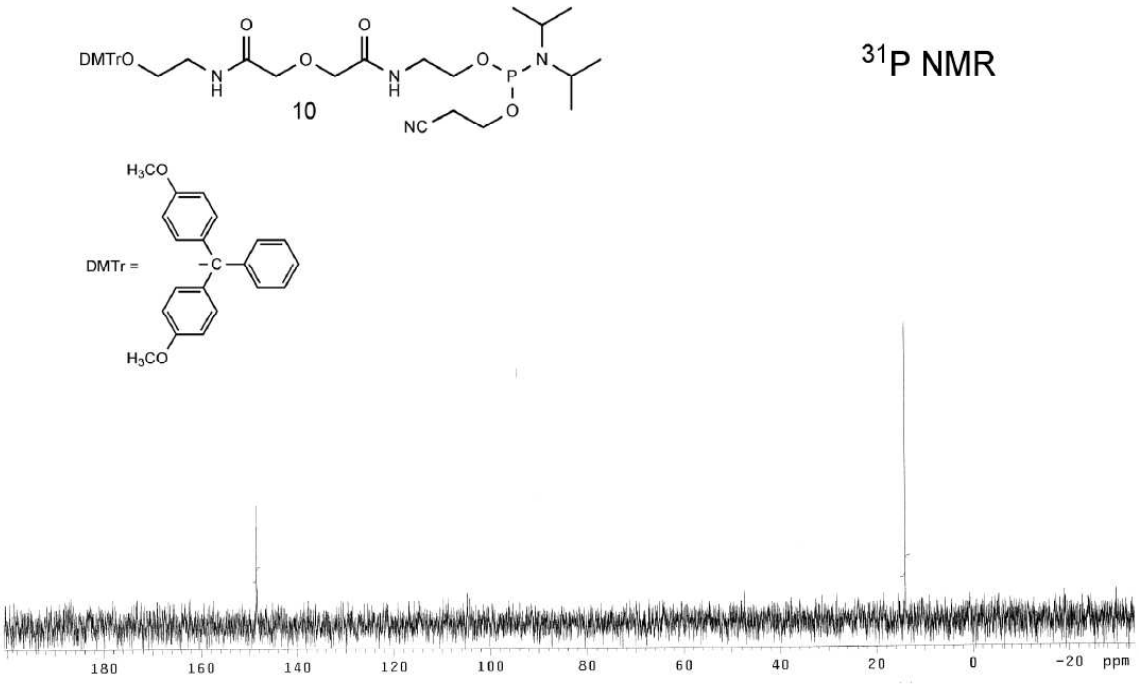

Figure S10: ^{31}P NMR spectrum of *N*-[3-O-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)ethyl]-*N*'-[2-(4,4' –dimethoxytrityl)oxyethyl]-2,2'-oxydiacetamide (**10**)

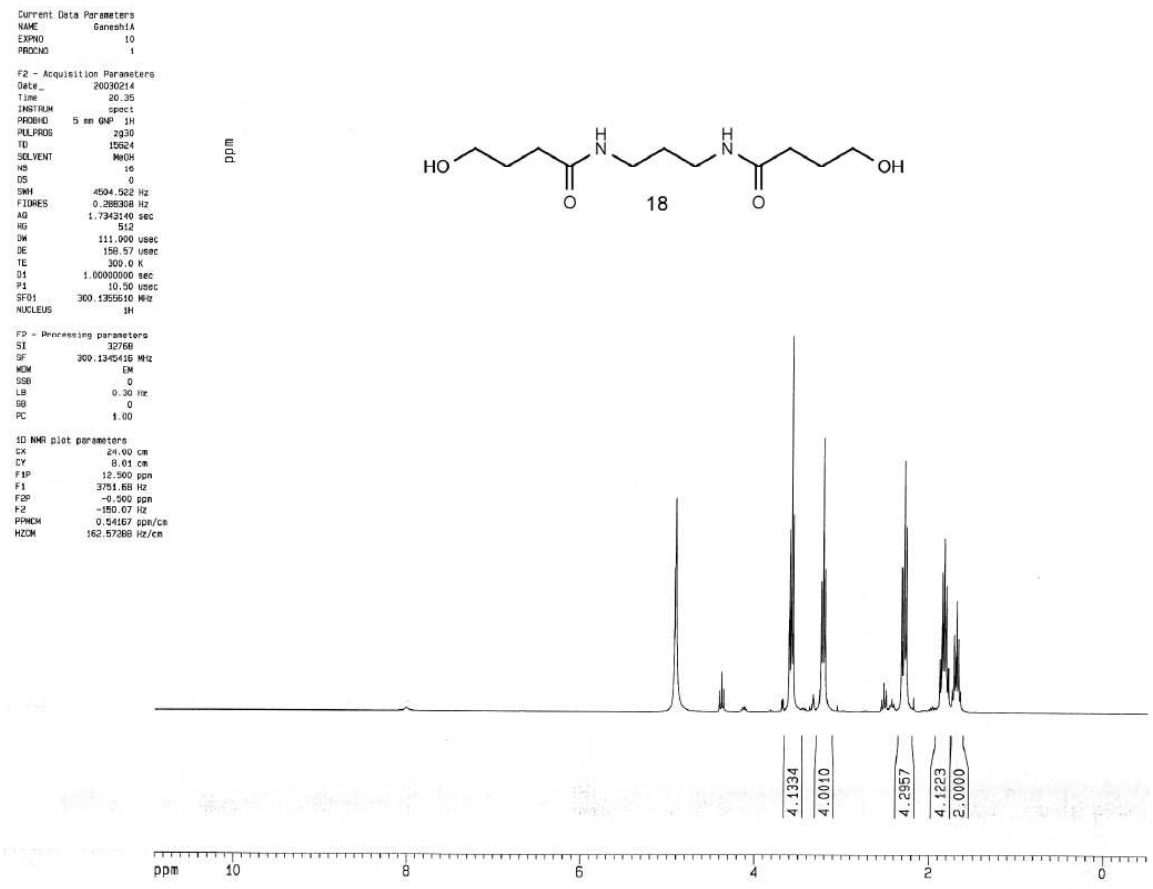

Figure S11: ^{1}H NMR spectrum of *N*, *N*'-Bis-(4-hydroxybutanoyl)-1,3-propanediamine (**18**)

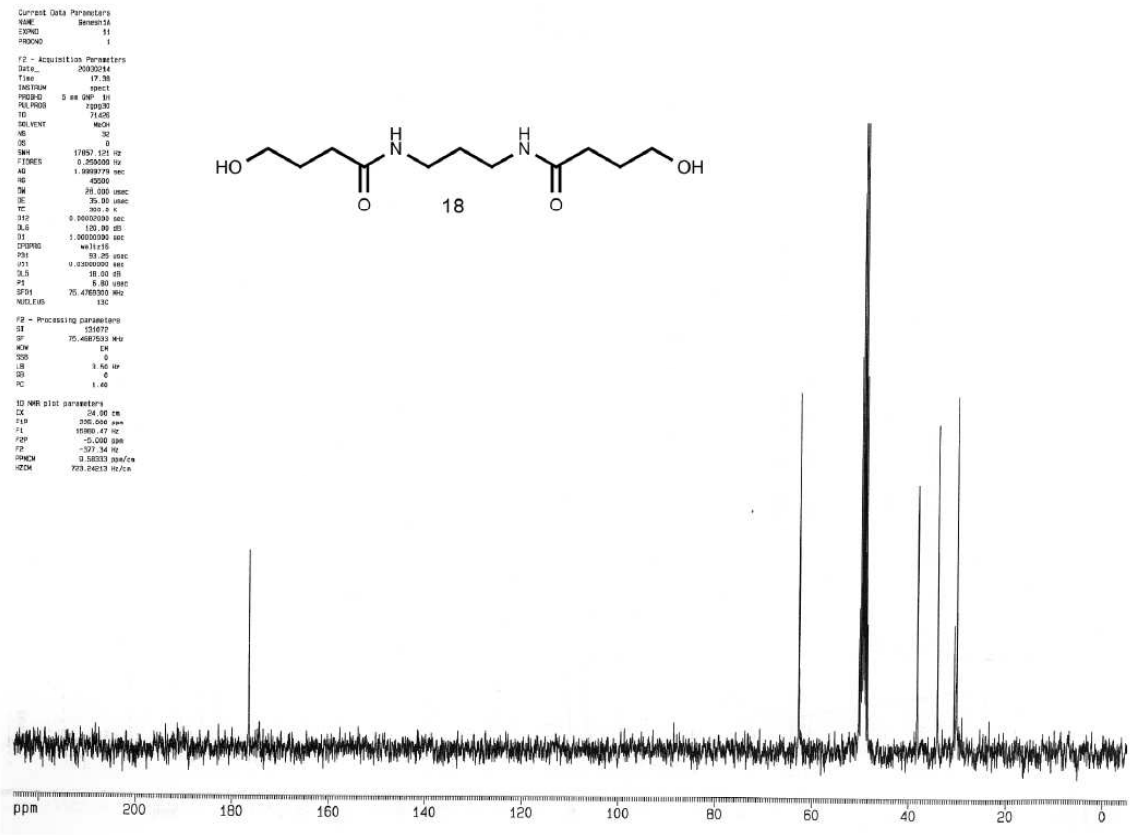

Figure S12: ^{13}C NMR spectrum of *N*, *N*'-Bis-(4-hydroxybutanoyl)-1,3-propanediamine (**18**)

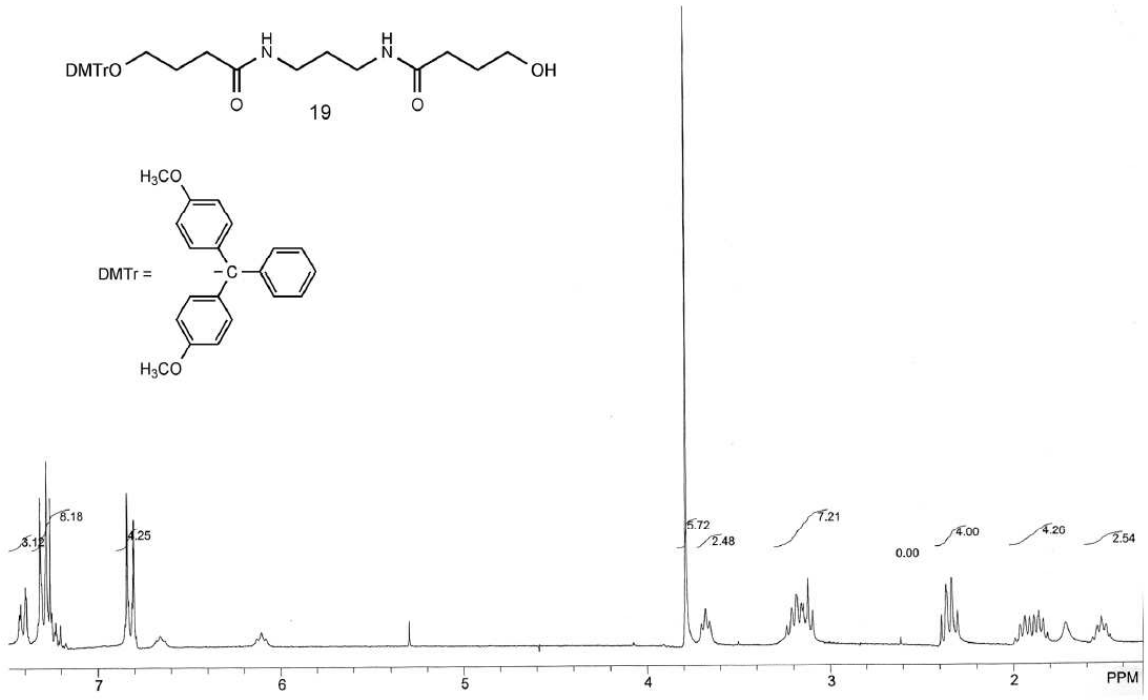

Figure S13: ^{1}H NMR spectrum of *N*-{4-[4,4'-(Dimethoxytrityl)oxy]butanoyl}-*N*'-4-hydroxybutanoyl-1,3-propanediamine (**19**)

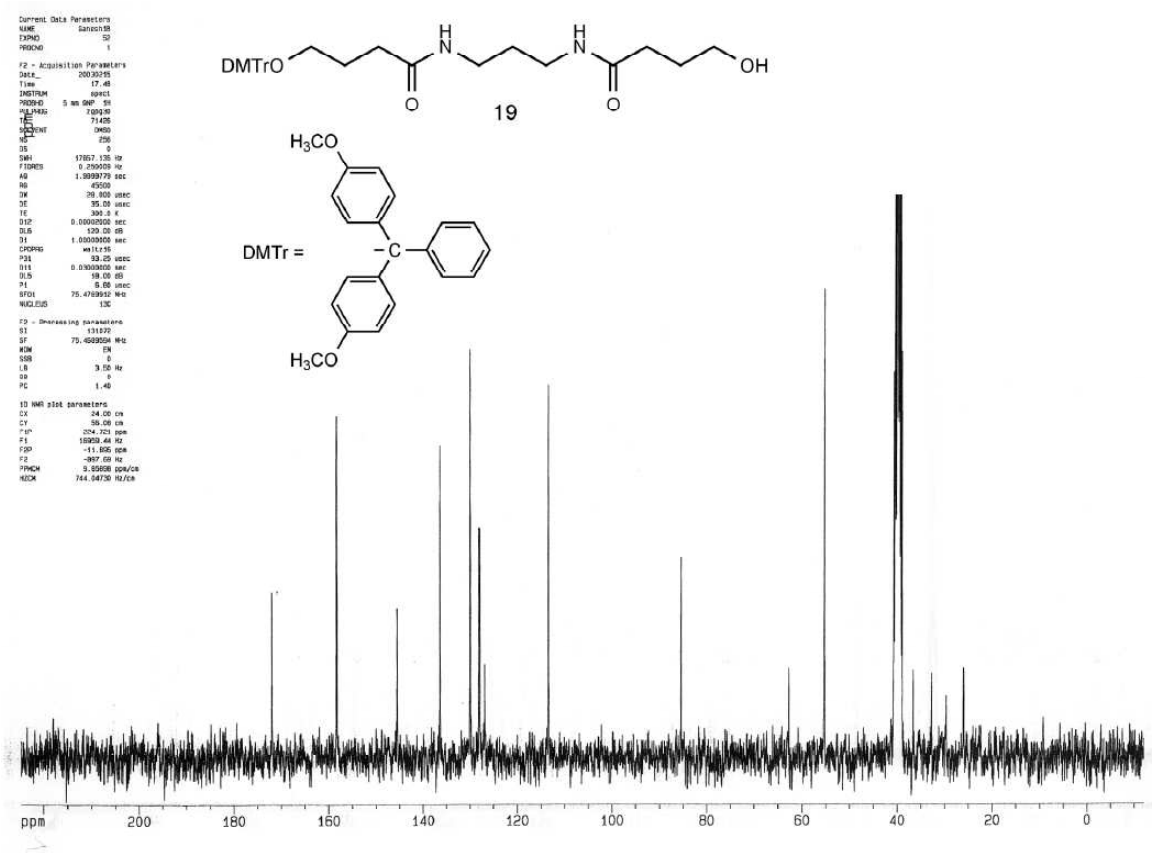

Figure S14: ^{13}C NMR spectrum of *N*-{4-[4,4'-(Dimethoxytrityl)oxy]butanoyl}-*N*'-4-hydroxybutanoyl-1,3-propanediamine (**19**)

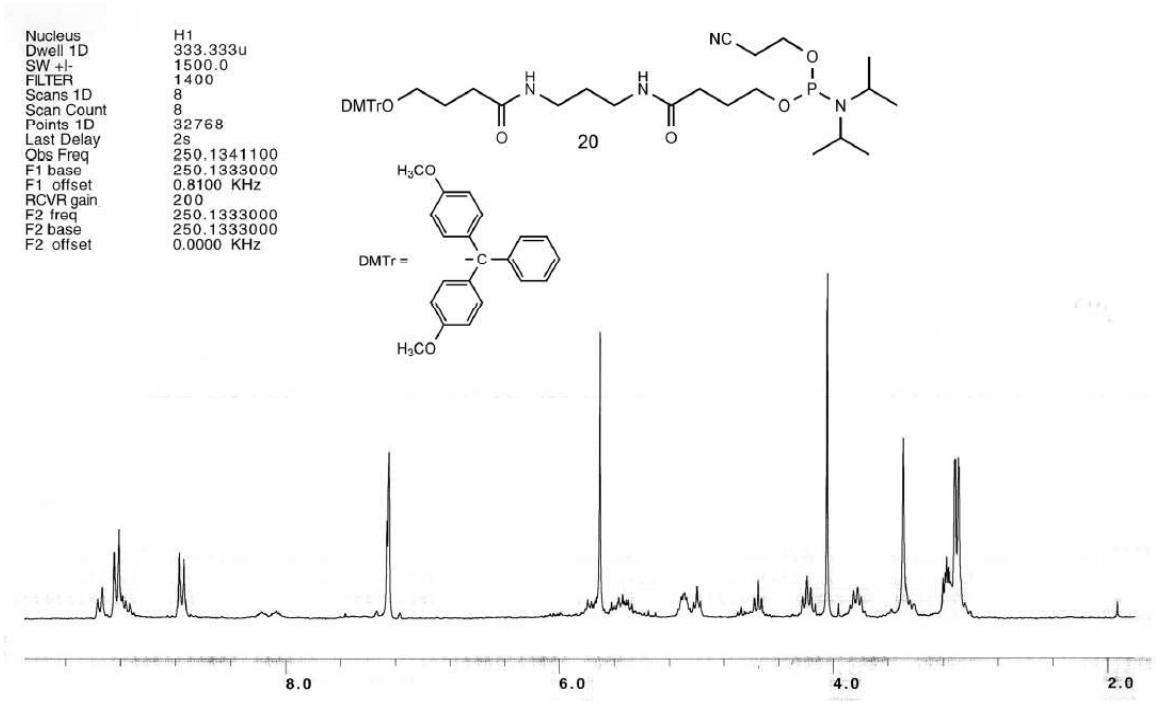

Figure S15: ^{1}H NMR spectrum of *N*-[4-O-(2-cyanoethyl-*N*,*N*-diisopropylphophoramidite)butanoyl]-*N*'-{4-[4,4'-(dimethoxytrityl)oxy]butanoyl}-1,3-propanediamine (**20**)

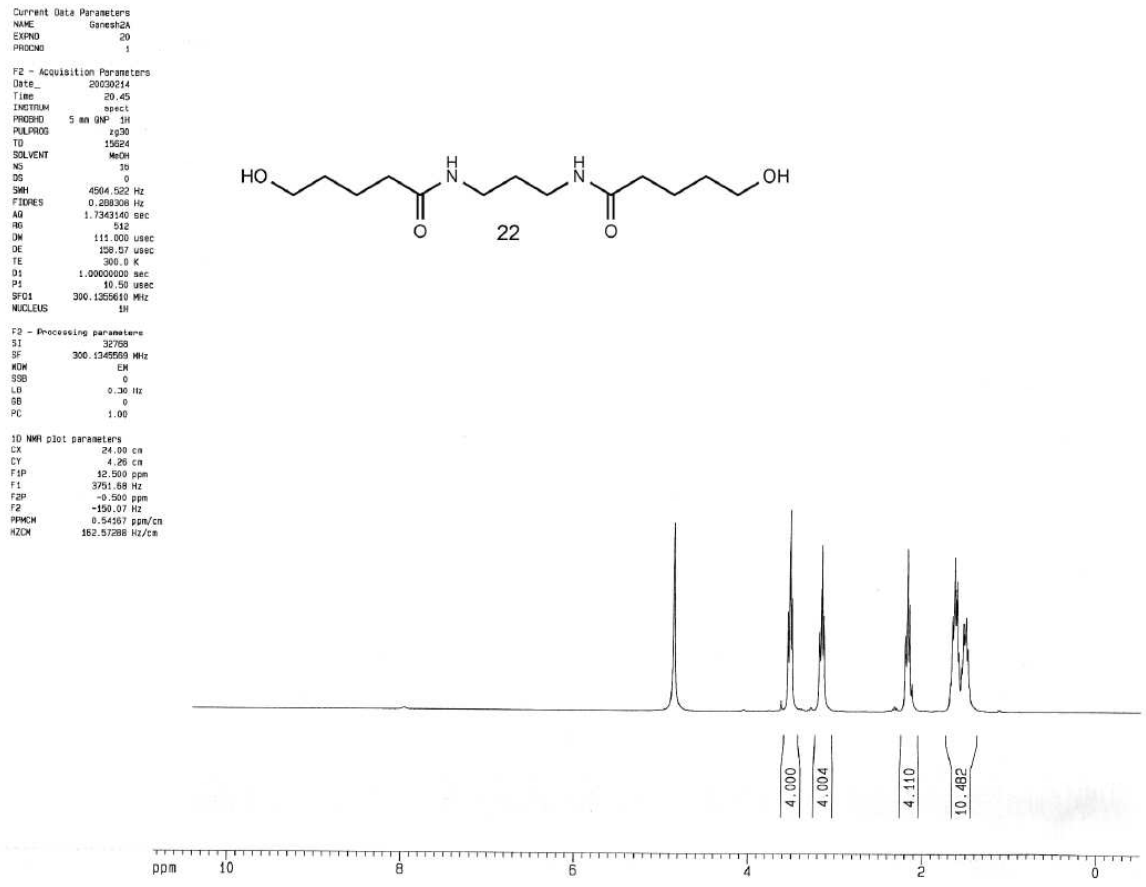

Figure S16: ^{1}H NMR spectrum of *N*, *N*'-Bis-(5-hydroxypentanoyl)-1,3-propanediamine (**22**)

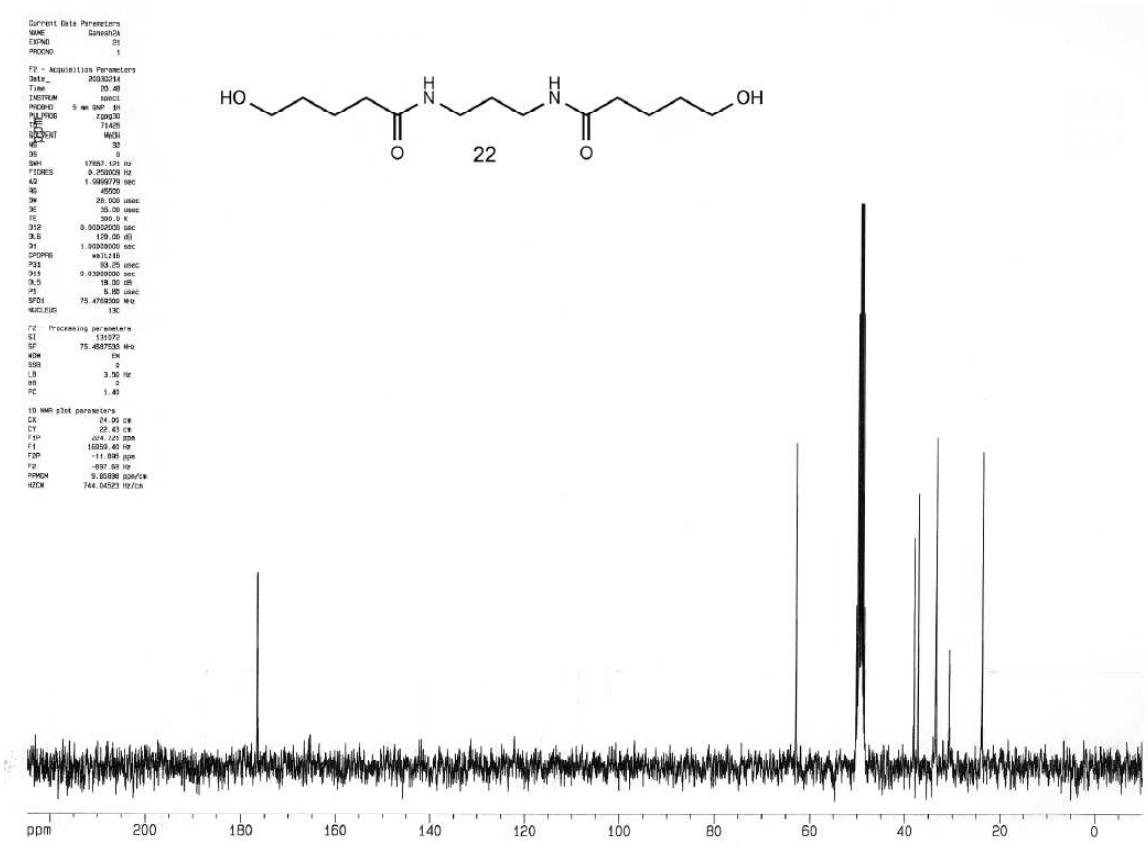

Figure S17: ^{13}C NMR spectrum of *N*, *N*'-Bis-(5-hydroxypentanoyl)-1,3-propanediamine (**22**)

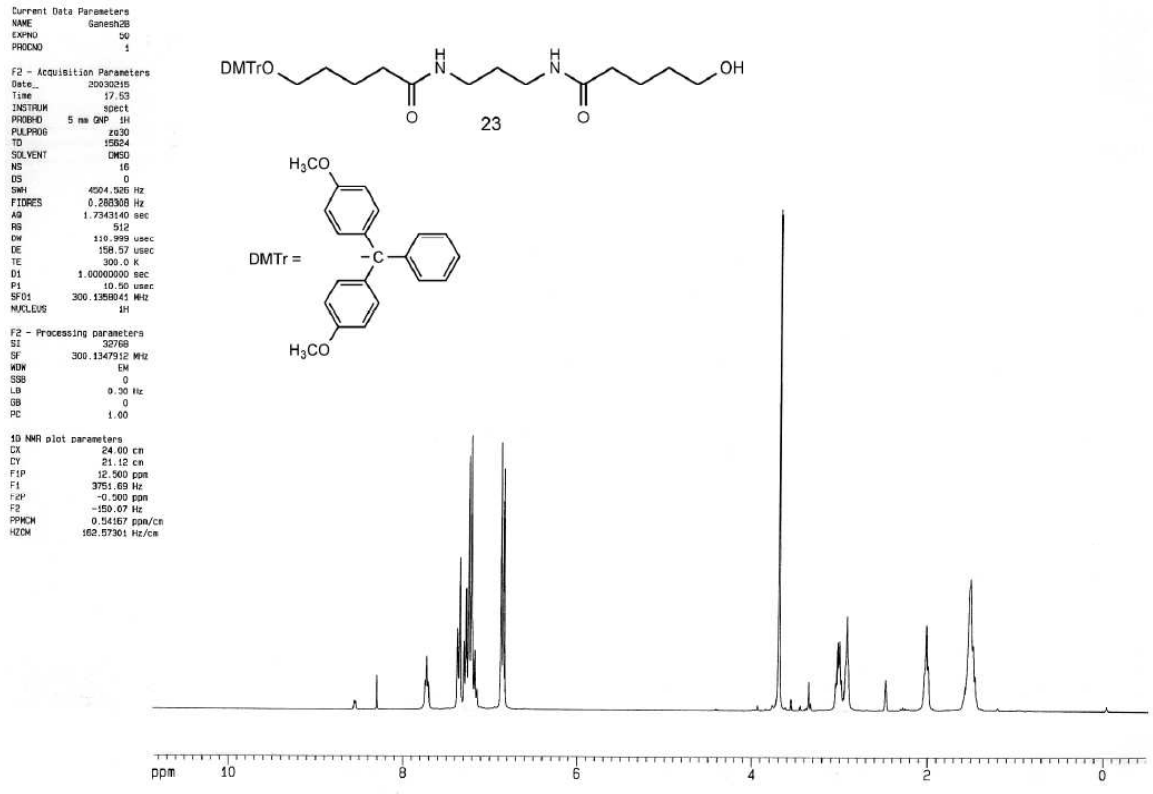

Figure S18: ^{1}H NMR spectrum of *N*-{5-[4,4'-(Dimethoxytrityl)oxy]pentanoyl}-*N*'-(5-hydroxypentanoyl]-1,3-propanediamine (**23**)

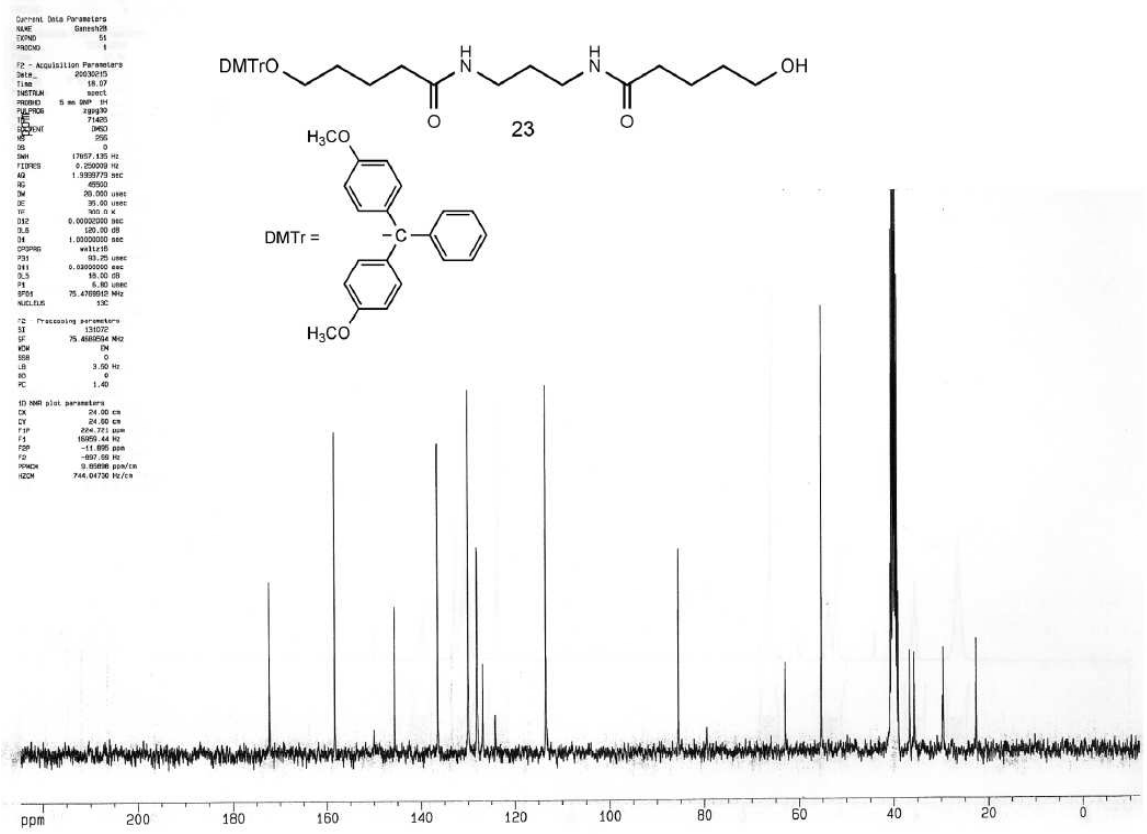

Figure S19: ^{13}C NMR spectrum of *N*-{5-[4,4'-(Dimethoxytrityl)oxy]pentanoyl}-*N*'-(5-hydroxypentanoyl]-1,3-propanediamine (**23**)

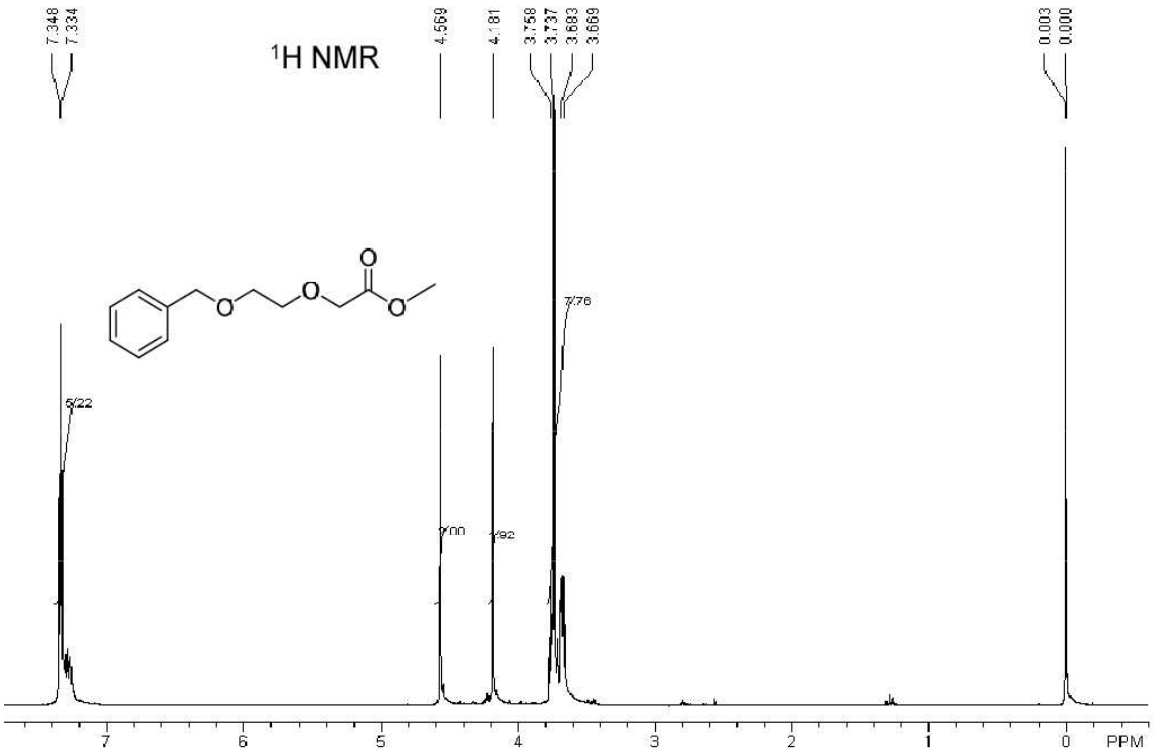

Figure S20: ^{1}HNMR spectrum of Methyl 2-(2-(benzyloxy)ethoxy)acetate (**26**)

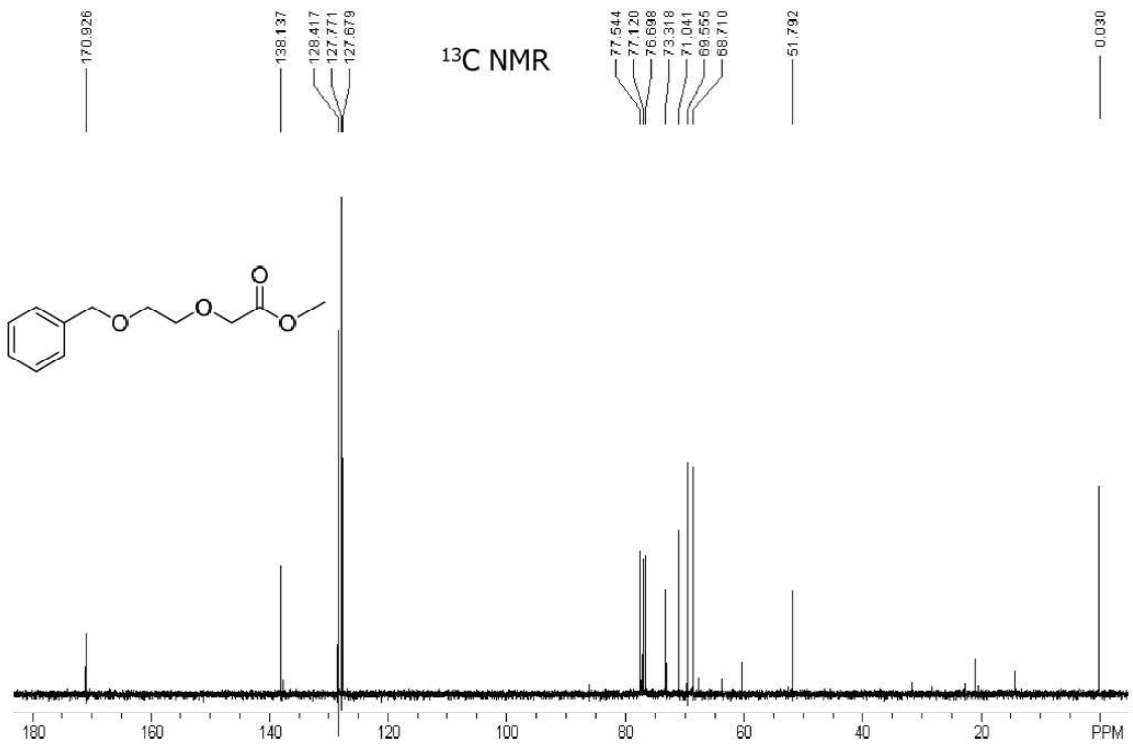

Figure S21: ^{13}C NMR spectrum of Methyl 2-(2-(benzyloxy)ethoxy)acetate (**26**)

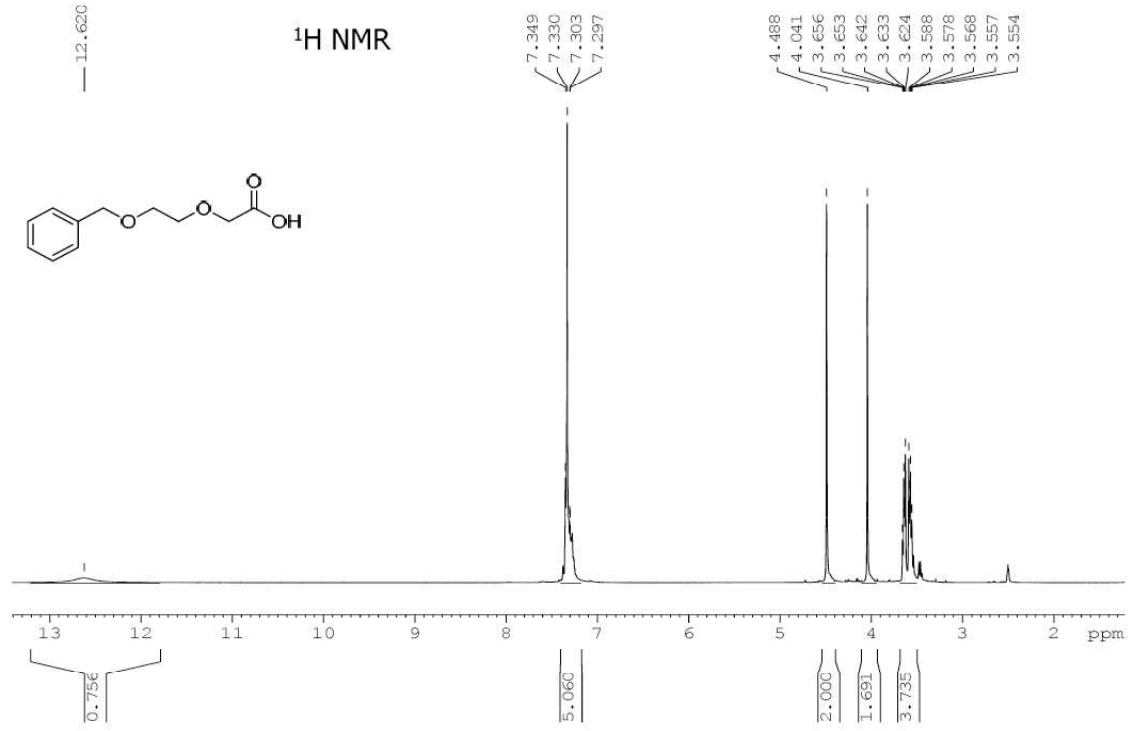

Figure S22: ^{1}H NMR spectrum of 2-(2-(Benzyloxy)ethoxy)acetic acid (**27**)

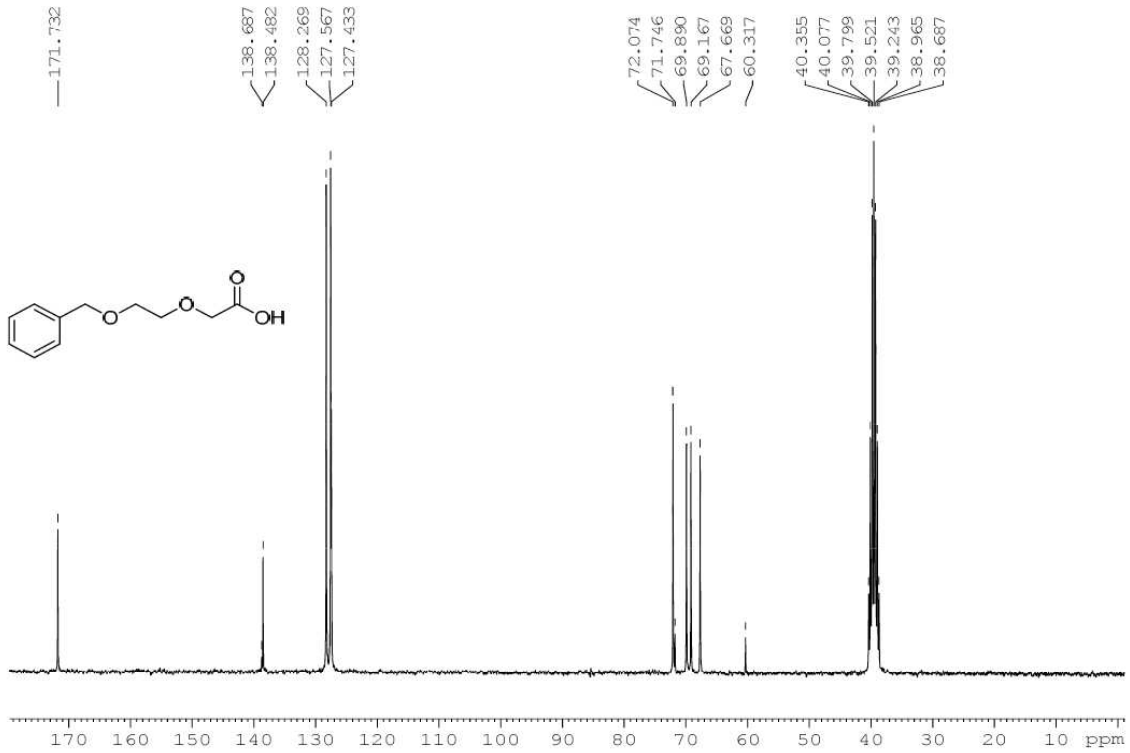

Figure S23: ^{13}C NMR spectrum of 2-(2-(Benzyloxy)ethoxy)acetic acid (**27**)

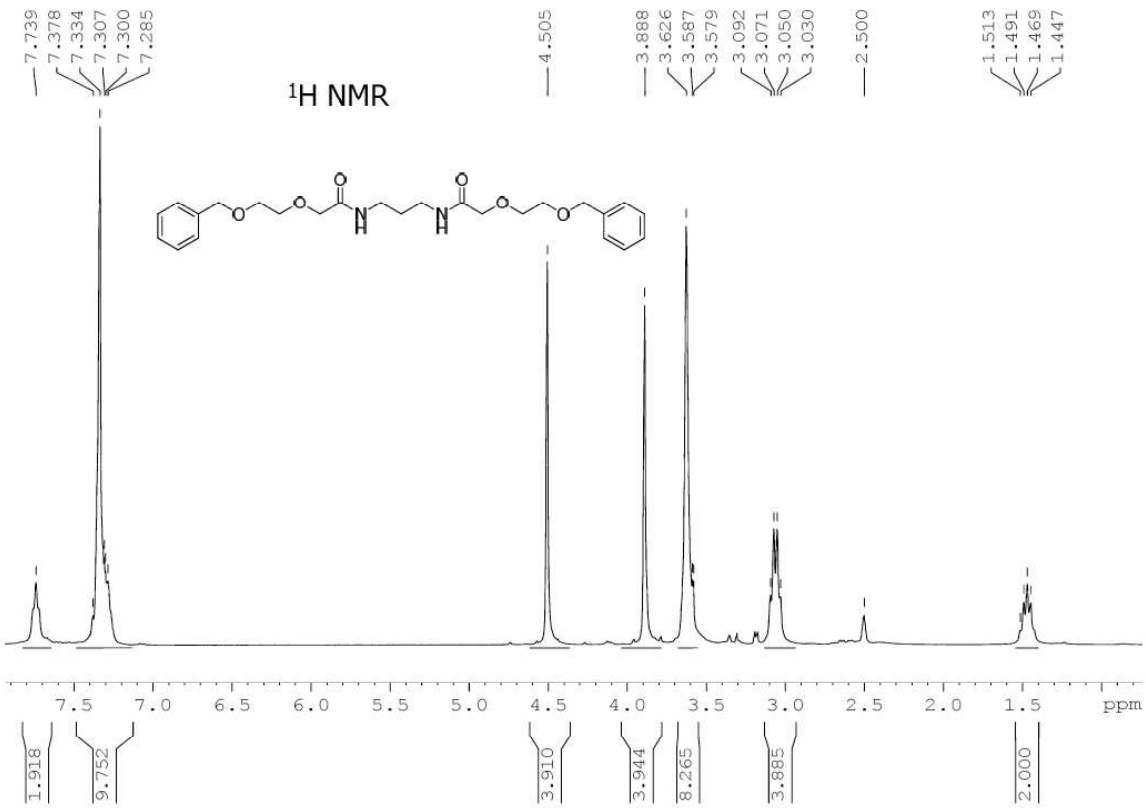

Figure S24: ^{1}H NMR spectrum of *N*, *N'*-Bis-(2-(2-benzyloxyethoxy)ethanoyl)-1,3-propanediamine (**28**)

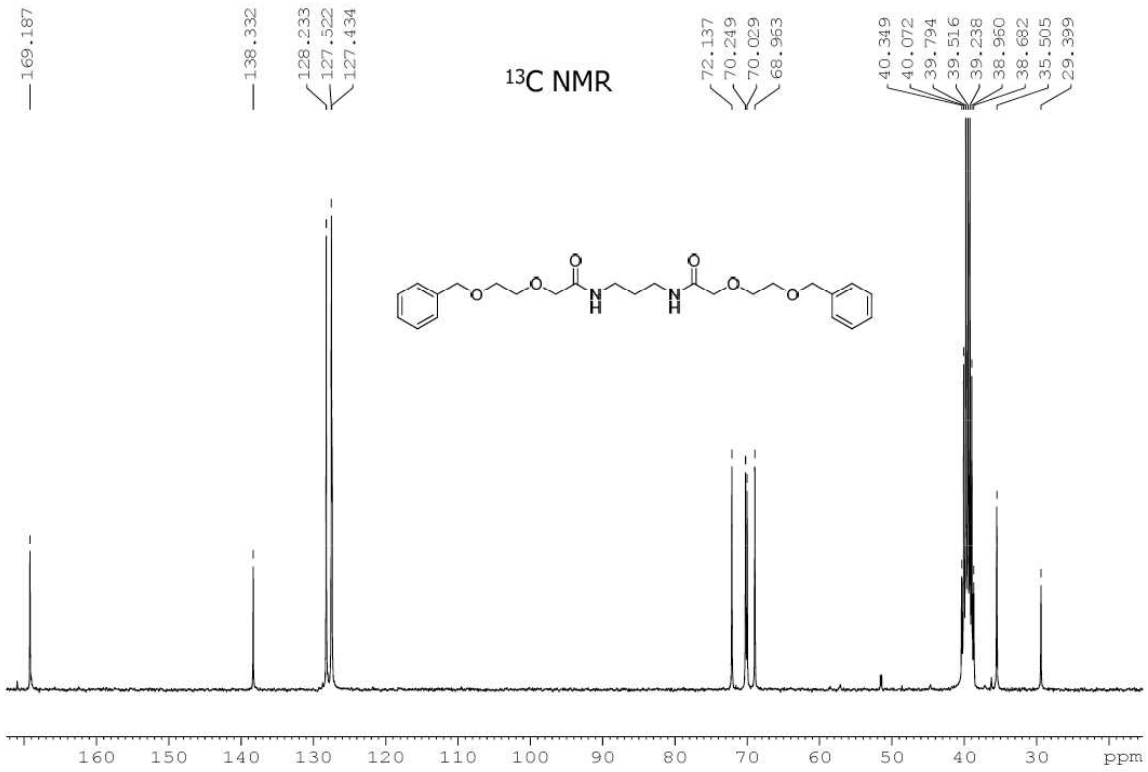

Figure S25: ^{13}C NMR spectrum of *N*, *N*'-Bis-(2-(2-benzyloxyethoxy)ethanoyl)-1,3-propanediamine (**28**)

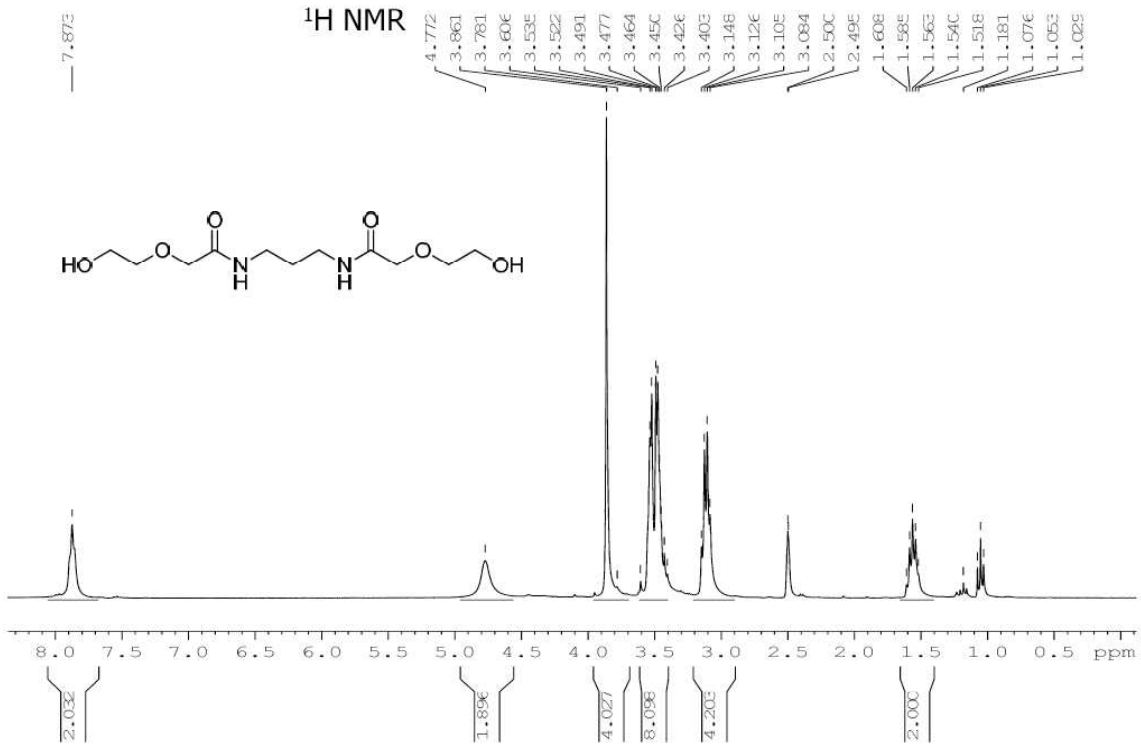

Figure S26: ^{1}H NMR spectrum of *N*, *N*'-Bis-(2-(2-hydroxyethoxy)ethanoyl)-1,3-propanediamine (**29**)

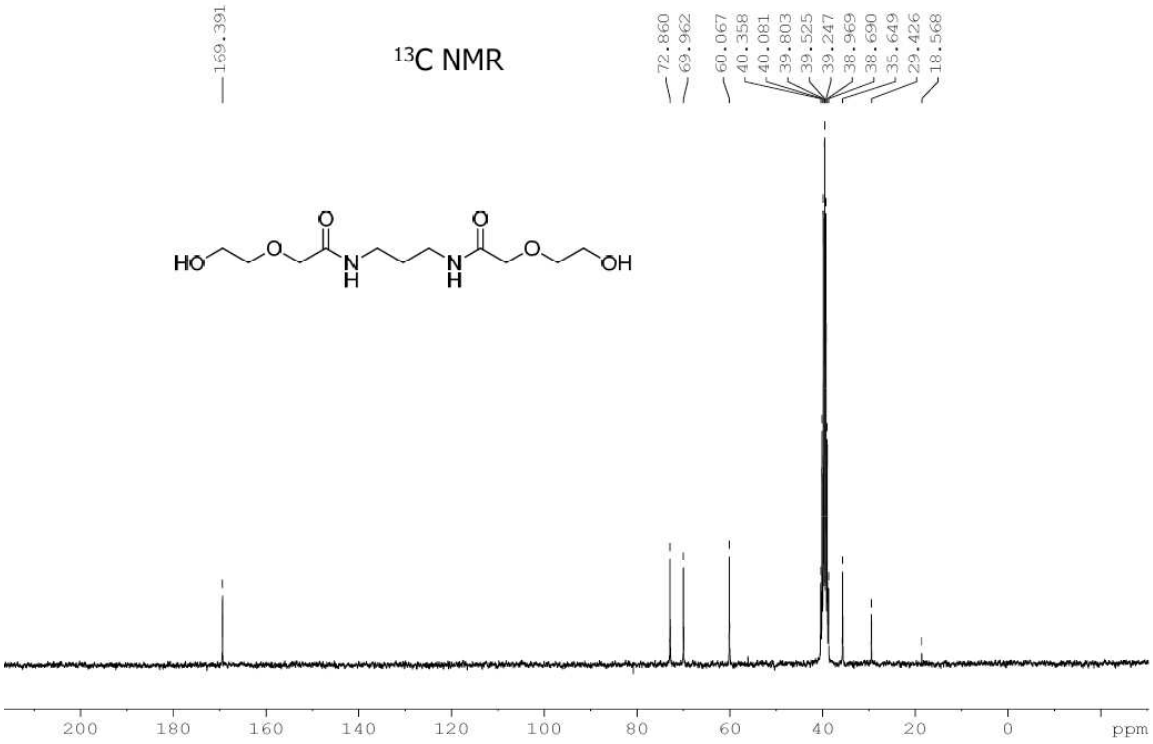

Figure S27: ^{13}C NMR spectrum of *N*, *N*'-Bis-(2-(2-hydroxyethoxy)ethanoyl)-1,3-propanediamine (**29**)

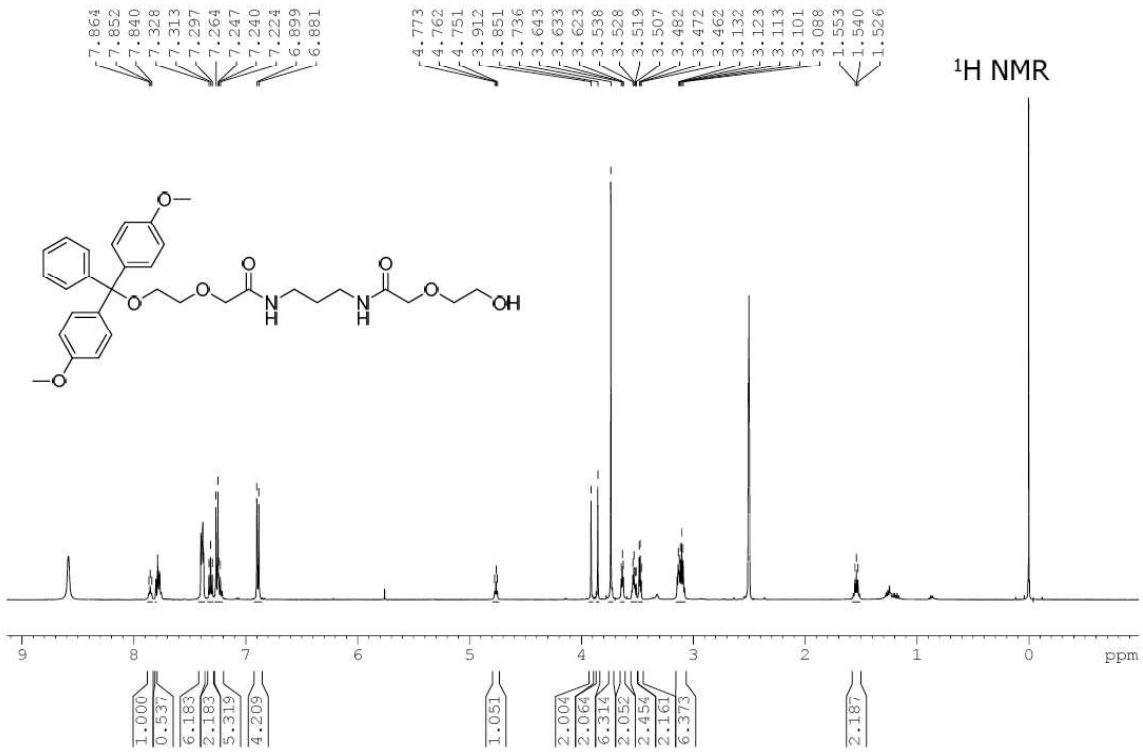

Figure S28: ^{1}H NMR spectrum of *N*-(2-(2-(4, 4'-dimethoxytrityloxy)ethoxy)ethanoyl)-*N*'-(2-(2-hydroxyethoxy)ethanoyl)-1,3-propanediamine (**30**)

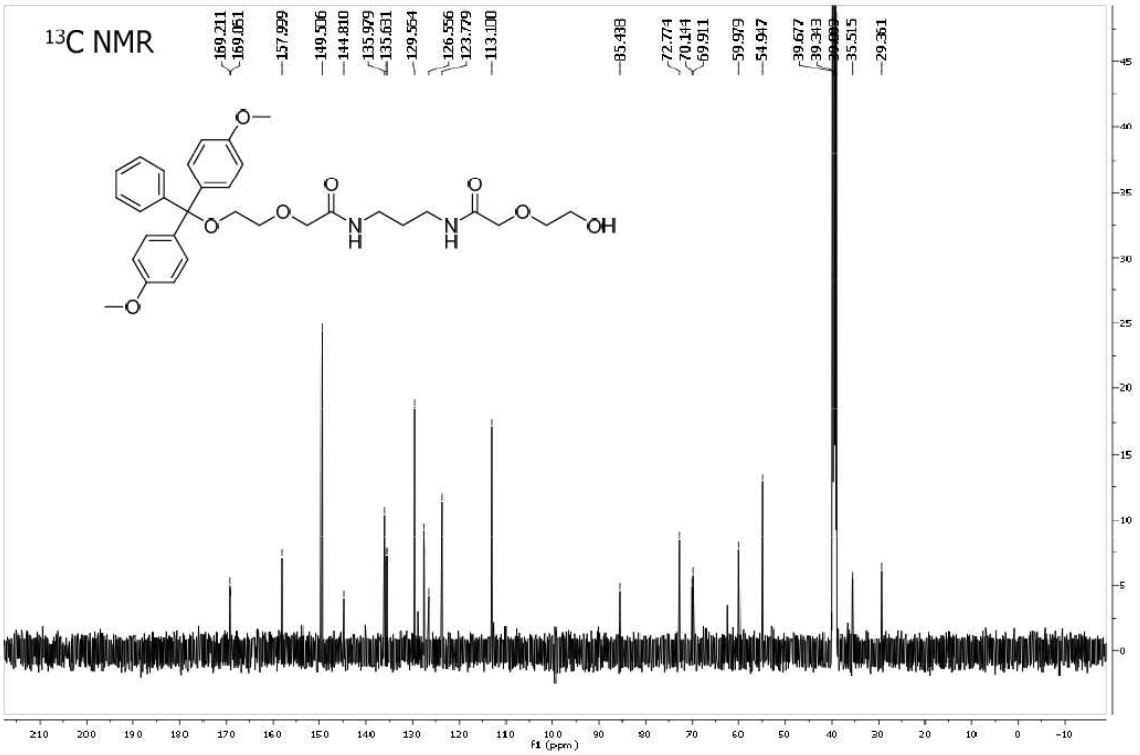

Figure S29: ^{13}C NMR spectrum of *N*-(2-(2-(4, 4'-dimethoxytrityloxy)ethoxy)ethanoyl)-*N*'-(2-(2-hydroxyethoxy)ethanoyl)-1,3-propanediamine (**30**)

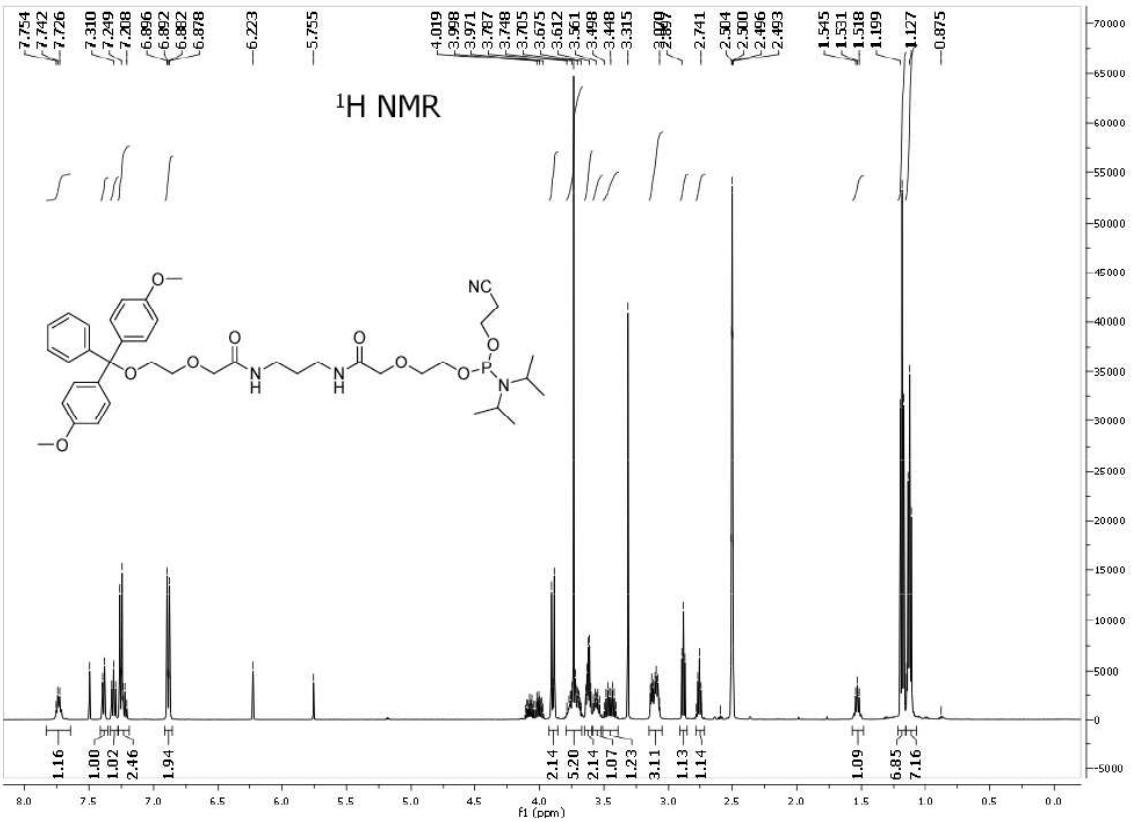

Figure S30: ^{1}H NMR spectrum of *N*-(2-(2-O-(2-cyanoethyl-*N*, *N*-diisopropylphosphoramidite)ethoxy)ethanoyl)-*N*'-(2-(2-(4, 4'-dimethoxytrityloxy)ethoxy)ethanoyl)-1,3-propanediamine (**31**)

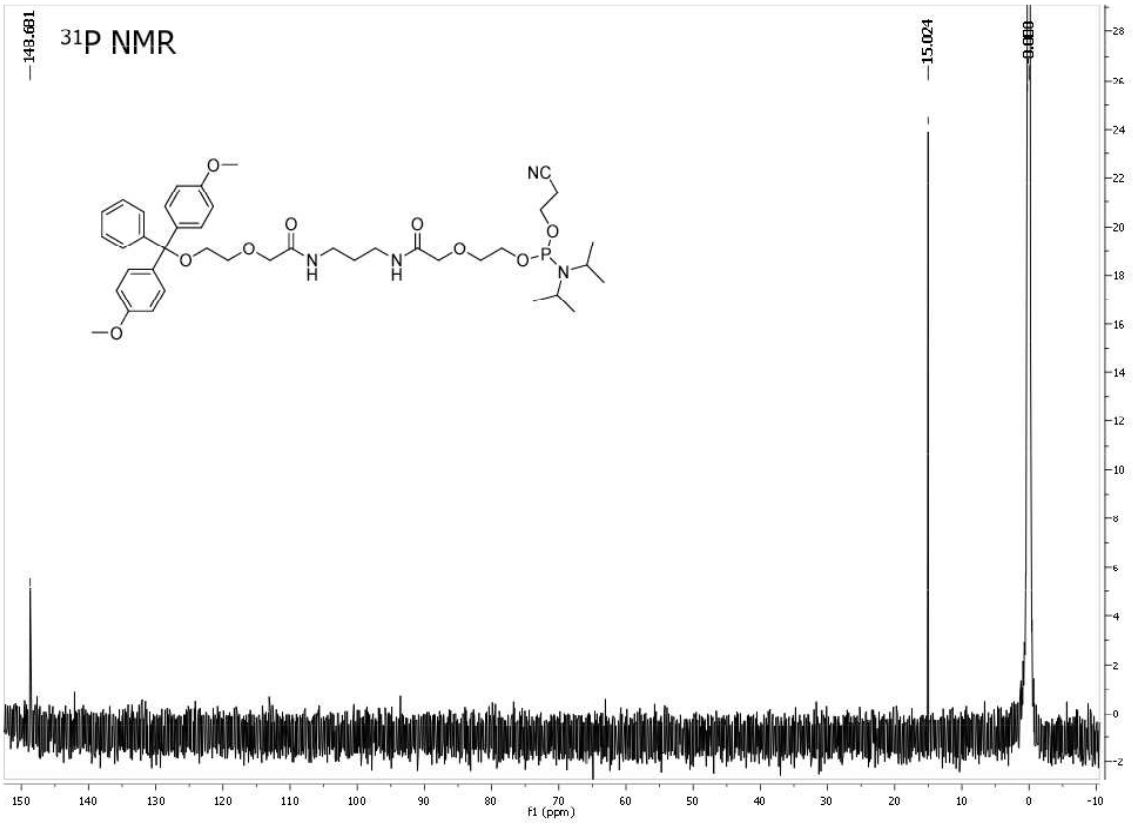

Figure S31: ^{31}P NMR spectrum of *N*-(2-(2-O-(2-cyanoethyl-*N*, *N*-diisopropylphosphoramidite)ethoxy)ethanoyl)-*N*'-(2-(2-(4, 4'-dimethoxytrityloxy)ethoxy)ethanoyl)-1,3-propanediamine (**31**)