

SUPPORTING INFORMATION

Synthesis and evaluation of new spacers for use as dsDNA endcaps

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List of contents:

Table S4 Oligonucleotide MALDI-TOFMS data

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

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

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

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

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

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

Figure S7: ^1H 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: ^1H 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: ^1H 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: ^1H 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: ^1H NMR spectrum of *N*-[4-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)butanoyl]-*N'*-{4-[4,4'-(dimethoxytrityl)oxy]butanoyl}-1,3-propanediamine (**20**)

Figure S16: ^1H 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: ^1H 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: ^1H NMR 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: ^1H 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: ^1H 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: ^1H 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: ^1H 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: ^1H 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**)

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

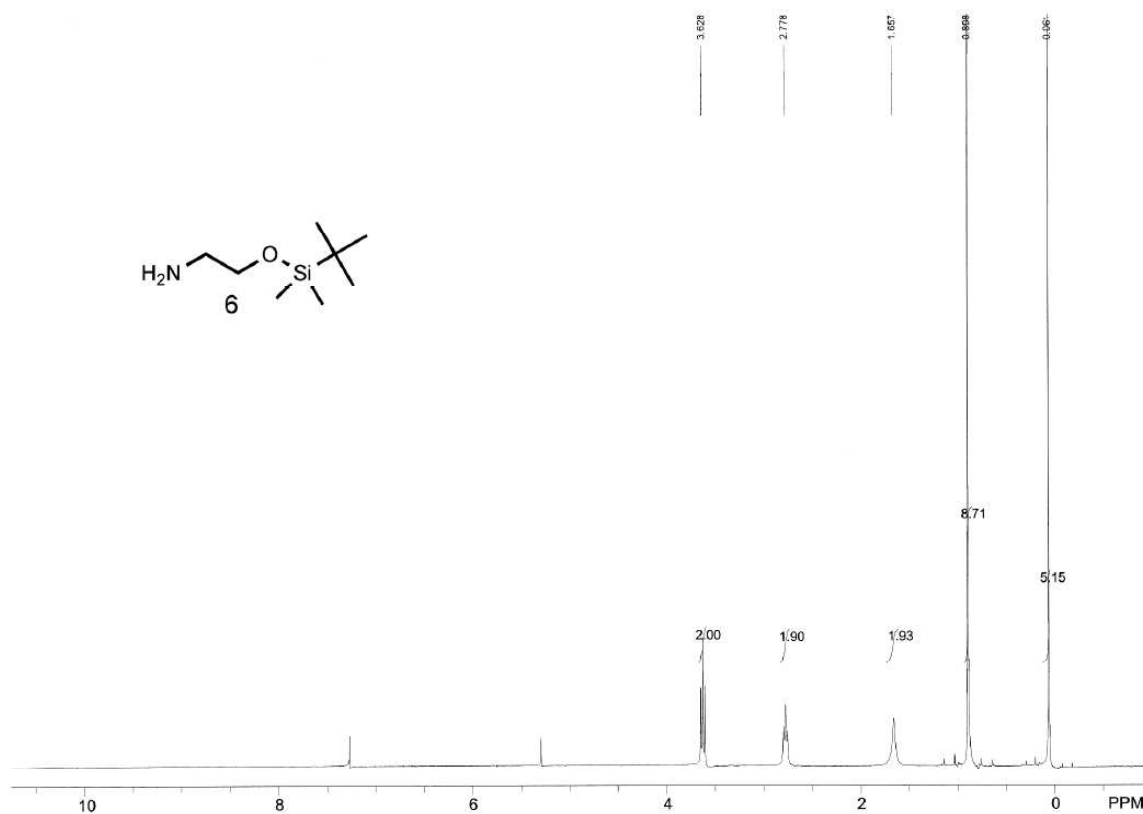


Figure S1: ¹H NMR spectrum of 2-(*tert*-Butyldimethylsilyloxy)ethylamine (**6**)

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PROCNO 1

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FIDRES 0.250008 Hz
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DM 26.000 usec
DE 35.00 usec
TE 300.0 K
D12 0.00000000 sec
DEC 120.00 dB
D1 1.00000000 sec
D11 33.25 usec
D11 0.03000000 sec
SLS 18.00 dB
P1 12.50 usec
SFO1 75.4760327 MHz
NUCLEUS 13C

F2 - Processing parameters
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LB 3.50 Hz
GB 0
PC 1.40

1D NMR plot parameters
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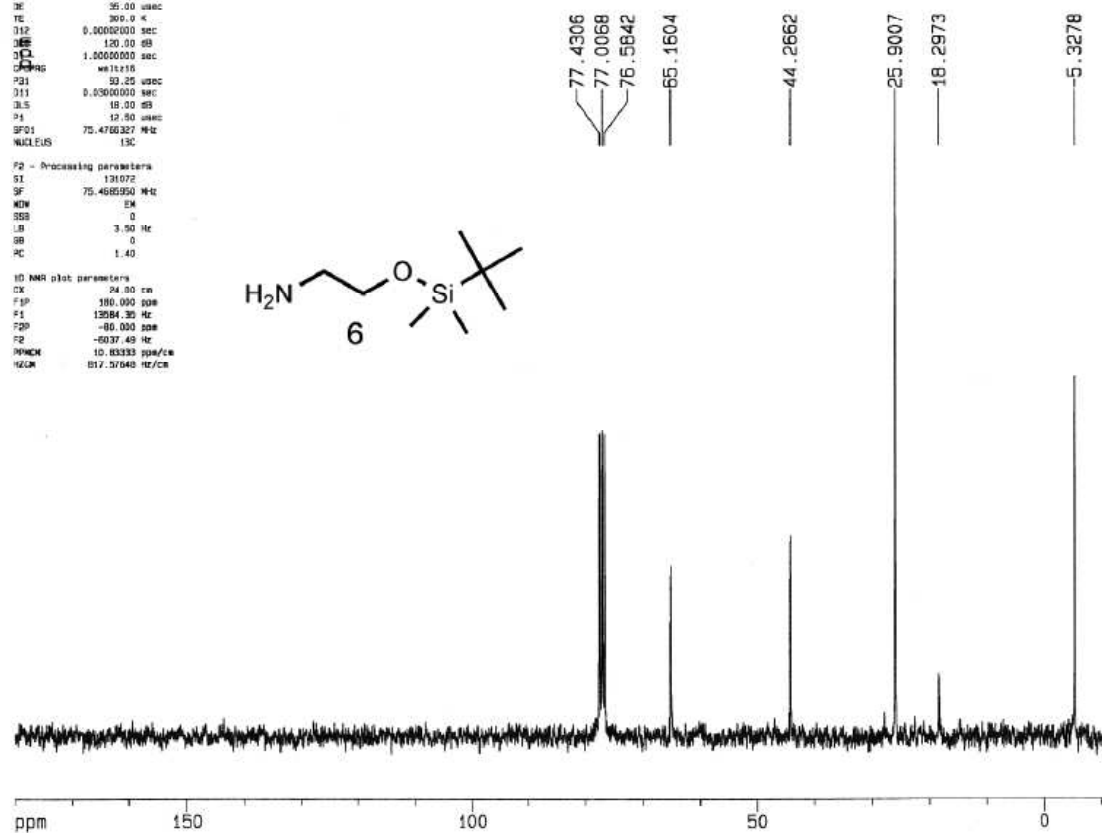
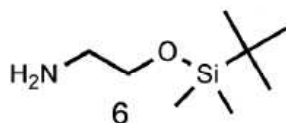


Figure S2: ^{13}C NMR spectrum of 2-(*tert*-Butyldimethylsilyloxy)ethylamine (**6**)

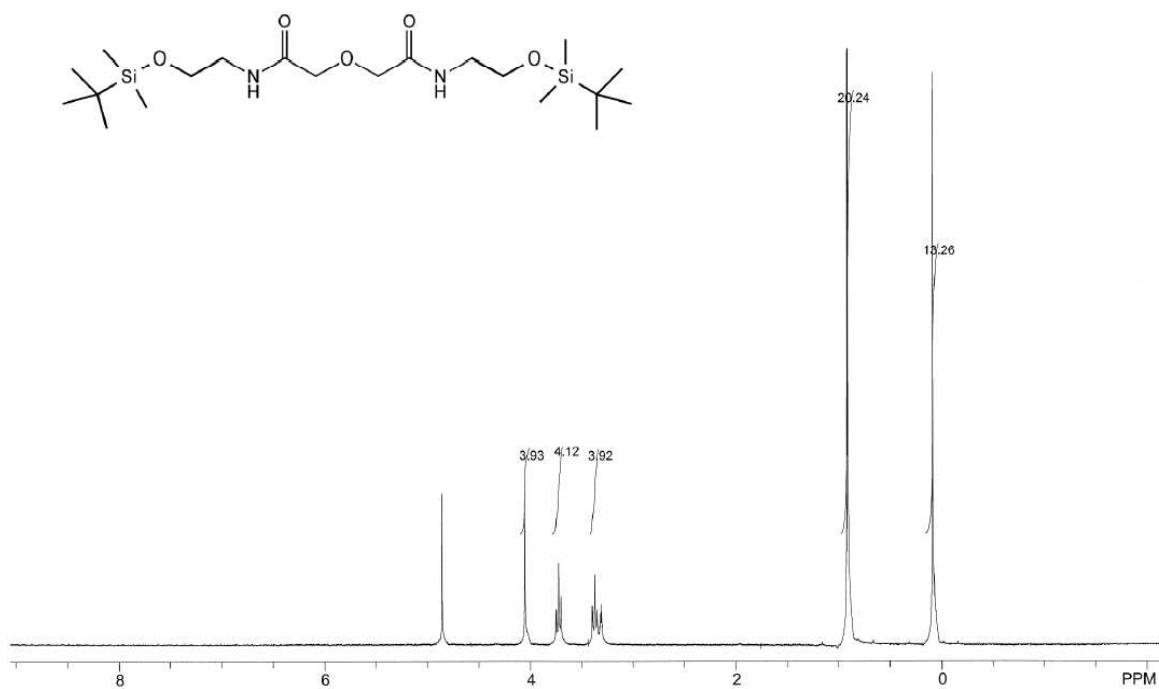


Figure S3: ¹H NMR spectrum of *N,N'*-Bis[2-(*tert*-butyl dimethylsilyloxyethyl)-2,2'-oxydiacetamide (**7**)

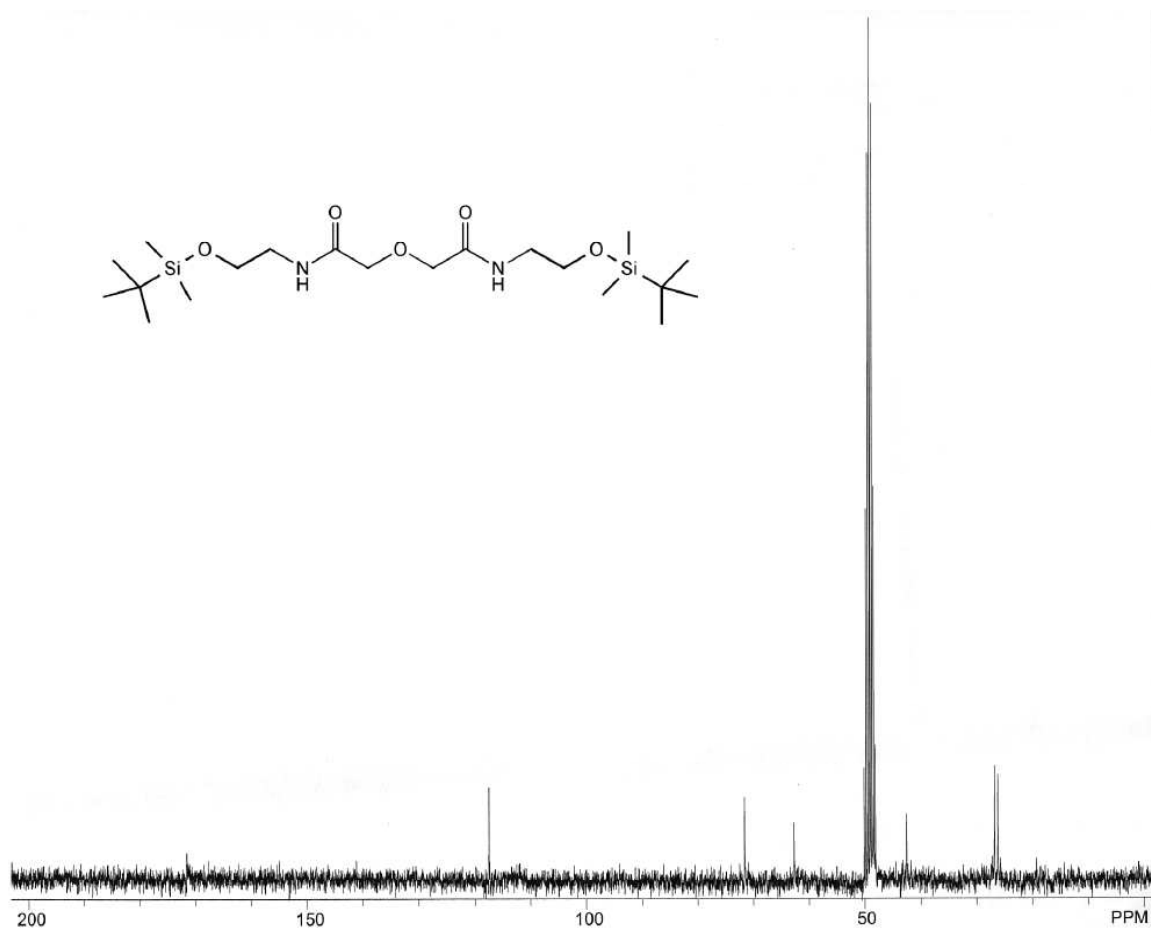


Figure S4: ^{13}C NMR spectrum of *N,N'*-Bis[2-(*tert*-butyldimethylsilyloxyethyl)-2,2'-oxydiacetamide (7)

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 Scan Count 16
 Points 1D 16384
 Last Delay 2s
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 F1 offset 1.5026 KHz
 RCVR gain 200
 F2 freq 250.1358000
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 SOLVENT: Methanol-d4

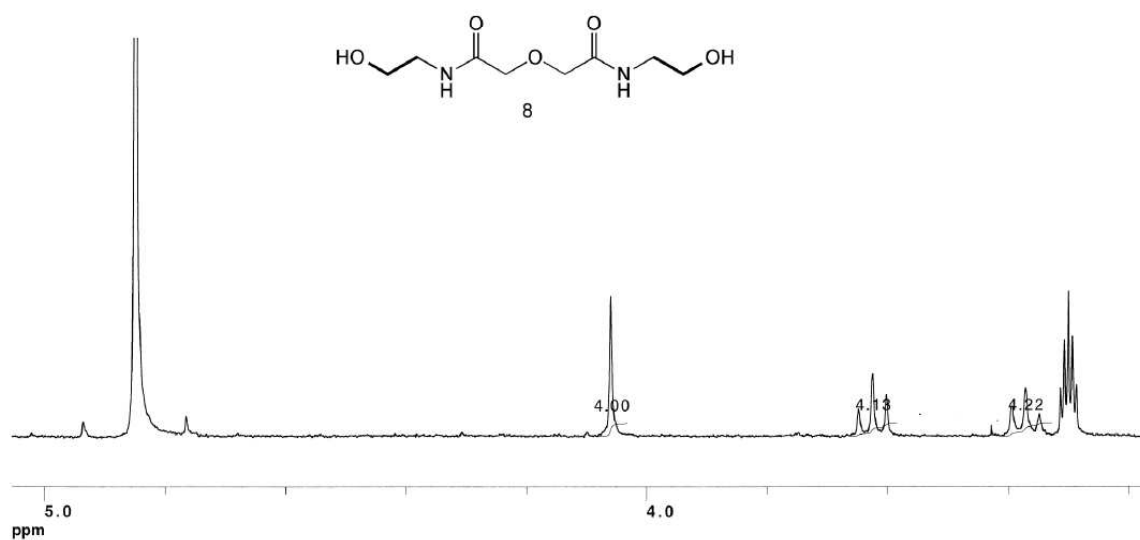


Figure S5: ¹H NMR spectrum of *N,N'*-Bis(2-hydroxyethyl)-2,2'-oxydiacetamide (**8**)

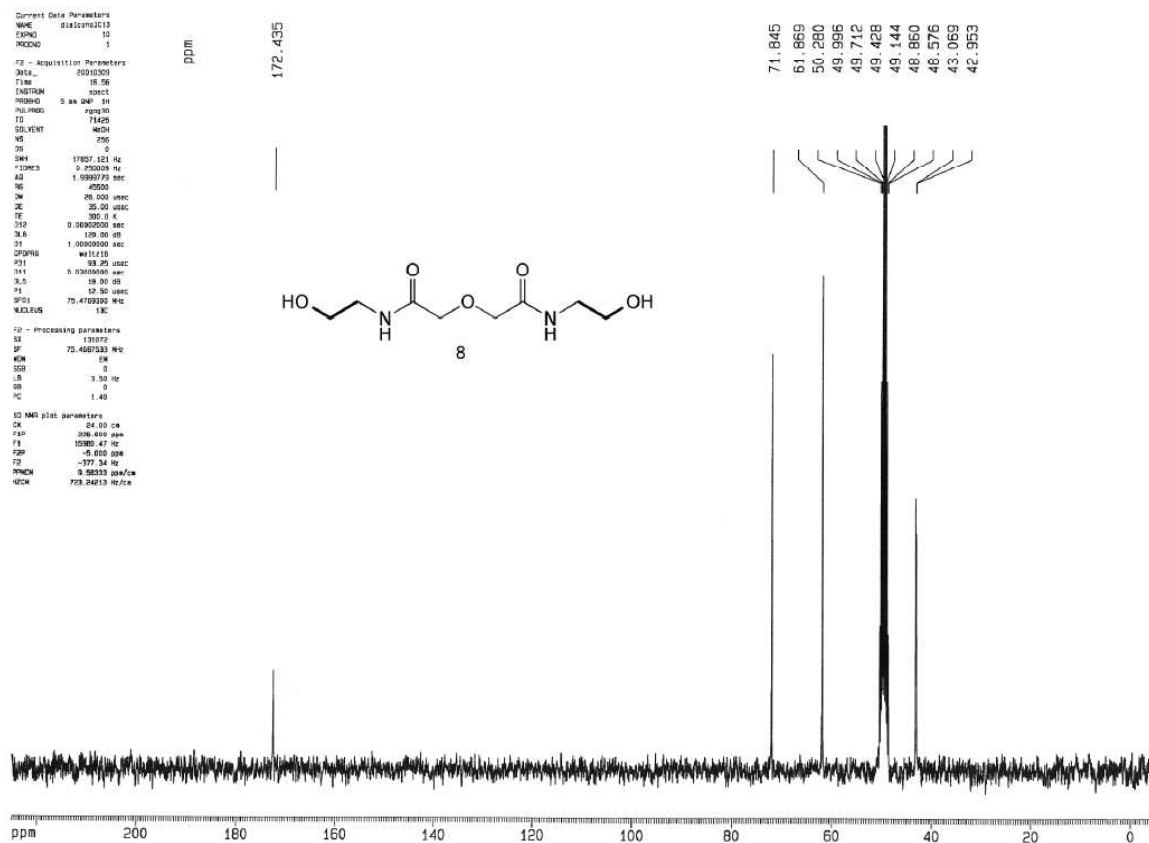


Figure S6: ¹³C NMR spectrum of *N,N'*-Bis(2-hydroxyethyl)-2,2'-oxydiacetamide (**8**)

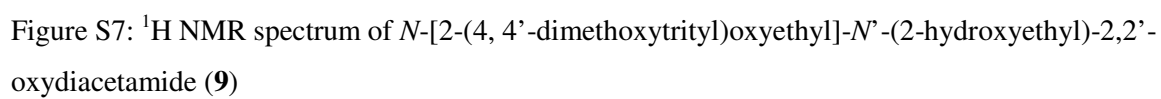


Figure S7: ^1H NMR spectrum of *N*-[2-(4, 4'-dimethoxytrityl)oxyethyl]-*N'*-(2-hydroxyethyl)-2,2'-oxydiacetamide (**9**)

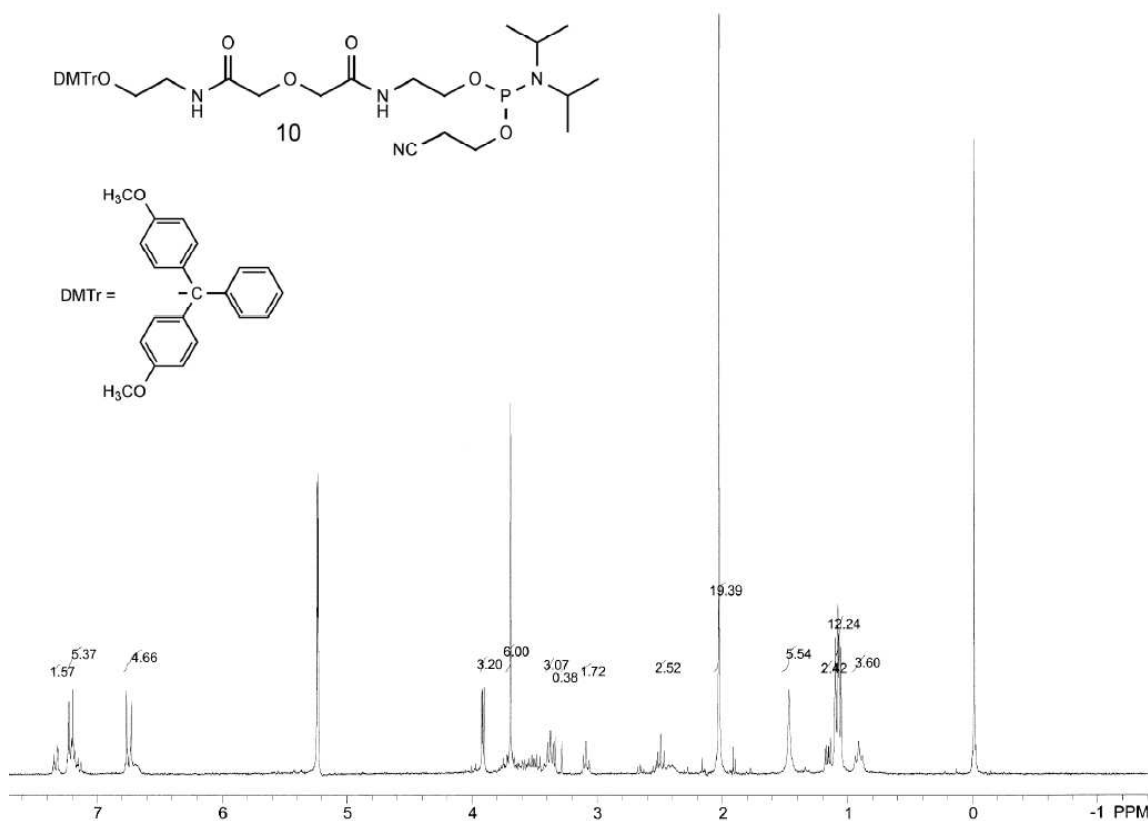


Figure S9: ¹H NMR spectrum of *N*-[3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)ethyl]-*N'*-[2-(4,4'-dimethoxytrityl)oxyethyl]-2,2'-oxydiacetamide (**10**)

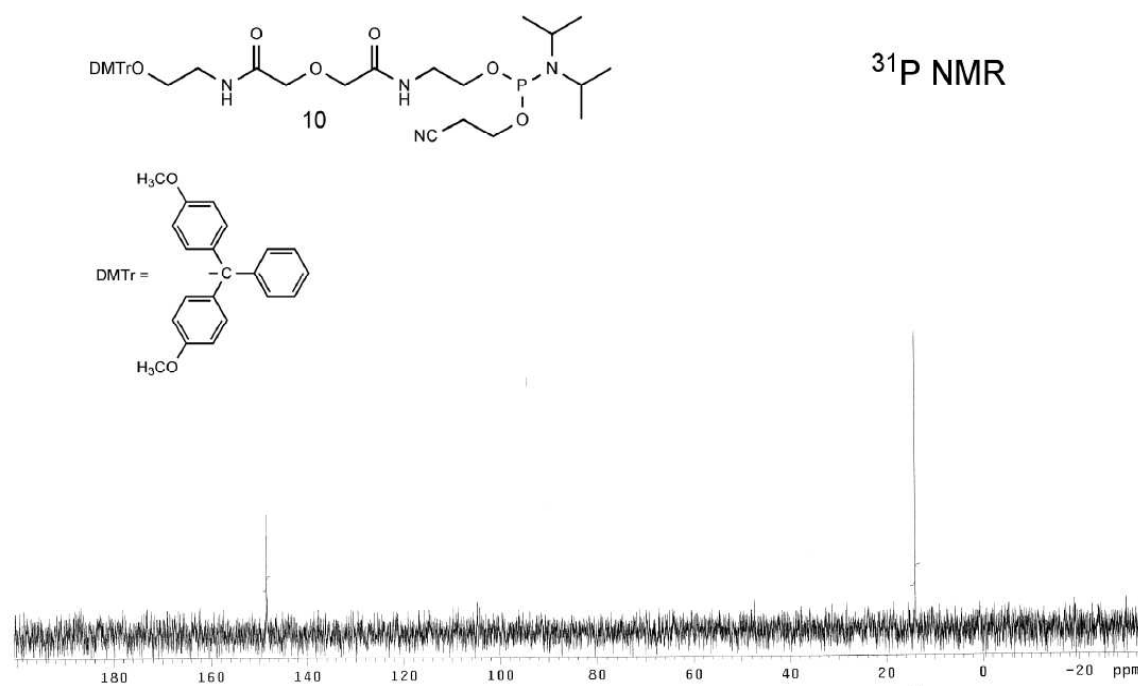


Figure S10: ³¹P NMR spectrum of *N*-[3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)ethyl]-*N'*-[2-(4,4'-dimethoxytrityl)oxyethyl]-2,2'-oxydiacetamide (**10**)

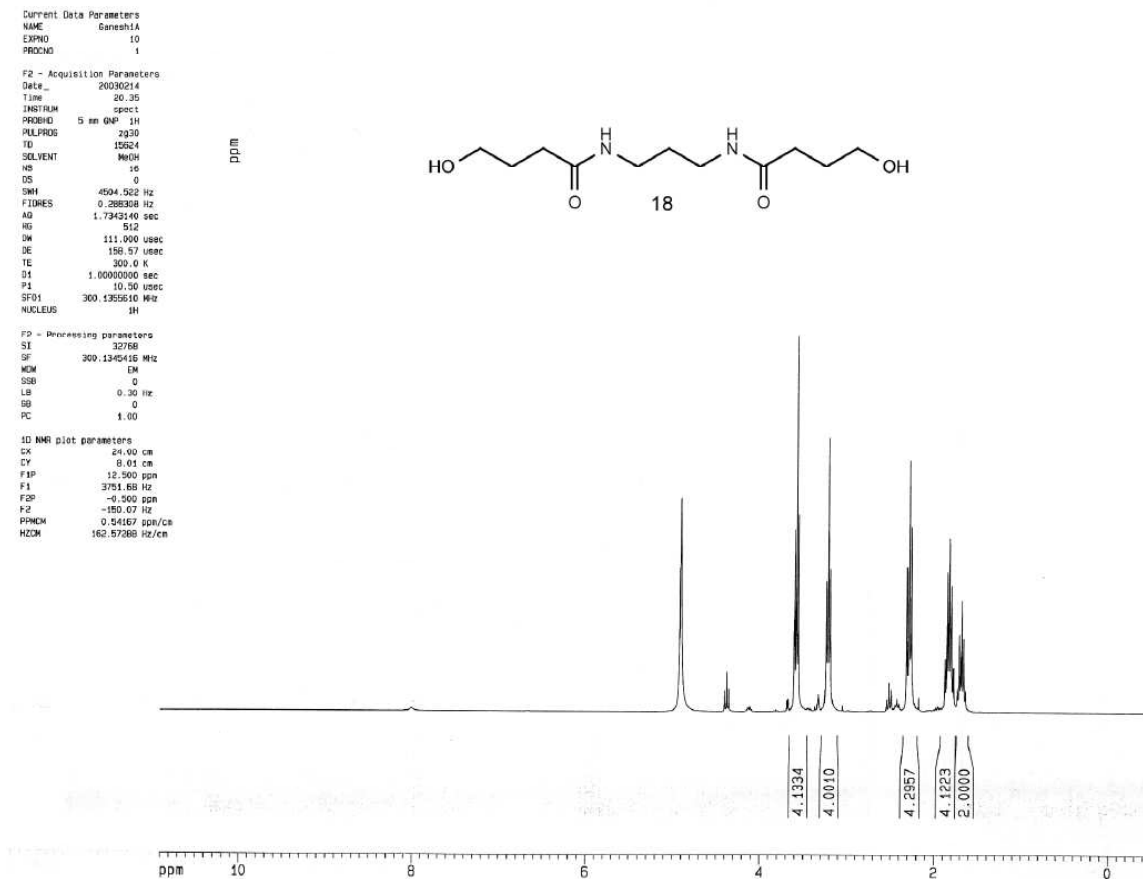


Figure S11: ^1H NMR spectrum of *N, N'*-Bis-(4-hydroxybutanoyl)-1,3-propanediamine (**18**)

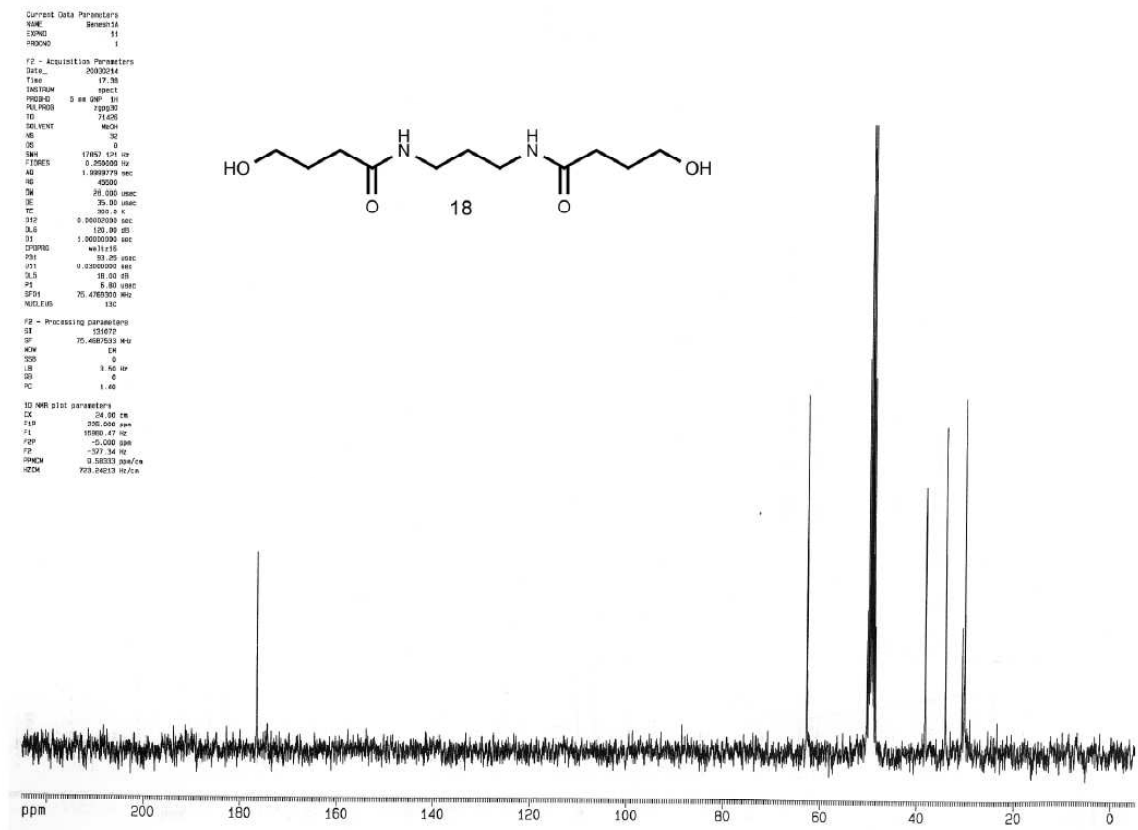


Figure S12: ¹³C NMR spectrum of *N,N*-Bis-(4-hydroxybutanoyl)-1,3-propanediamine (**18**)

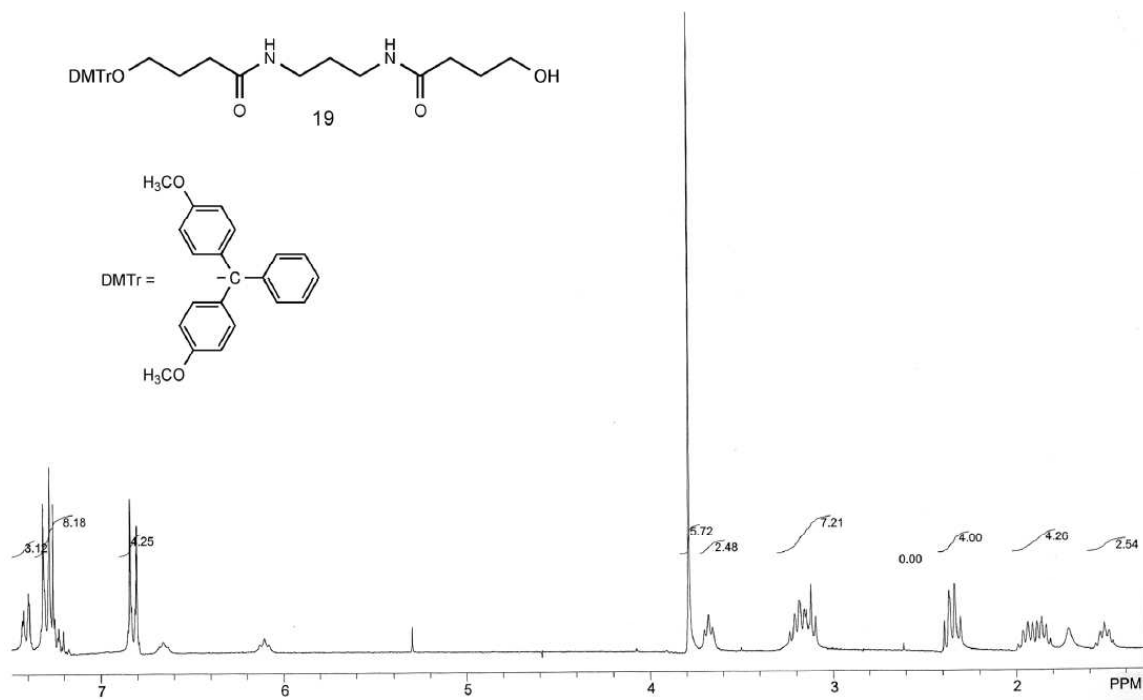


Figure S13: ^1H NMR spectrum of N -{4-[4,4'-(Dimethoxytrityl)oxy]butanoyl}- N' -4-hydroxybutanoyl-1,3-propanediamine (**19**)

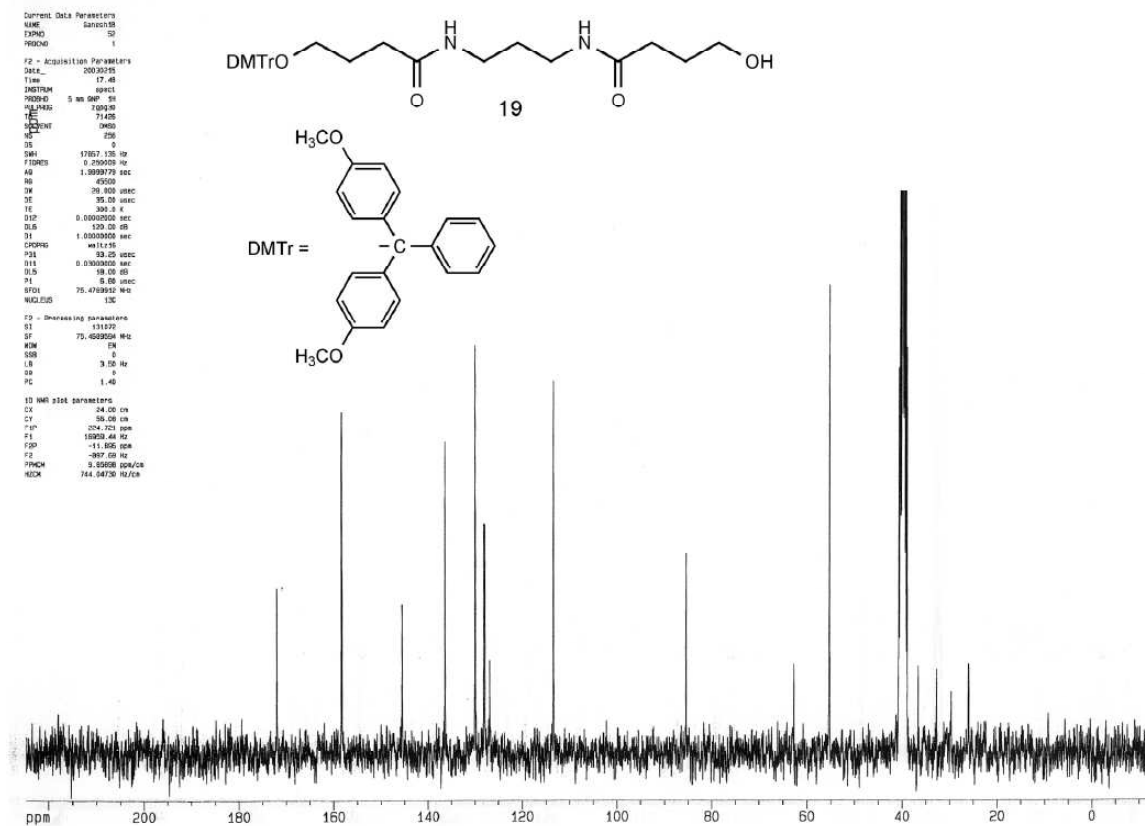


Figure S14: ^{13}C NMR spectrum of *N*-{4-[4,4'-(Dimethoxytrityl)oxy]butanoyl}-*N'*-4-hydroxybutanoyl-1,3-propanediamine (**19**)

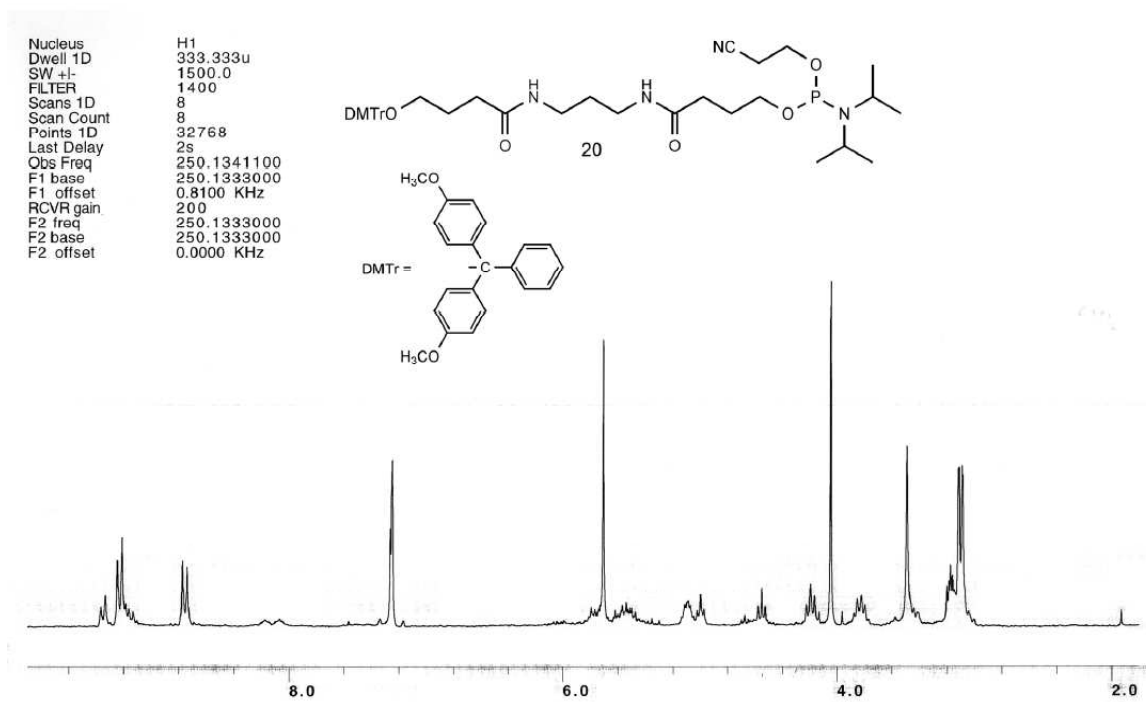


Figure S15: ^1H NMR spectrum of *N*-[4-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)butanoyl]-*N'*-(4-[4,4'-(dimethoxytrityl)oxy]butanoyl)-1,3-propanediamine (**20**)

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PROCNO 1

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RG 512
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DE 128.57 usec
TE 300.2 K
D1 1.0000000 sec
P1 10.50 usec
SFO1 300.1365610 MHz
NUCLEUS 1H

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LB 0.30 Hz
GB 0
PC 1.00

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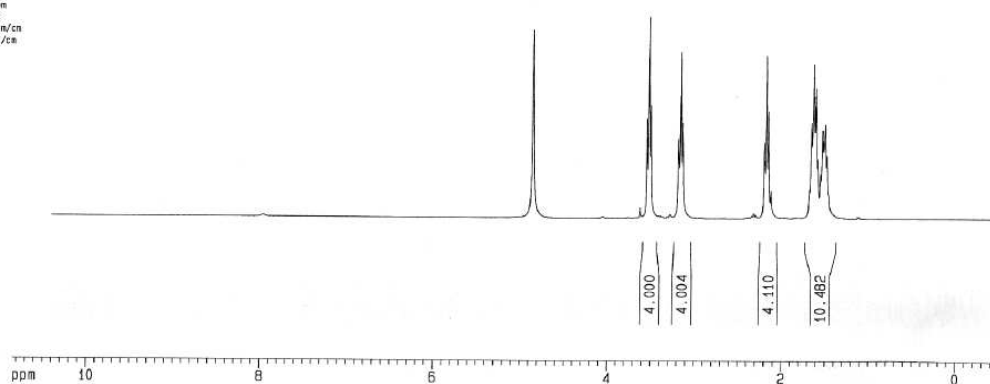
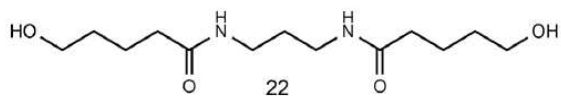


Figure S16: ¹H NMR spectrum of *N, N'*-Bis-(5-hydroxypentanoyl)-1,3-propanediamine (**22**)

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 SI: 0
 DS: 0
 SWH: 17867.124 Hz
 FIDRES: 0.253008 Hz
 AQ: 1.9999778 sec
 RG: 45500
 SW: 25.500 MHz
 SC: 35.50 MHz
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 S.F: 179.50 MHz
 FI: 1.5000000 sec
 SFOF2: 75.4758300 MHz
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 GB: 0
 PC: 1.40
 13 NMR plot parameters
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 CT: 00.00 deg
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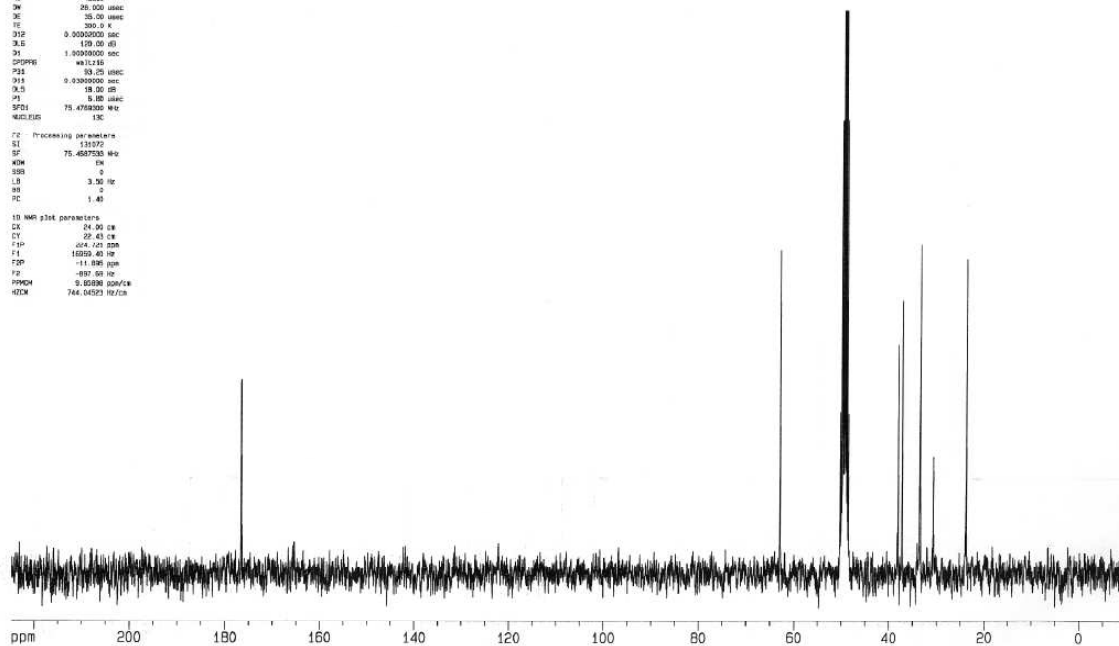
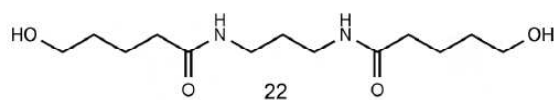


Figure S17: ^{13}C NMR spectrum of *N, N'*-Bis-(5-hydroxypentanoyl)-1,3-propanediamine (**22**)

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PROCNO 1

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FIDRES 0.280308 Hz
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RG 512
CW 110.999 usec
DE 158.57 usec
TE 300.0 K
D1 1.00000000 sec
P1 10.50 usec
SF01 300.136041 MHz
NUC1 1H

F2 - Processing parameters
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LB 0.30 Hz
GB 0
PC 1.00

1D NMR plot parameters
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CY 21.12 cm
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F2 -50.07 Hz
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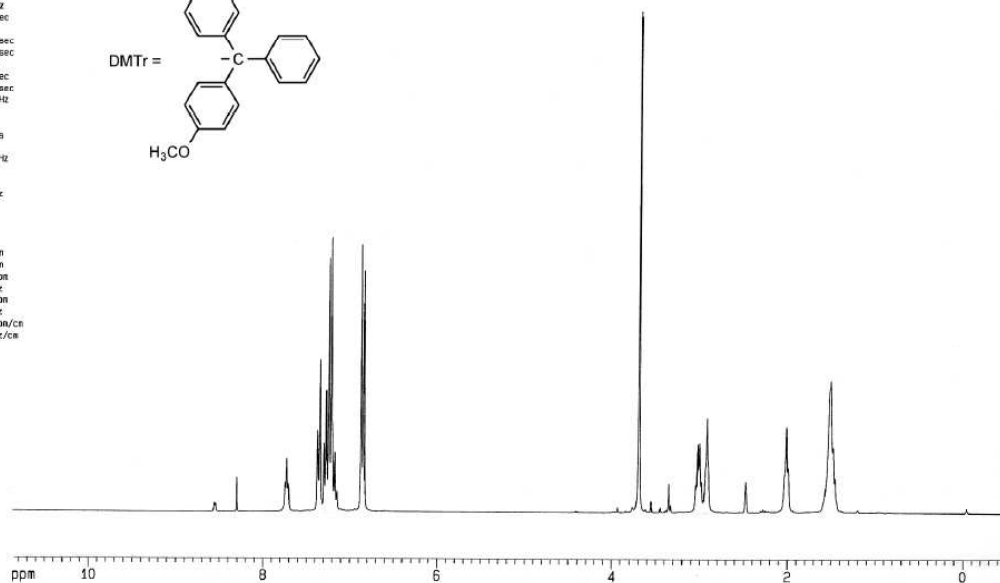
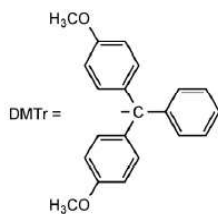
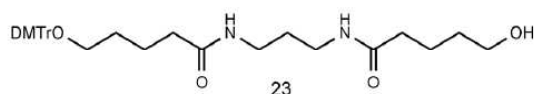


Figure S18: ¹H NMR spectrum of *N*-{5-[4,4'-(Dimethoxytrityl)oxy]pentanoyl}-*N'*-(5-hydroxypentanoyl)-1,3-propanediamine (**23**)

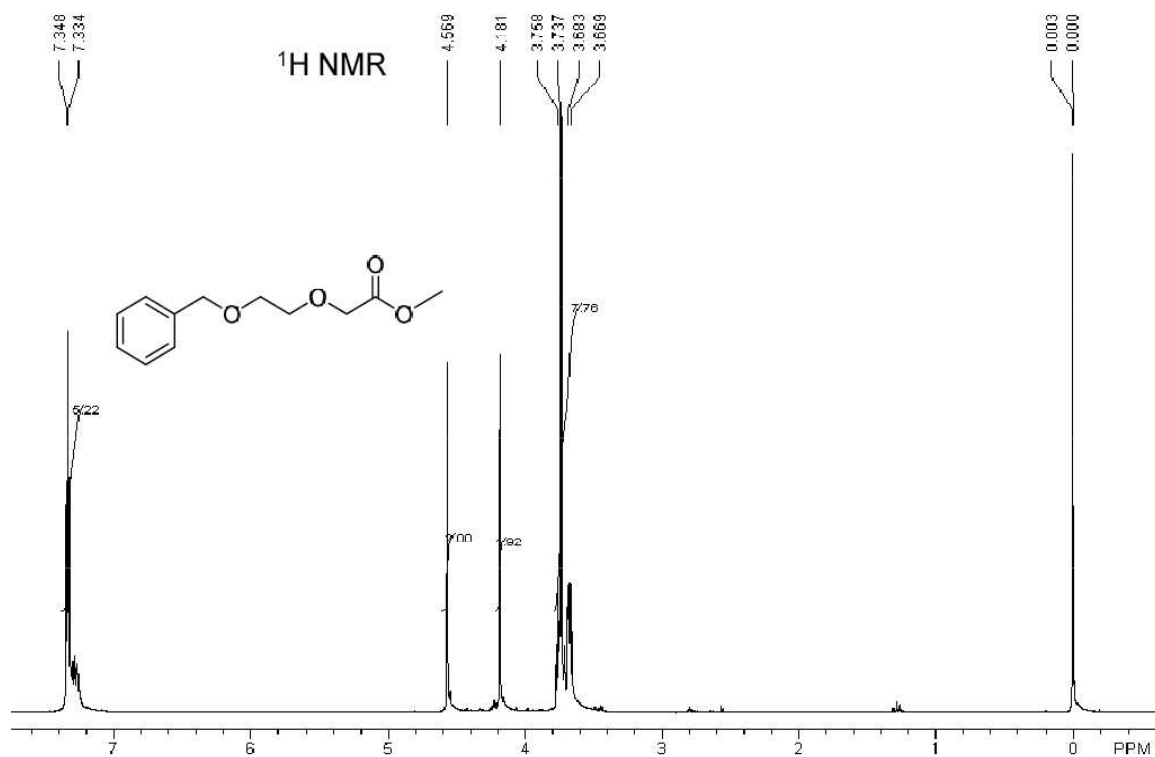


Figure S20: ¹H NMR spectrum of Methyl 2-(2-(benzyloxy)ethoxy)acetate (**26**)

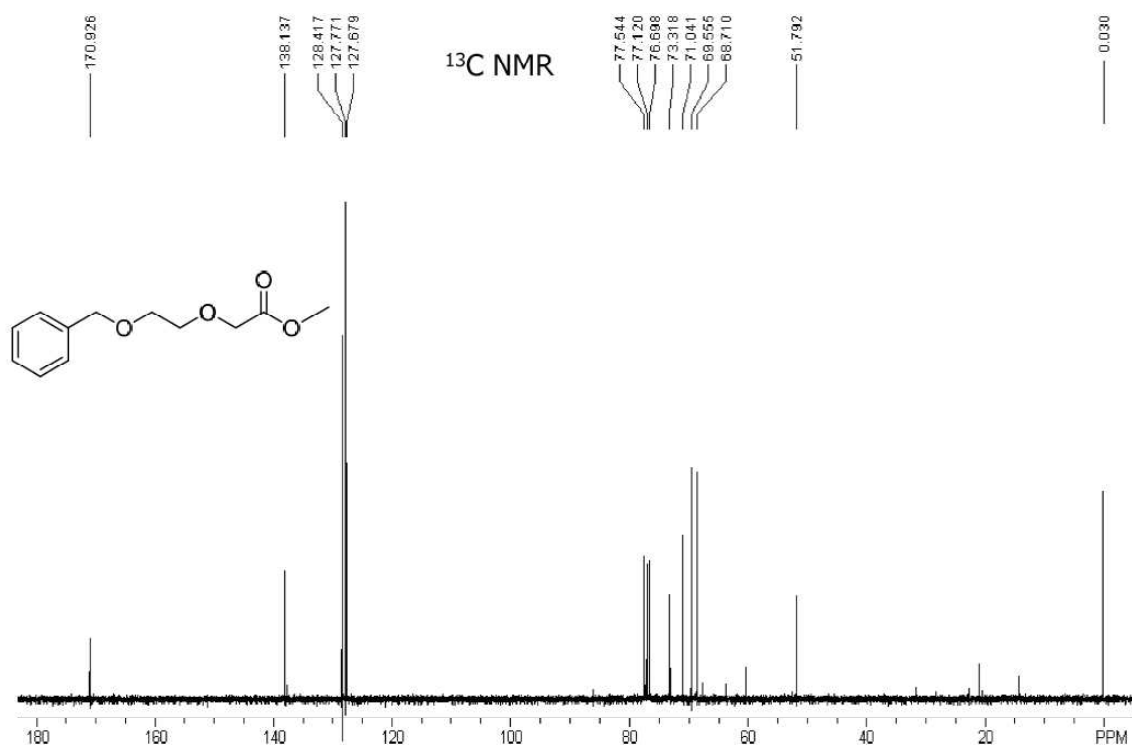


Figure S21: ¹³C NMR spectrum of Methyl 2-(2-(benzyloxy)ethoxy)acetate (**26**)

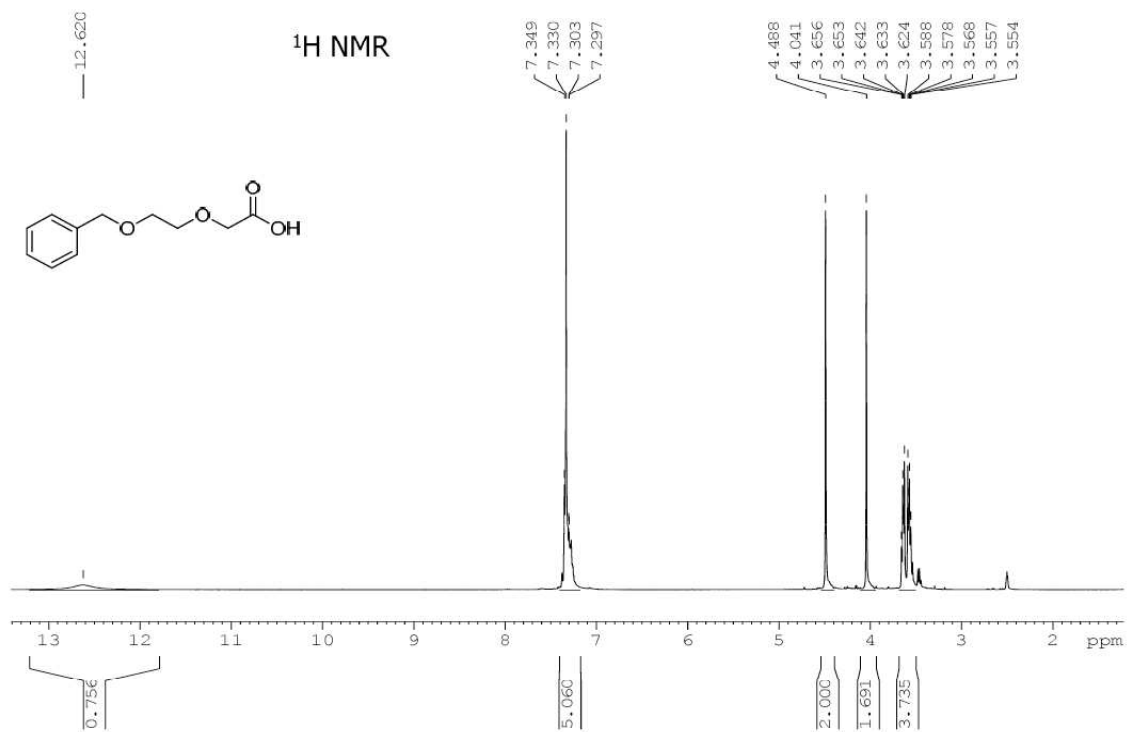


Figure S22: ¹H NMR spectrum of 2-(2-(Benzyloxy)ethoxy)acetic acid (**27**)

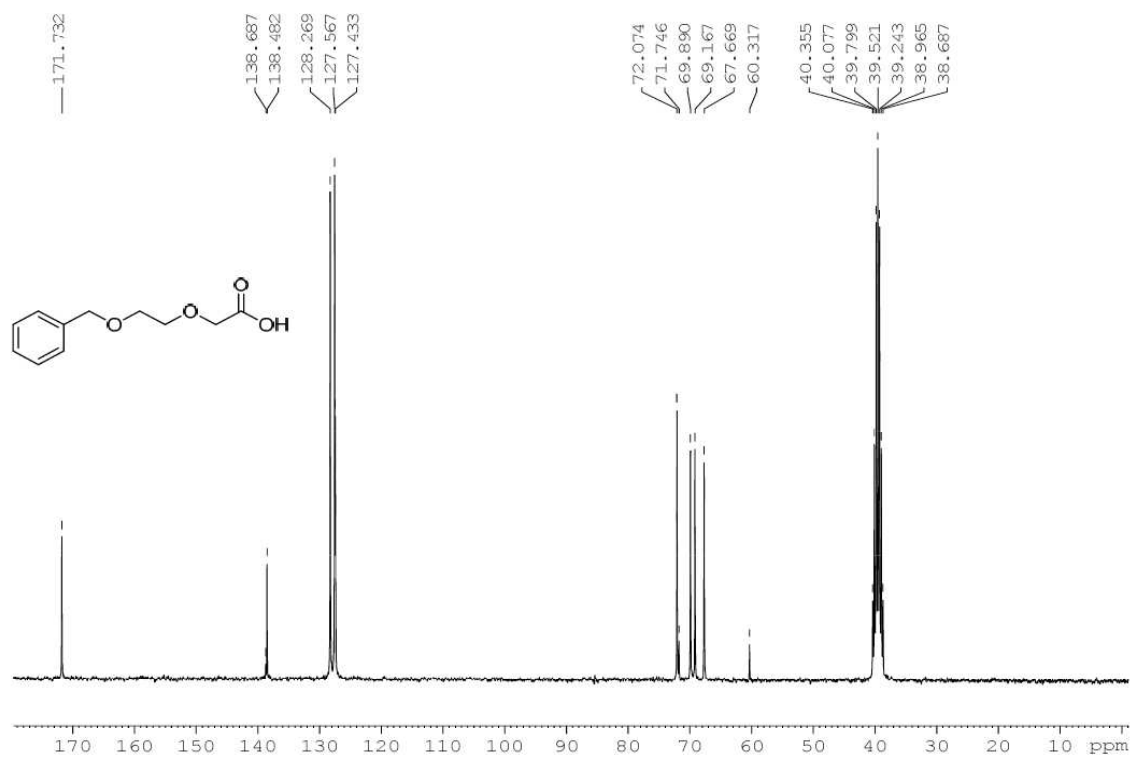


Figure S23: ¹³C NMR spectrum of 2-(2-(Benzyloxy)ethoxy)acetic acid (**27**)

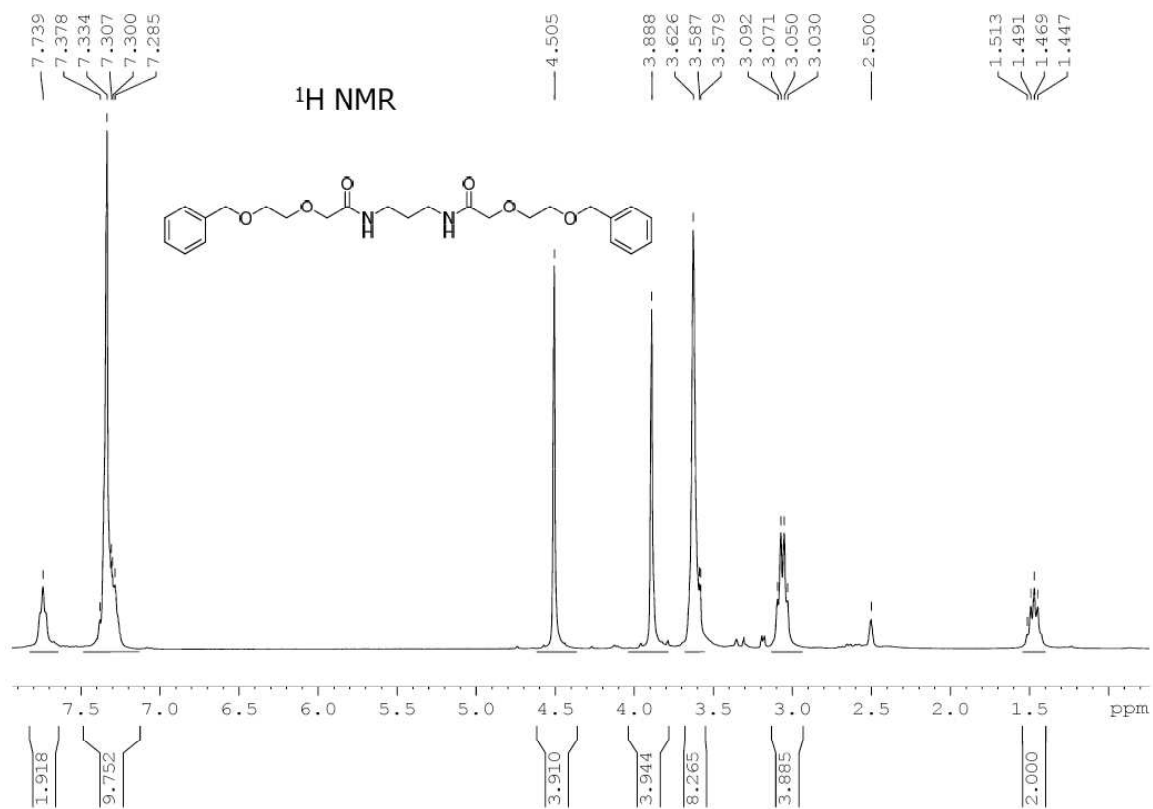


Figure S24: ¹H NMR spectrum of *N,N'*-Bis-(2-(2-benzyloxyethoxy)ethanoyl)-1,3-propanediamine (**28**)

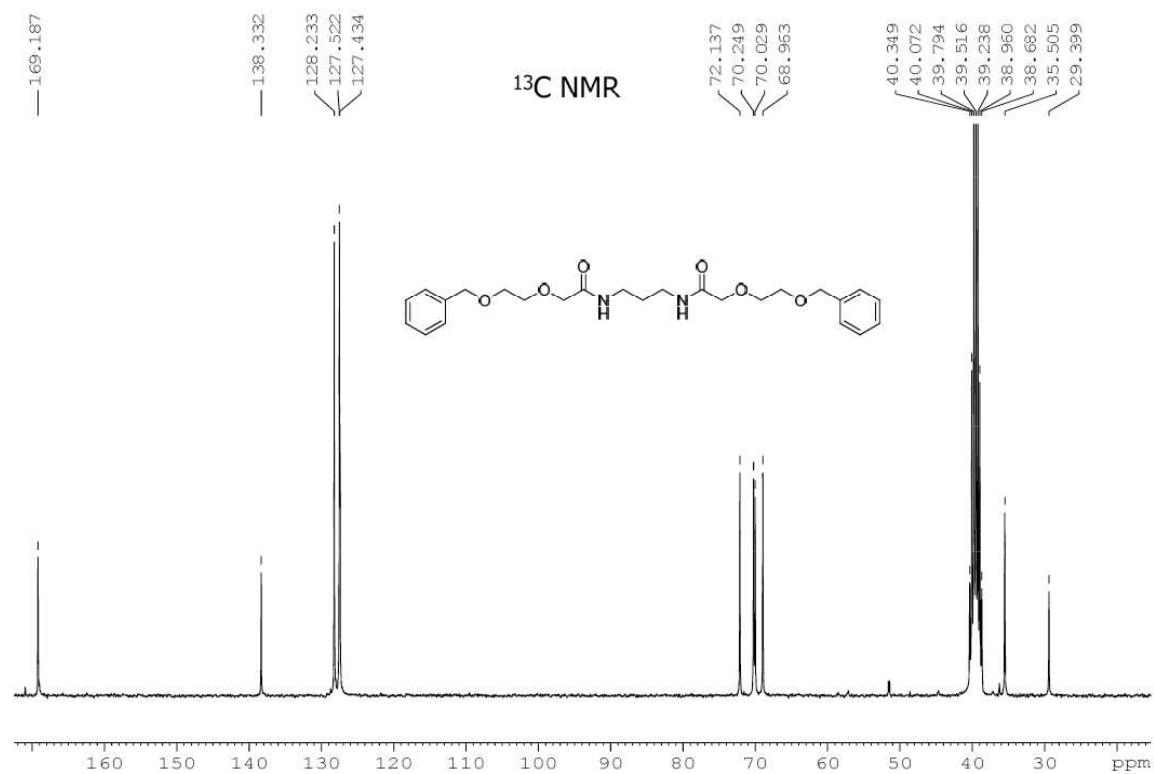


Figure S25: ¹³C NMR spectrum of *N,N'*-Bis-(2-(2-benzyloxyethoxy)ethanoyl)-1,3-propanediamine (**28**)

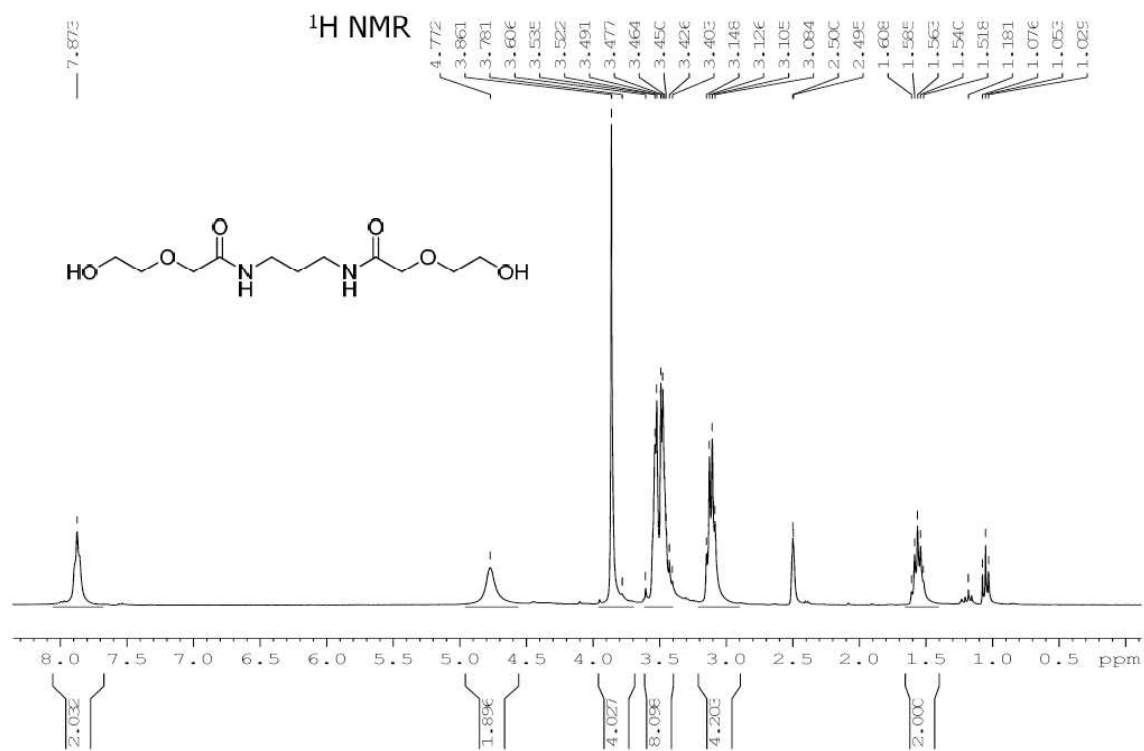


Figure S26: ¹H NMR spectrum of *N, N'*-Bis-(2-(2-hydroxyethoxy)ethanoyl)-1,3-propanediamine (**29**)

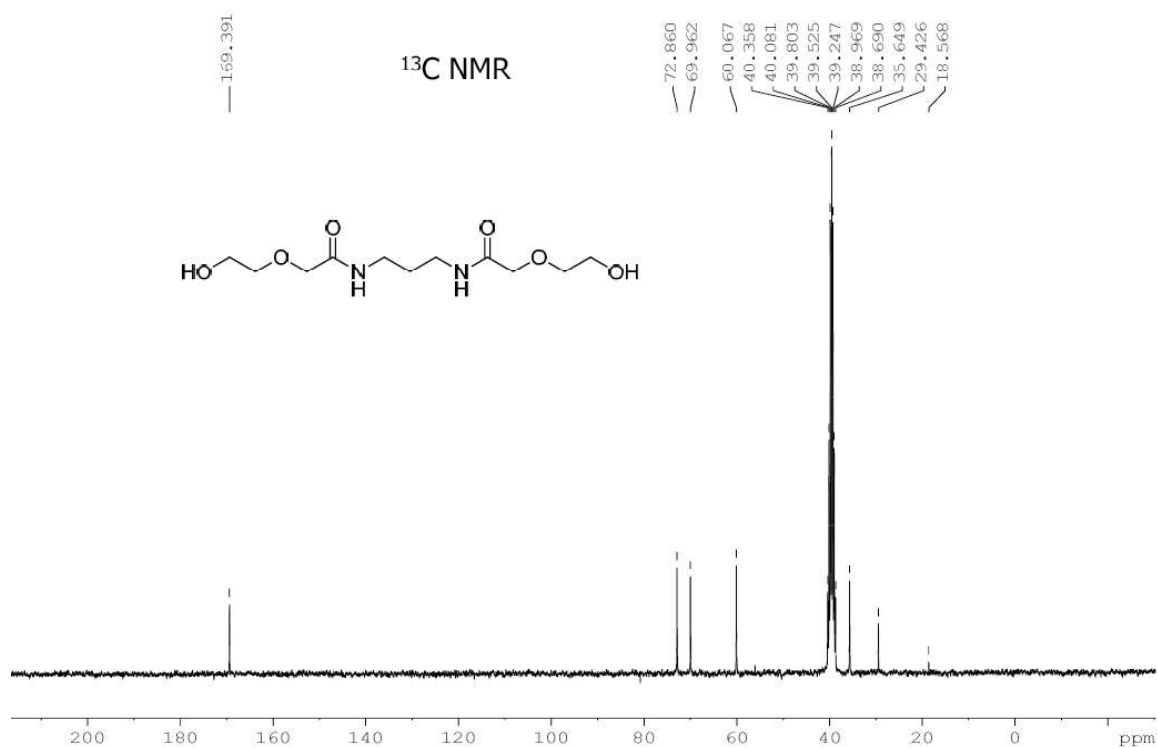


Figure S27: ¹³C NMR spectrum of *N,N'*-Bis-(2-(2-hydroxyethoxy)ethanoyl)-1,3-propanediamine (**29**)

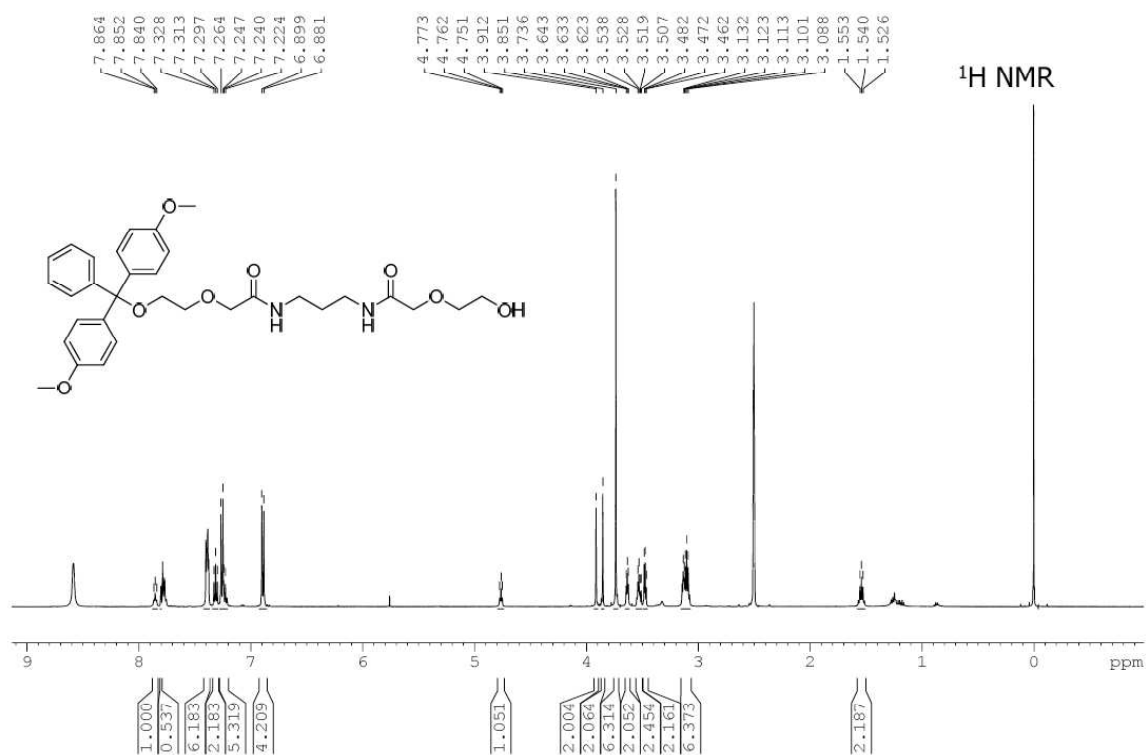


Figure S28: ¹H NMR spectrum of *N*-(2-(2-(4, 4'-dimethoxytrityloxy)ethoxy)ethanoyl)-*N'*-(2-(2-hydroxyethoxy)ethanoyl)-1,3-propanediamine (**30**)

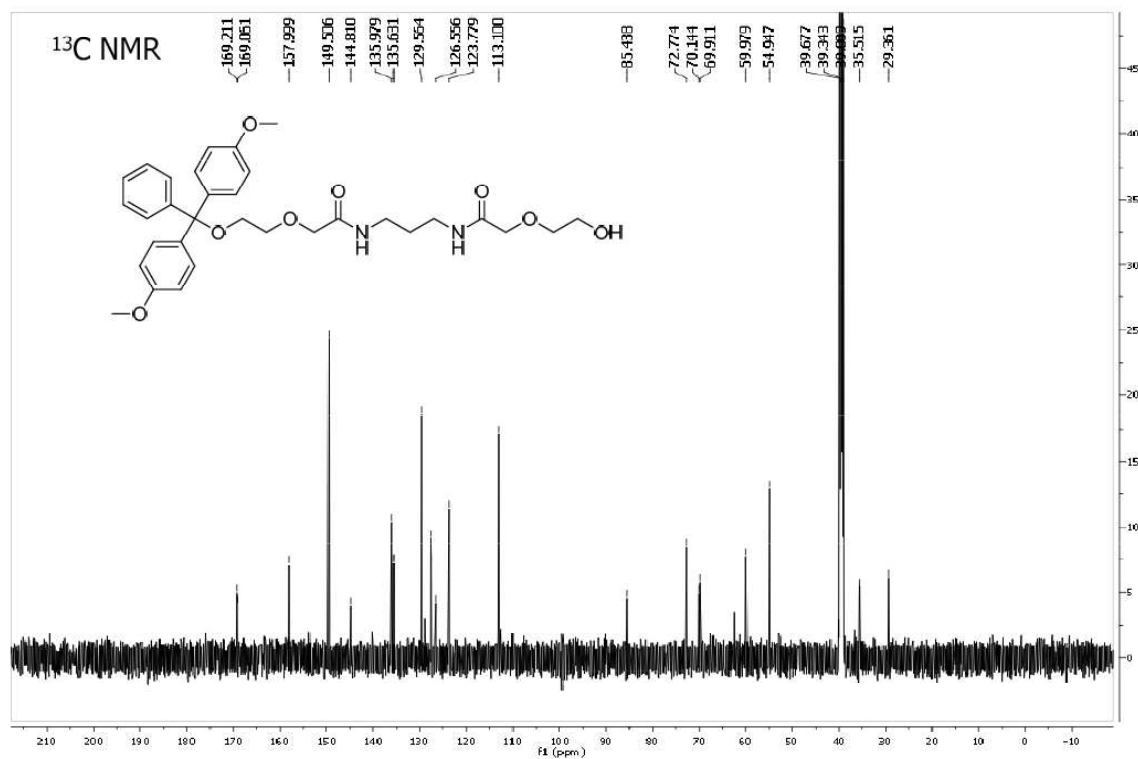


Figure S29: ¹³C NMR spectrum of *N*-(2-(2-(4,4'-dimethoxytrityloxy)ethoxy)ethanoyl)-*N'*-(2-(2-hydroxyethoxy)ethanoyl)-1,3-propanediamine (**30**)

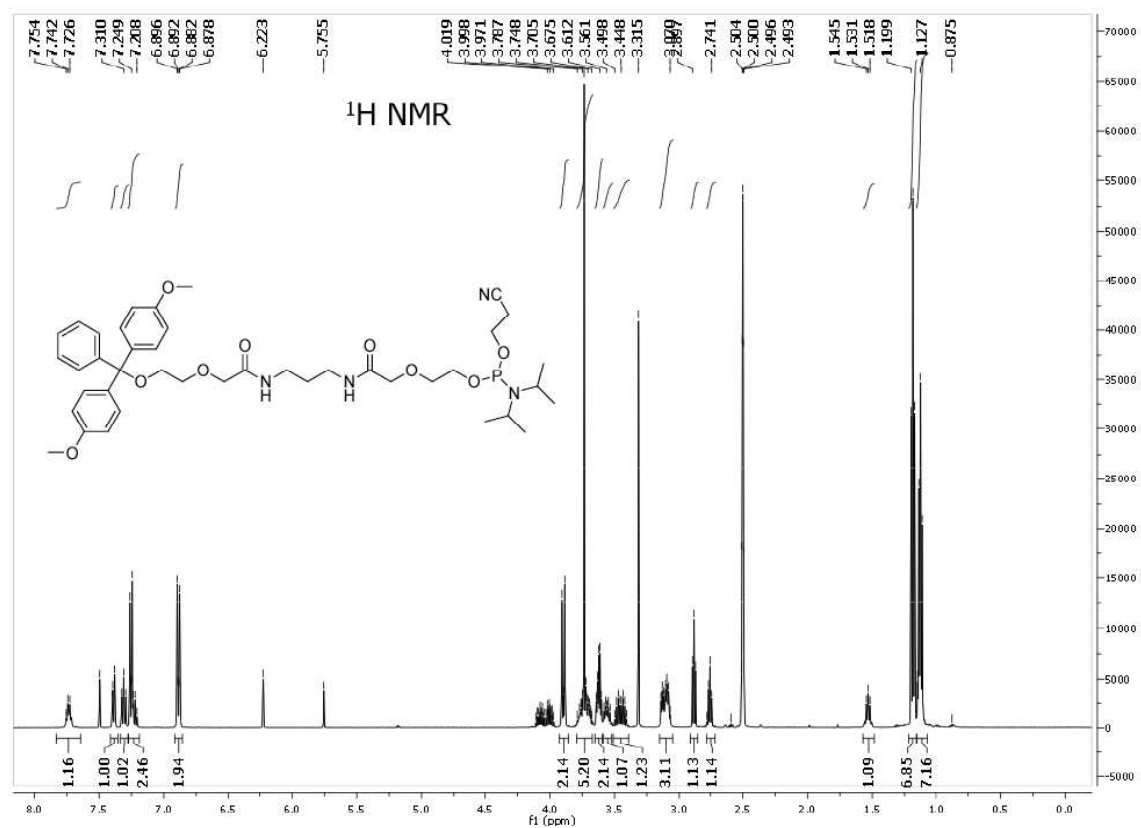


Figure S30: ¹H NMR spectrum of *N*-(2-(2-O-(2-cyanoethyl-*N*, *N*-diisopropylphosphoramidite)ethoxy)ethanoyl)-*N'*-(2-(2-(4, 4'-dimethoxytrityloxy)ethoxy)ethanoyl)-1,3-propanediamine (**31**)

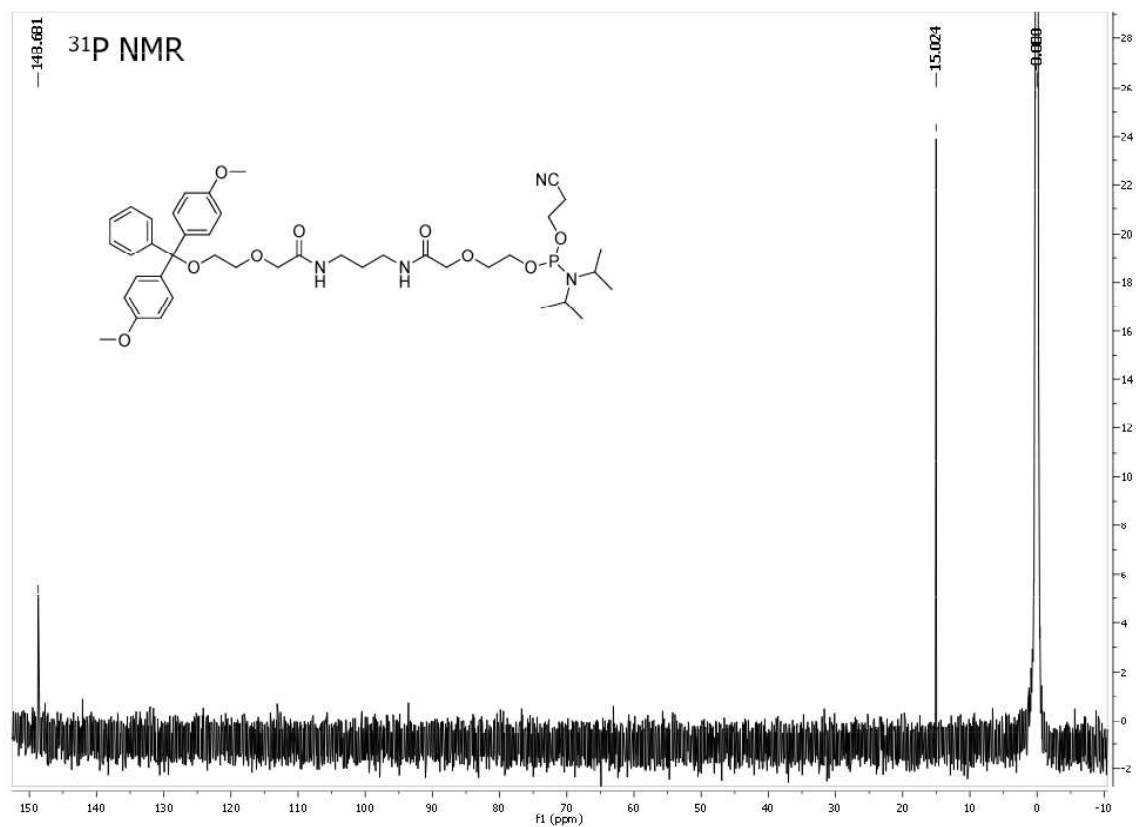


Figure S31: ³¹P NMR spectrum of *N*-(2-(2-O-(2-cyanoethyl-*N*, *N*-diisopropylphosphoramidite)ethoxy)ethanoyl)-*N'*-(2-(2-(4, 4'-dimethoxytrityloxy)ethoxy)ethanoyl)-1,3-propanediamine (**31**)