Synthesis of Glycol Nucleic Acids

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Abstract: Starting from glycidol, the synthesis of dimethoxytritylated glycol nucleoside phosphoramidites of adenine (A), thymine (T), uracil (U), guanine (G), and cytosine (C) is reported. These phosphoramidites are the building blocks for the automated solid phase synthesis of glycol nucleic acids (GNA) oligonucleotides and it is demonstrated that derived GNA duplexes with completely acyclic backbones considerably exceed the thermal stabilities of analogous DNA duplexes.

Key words: GNA, glycol nucleic acid, glycol nucleotides, acyclic nucleic acid backbone, epoxide ring opening

Since Watson and Crick unraveled the double helix structure of DNA more than 50 years ago, DNA has become a target of extensive chemical modification with the aim of understanding and altering its properties.[1–9] Our group is interested in designing structurally simplified artificial oligonucleotides that can still form stable duplexes in a programmable fashion. Along these lines, we recently succeeded in designing a glycol nucleic acid (GNA) with a stripped down acyclic backbone.[10] GNA may display the most atom economical solution for a functional nucleic acid backbone.

We here give a full account of the syntheses of dimethoxytritylated glycol phosphoramidites of adenine (A), thymine (T), uracil (U), guanine (G), and cytosine (C), which serve as the building blocks for the automated solid phase synthesis of GNA oligonucleotides (Figure 1). We demonstrate that GNA oligonucleotides can be synthesized in high yields and derived duplexes of antiparallel GNA strands considerably exceed the thermal stabilities of analogous DNA duplexes.[10–12]

The synthesis of 2,3-dihydroxypropyl derivatives of nucleobases (glycol nucleosides/nucleotides) has been reported from various starting materials,[12–22] most notably, from isopropylideneglycerol by tosylation of the hydroxyl group followed by nucleophilic substitution with a nucleobase or nucleobase derivative,[12–18] or in a different strategy, by direct ring-opening of glycidol with nucleobases or nucleobase derivatives.[19–21]

We were attracted by the shorter and more straightforward approach starting with 'spring-loaded' glycidol.[19–21] The presented synthetic schemes are related to a published

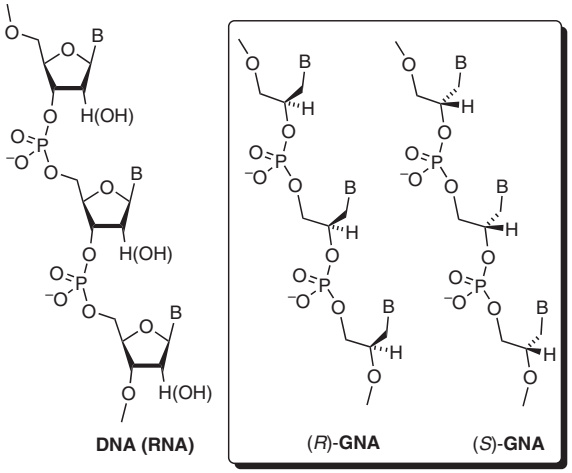

Figure 1 Comparison of the constitutions of DNA and RNA, with both enantiomers of glycol nucleic acid (GNA).

communication by Acevedo and Andrews, in which no experimental details were provided.[21]

The glycol nucleosides are accessible starting from the commercially available enantiomerically pure (*R*)-(+)- and (*S*)-(–)-glycidols. (*R*)-(+)-Glycidol yields (*S*)-glycol nucleotides and (*S*)-GNA, and (*S*)-(–)-glycidol yields (*R*)-glycol nucleotides and (*R*)-GNA.[10] The synthetic route starting with (*R*)-(+)-glycidol (**1**) is shown in Scheme 1. Tritylation of **1** with DMTrCl provides the tritylated (*S*)-glycidol **2** in quantitative yield.[23] We generally prefer to use the tritylated glycidol as a substrate for the following epoxide ring opening with nucleobases or their derivatives because it simplifies the purification. Accordingly, reactions of **2** with thymine (**3**), uracil (**4**), *N*-benzoylcytosine (**5**), and adenine (**6**), in the presence of around 0.2 equivalents of sodium hydride, afford in a regioselective and stereospecific fashion the compounds **7**[12] (49%), **8** (61%), **9** (59%), and **10** (51%), respectively (Schemes 1 and 2). The pyrimidine nucleosides are subsequently directly converted to the phosphoramidites (*S*)-**T**[12] (77%), (*S*)-**U** (65%), and (*S*)-**C** (66%) by reacting with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (**11**) in the presence of Hünig's base (Scheme 1). The adenine nucleoside **10** is first benzoylated at the exocyclic amino group to **12** (71%) before being converted to the phosphoramidite (*S*)-**A** (73%) (Scheme 2).

For the synthesis of the guanine glycol nucleoside, we were not able to obtain the desired nucleoside by ring opening of **2** with guanine or protected guanines. However, reaction of 2-amino-6-chloropurine (**13**) with (*R*)-(+)-

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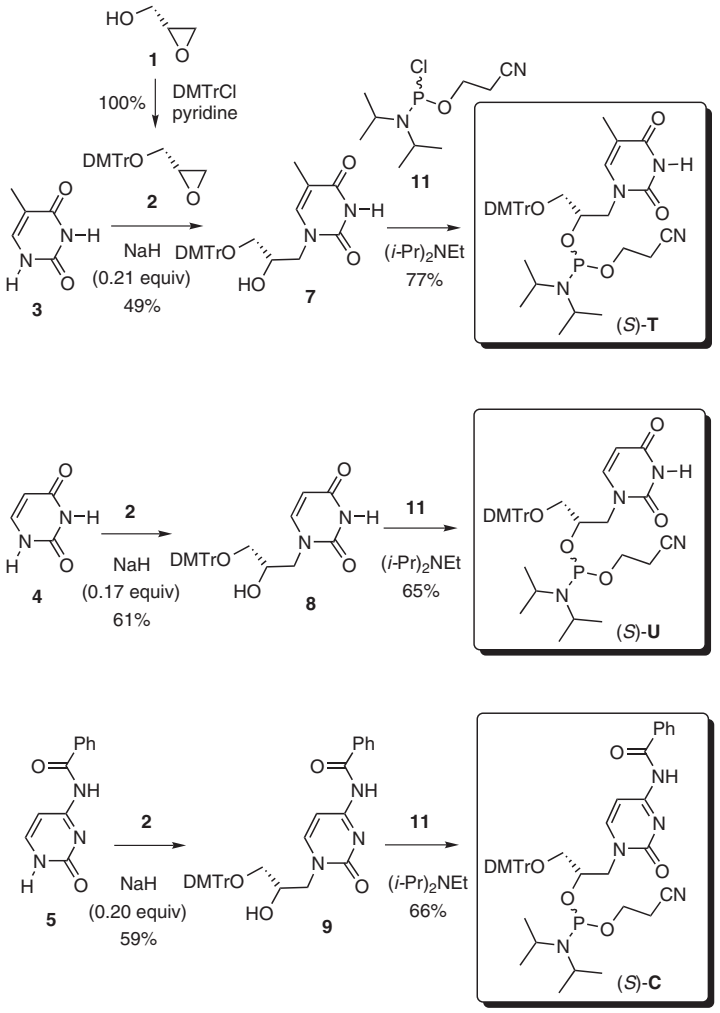


Scheme 1 Synthesis of pyrimidine glycol nucleotides. Shown is the route from (*R*)-(+)-glycidol leading to (*S*)-glycol nucleosides and (*S*)-phosphoramidites.

glycidol (**1**) in the presence of substoichiometric amounts of K_2CO_3 provides the ring-opened product **14** in 39% yield (Scheme 2). Acid hydrolysis of the chloride leads to **15**[15] (82%), followed by protection of the exocyclic amino group with an isobutyryl group to afford **16** (70%). After tritylation to **17** with DMTrCl in anhydrous pyridine in 75% yield, reaction with **11** in the presence of Hünig's base provides the final phosphoramidite (*S*)-**G**. It has to be noted that we were not able to run (*S*)-**G** over a silica gel column without decomposition. Instead, we developed a precipitation protocol which provides purified (*S*)-**G** in a yield of 45%.

Overall, the pyrimidine phosphoramidites (*S*)-**T**, (*S*)-**U**, and (*S*)-**C** can be synthesized from commercially available starting materials in three steps (overall yields of 38%, 40%, and 39%, respectively), the adenine glycol nucleotide (*S*)-**A** in four steps (overall yield of 26%), and the guanine glycol nucleotide (*S*)-**G** in five steps (overall yield of 8%). The enantiomeric compounds (*R*)-**T**, (*R*)-**U**, (*R*)-**C**, (*R*)-**A**, and (*R*)-**G** are accessible in the same fashion by starting from (*S*)-(–)-glycidol. Crystal structures of derivatized glycol nucleosides verified the desired regioselectivities of the epoxide ring openings (Figure 2).

In order to synthesize oligonucleotides entirely composed out of glycol nucleotides on solid support, we next derivatized long chain alkylamine controlled pore glass (LCAA–CPG) with glycol nucleosides. For this, the tritylated nucleosides **7**–**9**, **12**, and **17** were first converted to the succinates in a standard protocol by reacting with succinic anhydride and triethylamine, followed by amide coupling with LCAA–CPG under activation with 1-benzotriazole and 1,3-diisopropylcarbodiimide, yielding typical loadings of 60–70 μmol/g.[24,25]

Oligonucleotides **21**–**28** (Table 1) were synthesized on these derivatized CPG supports with standard protocols for 2-cyanoethyl phosphoramidites, except that the coupling time was increased to three minutes. Under these conditions, no differences in coupling efficiencies between the glycol nucleoside phosphoramidites and commercial 2′-deoxyribonucleosyl phosphoramidites could be detected. Typically, glycol oligonucleotides were synthesized in trityl-on mode and cleaved from the resin with concentrated ammonia at 55 °C for 12 hours. Tritylated

oligonucleotides were first purified by C18 reverse phase HPLC and then detritylated with 80% acetic acid. The strands were again purified, this time with a Waters XTerra column at elevated temperature (55–60 °C). This XTerra column combines reverse-phase with ion-pairing chromatography and gives superior resolution in eliminating shorter failure sequences. In our hands, regular reverse phase HPLC columns were not able to discriminate between glycol oligonucleotides that differ in the length by just one nucleotide unit. Typical yields of pure GNA strands were in the range of 20% to 40% based on the amount and loading of the solid supports.

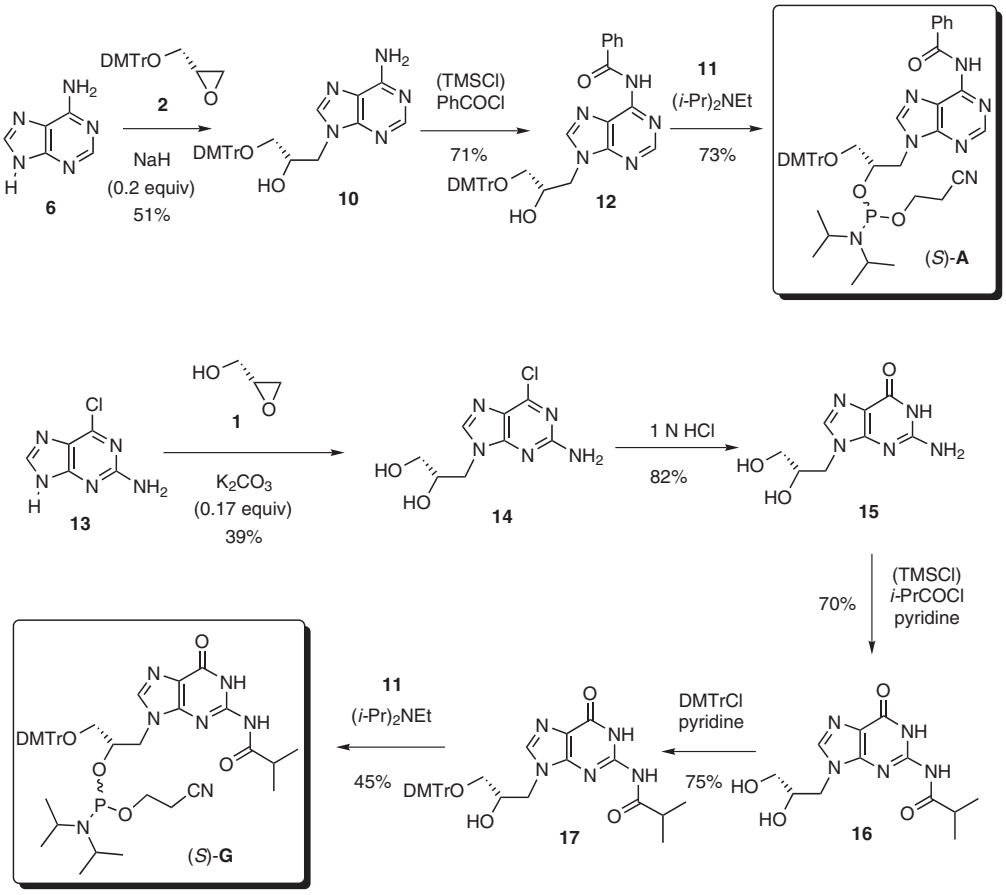
GNA strands are stable in buffered solution at room temperature. For example, no decomposition can be detected by HPLC with an analytical XTerra column of a T_6-GNA strand in 10 mM sodium phosphate buffer (pH 7.0) during a time period of two days.

We next investigated duplex formation of GNA strands with temperature-dependent UV spectroscopy at 260 nm. Mixtures (1:1) of complementary strands **21**:**22**, **23**:**24**, **25**:**26**, and **27**:**28** all yield characteristic sigmoidal melting curves, thus indicating cooperative melting of GNA duplexes. The UV-melting curve of the 15mer duplex **21**:**22** is displayed in Figure 3. For comparison, no sigmoidal melting and weaker hyperchromicities are observed with the single strands **21** and **22** alone (Figure 3, dashed and dotted curves).

These conclusions are confirmed by circular dichroism (CD) measurements. The CD spectra of a 1:1 mixture of GNA strands **21**:**22** and the individual strands are shown in Figure 4 and demonstrate strongly increased CD signals at around 205, 220, and 275 nm upon mixing of **21** and **22**, supporting the formation of a helical duplex.

It is intriguing that the thermal stabilities of GNA duplexes exceed the stabilities of analogous DNA duplexes (Table 1, entries a and b). For example, the GNA duplex **21**:**22** (Table 1, entry a) is thermally more stable with $\Delta T_M = 25$ °C under our experimental conditions (10 mM sodium phosphate, pH 7.0, 100 mM NaCl, 2 μM each strand) compared to a DNA duplex of the same sequence. Similarly, the GNA duplex **23**:**24** is thermally significantly more stable compared to the analogous DNA duplex ($\Delta T_M = 20$ °C). This is a surprising result considering the simplicity and acyclic nature of the backbone.

It has been widely assumed that nucleic acid analogues containing a phosphodiester backbone need to be cyclic in order to produce the required conformational preorganization for duplex formation.[26,27] This conclusion emerged



Scheme 2 Synthesis of purine glycol phosphoramidites for the automated solid phase oligonucleotide synthesis. Shown is the route from (*R*)-(+)-glycidol leading to (*S*)-glycol nucleosides and (*S*)-phosphoramidites.

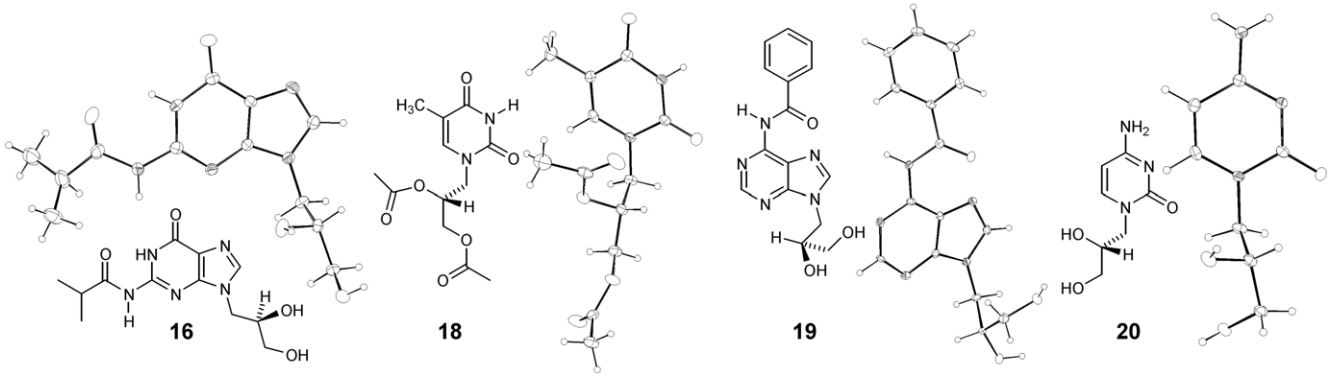


Figure 2 Crystal structures of derivatives of glycol nucleosides of G, T, A, and C. For the synthesis of **16** see Scheme 2. Crystals of **16** were grown from ethanol. Compound **18** was derived from **7** (Scheme 1) by detritylation with 5% trichloroacetic acid in CH_2Cl_2, followed by acetylation with acetic anhydride in pyridine. Crystals were grown from ethanol. Compound **19** was derived from **12** (Scheme 2) by detritylation with 80% aq acetic acid. Crystals were grown from methanol. Compound **20** was derived from **9** (Scheme 1) by detritylation with 5% trichloroacetic acid in CH_2Cl_2. Crystals were grown from hot ethanol, upon which the benzoyl group was cleaved off. X-ray intensity data were collected on a Rigaku Mercury CCD area detector employing graphite-monochromated Mo-K_α radiation at a temperature of 143 K and the structures were solved by direct methods.

partly from experimental observations in which single or multiple acyclic nucleotides incorporated into DNA resulted in a strong destabilization of the duplex structure. However, since this has been also observed for glycol nucleotides in DNA,[12] a reinvestigation of completely artificial backbones with different acyclic nucleotides may lead to interesting results.

Table 1 Synthesized GNA Oligonucleotides **21**–**28** and Thermal Stabilities of Derived GNA Duplexes[a]

Entries	Duplexes	T_M (°C)[b]
a	3′-CACATTATTGTTGTA-2′ (**21**) 2′-GTGTAATAACAACAT-3′ (**22**)	71 (46)
b	3′-AATATTATTATTTTA-2′ (**23**) 2′-TTATAATAATAAAAT-3′ (**24**)	51 (31)
c	3′-AAUAUUAUUAUUUUA-2′ (**25**) 2′-UUAUAAUAAUAAAAU-3′ (**26**)	32
d	3′-AAAAAAAAAAAAAAA-2′ (**27**) 2′-TTTTTTTTTTTTTTT-3′ (**28**)	62

[a] Measured in 10 mM sodium phosphate (pH 7.0) with 100 mM NaCl.
[b] Melting points of the analogous DNA duplexes are in brackets.

In summary, we have presented a full account of the syntheses of acyclic nucleic acids. Building blocks for automated solid phase synthesis are accessible in an economical fashion by nucleophilic ring opening of tritylated glycidol (A, T, C, U) or glycidol (G). GNA forms duplexes that are thermally significantly more stable compared to analogous DNA duplexes. The completely acyclic nature of the GNA backbone renders it currently the most economical solution for a phosphodiester bond containing nucleic acid backbone. Efforts to functionalize GNA by introducing artificial base pairs into this new duplex scaffold are underway.

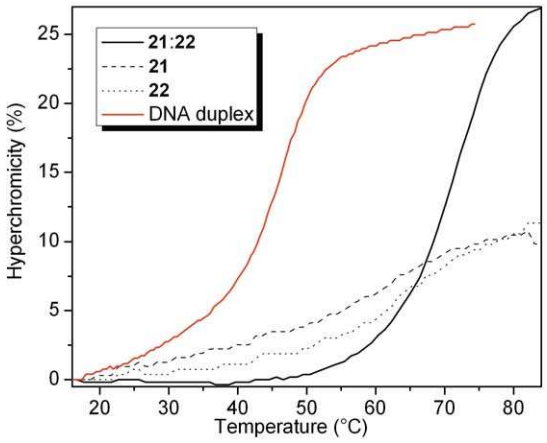


Figure 3 UV melting curves (260 nm) of a 1:1 mixture of GNA strands **21**:**22** (2 μM each), and the individual single strands **21** (2 μM) and **22** (2 μM). A DNA duplex (2 μM each strand) with the same sequence is shown in red. Experiments were performed in 10 mM sodium phosphate (pH 7.0) with 100 mM NaCl.

NMR spectra were recorded on a Bruker AM-500 (500 MHz) spectrometer. Low-resolution mass spectra were obtained on an LC platform from Micromass using ESI technique. High-resolution mass spectra were obtained with a Micromass AutoSpec instrument using either CI or ES ionization. Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer. Solvents and reagents were used as supplied from Aldrich or Acros. All non-aqueous operations were carried out under a dry argon atmosphere.

Compound 2[21]

To a solution of (*R*)-(+)-glycidol **1** (1.0 mL, 15.1 mmol) and Et_3N (5.4 mL, 40.7 mmol) in CH_2Cl_2 (34 mL) was added DMTrCl (6.45 g, 19 mmol). After 12 h, the reaction mixture was poured into sat. aq $NaHCO_3$ (50 mL). The organic layer was evaporated, the residue was dissolved in EtOAc, washed with aq sat. $NaHCO_3$, and purified by chromatography over silica gel eluting with hexanes–EtOAc–Et_3N (10:1:0.01), affording compound **2** as an oil (5.98 g, quantitative yield).

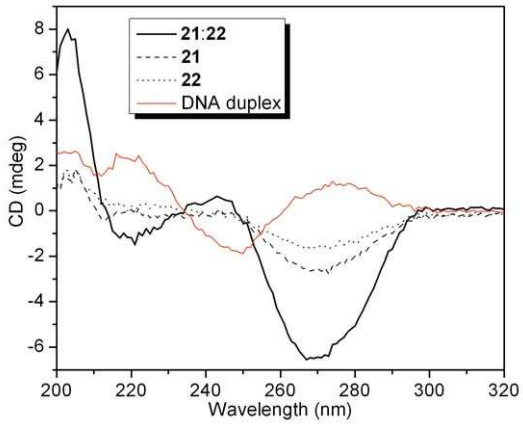


Figure 4 CD spectra of a 1:1 mixture of GNA strands **21**:**22** (4 μM each), and the individual single strands **21** (4 μM) and **22** (4 μM). A DNA duplex (4 μM each strand) with the same sequence is shown in red. Experiments were performed in 10 mM sodium phosphate (pH 7.0) with 100 mM NaCl.

IR (thin film): 1608, 1508, 1457, 1300, 1249, 1176, 1069, 1035, 830, 702 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.40 (m, 2 H), 7.29 (m, 4 H), 7.23 (m, 2 H), 7.15 (m, 1 H), 6.77 (d, *J* = 8.9 Hz, 4 H), 3.73 (s, 6 H), 3.26 (m, 1 H), 3.06 (m, 2 H), 2.71 (m, 1 H), 2.56 (dd, *J* = 2.2, 5.2 Hz, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 158.4, 144.7, 135.9, 135.8, 129.8, 128.0, 127.6, 126.6, 113.0, 86.0, 64.5, 54.9, 50.9, 44.3.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{24}H_{24}O_4Na$: 399.1572; found: 399.1581.

Compound 7[12]

A mixture of thymine (**3**; 0.63 g, 5.0 mmol) and NaH (60% in mineral oil, 42 mg, 1.06 mmol) in DMF (8 mL) was stirred at r.t. for 2 h. The tritylated glycidol **2** (1.79 g, 4.76 mmol) was added in DMF (10 mL) and the resulting mixture was heated at 110 °C for 20 h. The DMF was evaporated, the residue was extracted with EtOAc, and purified by silica gel chromatography eluting with hexanes–EtOAc–Et_3N (1:2:0.01), affording compound **7** as a colorless foam (1.22 g, 49%); $[\alpha]_D^{20}$ = –27.1 (*c* = 1, MeOH).

IR (thin film): 3444, 3199, 3061, 2933, 1681, 1607, 1510, 1464, 1302, 1249, 1177, 1034, 831, 582 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.35 (d, *J* = 8.0 Hz, 2 H), 7.23 (d, *J* = 8.5 Hz, 4 H), 7.19 (t, *J* = 7.8 Hz, 2 H), 7.12 (t, *J* = 7.1 Hz, 1 H), 6.99 (s, 1 H), 6.74 (d, *J* = 8.6 Hz, 4 H), 3.96–4.02 (m, 2 H), 3.69 (s, 6 H), 3.55 (dd, *J* = 7.3, 13.8 Hz, 1 H), 3.09 (m, 2 H), 1.72 (s, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 164.8, 158.8, 152.1, 144.9, 142.4, 136.0, 130.2, 128.3, 128.1, 127.1, 113.5, 110.1, 86.6, 69.4, 64.9, 55.4, 52.0, 12.4.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{29}H_{30}N_2O_6Na$: 525.2002; found: 525.2019.

Compound 8

A mixture of **4** (0.5 g, 4.5 mmol) and NaH (60% in mineral oil, 30 mg, 0.76 mmol) in DMF (10 mL) was stirred at r.t. for 1 h. The tritylated glycidol **2** (1.42 g, 3.77 mmol) in DMF (10 mL) was added, and the resulting mixture was heated at 110 °C for 20 h. The DMF was evaporated, the residue was extracted with EtOAc, and purified by chromatography over silica gel eluting first with hexane–EtOAc–Et_3N (1:2:0.01), and thereafter with EtOAc–Et_3N (100:1) to afford compound **8** as a colorless foam (1.04 g, 61%); $[\alpha]_D^{20}$ –33.4 (*c* = 1, MeOH).

IR (thin film): 1682, 1607, 1509, 1462, 1385, 1301, 1250, 1177, 1089, 1032, 912, 827, 728, 668, 590 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.35 (m, 2 H), 7.22 (m, 6 H), 7.16 (t, *J* = 7.2 Hz, 1 H), 7.10 (d, *J* = 7.9 Hz, 1 H), 6.77 (d, *J* = 8.8 Hz, 4 H), 5.49 (d, *J* = 7.9 Hz, 1 H), 3.99 (m, 2 H), 3.72 (s, 6 H), 3.57 (dd, *J* = 8.0, 14.7 Hz, 1 H), 3.13 (m, 2 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.7, 158.6, 151.5, 145.9, 144.3, 135.5, 129.9, 128.0, 127.9, 127.0, 113.3, 101.5, 86.5, 69.2, 64.2, 55.2, 51.5.

HRMS: *m*/*z* [M + Na$^+$] calcd for $C_{28}H_{28}N_2O_6Na$: 511.1845; found: 511.1853.

Compound 9

A mixture of **5** (2.1 g, 9.8 mmol) and NaH (60% in mineral oil, 30 mg, 1.98 mmol) in DMF (20 mL) was stirred at r.t. for 1 h. The tritylated glycidol **2** (3.29 g, 8.75 mmol) in DMF (20 mL) was added, and the resulting mixture was heated at 110 °C for 20 h. The DMF was evaporated, the residue was extracted with EtOAc, and purified by chromatography over silica gel eluting with hexanes–acetone–Et_3N (3:2:0.01) to afford compound **9** as a colorless foam (3.11 g, 59%); $[\alpha]_D^{20}$ –27.9 (*c* = 1, MeOH).

IR (thin film): 1654, 1621, 1607, 1558, 1508, 1484, 1357, 1297, 1250, 1178, 1091, 1027, 826, 796, 682, 668, 648, 594 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.85 (m, 2 H), 7.56 (d, *J* = 7.3 Hz, 1 H), 7.52 (tt, *J* = 2.1, 7.4 Hz, 1 H), 7.42 (t, *J* = 7.7 Hz, 1 H), 7.36 (m, 2 H), 7.32 (d, *J* = 7.1 Hz, 1 H), 7.20–7.26 (m, 7 H), 7.14 (tt, *J* = 1.6, 7.3 Hz, 1 H), 6.76 (d, *J* = 8.6 Hz, 4 H), 4.29 (dd, *J* = 2.9, 13.6 Hz, 1 H), 4.18 (m, 1 H), 3.75 (dd, *J* = 7.3, 13.6 Hz, 1 H), 3.71 (s, 6 H), 3.19 (dd, *J* = 5.1, 9.6 Hz, 1 H), 3.08 (dd, *J* = 5.8, 9.6 Hz, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 162.4, 158.6, 150.4, 144.5, 135.6, 135.5, 133.1, 133.0, 129.9, 128.92, 128.0, 127.9, 127.6, 126.9, 113.2, 96.5, 86.3, 68.9, 64.3, 55.16, 54.3.

HRMS: *m*/*z* [M + H$^+$] calcd for $C_{35}H_{34}N_3O_6$: 592.2448; found: 592.2454.

Compound 10

A mixture of **6** (1.3 g, 9.6 mmol) and NaH (60% in mineral oil, 78 mg, 1.95 mmol) in DMF (15 mL) was stirred at r.t. for 2 h. The tritylated glycidol **2** (3.4 g, 9.04 mmol) in DMF (25 mL) was added, and the resulting solution was heated at 105 °C for 15 h. The DMF was evaporated, the residue was extracted with EtOAc, and purified by chromatography over silica gel, first eluting with hexanes–EtOAc–Et_3N (1:2:0.01), then with EtOAc–Et_3N (100:1) affording compound **10** as a colorless foam (2.5 g, 51%); $[\alpha]_D^{20}$ = –7.8 (*c* = 1, MeOH).

IR (thin film): 3120, 1641, 1602, 1509, 1444, 1301, 1248, 1176, 1069, 1032, 829, 729 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 8.17 (s, 1 H), 7.64 (s, 1 H), 7.32 (d, *J* = 8.3 Hz, 2 H), 7.19 (m, 6 H), 7.13 (t, *J* = 7.7 Hz, 1 H), 6.73 (d, *J* = 8.9 Hz, 4 H), 5.94 (s, 2 H), 4.33 (dd, *J* = 2.4, 14.2 Hz, 1 H), 4.20 (dd, *J* = 7.0, 14.2 Hz, 1 H), 4.12 (m, 1 H), 3.71 (s, 6 H), 3.19 (dd, *J* = 5.4, 9.6 Hz, 1 H), 3.01 (dd, *J* = 6.2, 9.6 Hz, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 158.5, 155.5, 152.5, 150.0, 144.5, 141.6, 135.7, 135.6, 129.9, 128.0, 127.9, 126.9, 119.3, 113.2, 86.4, 69.4, 64.5, 55.2, 48.2.

HRMS: *m*/*z* [M + Na$^+$] calcd for $C_{29}H_{29}N_5O_4Na$: 534.2117; found: 534.2137.

Compound (*S*)-T[12]

To a solution of **7** (1.1 g, 2.2 mmol) and *N*,*N*-diisopropylethylamine (2.2 mL, 11.7 mmol) in CH_2Cl_2 (36 mL) was added 2-cyanoethyl

N,*N*-diisopropylchlorophosphoramidite (1 mL, 4.5 mmol). After 2 h, the reaction mixture was poured into sat. aq $NaHCO_3$ (35 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The organic layer was evaporated and the residue was purified by chromatography over silica gel eluting with hexanes–EtOAc–Et_3N (1:2:0.01), affording compound (*S*)-**T** as a colorless foam (1.18 g, 77%).

IR (thin film): 2965, 1685, 1607, 1508, 1463, 1364, 1300, 1250, 1178, 1033, 978, 885, 830, 704, 582 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.38–7.40 (m, 4 H), 7.27 (m, 8 H), 7.23 (m, 4 H), 7.16 (m, 2 H), 6.99 (d, *J* = 1.1 Hz, 1 H), 6.98 (d, *J* = 1.1 Hz, 1 H), 6.77 (m, 8 H), 4.16 (m, 3 H), 4.00 (dd, *J* = 4.7, 13.9 Hz, 1 H), 3.47–3.78 (m, 10 H), 3.74 (s, 6 H), 3.73 (s, 6 H), 3.24 (dd, *J* = 5.1, 9.9 Hz, 1 H), 3.19 (dd, *J* = 3.3, 9.9 Hz, 1 H), 3.14 (dd, *J* = 4.9, 10.0 Hz, 1 H), 3.09 (dd, *J* = 4.8, 10.0 Hz, 1 H), 2.53 (m, 2 H), 2.34 (t, *J* = 6.4 Hz, 2 H), 1.78 (d, *J* = 1.0 Hz, 3 H), 1.77 (d, *J* = 1.0 Hz, 3 H), 1.04–1.11 (m, 24 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 164.4, 164.4, 158.8, 151.1, 151.0, 144.9, 142.6, 142.5, 136.1, 136.00, 135.95, 130.3, 130.2, 128.4, 128.3, 128.1, 127.11, 127.09, 117.8, 117.6, 113.4, 109.9, 109.7, 86.5, 86.4, 71.4, 71.3, 70.8, 70.7, 64.4, 64.2, 58.6, 58.4, 58.4, 58.3, 55.47, 55.46, 51.91, 51.86, 51.1, 43.35, 43.45, 43.36, 24.92, 24.87, 24.82, 24.79, 24.77, 20.51, 20.45, 20.43, 20.37, 12.4.

^{31}P NMR (121 MHz, $CDCl_3$): δ = 150.08, 149.91.

HRMS: *m*/*z* [M + Na^+] calcd for $C_{38}H_{47}N_4O_7PNa$: 725.3080; found: 725.3054.

Compound (*S*)-U

To a solution of **8** (0.5 g, 1.0 mmol) and *N*,*N*-diisopropylethylamine (1.2 mL, 6.4 mmol) in CH_2Cl_2 (18 mL) was added 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.44 mL, 2 mmol). After 2 h, the reaction mixture was poured into sat. aq $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (2 × 15 mL). The organic layer was evaporated and the residue was purified by chromatography over silica gel eluting fast with hexanes–EtOAc–Et_3N (2:3:0.01), affording compound (*S*)-**U** as a colorless foam (0.46 g, 65%).

IR (thin film): 2968, 1682, 1607, 1507, 1462, 1302, 1251, 1179, 1031, 979, 826, 668, 648 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.39 (m, 4 H), 7.20–7.29 (m, 12 H), 7.16 (tt, *J* = 1.9, 7.0 Hz, 2 H), 7.12 (d, *J* = 7.9 Hz, 1 H), 7.09 (d, *J* = 7.9 Hz, 1 H), 6.77 (m, 8 H), 5.49 (d, *J* = 7.3 Hz, 1 H), 5.47 (d, *J* = 7.2 Hz, 1 H), 4.06–4.19 (m, 4 H), 3.47–3.78 (m, 10 H), 3.74 (s, 6 H), 3.73 (s, 6 H), 3.26 (dd, *J* = 5.2, 9.8 Hz, 1 H), 3.19 (dd, *J* = 3.4, 9.9 Hz, 1 H), 3.15 (dd, *J* = 5.2, 10.0 Hz, 1 H), 3.09 (dd, *J* = 4.5, 10.0 Hz, 1 H), 2.54 (m, 2 H), 2.36 (t, *J* = 7.5 Hz, 2 H), 1.05–1.11 (m, 24 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.6, 163.5, 158.6, 150.8, 150.6, 146.1, 146.0, 144.5, 144.4, 135.69, 135.65, 135.62, 135.60, 130.03, 129.95, 128.12, 128.05, 127.8, 126.9, 117.7, 117.4, 113.18, 113.15, 101.4, 101.1, 86.2, 86.1, 70.9, 70.8, 70.5, 70.4, 63.8, 63.5, 58.3, 58.2, 58.1, 58.0, 55.2, 51.5, 51.5, 50.9, 43.3, 43.20, 43.15, 43.06, 24.64, 24.61, 24.58, 20.28, 20.22, 20.18, 20.12.

^{31}P NMR (121 MHz, $CDCl_3$): δ = 150.06, 149.71.

HRMS: *m*/*z* [M + Na^+] calcd for $C_{37}H_{45}N_4O_7PNa$: 711.2924; found: 711.2943.

Compound (*S*)-C

To a solution of **9** (0.94 g, 1.6 mmol) and *N*,*N*-diisopropylethylamine (1.8 mL, 9.6 mmol) in CH_2Cl_2 (28 mL) was added 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.66 mL, 3 mmol) at r.t. After 2.5 h, the reaction mixture was poured into sat. aq $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The organic layer was evaporated and the residue was purified by chromatography over silica gel eluting fast with hexanes–acetone–Et_3N (2:1:0.01), affording compound (*S*)-**C** as a colorless foam (0.83 g, 66%).

IR (thin film): 2960, 1693, 1667, 1623, 1606, 1556, 1509, 1486, 1420, 1360, 1300, 1250, 1179, 1076, 1032, 979, 828, 705 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 8.64 (br, 2 H), 7.85 (d, *J* = 7.1 Hz, 4 H), 7.56 (m, 4 H), 7.40–7.46 (m, 8 H), 7.29 (m, 9 H), 7.20–7.25 (m, 5 H), 7.15 (m, 2 H), 6.77 (m, 8 H), 4. 25–4.37 (m, 4 H), 3.45–3.85 (m, 10 H), 3.73 (s, 6 H), 3.72 (s, 6 H), 3.35 (dd, *J* = 4.6, 10.0 Hz, 1 H), 3.21 (dd, *J* = 4.5, 9.9 Hz, 1 H), 3.16 (dd, *J* = 2.8, 10.0 Hz, 1 H), 3.09 (dd, *J* = 4.4, 10.0 Hz, 1 H), 2.62 (dt, *J* = 6.8, 16.8 Hz, 1 H), 2.51 (dt, *J* = 5.9, 16.8 Hz, 1 H), 2.37 (t, *J* = 6.5 Hz, 2 H), 1.08 (m, 24 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 162.3, 162.2, 158.5, 150.5, 150.4, 144.6, 135.8, 135.7, 135.63, 135.56, 133.1, 133.0, 130.1, 130.03, 129.99, 128.9, 128.1, 128.0, 127.8, 127.5, 126.81, 126.77, 117.8, 117.4, 113.1, 96.0, 95.8, 86.1, 86.0, 70.4, 70.3, 69.7, 69.6, 64.1, 63.8, 58.5, 58.3, 58.2, 58.1, 55.2, 53.6, 53.3, 43.3, 43.2, 43.1, 24.61, 24.55, 24.51, 20.13, 20.07, 20.01.

^{31}P NMR (121 MHz, $CDCl_3$): δ = 149.89.

HRMS: *m*/*z* [M + Na^+] calcd for $C_{44}H_{50}N_5O_7PNa$: 814.3346; found: 814.3340.

Compound 12

To compound **10** (2.15 g, 4.2 mmol) in anhyd pyridine (32 mL) was added trimethylsilyl chloride (2.2 mL, 17.3 mmol). After stirring for 2 h at r.t., the mixture was cooled to 0 °C and benzoyl chloride (0.75 mL, 6.5 mmol) was added dropwise. The mixture was allowed to warm slowly to r.t. and stirred for an additional 2 h. The reaction was stopped by the addition of H_2O (5 mL) at 0 °C with stirring, and then concd aq NH_3 (10 mL) was added after 15 min. After stirring for another 30 min, the mixture was extracted with CH_2Cl_2 (2 × 40 mL). The solvent was evaporated and the residue was purified by chromatography over silica gel eluting with hexanes–EtOAc–Et_3N (1:2:0.01), then with EtOAc–Et_3N (100:1), and finally with EtOAc–MeOH–Et_3N (25:1:0.01), affording compound **12** as a colorless foam (1.84 g, 71%).

IR (thin film): 3286 (br), 2933, 1704, 1609, 1582, 1510, 1454, 1301, 1250, 1176, 1073, 1033, 910, 828, 798, 727, 705 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 9.12 (br, 1 H), 8.68 (s, 1 H), 8.00 (d, *J* = 7.5 Hz, 2 H), 7.94 (s, 1 H), 7.57 (t, *J* = 7.3 Hz, 1 H), 7.47 (t, *J* = 7.6 Hz, 2 H), 7.37 (d, *J* = 7.6 Hz, 2 H), 7.25 (m, 6 H), 7.19 (t, *J* = 7.2 Hz, 1 H), 6.78 (d, *J* = 8.8 Hz, 4 H), 4.45 (dd, *J* = 2.4, 14.3 Hz, 1 H), 4.28 (dd, *J* = 7.2, 14.3 Hz, 1 H), 4.17 (m, 1 H), 3.75 (s, 6 H), 3.20 (dd, *J* = 5.6, 9.6 Hz, 1 H), 3.14 (dd, *J* = 5.8, 9.6 Hz, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 164.7, 158.6, 152.2, 152.0, 149.4, 144.4, 144.1, 135.5, 135.4, 133.7, 132.7, 129.9, 128.8, 127.91, 127.85, 127.0, 122.5, 113.2, 86.5, 69.3, 64.5, 55.2, 47.9.

HRMS: *m*/*z* [M + Na^+] calcd for $C_{36}H_{33}N_5O_5Na$: 638.2379; found: 638.2409.

Compound (*S*)-A

To a solution of **12** (1.20 g, 1.95 mmol) and *N*,*N*-diisopropylethylamine (2.1 mL, 11.2 mmol) in CH_2Cl_2 (33 mL) was added 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.88 mL, 3.97 mmol). After 2 h, the reaction mixture was poured into sat. aq $NaHCO_3$ (40 mL) and extracted with CH_2Cl_2 (2 × 35 mL). The organic layer was evaporated and the residue was purified by chromatography over silica gel eluting with hexanes–EtOAc–Et_3N (1:2:0.01) affording compound (*S*)-**A** as a colorless foam (1.16 g, 73%).

IR (thin film): 2966, 1700, 1608, 1509, 1456, 1302, 1250, 1179, 1034, 978, 830, 728, 582 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.04 (br, 2 H), 8.73 (s, 1 H), 8.71 (s, 1 H), 8.02 (s, 1 H), 7.98 (m, 4 H), 7.97 (s, 1 H), 7.54 (m, 2 H), 7.46 (m, 4 H), 7.39 (m, 4 H), 7.20–7.27 (m, 12 H), 7.16 (m, 2 H), 6.76 (m, 8 H), 4.53 (m, 2 H), 4.44 (m, 2 H), 4.31 (m, 2 H), 3.73 (s, 6 H), 3.72 (s, 6 H), 3.39–3.63 (m, 8 H), 3.24 (dd, *J* = 4.1, 9.8 Hz, 1 H), 3.15–3.20 (m, 2 H), 3.08 (dd, *J* = 5.9, 9.9 Hz, 1 H), 2.42 (q, *J* = 6.4 Hz, 2 H), 2.33 (t, *J* = 6.4 Hz, 2 H), 1.04 (d, *J* = 6.8 Hz, 12 H), 1.00 (d, *J* = 6.8 Hz, 6 H), 0.94 (d, *J* = 6.8 Hz, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 164.6, 164.5, 158.5, 152.5, 152.4, 149.2, 144.5, 144.2, 143.9, 135.63, 135.56, 133.8, 132.6, 130.0, 123.0, 128.81, 128.79, 128.1, 128.0, 127.8, 126.9, 126.8, 122.6, 122.5, 117.6, 117.3, 113.4, 86.7, 86.6, 71.5, 71.4, 71.3, 71.1, 64.2, 63.7, 58.1, 58.0, 57.9, 57.8, 55.2, 46.7, 46.6, 46.4, 43.2, 43.1, 43.0, 24.54, 24.51, 24.47, 24.4, 20.18, 20.12, 20.08.

^{31}P NMR (121 MHz, $CDCl_3$): δ = 150.37, 149.71.

HRMS: *m*/*z* [M + Na^+] calcd for $C_{45}H_{50}N_7O_6P$: 816.3638; found: 816.3648.

Compound 14

A mixture of **13** (2.4 g, 14.2 mmol), **1** (0.96 mL, 14.5 mmol), and K_2CO_3 (0.33 g, 2.4 mmol) in DMF (45 mL) was stirred at 90 °C for 14 h. The reaction mixture was concentrated in vacuo, dissolved in large amounts of MeOH, adsorbed onto silica gel, and then purified by chromatography over silica gel eluting with EtOAc–MeOH (10:1), and later with EtOAc–MeOH (20:3), affording compound **14** (1.4 g, 39%).

IR (KBr pellet): 3373, 3210, 2855, 1649, 1614, 1566, 1527, 1481, 1412, 1376, 1302, 1225, 1147, 1110, 1062, 1040, 999, 919, 783, 641 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.01 (s, 1 H), 6.86 (br, 2 H), 5.07 (d, *J* = 5.4 Hz, 1 H), 4.79 (t, *J* = 5.5 Hz, 1 H), 4.18 (dd, *J* = 3.4, 13.9 Hz, 1 H), 3.90 (dd, *J* = 8.6, 13.9 Hz, 1 H), 3.80 (m, 1 H), 3.40 (m, 1 H), 3.31 (m, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 159.6, 154.3, 149.1, 144.0, 123.2, 69.2, 63.5, 46.5.

Compound 15[15]

Compound **14** (5.4 g, 22.2 mmol) was stirred in 1 N HCl (195 mL) at 85 °C for 3 h and then cooled to r.t. The reaction solution was basified with concd NH_4OH to pH 9 and the precipitated product **15** was filtered off as an off-white solid (4.1 g, 82%).

IR (KBr pellet): 3130, 1693, 1641, 1542, 1480, 1401, 1234, 1192, 1075, 1042, 812, 779, 764, 696, 634 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 10.55 (s, 1 H), 7.59 (s, 1 H), 6.46 (br, 2 H), 5.04 (br, 1 H), 4.76 (br, 1 H), 4.07 (dd, *J* = 3.3, 13.6 Hz, 1 H), 3.80 (dd, *J* = 8.2, 13.5 Hz, 1 H), 3.74 (m, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 156.7, 153.5, 151.2, 138.2, 116.2, 69.7, 63.4, 46.0.

HRMS: *m*/*z* [M + Na^+] calcd for $C_8H_{12}N_5O_3$: 226.0940; found: 226.0949.

Compound 16

To a mixture of **15** (3.5 g, 15.5 mmol) in anhyd pyridine (100 mL) was added trimethylsilyl chloride (15 mL, 118 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and thereafter at r.t. for another 2 h. The mixture was cooled to 0 °C and isobutyryl chloride (12.9 mL, 77.5 mmol) was added dropwise. The mixture was allowed to warm slowly to r.t. and was stirred for an additional 4 h. The reaction was stopped by the addition of H_2O (20 mL) at 0 °C. After 15 min, concd aq NH_3 (20 mL) was added and incubated for 30 min. The reaction mixture was concentrated in vacuo. The residue was dissolved in MeOH, adsorbed onto silica gel, and purified by chromatography over silica gel eluting with EtOAc–MeOH (20:3), and then with EtOAc–MeOH (40:9), affording compound **16** (3.2 g, 70%).

IR (KBr pellet): 3462, 3198, 2933, 1696, 1617, 1562, 1480, 1410, 1323, 1253, 1199, 1164, 1113, 1056, 871, 843, 795, 746 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.04 (br, 1 H), 11.64 (br, 1 H), 7.88 (s, 1 H), 5.07 (d, *J* = 5.4 Hz, 1 H), 4.79 (t, *J* = 5.4 Hz, 1 H), 4.20 (dd, *J* = 3.5, 13.9 Hz, 1 H), 3.92 (dd, *J* = 8.7, 13.9 Hz, 1 H), 3.80 (m, 1 H), 3.40 (m, 1 H), 3.32 (m, 1 H), 2.77 (sept, *J* = 6.8 Hz, 1 H), 1.11 (d, *J* = 6.8 Hz, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 180.1, 154.9, 148.8, 147.7, 140.6, 119.9, 69.6, 63.6, 46.6, 34.7, 18.9.

HRMS: *m*/*z* [M + Na^+] calcd for $C_{12}H_{17}N_5O_4$: 296.1359; found: 296.1358.

Compound 17

To a solution of compound **16** (2.5 g, 8.5 mmol) in anhyd pyridine (37 mL) was added DMTrCl (3.5 g, 10.3 mmol) at r.t. After 3 h, the reaction mixture was concentrated in vacuo. The residue was purified by chromatography over silica gel first eluting with hexanes–EtOAc–Et_3N (1:2:0.01), then with EtOAc–Et_3N (100:1), and finally with EtOAc–MeOH–Et_3N (25:1:0.01) to afford compound **17** (3.8 g, 75%) as a colorless foam; $[\alpha]_D^{20}$ = –4.1 (*c* = 1, MeOH).

IR (thin film): 3153, 1675, 1606, 1563, 1510, 1406, 1250, 1176, 1034, 830 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.49 (s, 1 H), 7.39 (d, *J* = 7.6 Hz, 2 H), 7.26 (dd, *J* = 2.3, 8.9 Hz, 4 H), 7.20 (t, *J* = 7.6 Hz, 2 H), 7.13 (t, *J* = 7.2 Hz, 1 H), 6.74 (d, *J* = 8.8 Hz, 4 H), 4.39 (m, 1 H), 4.22 (dd, *J* = 2.2, 14 Hz, 1 H), 3.93 (dd, *J* = 8.6, 14 Hz, 1 H), 3.71 (s, 6 H), 3.24 (dd, *J* = 4.4, 9.4 Hz, 1 H), 3.12 (dd, *J* = 6.3, 9.4 Hz, 1 H), 2.62 (sept, *J* = 6.9 Hz, 1 H), 1.18 (m, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 179.0, 158.5, 154.9, 148.3, 147.3, 144.7, 140.7, 135.8, 135.8, 130.0, 128.1, 127.8, 126.8, 119.7, 113.2, 86.2, 68.3, 65.1, 55.2, 48.6, 36.3, 19.0, 18.8.

HRMS: *m*/*z* [M + H^+] calcd for $C_{33}H_{36}N_5O_6$: 598.2666; found: 598.2687.

Compound (*S*)-G

To a solution of **17** (0.5 g, 0.84 mmol) and *N*,*N*-diisopropylethylamine (0.22 mL, 1.2 mmol) in THF (7.5 mL) was added 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.2 mL, 0.9 mmol) dropwise at 0 °C. The reaction mixture was allowed to warm to r.t. and was continuously stirred overnight. The reaction mixture was then poured into sat. aq $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (2 × 10 mL). The organic layer was concentrated in vacuo, the residue was dissolved in a small amount of CH_2Cl_2 (5 mL), and the solution was added slowly to rapidly stirred pentane (350 mL). The white precipitate was collected by filtration, re-dissolved in CH_2Cl_2 (20 mL), and pentane was added to this solution until it started to become cloudy. The solution was subsequently stored at –20 °C for 2 h, during which a yellow oil formed. The remaining clear solution was decanted and evaporated to afford compound (*S*)-**G** as a light yellow foam (0.3 g, 45%).

IR (thin film): 2968, 1682, 1608, 1558, 1508, 1464, 1407, 1364, 1300, 1251, 1200, 1179, 1156, 1079, 1032, 978, 831, 790, 703 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.48 (s, 1 H), 7.43 (s, 1 H), 7.36 (m, 4 H), 7.11–7.23 (m, 14 H), 6.72 (m, 8 H), 4.45 (m, 1 H), 4.04–4.29 (m, 5 H), 3.40–3.80 (m, 10 H), 3.71 (s, 6 H), 3.70 (s, 6 H), 2.97–3.02 (m, 4 H), 2.49–2.57 (m, 4 H), 2.39 (t, *J* = 5.9 Hz, 1 H), 0.99–1.21 (m, 36 H)

^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.7, 178.5, 158.5, 155.61, 155.56, 148.7, 148.5, 147.2, 147.0, 144.64, 144.55, 140.0, 139.7, 135.7, 135.62, 135.56, 129.95, 129.85, 128.0, 127.9, 127.8, 127.7, 126.8, 120.9, 120.7, 118.1, 117.6, 113.1, 113.1, 113.0, 113.0, 86.3,

86.0, 71.2, 71.0, 63.6, 65.4, 57.4, 57.3, 57.2, 57.2, 55.2, 45.6, 43.1, 43.1, 43.03, 42.98, 36.2, 36.0, 24.6, 24.53, 24.49, 24.4, 20.3, 20.2, 18.8.

^{31}P NMR (121 MHz, $CDCl_3$): δ = 149.03, 148.98.

HRMS: *m*/*z* [M + Na^+] calcd for $C_{42}H_{52}N_7O_6PNa$: 820.3564; found: 820.3589.

Synthesis of the Solid Support

To compounds **7**–**9**, **12**, or **17** (0.5 mmol) were added succinic anhydride (0.75 mmol) and Et_3N (1.5 mmol) in anhyd CH_2Cl_2 (5 mL). The mixture was stirred at r.t. for 4 h. Then, the solution was washed with 4% citric acid, extracted with CH_2Cl_2 (2 × 5 mL), dried over $MgSO_4$, evaporated and used without further purification for the following coupling to the solid support. For this, a mixture of long chain alkylamine controlled pore glass (LCAA-CPG, nominal pore size 500 Å, mesh size 80–120; 500 mg), 1-hydroxybenzotriazole (0.015 mmol, 2 mg), 1,3-diisopropylcarbodiimide (0.15 mmol, 24 μL), pyridine (0.1 mL), and anhyd MeCN (2 mL) were shaken in a glass vial at r.t. for 30 min. Next, the succinates of **7**–**9**, **12**, or **17** (0.055 mmol) were added and the mixtures were shaken overnight. The solid support was filtered off, washed with MeOH and $CHCl_3$, and dried. Unreacted amino groups were acetylated with THF–lutidine–Ac_2O (8:1:1, 5 mL) and 10% methylimidazole (5 mL) in THF for 1 h, followed by washing with MeOH and $CHCl_3$. Trityl analysis yielded a loading of 65 μmol/g (for **7**), 69 μmol/g (for **8**), 68 μmol/g (for **9**), 71 μmol/g (for **12**), and 67 μmol/g (for **17**).

Oligonucleotide Synthesis and Purification

All oligonucleotides were prepared on an Expedite Nucleic Acid Synthesizer from ABI. Oligonucleotides were synthesized on 0.2 μmol or 0.5 μmol scales. A standard protocol for 2-cyanoethyl phosphoramidites (0.05 M) was used, except that the coupling of the phosphoramidites was extended to 3 min. After the trityl-on synthesis, the resin was incubated with concd aq NH_3 at 55 °C for 12 h and then evaporated. The tritylated oligonucleotides were purified by C18 reverse phase HPLC with 0.05 M aq TEAA and MeCN as the eluent (gradient: 5–80% MeCN in 20 min, Varian Dynamax 250 × 10 mm, Microsorb 300-10, C18). The oligonucleotides were then detritylated with 80% AcOH for 20 min, precipitated with *i*-PrOH after addition of NaOAc, and again purified by HPLC. For this purification step, best purities were obtained with a Waters XTerra column (MS C18, 4.6 × 50 mm, 2.5 μm) at 55–60 °C with 0.05 M aq TEAA and MeCN as the eluent (gradient: 3–12% MeCN in 40 min, or 2–11% in 40 min). The identities of all oligonucleotides were confirmed by MALDI–TOF MS.

21

MS (MALDI–TOF): *m*/*z* calcd for $C_{118}H_{157}N_{50}O_{76}P_{14}$: 3926; found: 3925.

22

MS (MALDI–TOF): *m*/*z* calcd for $C_{118}H_{154}N_{59}O_{70}P_{14}$: 3953; found: 3954.

23

MS (MALDI–TOF): *m*/*z* calcd for $C_{120}H_{159}N_{48}O_{76}P_{14}$: 3924; found: 3927.

24

MS (MALDI–TOF): *m*/*z* calcd for $C_{120}H_{156}N_{57}O_{70}P_{14}$: 3951; found: 3951.

25

MS (MALDI–TOF): *m*/*z* calcd for $C_{111}H_{141}N_{48}O_{76}P_{14}$: 3797; found: 3799.

26

MS (MALDI–TOF): *m*/*z* calcd for $C_{114}H_{144}N_{57}O_{70}P_{14}$: 3866; found: 3868.

27

MS (MALDI–TOF): *m*/*z* calcd for $C_{120}H_{150}N_{75}O_{58}P_{14}$: 4005; found: 4006.

28

MS (MALDI–TOF): *m*/*z* calcd for $C_{120}H_{165}N_{30}O_{88}P_{14}$: 3869; found: 3869.

DNA strands were purchased from Sigma Genosys (HPLC purified). Oligonucleotide concentrations were determined by measuring the UV absorbance of aqueous solutions. Extinction coefficients of oligonucleotides were calculated from increment values of the individual nucleobases.[28]

Thermal Denaturation

The melting studies were carried out in 1 cm path length quartz cells (total volume 325 μL; 200 μL sample solutions were covered by mineral oil) on a Beckman 800 UV-VIS spectrophotometer equipped with a thermo-programmer. Melting curves were monitored at 260 nm with a heating rate of 1 °C/min. Melting temperatures were calculated from the first derivatives of the heating curves. Experiments were performed in duplicate and mean values were taken.

Circular Dichroism

CD experiments were performed on an Aviv 62A DS spectrometer. A 1 cm path length quartz cuvette was used and the solutions were scanned from 200–320 nm at 25 °C with a time constant of 1 s and a wavelength step size of 1.0 nm. Each measurement was repeated 5 times and the average was taken.

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