Synthesis of Novel Phosphoramidite Building Blocks from Pentaerythritol

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Abstract: The synthesis of four types of phosphoramidites containing various protecting groups (DMTr, TBDMS, Lev) has been achieved starting from pentaerythritol. The protecting groups can be deprotected selectively in an orthogonal manner.

Key words: protecting groups, chemoselectivity, alcohols, DNA, oligonucleotides

The controllable formation of nanoscale architectures in solution and on solid supports is a central requirement for a range of activities in the emerging field of nanotechnology.[1] DNA molecules are well suited for these purposes because of their unique molecular recognition features. Linear DNA chains can be made to assemble into a range of non-linear structures by inducing branching of double helices through the incorporation of non-complementary sequences in the component strands.[2] More-versatile building blocks can be designed in which DNA chains are linked through components that take no part in double helix formation. For example, dendrimers[3] with arms terminating in oligodeoxyribonucleotides (ODNs) of the same[4] or different[5] sequences have been used to build cages, cryptands, tubes, nets, scaffolds and other more-complex three-dimensional (3-D) structures. For the construction of programmed nanostructures, there has been an increase in the importance of branched ODNs, especially heterosequence-containing branched ODNs, as vertices of nanostructures such as tetrahedra and cubes. For this purpose, both triply and quadruply branched ODNs have been synthesized using phosphoramidite monomers.[6] In this paper, we report not only the design and synthesis of novel symmetrical and unsymmetrical building blocks that are useful for constructing quadruply branched ODNs, but also the conditions for removing their several different protecting groups.

We designed the phosphoramidite molecules[7] **1**–**4** as branching points for ODNs (Figure 1). For the synthesis of **1**–**4**, we choose three protecting groups [dimethoxytrityl (DMTr), *tert*-butyldimethylsilyl (TBDMS), and levulinyl (Lev)] for the hydroxyl groups of pentaerythritol (**5**). Because it can be easily and virtually quantitatively deprotected by trichloroacetic acid, the DMTr group is used generally in ODN syntheses using the phosphoramidite method.[8] The TBDMS protecting group has been used to protect the 2′-hydroxyl group of RNA phosphoramidite, and here we have adapted it because we required a moiety having deprotection conditions that are different from those of the DMTr group. Finally, we selected the Lev group because it has been used, in conjunction with the DMTr group, for preparing asymmetric triply branched ODNs.[9] Based on these prior results, we designed and synthesized various phosphoramidite monomers using combinations – either the same or different – of these three protecting groups.

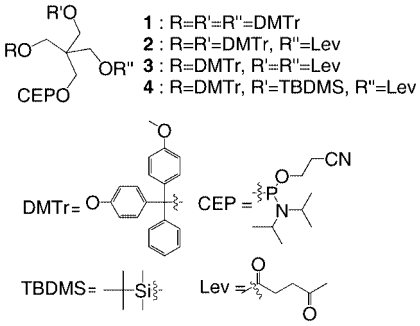

Figure 1 Phosphoramidite monomers.

The four pentaerythritol-derived phosphoramidite monomers were synthesized as shown in Scheme 1. Treatment of pentaerythritol (**5**) with DMTr-Cl (2.5 equiv) in the presence of DMAP in pyridine gave the mono-, bis-, and tris-O-DMTr-protected products in 7%, 12%, and 70% yields, respectively. The tris(DMTr)-protected compound was reacted with chloro-(2-cyanoethyl)-*N*,*N*-diisopropylaminophosphine (CEP-Cl) in the presence of DIPEA in THF to prepare the symmetrical phosphoramidite building block **1** in 35% yield. TBDMS-Cl was reacted with the bis(DMTr)-protected compound **7** to afford the bis(DMTr)-mono-TBDMS-protected compound. This compound could not, however, be made to react with CEP-Cl because of steric hindrance by the bulky protecting groups. For this reason, we chose to replace the TBDMS group with the Lev group, and with this change we were able to synthesize the desired phosphoramidite product **2** in 77% yield. When the mono-DMTr-protected pentaerythritol was reacted with levulinic acid (1.2 equiv), both the mono- and bis(Lev)-protected products were obtained. The bis(Lev)-protected compound **10** was converted to the phosphoramidite **3** in 61% yield. The mono-Lev-protected compound **11** was protected with the TBDMS group and then converted to the phosphoramidite **4** in 50% yield.

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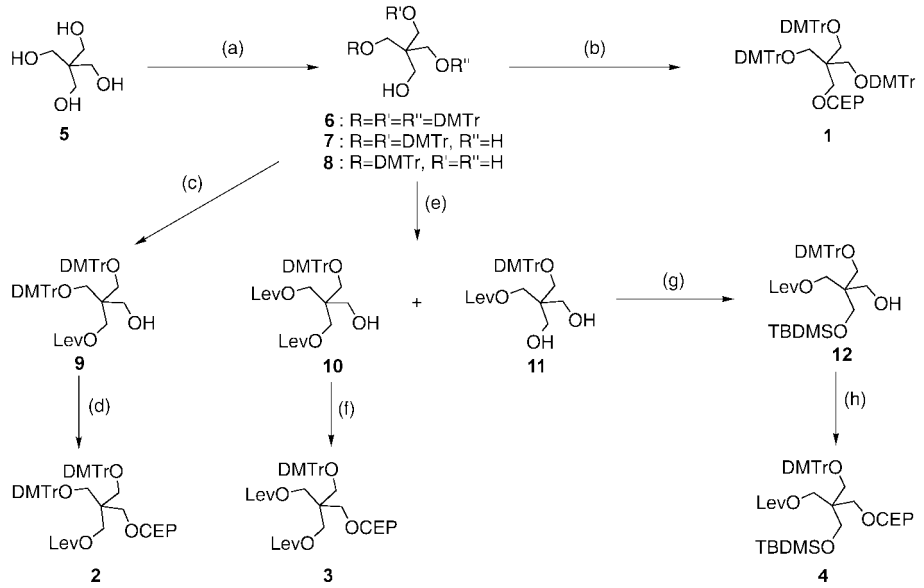
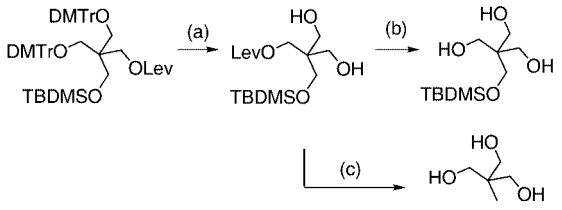


Scheme 1 Reagents and conditions: (a) DMTr-Cl, DMAP, Py; (b) **6**, CEP-Cl, DIPEA, THF; (c) **7**, levulinic acid, EDC, DMAP, CH_2Cl_2; (d) **9**, CEP-Cl, DIPEA, THF; (e) **8**, levulinic acid, EDC, DMAP, CH_2Cl_2; (f) **10**, CEP-Cl, DIPEA, THF; (g) **11**, TBDMS-Cl, Et_3N, CH_2Cl_2; (h) **12**, CEP-Cl, DIPEA, THF.

The DMTr, Lev, and TBDMS moieties are virtually orthogonal protecting groups; i.e., each one is susceptible to specific deprotection conditions. The DMTr group can be cleanly removed using trichloroacetic acid or $ZnBr_2$ in CH_2Cl_2 solution; the Lev and TBDMS groups are quite stable under either of these conditions. The Lev protecting group can be deprotected selectively by using buffered hydrazine hydrate.[6c,d,9] The TBDMS protecting group can be deprotected by using tetrabutylammonium fluoride; unfortunately, under this deprotection condition, the Lev group is also cleaved (Scheme 2). On the basis of these properties, we propose that branched ODNs containing four different base sequences should be amenable to synthesis based on the order of reactions shown in Figure 2. Additionally, it should be possible to synthesize symmetrical, quadruply branched ODNs[10] by using phosphoramidites **1**–**3**.



Scheme 2 Deprotection conditions: (a) Trichloroacetic acid, CH_2Cl_2; (b) $H_2NNH_2 \cdot H_2O$, Py, acetic acid; (c) TBAF, THF.

In summary, we have synthesized four different, non-nucleoside phosphoramidite monomers based on pentaerythritol. These compounds should be amenable to the preparation of a versatile range of compounds, such as quadruply branched ODNs, dendrimers, and materials for the construction of programmed nanostructures. Presently, we are using these compounds to prepare branched ODNs having homo- and hetero-sequences.

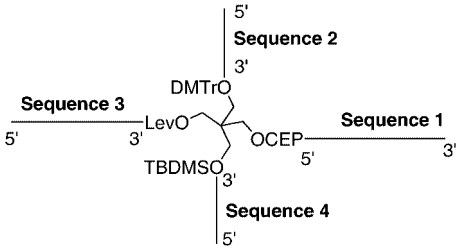


Figure 2 Order of reactions for the synthesis of quadruply branched ODNs.

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(7) Reaction conditions and selected spectroscopic data. **1**: CEP-Cl (297 μL, 1.34 mmol) was added to a solution of **6** (560 mg, 0.537 mmol) and DIPEA (187 μL, 1.074 mmol) in THF (6 mL). The reaction mixture was stirred at room temperature for 80 min and then 5% $NaHCO_3$ solution and EtOAc were added. The organic layer was separated, dried over $MgSO_4$, and concentrated under reduced pressure. Purification by flash chromatography provided **1** as a colorless oil (236 mg, 0.190 mmol, 35%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.27–7.14 (m, 27 H), 6.72–6.68 (m, 12 H), 4.11 (q, *J* = 6.7 Hz, 2 H), 3.75 (s, 18 H), 3.39–3.23 (m, 8 H), 2.23 (t, *J* = 6.3 Hz, 2 H), 2.03 (s, 2 H), 1.31–1.22 (m, 4 H), 1.09 (d, *J* = 6.7 Hz, 6 H), 0.95 (d, *J* = 6.7 Hz, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 157.7, 144.7, 135.8, 129.7, 127.8, 127.1, 126.0, 112.5, 85.1, 62.3, 57.7, 54.7, 42.5, 24.1, 24.0, 13.7; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 148.9; HR-ESIMS (*m/z*): $[M + Na]^+$ calcd for $C_{77}H_{83}N_2O_{11}PNa$, 1243.5852; found, 1243.5807. **2**: Reaction of CEP-Cl (166 μL, 0.74 mmol), **9** (319.7 mg, 0.37 mmol), and DIPEA (260 μL, 1.48 mmol) in THF (4 mL) for 90 min, followed by the workup described above, provided **2** as a yellow oil (302.8 mg, 0.29 mmol, 77%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.24 (d, *J* = 7.2 Hz, 4 H), 7.17–7.09 (m, 14 H), 6.67 (d, *J* = 8.4 Hz, 8 H), 4.02 (q, *J* = 10.7 Hz, 2 H), 3.68 (s, 12 H), 3.66–3.37 (m, 6 H), 3.17–3.14 (m, 4 H), 2.50 (t, *J* = 7.0 Hz, 2 H), 2.35 (t, *J* = 6.3 Hz, 2 H), 2.28 (t, *J* = 6.7 Hz, 2 H), 2.05 (s, 3 H), 1.21 (t, *J* = 5.7 Hz, 4 H), 1.05 (d, *J* = 6.7 Hz, 6 H), 0.94 (d, *J* = 6.7 Hz, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 172.5, 158.8, 145.2, 136.2, 130.5, 128.4, 127.9, 126.8, 117.9, 113.2, 86.0, 61.6, 60.6, 55.4, 43.3, 43.1, 38.0, 30.0, 28.0, 24.8, 24.7, 14.4; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 150.2; HR-FABMS (*m/z*): $[M + H]^+$ calcd for $C_{61}H_{72}N_2O_{11}P$, 1039.4874; found, 1039.4877. **3**: Reaction of CEP-Cl (74 μL, 0.33 mmol), **10** (141 mg, 0.222 mmol), and DIPEA (77 μL, 0.445 mmol) in THF (6 mL) for 30 min, followed by the workup described above, provided **3** as a yellow oil (114 mg, 0.135 mmol, 61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.38 (m, 2 H), 7.29–7.26 (m, 7 H), 6.82 (d, *J* = 8.7 Hz, 4 H), 4.17–4.12 (m, 4 H), 3.78 (s, 6 H), 3.73–3.65 (m, 2 H), 3.61–3.51 (m, 2 H), 3.15 (s, 2 H), 2.68 (t, *J* = 6.5 Hz, 4 H), 2.56 (t, *J* = 6.3 Hz, 2 H), 2.48 (t, *J* = 6.5 Hz, 4 H), 2.16 (s, 6 H), 1.25 (d, *J* = 6.7 Hz, 2 H), 1.16 (d, *J* = 6.7 Hz, 6 H), 1.10 (d, *J* = 6.7 Hz, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 205.8, 171.8, 158.0, 158.0, 144.2, 135.3, 135.0, 129.7, 127.7, 127.4, 127.3, 126.3, 117.2, 112.6, 112.5, 85.8, 85.5, 62.6, 61.6, 61.4, 60.2, 57.9, 57.7, 54.7, 46.9, 43.5, 42.7, 42.5, 37.3, 30.5, 29.3, 27.3, 24.2, 24.1, 22.1, 20.7, 19.9, 19.8; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 150.7; HR-FABMS (*m/z*): $[M + Na]^+$ calcd for $C_{45}H_{59}N_2O_{11}PNa$, 857.3754; found, 857.3759. **4**: Reaction of CEP-Cl (114 μL, 0.518 mmol), **12** (134.8 mg, 0.207 mmol), and DIPEA (144 μL, 0.828 mmol) in THF (4.2 mL) for 90 min, followed by the workup described above, provided **4** as a yellow oil (86.2 mg, 0.101 mmol, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.41–7.38 (m, 2 H), 7.29–7.17 (m, 7 H), 6.79 (d, *J* = 8.9 Hz, 4 H), 4.11–4.09 (m, 2 H), 3.76 (s, 6 H), 3.70–3.52 (m, 8 H), 3.12 (s, 2 H), 2.66–2.64 (m, 2 H), 2.53–2.45 (m, 4 H), 2.15 (s, 3 H), 1.14 (d, *J* = 6.8 Hz, 6 H), 1.08 (dd, J_1 = 6.7, J_2 = 1.4 Hz, 6 H), 0.08 (d, *J* = 1.1 Hz, 9 H), –0.03 (d, *J* = 2.4 Hz, 6 H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 205.9, 171.9, 157.9, 144.6, 135.7, 129.7, 127.2, 126.1, 117.2, 112.5, 85.3, 63.2, 61.0, 60.4, 57.7, 54,7, 45.0, 42.6, 42.5, 37.4, 29.4, 27.3, 25.3, 24.2, 24.1, 19.9, 19.8, 17.7, –6.12; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 150.3, 150.1; HR-FABMS (*m/z*): $[M + Na]^+$ calcd for $C_{46}H_{67}N_2O_9PSiNa$, 873.4251; found, 873.4252.
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