The Synthesis of Modified Achiral Internucleoside Linkages: -NHCH2CH2- Linked Oligonucleosides

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Abstract: A method for the synthesis of oligonucleosides uniformly linked by the NHCH2CH2 moiety is described. Syntheses of heterodimers and a thymidylate trimer along with relevant T_m and nuclease resistance data are described.

Analogs of antisense oligodeoxyribonucleotides are of interest as potential antiviral, antibacterial and anti-cancer agents.[1] To overcome the enzymatic lability and drug delivery limitations of natural phosphate-linked antisense oligomers, various oligonucleotide analogs have been evaluated.[2] In order for such compositions to be useful, they must possess good cell penetration properties, be reasonably resistant to degradation by nucleases, maintain sequence specific hybridization to target nucleic acids and be readily accessible by chemical synthesis.

We have explored the -NHCH2CH2- internucleoside linkage. Although linkages consisting of heteroatoms and carbon have been reported,[2] most studies have been limited to thymidine dimers. Adequate physicochemical examination of such linkages requires the synthesis of heterodimers and

Scheme 1[a,b] General Scheme for synthesis of NHCH2CH2-linked Oligonucleosides
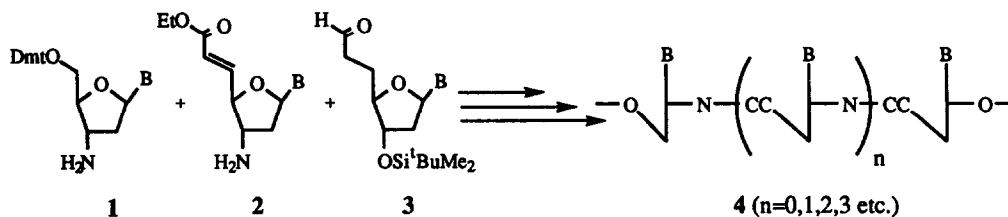
[a] **a**: B=T, **b**: B=A^Bz, **c**: B=C^Bz, **d**: B=G^iB; [b] Bz=Benzoyl, iB=isoButyryl and Dmt=Dimethoxytrityl

uniformly linked long oligomers. We describe the synthesis of heterodinucleosides containing the -NHCH$_2$CH$_2$- (henceforth referred as NCC) linkage. A strategy is also presented for the synthesis of uniformly linked oligonucleosides 4 (Scheme 1). The strategy for oligonucleosides 4 relies upon three key intermediates, a 5'-end synthon 1, a central bi-functional synthon 2, which can be repetitively coupled, and a 3'-end synthon 3. Thus reductive coupling of bifunctional nucleoside 2 with 7'-aldehyde 3 provides a 7'-functionalized dimer. This dimer, after synthetic elaboration to a 7'-aldehyde can either be coupled to the 5'-end synthon 1 to give a trimer or the chain extension cycle may be continued through repeated couplings with synthon 2 to prepare long chain oligomers 4 uniformly linked by the NCC backbone.

**Scheme 2**[a,b] Synthesis of 7'-aldehydes 3 for all bases
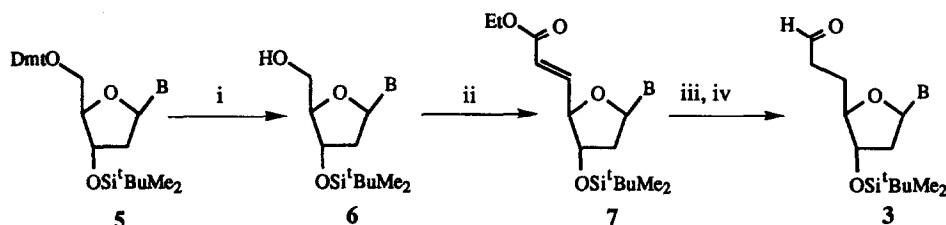
[a] a: B=T, b: B=A^{Bz}, c: B=C^{Bz}, d: B=G^{iB} [b]Reagents and conditions i) ZnBr$_2$, CH$_3$NO$_2$, 0 °C, 6h; 85-95%
ii) (COCl)$_2$, DMSO, Et$_3$N, -78 °C; Ph$_3$P=CHCO$_2$Et for 7a ,7b & 7c, 70-80%; Dess Martin periodinane;
NaH, (EtO)$_2$P(O)CH$_2$CO$_2$Et for 7d, 75% iii) H$_2$/Pd-C, 100% iv) i-Bu$_2$AlH, -78 °C, 70%.

The 3'-amines 1 are readily prepared from published procedures.[3] The 5'-hydroxy derivatives 6 were prepared in 1-100g scale by the zinc bromide-nitromethane deprotection[4] procedure in 85-95% yield. The 7'-aldehydes 3b and 3c were synthesized by 5'-homologation via Swern oxidation/Wittig condensation[5] followed by reduction of the 5',6' double bond and Dibal-H reaction (Scheme 2). The Swern/Wittig protocol failed to provide satisfactory yields of the guanosine derivative 7d. However the synthesis of this compound was accomplished by Dess-Martin oxidation[6] of 6d for the preparation of 5'-aldehyde, which was isolated and then subjected to Horner-Emmons reaction with triethylphosphonoacetate to give 7d in 75% isolated yield. No epimerization at the C-4' atom in 7b, 7c or 7d was seen under the conditions employed. The 7'-aldehydes 3b, 3c and 3d were reductively coupled (NaCNBH$_3$, pH 5.5, aq. ethanol) with amino-thymidine derivative 1a to give T-NCC-A^{Bz}, T-NCC-C^{Bz} and T-NCC-G^{iB} heterodimers in 60-65% yield. An additional heterodimer, A^{Bz}-NCC-T, was synthesized by similar reductive coupling between 1b and 3a. The internucleoside amino group was protected with the trifluoroacetyl group [(CF$_3$CO)$_2$O, Et$_3$N, CH$_2$Cl$_2$] in preparation for automated DNA synthesis.[7] The A^{Bz}-NCC-T dimer was incorporated into several oligodeoxyribo-nucleotide sequences with a mean coupling yield of 96.4%. The particular sequence, AGGTGT[A-ncc-T]CTCC[A-ncc-T]G, demonstrated nearly complete stability[8] towards

digestion by 3'-exonucleases.[9] Similar incorporation of the T-NCC-T dimer in a T_{11} sequence led to a drop of 3 °C in the melting temperature with complementary dA_{11} sequence. Such drops in T_m for hybridization to DNA are in keeping with literature precedents for similar linkages.[2g,10]

The steps in the synthesis of the protected trimer analog 11 are described in Scheme 3. Thus, readily available azidothymidine (AZT) was subjected to Swern oxidation followed by Wittig condensation to give the 7'-ester 8 in 76% yield after chromatographic purification. Reduction of the azido group by triphenyl-phosphine gave the bifunctional nucleoside 2a in 90% yield. Reductive amination with 7'-aldehyde 3a gave 5'-carbon functionalized dimer 9 in 60% yield after chromatography. Catalytic hydrogenation

Scheme 3[a] Synthesis of bifunctional intermediate 2 and thymidylate trimer analog 11
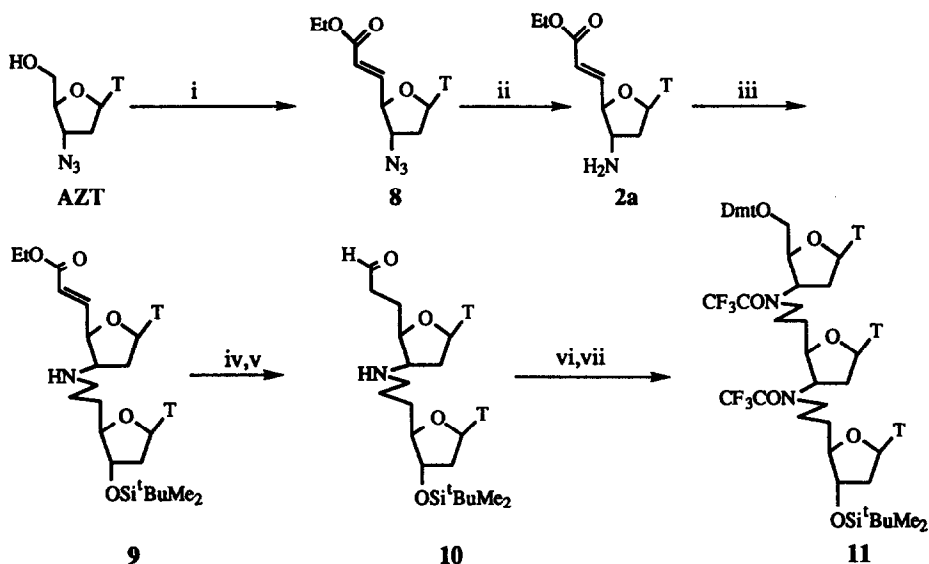
[a] i) (COCl)$_2$, DMSO, Et$_3$N, -78 °C; Ph$_3$P=CHCO$_2$Et, 76% ii) Ph$_3$P, THF-H$_2$O, 90% iii) 3a, NaCNBH$_3$, EtOH,

pH 5.5, 60% iv) H$_2$/Pd-C, MeOH v) i-Bu$_2$AlH, 60% vi) 1a, NaCNBH$_3$, EtOH, pH 5.5 vii) (CF$_3$CO)$_2$O, Et$_3$N, 50%.

followed by reaction with diisobutyl aluminium hydride at -78 °C gave the amino-aldehyde 10 in 60% yield. Reductive amination with an excess of 5'-synthon 1a followed by reaction with trifluoroacetic anhydride gave the fully protected trimer 11 in good overall yield. This synthesis provides short and convergent methodology for the preparation of analogous tetra, penta and longer oligomers of any sequence bridged uniformly with the NCC internucleoside linkage.[11] In addition, this strategy carries the potential for future development of an automated solid-support based synthesis.

In summary, a strategy for synthesis of oligodeoxynucleosides uniformly substituted by the NCC internucleoside backbone has been developed and exemplified by the synthesis of a trimer. Syntheses of various heterodimers containing the NCC internucleoside linker have been achieved and utility as potential antisense agents has been demonstrated.

References and Notes

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8. 95% of parent oligomer (minus the 3'-terminal G) remained undigested at 120 min as measured in a HPLC assay using a Gene Pak Fax (Waters) column eluting with phosphate buffer (0.05-0.5 M NaCl).
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11. Data for representative compounds. 3b: white foam; R_f 0.53 (silica, 4:1 EtOAc:hexane); ^{1}H NMR (CDCl$_3$, 300 MHz) δ 9.68 (s, 1 H), 9.44 (s, 1 H), 8.66 (s, 1 H), 8.04 (s 1 H), 7.94 (d, J=7 Hz, 2 H), 7.51-7.39 (m,3 H), 6.29 (t, J=3 Hz, 1 H), 4.37 (m, 1 H), 3.86 (m, 1 H), 2.89 (m, 1 H), 2.56-2.50 (m, 2 H), 2.40-2.32 (m, 1 H), 2.04-1.85 (m, 2 H), 0.85 (s, 9 H), 0.05 (s, 3 H), 0.04 (s, 3 H). ^{13}C NMR (CDCl$_3$, 75 MHz). δ 165.7, 153.1, 152.1, 150.4, 142.6, 134.2, 133.3, 129.3, 128.5, 122.2, 86.6, 84.9, 75.4, 40.4, 40.2, 25.9, 25.8, 18.1, -4.7. FAB-MS: (M+H)$^+$ = 497. 2a: R_f 0.3 (5% satd. NH$_3$/MeOH in EtOAc) ^{1}H NMR (CD$_3$OD, 300 MHz) δ 7.17 (s, 1 H), 6.84 (dd, J=15.5 Hz, 5.5 Hz, 1 H), 5.96 (dd, J=5 Hz, 3 Hz, 1 H), 5.72 (dd, J=15.5 Hz, 5.5 Hz, 1 H) 3.95 (q, J=7 Hz, 2 H), 3.2 (m, 1 H), 2.2-1.9 (m, 3 H), 1.66 (s, 3 H), 1.04 (t, J=7 Hz, 3 H). FAB-MS: (M+H)$^+$ = 310. 11: White foam; R_f 0.55 (5% satd. NH$_3$/MeOH in EtOAc) ^{1}H NMR (CDCl$_3$ 300 MHz): δ 7.55 (s, 1 H; 5' thymine 5-H), 7.10 (s, 1 H; thymine 5-H), 7.02 (s, 1 H; thymine 5-H), 6.38 (t, , J=6 Hz,1 H; 5'-ribose 1'-H),6.10 (t, J=6 Hz, 1 H; ribose 1'-H), 5.95 (t, J=6 Hz, 1 H; ribose 1'-H), 1.96 (s, 3 H; thymine 5-Me), 1.90(s, 3 H; thymine 5-Me), 1.6 (s, 3 H; 5' thymine-5-Me), 0.85(s, 9 H; ^{t}BuSi), 0.05 (S, 6 H; si-Me2). FAB-MS:(M+H)$^+$ = 1353; (M-H)$^-$ = 1351.