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SYNTHESIS OF PROTECTED GUANIDINIUM LINKED DINUCLEOSIDE INCORPORABLE INTO AN OLIGONUCLEOTIDE USING SOLID PHASE DNA METHODOLOGY

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Abstract: The synthesis of novel fully protected guanidinium linked dinucleoside for incorporation into oligonucleotide using solid-phase DNA synthesis methodology was developed. The three different protecting groups selected allow different deprotection conditions. © 1998 Elsevier Science Ltd. All rights reserved.

The use of antisense oligodeoxyribonucleotides (ODNs) to regulate gene products requires the development of modified ODNs possessing enhanced cellular uptake, nuclease resistance, and sequence specific hybridization to complementary RNAs. This has resulted in the synthesis of numerous DNA structural analogues with modified heterocycle, sugar, and phosphodiester backbone moieties.[1-3] Substantial progress has been made towards successful backbone modifications using phosphorus and non-phosphorus groups.[4,5] A number of modifications or replacements of phosphodiester linkages such as 2′-fluoro-N3′-P5′ phosphoramidates,[6] 3′-thioformacetals,[7,8] 2′-O-Me methylene(methylimino),[9] 2′-O-Me amide,[10] 2′-O-methylribonucleoside methylphosphonate,[11] and PNA[12] have been shown to complement with DNA and RNA with similar or higher stability while maintaining the sequence specificity. Except for the 2′-fluoro-N3′-P5′ phosphoramidates, these analogues are neutral and thus eliminate the electrostatic repulsion present in natural oligonucleotide complexes. An alternative approach, in which anionic phosphodiester groups are replaced by cationic linkages[13-17] or oligonucleotides conjugated with positively charged groups, such as zwitterionic DNA analogues,[18-20] show increased binding of these ODNs with complementary DNA or RNA. Conceptually, replacement of anionic phosphodiester linkage by neutral or positively charged dephosphono linkages can modulate the net charge of antisense ODN complex and thereby may enhance its antisense properties.[4]

Our ongoing research in this area is focused on the development of deoxynucleic guanidine (DNG) in which the negatively charged phosphodiester backbone of DNA is replaced by positively charged, achiral guanidinium linkage[15-17] to provide very stable complexes.[21,22] In this paper we propose to synthesize mixed backbone ODNs with guanidinium linkages at various places in otherwise DNA and RNA structures. This would allow a positive point charges to be inserted into otherwise negatively charged ODNs leading to possible increased binding ability. Also ODNs capped with DNG dinucleosides may be stable to exonucleases.

There are reports of the synthesis of N-substituted guanidinium internucleoside linkages that are neutral and can not be deblocked to give charged guanidinium.[23,24] In this paper we report the synthesis of protected guanidinium linked dinucleoside, which upon phosphitylation to an amidite can be incorporated into ODN using standard automated DNA solid-phase synthesis. The guanidinium linkage in these dinucleosides **1a–c**

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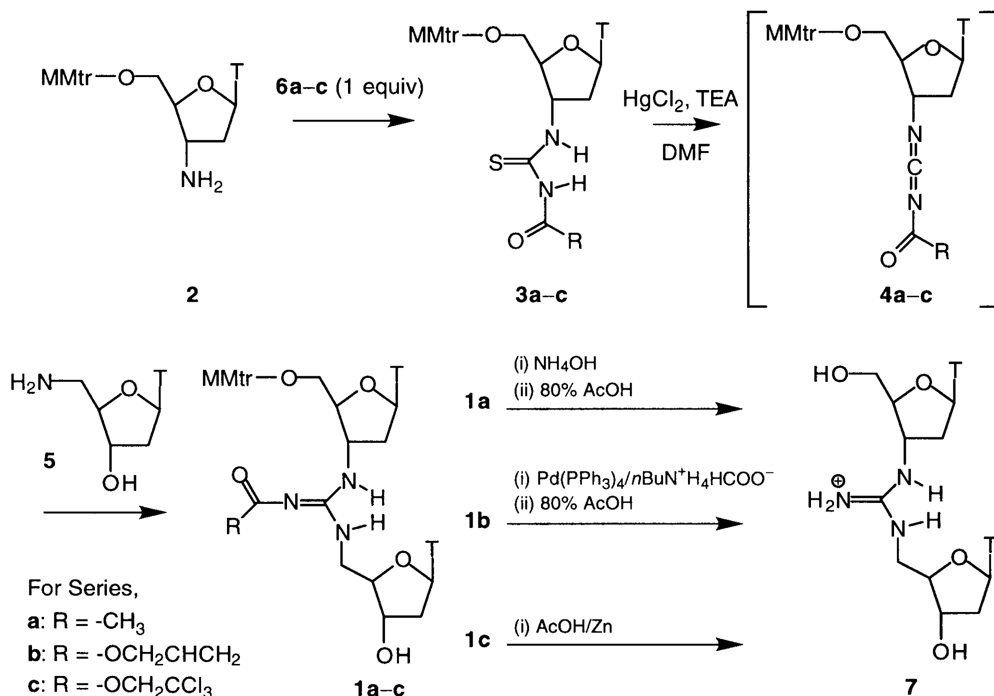
(Scheme 1)[25] remain protected during DNA synthesis and can be deprotected at the end of the synthesis. Three different protecting groups, **1a–c**, are selected for guanidinium protection and each is subject to different deprotection conditions.

Scheme 1.



The synthesis of dinucleosides, **1a–c**, involves coupling of the 5′-amino group of 5′-amino-5′-deoxythymidine **5** with in situ generated carbodiimides **4a–c** by reaction of the acyl protected thioureas **3a–c** with mercury (II) in the presence of TEA.[26] The various acyl protected thioureas **3a–c** were synthesized using 5′-O-monomethoxytrityl-3′-amino-3′-deoxythymidine **2** and the reagents acetylisothiocyanate **6a**, allyloxycarbonylisothiocyanate **6b**, or trichloroethoxycarbonylisothiocyanate **6c** in dichloromethane. The electron withdrawing nature of acyl groups on the thioureas **3a–c** activates the carbodiimide intermediate **4a–c**, facilitating the attack by the 5′-amine and then these acts as a protecting group on the resulting guanidinium linkage.

The acetyl protected dinucleoside **1a** remains stable to conditions required for DNA solid-phase synthesis and the acetyl is removed during usual final deprotection conditions of DNA synthesis. Hence, **1a** is suitable for standard automated DNA synthesis. Treatment of **1a** with 35% ammonium hydroxide at 55 °C for 45 h followed by detritylation gives the deprotected guanidinium dinucleoside **7** in quantitative yield (as determined by HPLC and characterized by FABMS $[(M + H)^+ = 508]$. The allyloxycarbonyl protected dinucleoside **1b** is stable to

ammonium hydroxide treatment. Deprotection of **1b** guanidinium linkages is selectively carried out with a brief treatment by a palladium(0) catalyst to which ODN protecting groups are stable.[27] Thus, the allyloxycarbonyl group can be removed while the ODN remains covalently linked to the solid support. The protecting group **1c** of internucleoside guanidinium linkage, when used along with phenoxyacetyl protected nucleobase phosphoramidites for ODN synthesis then final deprotection requires only mild ammonium hydroxide treatment and thus protecting group **1c** can be kept on throughout deprotection of ODN and trityl-on purification procedure. This has advantages in purification of oligos as guanidinium remains protected and finally it can be removed by treatment with acetic acid and Zinc powder,[28] which also detritylates the purified dinucleoside.

In summary, we provide the synthesis of novel protected DNG dinucleosides for incorporation into ODN using solid-phase synthesis. The three different protecting groups provides various deprotection conditions that can be performed at different stages of ODN deprotection and purification. These dinucleoside monomers **1a–c** upon phosphitylation will provide monomers for synthesis of a new class of mixed charge DNG/DNA antisense compounds using well established ODN solid-phase synthesis. Incorporation of these dinucleosides into oligonucleotides is now in progress, synthesis and physico-chemical properties of hybridization with complementary DNA and RNA will be published separately.

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25. **1a**: TLC (CH_2Cl_2:MeOH, 9:1) $R_f = 0.4$; ^{1}H NMR (400 MHz DMSO-d_6): δ (ppm) 1.4 (s, 3H, C*H*$_3$ Guan), 1.7 (s, 3H, C*H*$_3$ Thym), 1.8 (s, 3H, C*H*$_3$ Thym), 2–2.4 (m, 4H, 2×2′-H$_2$), 3.2–3.4 (m, 4H, 2×5′H$_2$), 3.75 (s, 3H, -O-C*H*$_3$ MMTr), 3.8 (m, 1H, 4′H), 3.95 (m, 1H, 4′H), 4.15 (m, 1H, 3′H), 4.8 (m, 1H, 3′H), 5.4 (m, 1H, exch, 3′-OH), 6.2 (t, 1H, 1′H), 6.4(t, 1H, 1′H), 6.9 (d, 2H, MMTr), 7.2–7.5 (m, 12H, MMTr), 7.6 (s, 1H, 6-H), 10.3 (s, 1H, exch, N*H* Thym), 11.4 (2s, 2H, N*H* Guan). HRMS (FAB) *m/z* 822.3384, calcd for $C_{43}H_{48}N_7O_{10}$ $(M + H)^+$ 822.3479.

1b: TLC (CH_2Cl_2:MeOH, 9:1) $R_f = 0.41$; ^{1}H NMR (400 MHz DMSO-d_6): δ (ppm) 1.44 (s, 3 H, C*H*$_3$ Thym), 1.76 48 (s, 3 H, C*H*$_3$ Thym), 2.10 (2 H, m, 2′-H$_2$), 2.24 (1 H, m, 2′-H), 2.35 (1 H, m, 2′-H), 3.28 (2 H, m, 5′-H$_2$), 3.45 (1 H, m, 5′-H), 3. (1 H, m, 5′-H), 3.72 (3 H, s, -O-C*H*$_3$ MMTr), 3.74 (1 H, q, J = 3.2 Hz, 4′-H), 3.82 (1 H, q, J = 5.1 Hz, 4′-H), 3.96 (1 H, m, 3′-H), 4.14 (1 H, m, 3′-H), 4.38 (2 H, d, J = 5.2 Hz, C*H*$_2$ allyl), 5.08 (1 H, d, J = 10.4 Hz, C*H* allyl), 5.16 (1 H, d, J = 17.2 Hz, C*H* allyl), 5.36 (1 H, d, J = 4.0 Hz, 3′-OH), 5.84 (1 H, m, C*H* allyl), 6.15 (1 H, t, J = 7.7 Hz, 1′-H), 6.24 (1 H, t, J = 7.8 Hz, 1′-H), 6.85 (2 H, d, J = 8.8 Hz, MMTr), 7.22 (4 H, m, MMTr), 7.28 (4 H, t, J = 7.2 Hz, MMTr), 7.38 (4 H, d, J = 7.4 Hz, MMTr), 7.39 (1 H, s, 6-H), 7.53 (1 H, s, 6-H), 11.26, (1 H, s, N*H* thym), 11.27, (1 H, s, N*H* thym); HRMS (FAB) *m/z*: 863.3499, calcd for $C_{45}H_{49}N_7O_{11}$ $(M + H)^+$ 863.3490

1c: TLC (CH_2Cl_2:MeOH, 9:1) $R_f = 0.44$; ^{1}H NMR (400 MHz DMSO-d_6): δ (ppm) 1.48 (s, 3 H, C*H*$_3$ Thym), 1.79 (s, 3 H, C*H*$_3$ Thym), 2.14 (4 H, m, 2×2′-H$_2$), 3.25 (2 H, m, 5′-H$_2$), 3.46 (1 H, m, 5′-H), 3.57 (1 H, m, 5′-H), 3.72 (3 H, s, -O-C*H*$_3$ MMTr), 3.73 (1 H, m, 4′-H), 3.83 (1 H, q, J = 4.4 Hz, 4′-H), 3.98 (1 H, m, 3′-H), 4.16 (1 H, m, 3′-H), 4.67 (2 H, s, troc-C*H*$_2$), 5.37 (1 H, s, 3′-OH), 6.15 (1 H, t, J = 7.8 Hz, 1′-H), 6.23 (1 H, t, J = 7.8 Hz, 1′-H), 6.85 (2 H, d, J = 8.8 Hz, MMTr), 7.22 (4 H, mMMTr), 7.28 (4 H, t, J = 7.2 Hz, MMTr), 7.37 (4 H, d, J = 7.4 Hz, MMTr), 7.38 (1 H, s, 6-H), 7.58 (1 H, s, 6-H), 11.27, (1 H, s, N*H* thym), 11.28, (1 H, s, N*H* thym); HRMS (FAB) *m/z*: 953.2330, calcd for $C_{44}H_{46}N_7O_{11}Cl_3$ $(M + H)^+$ 953.2321

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