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Multiple phosphate-linked nucleotide couplings via 5′ silyl ether protection in the phosphite triester and phosphoramidite approaches

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ABSTRACT

Phosphite triester and phosphoramidite coupling methodologies are described for performing fourfold solution phase installations of standard deoxynucleotides onto a single cavitand template molecule. The methodologies here are based on 5′ silyl ether protection of the appropriate nucleoside or nucleoside phosphoramidite. Synthesis of a novel water-soluble species incorporating four covalently-linked guanine nucleotides is shown.

Introduction

Template-assembled synthetic systems based on G-quartets and other nucleobase quartet motifs are increasingly becoming utilized to mimic biologically relevant structures and activities. In these systems, a template molecule is covalently decorated with nucleosides[1] or oligonucleotide strands[2] to reveal unconventional topologies and functions typically afforded from preorganized G-quartet and higher order G-quadruplex assemblies. G-quadruplex DNA is an essential and widely studied nucleic acid secondary structure owing to its localization in human telomeres and oncogenes and its putative role as a target for anticancer drug design.[3] For instance, single quartet templated systems have resulted in G-quartet-based G-quadruplex ligands,[1b–d] a cation-free G-quartet,[1f] and nanotube construction,[1g] while template-assembled G-quadruplex designs have revealed receptors for G-quadruplex ligands[2a,2d] and aptamers with anti-HIV activity.[2b] Expanding beyond guanine to other nucleobases has demonstrated the utility of template-assembled synthesis in stabilized U-quadruplex, U-quartet, and *i*-motif DNA structures.[4]

Linkage chemistries in these templated systems have been limited to only a few types including triazole production through copper(I) catalyzed azide alkyne cycloadditions (CuAAC), oxime and amide formation, and solid-phase DNA syntheses. The CuAAC owing in part to its bioorthogonal nature have found a widespread application in bioconjugation reactions and other modifications of nucleic acids and nucleosides.[5] However, one disadvantage of CuAAC mediated triazole formation is that solubilizing groups such as native phosphates for work in polar solvents or water are not incorporated directly.[6] Reactions capable of installing several guanine nucleotides at one time have not been described despite the attention that has been given to template-assembled synthetic G-quartet (TASQ) compounds.

We report here methodologies inspired by the phosphite triester and phosphoramidite approach toward DNA synthesis for performing fourfold phosphate-linked nucleotide couplings onto an individual substrate via a natural 3′ linkage in the solution phase. In these methodologies, conventional 5′ alcohol dimethoxytrityl (DMT) protection has been replaced by silyl ether protection with TBS. The scope of the phosphoramidite linkage methodology is adapted in order to accommodate four non-standard functionalized nucleobases. Furthermore, synthesis of an unprecedented water-soluble species incorporating four phosphate-linked guanine nucleotides is reported.

Results and discussion

Suitability of phosphorus(III) reagents to carry out multiple nucleotide forming reactions was explored. A fourfold coupling reaction was performed by modification of the phosphite triester DNA synthetic methodology (Scheme 1).[7] In situ conversion of base-protected 5′ TBS guanosine **1a**[8] to its activated 3′ phosphorus(III) intermediate and coupling to alcohol functionalized [4] cavitand template **2**[9] were carried out using 2,2,2-trichloroethylphosphorodichloridite as a coupling reagent in the presence of 2,6-lutidine in cooled THF solution. Minimization of steric bulk and avoidance of acid-catalyzed depurination side-reactions motivated the selection of the TBS group in place of the standard DMT group for 5′ alcohol protection.[10] Initial attempts with 5′ DMT

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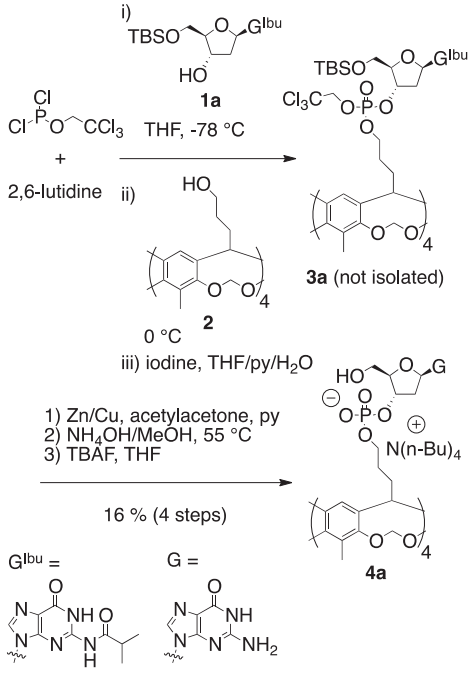


Scheme 1. Phosphite triester synthesis of tetra-guanine **4a**.

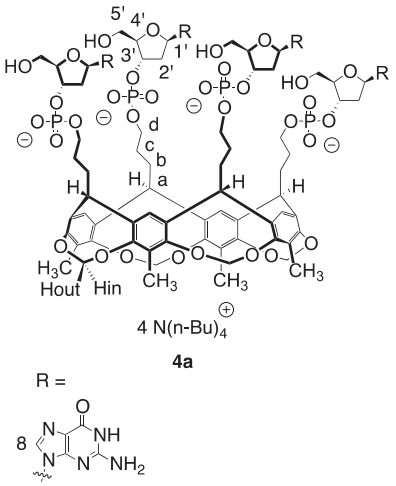
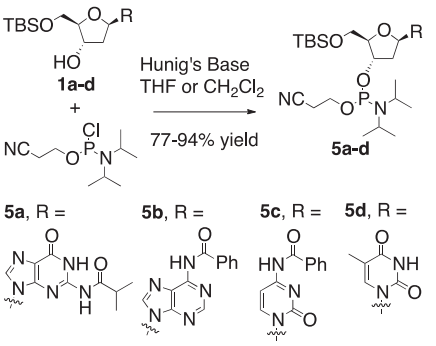


Figure 1. Structure of tetra-guanine **4a**.

nucleotide couplings followed by complete deprotection were unsuccessful. Any presence of partially substituted template products was found to be problematic for subsequent purifications and reactions. Thus, four equivalents of **1a** per alcohol function were observed to be sufficient to achieve complete conversion of starting material **2** to the fully coupled and protected intermediate conjugate, and the presence of starting material **2** or any mono-, di-, or tri-coupling intermediates could not be detected by TLC after several hours of reaction progress. After iodine oxidation, the fully protected fourfold coupled phosphate triester diastereomeric mixture **3a** was purified on silica gel.

Coupling and deblocking reactions were carried out in succession without isolation or characterization of the intermediates due to the problematic generation of diastereomeric mixtures when asymmetric phosphate triesters are formed and due to poor solubilities of intermediate deblocked compounds. Phosphate trichloroethyl protecting groups were removed with Zn/Cu couple

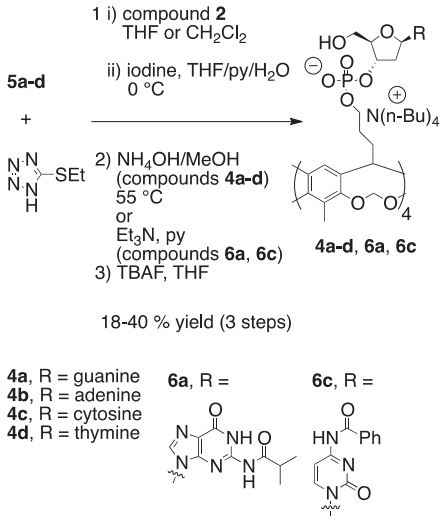


Scheme 2. Synthesis of phosphoramidites **5a–d**.

and acetylacetone in pyridine.[11] Ammonia treatment in a water/methanol mixture followed by fluoride cleavage facilitated deblocking of the respective base isobutyryl and 5′ silyl ether protecting groups. Purification on C_{18} reversed-phase silica afforded phosphate-linked conjugate **4a** in 16% yield over four steps as a *n*-tetrabutylammonium salt (Fig. 1).

Standard phosphoramidite based internucleotide bond forming reactions were also investigated as a potential way to carry out multiple nucleotide coupling reactions with guanine and other bases.[12] A new series of 2-cyanoethyl,-*N*,*N*-diisopropylamino phosphoramidite reagents of the canonical deoxynucleosides **5a–d** with standard *N*-isobutyryl or *N*-Bz base protection and 5′ TBS protection were prepared from deoxynucleosides **1a–d** (Scheme 2).[13,8] Phosphitylation was performed in THF at room temperature, and synthesis in 77–94% yield of the moisture and air sensitive phosphoramidites **5a–d** as diastereomeric pairs was confirmed by the appearance of two singlets at approximately 150 ppm in the ^{31}P NMR spectrum after work-up. These reagents were used to perform fourfold coupling reactions on template **2** in the presence of 5-(ethylthio)-tetrazole activator in THF or CH_2Cl_2 solvent (Scheme 3). 5-(Ethylthio)-tetrazole is noted to have greater solubility in THF solution and improved activity as a catalyst in the phosphoramidite reaction over 1*H*-tetrazole.[14] Equivalents and purification steps were in conformance to the preceding phosphite triester method. Simplified two step ammonia and TBAF deblocking and purification gave the phosphate-linked conjugates **4a–d** in up to 40% yield after three steps. In addition, a route toward compounds **6a** and **6c** that remained protected at their base position was achieved by selective phosphate deblocking with triethylamine instead of ammonia treatment, and this route is anticipated to facilitate additional base functionalization by corresponding substitution of the isobutyryl and Bz base-protecting groups in the starting material.

Installation of the 5′ TBS protecting group in place of routine 5′ DMT protection and subsequent TBAF cleavage proved to be advantageous as it provided a convenient route to incorporate a *n*-tetrabutylammonium counterion into the phosphate-linked conjugates **4a–d**, **6a**, and **6c**. Tetrabutylammonium salts **4a–d** and **6a** were found to have millimolar solubility in methanol and in water, while **6c** was found to have millimolar solubility in methanol only. For guanine conjugate **4a**, other counterions could not be found to impart solubility in water. Imparted water-solubility is an essential property of molecular assemblies inspired by or used to model Nature and becomes increasingly difficult at the less negative charge dense single guanine tetrad level with respect to G-quadruplex DNA. Furthermore, tetraalkylammonium counterions may be beneficial when utilized in NMR characterization studies as chemical exchange between counterions and substrate molecules that can potentially limit observation of exchangeable proton NMR signals is avoided. All high molecular weight tetra-coupled nucleotide target compounds **4a–d**, **6a**, and **6c** were characterized extensively



Scheme 3. Phosphoramidite synthesis of tetra-nucleotides **4a–d**, **6a**, and **6c**.

using ^{1}H, ^{31}P, ^{13}C, and ^{1}H–1H COSY NMR, and MALDI methods suitable for hydrid organic molecules between small molecule and oligonucleotide size.[15] Full signal assignments in the ^{1}H NMR spectra were made with assistance from ^{1}H–1H COSY cross-peaks.

Conclusions

In summary, we have shown the application of the atypical phosphite triester DNA synthesis approach and of a new class of 5′ TBS protected phosphoramidites for the construction of multiply-coupled phosphate-linked nucleotide compounds. The unprecedented synthesis reported here of a water-soluble tetra-coupled guanine nucleotide template species is of particular interest to the field of G-quartet/G-quadruplex chemistry, and may find application in the synthesis of model systems of human telomere structure or of artificial anticancer ligand receptors. In addition, multiple nucleotide coupled templates may aid in the discovery of unknown four motif nucleobase structures in Nature or in the design of new functional supramolecular systems.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.04.043.

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15. *Characterization data of tetra-guanine* ***4a****:* ^{1}H NMR (400 MHz, CD_3OD) δ 7.90 (s, 4H, H-8), 7.10 (s, 4H, aryl), 6.20 (t, J = 7.0 Hz, 4H, CH-1′), 5.66 (d, J = 7.0 Hz, 4H, H_{out}), 5.03 (br, 4H, CH-3′), 4.74 (t, J = 8.0 Hz, 4H, CH-a), 4.21 (br, 4H, CH-4′), 4.09 (d, J = 7.0 Hz, 4H, H_{in}), 4.00 (m, 8H, CH_2-d), 3.82 (m, 8H, CH_2-5′), 3.24 (m, 32H, $N(CH_2CH_2CH_2CH_3)_4$), 2.66 (m, 4H, CH_a-2′), 2.42 (m, 12H, CH_2-b, CH_b-2′), 1.80 (overlap, 8H, CH_2-c), 1.76 (s, 12H, methyl), 1.67 (m, 32H, $N(CH_2CH_2CH_2CH_3)_4$), 1.43 (m, 32H, $N(CH_2CH_2CH_2CH_3)_4$), 1.03 (t, J = 7.0 Hz, 48H, $N(CH_2CH_2CH_2CH_3)_4$); ^{13}C NMR (101 MHz, CD_3OD) δ 159.4, 155.1, 154.7, 152.3, 138.6, 138.4, 125.3, 119.2, 118.4, 99.8, 88.4, 86.1, 76.9, 65.7, 63.8, 59.5, 40.1, 37.0, 29.7, 26.2, 24.8, 20.7, 14.0, 10.0; ^{31}P NMR (162 MHz, CD_3OD) δ 2.3 (s); MS (MALDI-TOF) calculated for $C_{88}H_{103}N_{20}O_{36}P_4$ $(M-H)^-$: 2139.6; found: 2142.0.