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A Mild Method for the Syntheses of 3'-Thioformacetal Dinucleotides: The Development of Diphenylphosphinate as a Glycosyl Donor

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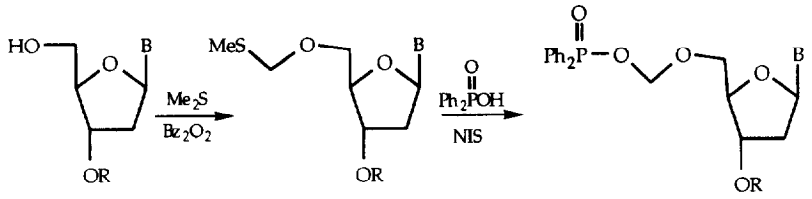
Abstract: A mild and efficient procedure for the syntheses of 3'-thioformacetal dinucleotides is presented. The glycosylation synthon agent, diphenylphosphinate formacetal was developed as the intermediate for the coupling reaction under mild basic conditions. This method is compatible with purine deoxyribosides, unprotected amino groups and acid labile functionalities.

The replacement of the phosphate diester linkage with neutral and achiral surrogates has been an area of interest for the identification of superior antisense and antigene oligonucleotide (ON) agents.[1] A host of these analogs has been synthesized aimed at improving the biochemical and pharmacological properties of ONs such as nuclease stability, hybridization affinity and cell membrane permeability.[2] Previously, we found that ONs containing 3'-thioformacetal-linked dinucleotides exhibited improved hybridization properties toward single stranded RNAs and double stranded DNAs relative to the native phosphate diester linkage.[3] The synthesis of the thioformacetal linkage, however, was of low yield and limited to pyrimidine bases. Further studies required improvement in the synthetic method for the thioformacetal linkages. Herein, we report a mild approach to 3'-thioformacetal nucleotide linkages using a thiol displacement reaction on a diphenyl phosphinate acetal under mild basic conditions.

The reported syntheses of 3'-thioformacetal dinucleotides employed an unstable chloromethyl ether intermediate which was generated *in situ* under strong acidic conditions.[3] The harsh acidity reduced the yields with purine bases due to depurination. Furthermore, this chloromethyl ether intermediate is too unstable to be fully characterized and conveniently stored. Desirable attributes of a new synthesis are yield, compatibility with purine deoxyribosides and stable intermediates. Dialkyl phosphate acetals are known intermediates in glycosylation reactions and have recently been described for the preparations of formacetal nucleotide linkages under Lewis acid coupling conditions.[4] Our attempts to apply these intermediates for the synthesis of thioformacetal linkages resulted in two problems. The first was the instability of dialkyl phosphate formacetal nucleoside intermediates. Substantial degradation was observed during column chromatography and slow decomposition resulted during storage. This problem has been solved by using a diphenyl phosphinate formacetal synthon. Diphenyl phosphinic acid is approximately 1 pH unit less acidic than dialkylphosphoric acid resulting in a more stable formacetal intermediate.[5] The second problem was the acidic nature of the coupling reaction and the concomitant depurination problem. The synthesis of the thioformacetal linkages can capitalize on the potent nucleophilicity of thioate anions. Consequently, mild basic conditions can be utilized for the coupling, eliminating depurination problems.

5'-Hydroxy-2'-deoxynucleosides (**1**–**3**), where the amino groups of adenine **2** and guanine **3** were protected with benzoyl and iso-butyryl amides respectively, were converted to their methoxythiomethyl (MTM) ether forms (**4**–**6**) following a known procedure.[6] Treatment of the MTM ether with *N*-iodosuccinimide (NIS) and diphenylphosphinic acid in $ClCH_2CH_2Cl$/ether (1/1) at ambient temperature afforded the phosphinate precursors (**7**–**9**) in good yields as outlined in Scheme **1**.[7]

Scheme 1



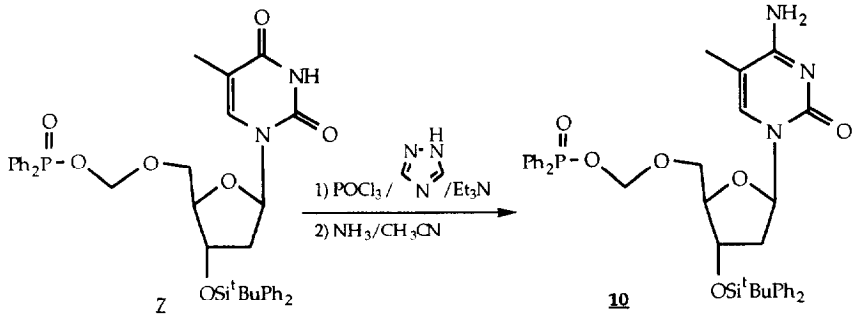
1. R = SitBuPh$_2$ B = T **4**. 78% **7**. 88%

2. R = SitBuPh$_2$ B = 6-N-Benzoyl-A **5**. 77% **8**. 63%

3. R = COPh B = 2-N-*iso* butyryl-G **6**. 75% **9**. 70%

Since 5-methyl cytosine (C^M) routinely replaces cytosine in antisense studies, the 5-methyl cytosine derivative was tested. This derivative was readily synthesized in 80% overall yield from the corresponding thymidine phosphinate by treatment with $POCl_3$ and triazole, followed by aminolysis with saturated ammonia in CH_3CN.[8] This 5-methyl cytosine precursor can also be directly obtained through the sequence of Scheme **1**, where the amino group can be masked as different amides. These diphenylphosphinyl nucleosides can be purified through a silica column and conveniently stored for months at -20°C without decomposition.[9]

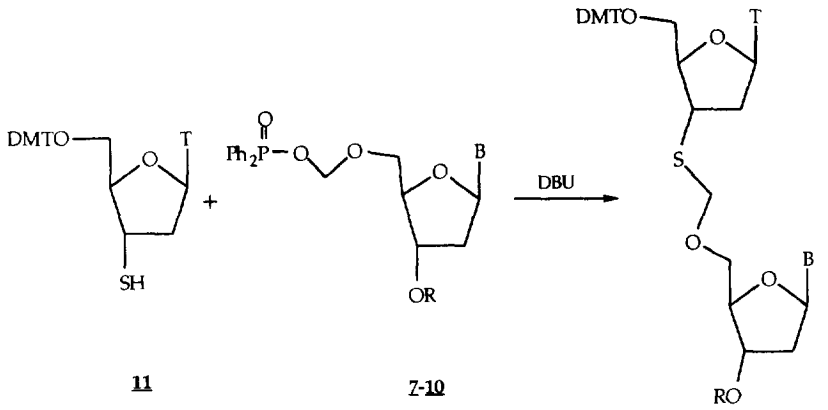
Scheme 2



7 **10**

Here, we exemplify the condensation of phosphinate with 3'-thiol nucleosides to form 3'-thioformacetal dinucleotides by preparing four dimers, TT, TC^M, TA^{Bz} and TG^{Ibu}, where different protecting moieties on the amino and hydroxy groups are present. The 3'-thio-thymidine **11** was obtained as previously described.[3]

Scheme 3



11 7-10

12. R = Si^tBuPh_2 B = T Y: 91%
13. R = Si^tBuPh_2 B = $5\text{-}C^M$ Y: 75%
14. R = Si^tBuPh_2 B = A^{Bz} Y: 71%
15. R = Bz B = G^{Ibu} Y: 55%

A variety of coupling conditions with different base and solvent combinations including DBU/DMF/formamide, NaOEt/EtOH, NaH/THF and NaOTMS/THF were tested for the formation of T•T dimer by coupling of **7** and **11**. The use of DBU as the base in DMF/formamide (1/1) was empirically determined to give the best results.[10] Then, we applied this condition to the syntheses of other dimers **13**, **14** and **15**. All couplings proceeded at ambient temperature under N_2 or Ar in predegassed solvent.[11] The yields are listed in Scheme **3** and are not fully optimized.[12] This reaction is tolerant of the presence of other mild nucleophiles as evidenced by the fact that the amino groups of 5-methyl cytosine, and presumably of adenosine and guanosine, can be left without protection.

In summary, the readily available diphenyl phosphinate acetals have been found to be desirable intermediates, which are not only suitable for the preparation of thioformacetal linkages, but are also expected to have a wide spectrum of applications in glycosylation reactions as general glycosyl donors. The synthesis of the 3'-thioformacetal linkage through this approach showed improved yields and was compatible with many functionalities, including purine deoxynucleosides. Further development of thioformacetal-linked nucleotides involving synthesis of larger analogs using this method is underway.

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7. NMR data for **7**, **8**, **9** and **10**. Compound **7**: ^{1}H NMR ($CDCl_3$): δ 7.93 (br s, 1H), 7.8–7.28 (br m, 21H), 6.40 (dd, J = 8.1, 5.8 Hz, 1H), 5.11 (m, 2H), 4.33 (m, 1H), 4.01 (m, 1H), 3.70 (dd, J = 10.7, 2.9 Hz, 1H), 3.29 (dd, J = 10.7, 2.6 Hz), 2.26 (m, 1H), 1.85 (m, 1H), 1.75 (s, 3H), 1.07 (s, 9H). ^{31}P NMR ($CDCl_3$): δ 31.71 (s). Compound **8**: ^{1}H NMR ($CDCl_3$): δ 8.97 (s, 1H), 8.77 (s, 1H), 8.19 (s, 1H), 8.01 (m, 2H), 7.80–7.28 (m, 23H), 6.59 (dd, J = 6.7, 6.7 Hz, 1H), 5.10 (d, J = 10.4 Hz, 2H), 4.51 (m, 1H), 4.13 (m, 1H), 3.69 (dd, J = 10.7, 3.5 Hz, 1H), 3.40 (dd, J = 10.7, 3.6 Hz, 1H), 2.49 (m, 2H), 1.09 (s, 9H). ^{31}P NMR ($CDCl_3$): δ 42.16 (s). Compound **9**: ^{1}H NMR ($CDCl_3$): δ 12.4 (s, 1H), 11.28 (s, 1H), 8.04 (m, 2H), 7.90–7.40 (m, 14H), 6.12 (dd, J = 10.7, 5.5 Hz, 1H), 5.46 (dd, J = 10.3, 5.5 Hz, 1H), 5.41 (m, 1H), 5.34 (dd, J = 13.5, 5.5 Hz, 1H), 4.38 (m, 1H), 3.94 (dd, J = 8.6, 3.2 Hz, 1H), 3.25 (m, 1H), 2.95 (m, 1H), 2.23 (dd, J = 13.6, 5.7 Hz, 1H), 1.29 (m, 1H), 1.20 (d, J = 6.8 Hz, 3H), 1.09 (d, J = 6.8 Hz, 3H). ^{31}P NMR ($CDCl_3$): δ 32.81. Compound **10**: ^{1}H NMR ($CDCl_3$): δ 7.90–7.28 (m, 21H), 6.43 (dd, J = 6.7, 6.2 Hz, 1H), 5.15 (dd, J = 9.9, 5.5 Hz, 1H), 5.00 (dd, J = 10.4, 5.5 Hz, 1H), 4.29 (m, 1H), 3.99 (m, 1H), 3.70 (dd, J = 10.8, 2.9 Hz, 1H), 3.23 (dd, J = 10.8, 3.2 Hz, 1H), 2.43 (ddd, J = 13.5, 6.0, 3.3 Hz, 1H), 1.83 (m, 1H), 1.74 (s, 3H), 1.05 (s, 9H). ^{31}P NMR ($CDCl_3$): δ 31.72 (s).
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9. Although stable at a lower temperature, it was found that partial decomposition of the phosphinates occurred after storage at room temperature for several weeks.
10. Without the presence of formamide, the reaction was slow and resulted in side products. The optimization of the solvent system by varying the volume ratio of DMF and formamide was not performed.
11. It is critical that the reaction atmosphere is oxygen free to prevent disulfide formation.
12. NMR data for compounds **12**, **13**, **14** and **15**. Compound **12**: ^{1}H NMR ($CDCl_3$): δ 8.22 (br s, 1H), 8.19 (br s, 1H), 7.62 (m, 5H), 7.39 (m, 8H), 7.25 (m, 7H), 7.10 (d, J = 1.0 Hz, 1H), 6.82 (d, J = 1.8 Hz, 2H), 6.79 (d, J = 1.8 Hz, 2H), 6.33 (dd, J = 7.6, 6.2 Hz, 1H), 6.13 (dd, J = 6.2, 6.2 Hz, 1H), 4.54 (d, J = 12.0 Hz, 1H), 4.44 (d, J = 12.0 Hz, 1H), 4.22 (m, 1H), 4.00 (m, 1H), 3.91 (m, 1H), 3.77(s, 6h), 3.72 (m, 1H), 3.49 (dd, J = 10.8, 2.3 Hz, 1H), 3.41 (dd, J = 10.8, 2.8 Hz, 1H), 3.25 (dd, J = 11.0, 2.8 Hz), 3.12 (dd, J = 11.0, 4.4 Hz, 1H), 2.40 (m, 2H), 2.27 (m, 1H), 1.81 (m, 1H), 1.79 (d, J = 1.0 Hz, 3H), 1.43 (d, J = 1.0 Hz, 3H), 1.07 (s, 9H). Compound **13**: ^{1}H NMR ($CDCl_3$): δ 7.61 (m, 5H), 7.38 (m, 8H), 7.23 (m, 8H), 6.82 (s, 2H), 6.79 (s, 2H), 6.38 (dd, J = 6.5, 6.5 Hz, 1H), 6.12 (dd, J = 5.5 Hz, 5.5 Hz, 1H), 4.54 (d, J = 12.0 Hz, 1H), 4.36 (d, J = 12.0 Hz, 1H), 4.21 (m, 1H), 3.98 (m, 1H), 3.92 (m, 1H), 3.77 (s, 6H), 3.59 (m, 1H), 3.49 (m, 2H), 3.28 (dd, J = 10.7, 2.3 Hz, 1H), 3.04 (dd, J = 10.7, 4.7 Hz, 1H), 2.42 (m, 3H), 1.80 (s, 3H), 1.76 (m, 1H), 1.42 (s, 3H), 1.05 (s, 9H). Compound **14**; ^{1}H NMR ($CDCl_3$): δ 9.40 (br s, 1H), 8.70 (br s, 1H), 8.12 (s, 1H), 8.07 (s, 1H), 8.05 (s, 1H), 7.80–7.10 (br m, 24 H), 6.81 (d, J = 2.1 Hz, 2H), 6.78 (d, J = 2.1 Hz, 2H), 6.52 (dd, J = 6.3, 6.3 Hz, 1H), 6.05 (m, 1H), 4.59 (d, J = 12.0 Hz, 1H), 4.54 (m, 1H), 4.30 (d, J = 12.0 Hz, 1H), 4.16 (m, 1H), 3.87 (m, 1H), 3.75 (s, 6H), 3.62 (m, 1H), 3.51 (m, 2H), 3.28 (m, 1H), 3.13 (m, 1H), 2.52 (m, 2H), 2.42 (m, 2H), 1.38 (s, 3H), 1.10 (s, 9H). Compound **15**: ^{1}H NMR ($CDCl_3$): δ 9.22 (br s, 1H), 9.10 (br s, 1H), 8.10–7.15 (br m, 16H), 6.82 (d, J = 2.6 Hz, 2H), 6.79 (d, J = 2.6 Hz, 1H), 6.29 (m, 1H), 6.15 (m, 1H), 5.57 (m, 1H), 4.74 (d, J = 12.1 Hz, 1H), 4.59 (d, J = 12.1 Hz, 1H), 4.38 (m, 1H), 3.98 (m, 1H), 3.87 (m, 1H), 3.75 (d, J = 2.9 Hz, 6H), 3.73 (m, 3H), 3.60 (m, 1H), 3.34 (m, 1H), 2.96 (m, 1H), 2.71–2.40 (br m, 4H), 1.40 (s, 3H), 1.26 (m, 6H).

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