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A New Synthetic Method for the Preparation of Nucleoside Phosphoramidate Analogues with the Nitrogen Atom in Bridging Positions of the Phosphoramidate Linkage

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Abstract: An efficient method for the preparation of nucleoside P3'→N5' and N3'→P5' phosphoramidates and their thio analogues results from generation of a pyridine adduct of a nucleoside metaphosphate or its analogue from a nucleoside H-phosphonate, nucleoside H-phosphonothioate or nucleoside H-phosphonodithioate monoester followed by its reaction with 5'- or 3'-aminonucleoside. © 1998 Elsevier Science Ltd. All rights reserved.

In the last decade a vast array of oligonucleotide analogues with modified internucleotidic linkages, modified sugar residues or with modifications in the heterocycle base moieties, have been designed for the use as artificial modulators of gene expression *via* antisense or antigene approach.[1]

Among these, oligonucleoside N3'→P5' phosphoramidates bearing the nitrogen atom in a bridging position of a phosphoramidate linkage, have recently attracted a considerable attention as a class of compounds of potential therapeutic value.[2-7] Oligonucleotide analogues uniformly modified with N3'→P5' functionality are resistant towards various nucleases[2] and hybridise to complementary DNA or RNA targets with much higher affinity than do their natural congeners.[3] This is in contrast to many other oligonucleotide analogues, where the increase in hydrolytic stability towards nucleases is often accompanied by the decrease in their binding ability to RNA or DNA.[8] These oligonucleotide analogues in solution adopt A-type helical structures,[3] in contradistinction to natural DNA fragments which prefer to reside in the B form. This can be related to a higher preference of the deoxyribose ring of 3'-amino-3'-deoxynucleosides to exist in the N-conformation.[3] In this respect, oligonucleoside N3'→P5' phosphoramidates possess more structural resemblance to RNA than to DNA.[3]

The first synthesis of nucleoside phosphoramidates with the P-N bond in a bridging position of the phosphate group was published by Jastroff and Hettler[9] in 1969. They prepared mono- and dinucleoside 5'-phosphoramidates *via* a stepwise phosphorylation of 5'-aminonucleosides with phenyl phosphorodichloride, followed by hydrolysis or the addition of a second nucleosidic component, respectively. Since then, such phosphoramidates have been synthesised using phosphotriester chemistry,[10] *via* the Staudinger type of reaction,[11-14] or by the phosphoramidite method.[15] More recently, oligonucleoside N3'→P5' phosphoramidates

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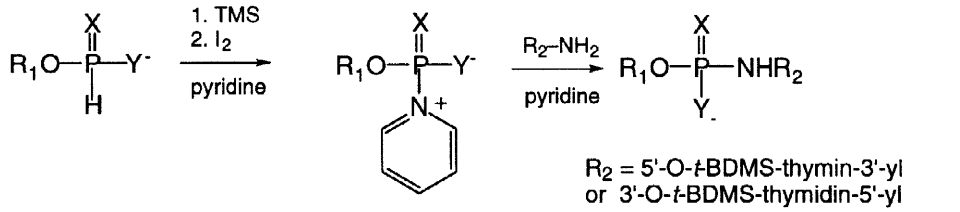
have been prepared *via* oxidative coupling of aminonucleosides with H-phosphonate diesters[5,16] under the Atherton-Todd oxidation conditions.[17]

As part of our research directed towards the development of synthetic methods for the introduction of heteroatoms at bridging positions of a phosphodiester function[18] using H-phosphonate methodology, we have investigated the possibility of preparing nucleoside phosphoramidates of types **6** and **7** (Scheme 2) *via* generation of the putative pyridine adduct of metaphosphates **1**, followed by their reactions with appropriate 5'- or 3'-aminonucleosides (Scheme 1).

Scheme 1



1

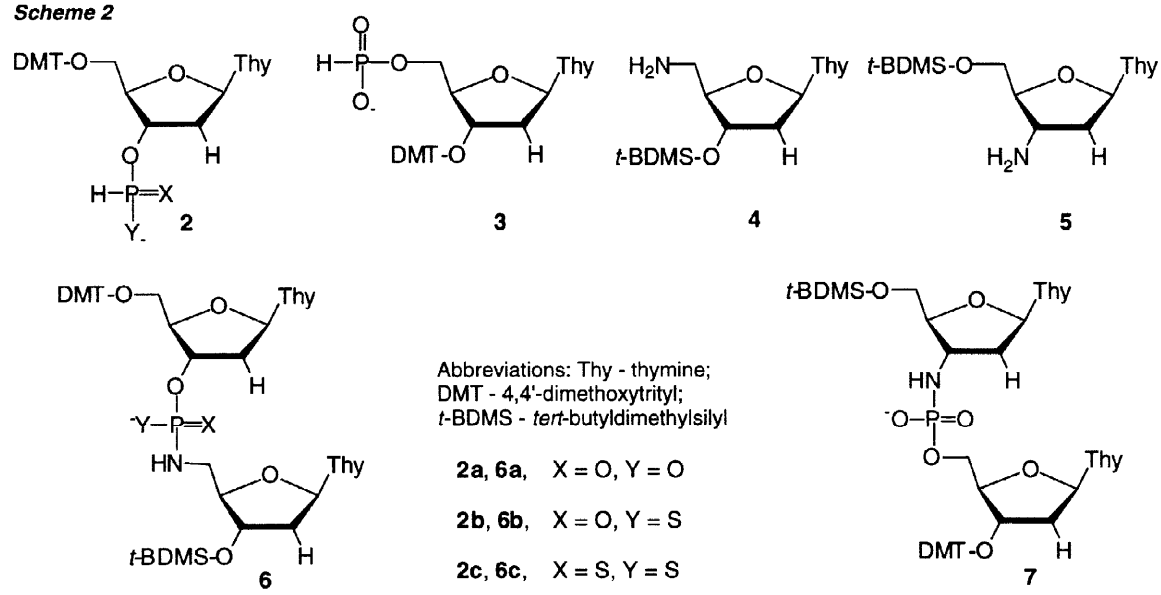
1a, X = Y = O, R_1 = 5'-O-DMT-thymidin-3'-yl **1c**, X = O, Y = S, R_1 = 5'-O-DMT-thymidin-3'-yl
1b, X = Y = O, R_1 = 3'-O-DMT-thymidin-5'-yl **1d**, X = S, Y = S, R_1 = 5'-O-DMT-thymidin-3'-yl

For abbreviations, see Scheme 2

Due to high nucleophilicity of amines towards the phosphorus centre, species **1a-d** should easily react with aminonucleoside **4** or **5** providing a new, general entry to nucleoside N3'→P5' and P3'→N5' phosphoramidates, as well as to the hitherto unknown mono- and dithio analogues (**6b** and **6c**). Since monoesters of phosphonic acid derivatives are used as substrates, the problem of removing phosphate protecting groups is alleviated in this approach.

To investigate the efficacy of the formation of phosphoramidates **6** or **7** *via* this metaphosphate approach (Scheme 1), a ^{31}P NMR study was undertaken. The putative pyridine adduct of metaphosphate **1a** or **1c** was generated analogously to a procedure described earlier,[19] and involved treatment of nucleoside 3'-H-phosphonate **2a**[20] [δ_P = 2.1 ppm, $^1J_{PH}$ = 599 Hz (d)] or nucleoside 3'-H-phosphonothioate **2b**[21] [δ_P = 53.2 & 53.8 ppm, $^1J_{PH}$ = 570 Hz (d)] in pyridine with trimethylsilyl chloride (TMS-Cl, 3 equiv), followed by the addition of iodine (1.5 equiv). The intermediates **1a** (δ_P = -5.1 ppm) and **1c** (δ_P = 54.6 ppm), respectively, were produced (5 min) as sole nucleotidic species (^{31}P NMR). The subsequent addition of 5'-aminonucleoside **4**[22,23] (1 equiv) together with triethylamine[24] (5 equiv) furnished a fast and quantitative formation of the corresponding silylated[25] dinucleoside phosphoramidate **6a** (δ_P = 2.0 ppm) or dinucleoside phosphoramidothioate **6b** (δ_P = 62.4 - 62.7 ppm). Analogously, the silylated nucleoside phosphoramidate **7** (δ_P = 0.8 & 0.9 ppm), with the nitrogen atom in the 3'-position of the internucleotide linkage (N3'→P5' analogue), was produced from nucleoside 5'-H-phosphonate **3**[20] [δ_P = 3.2 ppm, $^1J_{PH}$ = 599 Hz (d)] and aminonucleoside **5**,[26,27] *via* the pyridine adduct of metaphosphate **1b** (δ_P = -4.3 ppm) as an intermediate.

Scheme 2



In principle, less than the stoichiometric amount of TMS-Cl is needed for the formation of metaphosphates **1**, but to avoid a partial hydrolysis of this reactive intermediate by spurious water, it is recommended to use excess of a silylating agent for the reaction. Thus, TMS-Cl plays a dual role: (i) it facilities oxidation of H-phosphonate or H-phosphonothioate monoesters by iodine *via* converting them into the more susceptible silyl H-phosphonates (or bis-silyl phosphites) and (ii) it secures anhydrous reaction conditions.

The importance of TMS-Cl in keeping the reaction mixture anhydrous was particularly apparent in the synthesis of nucleoside phosphoramidodithioate **6c** from nucleoside H-phosphonodithioate **2c**[28] [δ_P = 85.0 ppm, $^1J_{PH}$ = 532 Hz (d)] and aminonucleoside **4**. Because of the ease of oxidation of **2c** with iodine in pyridine, the generation of metadithiophosphate **1d** (δ_P = 117.8 ppm) can be carried out either with or without the presence of TMS-Cl.[19] However, in the absence of TMS-Cl, besides the desired phosphoramidodithioate **6c**, considerable amounts of side products were formed (~30%), while in the presence of the silylating agent (3 equiv), **6c** (δ_P = 86.6 ppm) was produced exclusively.

Guided by the results obtained from ^{31}P NMR experiments, all the reactions described above were carried out on a preparative scale (0.5 grammes) and the products **6** and **7** were isolated and characterised.[29]

In conclusion, we have developed a new synthetic method that provides a facile access to nucleoside N3'→P5', nucleoside N5'→P3' phosphoramidates, and their new monothio and dithio analogues (**6b** and **6c**). The method is simple and efficient, and makes use of readily available nucleoside H-phosphonate, nucleoside H-phosphonothioate and nucleoside H-phosphonodithioate monoesters as starting materials. The lack of protection at the phosphorus centre simplifies further the synthetic protocols for the preparation of these compounds.

Acknowledgements

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References and Notes

1. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543-584.

2. Gryaznov, S.; Skorski, T.; Cucco, C.; Nieborowska-Skorska, M.; Chiu, C. Y.; Lloyd, D.; Chen, J.-K.; Koziolkiewicz, M.; Calabretta, B. *Nucleic Acids Res.* **1996**, *24*, 1508-1514.
3. Gryaznov, S. M.; Lloyd, D. H.; Chen, J.-K.; Schultz, R. G.; deDionisio, L. A.; Ratmeyer, L.; Wilson, W. D. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 5798-5802.
4. Giovannangeli, C.; Diviacco, S.; Labrousse, V.; Gryaznov, S.; Charneau, P.; Helene, C. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 79-84.
5. Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acids Res.* **1992**, *20*, 3403-3409.
6. Rigl, C. T.; Lloyd, D. H.; Tsou, D. S.; Gryaznov, S. M.; Wilson, W. D. *Biochemistry* **1997**, *36*, 650-659.
7. Escude, C.; Giovannangeli, C.; Sun, J.-S.; Llyod, D. H.; Chen, J.-K.; Gryaznov, S. M.; Garestier, T.; Helene, C. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 4365-4369.
8. Kibler-Herzog, L.; Zon, G.; Uznanski, B.; Whittier, G.; Wilson, W. D. *Nucleic Acids Res.* **1991**, *19*, 2979-2986.
9. Jastorff, B.; Hettler, H. *Chem. Ber.* **1969**, *102*, 4119-4127.
10. Mungall, W. S.; Greene, G. L.; Heavner, G. A.; Letsinger, R. L. *J. Org. Chem.* **1975**, *40*, 1659-1662.
11. Letsinger, R. L.; Heavner, G. A. *Tetrahedron Lett.* **1975**, 147-150.
12. Hata, T.; Yamamoto, I.; Sekine, M. *Chem. Lett.* **1976**, 601-604.
13. Gibbs, D. E. *Tetrahedron Lett.* **1977**, 679-682.
14. Maag, H.; Schmidt, B.; Rose, S. J. *Tetrahedron Lett.* **1994**, *35*, 6449-6452.
15. Bannwarth, W. *Helv. Chim. Acta* **1988**, *71*, 1517-1527.
16. Chen, J. K.; Schultz, R. G.; Lloyd, D. H.; Gryaznov, S. M. *Nucleic Acids Res.* **1995**, *23*, 2661-2668.
17. Atherton, F. R.; Openshaw, H. T.; Todd, A. R. *J. Chem. Soc.* **1945**, 660-663.
18. Sobkowska, A.; Sobkowski, M.; Cieslak, J.; Kraszewski, A.; Kers, I.; Stawinski, J. *J. Org. Chem.* **1997**, *62*, 4791-4794.
19. Bollmark, M.; Stawinski, J. *Tetrahedron Lett.* **1996**, *37*, 5739-5742.
20. Jankowska, J.; Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1994**, *35*, 3355-3358.
21. Stawinski, J.; Thelin, M.; Westman, E.; Zain, R. *J. Org. Chem.* **1990**, *55*, 3503-3506.
22. Yamamoto, I.; Sekine, M.; Hata, T. *J. Chem. Soc. Perkin Trans. 1* **1980**, 306-310.
23. Glinski, R. P.; Khan, M. S.; Kalamas, R. L.; Sporn, M. B. *J. Org. Chem.* **1973**, *38*, 4299-4305.
24. In the absence of triethylamine, the reactions did not go to completion, most likely due to a partial protonation of the amino function of nucleoside **4** by acidic species present in the reaction mixtures (pyridinium hydrochloride).
25. Due to the presence of TMS-Cl, the produced **6** and **7** may undergo trimethylsilylation under the reaction conditions, as it was apparent from the multiplicity of the corresponding ^{31}P NMR resonances and the observed changes in their chemical shift values after aqueous work up and isolation of compounds **6** and **7**.
26. Czernecki, S.; Valery, J.-M. *Synthesis* **1991**, 239-240.
27. Matsuda, A.; Satoh, M.; Ueda, T.; Machida, H.; Sasaki, T. *Nucleosides & Nucleotides* **1990**, *9*, 587-597.
28. Stawinski, J.; Szabó, T.; Thelin, M.; Westman, E.; Zain, R. *Collect. Czech. Chem. Commun.* **1990**, *55*, 141-144.
29. *Typical procedure:* **2** or **3** ($TEAH^+$ salt, 0.5 mmol) was rendered anhydrous by evaporation of added pyridine, dissolved in the same solvent (10 mL), and treated consecutively with TMS-Cl (1.5 mmol) and (after 5 min) with iodine (1.5 mmol, pyridine 1 mL). After 5 min the appropriate aminonucleoside (**4** or **5**, 0.5 mmol) together with triethylamine (2.5 mmol) in pyridine (2.5 mL), was added. The reactions went to completion within 5 min. The solvent was evaporated in *vacuo*, the residues partitioned between 10 % $Na_2S_2O_3$ in saturated brine (20 mL) and chloroform (4 x 20 mL). The organic phase was washed with 1 M TEAB (20 mL), dried over Na_2SO_4, concentrated, and purified on reversed phase silica gel using a stepwise gradient of methanol (0-80%) in water. The isolated compounds were precipitated from petroleum ether : diethyl ether (6:4, v/v). Yields: **6a** (72%), **6b** (89%), **6c** (69%), and **7** (79%). The identity of **6** and **7** was confirmed by ^1H, ^{13}C, ^{31}P NMR, (^1H,^1H) COSY, (^1H, ^{13}C) COSY, and TLC analyses.