Application of an Intramolecular Michaelis–Arbusov Reaction to the Synthesis of Nucleoside 3′-*S*,5′-*O*-Cyclic Phosphorothiolate Triesters

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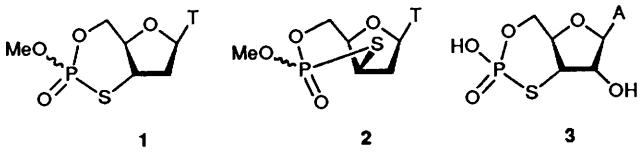
An intramolecular Michaelis–Arbusov reaction taking place between a 5′-phosphite and a 3′-*S*-disulfide can be used to prepare nucleoside 3′-*S*,5′-*O*-cyclic phosphorothiolate triesters.

The continuing search for effective anti-viral agents that are able to by-pass kinase-mediated activation, has maintained the interest in the study of nucleotide triester analogues as potential masked phosphate pro-drugs.[1] Partly for this reason we have become interested in the synthesis of nucleoside 3′-*S*,5′-*O*-cyclic phosphorothiolate triesters (*e.g.* **1** and **2**) which may breakdown



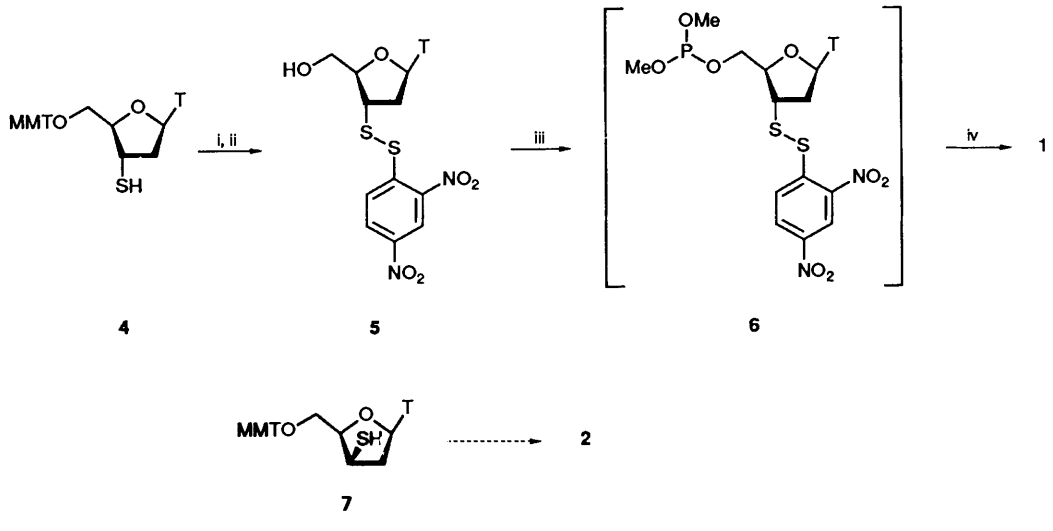
1 **2** **3**

T = Thymin-1-yl; A = Adenin-9-yl

intracellularly, by hydrolysis of the phosphorus–sulfur bond, to release the nucleoside 5′-monophosphate. However, perhaps more importantly, compounds such as **1** are interesting analogues of nucleoside 3′,5′-cyclic phosphates which do not appear to be readily available through previously published procedures. A search of the literature has revealed that whilst 3′-thioadenosine 3′-*S*,5′-*O*-cyclic phosphorothiolate **3** has previously been prepared by initial reaction of 3′-thioadenosine with phosphoryl chloride, the isolated yield was only 2–4%.[2] Here we report that an intramolecular Michaelis–Arbusov reaction taking place between a 5′-phosphite and a 3′-*S*-disulfide (Scheme 1) can be used to prepare nucleoside 3′-*S*,5′-*O*-cyclic phosphorothiolate triesters derived from 2′-deoxy-3′-thiothymidine **1** and 1-(2-deoxy-3-thio-β-D-xylofuranosyl)thymine **2**. An intermolecular variation of this reaction has previously been reported for the synthesis internucleoside 3′-*S*-phosphorothiolate linkages.[3]

Treatment of 5′-*O*-monomethoxytrityl-2′-deoxy-3′-thiothymidine[4] (**4**, Scheme 1) with an excess of 2,4-dinitrobenzenesulfenyl chloride in THF at 0 °C and subsequent detritylation with acetic acid gave 3′-(2,4-dinitrophenyldithio)-2′,3′-dideoxythymidine **5** in 79% yield after chromatography. Initial attempts to generate the phosphite **6** by reaction of *N*,*N*-diisopropylaminodimethoxyphosphine[5] with **5**, in the presence of tetrazole at room temperature, and effect its *in situ* cyclisation, resulted in a very poor yield of the cyclic phosphorothiolate **1**. The low yield appeared to be largely due to a side reaction in which *N*,*N*-diisopropylaminodimethoxyphosphine and the disulfide **5** participate directly in a Michaelis–Arbusov reaction. However, reaction of **5** with the less nucleophilic dimethoxychlorophosphine, in the presence of 2,6-lutidine at −70 °C, to form the intermediate **6** and reaction for a further four hours at room temp. gave **1** as a very unequal mixture (20 : 1) of diastereoisomers in a combined yield of 58%. Analysis of the mixture by ^{31}P NMR spectroscopy showed that the predominant isomer (17.93 ppm) resonated upfield from the minor isomer (21.42 ppm). From studies with the all oxygen-containing nucleoside 3′,5′-cyclic phosphate alkyl esters it is generally established that the axial isomers resonate upfield from the equatorial isomers in the ^{31}P NMR spectrum.[6] Comparison with our data strongly suggests, therefore, that the predominant, upfield, isomer has the axial orientation of the OMe group.

For the synthesis of the cyclic phosphorothiolate triester (**2**, Scheme 1), 1-(2-deoxy-5-*O*-monomethoxytrityl-3-thio-β-D-



4 **5** **6** **7**

Scheme 1 *Reagents and conditions:* i, 2,4-Dinitrobenzenesulfenyl chloride, THF, 0 °C to room temperature; ii, 80% AcOH; iii, dimethoxychlorophosphine, 2,6-lutidine, THF–toluene (1 : 9, v/v), −78 °C, 1 h; iv, room temperature, 4 h

xylofuranosyl)thymine* **7** was used as the starting material and the synthetic procedure was directly analogous to that described for the preparation of **1**. In this case, the yield in the cyclisation step was 50% and the two diastereoisomers of **2** were formed in equal proportions.

In conclusion, we have shown that an intramolecular Michaelis–Arbusov reaction provides, what appears to be, a generally applicable route to nucleoside cyclic phosphorothiolate triesters and this type of reaction should prove useful in the synthesis of a variety of cyclic nucleotide analogues.

Experimental

3′-(2,4-*Dinitrophenyldithio*)-2,3-*dideoxythymidine* **5**.—The nucleoside thiol **4** (530 mg, 1.0 mmol, dried by co-evaporation with pyridine to yield a thick oil) was dissolved in dry THF (10 cm^3) and added dropwise to a stirred and cooled (ice bath) solution of 2,4-dinitrobenzenesulfenyl chloride (469 mg, 2.0 mmol) in THF (20 cm^3). After a further 30 min at this temperature the mixture was allowed to warm to room temp. and was then stirred for 1 h. The THF was evaporated, the residue was dissolved in a solution of 80% acetic acid in water (20 cm^3) and stirred at room temp. overnight. The aqueous acid was removed under reduced pressure and the residue purified by flash chromatography on silica, eluting with dichloromethane containing methanol (3–5%). The appropriate fractions were combined and evaporated to yield the *disulfide* (361 mg, 79%) as a yellow solid; m.p. 202–203 °C (Found: C, 41.8; H, 3.57; N, 12.0. $C_{16}H_{16}N_4O_8S_2$ requires C, 42.10; H, 3.53; N, 12.3%); δ_H[200 MHz; $(CD_3)_2CO$] 10.0 (1 H, br, NH), 9.02 (1 H, d, *J* 2.0, ArH), 8.69 (2 H, m, ArH), 7.82 (1 H, s, 6-H), 6.13 (1 H, t, *J* 6.8, 1′-H), 5.62 (1 H, s, OH), 4.50 (1 H, m, 4′-H), 4.08 (1 H, m, 3′-H), 3.93 (2 H, m, 5′-H and 5″-H), 2.57 (2 H, m, 2′-H and 2″-H) and 1.76 (3 H, s, CH_3); *m/z* (FAB 2-nitrobenzylalcohol) 457 $(M + H)^+$.

General Procedure for Synthesis of Cyclic Phosphorothiolate Triesters.—The nucleoside disulfide **5** (200 mg, 0.438 mmol) was dried by co-evaporation with dry pyridine (2 × 5 cm^3) and dry toluene (2 × 5 cm^3) and dissolved in dry THF–toluene (1:9, v/v, 10 cm^3). The solution was cooled to −78 °C and 2,6-lutidine (175 mm^3, 1.53 mmol) and dimethoxychlorophosphine (150 mm^3, 1.31 mmol) added with stirring under a nitrogen atmosphere. After 1 h the reaction mixture was allowed to warm to room temp. and was maintained at this temperature for a further 4 h. The reaction mixture was diluted into dichloromethane (10 cm^3), extracted successively with sat. sodium hydrogen carbonate (10 cm^3) and brine (10 cm^3), dried and the organic solvents evaporated under reduced pressure. The residue was purified by flash chromatography on silica, eluting with dichloromethane containing methanol (4–5%) and the appropriate fractions were combined, evaporated and precipitated into light petroleum (40–60 °C) to yield the *cyclic phosphorothiolate* **1** as separate diastereoisomers in a combined yield of 58%. Analytical data for the major isomer, δ_H(200 MHz; $CDCl_3$) 8.77 (1 H, br, NH), 7.02 (1 H, s, 6-H), 6.03 (1 H, dd, *J* 8.2 and 2.6, 1′-H), 4.70–4.33 (2 H, m, 5′-H and 5″-H), 4.05 (1 H, m, 4′-H), 3.93 (3 H, d, *J* HP 12.9, OMe), 3.52 (1 H, m, 3′-H), 2.56–2.39 (2 H, m, 2′-H and 2″-H) and 1.95 (3 H, s, CH_3); δ_P(81 MHz; $CDCl_3$) 17.93; *m/z* (FAB 2-nitrobenzylalcohol) 335 $(M + H)^+$ [Found: $(M + H)^+$, 335.0475. $C_{11}H_{16}N_2O_6PS$ requires $(M + H)^+$, 335.0467].

Acknowledgements

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* This compound was prepared from 5′-*O*-monomethoxytrityl-3′-*O*-methanesulfonylthymidine by a procedure directly analogous to that described for the synthesis of **4**.[4]