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A new method for the synthesis of nucleoside 2′,3′-*O*,*O*-cyclic phosphorodithioates via aryl cyclic phosphites as intermediates

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Abstract—The reaction of 5′-*O*-protected ribonucleosides with tri(4-nitrophenyl) phosphite in the presence of pyridine furnished rapid formation of the corresponding 4-nitrophenyl 2′,3′-*O*,*O*-cyclic phosphites which upon sulfhydrolysis, followed by sulfurization of the resultant cyclic H-phosphonothioate and removal of the 5′-*O*-protecting groups, afforded nucleoside 2′,3′-*O*,*O*-cyclic phosphorodithioates in high yields.

Nucleoside 2′,3′-*O*,*O*-cyclic phosphates have been exploited for years in the biochemistry and molecular biology of nucleic acids as indispensable tools for establishing substrate specificity of various nucleases and in investigations of chemical and enzymatic aspects of ribonuclease catalysed reactions.[1] Although their biological significance is less clear than that of nucleoside 3′,5′-cyclic phosphates, recent studies implicate the involvement of five-membered cyclic phosphodiesters in various metabolic pathways.[2,3] Rapidly growing interest in self-splicing of RNA[4] and in the development of ribozymes for therapeutical purposes[5] again places nucleoside 2′,3′-*O*,*O*-cyclic phosphates in the forefront of synthetic and mechanistic chemistry,[6,7] with the aim to provide chemical support for diverse biological studies involving these compounds.

Due to the importance of nucleotide analogues in elucidation of mechanisms of enzymatic reactions,[8] we have initiated a program on the chemistry and biochemistry of nucleoside 2′,3′-*O*,*O*-cyclic phosphates and their analogues to study the effects of modification at the phosphorus centre on the conformational equilibria and flexibility of the fused five-membered rings in these compounds in the context of their interaction with RNases. In connection with these, we recently developed a new efficient method for the synthesis of nucleoside 2′,3′-*O*,*O*-cyclic phosphorothioates. This method was based on phosphonylation of suitably 5′-*O*-protected nucleosides with diphenyl H-phosphonate to produce nucleoside 2′,3′-*O*,*O*-cyclic H-phosphonates, which via oxidation with elemental sulfur provided the corresponding nucleoside 2′,3′-*O*,*O*-cyclic phosphorothioates in high yield.[9]

As part of this program, we became interested in a largely unexplored class of nucleotide analogues, nucleoside 2′,3′-*O*,*O*-cyclic phosphorodithioates for which only one representative, uridine 2′,3′-*O*,*O*-cyclic phosphorodithioate, was known. This compound was obtained from 5′-*O*-acetyluridine and P_2S_5 at elevated temperature,[10] but the reaction produced a complex mixture of products from which the desired cyclic phosphorodithioate was isolated in low yield (ca. 28%) after tedious ion-exchange chromatography on DEAE-cellulose.[10]

Inspired by the ease of formation of 2′,3′-*O*,*O*-cyclic H-phosphonates using diphenyl H-phosphonate in the synthesis of nucleoside 2′,3′-*O*,*O*-cyclic phosphorothioates,[9] we attempted the same strategy for the preparation of 2′,3′-*O*,*O*-cyclic phosphorodithioates, by making use of diphenyl H-phosphonothioate **2** to generate the 2′,3′-*O*,*O*-cyclic H-phosphonothioates **3** as a key intermediate (Scheme 1). Although the desired H-phosphonothioate **3a** always constituted the major product of the reaction of **1** and **2**, and could be easily converted into cyclic phosphorodithioate **4a** by treatment with elemental sulfur (vide infra), a parallel disproportiona-

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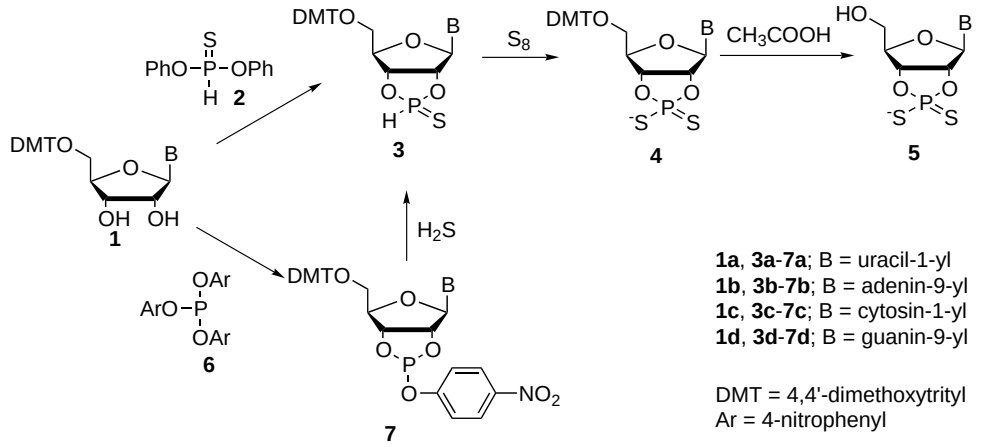

Scheme 1.

tion of diphenyl H-phosphonothioate **2** towards triphenyl phosphite and phenyl H-phosphonodithioate,[11] together with side-reactions involving **3a**, lowered the yield and made chromatographic purification of **4a** (or **5a**) troublesome.

To overcome these problems, we searched for another route to produce cyclic H-phosphonothioate intermediates of type **3** and considered sulfhydrolysis of 4-nitrophenyl cyclic phosphite **7** as a viable alternative for this purpose. We found that the required phosphite intermediate **7** can be efficiently produced by reacting 5′-*O*-protected nucleosides of type **1** with a crystalline reagent, tri(4-nitrophenyl) phosphite **6** (prepared in large scale by modification[12] of the Walsh method[13]). ^{31}P NMR spectroscopy showed that formation of phosphite **7a** from uridine derivative **1a** and reagent **6** (1.1 equiv.) in DMF/pyridine (9:1, v/v) was rapid (<3 min) and clean. It was apparent from the presence in the spectrum of only two resonances in the range of chemical shifts for cyclic phosphite derivatives [$\delta_P = 140.90$ ppm (s) and 146.40 ppm (t, $^3J_{HP} = 10.2$ Hz)] that two diastereoisomers of **7** had been formed. To ensure that the use of triaryl phosphite **6** is compatible with the presence of unprotected amino functions in nucleosidic substrates, in separate experiments we treated 3′,5′-*O*,*O*-di(*t*-butyldimethylsilyl) 2′-deoxyadenosine, 2′-deoxycytidine and 2′-deoxyguanosine with an excess (3 molar equiv.) of tris(4-nitrophenyl) phosphite **6**. No signals which could be assigned to *N*- or *O*-phosphitylated species produced from nucleobases and reagent **6** were observed within 20 min by ^{31}P NMR spectroscopy.

Next, the susceptibility of intermediate **7** to sulfhydrolysis was investigated. To this end, nucleoside 4-nitrophenyl 2′,3′-*O*,*O*-cyclic phosphite **7a** (produced in situ from nucleoside **1a** and **6** as described above) was treated with an excess of H_2S (5 molar equiv., 1 M stock solution in dioxane). The reaction was rapid (<3 min, ^{31}P NMR) and afforded a new compound (ca. 90%) [$\delta_P = 87.70$ ppm ($^1J_{HP} = 661.9$ Hz, brd) and 91.16 ppm ($^1J_{HP} = 659.0$ Hz, $^3J_{HP} = 10.2$ Hz, dd)], which was identical (chemical shifts, coupling constants and chemical reactivity) to that produced from nucleoside **1a** and diphenyl H-phosphonothioate (nucleoside 2′,3′-*O*,*O*-H-phosphonothioate **3a**, vide supra).[14] As the only side product in this reaction, we observed the formation of variable amounts (10–15%) of the corresponding nucleoside 2′,3′-*O*,*O*-cyclic H-phosphonate ($\delta_P = 23.22$ and 27.22 ppm, $^1J_{HP} = 659.0$ and $^1J_{HP} = 659.0$ Hz),[9] which we assumed to be due to the presence of adventitious water in the reaction mixture. To remedy this problem, we carried out the sulfhydrolysis of cyclic phosphite **7a** in the presence of trimethylsilyl chloride (TMSCl, 15 molar equiv.). This completely eliminated the interfering hydrolysis of the intermediate **7** and nucleoside 2′,3′-*O*,*O*-cyclic H-phosphonothioate **3a** could be produced as the sole nucleotidic species (^{31}P NMR). Addition of elemental sulfur (3 molar equiv.) directly to the reaction mixture furnished rapid (ca. 5 min, ^{31}P NMR) and clean sulfurisation to produce 2′,3′-*O*,*O*-phosphorodithioate **4a** (^{31}P NMR). Because phosphorodithioate **4a** was always the sole nucleotidic product present in the reaction mixture (ca. 90% of total phosphorus species, ^{31}P NMR), the final deprotection (80% acetic acid, 20 min)[15] was performed without prior purification. The unprotected uridine 2′,3′-*O*,*O*-cyclic phosphorodithioate **5a** was isolated by simple silica gel chromatography and obtained as a white amorphous solid after freeze-drying (95% yield).

When the reaction conditions developed for the synthesis of uridine derivative **5a** were applied to the other 5′-*O*-(4,4′-dimethoxytrityl)nucleosides **1b–d**, the corresponding 2′,3′-*O*,*O*-cyclic phosphorodithioates **5b–d** were also obtained in high yields (84–90%). In all instances investigated, high efficiency of the individual steps eliminated the need for chromatographic purification of the intermediates involved (compounds of type **3**, **4** or **7**) and the final products, nucleoside 2′,3′-*O*,*O*-cyclic phosphorodithioates of type **5**, could be isolated in excellent yields using silica gel chromatography (vide supra).

A typical procedure for the preparation of nucleoside 2′,3′-*O*,*O*-cyclophosphodithioates **5**: A solution of tri(4-nitrophenyl) phosphite **6** (1.1 molar equiv.) in DMF/

pyridine (9:1, v/v) (10 mL; sometimes gentle heating was necessary to dissolve **6**) was added to 5′-*O*-(4,4′-dimethoxytrityl)nucleoside of type **1** (1 mmol; made anhydrous by repeated evaporation of added anhydrous pyridine). The aryl nucleoside cyclic phosphite of type **7** formed was treated after 5 min with a mixture of H_2S (5 molar equiv., 1 M solution in dioxane) and trimethylsilyl chloride (15 molar equiv.) and then (after another 5 min) elemental sulfur (3 molar equiv.) was added. When the sulfurisation was complete (ca. 5 min, ^{31}P NMR and TLC), the reaction mixture was neutralised with 5% aq. $NaHCO_3$ (5 mL) and the solvents were removed by evaporation under reduced pressure. The residue was dissolved in methylene chloride containing triethylamine (1%, v/v; 30–40 mL), washed with 5% aq. $NaHCO_3$ (3×10 mL) and the organic layer evaporated. The removal of the 5′-*O*-dimethoxytrityl group was effected by treatment of the oily residue with 80% aq. acetic acid (10 mL) during 20 min. After evaporation of acetic acid, the crude product was dissolved in a minimum volume of methylene chloride/methanol (4:1, v/v) and applied on a silica gel column pre-equilibrated with methylene chloride/triethylamine (99:1, v/v). Chromatography was performed using a stepwise gradient (0–10%, v/v) of methanol in methylene chloride containing triethylamine (1%, v/v). The fractions containing pure product were collected, evaporated and freeze-dried from benzene/methanol (4:1, v/v). Cyclic phosphorodithioates **5** (triethylammonium salts) were obtained as white amorphous solids (purity >98%, ^1H NMR). Yields: **5a** 95%, **5b** 85%, **5c** 90%, **5d** 84%.[†]

In conclusion, we have developed a new, general protocol for the preparation of nucleoside 2′,3′-*O*,*O*-cyclic phosphorodithioates of type **5**. The method relies on sulfhydrolysis of 4-nitrophenyl cyclic phosphite **7** [accessible from ribonucleosides using the stable, crystalline, and readily available phosphitylating agent, tris(4-nitrophenyl) phosphite **6**], followed by oxidation of the produced cyclic H-phosphonothioate **3** with elemental sulfur. All transformations involved can be carried out as 'a one-pot reaction', and are compatible with nucleosidic substrates bearing unprotected amino functions. The method is very efficient and experimentally simple.

[†] Chemical identity of compounds **5a**–**d** was confirmed by, ^1H, ^{31}P NMR and HRMS. **5a**, δ_H (D_2O) 1.32 (9H, t, J=7.5 Hz, CH_2CH_3), 3.22 (6H, q, J=7.5 Hz, CH_2CH_3), 3.79 (1H, m, 5′, 5″-H_2), 4.42 (1H, m, 4′-H), 4.96 (1H, m, 3′-H), 5.07 (1H, m, 2′-H), 5.68 (1H, d, J=7.5 Hz, 5-H), 6.06 (1H, d, J=2.4 Hz, 1′-H), 7.78 (1H, d, J=7.5 Hz, 6-H); δ_P (D_2O) 138.22 (dd, $^3J_{HP}$=10.1 and 7.3 Hz); m/z 336.9740, calculated for $[C_9H_{10}N_2O_6PS_2]^-$ 336.9718. **5b**, δ_H (D_2O) 1.09 (9H, t, J=7.5 Hz, CH_2CH_3), 2.97 (6H, q, J=7.5 Hz, CH_2CH_3), 3.74 (2H, m, 5′, 5″-H_2), 4.44 (1H, m, 4′-H), 5.12 (1H, m, 3′-H), 5.40 (1H, m, 2′-H), 6.29 (1H, d, J=4.2 Hz, 1′-H), 8.06 (1H, s, 2-H), 8.16 (1H, s, 8-H); δ_P (D_2O) 137.65 (dd, $^3J_{HP}$=11.9 and 5.5 Hz); m/z 359.9992, calculated for $[C_{10}H_{11}N_5O_4PS_2]^-$ 359.9990. **5c**, δ_H (D_2O) 1.30 (9H, t, J=7.5 Hz, CH_2CH_3), 3.19 (6H, q, J=7.5 Hz, CH_2CH_3), 3.78 (2H, m, 5′, 5″-H_2), 4.44 (1H, m, 4′-H), 4.98 (1H, m, 3′-H), 5.03 (1H, m, 2′-H), 5.95 (1H, d, J=7.5 Hz, 5-H), 6.05 (1H, d, J=2.4 Hz, 1′-H), 7.81 (1H, d, J=7.5 Hz, 6-H); δ_P (D_2O) 137.10 (dd, $^3J_{HP}$= 11.9 and 6.4 Hz); m/z 335.9852, calculated for $[C_9H_{11}N_3O_5PS_2]^-$ 335.9878. **5d**, δ_H (D_2O) 1.30 (9H, t, J=7.3 Hz, CH_2CH_3), 3.22 (6H, q, J=7.3 Hz, CH_2CH_3), 3.80 (2H, m, 5′, 5″-H_2), 4.49 (1H, m, 4′-H), 5.18 (1H, m, 3′-H), 5.38 (1H, m, 2′-H), 6.24 (1H, d, J=3.9 Hz, 1′-H), 7.93 (1H, s, 8-H); δ_P (D_2O) 136.91 (t, $^3J_{HP}$=8.7 Hz); m/z 375.9947, calculated for $[C_{10}H_{11}N_5O_5PS_2]^-$ 375.9939.

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12. 4-Nitrophenol (4.2 g, 30 mmol; rendered anhydrous by repeated addition and evaporation of excess acetonitrile) and phosphorus trichloride (1.4 g, 10 mmol) were refluxed in acetonitrile (50 mL) for 3 days under slightly reduced pressure to evacuate the HCl evolved. After cooling to rt and concentration to half of the initial volume, white crystals of product **6** precipitated and were filtered off and dried. Yield 3.5 g (80%). ^{31}P NMR δ_P (DMF) 126.17 ppm; m/z 445.0301, calculated for $C_{18}H_{12}N_3O_9P$ 445.0311; mp=174–176°C.
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14. In separate experiments we showed that the integrity of the phospholane ring in **3a** remained intact upon prolonged (3–4 h) treatment with hydrogen sulfide (^{31}P NMR).
15. On this occasion we also studied the stability of the phosphorodithioates moiety in 2′,3′-cyclic phosphates under acidic conditions [see Ora et al. (Ref. 16) on the pH-dependent desulfurisation of nucleoside phosphorodithioates and their 2′,3′-*O*,*O*-phosphorodithioates]. When uridine 2′,3′-*O*,*O*-cyclic phosphorodithioate **5a**

was kept in 80% aqueous acetic acid for 8 h at 25°C, ^{31}P NMR spectroscopy and TLC analysis did not show the formation of any products of potential degradation of **5a** (desulfurisation, dephosphorylation, etc.). This indicated that the conditions used for the removal of the dimethoxytrityl group from **4** (80% aq. acetic acid, rt, 20 min) were not detrimental to 2′,3′-*O*,*O*-cyclic phosphorodithioates of type **5**.

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