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A new, efficient entry to nucleoside 2′,3′-*O*,*O*-cyclophosphorothioates

Jadwiga Jankowska,[a] Małgorzata Wenska,[a] Mariusz Popenda,[a] Jacek Stawiński [b] and Adam Kraszewski [a,*]

[a] *Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland*
[b] *Department of Organic Chemistry, Stockholm University, Arrhenius Laboratory, S-106 91 Stockholm, Sweden*

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Abstract

The reaction of 5′-protected ribonucleosides with diphenyl *H*-phosphonate in pyridine furnished rapid formation of the corresponding 2′,3′-cyclic *H*-phosphonates **3**, which upon sulfurisation and the subsequent removal of the 5′-protecting group, afforded nucleoside 2′,3′-*O*,*O*-cyclophosphorothioates **5** in high yields.

Keywords: nucleosides; nucleotides; cyclic phosphorothioates; synthesis.

Nucleoside 2′,3′-cyclic phosphates have, for a long time been in the focus of chemical research,[1] not least due to their synthetic utility[2] and the enormous acceleration observed for reactions that involve phosphorus in five-membered rings.[3] Although the biological significance of 2′,3′-cyclic phosphates per se is far from clear, the importance of ribonuclease-[4] and ribozyme-catalysed[5] reactions that involve cyclic phosphates as intermediates, make this class of phosphorus compounds an indispensable research tool in mechanistic bioorganic phosphorus chemistry and in molecular biology.

While a variety of nucleoside 3′,5′-cyclic phosphates have been investigated,[6–8] analogues of 2′,3′-cyclic phosphates are difficult to prepare and only nucleoside 2′,3′-*O*,*O*-phosphorothioates have received attention in conjunction with studies of stereochemical aspects of ribonuclease-catalysed reactions.[9,10] These compounds are usually prepared via the reactions of 5′-*O*,*N*-protected ribonucleosides with thiophosphoryl chloride,[10,11] cyclisation of nucleoside 2′(3′)-phosphorothioate derivatives,[12] or by using salicylic chlorophosphite as a phosphitylating agent.[13] Yields of these reactions (in most instances determined only by UV-spectroscopy) were invariably low (6–10%)[10–12] and with the most efficient, recent method,[13] did not exceed 40%.

During our studies on phosphonylation of 2′,3′-unprotected ribonucleosides with *H*-phosphonate monoesters in the presence of pivaloyl chloride we observed rapid formation of equimolar amounts

* Corresponding author. Tel: 48-61-852-85-03; fax 48-61-852-05-32; e-mail: akad@ibch.poznan.pl (A. Kraszewski)

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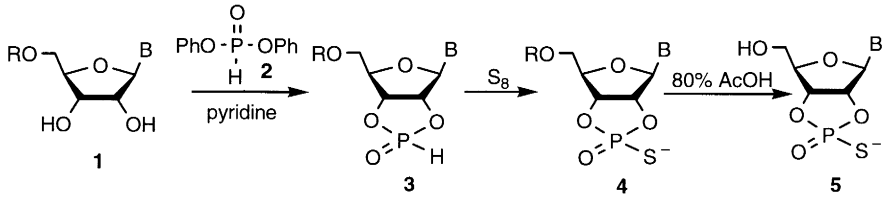
of symmetrical *H*-phosphonate diesters and nucleoside 2′,3′-cyclic *H*-phosphonates.[14] We attempted to exploit this finding for the development of a new method for the preparation of nucleoside 2′,3′-*O*,*O*-phosphorothioates using diphenyl *H*-phosphonate,[15] which is a commercially available, inexpensive phosphonylating reagent.

To this end, nucleoside **1a** in pyridine was treated with diphenyl *H*-phosphonate **2** (1.5 molar equiv.) (Scheme 1). The ^{31}P NMR spectroscopy revealed rapid formation of cyclic *H*-phosphonate **3a** [ca. 1:1 mixture of diastereomers; δ_P=23.22 ppm (ddd, $^1J_{PH}$=734.1 Hz, $^3J_{PH}$ 12.5 and 5.1 Hz) and 27.22 ppm (ddd, $^1J_{PH}$=731.3 Hz, $^3J_{PH}$=14.4 and 6.0 Hz)], which upon addition of elemental sulfur (3 molar equiv.), readily afforded the expected cyclic phosphorothioate **4a** [δ_P=76.74 ppm and 78.76 ppm (dd, $^3J_{PH}$=12.9 and 8.4 Hz)]. Using this approach the syntheses of 5′-*O*-protected nucleoside 2′,3′-*O*,*O*-cyclic phosphorothioates of type **4** were performed on a preparative scale (see below) and after removal of the 5′-*O*-dimethoxytrityl group (80% aqueous acetic acid, 10 min) the respective nucleoside cyclic phosphorothioates of type **5** were obtained in excellent yields (70–90%).



Scheme 1.

Typical procedure for the preparation of nucleoside 2′,3′-*O*,*O*-cyclophosphorothioates **5**: To a stirred solution of 5′-*O*-dimethoxytrityl nucleoside of type **1** (1 mmol; made anhydrous by repeated evaporation of added pyridine) in pyridine (10 mL) was added diphenyl *H*-phosphonate **2** (1.5 molar equiv). After 20 min (TLC analysis), the produced cyclic *H*-phosphonate of type **3** was oxidised with elemental sulfur (3 molar equiv.) for 5 min. The solvent was removed by evaporation and the residue was purified by silica gel column chromatography using a stepwise gradient of methanol (0–10%) in methylene chloride:triethylamine (95:5, v/v). The 5′-protected phosphorothioates **4** obtained (yields 85–95%) were treated with 80% acetic acid (10 mL) for 10 min, the mixtures were concentrated to dryness, co-evaporated with added isopropanol, dissolved in 0.1 M triethylammonium bicarbonate buffer (pH 7, 10 mL) and extracted with methylene chloride (3×20 mL). The aqueous phase was freeze-dried to afford **5** (triethylammonium salts) as white amorphous solids. Yields: **5a**, 93%; **5b**, 88%; **5c**, 78%; **5d**, 82%.†

In conclusion, the above protocol for the preparation of nucleoside 2′,3′-*O*,*O*-cyclophosphorothioates of type **5** represents a new, efficient and general entry to this class of compounds. It makes use of readily available starting materials, involves mild and efficient chemical transformations and does not require protection of the exocyclic amino groups in the substrates of type **1** nor use of phosphorus-protecting groups.

† Compounds **5a**–**d**, obtained as a mixture of diasteroisomers (ratio 1:1 for **5a**–**c** and 2:1 for **5d**) gave correct elemental analysis data and their chemical identity was confirmed by ^{1}H and ^{31}P NMR spectroscopy. Some diagnostic spectral data [compound, δ_P (D_2O), ($^3J_{PH}$)]: **5a**, 75.24 ppm (dd, 11.0 and 6.9 Hz), 76.60 ppm (dd, 12.4 and 7.3 Hz); **5b**, 75.20 ppm (dd, 11.1 and 6.5 Hz), 76.55 ppm (dd, 12.9 and 7.2 Hz); **5c**, 74.74 ppm (dd, 10.8, 6.7 Hz), 76.35 ppm (dd, 11.3, 8.8 Hz); **5d**, 74.71 ppm (dd, 10.2, 7.4 Hz), 76.22 ppm (t, 10.9 Hz).

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