Stereoselective formation of a P–P bond in the reaction of 2-alkoxy-2-thio-1,3,2-oxathiaphospholanes with *O*,*O*-dialkyl *H*-phosphonates and *H*-thiophosphonates†‡

Damian Błaziak, Piotr Guga, Agata Jagiełło, Dariusz Korczyński, Anna Maciaszek, Anna Nowicka, Aleksandra Pietkiewicz and Wojciech J. Stec*

Received 10th May 2010, Accepted 26th August 2010
DOI: 10.1039/c0ob00104j

A new method for the formation of organohypophosphates containing a P–P bond under mild conditions, based on the DBU-assisted reaction of 2-alkoxy-2-thio-1,3,2-oxathiaphospholanes with *O*,*O*-dialkyl *H*-phosphonates or *H*-thiophosphonates, has been elaborated. The resulting triesters of P^1-thio- and P^1,P^2-dithiohypophosphoric acids, respectively, having *O*-methyl or *O*-ethyl groups, can be selectively dealkylated to form the corresponding di- or monoesters. Appropriately protected 2′-deoxyguanosine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane) was converted into the corresponding P^1-thio- and P^1,P^2-dithiohypophosphate esters in a highly stereoselective manner (98%+ and 90%+, respectively).

Introduction

In spite of the wealth of chemical literature regarding the synthesis of compounds containing a P–P bond,[1,2] the number of methods for the synthesis of mono-, di-, tri- and tetraalkyl esters of hypophosphoric acids is limited. Monoesters of hypophosphoric acid were obtained by Setondji by DCC-assisted condensation of inorganic hypophosphate with an appropriate alcohol,[3] while tetraalkyl hypophosphates are available either by treatment of hypophosphoric acid with diazoalkanes,[4] or by condensation of sodium *O*,*O*-dialkyl phosphites with *O*,*O*-dialkyl phosphorochloridates. However, the latter process is accompanied by the formation of tetralkyl pyrophosphites, pyrophosphates and P^{III},P^{V}–mixed anhydrides, which are not easily separable from the desired hypophosphates.[5,6] P^1-Thio- and P^1,P^2-dithio-congeners of tetraalkyl hypophosphates have been obtained by condensation of *O*,*O*-dialkyl phosphorochloridothioates with sodium *O*,*O*-dialkyl phosphites or thiophosphites, respectively, but these reactions generate undesired side products.[7,8] The P–P bond can also be formed in the reaction of $(RO)_2P(O)Cl$ with diesters of phosphonic acids activated with the 1,3,2-dioxarsolan moiety ($(RO)_2P(O)$—$As(OCH_2)_2$).[9] In a recently developed approach, zirconium complexes have been prepared that effectively dehydrocouple primary and secondary phosphines *via* a σ-bond metathesis mechanism.[10] In general, such reactions require highly reactive substrates and rather harsh conditions. Therefore, a method for making the P–P bond starting from less reactive substrates under mild conditions would pave the way for synthesis of new compounds of biological importance. In the present paper we report on a method of synthesis of triesters of P^1-thio- and P^1,P^2-dithiohypophosphoric acid, *i.e.* compounds possessing (S)P—P(O) or (S)P—P(S) moieties, respectively. This approach is based on a reaction of P-chiral 2-alkoxy-2-thio-1,3,2-oxathiaphospholanes (including several nucleoside derivatives), having a tetracoordinate phosphorus atom with *O*,*O*-dialkyl *H*-phosphonates or *H*-thiophosphonates, respectively. The triesters carrying two methoxy or ethoxy groups at the P^2 atom can be sequentially dealkylated to form the corresponding 1,2-diesters and monoesters.

It should be mentioned that chirality of the P-atom in the oxathiaphospholane ring gives rise to racemic mixtures of the resulting esters of P^1-thio- and P^1,P^2-dithio-hypophosphoric acid. However, if the starting oxathiaphospholanes could be separated into pure enantiomers or P-diastereomers, the resulting hypophosphate products would be formed in a highly stereoselective manner, presumably with a stereoretentive outcome. This assumption is based upon the stereospecificity of the oxathiaphospholane ring opening condensation.[11]

The above mentioned method was used for the synthesis of nucleoside 3′-*O*-(P^1-thiohypophosphate)s and (P^1,P^2-dithiohypophosphate)s as well as their 5′-*O*- regioisomers, which can be further converted into corresponding nucleoside hypophosphates–the analogues of nucleoside *O*-diphosphates. Since ribonucleoside 5′-*O*-diphosphates are agonists of the $P2Y_n$ class of receptors and also are substrates for ribonucleotide reductase (RNR, an enzyme maintaining a proper level of 2′-deoxyribonucleotides in cells[12]), access to their analogues, which may be used as molecular tools for studies in molecular and cell biology, is of considerable interest.

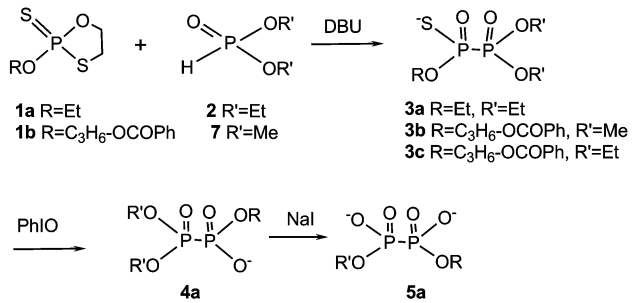
Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Department of Bioorganic Chemistry, Sienkiewicza 112, 90-363, Łódź, Poland. E-mail: wjstec@bio.cbmm.lodz.pl; Fax: +48-42-6815483; Tel: +48-42-6803248

† Electronic supplementary information (ESI) available: Synthesis of 3-*O*-benzoylpropyl ester of P^2-*O*,*O*-diethyl-P^1,P^2-dithiohypophosphoric acid, uridine 5′-*O*-hypophosphate and N^6-unprotected 5′-*O*-(2-thio-1,3,2-oxathiaphospholane)-2′,3′-*O*,*O*-diacetyladenosine, enzymatic phosphorylation of 5′-*O*-(P^1-thiohypophosphate) derivatives of nucleosides, measurement of inhibitory activity of nucleoside hypophosphates towards T7 RNA polymerase, calcium assay by fluorometric imaging plate reader (FLIPR), ^{31}P NMR proton decoupled spectra recorded for evaluation of stereochemistry of a P–P bond formation. See DOI: 10.1039/c0ob00104j

‡ Dedicated to Prof. Andrzej Zwierzak on the occasion of his 75th Birthday.

Results and discussion

In the course of the development of chemistry of P-substituted 2-thio-1,3,2-oxathiaphospholanes[11,13,14] it has been found that the DBU-assisted reaction of 2-ethoxy-2-thio-1,3,2-oxathiaphospholane (**1a**) with *O*,*O*-diethyl *H*-phosphonate (**2**) in CH_3CN (Scheme 1) provides P^1-*O*-ethyl-P^2-*O*,*O*-diethyl-P^1-thiohypophosphate (**3a**, R,R′ = Et), which is converted into triethyl hypophosphate (**4a**) upon treatment with iodosobenzene.[15,16] Finally, **4a** was dealkylated with sodium iodide[17] providing P^1-*O*-ethyl-P^2-*O*-ethyl hypophosphate (**5a**).

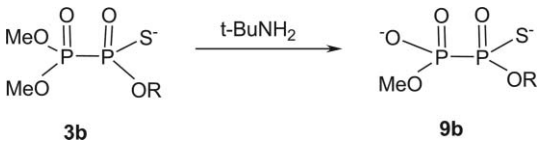


Scheme 1 Synthesis of P^1-*O*-alkyl-P^2-*O*,*O*-dialkyl-P^1-thiohypophosphates, P^1-*O*-ethyl-P^2-*O*,*O*-diethyl hypophosphate and P^1-*O*-ethyl-P^2-*O*-ethyl hypophosphate.

The molecular weight of **3a** was documented by FAB MS, and the presence of a P–P bond in **3a** and **4a** was confirmed by ^{31}P NMR spectra, which for each compound contained a pair of doublets with spin–spin coupling constants of 466 and 650 Hz, respectively.[18,19] Apparently, the P–P bond was formed by attack of the ambident, negatively charged phosphonyl ion (**6**, Scheme 2) at the phosphorus atom in compound **1**, followed by cleavage of the endocyclic P–S bond and elimination of thiirane.

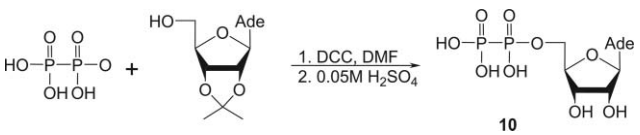
Since compounds **3a** and **4a** have poor UV-chromophores, their analysis by TLC and, especially, by HPLC techniques is cumbersome. Therefore, in next experiments we used a model substrate **1b** having the benzoyl moiety (Scheme 1). Compound **1b** was obtained by monobenzoylation of 1,3-propandiol in pyridine, followed by phosphitylation of the isolated material with 2-chloro-1,3,2-oxathiaphospholane (1.2 eq.) in the presence of elemental sulfur, and its identity was confirmed by ^{1}H NMR, ^{31}P NMR and FAB MS. The reaction of **1b** with an equimolar amount of $(MeO)_2P(O)H$ (**7**) and DBU (1.1 equiv.) in any of four anhydrous solvents, acetonitrile, diethyl ether, benzene, or dimethylformamide, after 3–12 h provided the expected P^1-*O*-(3-*O*-benzoylpropyl)-P^2-*O*,*O*-dimethyl-P^1-thiohypophosphate (**3b**) in 64–90% yield (by ^{31}P NMR). Similar reaction of **1b** with **2** in acetonitrile furnished P^2-*O*,*O*-diethyl-P^1-thiohypophosphoric derivative (**3c**) in 45% yield. Condensation of **1b** with $(EtO)_2P(S)H$ (**8**) in acetonitrile provided 3-*O*-benzoylpropyl ester of P^2-*O*,*O*-diethyl-P^1,P^2-dithiohypophosphoric acid (**3d**, Scheme 1S in ESI†) in 57% yield (by ^{31}P NMR).

In a typical workup, the compound **3b** was purified by ion-exchange chromatography on DEAE Sephadex A-25, eluted with a gradient 0,05–0,3M triethylammonium bicarbonate (TEAB), followed by conversion into sodium salt (DOWEX-Na^+) and lyophilization. Its identity was verified by ^{31}P NMR and FAB MS. Compound **3b** upon treatment with *t*-butylamine[20] (neat) was selectively demethylated to form P^2-*O*-methyl-P^1-thiohypophosphate derivative **9b** (Scheme 3). It was isolated on DEAE Sephadex A-25, converted into a sodium salt and lyophilized (total yield 25% calculated over **3b**).



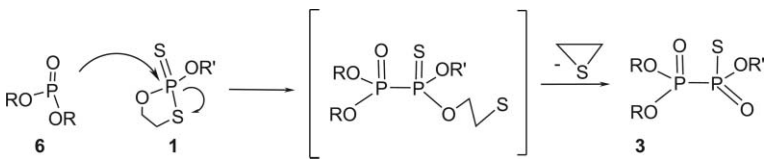
Scheme 3 Dealkylation of **3b** with *t*-butylamine: R = $CH_2CH_2CH_2$-OC(O)Ph.

Following this model study we turned to the nucleoside 5′-*O*-thiohypophosphate derivatives. Ebel and co-workers described a DCC-based method of synthesis of adenosine 5′-*O*-hypophosphate[3] (**10**, Scheme 4), which was proved in enzymatic tests to have substrate affinity towards pyruvate kinase.[21] This compound was also shown to be translocated into mitochondria, although it was not phosphorylated by the mitochondrial ATP synthetase.[22] In addition, attention has been paid to P^2,P^3-hypophosphate analogues of nucleoside 5′-*O*-triphosphates.[23]

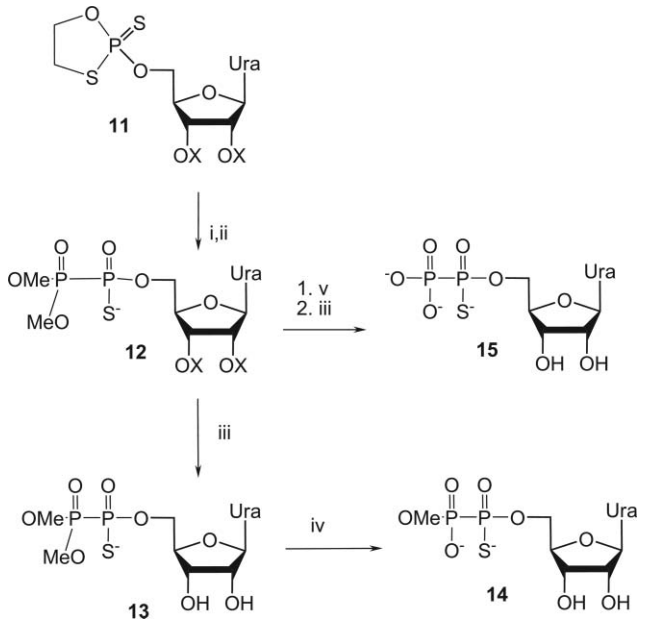


Scheme 4 Synthesis of adenosine 5′-*O*-hypophosphate by Setondji.[3]

Since Setondji's method is not suitable for synthesis of nucleoside 5′-*O*-(P^1-thiohypophosphates) we have used the oxathiaphospholane approach to obtain nucleoside 5′-*O*-(P^1-thiohypophosphates) and 5′-*O*-hypophosphates. As a model, 5′-*O*-(2-thio-1,3,2-oxathiaphospholane)-2′,3′-*O*,*O*-diisopropoxyacetyl-uridine (**11**, Scheme 5) was treated with **7.** The resulting 5′-*O*-(P^2-*O*,*O*-dimethyl-P^1-thiohypophosphate) derivative (**12**) upon ammonolytic removal of isopropoxyacetyl groups yielded uridine 5′-*O*-(P^2-*O*,*O*-dimethyl-P^1-thiohypophosphate) (**13**) in 38% yield (by ^{31}P NMR), further converted by treatment with *t*-butylamine into uridine 5′-*O*-(P^2-*O*-methyl-P^1-thiohypophosphate) (**14**) in virtually quantitative yield. Independently, treatment of **12** with trimethylsilyl bromide[24] and hydrolytic removal of trimethylsilyl and acyl groups with aqueous ammonia furnished uridine

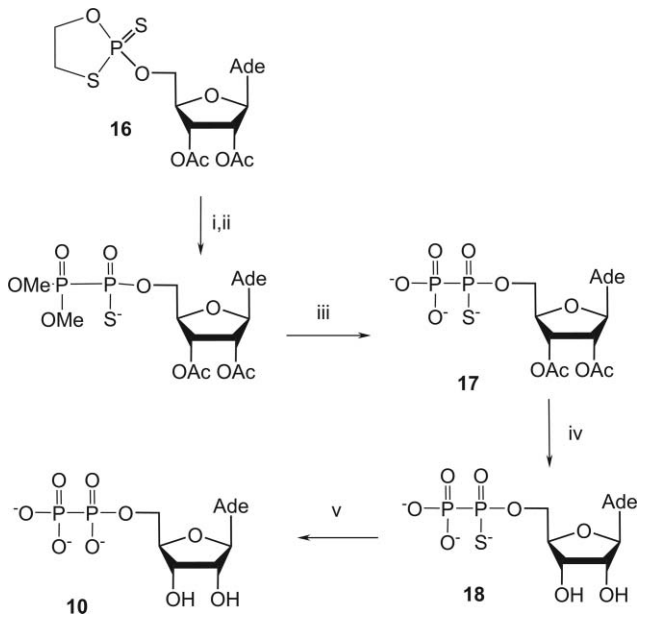


Scheme 2 Suggested mechanism of formation of the P–P bond.



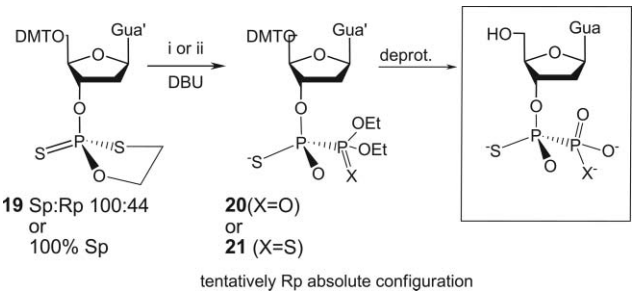
Scheme 5 Synthesis of uridine 5′-*O*-(P^1-thiohypophosphate) derivatives. X = isopropoxyacetyl. Reagents: (i) $(MeO)_2P(O)H$; (ii) DBU; (iii) $NH_4OH_{aq.}$; (iv) *t*-Bu-NH_2; (v) TMS-Br.

5′-*O*-(P^1-thiohypophosphate) (**15**). Next, N^6-unprotected 5′-*O*-(2-thio-1,3,2-oxathiaphospholane)-2′,3′-*O*,*O*-diacetyladenosine (**16**, Scheme 6, synthesis is described in the ESI†) was converted into adenosine 5′-*O*-(P^1-thiohypophosphate) (**18**). Oxidation of **18** with iodoxybenzene provided adenosine 5′-*O*-hypophosphate (**10**), identical to the compound described by Setondji.[3]



Scheme 6 Synthesis of adenosine 5′-*O*-(P^1-thiohypophosphate) and hypophosphate derivatives. Reagents: (i) $(MeO)_2P(O)H$; (ii) DBU; (iii) TMS-Br.; (iv) NH_4OH_{aq}; (v) $PhIO_2$.

As indicated earlier, the thiohypophosphate compounds **3** and **9** have a chiral phosphorus atom derived from their oxathiaphospholane precursor. Since the P-diastereomerically pure 3′-*O*-(1,3,2-oxathiaphospholane) derivatives of nucleosides (*e.g.* **19**, Scheme 7) were successfully used for synthesis of stereodefined oligonucleoside phosphorothioates (without epimerization at phosphorus),[14] we checked the stereoselectivity of the oxathiaphospholane ring opening in **19** with a negatively charged phosphonyl ion **6** and its phosphonothioyl counterpart. We prepared diastereomerically enriched (*fast* (S_P):*slow* (R_P) 100 : 44, by ^{31}P NMR, Figure 1S in the ESI†) 5′-*O*-DMT-N^{iBu},O^{DPC}-deoxyguanosine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane) (**19**, DPC = diphenylcarbamoyl).



Scheme 7 Synthesis of stereodefined nucleoside 3′-*O*-(P^1-thiohypophosphate) or 3′-*O*-(P^1,P^2-dithiohypophosphate). Gua′ = $Gua^{iBu,DPC}$; Reagents: X = O, (i) $(EtO)_2P(O)H$; X = S, (ii) $(EtO)_2P(S)H$. (The major S_P isomer of **19** is shown.).

This diastereomeric ratio range was selected because the content of the minor component is high enough to observe the resonances of interest for the P^1 atoms in both P-diastereomers of each product **20** and **21** (a pair of doublets, the resonances split due to the direct ^{31}P–^{31}P spin–spin coupling), and the presence of four signals provides evidence for sufficient resolution of the spectrum. Substrate **19** was reacted with **2** in the presence of DBU to give **20**. Its ^{31}P NMR spectrum (Fig. 1) contained resonances for P^1 atoms at δ 54.0 ppm (d, $^1J_{P-P}$ = 469 Hz, CD_3CN) and δ 54.4 ppm (d, $^1J_{P-P}$ = 466 Hz) at relative intensity 100 : 43, *i.e.* close to the diastereomeric ratio 100 : 44 of the substrate **19**.

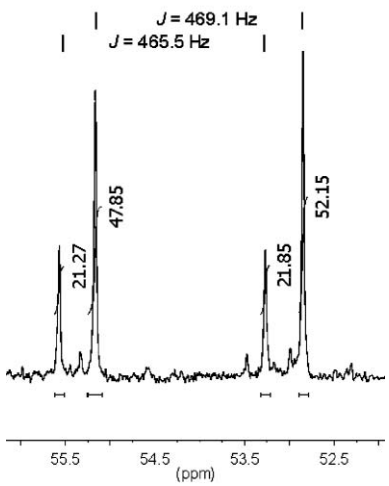


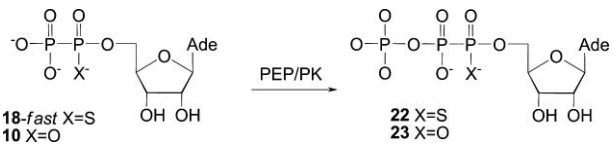
Fig. 1 A region of the ^{31}P NMR spectrum for the P^1 atoms in both P-diastereomers of 5′-*O*-DMT-N^{iBu},O^{DPC}-deoxyguanosine-3′-*O*-(P^2-*O*,*O*-diethyl-P^1-thiohypophosphate) (**20**). The vertical numbers represent integration data.

To assess precisely the stereoselectivity of the reaction, the same experiment was executed using diastereomerically pure **19** (100% *fast*-isomer) and the relevant region of the ^{31}P NMR spectrum recorded on a 500 MHz spectrometer (Figure 2S, see ESI†) did not contain any measurable signals of the epimerized product (to be seen as a doublet centered at δ 54.4 ppm (d, $^{1}J_{P-P}$ = 466 Hz). This result indicates that the DBU-assisted ring-opening condensation of 2-alkoxyl-2-thio-1,3,2-oxathiaphospholanes with *O*,*O*-dialkyl *H*-phosphonates is highly stereoselective (>98%). The presumed stereoretentive outcome (S_P-**19**→R_P-**20**/**21**) needs to be confirmed by X-ray or additional NMR measurements.

An analogous reaction of **19** (the mixture 100 : 44) with **8** yielded P^2-*O*,*O*-diethyl-P^1,P^2-dithiohypophosphate derivative **21** and the relevant region of the ^{31}P NMR spectrum (Figure 3S, see ESI†) contained resonances for the P^1 atoms at δ 58.5 ppm (d) and δ 58.9 ppm (d), $^{1}J_{P-P}$ = 352 Hz. However, their relative intensity 100 : 53, indicates that the reaction was slightly less stereoselective (*ca.* 90%).

When diastereomerically pure (or highly enriched) 5′-*O*-(2-thio-1,3,2-oxathiaphospholane) derivatives of nucleosides become available, it can be assumed that the similar stereochemistry and stereoselectivity will be observed leading to the desired stereodefined thiohypophosphate congeners of NDPs.

For preliminary biological evaluation, 5′-*O*-(P^1-thiohypophosphate) derivatives of adenosine (**18**), uridine (**15**) and cytidine (obtained analogously to the uridine derivative **15**) were used as mixed diastereoisomers. Only the adenosine derivative was accepted as a substrate by the purine nucleotide preferring[25] phosphoenolpyruvate/pyruvate kinase system (see ESI†) to form the hypo-analogue of ATPαS (**22**) (Scheme 8). Moreover, from the two P-diastereomers of adenosine 5′-*O*-(P^1-thiohypophosphate) (separated by RP-HPLC) only the *fast*-eluting diastereomer was transformed into the corresponding adenosine 5′-*O*-(P^1-thio-P^1,P^2-hypotriphosphate), *i.e.* P^1,P^2-hypophosphate analogue of ATPαS.[26] Its identity was confirmed by MALDI TOF mass spectrometry.



Scheme 8 Enzymatic conversion of adenosine 5′-*O*-(P^1-thiohypophosphate) or 5′-*O*-hypophosphate into the corresponding P^1,P^2-hypotriphosphate analogues (**22** or **23**) of ATPαS or ATP, respectively. PEP/PK, phosphoenolpyruvate/pyruvate kinase.

Diastereomerically pure **18**-*fast*, **18**-*slow*, **22** (derived from **18**-*fast*) and P-achiral adenosine 5′-*O*-(P^1,P^2-hypotriphosphate) (**23**, obtained from **10** analogously to **22**) were screened for their inhibitory activity towards T7 RNA polymerase (see ESI†). For the transcription reaction, the inhibitory concentrations IC_{50} of 2.90, 2.36, 0.58 and 0.55 mM, respectively, were determined, thus P^1,P^2-hypotriphosphate analogues of ATP and ATPαS were the most potent inhibitors of virtually the same activity.[27]

Interesting preliminary results were also obtained on the interactions of the sodium salt of uridine 5′-*O*-hypophosphate (synthesis is described in ESI†) and uridine 5′-*O*-(P^1-thiohypophosphate) **15** with the $P2Y_6$ receptor, which is a subtype of the human pyrimidinergic P2Y receptor. The assay consisted of measurements of intracellular calcium ion concentrations in mammalian cells (1321N1 astrocytoma cell line) that stably express the human receptor. Both compounds fully activated the $P2Y_6$ receptor (the thio analogue was slightly less potent than the oxo analogue, EC_{50} = 16 μM and 9 μM, respectively), although less potent than the endogenous agonist $U_{P3}U$, for which EC_{50} = 0.4 μM (Calcium assay description and data given in ESI†).[28] One can argue that 40- and 22-fold reductions in activity, respectively, are discouraging, especially when a remarkable family of UDP derivatives and analogues were synthesized and evaluated at $P2Y_2$, $P2Y_4$, and $P2Y_6$ subtypes of the P2Y receptor. For example, a large phenacyl substituent at N3 of UDP was well tolerated by the $P2Y_6$ receptor, yielding a potent and selective $P2Y_6$ receptor agonist (EC_{50} = 70 nM).[29] However, there could be an advantage of the hypophosphate analogues for their use in pharmacology experiments due to significantly higher nucleolytic stability of the P–P bond over the P–O–P bond while the unchanged nucleoside moieties may interact with proteins in virtually unaltered way. It was found that **10** was refractory to several hydrolases as bacterial alkaline phosphatase (phosphate-monoester phosphohydrolase, EC 3.1.3.1), lupin apyrase (ATP diphosphatase, EC 3.6.1.5) and snake venom phosphodiesterase (EC 3.1.15.1). Only snake venom 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) slowly hydrolyzed this compound, releasing adenosine and hypophosphate anion.[30]

Conclusion

In this report we present an extension of the oxathiaphospholane method to P–P bond formation under relatively mild conditions, and have applied it to the synthesis of hypophosphorylated alcohols (including nucleosides) and their P^1-thio- and P^1,P^2-dithio-congeners in moderate yield. The process of DBU-assisted oxathiaphospholane ring opening by *O*,*O*-dialkyl *H*-phosphonates or *O*,*O*-dialkyl *H*-thiophosphonates is highly stereoselective. Enhanced stability of the P–P bond in nucleoside hypophosphates and their P^1-thiohypophosphate congeners makes them potentially interesting models for studies on the biochemistry of nucleoside polyphosphates.

Experimental section

Nuclear magnetic resonance spectra (^{31}P NMR) were recorded on a Bruker AC-200 instrument (200 MHz for ^{1}H), or DRX-500 (500 MHz); 85% H_3PO_4 was used as an external standard. All chemical shifts are measured in units of ppm. FAB-MS spectra (13keV, Cs^+) were recorded on a Finnigan MAT 95 spectrometer, negative ion MALDI TOF mass spectra were recorded on a Voyager-Elite instrument (PerSeptive Biosystems Inc., Framingham, MA). HPLC analyses were performed using a binary HPLC system (ProStar, Varian Inc.). Anhydrous acetonitrile and 1,4-diazabicyclo[5.4.0]undec-7-ene (DBU) were supplied by Fluka and Acros Organics, respectively. *O*,*O*-Dialkyl *H*-phosphonates were purchased from Aldrich. 5′-*O*-(2-Thio-1,3,2-oxathiaphospholane) derivatives of *N*-protected nucleosides were synthesized as described earlier.[31] Nucleoside oxathiaphospholanes **19** were synthesized according to published methods.[14] Iodosobenzene was

a gift from Dr A. Łopusiński. Iodoxybenzene was synthesized according to the published procedure.[32]

3-Hydroxypropyl benzoate: To a cooled solution (0 °C) of 1,3-propandiol (1.89 mL; 0.026 mol) in pyridine (5 mL) benzoyl chloride (3 mL; 1 eq) was added dropwise. The mixture was stirred overnight at room temperature and the product isolated on a silica gel column eluted with $CHCl_3$:acetone (9 : 1, v/v) (1.79 g, 40%). ^{1}H NMR δ 1,97 (quintet, 2H); 3.09 (s, 1H); 3.74 (t, 2H); 4.43 (t, 2H); 7.21–8.54 (m, 5H).

2-(3-*O*-Benzoylpropyloxy)-2-thio-(1,3,2)-oxathiaphospholane (**1b**): Into a solution of 3-hydroxypropyl benzoate (1.79 g; 0.010 mol) in anhydrous pyridine (10 mL), elemental sulfur was added (0.64 g; 2 eq.) followed by 2-chloro-1,3,2-oxathiaphospholane (1 mL; 1.2 eq., dropwise). The mixture was stirred overnight at room temperature and the product was isolated (1.58 g, 51%) on a silica gel column eluted with a chloroform/acetone mixture (9 : 1, v/v). δ ^{31}P NMR ($CDCl_3$) 104.19 (s) FAB MS $C_{12}H_{15}O_4PS_2$, calc. 318, $[M - H]^-$ found *m/z* 317.

P^1-*O*-ethyl-*P*^{2}-*O*,*O*-diethyl-*P*^{1}-thiohypophosphate (**3a**): To a solution of 2-ethoxy-2-thio-1,3,2-oxathiaphospholane (**1a**) (38 mg, 0.21 mmol) in dry MeCN (1 mL), $(EtO)_2P(O)H$ (**2**, 13 µL, 0.10 mmol) and DBU (46 µL, 0.31 mmol) were added. After 8 h the mixture was evaporated to dryness and the product **3a** ($DBUH^+$ salt, M.W. 414) was isolated on a silica gel column eluted with a chloroform–methanol mixture (1 : 1, v/v), (17 mg, 0.041 mmol, 19%). δ ^{31}P NMR (D_2O) 50.31 (d), 12.08 (d), $^1J_{P-P}$ = 466 Hz; FAB MS *m/z* 261, $[M - H]^-$.

Triethyl hypophosphate (**4a**): To a solution of *P*^{1}-*O*-ethyl-*P*^{2}-*O*,*O*-diethyl-*P*^{1}-thiohypophosphate (**3a**, 13 mg, 0.031 mmol) in 1 mL of MeCN, iodoxybenzene ($PhIO_2$, 7 mg, 0.031 mmol) was added. After 48 h, the reaction mixture was filtered and the filtrate was evaporated to dryness to yield **4a** quantitatively. δ ^{31}P NMR (D_2O) 15.80 (d), –3.17 (d), $^1J_{P-P}$ = 650 Hz.

P^1-*O*-ethyl-*P*^{2}-*O*-ethyl-hypophosphate (**5a**): To a solution of crude triethyl hypophosphate (**4a**, 12 mg, 0.03 mmol) in 1 mL of acetone, sodium iodide (4.7 mg, 0.031 mmol) was added. Reaction mixture was refluxed for 24 h and cooled down. The crystalline product was washed with acetone and dried (5 mg, 61%). δ ^{31}P NMR (D_2O) 9.39 (s); FAB MS *m/z* 217.1.

P^1-*O*-(3-*O*-Benzoylpropyl)-*P*^{2}-*O*,*O*-dimethyl-*P*^{1}-thiohypophosphate (**3b**): To a solution of 2-(3-*O*-benzoylpropyloxy)-2-thio-1,3,2-oxathiaphospholane (**1b**, 67 mg; 0.21 mmol) in dry MeCN (1 mL), $(MeO)_2P(O)H$ (**7**, 19 µL, 0.21 mmol) and DBU (34 µL, 0.23 mmol) were added. After 3 h the reaction mixture was concentrated to dryness and the product **3b** was isolated on a DEAE Sephadex A-25 column eluted with a triethylammonium bicarbonate buffer (TEAB) using a gradient 0.05–0.3 M. The product was >92% pure by ^{31}P NMR. δ (CH_3OD) 54.63 (d), 16.31 (d), $^1J_{P-P}$ = 496 Hz; FAB MS *m/z* 367, $[M-1]^-$. Finally, the product was converted into the sodium salt (DOWEX, Na^+) and lyophilized (37 mg, 45%).

P^1-*O*-(3-*O*-Benzoylpropyl)-*P*^{2}-*O*-methyl-*P*^{1}-thiohypophosphate (**9b**): Sodium salt of *P*^{1}-*O*-(3-*O*-benzoylpropyl)-*P*^{2}-*O*,*O*-dimethyl thiohypophosphate (**3b**, 37 mg, 0.095 mmol) was dissolved in *t*-$BuNH_2$ (1 mL) and kept for 72 h at 45 °C. The reaction mixture was analyzed by ^{31}P NMR (C_6D_6) δ 64.86 (d), 10.18 (d), $^1J_{P-P}$ = 537 Hz (62% by ^{31}P NMR). The product **9b** was isolated on a DEAE Sephadex A-25 column eluted with TEAB (a gradient 0.05–0.3 M). Finally, the product was converted into the disodium salt (DOWEX, Na^+) and lyophilized (21 mg, 53%). MALDI TOF MS *m/z* 353, M^-, *m/z* 375, $[M+Na^+]^-$.

P^1-*O*-(3-*O*-Benzoylpropyl)-*P*^{2}-*O*,*O*-diethyl-*P*^{1}-thiohypophosphate (**3c**): To a solution of 2-(3-*O*-benzoylpropyloxy)-2-thio-(1,3,2-oxathiaphospholane) (**1b**, 68 mg; 0.21 mmol) in dry MeCN (1 mL), $(EtO)_2P(O)H$ (**2**, 30 µL, 0.21 mmol) and DBU (34 µL, 0.23 mmol) were added. After 12 h the ^{31}P NMR spectrum showed the presence of the product **3c** (45%, not isolated). δ ^{31}P NMR (CH_3OD) 53.53 (d), 14.39 (d), $^1J_{P-P}$ = 475 Hz.

Uridine 5′-*O*-(*P*^{2}-*O*,*O*-dimethyl-*P*^{1}-thiohypophosphate) (**13**): To a solution of 5′-*O*-(2-thio-1,3,2-oxathiaphospholane)-2′,3′-*O*,*O*-diisopropoxyacetyluridine (**11**, 29 mg, 0.05 mmol) in 0.5 mL of dry MeCN, $(MeO)_2P(O)H$ (**7**, 5.5 mg, 0.05 mmol) and DBU (7.8 µL, 0.055 mmol) were added. After 2.5 h, the reaction mixture was evaporated, the residue was dissolved in 3 mL of concentrated NH_4OH_{aq} and kept at room temperature. After 1 h, the mixture was evaporated to dryness. The product **13** (triethylammonium salt) was isolated using ion-exchange chromatography on DEAE Sephadex A-25, with a linear gradient 0.1 to 0.6 M TEAB buffer (pH 7.5), (5 mg, 20%). δ ^{31}P NMR (D_2O) 55.18 (d), 15.65 (d), $^1J_{P-P}$ = 501 Hz; MALDI TOF MS *m/z* 431.0, M^-.

Uridine 5′-*O*-(*P*^{2}-*O*-methyl-*P*^{1}-thiohypophosphate) (**14**): Uridine 5′-*O*-(*P*^{2}-*O*,*O*-dimethyl-*P*^{1}-thiohypophosphate) (**13**, 2.5 mg, 0.005 mmol) was dissolved in 0.5 mL of t-butylamine. After 4 d, t-butylamine was evaporated under high vacuum and the product **14** was obtained in near quantitative yield. δ ^{31}P NMR (D_2O) 65.11 (d), 9.81 (d), $^1J_{P-P}$ = 531 Hz; MALDI TOF MS *m/z* 416.9 M^-.

Uridine 5′-*O*-(*P*^{1}-thiohypophosphate) (**15**): To a solution of 5′-*O*-(2-thio-1,3,2-oxathiaphospholane)-2′,3′-*O*,*O*-diisopropoxyacetyluridine (**12**, 29 mg, 0.05 mmol) in 0.5 mL of MeCN, $(MeO)_2P(O)H$ (**7**, 5.5 mg, 0.05 mmol) and DBU (7.8 µL, 0.055 mmol) were added. After 2.5 h, the reaction mixture was cooled to –40 °C and trimethylsilyl bromide (26 µL, 0.2 mmol) added dropwise with a gas-tight syringe. The mixture was gradually warmed up to a room temperature and stirred overnight. Then, the mixture was evaporated and the residue was dissolved in 3 mL of concentrated NH_4OH_{aq}. After 1 h, solvent was evaporated and the product **15** (containing three triethylammonium counterions) was isolated using ion-exchange chromatography on DEAE Sephadex A-25 with a linear gradient 0.1 to 0.6 M TEAB buffer, followed by gel filtration on Sephadex LH-20 (3.6 mg, 14%). δ ^{31}P NMR (D_2O) 66.37 (d), 7.64 (d), $^1J_{P-P}$ = 543 Hz; MALDI TOF MS *m/z* 403.0 M^-.

Adenosine 5′-*O*-(*P*^{1}-thiohypophosphate) (**18**): To a solution of N^6-unprotected 2′,3′-*O*,*O*-diacetyladenosine-5′-*O*-(2-thio-1,3,2-oxathiaphospholane) (**16**, 98 mg, 0.2 mmol, see ESI†) in 3 mL of dry MeCN, $(MeO)_2P(O)H$ (**7**, 22 mg, 0.2 mmol) and DBU (33 µL, 0.22 mmol) were added. After 2 h, the reaction mixture was cooled to –40 °C and trimethylsilyl bromide (105 µL, 0.8 mmol) was added dropwise *via* gas-tight syringe. The mixture was gradually warmed up to room temperature and stirred overnight. The mixture was evaporated and the residue containing diacylated monoester intermediate **17** dissolved in 3 mL of concentrated NH_4OH_{aq}. After 2 h, solvent was evaporated and the product **18** (containing three triethylammonium counterions) was isolated using ion-exchange chromatography on DEAE Sephadex A-25 with a linear gradient 0.1 to 0.6 M TEAB buffer (35 mg, 24%). δ ^{31}P NMR (D_2O) 68.22 (d), 68.05 (d) (resonances for a pair of

P-diastereoisomers), 7.64 (d), $^1J_{P-P}$ = 530 Hz; MALDI TOF MS *m/z* 426.0, M^-.

Adenosine 5′-*O*-hypophosphate (**10**): To a stirred solution of adenosine 5′-*O*-(P^1-thiohypophosphate) (**18**) (22 mg, 0.05 mmol) in water (0.8 mL), iodoxybenzene ($PhIO_2$, 6 mg, 0.025 mmol) was added. After 10 min, the mixture was diluted with water (9 mL) and extracted with chloroform. The aqueous layer was concentrated and the product **10** was isolated using ion-exchange chromatography on DEAE Sephadex A-25, with a linear gradient 0.05 to 0.6 M TEAB buffer. Finally, the product was converted into the sodium salt (DOWEX Na^+) and lyophilized (9 mg, three sodium counterions, 63%). δ ^{31}P NMR (D_2O) 17.09 (d), 4.93 (d), $^1J_{P-P}$ = 660 Hz; MALDI TOF MS *m/z* 410.0 M^-.

5′-*O*-DMT-N^{iBu},O^{DPC}-2′-deoxyguanosine-3′-*O*-(2-thio-1,3,2-oxathiaphospholane) (**19**): the compound was synthesized and enriched in the *fast* diastereomer (final ratio *fast*:*slow* 100 : 44 or 100% *fast*) by means of chromatography on a silica gel column, according to the published method.[14]

5′-*O*-DMT-N^{iBu},O^{DPC}-2′-deoxyguanosine-3′-*O*-(P^2-*O*,*O*-diethyl-P^1-thiohypophosphate) (**20**): To a solution of 5′-*O*-DMT-N^{iBu},O^{DPC}-2′-deoxyguanosine-(2-thio-1,3,2-oxathiaphospholane) (**19**, 40 mg, 0.04 mmol, *fast*:*slow* 100 : 44 or 100% *fast*) in 0.5 mL of anhydrous MeCN, $(EtO)_2P(O)H$ (**2**, 5μL, 0.04 mmol) and DBU (8 μL, 0.045 mmol) were added. After 12 h, the reaction mixture was evaporated and the residue was dissolved in CD_3CN. The ^{31}P NMR spectrum (500 MHz instrument) contained resonances for P^1 atoms at δ 54.0 (d, $^1J_{P-P}$ = 467 Hz, CD_3CN) and δ 54.4 (d, $^1J_{P-P}$ = 466 Hz) at relative intensity 100 : 40 (only one doublet at δ 54.4 ($^1J_{P-P}$ = 470 Hz) for the diastereomerically pure substrate).

5′-*O*-DMT-N^{iBu},O^{DPC}-2′-deoxyguanosine-3′-*O*-(P^2-*O*,*O*-diethyl-P^1,P^2-dithiohypophosphate) (**21**): To a solution of 5′-*O*-DMT-N^{iBu},O^{DPC}-2′-deoxyguanosine-(2-thio-1,3,2-oxathiaphospholane) (**19**, 40 mg, 0.04 mmol, *fast*:*slow* 100 : 44) in 0.5 mL of anhydrous MeCN, $(EtO)_2P(S)H$ (**8**, 6 μL, 0.04 mmol) and DBU (8 μL, 0.045 mmol) were added. After 12 h, the reaction mixture was evaporated and the residue dissolved in CD_3CN. The relevant ^{31}P NMR spectrum (500 MHz) showed the presence of **21** in 17% yield; δ 58.5 (d, $^1J_{P-P}$ = 354 Hz) and δ 58.9 (d, $^1J_{P-P}$ = 352 Hz), relative intensity 100 : 53.

Acknowledgements

This work was financially supported by Polish Ministry of Science and Higher Education, grant 3 T09A 059 28 (to WJS). Authors are indebted to Prof. K.A. Jacobson of NIH, Bethesda, MD., for sharing with us the results of preliminary studies on interaction of nucleoside hypophosphates with $P2Y_6$ receptor. Critical comments of Prof. G. M. Blackburn on the manuscript are highly appreciated.

Notes and references

1 A. H. Cowley, *Chem. Rev.*, 1965, **65**, 617–634.
2 I. F. Lutsenko and M. V. Proskurnina, *Russ. Chem. Rev.*, 1978, **47**, 880–895.
3 J. Setondji, P. Remy, G. Dirheimer and J. P. Ebel, *Biochim. Biophys. Acta, Nucleic Acids Protein Synth.*, 1970, **224**, 136–143.
4 M. Baudler, *Z. Anorg. Allg. Chem.*, 1956, **288**, 171.
5 J. Michalski and A. Zwierzak, *Bull. Acad. Polon. Sci. Ser. Sci. Chim.*, 1965, **13**, 253–259.
6 W. Stec, A. Zwierzak and J. Michalski, *Bull. Acad. Polon. Sci. Ser. Sci. Chim.*, 1970, **18**, 23–29.
7 L. Almasi and L. Paskucz, *Chem. Ber.*, 1963, **96**, 2024–2028.
8 J. Michalski, W. Stec and A. Zwierzak, *Bull. Acad. Polon. Sci. Ser. Sci. Chim.*, 1965, **13**, 677–682.
9 R. C. Mehrotra, G. Srivastava and P. N. Nagar, *Phosphorus Sulfur Relat. Elem.*, 1983, **18**, 145–148.
10 R. Waterman, *Organometallics*, 2007, **26**, 2492–2494.
11 W. J. Stec, B. Karwowski, M. Boczkowska, P. Guga, M. Koziołkiewicz, M. Sochacki, M. Wieczorek and J. Błaszczyk, *J. Am. Chem. Soc.*, 1998, **120**, 7156–7167.
12 J. Herrick and B. Sclavi, *Mol. Microbiol.*, 2007, **63**, 22–34.
13 P. Guga, A. Okruszek, W. J. Stec, *Topics in Current Chemistry.* Vol. 220, Springer Verlag, 2002, J. P. Majoral, Ed., pp. 169-200.
14 P. Guga, W. J. Stec,(2003) *Synthesis of Phosphorothioate Oligonucleotides with Stereodefined Phosphorothioate Linkages. In Current Protocols in Nucleic Acid Chemistry* (S. L. Beaucage, D. E. Bergstrom, G. D. Glick and R. A. Jones, ed.) pp. 4.17.1–4.17.28. John Wiley & Sons, Hoboken, N.J.
15 A. Łopusiński and G. Mielniczak, *Synlett.*, 2001, **4**, 505–508.
16 B. Nawrot, B. Rębowska, O. Michalak, M. Bulkowski, D. Błaziak, P. Guga and W. J. Stec, *Pure Appl. Chem.*, 2008, **80**, 1859–1871.
17 J. Michalski, W. Stec and A. Zwierzak, *Chem. & Ind.*, 1966, 856.
18 R. K. Harris, J. R. Woplin and W. J. Stec, *J. Chem. Soc. Chem. Commun.*, 1970, 1391.
19 W. J. Stec, J. R. Van Wazer and N. Goddard, *J. Chem. Soc., Perkin Trans. 2*, 1972, 463.
20 M. D. M. Gray and D. J. H. Smith, *Tetrahedron Lett.*, 1980, **21**, 859–860.
21 J. Setondji, P. Remy, G. Dirheimer and J. P. Ebel, *Biochim. Biophys. Acta, Nucleic Acids Protein Synth.*, 1971, **232**, 585–594.
22 P. V. Vignais, J. Setondji and J. P. Ebel, *Biochimie*, 1971, **53**, 127–129.
23 (*a*) P. Remy, G. Dirheimer and J. P. Ebel, *Biochim. Biophys. Acta, Gen. Subj.*, 1967, **136**, 99–107; (*b*) P. Remy, J. Setondji, G. Dirheimer and J. P. Ebel, *Biochim. Biophys. Acta, Nucleic Acids Protein Synth.*, 1970, **204**, 31–38; (*c*) M. K Kukhanova, N. F Zakirova, A. V. Ivanov, L. A. Alexandrova, M. V. Jasco and A. R. Khomutov, *Biochem. Biophys. Res. Commun.*, 2005, **338**, 1335–1341.
24 J. Chojnowski, M. Cypryk and J. Michalski, *Synthesis*, 1978, 777–779.
25 J. Bao and D. D. Y. Ryu, *Biotechnol. Bioeng.*, 2007, **98**, 1–11.
26 Analogous P-diastereoselectivity was observed in phosphorylation of a mixture of isomers of ADPαS with pyruvate kinase, which yielded only one P-diastereomer of ATPαS. F. Eckstein and R. S. Goody, *Biochemistry*, 1976, **15**, 1685–1691.
27 For the T7 RNA polymerase/ATP transcription system (pH 7.9), K_m values of 0.0095 mM and 0.14 mM were reported in the literature: (*a*) D. Imburgio, M. Anikin and W. T. McAllister, *J. Mol. Biol.*, 2002, **319**, 37–51; (*b*) D. Patra, E. M. Lafer and R. Sousa, *J. Mol. Biol.*, 1992, **224**, 307–318.
28 K. A. Jacobson (Laboratory of Bioorganic Chemistry & Molecular Recognition Section, NIH, Bethesda, MD – unpublished results.
29 A. El-Tayeb, A. Qi and C. E. Muller, *J. Med. Chem.*, 2006, **49**, 7076–7087.
30 A. Guranowski, A. M. Wojdyła, J. Zimny, A. Wypijewska, J. Kowalska, M. Łukaszewicz, J. Jemielity, E. Darżynkiewicz, A. Jagiełło and P. Bieganowski, *New J. Chem.*, 2010, **34**, 888, DOI: 10.1039/b9nj00660e.
31 K. Misiura, D. Szymanowicz and W. J. Stec, *Org. Lett.*, 2005, **7**, 2217–2220.
32 P. Kaźmierczak, L. Skulski and Ł. Kraszkiewicz, *Molecules*, 2001, **6**, 881–891.

