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One-pot synthesis of nucleoside 3′-*O*-(*S*-phenyl methanephosphonothioates)

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Abstract

The one-pot reaction of nucleosides with $MeP(O)Cl_2$ and thiophenol, providing nucleoside 3′-*O*-(*S*-phenyl methanephosphonothioate), potential monomers for the synthesis of oligonucleoside methanephosphonate, is described.

Keywords: nucleoside; methanephosphonothioates; *S*-aryl esters.

Former reports from this laboratory presented a one-pot reaction of appropriately protected 3′-OH-nucleosides (**1**) with methanephosphonodichloridate (**2**) and aniline. This provided in satisfactory yields the corresponding nucleoside 3′-*O*-methanephosphonanilidates [R*O*$^{3'}$-PMe(O)NHPh, R=5′-*O*-DMT-nucleoside, **3**].[1] Under treatment with sodium hydride/carbon disulfide, anilidates **3** were converted into sodium nucleoside 3′-*O*-methanephosphonothioates [R*O*$^{3'}$-PMe(O)SNa, R=5′-*O*-DMT-nucleoside, **4**].[2] Regioselective *S*-alkylation of **4** with alkyl halides afforded nucleoside 3′-*O*-(*S*-alkyl methanephosphonothioates) [R*O*$^{3'}$-PMe(O)SAlk, R=5′-*O*-DMT-nucleoside, **5**], convenient substrates for the stereocontrolled synthesis of dinucleoside (3′,5′)-methanephosphonates (**6**).[3] Since the ***PN→PS*** conversion **3→4**, as well as the *S*-alkylation of **4**, are stereospecific reactions, diastereomerically pure methanephosphonothioates **5** are available either via separation of the diastereomeric mixture of anilidates **3** followed by their regioselective and stereospecific conversion into *S*-alkyl esters **5**, or by a separation of the diastereomeric mixture of **5** into individual diastereomeric species. We have also demonstrated that the reaction of diastereomerically pure esters **5** with 5′-OH nucleosi(ti)des in the presence of DBU/LiCl is stereospecific, and affords dinucleoside (3′,5′)-methanephosphonates (**6**) with a predetermined sense of chirality at the phosphorus centre.[4]

During the studies we conducted for further development of this methodology, it was tempting to prepare the corresponding nucleoside 3′-*O*-(*S*-aryl methanephosphonothioate)s (**7**), and to compare their reactivity with the previously studied[3] *S*-alkyl substrates **5**. In spite of a report on the successful

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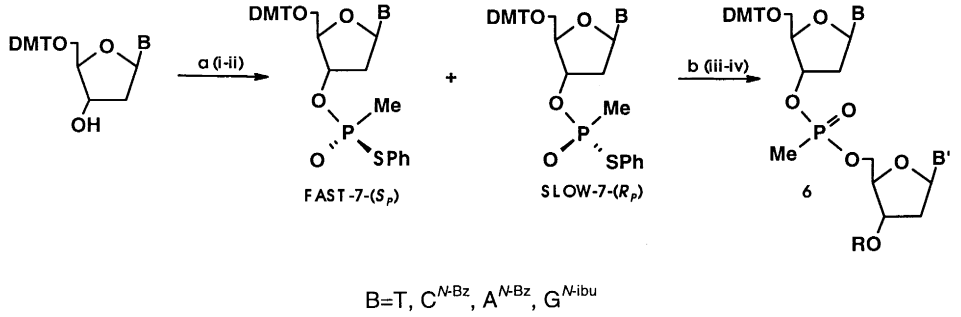
S-arylation of *O*-alkyl alkanephosphonothioates with benzenediazonium chloride, providing in 50% yield *O*-ethyl *S*-phenyl ethanephosphonothioate,[5] our efforts at the *S*-arylation of methanephosphonothioates **4** with benzenediazonium chloride have failed.[6,7] Although nucleoside 3′-*O*-(*S*-aryl methanephosphonothioate)s were described by Brill and Caruthers,[8] their preparation involved either 3′-*O*-methanephosphonylimidazolidates and oxybenzotriazolidates,[9] or P(III) derivatives,[10] and required the use of highly reactive phosphonylating agents.

Therefore, we decided to adapt the methodology developed previously for preparation of anilidates **3**, and apply it to the synthesis of nucleoside 3′-methanephosphonothioates **7** (Scheme 1a).



Scheme 1. *Reaction conditions*: (i) $MeP(O)Cl_2$ (**2**), pyridine, rt, 15 min (ii) thiophenol, 30 min; (iii) separation into diastereomers; (iv) single diastereomer **7**, 3′-*O*-acetyl thymidine, DBU/LiCl

We have found that the one-pot reaction of an appropriately protected nucleoside **1** with methanephosphonodichloridate and thiophenol can be used as a fast and effective route to the diastereomeric mixture of **7**, easily separable into pure diastereomers via silica gel column chromatography.

The typical procedure is as follows: Into a stirred solution of *N*-protected (except thymine) nucleoside **1** (1 mmol) in dry pyridine (10 mL) at ambient temperature was added in one portion a solution of methanephosphonodichloridate (2 mmol) in pyridine (2 mL). After 15 min an excess of thiophenol (1 mL) was added in one portion, and stirring was continued for additional 30 min. The reaction mixture was partially concentrated to a total volume of ca. 5 mL, diluted with chloroform (50 mL), washed twice with H_2O, and dried over $MgSO_4$. After concentration of the organic fraction under reduced pressure the crude product was purified by flash column chromatography (230–400 mesh, chloroform–ethanol, 98:2 v/v). Purified and separated diastereomers FAST **7** and SLOW **7** (quantitative separation of diastereomers) were dissolved in CH_2Cl_2, precipitated from hexane (petroleum ether) and stored as stable, white powders.[11] The yields of compounds **7** and selected spectroscopic characteristics are given in Table 1.

The absolute configuration at the *P*-chiral centre in **7** was not assigned directly. However, one can assume it is based upon the relative mobility/NMR relationship.[12] As reported by Caruthers et al.[13] the X-ray data for FAST thymidine 3′-(*S*-phenyl methanephosphonothioate)[13] demonstrates that FAST **7** has (S_P) configuration, while SLOW **7** possesses the (R_P) configuration.

Both the FAST **7** and SLOW **7** (B=Thy) diastereomers were condensed in the presence DBU/LiCl with 3′-*O*-acetylthymidine under conditions comparable with those used in earlier experiments with **5** (alkyl=CH_3-, $C_6H_5CH_2-$).[3] End-protected dithymidylyl (3′,5′)-methanephosphonates **6** were obtained in moderate yields (53% and 42%, respectively), but the process of condensation was accompanied by partial *P*-epimerisation.[14] That loss of stereospecificity can be explained by enhanced *P*-epimerisation of **7** in the presence of DBU. Such an effect of DBU was previously observed in the DBU-promoted transesterification of nucleoside 3′-*O*-*p*-nitrophenyl methanephosphonates[15] and the DBU-assisted solvolysis reactions of nucleoside 3′-*O*-(*O*-2,4,6-trimethylbenzoyl methanephosphonates).[16] Studies involving the

Table 1

B	Yield *	Mobility**	^{31}P NMR	^{1}H NMR (P-CH_3)	$^{2}J_{P\text{-}CH3}$
Thy	63 (3:2)	FAST	54.73	1.72	15.56
		SLOW	55.00	1.76	15.62
$C^{N\text{-Bz}}$	60 (1:1)	FAST	54.92	1.71	15.58
		SLOW	54.17	1.75	15.60
$A^{N\text{-Bz}}$	53 (1:1)	FAST	54.26	1.77	15.50
		SLOW	54.43	1.79	15.61
$G^{N\text{-ibu}}$	57 (1:1)	FAST	54.74	1.69	15.48
		SLOW	55.00	1.76	15.57

* Total yield of both separated isomers **7**; in parentheses a ratio of diastereomers FAST:SLOW is given.

** Relative mobility: Silica gel HP TLC plate (silica gel 60 F_{254}-Merck), eluent: chloroform-EtOH (95:5).

search for conditions for the condensation of **7** with 5′-OH nucleosides in the presence of catalysts other than DBU are presently in progress**.**

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7. Modifications of the purines, which further decrease the yields of **7**, could be expected, as indicated by Hiramoto, K.; Kaku, M.; Sueyoshi. A.; Fujise, M.; Kikugawa, K. *Chem. Res. Toxicol.* **1997**, *8*, 356–362 and references cited therein.
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1H, H4′), 6.12 (dd, J=6.4, 6.5, 1H, H1′); $C_{44}H_{41}O_8N_3P_1S_1$, HR FAB MS [M−H]: 802.235. 5′-*O*-DMT-Thymidine 3′-*O*-(*S*-phenyl methanephosphonothioate (**7**, B=Thy) was obtained from 5′-*O*-DMT-thymidine (1 mmol). Yield 63% (FAST:SLOW 3:2). SLOW (R_P). ^{31}P NMR ($CDCl_3$): 55.0, ^{1}H NMR ($CDCl_3$): 1.76 (d, J=15.6, 3H, P-CH_3), 2.47 (m, 1H, H2′), 2.71 (m, 1H, H2′′), 3.48 (m, 2H, H5′, H5′′), 4.16 (m, 1H, H3′), 5.43 (dd, J=5.8, 10.0, 1H, H4′), 6.46 (dd, J=5.4, 9.0, 1H, H1′); $C_{38}H_{38}O_8N_2P_1S_1$, HR FAB MS [M−H]: 713.212, calcd 713.216. FAST (S_P): ^{31}P NMR ($CDCl_3$): 54.73, ^{1}H NMR ($CDCl_3$): 1.72 (d, J=15.6, 3H, P-CH_3), 2.54 (m, 2H, H2′, H2′′), 3.49 (m, 2H, H5′, H5′′), 4.34 (m, 1H, H3′), 5.40 (m, 1H, H4′), 6.43 (dd, J=5.6, 8.9, 1H, H1′); $C_{38}H_{38}O_8N_2P_1S_1$, FAB MS [M−H]: 713.2. 5′-*O*-DMT-N^6-Benzoyl-2′-deoxyadenosine 3′-*O*-(*S*-phenyl methanephosphonothioate) (**7**, B=A^{Bz}) was obtained from N^6-benzoyl-2′-deoxyadenosine (1 mmol). Yield 53%. SLOW (R_P): ^{31}P NMR ($CDCl_3$): 54.43, ^{1}H NMR ($CDCl_3$): 1.79 (d, J=15.6, 3H, P-CH_3) 2.90 (m, 1H, H2′), 3.12 (m, 1H, H2′′), 3.5 (m, 2H, H5′, H5′′), 4.35 (m, 1H, H4′), 5.55 (m, 1H, H3′), 6.53 (dd, 1H, J=5.5, 5.8, H1′); $C_{45}H_{41}O_7N_5P_1S_1$, HR FAB MS [M−H]: 826.234, calcd 827.237. FAST (S_P): ^{31}P NMR ($CDCl_3$): 54.26, ^{1}H NMR ($CDCl_3$): 1.77 (d, J=15.5, 3H, P-CH_3), 2.69 (m, 1H, H2′), 3.03 (m, 1H, H2′′), 3.6 (m, 2H, H5′, H5′′), 4.54 (m, 1H, H3′), 5.45 (m, 1H, H4′), 6.52 (dd, 1H, J=5.4, 5.7, H1′); $C_{45}H_{41}O_7N_5P_1S_1$, HR FAB MS [M−H]: 826.234. 5′-*O*-DMT-N^4-Isobutyryl-2′-deoxyguanosine 3′-*O*-(*S*-phenyl methanephosphonothioate) (**7**, B=G^{ibu}) was obtained from N^4-isobutyryl-2′-deoxyguanosine (1 mmol). Purified by a column chromatography (twice) ($CHCl_3$-3% EtOH), followed by precipitation from petroleum ether. Total yield: 57%. SLOW (R_P): ^{31}P NMR ($CDCl_3$): 55.00, ^{1}H NMR ($CDCl_3$): 1.76 (d, J=15.6, 3H, P-CH_3), 2.80 (m, 2H, H2′, H2′′), 3.25 (m, 1H, H5′′), 3.47 (dd, J=3.1, 10.6, 1H, H5′′), 4.16 (m, 1H, H4′), 5.80 (m, 1H, H3′), 6.14 (dd, J=5.9, 6.9, 1H, H1′); $C_{42}H_{43}N_5O_8P_1S_1$, HR FAB MS [M−H]: 808.253, calcd 808.257. FAST (S_P): ^{31}P NMR ($CDCl_3$): 54.74, ^{1}H NMR ($CDCl_3$): 1.69 (d, J=15.2, 3H, P-CH_3), 2.13 (m, 1H, H2′), 2.55 (m, 1H, H2′′), 3.20 (dd, J=3.1, 10.7, 1H, H5′), 3.42 (dd, 1H, J=2.9, 10.9, H5′′), 4.21 (m, 1H, H4′), 6.76 (m, 2H, H3′, H1′); $C_{42}H_{43}N_5O_8P_1S_1$, HR FAB MS [M−H]: 808.253.

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