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SYNTHESIS AND ANTIVIRAL ACTIVITY OF NEW PHOSPHONOBUTOXYPURINES

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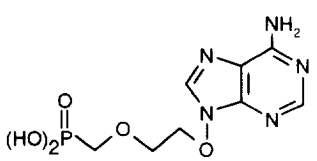
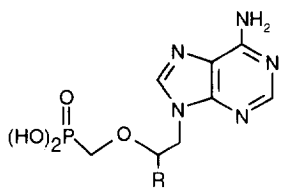
Abstract: A series of new 9-(4-phosphonobutoxy)purines was synthesized and evaluated as antiviral agents. 9-(4-Phosphonobutoxy)guanine displayed potent and selective activity against HIV-1 in peripheral blood lymphocytes.

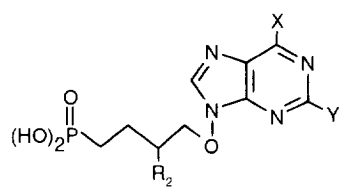
Considerable interest has been focussed on the synthesis and biological evaluation of acyclic nucleoside phosphonates and this research has led to the discovery of several potent antiviral agents with broad spectrum activity.[1-5] Acyclic purine phosphonates such as 9-[2-(phosphonomethoxy)ethoxy]adenine **1**, 9-(2-phosphonomethoxyethyl)adenine **2a** (PMEA, adefovir) and (*R*)-9-(2-phosphonomethoxypropyl)adenine **2b** (PMPA) have been shown to possess potent and selective activity against HIV and a wide range of other retroviruses *in vitro* and *in vivo*.[6-8] The orally bioavailable diphenyl ester of 9-[2-(phosphonomethoxy)ethoxy]adenine has also proved effective in inhibiting splenomegaly and viraemia in mice infected with Rauscher murine leukemia virus.[9]
Recently, the report that PMPA prevented simian immunodeficiency infection without toxicity in macaques[10] has given a fresh impetus to the research on this important class of compounds.[11] Here, we describe the synthesis and antiviral activity of some phosphonobutoxy purines in which the phosphonobutoxy side chain is both isomeric with the PMPA side chain and isosteric with the acyclic moiety of **2a**.



1



2a R=H
2b R=CH_3



3a R_2=H X=OH Y=NH_2
3b R_2=CH_2OH X=OH Y=NH_2
3c R_2=H X=Y=NH_2
3d R_2=CH_2OH X=Y=NH_2
3e R_2=H X=NH_2 Y=H
3f R_2=CH_2OH X=NH_2 Y=H

The 9-(4-phosphonobutoxy)purines **3a**,**3b**,**3c**,**3d**,**3e** and **3f** were prepared as presented in Scheme 1. The alkoxyamines **9a**,**9b**,**9c** were required as key intermediates in the synthesis. Thus, the alkoxyamine **9a** was obtained in good overall yield by the Arbuzov reaction of the protected 4-iodoalcohol[12] **7** with triethyl

phosphite, subsequent removal of the *tert*-butyldimethylsilyl group, reaction of phosphonate **8** with N-hydroxyphthalimide under Mitsunobu conditions[13] and finally cleavage of the resultant N-alkoxyphthalimide with methyl hydrazine in dichloromethane. The alkoxyamines **9b** and **9c** were prepared in a similar way. Treatment of the diacetate[14] **4** with triethyl phosphite followed by hydrolysis of the acetyl groups under acidic conditions afforded the corresponding phosphonate **5**. Differentiation of the two hydroxyl functions of **5** was achieved either by formation of the monoacetate **6a** via a cyclic orthoester,[14] or by reaction of **5** with an equimolar amount of *tert*-butyldimethylsilyl chloride to give **6b**. Compounds **6a** and **6b** were subsequently converted into alkoxyamines **9b** and **9c** in the same manner as described for the synthesis of **9a**.

Reaction of the phosphonoalkoxyamines **9a**,**9c** with 4,6-dichloro-5-formamidopyrimidine, and **9a**,**9b** with 4,6-dichloro-2,5-diformamidopyrimidine in the presence of N,N-diisopropylethylamine afforded the 6-alkoxyaminopyrimidines **10a**,**10d** and **10b**,**10c**, respectively, in 64-81% yield. Compounds **10a**,**10d** and **10b**,**10c** were heated in diethoxymethyl acetate to provide the corresponding 6-chloro- and 6-chloro-2-formamidopurines **11a**,**11c** and **13a**,**13b**. Displacement of the 6-chloro substituent in **11a**,**11c** was achieved with ethanolic ammonia to give the 6-amino intermediates **11b**,**11d** in 93 and 85% yield, respectively. Compound **11b** upon treatment with trimethylsilyl bromide afforded the adenine analogue **3e**. In the case of the phosphonoalkoxyadenine **11d**, the *tert*-butyldimethylsilyl group was removed prior to the de-esterification and compound **3f** was isolated in 64% yield, after two steps. The 2,6-diaminopurine derivatives **3c** and **3d** were obtained from **13a** and **13b** in 33-45% overall yield by reaction with ethanolic ammonia, and subsequent de-esterification with trimethylsilyl bromide.[15]

Hydrolysis of the 6-chloro-2-formamidopurines **13a**,**13b** with 80% formic acid, followed by 0.2M HCl, provided the guanine derivatives **14a**,**14b**, which in turn were converted into the corresponding phosphonic acids **3a**,**3b** in 64-70% yield.[15]

The new acyclic nucleoside phosphonates were evaluated *in vitro* against human immunodeficiency virus type-1 (HIV-1), herpes simplex virus (HSV) types 1 and 2 and varicella zoster virus (VZV). Two compounds,

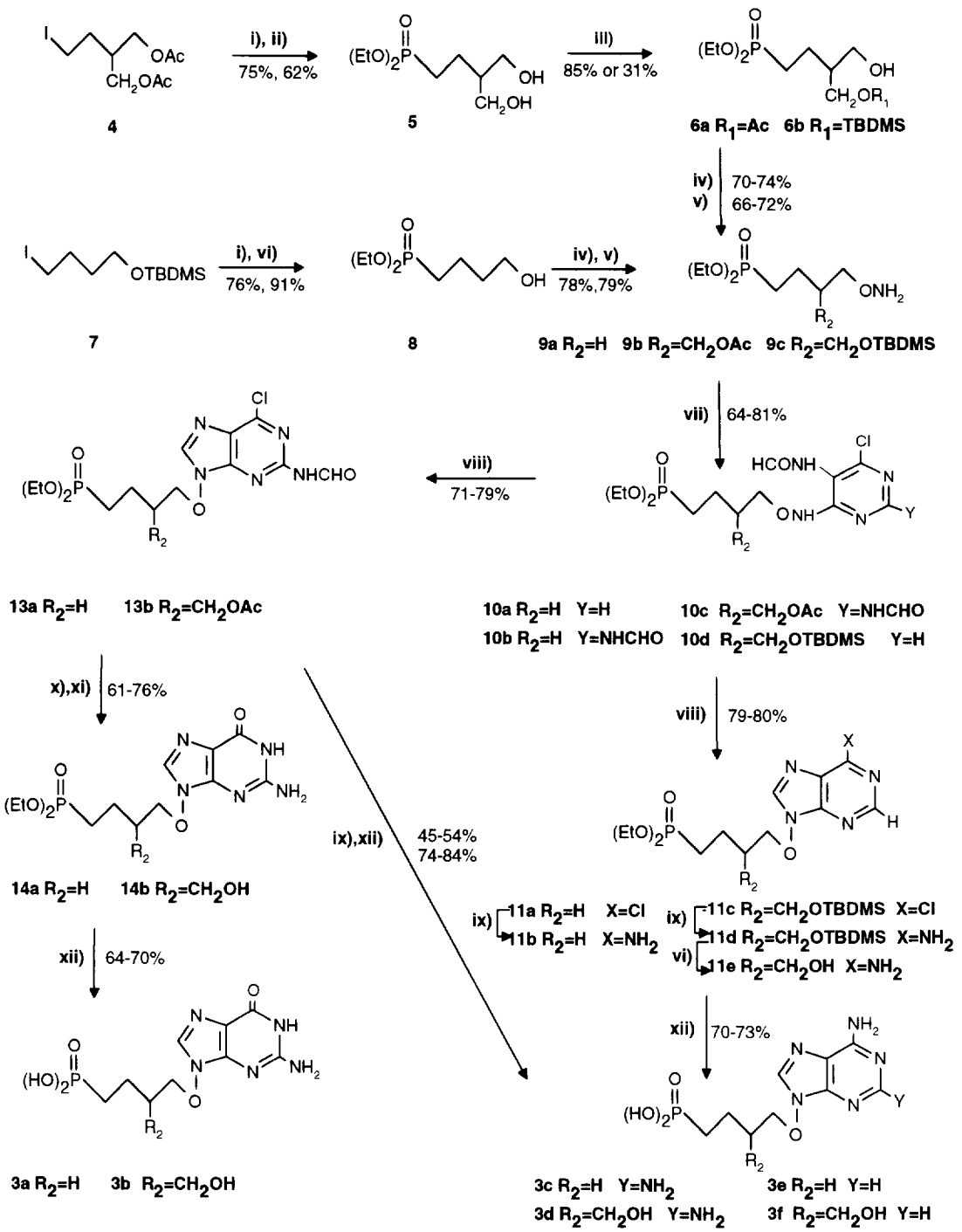
	IC_{50} (μg ml^{-1})[a,b]					
compound no	HSV-1 (SC16)	HSV-2 (MS)	VZV (Ellen)	MRC-5 cells ^{3}H-dT	PBL MTT IC_{50}(μM)	HIV[c] (D34) IC_{50}(μM)
3a	>100	23	6	1.6	>100	0.01
3b	>100	>100	5	8.8	NT	>100
3c	>100	>100	17	26	>100	1.0
3d	>100	>100	17	78	NT	NT
3e	>100	>100	>100	58	NT	>100
3f	>100	>100	>100	>100	NT	>100

Table 1. Antiviral activity

a. Carried out in human fibroblast (MRC-5) cells. At concentrations up to 100 μg ml^{-1}, none of the compounds was cytotoxic to the cell monolayers used in the tests.

b. Concentration of compound which inhibited by 50% the number of plaques (HSV-2, VZV) or cytopathic effect (HSV-1) in infected cells or incorporation of ^{3}H-dT into uninfected cells.

c. Carried out in Diagen Laboratory



SCHEME 1. i) $P(OEt)_3$,130°C; ii) 2.2M HCl/EtOH, 80°C; iii) $CH_3C(OMe)_3/CF_3COOH$ or TBDMSCl/Im/DMF; iv) N-hydroxyphthalimide/DEAD/ Ph_3P/THF; v) $MeNHNH_2/CH_2Cl_2$; vi) 80% AcOH; vii) 4,6-dichloro-5-formamidopyrimidine or 4,6-dichloro-2,5-diformamidopyrimidine/N^iPr_2Et/diglyme; viii) diethoxymethyl acetate/120°C; ix) NH_3/EtOH; x) 80% HCOOH; xi) 0.2M HCl; xii) TMSBr/DMF.

9-(4-phosphonobutoxy)guanine **3a** and the corresponding 2,6-diaminopurine **3c** showed good activity against HIV replication in human peripheral blood lymphocytes (PBLs) with IC_{50} values of 0.01 μM and 1 μM, respectively. Using an MTT-based cytotoxicity assay in replicating PBLs cells, the 50% cytotoxic concentration for these compounds was >100 μM. Compound **3a** also displayed moderate activity against herpes viruses (HSV-2, VZV) in human fibroblast (MRC-5) cells. None of the acyclonucleotides **3b**,**3f** with a hydroxymethyl group on the acyclic 9-substituent showed anti-HIV activity. The 2-hydroxymethyl analogues **3b** and **3d** were, however, active against VZV at 5 μM and 17 μM concentrations, respectively.

Although acyclic nucleoside phosphonates are poorly transported through cell membranes, results from our recent study of bioavailable esters of **1** indicate,[9] that it should be possible to achieve good *in vivo* activity of **3a** and **3c** by preparing prodrugs of these compounds.

Acknowledgements

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15. Data for **3a**: mp 208°C; ^{1}H NMR (DMSO-d_6, 270 MHz) δ 1.41 (m, 2H), 1.68 (br s, 4H), 4.21 (m, 2H), 6.92 (s, D_2O exchangeable, 2H), 7.89 (s, 1H); Anal. Calcd for $C_9H_{17}N_6O_5P.0.6HBr$: C, 29.31; H, 4.80; N, 22.78. Found: C, 29.35; H, 4.79; N, 23.04. **3b**: ^{1}H NMR (DMSO-d_6, 270 MHz) δ 1.50 (m, 3H), 1.82 (m, 2H), 3.46 (m, 2H), 4.20 (m, 2H), 6.94 (br s, D_2O exchangeable, 2H), 7.90 (s, 1H); Anal. Calcd for $C_{10}H_{19}N_6O_6P.0.2$ HBr.H_2O: C, 31.24; H, 5.55; N, 21.85. Found: C, 31.40; H, 5.62; N, 21.98. **3c**: mp 295-297°C; ^{1}H NMR (DMSO-d_6/D_2O, 270 MHz) δ 1.68 (m, 6H), 4.31 (t, J = 5.9 Hz, 2H), 8.35 (s, 1H); Anal. Calcd for $C_9H_{15}N_6O_4P.HBr$: C, 28.21; H, 4.21; N, 21.93. Found: C, 28.58; H, 4.47; N, 21.86. **3e**: mp 265-268°C; ^{1}H NMR (DMSO-d_6, 270 MHz) δ 1.76 (m, 6H), 4.46 (t, J = 6.2 Hz), 7.47 (br s., D_2O exchangeable, 2H), 8.24 (s, 1H), 8.48 (s, 1H); Anal. Calcd for $C_9H_{14}N_5O_4P.0.8HBr.\ 2H_2O$: C, 27.87; H, 4.88; N, 18.05. Found: C, 28.00; H, 4.87; N, 17.85.

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