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SYNTHESIS AND ANTIVIRAL ACTIVITY OF 9-(PHOSPHONOALKYNYLOXY)PURINES: NOVEL ACYCLONUCLEOTIDES

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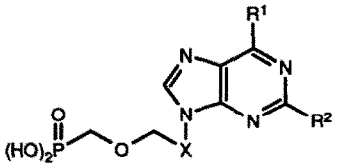
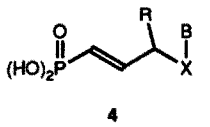
Abstract. 9-(3-Phosphonoprop-2-ynyloxy)adenine (**5**) and -guanine (**6**) were synthesised and found to have anti-herpesvirus activity. Attempted Arbusov reaction of a chloroalkyne (**17**) with triethyl phosphite unexpectedly gave the (*Z*)-2-chloroalkenylphosphonate (**19**).

The phosphonomethoxy group has proved effective as a stable phosphate mimic and has recently been widely employed in the synthesis of nucleotide analogues as antiviral agents.[1-3] For example both 9-[2-(phosphonomethoxy)ethyl]adenine[4,5] (PMEA, **1**) and 9-[2-(phosphonomethoxy)ethyl]guanine[4] (PMEG, **2**) have broad spectrum antiviral activity and 9-[2-(phosphonomethoxy)ethoxy]adenine[6-8] (BRL 47923, **3**) has potent and selective anti-retrovirus activity including activity against human immunodeficiency virus (HIV). We have recently reported[9] the synthesis and antiviral activity of a series of acyclonucleotide analogues (**4**) incorporating the alkenylphosphonic acid moiety as the phosphate mimic. The biological activity exhibited by some of these compounds suggested that we should extend our studies to include novel analogues in which the alkynylphosphonic acid group acted as the phosphate mimic. As far as we are aware this moiety has not been used previously as a phosphate mimic in biologically active molecules. Acyclonucleotide analogues in which the acyclic substituent is linked to the purine by a nitrogen-oxygen bond have shown potential as potent and selective antiviral agents,[6-8,10] therefore the N-O linked alkynylphosphonic acid analogues (**5** and **6**) of PMEA and PMEG were chosen as initial targets.



1 R^1=NH_2, R^2=H X=CH_2
2 R^1=OH, R^2=NH_2 X=CH_2
3 R^1=NH_2, R^2=H X=CH_2O



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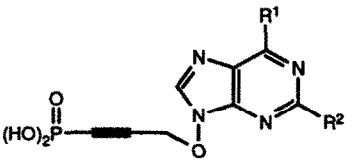
B = Nucleic acid base
X = O, CH_2, CH_2O
R = H, CH_2OH

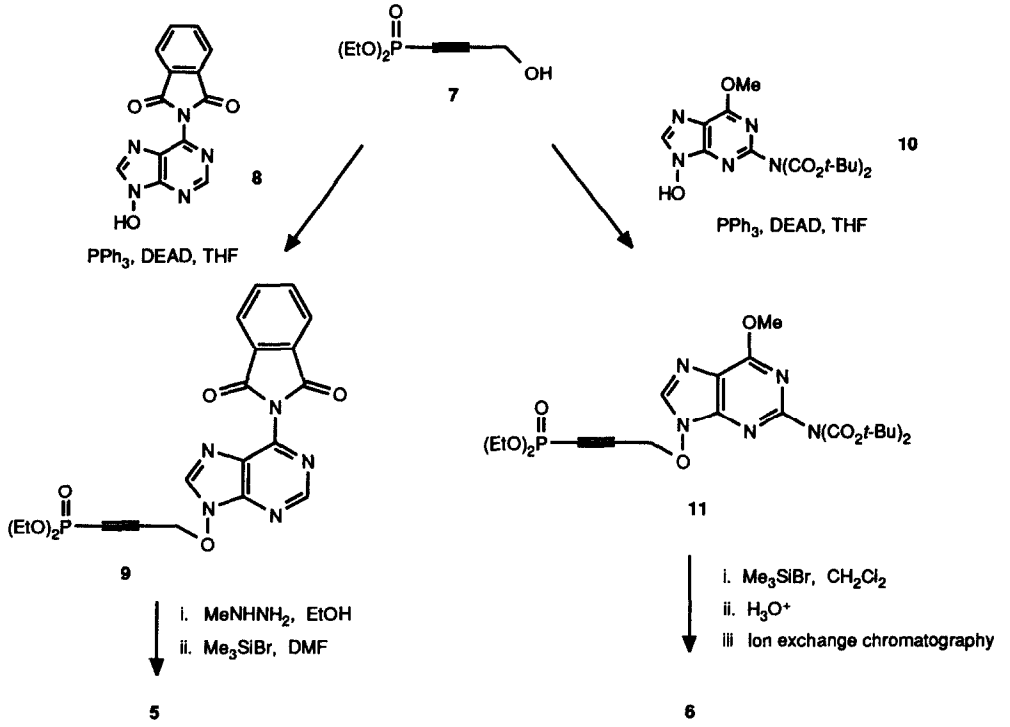


5 R^1=NH_2, R^2=H
6 R^1=OH, R^2=NH_2

Mitsunobu coupling of the alcohol **7**[11] with 9-hydroxy-6-*N*-phthalimidopurine[7,12] (**8**) afforded a 62% yield of the purine derivative **9** which was deprotected by sequential treatment with methylhydrazine and bromotrimethylsilane to give the phosphonic acid **5** in 18% overall yield (Scheme I). Similar coupling of the alcohol **7** with 2-[bis(*tert*-butoxycarbonyl)amino]-9-hydroxy-6-methoxypurine[7,12] (**10**) gave the intermediate **11** in 67% yield. Treatment of compound **11** with bromotrimethylsilane followed by mild acid treatment

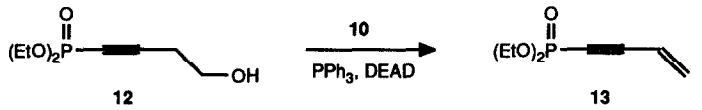
afforded a 75% yield of the phosphonic acid **6** (isolated as the mono-triethylammonium salt after purification by ion exchange chromatography on DEAE Sephadex eluting with triethylammonium bicarbonate buffer).

Scheme I



In an attempt to prepare chain extended analogues (related to **3**) the alcohol **12** was treated under Mitsunobu conditions with **10**. However, in contrast to the analogous alkenylphosphonate[9], the alcohol **12** did not couple successfully with **10**, the eliminated material **13** being the sole product (Scheme II).

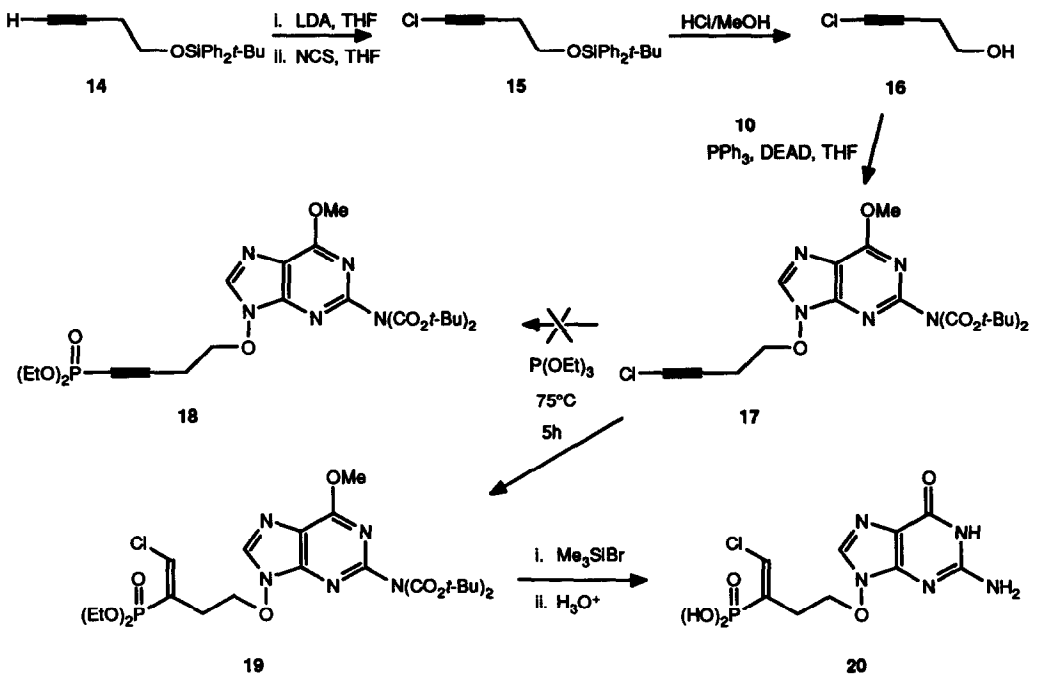
Scheme II



An attempt was made to introduce the phosphonyl moiety directly into a 9-alkynylpurine via the chloroalkyne **17** (Scheme III). Treatment of the alkyne **14** with lithium diisopropylamide followed by *N*-chlorosuccinimide[13] gave an 82% yield of the chloroalkyne **15** which was deprotected using methanolic hydrogen chloride to afford the volatile alcohol **16**[14] in 93% yield. Mitsunobu coupling of **16** with **10** then gave the 9-(chloroalkynyl)purine derivative **17** in 79% yield. Halogenoalkynes are known to undergo an apparent Arbusov reaction with trialkyl phosphites to give alkynylphosphonates when the other alkyne substituent is an α-anion stabilising group [e.g. Ph, CH_2=CH, $(EtO)_2P(O)$, Cl, Me_3Si or Et_3Sn][15], although to our knowledge, the outcome of the reaction with simple alkyl substituted halogenoalkynes has not been

reported. Treatment of **17** with triethyl phosphite did not give the desired alkynylphosphonate **18**, however, but only a 38% yield of the (*Z*)-2-chloroalkenylphosphonate[16] **19**.[17] Compound **19** was subsequently deprotected using bromotrimethylsilane followed by mild acid treatment to afford the phosphonic acid **20** in 67% yield.

Scheme III



In cell culture assays, the phosphonic acids **5** and **20** had no effect on the replication of herpes simplex type 1 (HSV-1) or cytomegalovirus (CMV) at concentrations up to 100μg/ml, but both compounds showed moderate activity against herpes simplex virus type 2 (HSV-2) (IC_{50} 14 and 25μg/ml respectively). By contrast, compound **6** showed activity against HSV-1, HSV-2, varicella zoster virus and CMV at similar concentrations (IC_{50} 6.5, 3.9, 3.2 and 3.8μg/ml respectively). In the herpesvirus tests no toxicity for the cell monolayers was observed, except in the HSV-1 test where cytotoxicity was observed for **6** at 30μg/ml. However, in a secondary assay of cytotoxicity in uninfected MRC-5 cells, compounds **6** and **20** inhibited DNA synthesis (IC_{50} 4.2 and 17μg/ml respectively, as measured by incorporation of tritiated thymidine) indicating that these compounds are toxic to replicating cells. Compound **5** showed only slight cytotoxicity in this assay (IC_{50} 98μg/ml). The biological properties of compounds **5** and **6** suggest that the phosphonoalkynyl group can act as an effective stabilised phosphate mimic which could find application in other areas.

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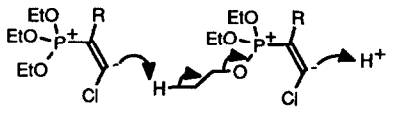
References and Notes.

1. De Clercq, E.; Holý, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature*, **1986**, *323*, 464.
2. De Clercq, E. *Biochem. Pharmacol.*, **1991**, *42*, 963.
3. Harnden, M. R.; Jarvest, R. L.; Parratt, M. J. *J. Chem. Soc., Perkin Trans. I*, **1992**, 2259 and references cited therein.
4. De Clercq, E.; Sakuma, T.; Baba, M.; Pauwels, R.; Balzarini, J.; Rosenberg, I.; Holý, A. *Antiviral Res.*, **1987**, *8*, 261.
5 Pauwels, R.; Balzarini, J.; Schols, D.; Baba, M.; Desmyter, J.; Rosenberg, I.; Holý, A.; De Clercq, E. *Antimicrob. Agents Chemother.*, **1988**, *32*, 1025.
6. Duckworth, D. M.; Harnden, M. R.; Perkins, R. M.; Planterose, D. N. *Nucleosides and Nucleotides*, **1991**, *10*, 427.
7. Duckworth, D. M.; Harnden, M. R.; Perkins, R. M.; Planterose, D. N. *Antiviral Chem. Chemother.*, **1991**, *2*, 229.
8. Perkins, R. M.; Patience, C.; Ashton, R. J.; Love, S.; Barnes, K.; Jenkins, O.; Donker, G.; Reynolds, K.; Elphick, L. M.; Planterose, D. N.; Kenig, M. D.; Darlison, S. J.; Vere Hodge, R. A.; Duckworth, D. M.; Serafinowska, H. T.; Harnden, M. R.; Weiss, R. A.; Brown, A. G. *J. Cell. Biochem., Suppl. 16E*, **1992**, 86.
9. Harnden, M. R.; Parkin, A.; Parratt, M. J.; Perkins, R. M. manuscript submitted for publication.
10. Kim, C. U.; Luh, B. Y.; Martin, J. C. *Tet. Lett.*, **1992**, *33*, 25.
11. Poss, A. J.; Belter, R. K. *J. Org. Chem.*, **1987**, *52*, 4810.
12. Harnden, M. R.; Wyatt, P. G. *Tet. Lett.*, **1990**, *31*, 2185.
13. Murray, R. E., *Synth. Commun.* **1980**, *10*, 345.
14. Compound **16** was isolated as follows: The reaction mixture was neutralised by addition of saturated aqueous sodium bicarbonate solution then the product was extracted into dichloromethane. The organic portion was dried ($MgSO_4$) and filtered then the solvent was removed carefully under reduced pressure. The residue was purified by column chromatography on silica gel eluting with pentane-diethyl ether mixtures.
15. Burt, D. W.; Simpson, P. *J. Chem. Soc. (C)*, **1969**, 2273 and references cited therein.
16. Spectroscopic data for **19**: ν_{max} (film) 2910, 1795, 1730, 1590, 1475, 1370, 1250, 1155, 1100, 1050 and 970 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.38 (6H, t, *J* 7.5 Hz, 2 x CH_2CH_3), 1.47 (18H, s, 2 x $C(CH_3)_3$), 2.88 (2H, dt, $^3J_{HH}$ 6.1 Hz and $^3J_{PH}$ 15.5 Hz, OCH_2CH_2), 4.17 (7H, m, 2 x CH_2CH_3 and OCH_3), 4.59 (2H, t, *J* 6.1 Hz, $NOCH_2$), 7.03 (1H, d, *J* 37.4 Hz, PC=CH), 8.04 (1H, s, 8-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.33 (d, $^3J_{PC}$ 6.1 Hz, $POCH_2CH_3$), 27.94 (2 x $C(CH_3)_3$), 33.54 (d, $^2J_{PC}$ 8.5 Hz, $POCH_2CH_3$), 78.75 ($NOCH_2CH_2$), 83.18 (2 x $C(CH_3)_3$), 116.31, 128.21 (d, $^1J_{PC}$ 180.1 Hz, PC=CH), 132.98 (PC=CH), 138.64, 148.51, 150.88, 152,60, 161.77 (C=O); HRMS calculated for $C_{24}H_{37}ClN_5O_9P$ 606.2095, found 606.2098.
17. *Trans*-addition to the alkyne must give the intermediate **21** (preferentially formed due to stabilisation of the negative charge by the chloro substituent) which presumably breaks down to the phosphonate **19** by protonation, and elimination of ethene or vice versa. The scheme below indicates the overall process only and is not intended to imply concertedness.



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