SHORT PAPER

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Facile synthesis of bivalent ligand-based phosphoramidates of AZT†

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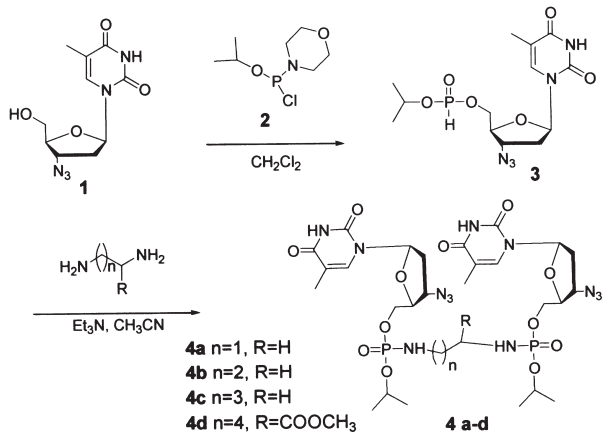
A series of bivalent ligand-based phosphoramidates of AZT were synthesised in a facile way using a modified Atherton-Todd reaction.

Keywords: phosphoramidate, AZT, anti-HIV, Atherton-Todd reaction, *H*-phosphonate

AZT (Zidovudine) **1** was the first 2',3'-dideoxynucleosides (ddN) that was approved by the Food and Drug Administration (FDA) for the treatment of patients suffering from AIDS (acquired immunodeficiency syndrome).[1] It can be converted into corresponding 5'-O-triphosphate, and it then may inhibit the replication of the virus by competitive inhibition of the viral reverse transcriptase (RT) and/or by incorporation and subsequent chain termination of the growing viral DNA strand.[2] However, a significant dose-related toxicity associated with the administration of AZT, resulting in anemia and leucopenia, remains a limit factor for its use.[3] Moreover, the necessity of administrating high doses of AZT to achieve adequate concentrations in the cerebrospinal fluid (CSF) results in bone marrow toxicity. In attempts to increase its therapeutic efficacy, numerous AZT prodrugs have been reported.[4] The expected advantage of the AZT prodrugs can be many including, synergistic drug interactions, enhancement of AZT intracellular uptake, increasing of AZT brain delivery, bypass the first AZT phosphorylation step into the cells and a decreasing toxicity.[5] In the present paper, we report the synthesis a new class of bivalent ligand-based phosphoramidate prodrugs.



Scheme 1

In the first step, AZT was synthesised from thymidine by a two-step route.[6] The phosphoryl reagent **2** (^{31}P NMR δ 168.3 ppm) was prepared using a standard procedure.[7] Then, AZT was phosphorylated by reagent **2** in dichloromethane. When the ^{31}P NMR showed that the reaction was over, the resulting phosphoramidite was not separated but it was hydrolysed directly with l*H*-tetrazole in the presence of water. After purification on a silica gel column, the key intermediate *H*-phosphonate **3** was obtained in 85% yield. It displayed the characteristic ^{31}P NMR spectrum of *H*-phosphonate ($^1J_{P-H}$=702 Hz). It was a pair of diastereomers due to the chirality of the AZT. The ratio was about 1:l. Their signals in the ^{31}P NMR spectrum appear at δ 7.8ppm and 6.8ppm respectively. Using our modified Atherton–Todd reaction,[8] the target bivalent phosphoramidates **4a–d** were then obtained in high yield by the reaction of *H*-phosphonate **3** with the corresponding diamine. It is worth mentioning that in compound **4d**, the diamine is lysine methyl ester, and is also an analogue of nucleotide kinase inhibitor.[9]

In conclusion, a new class of bivalent ligand-based phosphoramidates of AZT has been synthesised in a simple way. The structures were confirmed on the basis of the NMR, ESI-MS and HRMS. The methodology used herein can be extended to other biological significant molecules such as carbohydrates and steroids.

Experimental

All ^{1}H NMR spectra were recorded in CD_3OD on an INOVA 500 and INOVA 300 NMR spectrometers and the chemical shifts were reported in ppm and TMS as internal standard. ^{31}P NMR was recorded on a Bruker APC 200 spectrometer at 80 MHz under decoupling conditions, with 85% phosphoric acid (δ =O.O) as external standard. ES1 mass spectra were obtained on a Bruker Esquire-LC ion trap mass spectrometer operated in positive ion mode. The ESI HRMS were measured on a Bruker APEX II spectrometer in positive ion mode.

Preparation of 5'-O-hydrogen phosphonate **3**: The phosphorylation reagent **2** (253 mg, 1.2mmol) was added to a stirred suspension of AZT **1**(267 mg, lmmol) in anhydrous dichloromethane (10ml) containing Et_3N (151 mg, 1.5mmol) over 10 min. The mixture was stirred for an additional 15 min. The solvent was evaporated and the residue was dissolved in CH_3CN (15ml). 1 *H*-tetrazole (100mg) and H_2O (0.15ml) were added to hydrolyse the obtained phosphoramidite. After 15min, the reaction mixture was concentrated. The residue was isolated on a silica gel column with $CHCl_3$: CH_3OH (30:1) as the eluent. *H*-phosphonate **3** was obtained in 85% overall yield as a colourless oil.

Spectral data of **3** (a pair of diastereomers: different chemical shifts assigned to the same centre are marked with asterisk): ^{31}P NMR ($CDCl_3$, 80MHz) δ 8.02, 7.18*, ^{1}H NMR (500MHz, $CDCl_3$): 9.37 (br, lH, NH, H-3), 7.40, 7.37* (d, lH, H-6, *J*=l.5Hz), 6.94, 6.93* (d, 1H, P-H, $^1J_{P-H}$=703Hz), 6.22 (m, lH, H-l'), 4.83 (m, lH, $(CH_3)_2CHO$), 4.32–4.37 (m, 3H, H-5' and H-4'), 4.04 (m, lH, H-3'), 2.46 (m, lH, H-2'), 2.37 (m, 1H, H-2') 2.36 (d, 1H, H-5, *J*=l.5Hz), 1.39 (m, 6H, $(CH_3)_2CHO$); ^{13}C NMR (125Hz, $CDC1_3$): 163.86 (C-4), 150.26 (C-2), 135.15, 135.07* (C-6), 111.06, 11l.0l* (C-5) 84.59

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(C-l'), 81.80, 81.75* (C-4'), 71.83 (^2J=4.3Hz), 71.89* (^2J=5.3Hz, $CH_3)_2CHO$), 63.86 (C-5'), 59.83, 59.78* (C-3'), 36.84 (C-2'), 23.63, 23.39* ($(CH_3)_2CHO$), 12.10 (5-CH_3) ESI-MS(+): *m/z* 396 $[M+Na]^+$, 374 $[M+H]^+$.

General procedure for the preparation of bivalent ligand-based phosphoramidates **4a–d**: A solution of *H*-phosphonate **3** (lmmol) in CH_2C1_2 (2ml) was slowly added to a solution of corresponding diamine (0.5 mmol) in Et_3N (3mmol), $CC1_4$ (0.5ml) and CH_3CN (5ml) at 0°C. The reaction mixture was stirred at room temperature for 10min. Then the solvent was concentrated under reduced pressure at 40'C. The residue was puritied on a silica gel column (CH_2C1_2:CH_3OH =15:1) to give the corresponding phosphoramidates **4a–d.**

4a, colourless oil, spectral data (a pair of diastereomers): ^{31}P NMR (CD_3OD) δ 10.60; ^1H NMR (500MHz, CD_3OD) δ 7.55, 7.53* (d, 2H, *J*=l.5 Hz, H-6), 6.15 (m, 2H, H-1') 4.61 (m, 2H, $(CH_3)_2CHO$), 4.41 (m, 2H, H-4'), 4.17 (m, 4H, H-5'), 4.04 (m, 2H, H-3'), 2.98 (m, 4H, CH_2NH), 2.43 (m, 4H, H-2'), 1.90 (s, 6H, 5-CH_3), 1.33 (d, 12H, *J*=6.0Hz, $(CH_3)_2CHO$); ^{13}C NMR (125MHz, CD_3OD) δ 166.23 (C-4) 152.13 (C-2), 137.84, (C-6), 111.90 (C-5), 86.56, 86.41* (C-l'), 83.79, 83.72* (C-4'), 73.29($(CH_3)_2CHO$), 66.67, 66.49* (C-5'), 61.88, 61.82* (C-3'), 43.61(CH_2NH), 38.04, 37.68* (C-2'), 24.13 ($(CH_3)_2CHO$), 12.64 (5-CH3); ESI-MS (+): *m/z* 803 $[M+H]^+$, 825 $[M+Na]^+$, 841 $[M+K]^+$. HR-MS: 803.2750 (Found) for $C_{28}H_{45}N_{12}O_{12}P_2$ $[M+H]^+$, 803.2750 (Calcd).

4b, colourless oil, spectral data (a pair of diastereomers): ^{31}P NMR (CD_3OD) δ 10.76; ^1H NMR (300MHz, CD_3OD) δ 7.55, 7.53* (s, 2H, H-6), 6.15 (m, 2H, H-l'), 4.60 (m, 2H, $(CH_3)_2CHO$), 4.41 (m, 2H, H-4'), 4.17 (m, 4H, H-5'), 4.05 (m, 2H, H-3'), 2.98 (m, 4H, CH_2NH), 2.43 (m, 4H, H-2'), 1.90 (d, 6H, 1.5 Hz, 5-CH_3), 1.68(m, 2H, CH_2CH_2NH), 1.32 (d, 12H, *J*=6.0Hz, $(CH_3)_2CHO$); ^{13}C NMR (125MHz, CD_3OD) δ 166.13 (C-4), 152.10 (C-2), 137.70, 137.62* (C-6), 111.89 (C-5), 86.35, 86.22* (C-l'), 83.78, 83.70* (C-4'), 73.05($(CH_3)_2CHO$), 66.55, 66.39* (C-5'), 61.89 (C-3'), 39.49 (CH_2NH), 37.76, 37.72* (C-2'), 34.60 ($HNCH_2CH_2CH_2NH$), 24.15, 24.10* ($(CH_3)_2CHO$), 12.76, 12.71* (5-CH_3); ESI-MS (+): *m/z* 817 $[M+H]^+$, 839 $[M+Na]^+$, 855 $[M+K]^+$. HR-MS (ESI) *m/z*: 817.2913 (Found) for $C_{29}H_{47}N_{12}O_{12}P_2$ $[M+H]^+$, 817.2906 (Calcd).

4c, colourless oil, spectral data (a pair of diastereomers): ^{31}P NMR (CD_3OD) δ 10.74: ; ^1H NMR (500MHz, CD_3OD) δ 7.56, 7.54* (s, 2H, H-6), 6.16 (m, 2H, H-1'), 4.60 (m, 2H, $(CH_3)_2CHO$), 4.42 (m, 2H, H-4'), 4.17 (m, 4H, H-5'), 4.05 (m, 2H, H-3'), 2.88 (m, 4H, CH_2NH), 2.43, 2.42* (m, 4H, H-2'), 1.90 (s, 6H, 5-CH_3), l.52 (m, 4H, CH_2CH_2NH), l.32 (d, 12H, $(CH_3)_2CHO$); ^{13}C NMR (125MHz, CD_3OD) δ ppm 166.09 (C-4), 152.05 (C-2), 137.64, 137.58* (C-6), 111-81 (C-5), 86.35, 86.19*, (C-1'), 83.76, 83.70* (C-4'), 72.9l(($(CH_3)_2CHO$), 66.40, 66.18* (C-5'), 61.86, 61.80* (C-3'), 41.94(CH_2NH), 37.97, 37.74* (C-2'), 29.9 1($HNCH_2CH_2$ CH_2CH_2NH), 24.16, 24.08* ($(CH_3)_2CHO$), 12.69 (5-CH_3); ESI-MS (+): *m/z* 831 $[M+H]^+$, 853 $[M+Na]^+$, 869 $[M+K]^+$. HR-MS (ESI) *m/z*: 831.3057 (Found) for $C_{30}H_{48}N_{12}O_{12}P_2$ $[M+H]^+$, 831.3063 (Calcd).

4d, gum, spectral data (two pair of diastereomers): ^{31}P NMR (CD_3OD): δ 10.65, 10.55, 8.56; ^1H NMR (500MHz, CD_3OD) δ 7.53 (s, 2H, H-6), 6.17 (m, 2H, H-l'), 4.56 (m, 2H, $(CH_3)_2CHO$), 4.45 (m, 2H, H-4'), 4.20 (m, 4H, H-5'), 4.08 (m, 2H, H-3'), 3.65, 3.60* (3H, s, OCH_3), 3.58 (lH, m, H-α), 2.80 (m, 2H, H-ε), 2.43 (m, 4H, H-2'), 1.90, 1.89* (s, 6H, 5-CH_3), 1.52 (m, 4H, H-β), 1.40 (m, 4H, H-δ and H-γ), 1.32 (d, 12H, $(CH_3)_2CHO$); ^{13}C NMR (125MHz, CD_3OD) δ 175.50, 175.42 ($COOCH_3$), 166.09 (C-4), 152.05 (C-2), 137.64, 137.58 (C-6), 111.81, 111.50 (C-5), 86.50, 86.23 (C-l'), 83.94, 83.82* (C-4'), 72.9l ($(CH_3)_2CHO$), 66.56 (m, C-5'), 61.86, 61.80 (C-3'), 56.05 (C-α), 53.35 (OCH_3), 43.20 (C-ε), 37.97, 37.74 (C-2'), 34.56 (C-β), 32.25 (C-δ), 24.16, 24.08* ($(CH_3)_2CHO$), 23.86 (C-γ), 12.59 (5-CH_3); ESI-MS (+): *m/z* 903 $[M+H]^+$, 925 $[M+Na]^+$; HR-MS (ESI) *m/z*: 903.3269 (Found) for $C_{33}H_{53}N_{12}O_{14}P_2$ $[M+H]^+$, 903.3274 (Calcd).

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