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Research Paper

Synthesis of methoxypoly(ethylene glycol) carbonate prodrugs of zidovudine and penetration through human skin *in vitro*

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Abstract

Objectives The aim of this study was to synthesise a series of novel methoxypoly (ethylene glycol) carbonate prodrugs of the antiretroviral drug zidovudine (azidothymidine, AZT) in an attempt to enhance the physicochemical properties for transdermal delivery, which may reduce the severe side-effects and toxicity associated with high oral doses of AZT.

Methods Methoxypoly(ethylene glycol) carbonates of AZT were synthesised in two steps: activation of the relevant methoxypoly(ethylene glycol) with *p*-nitrophenyl chloroformate, followed by reaction with AZT. Analysis of the hydrolytic stability in phosphate buffer at pH 5.0 and 7.4 revealed that all the carbonates were markedly more stable at pH 5.0 than at pH 7.4 (0.01 M), with half-lives ranging from 15 to 44 days at pH 5.0 and from 6 to 24 days at pH 7.4. The potential of the series to penetrate the skin was evaluated *in vitro* by measuring diffusion through excised abdominal female human skin at pH 5.0.

Key findings Prodrugs with 1–3 or 8 oxyethylene units in the methoxypoly(ethylene glycol) moiety were found to permeate the skin whereas those with 12 or 17 units did not. The prodrug with eight oxyethylene units was the most effective penetrant, permeating the skin with a mean flux of 53.3 ± 46.5 nmol/cm^2 per h, which is 2.4–10.1 times that of AZT (8.55 ± 5.3 nmol/cm^2 per h).

Conclusions The bioreversible conjugation of the methoxypoly(ethylene glycol) promoiety to AZT appears to be a promising strategy for the transdermal delivery of AZT at a therapeutic dose.

Keywords AZT; methoxypoly(ethylene glycol) (MPEG); oxyethylene; prodrug; transdermal penetration

Introduction

Zidovudine (3′-azido-3′-deoxythymidine, AZT; compound (**4**)) is an antiretroviral agent that is a synthetic analogue of the naturally occurring nucleoside thymidine; its virustatic activity results from inhibition of nucleoside reverse transcriptase. AZT was the first and is still the most important drug used for antiretroviral (ARV) therapy, either alone, particularly for the prevention of perinatal transmission of HIV from an HIV-infected mother to her child, or in combination with other ARV agents. A number of clinical benefits have been reported for patients with AIDS or AIDS-related complex receiving AZT, including increased survival and decreased opportunistic infections.[1] However, despite these undeniable benefits, AZT shows dose-related toxic effects, especially on bone marrow, which can necessitate dose reduction or discontinuation of treatment.[2]

After oral administration, AZT is rapidly absorbed from the gastrointestinal tract, with a peak serum concentration occurring in about 1 h. In order to maintain therapeutic levels (> 1 μM), large doses are frequently administered (200 mg every 4 h).[3] This dosage leads to blood levels that exceed toxic levels, resulting in severe adverse effects such as granulocytopenia and anaemia. Hence, the controlled delivery of AZT through non-oral pathways such as the transdermal route can be a useful way to circumvent these problems. In reviews, Kim and Chien[4] and Panchagnula and Patel[5] reported that transdermal delivery of AZT provided sustained plasma concentrations for a prolonged period and consequently improved patient compliance, as well as reducing the frequency and severity of side-effects. However, because of its hydrophilicity (log *P* –0.9),[6] the passive

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permeation rate of AZT is very poor and is below the rate sufficient to achieve a therapeutic effect.

Transdermal permeation can be increased by physical techniques (e.g. iontophoresis, electroporation) and the use of chemical penetration enhancers. Previous research has reported that transdermal flux of AZT across excised rat skin is enhanced in the presence of several vehicles such as terpenes, fatty acids and their combination. However, these penetration enhancers also alter lipid bilayer fluidity and induce swelling of polar pathways of the skin,[7] which can lead to epidermal dehydration.[8]

An alternative promising strategy to enhance a drug's skin permeability is to develop a prodrug[9] by chemical modification of the drug into a bioreversible form, in order to change its physicochemical characteristics and thus enhance its skin permeation. Generally, regeneration of the parent drug occurs in the skin by enzymatic processes. Since the skin is a highly metabolic organ,[10] this approach has been used increasingly in the past 15 years to optimise the dermal and transdermal delivery of drugs.[11–14] When developing a transdermal drug delivery system, it is important to evaluate the skin permeation characteristics of drugs *in vitro* before conducting studies *in vivo*.

Here we report the synthesis of a series of novel methoxypoly(ethylene glycol) (MPEG) carbonate prodrugs of AZT, their physicochemical properties and their transdermal flux through excised human skin *in vitro*.

Materials and Methods

Materials

Methoxyethanol, di- and tri(ethylene glycol) monomethyl ethers and poly(ethylene glycol) monomethyl ethers (number average molecular weight 350, 550 and 750) were purchased from Fluka (Johannesburg, South Africa). Zidovudine (AZT) was kindly donated by Aspen Pharmacare (Port Elizabeth, South Africa). *para*-Nitrophenyl chloroformate (*p*-NPCF) was purchased from Sigma-Aldrich (Johannesburg, South Africa) and HPLC-grade methanol was obtained from Merck (Johannesburg, South Africa). All reagents and chemicals were of analytical grade.

General procedures

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer (Varian, Palo Alto, CA, USA) in $CDCl_3$, at a frequency of 300.075 and 75.462 MHz, respectively. Chemical shifts are reported in parts per million δ (ppm) using tetramethylsilane as internal standard. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet) and m (multiplet). The MS spectra were recorded on an analytical VG 7070E mass spectrometer (Fisons-VG Analytical, Manchester, UK) using electron impact (EI) at 70 eV as the ionisation technique. Melting points were determined by differential scanning calorimetry (DSC) using a Shimadzu DSC-50 instrument (Shimadzu Corp. Kyoto, Japan). Thin-layer chromatography was performed using silica gel plates ($60F_{254}$ Merck). Preparative flash column chromatography was carried out on silica gel (230–240 mesh, G60 Merck).

HPLC analysis

The HPLC system consisted of Agilent 1100 series auto sampler, variable wavelength detector and isocratic pump (Hewlett Packard, Palo Alto, CA, USA). The Agilent Chemstation for LC systems software package was used for data analysis. Separation was achieved using a Luna C_{18} column, 5 μm, 250 mm × 4.60 mm (Phenomenex, Torrance, CA, USA), protected with a Securityguard precolumn (C_{18}, 4 mm × 3 mm) insert (Phenomenex). The compounds were quantified using a gradient method at a flow rate of 1 ml/min. The gradient consisted of 15% solvent B in A to 85% B in 10 min (A = 0.1% acetic acid in water; B = methanol). A calibration curve of peak area versus drug concentration was constructed for each compound. The working concentrations were in the range 0.1–200 μg/ml. Correlation coefficients (r^2) were in the range 0.998–1, indicated good linearity. The absorption maximum for all the prodrugs studied was at 267 nm; this wavelength was therefore used for the HPLC detection. A standard volume of 50 μl was injected for each sample. Recycling of the mobile phase did not adversely affect HPLC analysis. The retention times were 4.5 min for **4** (AZT), 5.3 min for **5a**, 5.4 min for **5b**, 5.5 min for **5c**, 5.7 min for **5d**, 5.9 min for **5e** and 6.2 min for **5f**.

LC–MS analysis

LC–MS was performed using a Hewlett Packard HP1100 series HPLC with binary gradient pump, autosampler and vacuum degasser, coupled to an Applied Biosystems API 2000 triple quadrupole mass spectrometer and analyte data acquisition and analysis software. The column was a Gemini C_{18}, 150 × 2 mm, 5 μm (Phenomenex). The gradient consisted of 90% A (0.1% formic acid in water) and 10% B (0.1% formic acid in acetonitrile) initially for up to 3 min, then 10% A / 90% B up to 6 min, followed by 90% A / 10% B up to 10 min. The flow rate was 250 μl/min and the injection volume was 5 μl.

The mass spectrometer used atmospheric pressure electron ionisation (turbo ion spray source) in positive-ion mode. The full scan from 100 to 200 amu was performed in 1 s. The declustering, focusing and entrance potentials were 80, 400 and 10 V, respectively; the ion spray voltage was 5500 V. The curtain gas and ion source gases 1 and 2 were all used at a flow rate of 20 l/h; the temperature was 300°C.

General procedure for activation of MPEG

Activation of MPEG (Figure 1) was achieved using the method reported by Bodansky[15] with slight modifications. Thus, to a solution of MPEG (18.85 mmol) dissolved in 50 ml dry dichloromethane (DCM) and stirring at room temperature was added triethylamine (TEA; 20.73 mmol, 1.1 equiv. relative to MPEG). After 10 min, *p*-NPCF (19.85 mmol, 4 g) was added portionwise, and the stirring continued for 2 h. Evaporation of the solvent under reduced pressure resulted in the appearance of triethylammonium chloride as a white precipitate, which was washed several times with diethyl ether and filtered off. The filtrate was concentrated to an oily residue, which was further purified by flash chromatography to afford the activated MPEG. ^{1}H and

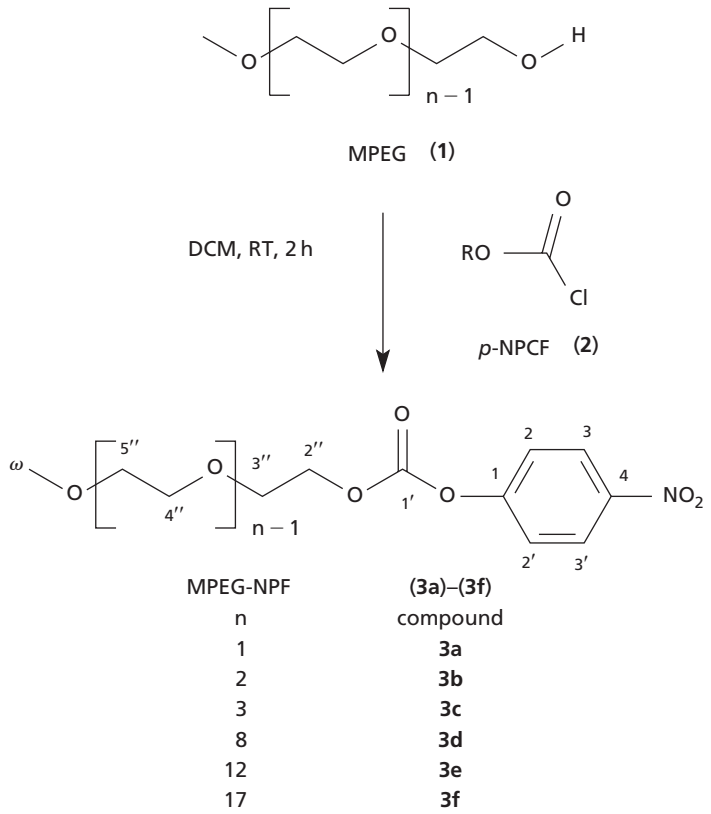

Figure 1 Activation of methoxypoly(ethylene glycol) (MPEG) using *p*-nitrophenyl chloroformate (*p*-NPF). DCM, dichloromethane.

^{13}C NMR chemical shifts and MS data for compounds **3a–3f** are reported.

Methoxyethylene glycol p-*nitrophenyl carbonate (n = 1, 3a)*

Carbonate **3a** was purified by flash silica gel column chromatography eluting with DCM : EtOAc (10 : 1) and obtained as white crystals with yield of 4.35 g (91%) after crystallisation in hexane : EtOAc (5 : 1). m.p. 47.8°C, $C_{10}H_{11}NO_6$. ^{1}H NMR δ (ppm) 3.40 (s, 3H, H-ω), 3.66–3.69 (m, 2H, H-3″), 4.39–4.3 (m, 2H, H-2″), 7.37 (d, 2H, H-3 and -3′, J = 9.26 Hz), 8.25 (d, 2H, H-2 and -2′, J = 9.26 Hz). ^{13}C NMR δ (ppm): 59.02 (C-ω), 68.10 (C-3″), 69.83 (C-2″), 115.53 (C-4), 121.68 (C-3′), 125.47 (C-3), 126.09 (C-2), 145.37 (C-2′), 152.45 (C-1), 155.47 (C-1′). MS FAB 242 ((M + H$^+$) 70%), 289 (10%), 210 (20%), 165 (12%), 154 (90%), 137 (100%), 123 (32%), 119 (20%), 107 (44%).

Methoxydi(ethylene glycol) p-*nitrophenyl carbonate (n = 2, 3b)*

Compound **3b** was purified by flash silica gel column chromatography eluting with DCM : EtOAc (10 : 1) and obtained as a yellowish oil with a yield of 4.9 g (87%). $C_{12}H_{15}NO_7$. ^{1}H NMR δ (ppm): 3.36 (s, 3H, H-ω), 3.50–3.54 (m, 2H, H-5″), 3.55–3.60 (m, 2H, H-4″), 3.8–3.85 (m, 2H, H-3″), 4.40–4.50 (m, 2H, H-2″), 7.36 (d, 2H, H-3 and -3′, J = 9.13 Hz), 8.25 (d, 2H, H-2′ and -2, J = 9.25 Hz). ^{13}C NMR δ (ppm): 57.60 (C-ω), 68.25 (C-5″), 68.37 (C-4″), 69.35 (C-3″), 71.50 (C-2″), 121.57 (C-4), 124.85 (C-3′), 126.09 (C-3), 144.95 (C-2′), 152.25 (C-2), 154.97 (C-1′). MS FAB 286 ((M + H$^+$) 40%), 210 (72%), 165 (12%), 154 (22%), 137 (42%), 123 (22%), 119 (16%), 107 (26%), 103 (100%).

Methoxytri(ethylene glycol) p-*nitrophenyl carbonate (n = 3, 3c)*

Purification by flash silica column chromatography eluting with DCM : EtOAc (10 : 1) yielded 3.73 g (57.1%) carbonate **3c** as a yellowish oil. $C_{14}H_{19}NO_8$. ^{1}H NMR δ (ppm): 3.28 (s, 3H H-ω), 3.44–3.47 (m, 2H, end chain H-5″), 3.55–3.63 (m, 6H, H-4″, mid-chain H-5″), 3.71–3.74 (m, 2H, H-3″), 4.33–4.36 (m, 2H, H-2″), 7.37 (d, 2H, H-3 & -3′, J = 9.31 Hz), 8.25 (d, 2H, H-2′ and -2, J = 9.23 Hz). ^{13}C NMR δ (ppm): 58.69 (C-ω), 68.07 (C-5″), 68.37 (C-4″), 70.32 (C-3″), 71.69 (C-2″), 121.55 (C-4), 125.04 (C-3′), 126.09 (C-3), 145.15 (C-2′), 152.20 (C-2), 155.33 (C-1′). MS FAB 330 ((M + H$^+$) 30%), 392 (30%), 210 (66%), 167 (18%), 154 (34%), 149 (100%), 137 (42%), 103 (50%).

Methoxyocta(ethylene glycol) nitrophenyl carbonate (n = 8, 3d)

Compound **3d** was purified by flash silica gel column chromatography eluting with DCM : EtOAc (5 : 1) and EtOAc, successively, and obtained as a yellowish oil, with a yield of 3.5 g (34.5%). $C_{22}H_{35}NO_{12}$. ^{1}H NMR δ (ppm): 3.33 (s, 3H H-ω), 3.49–3.52 (m, 2H, end chain H-5″), 3.62 (bs, 24H, H-4″, mid-chain H-5″), 3.75–3.79 (m, 2H, H-3″), 4.38–4.41 (m, 2H, H-2″), 7.37 (d, 2H, H-3 and -3′, J = 9.31

Hz), 8.26 (d, 2H, H-2′ and -2, J = 9.31 Hz). ^{13}C NMR δ (ppm): 58.45 (C-ω), 68.17 (C-5″), 68.32 (C-3″), 70.22 (C-4″), 71.65 (C-2″), 121.45 (C-4), 125.14 (C-3′), 126.01 (C-3), 145.12 (C-2′), 152.25 (C-2), 155.32 (C-1′). MS *m/z* 342 (9), 298 (9), 210 (72), 147 (46), 133 (10), 122 (26), 102 (100).

Methoxydodeca(ethylene glycol) nitrophenyl carbonate (n = 12, 3e)

Purification by flash silica column chromatography eluting with DCM : EtOAc (3 : 1) and EtOAc, successively, yielded 5 g (34.7%) of the target compound as a yellowish oil. $C_{32}H_{55}NO_{17}$. ^{1}H NMR δ (ppm) 3.28 (s, 3H, H-ω), 3.44–3.46 (m, 2H, end chain H-5″), 3.54 (bs, 42H, H-4″, mid-chain H-5″), 3.70–3.73 (m, 2H, H-3″), 4.32–4.36 (m, 2H, H-2″), 7.30 (d, 2H, H-3 and -3′ , J = 9.29 Hz), 8.18 (d, 2H, H-2′ and -2, J = 9.31 Hz). ^{13}C NMR δ (ppm): 58.72 (C-ω), 68.09 (C-5″), 68.38 (C-3″), 70.32 (C-4″), 71.69 (C-2″), 121.55 (C-4), 125.04 (C-3″), 126.09 (C-3), 145.15 (C-2′), 152.22 (C-1), 155.33 (C-1′). MS *m/z* 664 (14), 648 (20) 342 (10), 279 (14), 210 (70), 191 (18), 167 (33), 147 (54), 133 (30), 102 (100).

Methoxyheptadeca(ethylene glycol) nitrophenyl carbonate (n = 17, 3f)

Carbonate **3f** was purified by flash silica gel column chromatography eluting with DCM : EtOAc (1 : 3) and EtOAc, successively, and was obtained as a yellowish oil, with a yield of 4.32 g (23%). $C_{42}H_{75}NO_{22}$. ^{1}H NMR δ (ppm) 3.19 (s, 3H, H-ω), 3.37–3.44 (m, 2H, end chain H-5″), 3.46 (bs, 62H, H-4″, mid-chain H-5″), 3.60–3.70 (m, 2H, H-3″), 4.20–4.30 (m, 2H, H-2″), 7.23 (d, 2H, H-3 and -3′, J = 9.31 Hz), 8.10 (d, 2H, H-2′ and -2, J = 9.28 Hz). ^{13}C NMR δ (ppm): 58.42 (C-ω), 67.83 (C-5″), 69.12 (C-3″), 70.06 (C-4″), 71.44 (C-2″), 121.37 (C-4), 125.04 (C-3′), 126.09 (C-3), 145.15 (C-2′), 152.22 (C-1), 155.33 (C-1′). MS *m/z* 211 (84), 149 (49), 137 (19), 115 (25), 103 (100).

General procedure for the synthesis of MPEG carbonates of AZT

MPEG carbonates of AZT, compounds **5a**–**5f** were synthesised by following the general method outlined in Figure 2. TEA (16.58 mmol, 2 equiv.) was added to a solution of activated methoxypoly(ethylene glycol) and MPEG-NPF

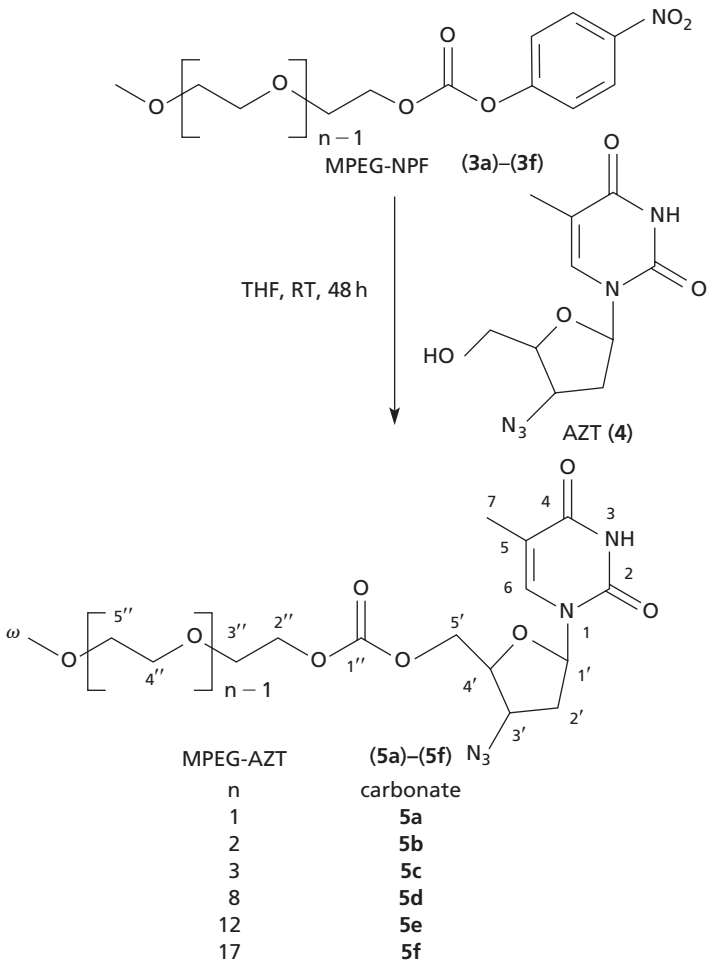

Figure 2 Synthesis of methoxypoly(ethylene glycol) (MPEG) zidovudine (AZT) prodrugs. DCM, dichloromethane; *p*-NPF, *p*-nitrophenyl chloroformate; RT, room temperature; THF, tetrahydrofuran.

(8.29 mmol, 2 g) in dry tetrahydrofuran (THF) (20 ml) whilst stirring at room temperature. AZT (9.95 mmol, 2.66 g, 1.2 equiv.) dissolved in THF (5 ml) was added dropwise over 5–10 min, followed by a catalytic amount of *N*,*N*-dimethylaminopyridine (0.83 mmol, 0.1 g, 0.1 equiv). After 48 h' stirring at room temperature, the solvent was evaporated *in vacuo*. Purification of the residue by silica gel flash chromatography afforded the target compounds.

*3′-Azido-3′-deoxythymidin-5′-yl-*O*-(methoxy(ethylene glycol)) carbonate (5a)*

A yield of 1.44 g (47%) of white crystalline compound was obtained. m.p. 84.41°C, $C_{14}H_{19}N_5O_7$; ^{1}H NMR δ (ppm): 1.89 (s, 3H, H-7), 2.31–2.58 (m, 2H, H-2′a, b), 3.34 (s, 3H, H-ω), 3.58 (t, 2H, H-3″, J = 4.26 Hz), 4.00–4.04 (m, 1H, H-4′), 4.20–4.44 (m, overlapping, 5H, H-3′, H-2″, H-5′a, b), 6.19 (t, 1H, H-1′, J = 6.18 Hz), 7.38 (s, 1H, H-6), 9.39 (s, 1H, H-3). ^{13}C NMR δ (ppm): 12.3 (C-7), 37.63 (C-2′), 58.83 (C-ω), 59.87 (C-3″), 66.25 (C-3′), 67.43 (C-2″), 69.96 (C-5′), 81.57 (C-1′), 84.87 (C-4′), 111.38 (C-5), 135.2 (C-6), 150.28 (C-2), 154.60 (C-4), 163.76 (C-1″). *m/z* (EI+, %): M^+ 369 (15), 244 (33), 126 (50), 81 (95), 59 (100), 45 (50), 28 (51).

*3′-Azido-3′-deoxythymidin-5′-yl-*O*-(methoxydi(ethylene glycol)) carbonate (5b)*

Compound **3b** (7.01 mmol, 2 g) afforded 1.88 g (64.9%) of **5b** as yellowish oil after purification by flash silica gel column chromatography eluting with DCM : EtOAc : MeOH (10 : 1 : 0.5). $C_{16}H_{23}N_5O_8$. ^{1}H NMR δ (ppm): 1.91 (s, 3H, H-7), 2.41–3.36 (m, 2H, H-2′a, b), 3.35 (s, 3H, H-ω), 3.50–3.60 (t, 2H, H-5″, J = 3.75), 3.61–3.69 (m, 2H, H-4″), 3.70 (t, 2H, H-3″, J = 4.67 Hz), 4.01–4.05 (m, 1H, H-4′), 4.25–4.44 (m, overlapping, 5H, H-3′, -5′a, b, -2″), 6.16 (t, 1H, H-1′, J = 6.08 Hz), 7.35 (s, 1H, H-6), 8.92 (s, 1H, H-3). ^{13}C NMR δ (ppm): 12.41 (C-7), 37.67 (C-2′), 58.99 (C-ω), 59.90 (C-4″), 66.22 (C-3′), 67.62 (C-5″), 68.78 (C-3″), 70.52 (C-2″), 71.87 (C-5′), 81.66 (C-1′), 85.04 (C-4′), 111.37 (C-5), 135.33 (C-6), 150.13 (C-2), 154.63 (C-4), 163.56 (C-1″). *m/z* (EI+, %): M^+ 413 (10), 245 (28), 126 (37), 103 (71), 81 (100), 59 (92), 45 (51), 28 (55).

*3′-Azido-3′-deoxythymidin-5′-yl-*O*-(methoxytri(ethylene glycol)) carbonate (5c)*

Compound **3c** (6.07 mmol, 2 g) afforded **5c** (1.67 g, 60.1%) as a yellowish oil after purification by flash silica gel column chromatography eluting with DCM : EtOAc : MeOH (10 : 1 : 0.5). $C_{18}H_{27}N_5O_9$; ^{1}H NMR δ (ppm): 1.88 (s, 3H, H-7), 2.35–2.43 (m, 2H, H-2′a, b), 3.33 (s, 3H, H-ω), 3.34–3.49 (m, 2H, end chain H-5″, J =3.25 Hz), 3.50–3.62 (m, 6H, H-4″, mid-chain H-5″), 3.69 (t, 2H, H-3″, J = 4.55 Hz), 4.01–4.04 (m, 1H, H-4′), 4.24–4.42 (m, overlapping, 5H, H-3′, -5′a, b, -2″), 6.17 (t, 1H, H-1′, J = 6.19 Hz), 7.35 (s, 1H, H-6), 9.26 (s, 1H, H-3). ^{13}C NMR δ (ppm): 12.37 (C-7), 37.59 (C-2′), 58.90 (C-ω), 59.91 (C-4″), 66.25 (C-3′), 67.60 (C-5″), 68.71 (C-3″), 70.53 (C-2″), 71.84 (C-5′), 81.59 (C-1′), 84.97 (C-4′), 111.32 (C-5), 135.27 (C-6), 150.22 (C-2), 154.58 (C-4), 163.69 (C-1″). *m/z* (EI+, %): M^+ 457 (10), 289 (36), 126 (35), 103 (51), 81 (59), 59 (100), 45 (56), 28 (60).

*3′-Azido-3′-deoxythymidin-5′-yl-*O*-(methoxyocta(ethylene glycol)) carbonate (5d)*

Compound **5d** (0.952g) was purified by flash silica gel column chromatography eluting with DCM : MeOH (10 : 1) then EtOAc : MeOH (5 : 1), as a yellowish oil. $C_{28}H_{47}N_5O_{14}$. ^{1}H NMR δ (ppm): 1.88 (s, 3H, H-7), 2.40–2.50 (m, 2H, H-2a, b′), 3.35 (s, 3H, H-ω), 3.50–3.54 (m, 2H, end chain H-5″), 3.62 (bs, 26H, H-4″, mid-chain H-5″), 3.70 (t, 2H, H-3″, J = 4.60 Hz), 4.01–4.05 (m, 1H, H-4′), 4.24–4.45 (m, overlapping, 5H, H-3′, -5′a, b, -2″), 6.17 (t, 1H, H-1′, J = 6.19 Hz), 7.33 (s, 1H, H-6), 9.04 (s, 1H, H-3). ^{13}C NMR δ (ppm): 12.35 (C-7), 37.52 (C-2′), 58.80 (C-ω), 60.00 (C-4″), 66.20 (C-3′), 67.45 (C-5″), 68.85 (C-3″), 70.75 (C-2″), 72.84 (C-5′), 82.47 (C-1′), 85.07 (C-4′), 111.45 (C-5), 136.25 (C-6), 150.20 (C-2), 154.85 (C-4), 163.78 (C-1″). *m/z* (EI+, %): M^+ 678 (10), 429 (34), 177 (50), 147 (65), 126 (61), 133 (77), 103 (86), 89 (92), 58 (75), 45 (100).

*3′-Azido-3′-deoxythymidin-5′-yl-*O*-(methoxydodeca(ethylene glycol)) carbonate (5e)*

Compound **5e** was obtained as yellowish oil with a yield of 1.31 g (55.7%) after flash chromatography eluting successively with DCM : MeOH (10 : 1) and EtOAc : MeOH (5 : 1). $C_{36}H_{63}N_5O_{18}$; ^{1}H NMR δ (ppm): 1.85 (s, 3H, H-7), 2.37–3.40 (m, 2H, H-2′a, b), 3.32 (s, 3H, H-ω), 3.48–3.58 (m, 2H, end chain H-5″), 3.59 (bs, 42H, H-4″, mid-chain H-5″), 3.80 (t, 2H, H-3″, J = 4.67 Hz), 4.00–4.02 (m, 1H, H-4′), 4.23–4.38 (m, overlapping, 5H, H-3′, -5′a, b, -2″), 6.15 (t, 1H, H-1′, J = 6.15 Hz), 7.28 (s, 1H, H-6), 9.20 (s, 1H, H-3). ^{13}C NMR δ (ppm): 12.36 (C-7), 37.56 (C-2′), 58.88 (C-ω), 59.90 (C-4″), 66.23 (C-3′), 67.57 (C-5″), 68.69 (C-3″), 70.46 (C-2″), 71.84 (C-5′), 81.57 (C-1′), 84.97 (C-4′), 111.25 (C-5), 135.25 (C-6), 150.10 (C-2), 154.55 (C-4), 163.53 (C-1″). LC-MS, M^+ (*m/z* 854).

*3′-Azido-3′-deoxythymidin-5′-yl-*O*-(methoxyheptadeca(ethylene glycol)) carbonate (5f)*

Compound **5f** was purified by flash silica gel column chromatography eluting with DCM : MeOH (10 : 1) and EtOAc : MeOH (4 : 1) and was isolated as yellowish oil with a yield of 1.02 g (44.9%). $C_{46}H_{83}N_5O_{23}$. ^{1}H NMR δ (ppm): 1.87 (s, 3H, H-7), 2.38–2.50 (m, 2H, H-2′a, b), 3.34 (s, 3H, H-ω), 3.49-3.59 (m, 2H, end chain H-5″), 3.60 (bs, 60H, H-4″, mid-chain H-5″), 3.70 (t, 2H, H-3″, J = 4.67 Hz), 4.00–4.05 (m, 1H, H-4′), 4.20–4.38 (m, overlapping, 5H, H-3′, -5′a, b, -2″), 6.17 (t, 1H, H-1′, J = 6.13 Hz), 7.35 (s, 1H, H-6), 8.84 (s, 1H, H-3). ^{13}C NMR δ (ppm): 12.39 (C-7), 37.61 (C-2′), 58.93 (C-ω), 59.93 (C-4″), 66.25 (C-3′), 67.61 (C-5″), 68.72 (C-3″), 70.51 (C-2″), 71.89 (C-5′), 81.61 (C-1′), 85.01 (C-4′), 111.27 (C-5), 135.26 (C-6), 150.25 (C-2), 154.55 (C-4), 163.45 (C-1″). LC-MS; M^+ (*m/z* 1074).

Physicochemical properties

Determination of chemical stability

The hydrolytic stability of MPEG-AZT prodrugs **5a**–**5f** in phosphate buffer (0.01 M) at pH 5.0 and pH 7.4 was determined using the method reported by Vlieghe *et al.*[16,17] Thus, 50 μl prodrug (10 mg/ml in DMSO) was added to 4950 μl phosphate buffer and the mixture incubated

at 32°C in a water bath. At various time points, a 400 μl sample was withdrawn and added immediately to 1600 μl ice-cold methanol. The resulting samples were thoroughly mixed, filtered through acrodisc filters (0.2 μm) and then analysed by the HPLC method described above.

To determine the relative rate of hydrolytic decomposition of the prodrugs, the logarithm of the concentration remaining was plotted against time. The half-life ($t_{1/2}$) of each compound **5a–5f**, compiled in Table 1, was calculated from the slope of the kinetic curves.

Solubility determination

The aqueous solubility of solid compounds **4** and **5a** was determined by preparing saturated solutions in phosphate buffer pH 5.0 (0.01 M). The slurries were stirred with magnetic bars in a water bath at 32°C for 24 h. An excess of solute was present at all times to ensure solutions were saturated. After 24 h, the solutions were filtered through acrodisc filters (0.2 μm), diluted with phosphate buffer and analysed by HPLC to determine the concentration of solute dissolved in the solvent. The experiment was performed in triplicate (Table 2).

Experimental log *D*

Equal volumes of *n*-octanol and phosphate buffer pH 5.0 (0.01 M) were saturated with each other under vigorous stirring for at least 24 h. Each compound (30 mg) was dissolved in 3 ml presaturated *n*-octanol, stoppered and agitated for 10 min in a 10 ml graduated tube (0.5 ml division), after which 3 ml of presaturated buffer was added. The tubes were stoppered and agitated for 45 min, then centrifuged at 2789*g* for 30 min. The *n*-octanol and aqueous phases were allowed to separate at room temperature for 5 min, after which volume ratios (v/v; *n*-octanol : buffer) were determined, and were found to be 1 in all experiments. The *n*-octanol phases were diluted with methanol, analysed by HPLC and the concentrations determined. The concentrations in the aqueous phases could then be deduced. This method had previously been used by Taylor and Sloan.[18] The log *D* value [log *n*-octanol : pH 5.0 buffer partition coefficient)] was calculated as the logarithmic ratio of the compound concentration in the *n*-octanol phase and the buffer. The experiment was performed in triplicate. The mean results are listed in Table 2.

Table 1 Chemical stability of compounds **5a–5f** in 0.01 M phosphate buffer at 32°C

Compound	pH 5.0		pH 7.4	
	k	$t_{½}$	k	$t_{½}$
5a	2.0	14.5	5.0	5.8
5b	1.9	14.8	4.6	6.3
5c	1.5	19.0	3.7	7.8
5d	1.1	25.3	3.3	8.9
5e	0.8	34.5	1.4	21.4
5d	0.7	43.8	1.2	23.8

k, rate of hydrolysis in 10^{-3}/h; $t_{½}$, half-life in days.

Diffusion experiments

Preparation of donor solution

Donor solutions (0.05 M) of AZT and its carbonates were obtained by dissolving the exact amount of each compound in phosphate buffer (0.01 M, pH 5.0). The process was carried out in stoppered flasks in a water bath at 32°C over a period of 24 h. The donor of carbonate **5a** was a suspension (with visible solute), donors of prodrugs **5b** and **5c** were colloids (suspension of non-visible solute) while **4** and derivatives **5d–5f** led to homogenous solutions.

Preparation of skin

The project '*In vitro* transdermal delivery of drugs through human skin' was approved by the Ethics Committee of the North-West University (Potchefstroom campus, South Africa).

Samples of skin for permeation studies were obtained from a white female undergoing cosmetic abdominoplasty. Samples from this donor were used for all experiments. The donor gave written informed consent, and her identity was masked to ensure anonymity.

A scalpel was used to separate the skin from the fat layer. The epidermis was then removed by immersion in 60°C HPLC-grade water for 60 s and was gently teased away from the skin with forceps. Special care was taken to ensure that the integrity of the epidermis was maintained. The epidermis

Table 2 Oxyethylene unit (n), molecular weight (Mw), melting point (Mp), aqueous solubility (S_w), lipid solubility (S_{OCT}), partition coefficient *D* (*n*-octanol : phosphate buffer, pH 5.0), steady-state flux (J_{ss}) and enhancement factor (EF) of zidovudine (AZT) and its carbonates **5a–5d**

Compound	n	Mw (g/mol)	Mp (°C)	Log S_W (mM)	Log S_{OCT} (mM)[d]	Log *D*[e]	J_{ss} (nmol/cm^2 per h)[f]	EF [Interval][g]
4 (AZT)		267.2	124	0.51[a] (1.98[b])	–0.19 (1.28 [b])	–0.7 ± 0.04	8.55 ± 5.27	1
5a	1	363.3	84.4	0.41[a] (1.27[b])	0.21 (1.07 [b])	–0.2 ± 0.01	38.5 ± 10.34	4.5 ± 1.75 [2.75;6.25][i]
5b	2	413.4	oil	0.99[c]	0.69	–0.3 ± 0.02	3.39 ± 2.78	0.4 ± 0.24 [0.17;0.63]
5c	3	457.4	oil	1.32[c]	0.72	–0.6 ± 0.04	10.59 ± 5.93	1.24 ± 0.6 [0.64;1.83]
5d	8	677.7	oil	1.85[c]	0.75	–1.1 ± 0.05	53.33 ± 46.51	6.23 ± 3.84 [2.39;10.08][i]
5e	12	853.9	oil	2.07[c]	0.77	–1.3 ± 0.02	nd	nd
5f	17	1074.2	oil	2.39[c]	0.79	–1.6 ± 0.06	nd	nd

[a]Log S_W for aqueous compounds was calculated from Log S_w = –log *D* – 0.01Mp + 1.05; [b]Determined experimentally; [c]Log S_w for oils was calculated from Log S_w = –1.072log *D* + 0.672; [d]Calculated from $S_{OCT} = D \times S_W$; [e]Means ± SD (*n* = 3 experiments); [f] Each experiment was run on three different cells; values are means ± SD; [g]EF expressed as ratio of flux of each carbonate **5a–5d** over that of the parent drug **4**; Values are means ± SE; [i]observed enhancement; nd, not detected.

was placed in a bath filled with HPLC water, then carefully set on Whatman filter paper, left to air dry and then wrapped in foil and stored at −20°C for no longer than 6 months. Before use, the epidermis was thawed and examined before being mounted on the Franz diffusion cells.

Skin permeation

Permeation studies were done in vertical Franz diffusion cells with a 2.0 ml receptor compartment and 1.0751 cm^2 effective diffusion area. The skin epidermis was carefully mounted on the lower half of the Franz cell, with the stratum corneum facing upwards. A clamp was used to fasten the upper and lower parts of the Franz cell together, with the epidermis separating the donor and the receptor compartments. The receptor compartments were filled with phosphate buffer (pH 5.0). Special care was taken to ensure that there were no air bubbles between the buffer solution and the epidermis. Franz cells containing the buffer solution were equilibrated for 1 h in the water bath at 32°C before addition of the 0.05 M solutions to the donor compartments. Only the receptor compartments were submerged in the water and were equipped with stirring magnets. After a period of 1 h, 1.0 ml freshly prepared solution of solute was added to each donor compartment, which was immediately covered with Parafilm to prevent evaporation of any constituent during the experiment. Each compound was tested in three cells.

The entire receptor volume was withdrawn after 2, 4, 6, 8, 10 and 24 h and replaced with fresh buffer solution (pH 5.0) prewarmed to 32°C. The entire receptor volume was withdrawn to mimic the sink conditions that occur in the human body.

The withdrawn samples were assayed immediately by HPLC: 10 μl of each sample was injected onto the column in duplicate. The results, expressed as means, allowed determination of the concentration of parent drug **4** and intact prodrugs **5a**–**5e** that had permeated the epidermis. The cumulative amount of intact prodrug collected in the receptor compartment was plotted as a function of the time. The flux value for a given experiment was obtained from the slope of the steady-state portion of the graph (0–8 h).

Statistical analysis

Each compound was tested in three different cells (different skin samples from same donor) and two measurements per cell were done by HPLC analysis. To exclude the effect of pseudo replicates, the mean of the two measurements was used in the statistical analysis. A Kruskal–Wallis test was done with Statistica 8 sofware (StatSoft Inc., Tulsa, OK, USA) to test the statistical significance of differences between the median flux of different compounds at a 5% level. Multiple comparisons on the mean flux of individual compounds were performed.[19]

An interval for the enhancement factor was determined by dividing the mean flux of a specific derivative by the mean flux of the parent drug and adding or subtracting the standard error for a proportion.[20]

Results

The series of prodrugs can be divided into two homologous subseries, namely I and II, on the basis of the oxyethylene index (n): I refers to the oligomeric subseries comprising compounds **5a**, **5b** and **5c**; the polymeric subseries II comprised prodrugs **5d**, **5e** and **5f**.

Chemical stability

The hydrolytical stability of prodrugs **5a**–**5f** was evaluated at physiological pH 7.4 and at pH 5.0 (0.01 M phosphate buffer) and skin temperature (32°C). pH 5.0 was selected for determination of physicochemical parameters and diffusion experiments to reflect the acidic conditions on the outer surface of the skin (pH ~ 5).[21]

The kinetics of the chemical hydrolysis of each carbonate followed pseudo first-order kinetics. The chemical stability was substantially higher at pH 5.0 than at pH 7.4, with half-lives ranging from 14 to 44 days at pH 5.0 and from 6 to 24 days at pH 7.4 (Table 1).

Hydrophilicity and lipophilicity

All the components of the series are water-miscible oils with the exception of parent drug **4** and its derivative **5a**, which were crystalline. We were unable to determine the aqueous solubility of these carbonates using the classic method described above, so the aqueous solubility values (S_W) for compounds **5b**–**5f** were estimated using the theoretical method reported by Yalkowsky and co-workers,[22,23] which is widely used.[12,24,25] The water solubility of solid compounds is calculated from Log $S_w = -\log D - 0.01Mp + 1.05$ and that of oils from Log $S_w = -1.072\log D + 0.672$, where Mp is the melting point and D the octanol : buffer (pH 5.0) partition coefficient (Figure 3).

The solubility in octanol (S_{OCT}) was then calculated from S_W and log D data, given that $S_{OCT} = D \times S_W$ (Figure 4). The physicochemical properties of AZT and its various MPEG carbonate prodrugs are summarised in Table 2.

Skin permeation

Stratum corneum and epidermis from excised female abdominal skin was used to examine the permeation of AZT **4** and its prodrugs **5a**–**5f**. Each donor was applied to maintain constant diffusion. The profiles of cumulative permeated amount (in nmol) of AZT and intact prodrugs **5a**–**5d** are shown in Figure 5.

Measurements across cells showed large variations, as also experienced previously,[26] so it was not possible to determine whether differences between the mean flux of a specific prodrug and the parent drug were statistically significant. However, conclusions were made by evaluating the interval of the enhancement factor. Thus, a specific prodrug with the lower limit of its enhancement factor interval greater than 1 is considered to enhance the skin permeation of the parent drug. The enhancement factor interval of derivative **5a** (2.75; 6.25) thus indicates a significant enhancement, meaning that compound **5a** penetrates the skin between 2.75 and 6.25 times better than the

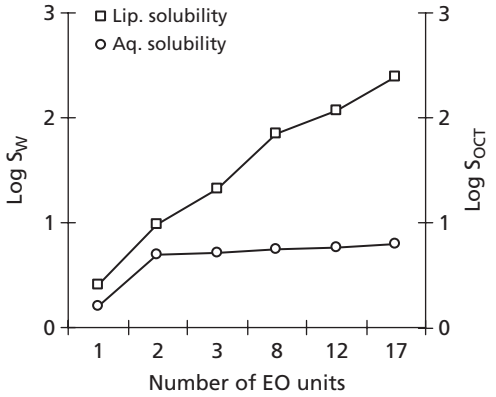

Figure 3 Relationship between lipid and aqueous solubility parameters and number of oxyethylene (EO) units in the carbonate series **5a**–**5f**.

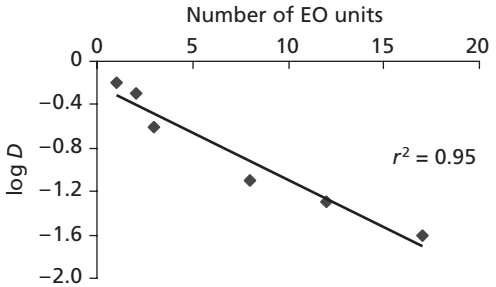

Figure 4 Relationship between log *D* (*n*-octanol : PBS, pH 5.0 partition coefficient) and number of oxyethylene (EO) units in the carbonate series **5a**–**5f**.

parent drug **4**. Similarly, compound **5d** penetrates the skin 2.39–10.08 times better than compound **4**.

Discussion

Chemistry

Poly(ethylene glycol) (PEG) is one of the most extensively investigated biocompatible polymers, following initial success achieved with the pegylation of proteins.[27] Extension of the pegylation procedure to other biomaterials is well on its way to becoming a standard component of the pharmaceutical tool box, since PEG (also MPEG) possesses a unique set of properties, including absence of toxicity, immunogenicity and antigenicity, low mass-dependent elimination via the kidney, and high solubility in water and other organic media (amphiphilicity).[28] This amphiphilicity provides the rationale for choosing MPEG as the linked promoiety in this project, because aqueous and lipid solubilities of prodrugs are important criteria when enhancing topical delivery.[29] The monofunctional MPEG was preferred to the native bifunctional PEG for reasons of homogeneity.

5′-*O*-MPEG carbonate prodrugs of AZT (**5a**–**5f**) were prepared in 38–65% yields in a two-step process by carbonylation using *p*-NPCF as the alkoxy-carbonylating agent. First, MPEG was activated at its remaining terminal hydroxyl group by *p*-NPCF, resulting in unsymmetrical alkoxy aryl carbonates with a good leaving group (*p*-nitrophenoxide). The presence of this electron-withdrawing group increases the susceptibility of such carbonates to carbonylation exchange reactions.[30] The exchange reaction between the activated carbonate and AZT led to the desired compounds as result of the most nucleophilic group (5′-OH of AZT) displacing the nucleofuge, and was favoured by the basic environment provided by TEA and the use of 4-(dimethylamino)pyridine as catalyst. The chemical structures of MPEG-AZT carbonates were confirmed by NMR and MS data. Indeed, comparison of the integral of the peak at δ 3.32 ppm assignable to $-OCH_3$ of MPEG with the integral of the peak related to protons at 1.85 ppm assignable to $-CH_3$ (methyl) that belong to the drug showed a 1 : 1 ratio. This indicates that one molecule of MPEG was linked to one drug molecule via a carbonate linker. The presence of such a linker was confirmed by the resonance of carbon C-1″ at 163 ppm in ^{13}C NMR. ^{1}H NMR spectra of all the carbonates exhibited resonance in the 3.48–3.70 ppm region characteristic of methylene protons $-OCH_2$-CH_2O- belonging to MPEG. The MS data for the compounds confirmed the presence of molecular ions (M^+) at 363, 413, 457 and 678, corresponding to the molecular formulae $C_{14}H_{19}N_5O_7$ (**5a**), $C_{16}H_{23}N_5O_8$ (**5b**), $C_{18}H_{27}N_5O_9$ (**5c**) and $C_{27}H_{47}N_5O_{14}$ (**5d**), respectively. These formulae in turn indicate the oxyethylene unit (n) to be 1, 2, 3 and 8 for **5a**, **5b**, **5c** and **5d**, respectively. LC–MS analysis also showed the presence of molecular ions of **5e** (*m/z* 854) and **5f** (*m/z* 1074). Thus, the correlation with ^{1}H NMR data revealed n values for **5e** and **5f** to be 12 and 17, respectively.

Chemical stability

The chemical stability result is in excellent agreement with the literature, as the carbonate linker is commonly cleaved under basic conditions.[31] Regardless of the pH value of the medium, the stability of the prodrugs increased as the chain length increased. This increase is not significant across each subseries (I or II), but increased substantially when comparing the two subseries. Thus, the stability of subseries II at pH 5.0 and pH 7.4 was on average 2- and 2.5-fold higher than that of compounds in subseries I, respectively. The hydrolysis rate of compounds in subseries I also decreased as chain length increased, in accordance with the results from Puglia and colleagues.[24] This increase in stability with increased chain length, however small, is likely to be due to steric hindrance of the carbonate linker by the MPEG chain. Indeed, as the (n) value increases, the resulting flexible chain presumably coils the carbonate bond, thus temporarily protecting it from hydrolysis. Such behaviour of polymeric drug delivery agents has already been reported.[32] The derivatives **5a**–**5f** were chemically more stable under weakly acidic conditions, so this medium was selected for subsequent experiments.

Hydrophilicity and lipophilicity

Lipophilicity is an important property for topically delivered drugs because the stratum corneum, the major barrier to drug

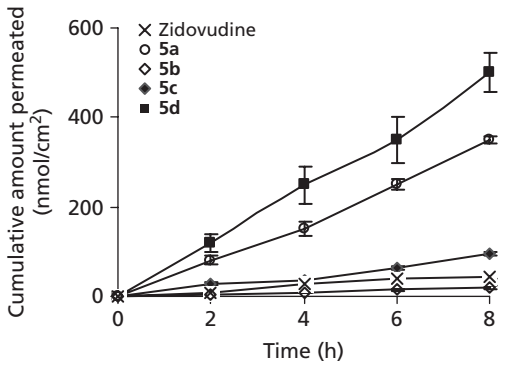

Figure 5 Skin permeation of zidovudine and prodrugs. Permeation profiles of 50 mM solutions of zidovudine and its carbonates, **5a**, **5b**, **5c** and **5d** through human skin in phosphate buffer (0.01 M, pH 5).

permeation, is lipophilic in nature and generally favours the permeation of lipophilic drugs. The aqueous solubility (hydrophilicity) of a drug molecule has also been reported to be important, particularly for very lipophilic compounds.[9,33] Thus, to readily diffuse through the skin a drug molecule should possess both hydrophilic and lipophilic properties.[18,34] MPEG readily dissolves in both aqueous and lipid media and is therefore an ideal promoiety in the hope that once conjugated it would impart this unique characteristic to the ultimate prodrugs. The selection of MPEG was further motivated by the report of poor skin permeation results displayed by lipophilic *O*-acyl derivatives of AZT[35] and *N*-acyl prodrugs of lamivudine.[26]

One striking observation of these data is that both hydrophilicity and lipophilicity parameters increase as the chain lengthens (see Figure 3). However, the increased solubility in water is greater than that in lipid, and can be seen by the decrease in the partition coefficient (Figure 4). The increase in lipophilicity as the chain lengthens regardless of the subseries agrees with the increasing number of lipid-soluble ethylene units introduced into the prodrug structures. All the carbonate prodrugs are more lipophilic than the parent drug AZT. Moreover, prodrug **5a**, the first member of the carbonate series, exhibits greater solubility in octanol than AZT, which is in accordance with a previous report [18] that, generally, the initial increase in lipid solubility exhibited by members of a homologous series occurs because the promoiety masks a hydrogen bond donor, in this case 5′-OH of AZT.

The increase in hydrophilicity throughout all series of carbonates also corroborates the increase in hydration as a result of hydrogen-bond formation between the intrachain oxygen atoms and water molecules in the aqueous medium. The behaviour of these MPEG-AZT prodrugs thus confirms the amphiphilic character of PEG.

The molecular weights of AZT and its carbonate prodrugs **5a**, **5b** and **5c**, ranging from 363.3 to 457.4 g/mol, fall in the appropriate range for transdermal delivery.[36,37] In light of these physicochemical data, the decreasing order of balance between aqueous and lipid solubilities is as follows: **5b**, **5c**, **5a**, **5d**, **5e**, **5f** and **4**. Carbonates **5b**, **5c** and **5a** possess the best balance between hydrophilicity and lipophilicity and also have molecular weights in the appropriate range for transdermal delivery and are therefore expected to exhibit the highest skin permeation rate as predicted by the literature.[9,13]

AZT has a reported solubility of 25 mg/ml (log S_W = 1.97 mM) at 25°C in water,[38] which is in agreement with the experimental value of 25.5 mg/ml (log S_W = 1.98 mM) determined in phosphate buffer (pH 5.0) at 32°C in this study. The estimated log S_W for **4** and **5a** are 0.51 and 0.41 mM, respectively, whereas the experimental values are 1.98 and 1.27 mM, respectively.

Skin permeation

Passive diffusion is driven by a high drug concentration in the aqueous vehicle, which prompted the use of saturated solutions with visibly excess solute in diffusion studies. In order to interpret the results of the diffusion cell experiments, 0.05 M (50 mM; log (50 mM) = 1.7 mM) standard solution of each compound was used as donor. The approach was also adopted to palliate the difference in physical state of the compounds; some being crystalline (**4** and **5a**) whereas **5b**–**5f** were water-miscible oils. Assuming the calculated aqueous solubilities S_W listed in Table 2 are reasonably accurate, the parent drug **4** and its derivatives **5a**, **5b** and **5c** would all be tested as suspensions since their log S_W values are below 1.7 mM, while **5d**, **5e** and **5f** would be tested as homogenous solutions. In actual fact, the donor phase of carbonate **5a** was a suspension with excess visible solute, those of prodrugs **5b** and **5c** were colloids (suspension of non-visible solute) while **4** and prodrugs **5d**–**5f** led to homogenous solutions. The parent drug tested as homogenous solution is not surprising since its experimental log S_W of 1.98 is above the standard value.

AZT and prodrugs **5a**–**5d** permeated the skin, whereas carbonates **5e** and **5f** were not detected in the receptor solution. These latter two carbonates possess the highest solubility in both aqueous and lipid media, and showed the poorest balance between the two parameters, so this result was not unexpected. Furthermore, one would not expect these carbonates to permeate the skin because their effective molecular weights are beyond the transdermal delivery range, and solute diffusivity decreases exponentially as molecular volume (and hence molecular weight) increases.[32] Furthermore, MPEG is a polyether and therefore forms tight hydrogen bonds with molecules of the vehicle (water) through its intrachain oxygen atoms. Studies of PEG moieties in solution have shown that each oxyethylene unit is tightly associated with two or three water molecules.[39] Thus, once in solution each prodrug forms a hydrated entity with increased effective MW by 36 × n if one considers that a minimum of two water molecules hydrate each oxyethylene (MW for water 18 g/mol). The hydrated prodrugs would have the following MW values (in g/mol): **5a** (n = 1) 399.3; **5b** (n = 2) 485.4; **5c** (n = 3) 565.4; **5d** (n = 8) 965.7; **5e** (n = 12) 1285.9; **5f** (n = 17) 1686.2. The increase in effective MW would most probably lead to a decrease in skin permeation and could further explain the absence of prodrugs **5e** and **5f** in the receptor cells. Prodrugs **5d** and **5a** were two most efficient penetrants of the series: they permeated the skin with mean fluxes of 53.3 and

38.5 nmol/cm^2 per h, respectively, therefore enhancing the skin permeation of AZT 2.75–6.25 and 2.39–10.08 times, respectively. These flux data did not follow the prediction order of the series, as one would expect prodrugs **5b** and **5c** to be the two best penetrants, followed by derivatives **5a** then **5d**. However, similar skin permeation behaviour of homologous series of AZT prodrugs had already been reported.[35] Prodrug **5d** has an effective MW of 677.7 g/mol, which is above the appropriate range for transdermal delivery, and yet is the best penetrant of the series. This corroborates the literature and confirms that molecular weight is not as rigorous a parameter as partition coefficient and solubility parameters are in influencing skin permeation.[40]

Conclusions

Our result show that the permeation of the antiretroviral drug AZT through human skin can be improved, at least *in vitro,* by bioreversible conjugation to MPEG promoieties of various oxyethylene units. The carbonates resulting from such a conjugation are stable in phosphate buffer (0.01 M) at pH 5.0 and at pH 7.4. Among them, two prodrugs (**5d** and **5a**), which feature eight and one oxyethylene units, respectively, combine adequate aqueous and lipid solubilities. They enhanced the skin permeation rate of AZT 2.75–6.25 and 2.39–10.08 times, respectively, and therefore merit further investigation for stability in biological tissue and therapeutic delivery of AZT.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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