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ORIGINAL ARTICLE

Synthesis and transdermal permeation of novel N^4-methoxypoly(ethylene glycol) carbamates of cytarabine

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Abstract

Background: Cytarabine is a deoxycytidine analogue commonly used in the treatment of hematological malignant diseases. Its clinical utility, however, is severely limited by its short plasma half-life because of the catabolic action of nucleoside deaminases. *Method*: In this study, N^4-carbamate derivatives of cytarabine (**1**) were synthesized and evaluated for transdermal penetration because this mode of administration may circumvent its limitations. The synthesis of these compounds was achieved in a two-step process. First, the methoxypoly(ethylene glycol) was activated by *p*-nitrophenyl chloroformate. Second, the activated intermediates were reacted with cytarabine in the presence of *N*-hydroxysuccinamide to give the N^4-methoxypoly(ethylene glycol) carbamate derivatives. The transdermal flux values of the N^4-carbamates of cytarabine were determined in vitro by Franz diffusion cell methodology. Aqueous solubility and log *D* (pH 7.4) values were determined and assessed for correlation with transdermal flux values. *Results*: The synthesized carbamates, particularly, (**9**)–(**13**), showed increased solubility in both aqueous and lipid media. Log *D* values decreased as the oxyethylene chain lengthened. *Conclusion*: Although none of the derivatives showed significantly higher transdermal penetration than cytarabine (**1**), it should be mentioned that the mean for cytarabine N^4-methoxyethyleneoxycarbamate (**8**) was 10 times higher and the median was 2 times higher.

Key words: *Carbamate; cytarabine; derivatives; log D; methoxypoly(ethylene glycol); percutaneous absorption; physicochemical properties; skin penetration; transdermal delivery systems*

Introduction

Cytarabine is a deoxycytidine analogue commonly used in the treatment of hematological malignant diseases. This pyrimidine nucleoside analogue is one of the most active single agents in the treatment of myeloid leukemia[1]. However, its clinical utility is severely limited by the catabolic action of nucleoside deaminases widely distributed in both normal and tumor tissues, which give rise to the inactive metabolite 1-(β-D-arabinofuranosyl)uracil (ara-U)[2]. As a result, cytarabine (**1**) exhibits a very short plasma half-life. Because of its cell cycle (S-phase) specificity, a prolonged exposure of cells to cytarabine's cytotoxic concentrations is essential to achieve maximum activity[3,4]. In practice, it is administered intravenously by repetitive schedules or by continuous infusion to achieve sustained supply. These regimens, however, are associated with adverse effects such as myelosuppression, vomiting, and stomatitis at conventional dose[1,5,6]. Because of these shortcomings, cytarabine has been a subject of many studies aiming to circumvent these problems. In particular, many prodrug approaches have been explored with varied degrees of success[7–11].

Over the years, the prodrug approach for transdermal delivery has been explored with some success stories[12–14]. When compared to more conventional drug delivery strategies, transdermal drug delivery offers several important advantages over more traditional

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dosage forms. These include the potential for sustained release, which is useful for drugs with short biological half-lives requiring frequent oral or parenteral administration, and controlled input kinetics which is particularly indispensable for drugs with narrow therapeutic indices[15]. To date, however, transdermal drug delivery has received scanty attention in a quest to improve pharmacokinetics of cytarabine. Despite the many advantages of the skin as site of drug delivery only a small number of drugs are currently in a transdermal delivery system on the market, inter alia clonidine, estradiol, nitroglycerin, fentanyl, testosterone, scopolamine, nicotine, and oxybutinin. The most important reason for this is the low permeability of drugs through the stratum corneum that acts as a barrier and penetration is affected by the physicochemical properties of the permeant and the possible skin sensitization reactions which can be caused by the drug[16]. For instance, many drugs with hydrophilic structures permeate the skin too slowly to be of therapeutic benefit. Hydrogen-bonding functionality on the permeant is reported to drastically retard permeation[17–19]. Against this background, cytarabine with its inherent high hydrophilicity and plurality of hydrogen-bonding functionalities would not easily penetrate the skin. Prodrug approaches could be used to transiently modify the physicochemical properties of a therapeutic agent for optimum transdermal penetration[13]. The success of this depends on factors including the inherent nature of a drug and choices of promoieties and linkers. It is known that the balance between lipid and aqueous solubility is essential to optimize flux[12,13]. The choice of functional groups as carriers in transdermal prodrugs will of course depend on, among others, whether increased lipophilicity or hydrophilicity is aimed at. Generally, lipophilicity of the parent drug could be increased by conjugation with an aliphatic or aromatic promoiety, whereas for increased hydrophilicity promoieties containing free hydrophilic groups such as hydroxyl, carboxylic, and amino groups (in addition to a group involved in conjugation) are employed[20]. With appropriate choice of poly(ethylene glycol) (PEG) promoieties, in particular, both lipophilicity and hydrophilicity could be enhanced[21,22].

PEGs possess a unique set of advantageous properties, including absence of toxicity, immunogenicity, antigenicity, low-mass-dependent elimination through the kidney, and high amphiphilicity (solubility in water and organic media)[23]. The choice of methoxypoly(ethylene glycol) (mPEG) as a promoiety is due to its amphiphilic nature because water solubility and enhanced lipid solubility are important factors to consider in any attempt to optimize a particular derivative approach to enhancing transdermal penetration and hence topical delivery[9,24]. It is generally accepted that compounds with inter alia low molecular weight (<600 Da) penetrate the skin better than high molecular weight permeants[25]. The choice of mPEG with higher molecular weights (350, 550, and 750) was to determine what the influence of these groups on the transdermal penetration would be given that previous research has found that compounds with high molecular weight could be delivered by passive diffusion through the skin[26,27]. In this study, N^4-carbamate mPEG derivatives of cytarabine were synthesized and evaluated for transdermal penetration. The log *D* and solubility of these compounds in water and octanol were determined and assessed for correlation with transdermal flux values.

Materials and methods

Materials

Cytarabine was purchased from Jingma Chemicals Ltd. (Longyou Zhe Jiang, China) *para*-Nitrophenyl chloroformate (*p*-NPCF) was purchased from Sigma-Aldrich (Johannesburg, South Africa). Methoxyethanol, di- and tri(ethylene glycol) monomethyl ethers, and PEG monomethyl ethers (mPEG) (average molecular weight 350, 550, and 750) were purchased from Fluka (Johannesburg, South Africa). A high-performance liquid chromatography (HPLC) grade methanol was obtained from Labchem South Africa Ltd. (Johannesburg, Gauteng, South Africa). All other reagents were of analytical grade and were used without further purification.

General procedures

The ^{1}H, ^{13}C, COSY, HSQC, and HMBC spectra were recorded on a Bruker 600 spectrometer, using deuterated dimethyl sulfoxide (DMSO) as solvent. The ^{1}H and ^{13}C spectra were recorded at frequencies of 600.17 and 151.92 MHz, respectively. All the chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane ($\delta = 0$). The splitting pattern abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). The melting points of solid products were determined by Shimadzu DSC-60A using TA60 (Version 2.11) software. Mass spectrometer (MS) spectra were recorded on an analytical VG 7070E MS using fast atom bombardment (FAB) as ionization technique. Thin-layer chromatography was performed using silica gel plates (60F254 Merck) and flash column chromatography on silica gel (70–240 mesh, G60 Merck). Mobile phases were mixed in a volume-to-volume ratio.

High-pressure liquid chromatography

The HPLC system consisted of a Hewlett–Packard (HP) Agilent 1100 series autosampler, HP Agilent 1100 series

variable wavelength detector, and HP Agilent 1100 series pump (Agilent, Palo Alto, CA, USA). A Phenomenex (Luna C-18, 150 × 4.60 mm, 5 µm) column was used with a security guard pre-column (C-18, 4 × 3 mm) insert (Phenomenex, Torrance, CA, USA) to prolong column life. Elution was accomplished with gradient at a flow rate of 1 mL/min with a mobile phase consisting of methanol (A) and 0.005 M heptane sulfonic acid-Na in water adjusted to pH 3.5 with orthophosphoric acid (B). The gradient was started with 30% A, increased linearly to 95% A in 8 minutes, held until 11 minutes, where after the column was reequilibrated at the starting conditions. A standard volume of 50 mL was injected for each sample. The working concentrations were in the range 0.2–400 µg/mL. Correlation coefficients (r^2) were in the range of 0.997–1, indicating good linearity. These experiments were done in three different cells. The steady-state flux (J_{ss}) was determined as the slope of the plot of cumulative drug permeating per unit area versus time in the steady state.

LC–MS analysis

LC–MS was performed using an HP 1100 series HPLC with binary gradient pump, autosampler, and vacuum degasser, coupled with an Applied Biosystems API 2000 triple quadruple MS and analyte data acquisition and analysis software. The column was a Gemini C-8, 150 × 2 mm, 5 mm (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 minutes, then 10% A/90% B up to 6 minutes, followed by 90% A/10% B up to 10 minutes. The flow rate was 250 mL/min and the injection volume 5 mL. The MS used atmospheric pressure electron ionization (turbo ion spray source) in positive-ion mode. Full scan from 100 to 200 amu was performed in 1 second. 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 nebulizer gas (1) and heater gas (2) were all used at a flow rate of 20 L/h; the temperature was set at 300°C.

Chemical synthesis

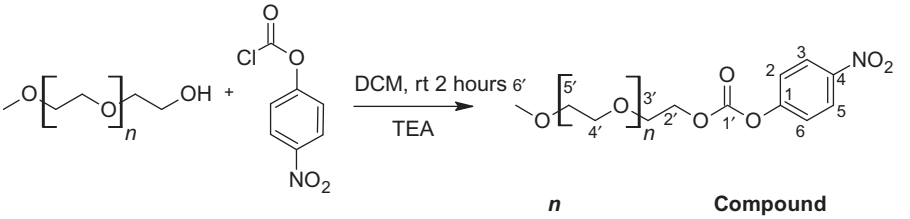
The syntheses of the compounds were generally achieved in a two-step process. First, mPEGs were activated by *p*-NPCF. Second, the activated intermediates were reacted with cytarabine in the presence of *N*-hydroxysuccinamide to give N^4-mPEG carbamates of cytarabine.

Activation of mPEGs (Scheme 1)

To react with the N^4-amino group on pyrimidine ring of cytarabine, terminal hydroxyl groups of monomethyl ethylene glycol oligomers and mPEGs were converted to their corresponding active *p*-nitrophenyl carbonate derivatives (**2**)–(**7**)[28,29]. For simplicity, only the synthesis of methoxyethylene glycol-(*p*-nitrophenyl carbonate) (**2**) is described here: Methoxyethylene glycol (22.32 mmol, 1.7 g) and *p*-NPCF (24.80 mmol, 5.0 g) were dissolved in 50 mL anhydrous dichloromethane (DCM). To this solution, triethylamine (24.80 mmol, 3.4 mL) was added and the mixture was stirred at room temperature for 2 hours. The solvent was removed under vacuum. The residue was suspended in 100 mL of diethyl ether to precipitate triethylammonium chloride salt and filtered. The filtrate was concentrated and separated by silica column chromatography using DCM:EtOAc (20:1) to yield the product as white crystals (18.03 mmol, 4.4 g).

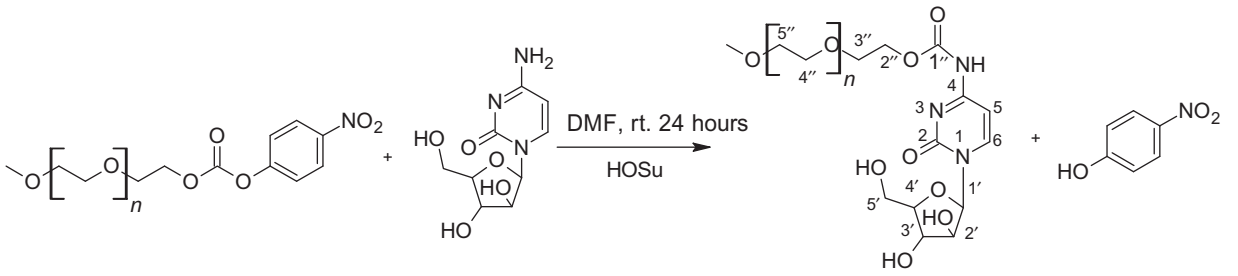
Synthesis of N^4-mPEG-cytarabine carbamates (Scheme 2)

The mPEG N^4-carbamates of cytarabine were synthesized according to the method depicted in Scheme 2 and described as follows to exemplify the preparation of carbamate (**8**). To a solution of (**2**) (6.26 mmol, 1.5 g, 1 equiv) and cytarabine (6.91 mmol, 1.7 g, 1.1 equiv) dissolved in 20 mL anhydrous dimethylformamide was added *N*-hydroxysuccinamide (0.59 mmol, 0.07 g, 0.9 equiv) and the reaction was stirred at room temperature.



n	Compound
0	(**2**)
1	(**3**)
2	(**4**)
7	(**5**)
11	(**6**)
16	(**7**)

Scheme 1. Activation of mPEGs.



n	Compound
0	(**8**)
1	(**9**)
2	(**10**)
7	(**11**)
11	(**12**)
16	(**13**)

Scheme 2. Conjugation of mPEG with cytarabine.

The reaction progress was monitored by silica thin-layer chromatography using DCM:MeOH (8:1). After 48 hours of stirring at room temperature, the solvent was removed in vacuo to yield a yellow residue. The residue was purified by flash chromatography with silica gel as stationary phase and DCM : MeOH (8 : 1) as an eluant to give 1.4 g of product as white powder. The structure of the product was elucidated by NMR and MS data presented below.

Cytarabine N^4*-methoxyethyleneoxycarbamate (**8**)*. Compound (**8**) was purified by flash silica gel chromatography eluting with DCM : MeOH (8:1) to give 1.4 mg (64%) of white powder. m.p. 121.3°C. ^{1}H NMR (600 MHz, DMSO) δ 3.27 (s, 3H, OCH_3), 3.57–3.52 (m, 2H, H-3″), 3.61 (t, J = 5.3, 2H, H-5′), 3.82 (td, J = 5.4, 2.9, 1H, H-4′), 3.92 (dd, J = 6.0, 2.7, 1H, H-3′), 4.05 (t, J = 5.5, 1H, H-2′), 4.30–4.17 (m, 2H, H-2″), 5.07 (t, J = 5.4, 1H, OH-5′), 5.48 (d, J = 4.1, 1H, OH-2′), 5.50 (d, J = 5.3, 1H, OH-3′), 6.04 (d, J = 3.9, 1H, H-1′), 7.00 (d, J = 7.4, 1H, H-5), 8.04 (d, J = 7.5, 1H, H-6), 10.68 (s, 1H, HNCOO). ^{13}C NMR (151 MHz, DMSO) δ 58.02 (C-ω), 60.99 (C-5′), 64.21 (C-2″), 69.72 (C-3″), 74.56 (C-2′), 76.09 (C-3′), 85.68 (C-4′), 86.85 (C-1′), 93.19 (C-5), 146.31 (C-6), 154.28 (C-2), 162.58 (C-4), 165.29 (C-1″). MS FAB 345.7 $(M + H)^+$, 368.0 $(M + Na)^+$, 11.8, 214.5.

Cytarabine N^4*-methoxydiethyleneoxycarbamate* (***9***). Carbamate (**9**) was purified by column chromatography using DCM:MeOH (8:1) as mobile phase to give 1.8 g (75%) of white powder. m.p. 132.9°C. ^{1}H NMR (600 MHz, DMSO) δ 3.24 (s, 3H, H-ω), 3.46–3.41 (m, 2H, H-5″), 3.58–3.52 (m, 2H, H-4″), 3.62 (ddd, J = 13.1, 7.8, 4.3, 4H, H-5′ and 3″), 3.82 (td, J = 5.4, 2.9, 1H, H-4′), 3.92 (dd, J = 6.2, 2.7, 1H, H-3′), 4.05 (dd, J = 7.5, 4.0, 1H, H-2′), 4.23 (dd, J = 5.4, 3.8, 2H, H-2″), 5.06 (t, J = 5.5, 1H, OH-5′), 5.48 (d, J = 4.1, 1H, OH-3′), 5.50 (d, J = 5.4, 1H, OH-2′), 6.04 (d, J = 3.9, 1H, H-1′), 7.01 (d, J = 7.4, 1H, H-5), 8.04 (d, J = 7.5, 1H, H-6), 10.69 (s, 1H, HNCOO). ^{13}C NMR (151 MHz, DMSO) δ 58.04 (C-ω), 60.99 (C-5″), 64.50 (C-2″), 68.25 (C-3″), 69.56 (C-4″), 71.21 (C-5′), 74.56 (C-2′), 76.10 (C-3′), 85.68 (C-4′), 86.84 (C-1′), 93.20 (C-5), 146.29 (C-6), 153.20 (C-1″), 154.27 (C-2), 162.58 (C-4). MS FAB $(M + H)^+$ 390.0, 279.9, 258.0, 137.6.

Cytarabine N^4*-methoxytriethyleneoxycarbamate (**10**)*. Carbamate (**10**) was purified by column chromatography using DCM:MeOH (10:1) as mobile phase to give 1.5 g (53%) of yellowish viscous oil. ^{1}H NMR (600 MHz, DMSO) δ 3.23 (s, 3H, H-ω), 3.45–3.39 (m, 2H, end-chain H-5″), 3.53–3.48 (m, 4H, mid-chain H-5″ and end-chain H-4″), 3.55 (td, J = 3.9, 0.9, 2H, mid-chain H-4″), 3.61 (t, J = 5.5, 2H, H-5′), 3.67–3.63 (m, 2H, H-3″), 3.82 (td, J = 5.4, 2.9, 1H, H-4′), 3.92 (dd, J = 6.3, 2.6, 1H, H-3′), 4.08–4.01 (m, 1H, H-2′), 4.23 (dd, J = 5.4, 3.8, 2H, H-2″), 5.07 (t, J = 5.6, 1H, OH-5′), 5.48 (d, J = 4.1, 1H, OH-3′), 5.50 (d, J = 5.4, 1H, OH-2′), 6.04 (d, J = 3.9, 1H, H-1′), 7.01 (d, J = 7.4, 1H, H-5), 8.04 (d, J = 7.5, 1H, H-6), 10.68 (s, 1H, HNCOO). ^{13}C NMR (151 MHz, DMSO) δ 58.00 (C-ω″), 60.99 (C-5′), 64.49 (C-2″), 68.27 (C-3″), 69.55 (C-4″), 69.72 (C-5″), 69.77 (end-chain C-4″), 71.23 (end-chain C-5″), 74.55 (C-2′), 76.10 (C-3′), 85.68 (C-4′), 86.85 (C-1′), 93.19 (C-5), 146.29 (C-6″), 153.21 (C-1″), 154.26 (C-2), 162.58 (C-4). MS FAB $(M + H)^+$ 433.8, 323.7, 301.8, 229.9, 186.6, 137.9.

Cytarabine N^4*-methoxyoctaethyleneoxycarbamate (**11**)*. Carbamate (**11**) was purified by column chromatography using DCM:MeOH (8:1) as mobile phase to give 1.0 g (41%) of light yellow oil. ^{1}H NMR (600 MHz, DMSO) δ 3.23 (s, 3H, H-ω), 3.45–3.40 (m, 2H, end-chain H-5″), 3.54–3.46 (m, 30H, H-5″ and mid-chain 4″ and mid-chain 5″ and end-chain 4″), 3.56 (dd, J = 5.9, 3.4, 2H, start-chain H-4″), 3.61 (t, J = 5.4, 2H, H-5′), 3.67–3.63

(m, 2H, H-3″), 3.82 (dd, *J* = 8.1, 5.3, 1H, H-4′), 3.92 (s, 1H, H-3′), 4.05 (s, 1H, H-2′), 4.23 (d, *J* = 2.8, 2H, H-2″), 5.07 (t, *J* = 5.5, 1H, OH-5′), 5.49 (d, *J* = 4.1, 1H, OH-3′), 5.50 (d, *J* = 5.5, 1H, OH-2′), 6.04 (d, *J* = 3.9, 1H, H-1′), 7.01 (d, *J* = 7.2, 1H, H-5), 8.04 (d, *J* = 7.5, 1H, H-6), 10.68 (s, 1H, HNCOO). ^{13}C NMR (151 MHz, DMSO) δ 58.01 (C-5), 60.99 (C-4′), 64.51 (C-3′), 68.26 (C-2′), 69.53 (C-end-chain 5″), 69.73 (C- H-5″ and mid-chain 4″ and mid-chain 5″ and end-chain 4″), 71.23 (C-4″), 74.54 (C-3″), 76.09 (C-2″), 85.69 (C-5′), 86.85 (C-ω). LC-MS, M^+ (*m/z* 653).

Cytarabine N^4*-methoxydodecaethyleneoxycarbamate (**12**)*. Carbamate (**12**) was purified by column chromatography using DCM:MeOH (8:1) as mobile phase to give 2.9 g (35%) of light yellow oil. ^{1}H NMR (600 MHz, DMSO) δ 3.24 (s, 3H, H-ω), 3.44–3.40 (m, 2H, end-chain H-5″), 3.54–3.46 (m, 48H, H-5″ and mid-chain 4″ and mid-chain 5″ and end-chain 4″), 3.56 (dd, *J* = 5.9, 3.3, 2H, start-chain H-4″), 3.61 (t, *J* = 5.2, 2H, H-5′), 3.68–3.63 (m, 2H, H-3″), 3.82 (td, *J* = 5.4, 2.9, 1H, H-4′), 3.92 (s, 1H, H-3′), 4.04 (d, *J* = 5.5, 1H, H-2′), 4.27–4.19 (m, 2H, H-2″), 5.08 (t, *J* = 5.4, 1H, OH-5′), 5.49 (t, *J* = 5.4, 2H, OH-2′ & 3′), 6.04 (d, *J* = 3.9, 1H, H-1′), 7.00 (d, *J* = 7.4, 1H, H-5), 8.04 (d, *J* = 7.5, 1H, H-6), 10.59 (s, 1H, HNCOO), LC-MS, M^+ (*m/z* 830).

Cytarabine N^4*-methoxyheptadecaethyleneoxycarbamate (**13**)*. Carbamate (**13**) was purified by column chromatography using DCM:MeOH (8:1) as mobile phase to give 1.2 g (30%) of light yellow oil. ^{1}H NMR (600 MHz, DMSO) δ 3.24 (s, 3H, H-ω), 3.46–3.40 (m, 2H, end-chain H-5″), 3.54–3.46 (m, 43H, H-5″ and mid-chain 4″ and mid-chain 5″ and end-chain 4″), 3.58–3.54 (m, 2H, start-chain H-4″), 3.61 (t, *J* = 5.4, 2H, H-5′), 3.67–3.63 (m, 2H, H-3″), 3.82 (d, *J* = 2.8, 1H, H-4′), 3.93 (s, 1H, H-3′), 4.05 (s, 1H, H-2′), 4.23 (d, *J* = 2.9, 2H, H-2″), 5.09 (t, *J* = 5.5, 1H, OH-5′), 5.50 (t, *J* = 4.4, 2H, OH-2′ & 3′), 6.04 (d, *J* = 3.9, 1H, H-1′), 7.01 (s, 1H, H-5), 8.04 (d, *J* = 7.5, 1H, H-6), 10.85–10.53 (m, 1H, HNCOO). LC-MS, M^+ (*m/z* 1094).

Physicochemical properties

Solubility

The aqueous solubility of solid compounds (**1**), (**8**), and (**9**) was determined by preparing saturated solutions in phosphate buffer solution (PBS) at pH 7.4. The slurries were stirred with magnetic bars in a water bath at 32°C for 24 hours. It was ensured that an excess of solute is present at all times to provide saturation. The solutions were filtered through 0.2 µm filter, diluted appropriately in PBS (7.4), and analyzed by HPLC to determine the concentration of dissolved solutes in PBS. The experiment was done in triplicate. Carbamates (**10**)–(**13**) were water-miscible oils and therefore experimental aqueous solubility could not be determined. The water solubility of these compounds was estimated using the theoretical method reported by Magnusson and co-workers[30] assuming that the solubility of oils in water is that of the pure oils. In the estimation 1/molecular weight was used to get a reasonably quick estimation. The concentration µmol/mL can be estimated as approximately (1 g/MW)/mL. The estimated water solubility values for compounds (**10**)–(**13**) are reported in Table 1.

The solubility in octanol was calculated by substituting S_w and D data in $S_{OCT} = D \times S_w$. The routinely used and well-documented experimental method[31,32], which involves adding an excess quantity of compound to the aqueous medium, could not be applied for all the compounds, some being crystalline whereas others are water-miscible oils (see Table 1).

Experimental log *D*

The partition coefficients were determined by a method reported by Taylor and Sloan[33]. Equal volumes of *n*-octanol and PBS of pH 7.4 were saturated with each other under vigorous stirring for at least 24 hours. Accurately weighed 30 mg of (**1**) as well as of each derivative (**8**)–(**13**) was dissolved in 3 mL of presaturated *n*-octanol, stoppered and agitated for 10 minutes in a

Table 1. Oxyethylene unit (*n*), molecular weight (Mw), melting point (Mp), partition coefficient *D* (*n*-octanol:phosphate buffer, pH 7.4), aqueous solubility (S_w), octanol solubility (S_{OCT}), and steady-state flux (J_{ss}) of cytarabine and its N^4-carbamates (8)–(13).

Compound	*n*	Mw (g/mol)	Mp (°C)	Log D[a]	SD	S_W (mol/L)	Log S_w	S_{OCT}[b]	Log S_{OCT}	J_{ss} (nmol/cm^2/h)	SD	J_{ss} (nmol/cm^2/h)[c]	P-value[d]	P-value[e]
1	0	243.2	212.0	−1.93	0.02	0.73[f]	−0.14	0.009	−2.07	3.70[g]	1.37	3.70		
8	1	345.3	173.6	−1.20	0.03	0.23[f]	−0.64	0.015	−1.84	35.18[g]	59.88	8.30	0.019	1.000 (1–8)[h]
9	2	389.4	132.9	−1.38	0.02	0.33[f]	−0.48	0.014	−1.86	nd[i]	—	nd	—	—
10	3	433.4	Oil	−1.66	0.01	2.31[j]	0.36	0.051	−1.30	nd[i]	—	nd	—	—
11	8	653.7	Oil	−1.83	0.02	1.53[j]	0.18	0.023	−1.65	3.88[g]	3.20	3.15	0.019	1.000 (1–11)[h]
12	12	829.9	Oil	−1.90	0.01	1.20[j]	0.08	0.015	−1.82	1.43[g]	0.90	1.20	0.019	0.853 (1–12)[h]
13	17	1050.2	Oil	−1.94	0.02	0.91[j]	−0.04	0.011	−1.98	2.23[g]	0.51	2.10	0.019	1.000 (1–13)[h]

[a]Mean ± SD (n = 5 experiments). [b]Calculated from $S_{OCT} = D \times S_W$. [c]Flux medians. [d]Kruskal–Wallis P-value. [e]Multiple comparison P-value. [f]Determined experimentally. [g]Each experiment was run on three different cells, values are means. [h]Not statistically significant. [i]Each experiment was run on six different cells, values are means. [j]Determined by method as described by Magnusson et al.[30] nd = not detected.

10-mL graduated tube (0.5 mL division). Subsequently, 3 mL of presaturated buffer was transferred to the tubes containing before-mentioned solutions. The tubes were stoppered and agitated for 45 minutes, then centrifuged at $2879 \times g$ for 30 minutes. The volume ratio (octanol:buffer) was not discernibly different from 1. The octanol and buffer phases were each diluted with methanol and the concentrations of (**8**)–(**13**) were measured by HPLC. The log *D* values were calculated as logs of the concentration ratios in the two phases. The results expressed as means are listed in Table 1.

In vitro skin permeation

Preparation of donor phase

The effect of drug concentration and solubility has been thoroughly studied for transdermal drugs. Generally the flux is concentration dependent and increases as the concentration of the drug in formulation increases[34] up to saturation. To compare the flux of different members of a homologous series a standard concentration at which all members are in solution had to be used. Donor solutions (0.2 M) of compounds were prepared by dissolving each compound in PBS at pH 7.4 and 32°C. All the compounds were in solution at this concentration.

Skin preparation

Caucasian female human abdominal skin used for permeation studies was obtained (Human ethics approval reference number 04D08, North-West University) with written informed consent after cosmetic abdominoplasty at Sunwardpark Clinic (Boksburg, South Africa). The skin was kept in a cooler box during transportation and stored in a freezer (at −20°C) until time of use. A scalpel was used to separate the skin from the fat layer. The epidermis was removed by first immersing the skin in 60°C HPLC water for 60 seconds[35]. The epidermis was then gently teased away from the skin with forceps. The epidermis was placed in a bath with HPLC water, with the outer side facing up and carefully set on Whatman® filter paper, left to dry at room temperature, and wrapped in foil. The foil containing epidermis was stored in a freezer at −20°C and was used within 3 months. Prior to use, the epidermis was thawed and examined with a light microscope for any defects, before mounting on the Franz diffusion cells.

Skin permeation

Vertical Franz diffusion cells with 2.0-mL receptor compartments and 1.0751 cm^3 effective diffusion areas were used for permeation studies. The epidermis skin layer prepared above was placed on the lower half of the Franz cell with the stratum corneum facing upwards; the upper half of the cell was mounted ensuring no possible leakage with the epidermis separating the donor and receptor compartments. The receptor compartment was filled with PBS (pH 7.4) taking special care that no air bubbles came between the receptor vehicle and epidermis, as this would reduce the effective diffusion area. The donor compartment was filled with 1.0 mL of PBS and the system was equilibrated at 32°C for 1 hour in water bath. Only the receptor compartments were submerged in water and were equipped with magnetic stirrers. After a period of 1 hour, 1 mL of freshly prepared solutions (0.2 M) was added to each donor compartment, which was immediately covered with Parafilm® to prevent evaporation of any constituent within the solution for the duration of the experiment. On scheduled times (i.e., after 2, 4, 6, 8, 10, and 12 hours), the total receptor phase was withdrawn and replaced with 32°C fresh buffer solution (pH 7.4) to mimic sink conditions as they occur in the human body. The withdrawn samples were immediately analyzed by HPLC to determine the concentrations of the compounds that permeated the epidermis. The flux was determined by plotting cumulative drug permeating per unit area versus time to determine the slope of the steady-state region. The experiments were performed in triplicate. The flux values are summarized in Table 1.

Data analysis

The following statistical procedures were used to test if there were statistically significant differences between the parent compound's median flux value and that of the newly synthesized derivatives. The reason for comparing the medians instead of the means of the flux values was that nonparametric tests were done, because normality of the distribution of the data of this study could not be assumed. The usual parametric test to compare means in the case of samples of less than 30 items can only be applied when normality is assured[36].

The nonparametric Kruskal–Wallis test was therefore done with Statistica[37] to test the statistical significance of differences between the medians of different compounds on a 5% level. Multiple comparisons on the mean ranks of individual groups were performed to determine where the differences occurred. Note that the compounds where no fluxes were detected were not included in the statistical analysis, because the conclusion that they were much less efficient skin penetrant than the parent compound is trivial.

Results and discussion

Chemistry

The carbamates (**8**)–(**13**) were successfully prepared with 30–75% yields. Carbamates (**8**)–(**10**) have oligomeric

ethylene moieties whereas (**11**)–(**13**) are polymeric. All the carbamates were synthesized by the method described by Bodansky[28] with modifications.

Compounds (**8**) and (**9**) were obtained as solids whereas (**10**)–(**13**) were oils. This reaction shows selectivity to the N^4-amino group of cytosine in cytarabine as the major product is the N^4-substituted derivative. The structures of the compounds were confirmed by NMR and MS. Comparison of the integral of peak at 3.20 ppm assignable to OCH_3 (H-ω) of mPEG moiety with the integral of the peak at 6.04 ppm assignable to H-1′ that belongs to the drug moiety showed 3:1 ratio. This indicates that one molecule of the native drug is linked to one molecule of the mPEG. The carbamate linker was confirmed by the peak at 165 ppm assignable to carbamate carbonyl carbon (C-1″). Because of the electron-withdrawing nature of carbamate linker, H-5 and H-6 signals were deshielded from 5.60 and 7.50 ppm to 7.00 and 8.00 ppm, respectively. This confirms that acylation took place at N^4. The ^{1}H NMR spectra of all the carbamates showed resonances in the 3.41–3.65 ppm region characteristic of methylene protons, –OCH_2–CH_2O–, of the mPEG part of the molecule. The MS data for the compounds confirmed the presence of molecular ions (M^+) at 345.3, 389.4, and 433.4 corresponding to the molecular formulae $C_{13}H_{19}N_3O_8$ (**8**), $C_{15}H_{23}N_3O_9$ (**9**), and $C_{17}H_{27}N_3O_{10}$ (**10**), respectively, which was confirmed with accurate mass values of 345.3, 389.4, and 433.4 MS. These formulae in turn indicate the number of oxyethylene (OE) unit(s) (*n*) in (**8**), (**9**), and (**10**) to be 1, 2, and 3, respectively. LC–MS analysis also showed the presence of molecular ions of (**11**) (*m/z* 653), (**12**) (*m/z* 829), and (**13**) (*m/z* 1050). Thus, the correlation with ^{1}H NMR data revealed (*n*) values for (**11**), (**12**), and (**13**) to be 8, 12, and 17, respectively.

Hydrophilicity and lipophilicity

Generally, prodrugs with enhanced biphasic (both lipophilic and hydrophilic) solubilities are reported to show better transdermal penetration than the parent drugs[12,38]. To assess solubility parameters of carbamates (**8**)–(**13**), log *D* and aqueous solubility were determined. Cytarabine carbamates (**9**)–(**10**) exhibited increased hydrophilicity and lipid solubility compared with the parent compound with the increasing number of OE units. The trend was expected because mPEG is expected to impose its properties as the polymeric index increases. As the chain length increases beyond three OE units, the change in both lipophilicity and hydrophilicity becomes marginal. In the oligomeric series (**8**)–(**10**), log *D* decreases abruptly as OE chain lengthens whereas in polymeric series (**11**)–(**13**) the change is marginal. It appears that the mPEG moiety imposed its amphiphilic nature by increasing both aqueous and lipid solubilities of the carbamates. All the carbamate (**8**)–(**13**) prodrugs are more lipophilic than the parent drug cytarabine. Moreover, carbamate (**8**), the first member of the homologous series, exhibits greater solubility in octanol than cytarabine, which is in accordance with a previous report[33] 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, N^4-amino group of cytarabine. As may be seen in Figure 1, the aqueous solubilities increase as the ethylene oxide chain lengthens but the octanol solubilities remain relatively constant. The hydrophilicity increases as a result of increased association of water molecules with the intrachain oxygen atoms of mPEG[39]. Although a lipophilic ethylene group was added with each oxygen atom, the additional associated water molecules mask the lipophilic ethylene group from association with the lipophilic octanol (Figure 2).

Skin permeation

The first member of the series (**8**) was found to have a higher flux than others. Compounds (**9**) and (**10**) were not detected in the receptor phase. There is no clear trend between flux values and molecular weights of compounds. Compounds (**11**)–(**13**) penetrated the skin much better than (**9**) and (**10**), despite having higher molecular weights. These corroborates previous findings in which some members of homologous series with

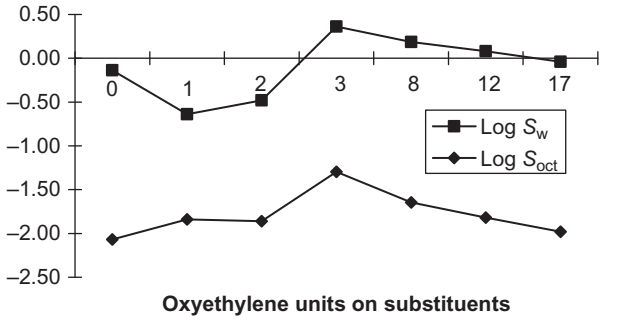


Figure 1. Relationship between the octanol and water solubility and the number of oxyethylene (EO) units in the carbamate series.

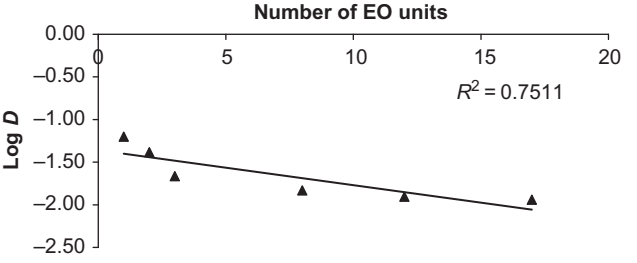


Figure 2. Relationship between log *D* (*n*-octanol:PBS, pH 7.4) and the number of EO in the carbamate series.

higher molecular weights penetrated the skin better than members with lower molecular weights[21,40]. Drug derivatives with enhanced biphasic (both lipophilic and hydrophilic) solubilities are reported to show better transdermal penetration than the parent drugs[12,13,38]. The use of amphiphilic promoieties such as PEG and mPEG has been reported with variable success[21,29,40]. In this study, however, no significant transdermal penetration enhancement was found by using the N^4-mPEG carbamates of cytarabine (**1**). As compared to ketoprofen, naproxen, and diclofenac for which PEG was successfully used as a promoiety for transdermal prodrug design[21], cytarabine (**1**) and its carbamate derivatives in this study are very polar and have more hydrogen-bonding functional groups. The hydrogen-bonding functional groups in the permeant tend to slow down transdermal diffusion because of their interaction with polar head groups of the intercellular lipids present in the skin[17,18]. Although the apparent molecular weights of N^4-oligomeric ethylene carbamates (**8**)–(**10**), ranging from 243.2 to 433.4 g/mol, fell in the appropriate range for transdermal delivery[41], no significant enhancement in transdermal penetration of the parent compound was observed (Table 1). This could be attributed to inter alia the fact that PEG moieties in solution have higher effective MW than is apparent from the molecular formula because of the association of water molecules with the OE chain. Studies of PEG moieties in solution have shown that each OE unit is tightly associated with two or three water molecules[41]. Bieze et al.[42] used neutron diffraction to show the number is 2–6 H_2O. Thus, once in solution each prodrug forms a hydrated entity with increased effective MW by $54 \times n$ if one considers that a maximum of three water molecules hydrate each OE (MW for water 18 g/mol).

Conclusion

The N^4-mPEG carbamates of cytarabine were successfully synthesized and their structures elucidated by MS and NMR spectroscopy. Lipid solubility, aqueous solubility, and transdermal fluxes of the carbamates were determined. There was no significant increase in transdermal delivery of cytarabine by its derivatives and no clear relationship between lipid and aqueous solubility and transdermal flux values were observed. mPEG as a promoiety exhibited the ability to increase both aqueous and lipid solubilities of carbamates of cytarabine (**1**).

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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