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Synthesis, Stability and In Vitro Dermal Evaluation of Aminocarbonyloxymethyl Esters as Prodrugs of Carboxylic Acid Agents

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Abstract—Aminocarbonyloxymethyl esters **3** based on (*S*)-amino acid carriers were synthesised and evaluated as potential prodrugs of carboxylic acid agents. In addition, the compounds were evaluated as topical prodrugs with the aim of improving the dermal delivery of two non-steroidal anti-inflammatory agents: naproxen and flufenamic acid. The lipophilicities of these compounds were determined and their hydrolyses in aqueous solutions and in human plasma were examined. Compounds **3** containing a secondary carbamate group were hydrolysed at pH 7.4 by two different routes: (i) direct nucleophilic attack at the ester carbonyl carbon leading to the release of the parent carboxylic acid and (ii) intramolecular rearrangement involving an O→N acyl migration, leading to the formation of the corresponding amide. The rearrangement pathway is highly dependent on the size of the carboxylic acid and amino acid substituents, being eliminated when the amino acid is valine or leucine. In contrast, compounds **3** decomposed in plasma exclusively through ester hydrolysis, most releasing the parent carboxylic acid quantitatively with half-lives shorter than 5 min. The permeation of selected prodrugs across excised postmortem human skin was studied in vitro. All prodrugs evaluated exhibited a lower flux than the corresponding parent carboxylic acid. The poor skin permeation observed for compounds **3** is most probably due to their low aqueous solubility and high partition coefficient. © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

Many drugs containing the carboxylic acid group are poorly absorbed from the gastrointestinal (GI) tract as a result of unfavourable physicochemical properties.[1] The prodrug strategy has been widely used to improve oral delivery of such drugs by appropriate modulation of properties, such as lipophilicity and aqueous solubility.[2] Moreover, for non-steroidal anti-inflammatory drugs (NSAIDs), such as naproxen and diclofenac, which contain the carboxylic acid functional group, the prodrug concept has also been used to minimise the GI side effects that constitute the most frequent adverse reactions of this class of drug.[3–6] Further, for NSAIDs, topical application can be considered as an alternative delivery method to oral use, especially in the treatment of local inflammatory and pain processes, where a high local drug concentration is needed.[7–9] However, because most NSAIDs also display limited skin permeation, the prodrug approach has also been suggested as an attractive method of enhancing the skin permeability of several members of this drug class such as naproxen and ketoprofen.[10–13]

A commonly used prodrug approach is to prepare acyloxymethyl derivatives **1**. These offer the possibility of modulating the rate of release of the parent drug and simultaneously modifying the physicochemical properties, such as water solubility and lipophilicity, by varying the R group in the acyl pro-moiety. However, neutral acyloxymethyl ester prodrugs **1** still present oral and dermal delivery problems due to very low water solubility and very high lipid solubility. For example, α-(acyloxy)alkyl esters of neutral β-lactam antibiotics are inefficiently absorbed from the GI tract[14] and thus taken orally are poorly active because they have very low aqueous solubility. It has also been recently reported that acyloxymethyl esters of naproxen present low

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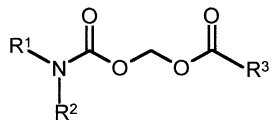
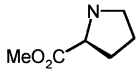
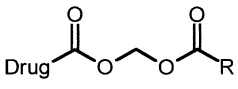
Table 1. Structures of compounds **3**



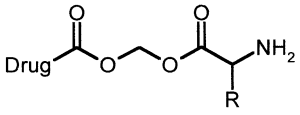
Compound	R^1	R^2	R^3COOH
3a	H	CH_2CO_2Et	Benzoic acid
3b	H	$CH(Me)CO_2Et$	Benzoic acid
3c	H	$CH(CH_2Ph)CO_2Et$	Benzoic acid
3d	H	$CH(CHMe_2)CO_2Et$	Benzoic acid
3e	H	$CH(CH2CHMe_2)CO_2Et$	Benzoic acid
3f	H	CH_2CO_2Et	Diclofenac
3g	H	CH_2CO_2Et	Clofibric acid
3h	H	CH_2CO_2Et	Nicotinic acid
3i	H	CH_2CO_2Et	Flufenamic acid
3j	Me	CH_2CO_2Et	Benzoic acid
3k	Me	CH_2CO_2Et	Naproxen
3l			Benzoic acid

dermal delivery through excised human skin, probably due to their poor aqueous solubility and high partition coefficients that are above the optimal range for skin permeation.[12] The introduction of a basic α-amino group into the acyl pro-moiety of **1**, for example by using an α-aminoacid carrier such as **2**, significantly increased the water-solubility but led to unstable compounds.[13] This is due both to the strong electron-withdrawing effect of the protonated amino group at physiological pH and also to the ability of the unprotonated amino group to enhance hydrolysis via nucleophilic and general-base catalysis.[2] A further disadvantage of aminoacyloxymethyl esters **2** is their potential to undergo intramolecular rearrangement, involving the amino group of the amino acid fragment, to form an amide, as has been reported for such derivatives of cefamandole.[14] Even so, because they offer enormous potential in terms of low toxicity, amino acids have been considered the ideal carriers for the development of prodrugs. Thus, a potentially valuable strategy employing amino acid carriers is undone by the presence of the free amino group derived from the amino acid moiety.

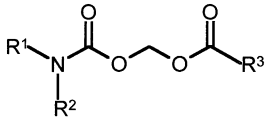
Gogate has previously discussed the rationale behind the use of (acyloxy)alkyl carbamates as prodrugs of amines.[15,16] Indeed, modified carbamates with an enzymatically hydrolyzable ester function were also suggested as prodrugs for amines[17] and this is now probably one of the most versatile prodrug approaches for an amino containing compound. By adapting and combining the acyloxymethyl and carbamate strategies, we now propose a novel and versatile approach for the design and synthesis of natural-amino-acid-carrier prodrugs of drugs containing the carboxylic acid functionality. Conceptually, this approach corresponds to an inversion of the amino acid position in the potentially unstable prodrugs **2**, and the rationale for this approach is based on the low chemical reactivity of carbamates in neutral aqueous media.[18] The present study reports the synthesis of a series of aminocarbonyloxymethyl esters **3** (Table 1) and a kinetic study that evaluates the influence of the aminoacid and carboxylic acid moieties on the chemical reactivity and stability in human plasma. The permeation of selected aminocarbonyloxymethyl esters **3** across excised human skin was also evaluated in vitro. Recent studies have suggested that an effective prodrug for dermal drug delivery must possess good biphasic solubility characteristics: that is, adequate aqueous solubility together with enhanced lipophilicity over the parent drug.[19, 20]



1



2



3

Experimental

Melting points were determined using a Kofler camera Bock Monoscop M and are uncorrected. Elemental analyses were performed either by Medac Ltd, Brunel Science Park, Englefield Green, Egham, Surrey, UK or Instituto Tecnológico Nuclear, Sacavém, Portugal. FTIR spectra were recorded using a Nicolet Impact 400 spectrophotometer^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions with Me_4Si as internal standard, using a Jeol LA300 or General Electric QE-300 spectrometers. Coupling constants, *J*, are expressed in Hz. The carboxylic acids were purchased from Sigma. All other chemicals and solvents were of reagent grade, except buffers substances and HPLC solvents which were analytical grade and LiChrosolv© (Merck) grade, respectively. Column chromatography was performed using silica gel 60 mesh 70–230 (Merck).

General method for the synthesis of aminocarbonyloxymethyl esters (3)

A dichloromethane solution (20 mL) of the amino acid ethyl or methyl ester hydrochloride (11.3 mmol) and triethylamine (10.8 mmol) was added to a solution of chloromethyl chloroformate (0.95 mL; 10.8 mmol) in dichloromethane (25 mL) at −10 °C. After stirring at −10 °C for 25 min and 75 min at room temperature, the reaction mixture was filtered, and the filtrate washed with water, dried and evaporated to obtain the corresponding chloromethylcarbamate **4** in ca. 90% yield. A

solution of the sodium salt of the appropriate carboxylic acid (6.4 mmol) and tetrabutylammonium bromide (6.4 mmol) in tetrahydrofuran (10 mL) was added to a solution of the intermediate **4** (6.4 mmol) in tetrahydrofuran (5 mL). After stirring for 24 h at room temperature, the solvent was evaporated and the resultant solid purified by silica gel column chromatography using diethyl ether as eluent.

3a. Synthesised using ethyl glycinate hydrochoride and sodium benzoate. Yield 15%. Mp 68–70 °C; ν_{max}/cm^{-1} 3312, 1740, 1720 (br), 1603; δ_H 1.26 (3H, t, $J=7.0$, CH_3CH_2), 3.96 (2H, d, $J=5.4$, NCH_2), 4.16 (2H, q, $J=7.0$, CH_3CH_2), 5.63 (1H, t, $J=5.4$, NH), 5.96 (2H, s, OCH_2O), 7.36–8.16 (5H, m, ArH). Found: C, 55.3; H, 5.3; N, 4.8. Calcd for $C_{13}H_{15}NO_6$: C, 55.5; H, 5.3; N, 4.9.

3b. Synthesised using ethyl alaninate hydrochloride and sodium benzoate. Yield 50%. Oil; ν_{max}/cm^{-1} 3348, 1741 (v br), 1600 δ_H 1.19 (3H, t, $J=7.1$, CH_3CH_2), 1.36 (3H, d, $J=7.1$, $CHCH_3$), 4.13 (2H, q, $J=7.1$, CH_3CH_2), 4.30 (1H, quint, $J=7.5$, $CHCH_3$), 5.47 (1H, d, $J=7.3$, NH), 5.92 (2H, 2 × d, $J=5.86$, OCH_2O), 7.34–8.03 (5H, m, ArH). Found: C, 57.0; H, 5.9; N, 4.6. Calcd for $C_{14}H_{17}NO_6$: C, 57.0; H, 5.8; N, 4.7.

3c. Synthesised using ethyl phenylalaninate hydrochloride and sodium benzoate. Yield 35%. Mp 90–92 °C; ν_{max}/cm^{-1} 3333, 1749, 1705 (br), 1600; δ_H 1.22 (3H, t, $J=7.2$, CH_3CH_2), 3.17 and 3.10 (2H, 2× dd, $J=14.7$, 6.0 Hz, Ar–CH_2), 4.17–4.12 (2H, m, CH_3CH_2), 4.64 (1H, dt, $J=8.1$; 6.0, α-CH), 5.40 (1H, d, $J=8.1$, NH), 5.97 (2H, s, OCH_2O), 7.08–8.09 (10H, m, ArH). Found: C, 65.0; H, 6.2; N, 3.8. Calcd for $C_{20}H_{21}NO_6$: C, 64.7; H, 5.7; N, 3.8.

3d. Synthesised using ethyl valinate hydrochloride and sodium benzoate. Yield 30%. Oil; ν_{max}/cm^{-1} 3380, 1730 (v br), 1600. δ_H 0.87 (3H, d, $J=7.5$, CH_3CHCH_3), 0.94 (3H, d, $J=6.9$, CH_3CHCH_3), 1.24 (3H, t, $J=6.9$, CH_3CH_2O), 2.10–2.22 (1H, m, MeCH), 4.17 (2H, m, CH_3CH_2O), 4.27 (1H, dd, $J=9.0$, 4.7, α-CH), 5.48 (1H, d, $J=9.0$, NH), 5.97 (2H, s, OCH_2O), 7.42–8.06 (5H, m, ArH). Found: C, 59.3; H, 6.9; N, 4.4. Calcd for $C_{16}H_{21}NO_6$: C, 59.4; H, 6.5; N, 4.1.

3e. Synthesised using ethyl leucinate hydrochloride and sodium benzoate. Yield 40%. Oil; ν_{max}/cm^{-1} 3370, 1740 (v br), 1600; δ_H 0.93 (6H, t, $J=6.6$, $(CH_3)_2CH$), 1.25 (3H, t, $J=6.9$, CH_3CH_2O), 1.49–1.70 (3H, m, (Me_2CHCH_2), 4.17 (2H, q, $J=7.0$, CH_3CH_2O), 4.37 (1H, m, α-CH), 5.37 (1H, d, $J=8.1$, NH), 5.98 (2 H, s, OCH_2O), 7.44–8.07 (5H, m, ArH). Found: C, 60.2; H, 7.2; N, 4.3. Calcd for $C_{17}H_{23}NO_6$: C, 60.5; H, 6.9; N, 4.1.

3f. Synthesised using ethyl glycinate hydrochloride and diclofenac (sodium salt). Yield 20%. Mp 63–65 °C; ν_{max}/cm^{-1} 3370, 3358, 1760, 1744, 1720; δ_H 1.28 (3H, t, $J=7.0$, CH_3CH_2), 3.87 (2H, s, CH_2Ar), 3.98 (2H, d, $J=5.7$, NCH_2), 4.22 (2H, q, $J=7.6$, CH_3CH_2), 5.39 (1H, t, $J=5.2$, CH_2NH), 5.80 (2H, s, OCH_2O), 6.56 (1H, d, $J=7.2$, ArH), 6.74 (1H, s, NH), 6.94–7.34 (6H, m, ArH). Found: C, 52.7; H, 4.4; N, 5.7. Calcd for $C_{20}H_{20}N_2Cl_2O_6$: C, 52.8; H, 4.4; N, 6.1.

3g. Synthesised using ethyl glycinate hydrochloride and clofibric acid (sodium salt). Yield 40%. Oil; ν_{max}/cm^{-1} 3384, 1730 (v br), 1605; δ_H 1.29 (3H, d, $J=7.0$, CH_3CH_2), 1.62 (6H, s, Me_2), 3.92 (2H, d, $J=5.1$, NCH_2), 4.20 (2H, q, $J=7.0$, CH_3CH_2), 5.44 (1H, t, $J=5.1$, NH), 5.84 (2H, s, OCH_2O), 6.80 (2H, d, $J=8.8$, ArH), 7.19 (2H, d, $J=8.8$, ArH). Found: C, 52.0; H, 5.7; N, 3.8. Calcd for $C_{16}H_{20}NO_7Cl$: C, 51.4; H, 5.4; N, 3.7.

3h. Synthesised using ethyl glycinate hydrochloride and nicotinic acid (sodium salt). Yield 34%. Mp 80–81 °C; ν_{max}/cm^{-1}3230, 1750 (v br), 1600; δ_H 1.24 (3H, d, $J=7$, CH_3CH_2), 3.97 (2H, d, $J=5.7$, NCH_2), 4.18 (2H, q, $J=7.0$, CH_3CH_2), 5.75 (1H, t, $J=5.1$, NH), 5.99 (2H, s, OCH_2O), 7.36–7.40 (1H, m, ArH), 8.28–8.31 (1H, m, ArH), 8.76–8.78 (1H, m, ArH), 9.22 (1H, s, ArH). Found: C, 51.5; H, 5.1; N, 10.2. Calcd for $C_{12}H_{14}N_2O_6$: C, 51.1; H, 5.0; N, 9.9.

3i. Synthesised using ethyl glycinate hydrochloride and flufenamic acid (sodium salt). Yield 44%. Mp 84–85 °C; ν_{max}/cm^{-1} 3404, 3328, 1753, 1725 (br), 1696; δ_H 1.26 (3H, t, $J=7.5$, CH_3CH_2), 4.04 (2H, d, $J=5.4$, NCH_2), 4.20 (2H, q, $J=7.2$, CH_2CH_3), 5.57 (1H, t, $J=5.4$, CH_2NH), 6.00 (2H, s, OCH_2O), 6.80–8.03 (8H, m, ArH), 9.45 (1H, s, NH). Found: C, 54.9; H, 4.3; N, 6.0. Calcd for $C_{20}H_{19}N_2O_6F_3$: C, 54.5; H, 4.3; N, 6.4.

3j. Synthesised using ethyl sarcosine hydrochloride and sodium benzoate. Yield 58%. Oil; ν_{max}/cm^{-1} 1700 (v br), 1600; δ_H (mixture of rotamers): 1.20 and 1.26 (3H, 2 × t, $J=7.0$, CH_3CH_2), 3.06 (3H, s, NCH_3), 3.98 and 4.05 (2H, 2 × s, CH_2N), 4.11 and 4.19 (2H, 2 × q, $J=7.0$, CH_3CH_2), 5.99 and 6.04 (2H, 2 × s, OCH_2O), 7.50–8.30 (5H, m, ArH). Found: C, 57.0; H, 5.7; N, 4.9. Calcd for $C_{14}H_{17}NO_6$: C, 56.9; H, 5.8; N, 4.7.

3k. Synthesised using ethyl sarcosine hydrochloride and naproxen (sodium salt). Yield 45%. Oil; ν_{max}/cm^{-1} 1710 (v br), 1600; δ_H (mixture of rotamers) 1.20 and 1.25 (3H, 2 × t, $J=7.0$, CH_3CH_2), 1.43 and 1.46 (3H, 2 × d, $J=6.0$, $CHCH_3$), 2.68 and 2.76 (3H, 2 × s, NCH_3), 3.91 (3H, s, OMe), 4.10 and 4.17 (2H, 2 × q, $J=7.0$, CH_3CH_2), 3.93 (2H, m, NCH_2), 5.55–5.66 (2 H, 4 × d, $J=5.7$, OCH_2O), 6.95–7.27 (6H, m, ArH); δ_C 14.07 and 14.10 (CH_3CH_2), 18.16 (α-CH_3), 35.86 and 35.18 (N–CH_3), 45.15 and 45.13 (CH), 50.52 and 50.24 (CH_3CH_2), 52.23 (CH_3O), 61.18 (NCH_2), 80.36 and 80.65 (OCH_2O), 105.5 (CHAr), 118.9–134.9 (CHAr and CAr), 157.7 (COMe), 154.3 and 154.9 (C=O), 168.9 and 169.0 (C=O), 173.5 and 173.5 (C=O). Found: C, 61.5; H, 6.4; N, 3.4. Calcd for $C_{21}H_{25}NO_7$: C, 61.5; H, 6.2; N, 3.5.

3l. Synthesised using methyl prolinate hydrochloride and sodium benzoate. Yield 77%. Oil; ν_{max}/cm^{-1} 1740 (v br), 1607; δ_H (mixture of rotamers) 1.79–2.31 (4H, m, 2 × CH_2), 3.44–3.69 (2H, m, NCH_2), 3.61 and 3.74 (3H, 2 × s, CH_3O), 4.39 and 4.36 (1H, 2 × dd, $J=11.1$, 8.7

and $J = 11.1$, 8.4, α-C*H*), 5.95, 5.98 and 6.06 (2H, 4 × overlapping doublets, $J = 5.6$, OCH_2O), 7.42–8.10 (5H, m, ArH); δ_C 23.35 and 24.22 (CH_2 proline), 29.85 and 30.77 (CH_2 proline), 46.62 and 46.95 (NCH_2 proline), 52.19 and 52.28 (CH_3O), 58.79 and 59.23 (α-*C*H), 80.24 and 80.64 (OCH_2O), 128.45 (Ar*C*H), 129.97 and 130.03 (Ar*C*H), 133.52 and 133.56 (Ar*C*H), 152.58 and 153.17 (*C*=O), 165.28 and 165.33 (C=O); 172.61 and 172.65 (*C*=O). Found: C, 58.6; H, 5.5; N, 4.5. Calcd for $C_{15}H_{17}NO_6$: C, 58.6; H, 5.5; N, 4.5.

Apparent partition coefficients

These were determined at 22 °C using octanol–pH 7.4 phosphate buffer and octanol–pH 5.0 acetate buffer. Each phase was mutually saturated before the experiment. The volumes of each phase were chosen so that solute concentrations in the aqueous phase after distribution were readily measurable. The compounds were dissolved in 1-octanol and the octanol–phosphate buffer mixtures were shaken for 30 min to reach an equilibrium distribution. Each phase was analysed separately by HPLC. Partition coefficients, P, were calculated from the ratio of the peak area in octanol to the peak area in buffer.

Hydrolysis in aqueous solution

All kinetic experiments carried out in aqueous systems were buffered (phosphate, acetate, etc.) with the ionic strength maintained at 0.5 M using $NaClO_4$. Reaction mixtures were analysed using UV spectrophotometry and HPLC. Usually, a 10 μL aliquot of a 10^{-2} M stock solution of substrate in acetonitrile was added to 10 mL of the appropriate thermostatted buffer solution. At regular intervals, samples of the reaction mixture were analysed by HPLC using the following conditions: a Merck LiChrospher© 100 RP-18 5 μm 125 × 4 mm column; mobile phase, acetonitrile/water (the composition of which depended on the compound); flow rate 1.0 mL/min; detector wavelength, 230 nm.

Hydrolysis in human plasma

Human plasma was obtained from the pooled, heparinised blood of healthy donors, and was frozen and stored at −70 °C prior to use. For the hydrolysis experiments, the substrates **3** were incubated at 37 °C in human plasma that had been diluted to 80% (v/v) with pH 7.4 isotonic phosphate buffer. At appropriate intervals, aliquots were added to acetonitrile to both quench the reaction and precipitate plasma proteins. These samples were centrifuged and the supernatant analysed by HPLC for the presence of substrate and products.

In vitro skin permeation study

Samples of human skin were obtained from the abdominal region of adult human cadavers from the Kuopio University Hospital (Kuopio, Finland). The epidermis was isolated from the underlying dermis by heat separation at 60 °C in distilled water for 2 min, after which the skin specimens were dried and frozen prior to use. The permeation studies were carried out using the Franz-type diffusion cell (PermeGear, Inc., Riegel, PA, USA) as previously described.[12] Skin specimens were rehydrated before being mounted in the diffusion cell. The receptor medium (0.05 M pH 7.4 isotonic phosphate buffer solution) was stirred and kept at 37 °C throughout the study. The compounds were applied as suspensions in 0.05 M pH 5.0 acetate buffer. At specified time intervals 250 μL aliquots were withdrawn from the receptor compartment and replaced with fresh buffer. The drug concentrations were assayed by HPLC. The steady-state flux for parent compounds and their prodrugs was determined by plotting the cumulative amount (in nmol) of the parent drug and intact prodrug as measured in the receptor phase against time, and dividing the slope of the steady-state position by the surface area of the diffusion cell (0.71 cm^2).

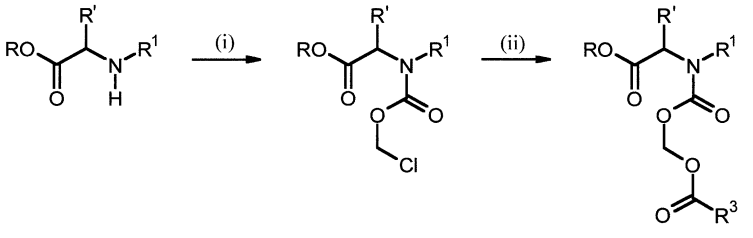
Statistical analysis

A one-factor analysis of variance (ANOVA factorial) was used to test the statistical significance of differences between parent acid and prodrugs. Significance in the differences in the means was tested using Fisher's protected least significance difference (PLSD) at 95% confidence.

Results and Discussion

Synthesis

The synthesis of *N*-aminocarbonyloxymethyl esters **3a–l** (Table 1) involves the preparation of *N*-[(chloromethyl)oxy]carbonyl derivatives **4** by reaction of the appropriate amino acid ester with chloromethyl chloroformate (Scheme 1). Reaction of intermediates **4** with the sodium salt of the appropriate carboxylic acid agent afforded **3** in 15–77% yield. As the main aim of the present work was to study the stability and skin permeability of these compounds, we made no attempt to optimise these yields. However, two trends are worthy



Scheme 1. $ClCH_2OCOCl/Et_3N/CH_2Cl_2$; R^3CO_2Na/TBAB/THF.

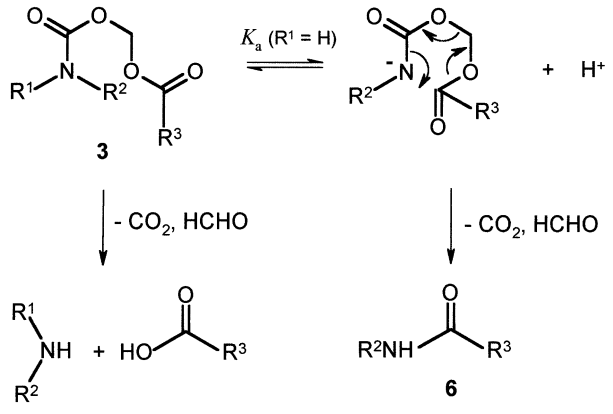
of mention. First, the yields are higher the more bulky the amino acid moiety. This suggests that the carboxylate salt can attack more than one site in the intermediate **4** and that steric bulk directs it to the less-hindered chloromethyl site. Second, tertiary carbamates also give rise to higher yields. One possible explanation for this is that those intermediates **4** that contain the secondary carbamate functionality undergo a competetive E1cb elimination reaction catalysed by the carboxylate ion.

The structure of compounds **3a**–**l** follows from their spectroscopic and analytical data. In particular, the ^{1}H NMR spectra of *N*-aminocarbonyloxymethyl esters **3a** and **3c**–**i** exhibit a characteristic singlet at ca. δ 6.0–6.6 ppm due to the OCH_2O group. Interestingly despite the diastereotopic nature of the OCH_2O protons in compounds **3b**–**e**, only **3b** revealed the presence of a pair of doublets ($J = 5.9$ Hz). The ^{13}C and ^{1}H NMR spectra of derivatives **3j**–**l**, which contain a tertiary carbamate group, reveal them to be a mixture of *E* and *Z* rotamers due to restricted rotation about carbamate C–N bond. Moreover, for the proline derivative, **3l**, the OCH_2O signal appears as two pairs of overlapping doublets (the central signal of which at δ 5.98 is twice as intense as the other two signals), reflecting the diastereotopic nature of the methylene protons ($J = 6.0$ Hz) as well as the presence of two rotamers. The spectroscopic detection of rotamers has been reported for other prolyl carbamates and is consistent with a barrier to rotation for the carbamate N-C=O framework similar to the barrier of acyclic tertiary amides.[21,22]

Kinetics and products of chemical hydrolysis

The hydrolysis of compounds **3a**–**l** was studied in isotonic pH 7.4 phosphate buffer at 37 °C, and the corresponding half-lives are given in Tables 1 and 2. All reactions displayed strict first-order kinetics over at least four half-lives. Compounds **3** are reasonably stable in pH 7.4 buffer, with half-lives ranging between 9 and 169 h, except the diclofenac and nicotinic acid derivatives, **3f** and **3h**, which hydrolyse with half-lives of ca. 1 and 2 h, respectively.

The hydrolysis of derivative **3a** was also studied over a range of pH values using HCl and NaOH solutions as



Scheme 2.

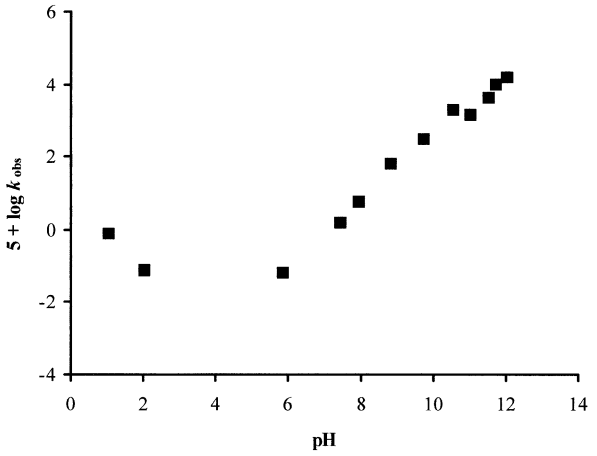
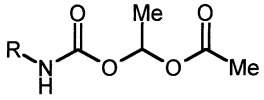


Figure 1. pH-Rate profile for aminocarbonyloxymethyl benzoate **3a** at 37 °C.

well as acetate, phosphate and borate buffers. The pseudo-first-order rate constants, k_{obs}, were found to vary with buffer concentration. The pH-rate profile (Fig. 1) was constructed using the intercepts of plots of k_{obs} versus [buffer], together with the k_{obs} values determined in HCl and NaOH solutions. Between pH 2 and pH 6 reactions were too slow to monitor accurately. A similar pH-rate profile, indicative of the presence of acid-catalysed and base-catalysed processes has been described for the related *N*-acyloxyalkoxycarbonyl derivatives of aniline, **5** (R = Ph) studied by Gogate.[15] The pH-rate profile can be described by eq (1),

$$k_{obs} = k^{app}_{OH-}[OH-] + k^{+}_{H}[H^+] \quad (1)$$

where k^{app}_{OH-} and k_{H^+} are the OH^- catalysed and H^+ catalysed second-order rate constants, respectively. The best computer fit (solid line) to the experimental data (individual points) using eq (1), gave values for the catalytic second-order rate constants, k_{H^+} and k_{OH^-}, of $9.0\times10\times^5$ and $16.94\,M^{-1}\,s^{-1}$, respectively.



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As revealed by the HPLC analysis of the reaction mixtures, the hydrolysis of *N*-aminocarbonyloxymethyl ester **3a** proceeds with quantitative formation of the parent carboxylic acid at pH < 3 (Scheme 2). However, for pH > 5, ethyl *N*-benzoylglycinate **6** (R^2 = EtO-$COCH_2NH$, R^3 = Ph) was also detected as a major reaction product, presumably arising from a rearrangement involving a O→N intramolecular acyl transfer from the carbamate conjugate base (Scheme 2). As revealed from the data in Table 2, the parent carboxylic acid and the *N*-acylamine rearrangement product were also observed in the hydrolysis of secondary carbamates **3a**–**c** and **3f**–**i** in pH 7.4 phosphate buffer (see also

Table 2. Kinetic and product data for the hydrolysis of esters **3** in pH 7.4 isotonic phosphate buffer and in 80% human plasma at 37 °C, together with their apparent partition coefficients ($logP_{app}$, mean + SD; $n = 3$)

Compound	pH 7.4 phosphate buffer			80% Human plasma		$logP_{app}$	
	$t_{1/2}$ / (h)	% Rearrangement	% carboxylic acid	$t_{1/2}$ / (min)	% carboxylic acid	pH 7.4	pH 5.0
3a	9.04	45.4	54.6	0.24	100	2.02±0.05	1.88±0.04
3b	30.6	34.8	65.2	1.90	100	2.45±0.03	2.26±0.03
3c	15.5	2.3	97.7	4.87	100	3.74±0.18	3.57±0.09
3d	70.3	ND	100	2.85	100	3.17±0.06	3.02±0.03
3e	29.2	ND	100	2.50	100	3.61±0.17	3.63±0.01
3f	0.98	7.0	93.0	4.17	100	4.16±0.15	3.92±0.05
3g	37.0	8.8	91.2	1.63	100	2.94±0.01	2.91±0.03
3h	2.24	82.0	18.0	0.43	100	0.68±0.02	0.63±0.09
3i	19.0	19.4	80.6	21.8	100	3.99±0.17	4.29±0.30
3j	34.3	ND	100	0.41	100	2.09±0.10	2.07±0.02
3k	39.1	ND	100	2.72	100	3.52±0.10	3.35±0.17
3l	169	ND	100	3.48	100	2.12±0.09	2.14±0.08

ND: Not detected

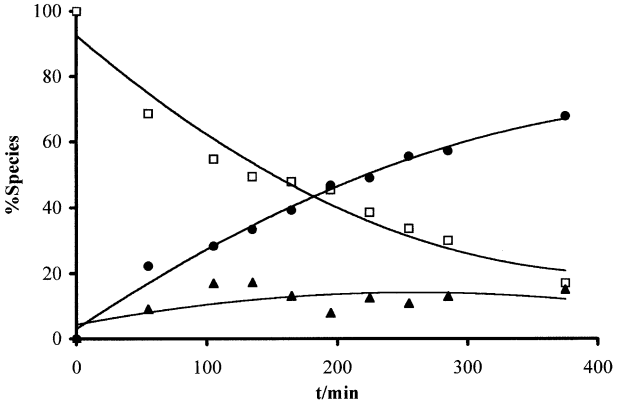


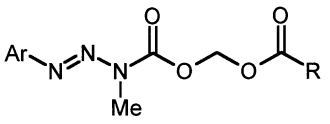
Figure 2. HPLC analysis of the hydrolysis of **3h** in pH 7.4 phosphate buffer: **3h** (□); ethyl *N*-benzoylglycinate (●); nicotinic acid (▲).

Fig. 2). Consistent with rearrangement arising from the conjugate base, hydrolysis of tertiary carbamates **3j–l** proceeded with quantitative formation of the parent carboxylic acid. Inspection of the data in Table 2 suggests that the extent of the rearrangement depends on the steric hindrance of the amino acid and carboxylic acid moieties. Thus, intramolecular acyl transfer is an important route for benzoates **3a** and **3b**, whereas for the more sterically hindered valine, **3d**, and leucine derivatives, **3e**, only direct ester hydrolysis was observed. For the nicotinic acid derivative **3h** there is a predominance of the intramolecular acyl transfer when compared to diclofenac, **3f**, and clofibrate, **3g**, derivatives. Moreover, the more reactive nicotinate derivative **3h** displays a greater level of rearrangement than the corresponding benzoate **3a**, as might be expected for a more electrophilic acyl group that is the result of the presence of the nitrogen atom in the aryl ring.

Hydrolysis in human plasma

Hydrolysis of compounds **3a–l** in human plasma also followed strict first-order kinetics for at least four half-lives, ($r^2 > 0.95$ in all cases). Product analysis revealed that the plasma catalysed hydrolysis of these esters quantitatively afforded the corresponding parent carboxylic acid; no rearrangement product was detected for any compound. This observation contrasts sharply with the corresponding product profiles in pH 7.4 buffer, especially for the benzoates **3a**,**b** and for the nicotinate **3h**. These product profiles in human plasma also contrast with those for the closely related secondary *N*-acyloxyalkoxycarbonyl derivatives of aniline **5** (R = Ph) which were reported to give between 15 and 30% of rearrangement product, but compare with that for the benzylamine counterpart **5** ($R = CH_2Ph$) which hydrolysed in human plasma almost exclusively to the parent amine.[16]

The susceptibility of compounds **3** to undergo enzymatic plasma activation was assessed in vitro by comparing the half-lives for hydrolysis in 80% human plasma with those in pH 7.4 phosphate buffer at 37 °C (Table 2). With half-lives ranging from 0.2 to ca. 20 min, it is clear that human plasma enzymes markedly accelerated the rate of hydrolysis of aminocarbonyloxymethyl esters **3a–l.** Reactivity in human plasma appears to be dependent on the size of the substituents in both the amino acid carrier and the parent carboxylic acid. Indeed, the benzoate **3a** hydrolysed ca. 17, 7 and 90 times faster than the more sterically hindered esters **3f**, **3g** and **3i**, respectively. Similarly, compound **3a**, a glycine derivative, hydrolysed ca. 8 and 20 times faster than its alanine and phenylalanine counterparts, **3b** and **3c**, respectively. The rate data in Table 2 were analysed for quantitative relationships with several physicochemical parameters, including Charton's steric parameter ν for the amino acid carrier, the pK_a of the parent carboxylic acid and the logP values of the prodrugs. No clear correlation between the rate data encompassing all aminocarbonyloxymethyl esters **3a–l** and the corresponding physicochemical parameters emerged, although a slight correlation ($r^2 = 0.62$) was observed between log $t_{1/2}$ and logP, indicating that increasing substrate lipophilicity lead to a reduction in reactivity (Fig. 3). A similar trend has been reported for the closely related acyloxymethyl carbamates of triazenes, **7**,[23] as well as for other different types of prodrugs, for example amidomethyl esters,[24] and can be ascribed to the dependence of esterase activity on substrate lipophilicity.



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In vitro skin permeation study

In vitro skin permeation studies through excised post-mortem human skin were carried out using suspensions in phosphate buffer to maintain constant diffusion and maximum flux. Each parent carboxylic acid and corresponding prodrug was applied in 0.05 M pH 5.0 isotonic phosphate buffer to avoid significant hydrolysis (see Fig. 1). The cumulative amount (in nmol/cm^2) of permeated parent acid or intact prodrug generally displayed a linear relationship with time (data not shown). The steady-state flux (J_{ss}) was calculated from the slope of the linear portions of these plots and is presented in Table 3. All prodrugs tested permeated the skin intact, reflecting their expected stability in pH 5.0 phosphate buffer. Unfortunately, all the prodrugs evaluated exhibited a lower flux than the corresponding parent carboxylic acid. The poor skin permeation observed for compounds **3** is most probably due to their high partition coefficient (Table 2) and, more especially, their low aqueous solubility (Table 3). Similar poor skin permeation has been reported for the neutral pivaloyloxymethyl prodrug of naproxen (**1**, R = CMe$_3$).[12] This compound has similar logP_{app} (2.8) and aqueous solubility (0.4 μM) values[12] to the naproxen derivative **3k** (Tables 2 and 3). Not surprisingly, therefore, it displays a similar J_{ss} value (0.02 nmol/cm^2/h) to **3k**. This is consistent with recent reports suggesting that an effective topical prodrug must possess good biphasic solubility characteristics (i.e., good balance between lipid and aqueous solubility).[20,25] Moreover, optimal logP values ranging between 2 and 3 for maximal fluxes have been reported for several NSAIDs.[26,27]

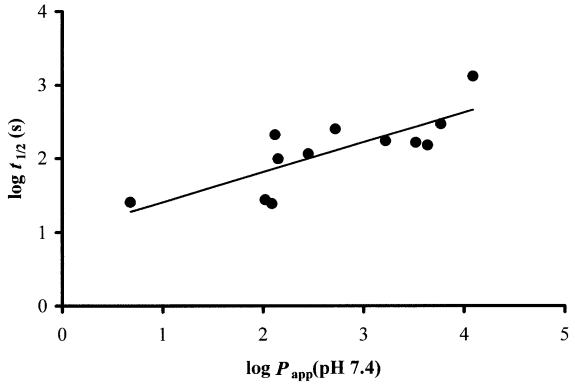

Figure 3. Plot of the plasma hydrolysis half-lives of compounds **3** against the corresponding pH 7.4 logP_{app} values.

Table 3. Steady-state fluxes (J_{ss}) (mean ± SE; n = 2–12) for the delivery of total parent compound species through excised human skin in vitro in 0.05 M pH 5.0 isotonic phosphate buffer at 37 °C

Compound	Aqueous solubility (pH 5.0)/(μM)	J_{SS} (nmol cm^{-2} h^{-1})
Naproxen	460 ± 20	1.6 ± 0.2[a]
3k	2 ± 0.3	0.06 ± 0.01[b]
Flufenamic acid	41 ± 0.6	0.83 ± 0.16
3i	—[d]	—[c]
Benzoic acid	39,000 ± 5000	51.24 ± 4.40
3b	1301 ± 106	3.54 ± 0.71[b]
3c	3 ± 0.5	—[c]
3d	166 ± 20	0.50 ± 0.15[b]
3e	34 ± 2	0.91 ± 0.28[b]

[a]Ref 20.
[b]$p < 0.05$ compared to parent compound (ANOVA, Fisher's PLSD test).
[c]No detectable amount was found in the receptor chamber.
[d]Compound bellow the limit of quantitation in the solution. Calculated solubility 0.56 μM (ALOGPS v2.0 available at http://www.lnh.unil.ch/appl/cchem00.html).

In conclusion, aminocarbonyloxymethyl esters **3** based on non-toxic amino acid carriers provide a new and potentially attractive approach to the preparation of prodrugs of carboxylic acid drugs. The present study shows that it is possible to prepare aminocarbonyloxymethyl esters **3** with a wide range of lipophilicities and that simultaneously combine a high susceptibility to plasma hydrolysis with good stability in aqueous buffers. More importantly, compounds **3** can liberate quantitatively in a few min a parent carboxylic acid containing bulky substituents. This is a significant improvement over the amidomethyl esters, based on the ethyl hippurate carrier, of sterically hindered carboxylic acids, which are only slowly hydrolysed in human plasma.[24] Despite their potential as prodrugs, the current series of compounds displayed poor skin permeation, probably due to their high lipophilicity and low aqueous solubility. We are currently evaluating similar derivatives containing an ionisable functionality that should increase aqueous solubility.

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