Synthesis and *In Vitro* Evaluation of Aminoacyloxyalkyl Esters of 2-(6-methoxy-2-naphthyl)propionic Acid as Novel Naproxen Prodrugs for Dermal Drug Delivery

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Purpose. To synthesize and evaluate various novel aminoacyloxyalkyl esters of naproxen (**3a-i**) and naproxenoxyalkyl diesters of glutamic and aspartic acids (**3j-m**) as potential dermal prodrugs of naproxen.
Methods. The prodrugs **3a-m** were synthesized, and their aqueous solubilities, lipophilicities and hydrolysis rates were determined in a buffered solution and in human serum. The permeation of selected prodrugs across excised postmortem human skin was studied *in vitro*.
Results. The aminoacyloxyalkyl prodrugs showed higher aqueous solubilities and similar lipid solubilities, in terms of octanol-buffer partition coefficients (log P_{app}) at pH 5.0, when compared with naproxen. At pH 7.4 the prodrugs were significantly more lipophilic than naproxen. Prodrugs **3a-i** showed moderate chemical stability in aqueous solutions at pH 5.0 and were rapidly converted to naproxen in human serum ($t_{1/2}$ = 4–19 min). The selected aminoacyloxyalkyl prodrugs possessed a higher flux across the skin than naproxen, with a maximum enhancement of 3-fold compared to naproxen. Prodrugs **3j-m** showed poor aqueous solubility and permeation across the skin.
Conclusions. Combinations of adequate aqueous solubility and lipophilicity of naproxen aminoacyloxyalkyl prodrugs having fast rates of enzymatic hydrolysis resulted in improved dermal delivery of naproxen.

KEY WORDS: naproxen; prodrugs; solubility; lipophilicity; hydrolysis kinetics; skin permeation.

INTRODUCTION

Dermal administration currently holds a high level of interest in pharmaceutical research because it has a number of advantages over the conventional methods, such as oral drug delivery. For example, dermal application can provide higher local drug levels than those safely obtainable with oral delivery (1–3). However, the barrier function of the skin and undesirable physicochemical properties of drugs limit the skin permeation of a wide range of substances which have led to the development of various strategies to enhance drug-skin permeation. Approaches have included formulation additives, such as chemical penetration enhancers, and vehicles which maximize partitioning of drugs into the skin. In particular, the prodrug approach has been studied to enhance the skin permeation of drugs.

2-(6-Methoxy-2-naphthyl)propionic acid (naproxen) is a nonsteroidal anti-inflammatory drug (NSAID) that is widely used in the treatment of rheumatic diseases and related painful conditions (4). The bioavailability through percutaneous absorption of naproxen in humans is only 1–2% (1,3,5,6), and a few reports have been published concerning the use of the prodrug approach to increase the dermal permeation of naproxen (7–9). Various alkyl esters of naproxen have been reported to be possible dermal prodrugs (7–9). The esters increased the skin permeation of naproxen, but they released naproxen very slowly in both human serum and skin-serum homogenate (7), or no information on the hydrolysis kinetics was reported (8,9). 1-Alkylazacycloalkan-2-one esters of naproxen were hydrolyzed *in vitro* by porcine esterases to give a moderate enhancement in skin permeation compared to naproxen (10). Recently, we synthesized and evaluated a number of various acyloxyalkyl esters of naproxen, which readily hydrolyzed to naproxen *in vitro*, both in human skin homogenate and human serum (11). However, these highly lipophilic prodrugs did not enhanced dermal permeation of naproxen, probably due to the low aqueous solubilities. This finding led us to investigate various aminoacyloxyalkyl esters (amino acids as a promoiety) as prodrugs of naproxen to overcome the poor aqueous solubility of the previously studied acyloxyalkyl derivatives. The present study describes the synthesis and *in vitro* evaluation of various novel aminoacyloxyalkyl prodrugs of naproxen and naproxenoxyalkyl diesters of glutamic and aspartic acids for improved dermal drug delivery.

MATERIALS AND METHODS

Chemicals

Naproxen was obtained from Orion Pharma (Espoo, Finland) and BOC-protected amino acids were purchased from Sigma (St. Louis, MO, USA). *N*-BOC-L-aspartic acid was obtained from Calbiochem-Novabiochem (Läufinger, Switzerland). Other reagents were obtained from Aldrich (Steinheim, Germany). The bulk solvents were purchased from Merck (Darmstadt, Germany). The hydroxyalkyl esters of naproxen (**2a-c**) were synthesized and identified as described earlier (11). Naproxen was converted to the acid chloride with thionyl chloride as described earlier (12). The purities of the prodrug substances were determinated by HPLC and NMR and they were >98% (mol %) for each prodrug.

Methods

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM 400 WB operating at 400.1 and 100.6 MHz, respectively, using TMS as a reference. On request, the ^{13}C shifts are available from authors. Electron impact (E.I.) mass spectra of the prodrugs were determined by a VG 70-250SE magnetic sector mass spectrometer (VG Analytical, Manchester, UK). The analytical HPLC system for the determination of prodrug physicochemical properties consisted of a Beckman model 116 pump with a

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model 166 UV detector operated at a fixed wavelength (230 nm), a Marathon automatic sample injector, and a Osborne MD 700 computer (method 1). The analytical HPLC system for determination of drug in skin permeation samples consisted a Merck Hitachi L-6200A intelligent pump, Hewlett Packard HP 1046A programmable fluorescence detector (exitation 226 nm; emission 368 nm), a Merck Hitachi D-6000A interface module, a Merck Hitachi AS-2000 autosampler, and a Merck LaChrom column oven L-7350 (method 2). All sample separations were made a Purospher RP-C18 column (125 × 4 mm, 5 μm). Flash chromatography was accomplished over silica gel (J. T. Baker, 30–60 μm). Thin-layer chromatography (TLC) analyses of reactions were run on aluminium foil plates coated with silica gel 60 F_{254} (Merck).

General Procedures for the Synthesis of Naproxen Prodrugs

The synthesis of the naproxen aminoacyloxyalkyl ester prodrugs **3a-i** is illustrated in Scheme I. Derivatives **3a-g** were prepared by coupling the corresponding naproxen hydroxyalkyl ester **2a-c** with the *N-tert*-butyloxycarbonyl (BOC) protected amino acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in dry dichloromethane. Deprotection of the BOC-protected naproxen aminoacyloxyalkyl ester with trifluoroacetic acid (TFA) afforded the desired naproxen aminoacyloxyalkyl esters **3a-g**, which were converted to their hydrochloride salts with HCl (g) in diethyl ether (Et_2O), except **3e** and **3g** which were obtained as free amines. The 1-(aminoacyloxy)ethyl esters **3h** and **3i** were synthesized by the reaction of the naproxen 1-chloroethylester **2d** and sodium salt of the corresponding BOC-amino acid. The naproxen 1-chloroethylester **2d** was prepared from naproxen acid chloride **2b** and acetaldehyde in the presence of anhydrous $ZnCl_2$. The BOC-protecting groups were removed with HCl/dioxane to afford **3h** and **3i** as HCl salts. The naproxenoxyalkyl diesters of glutamic and aspartic acids **3j-m** were synthesized as **3a-g** (Scheme II).

1-chloroethyl 2-(6-methoxy-2-naphthyl)propanoate (2d)

To a cooled (0–5°C) mixture of acetaldehyde (3 mL), anhydrous $ZnCl_2$ (200 mg) in dry dioxane (10 mL) was added dropwise the naproxen acid chloride (10.3 mmol) over period of 1h. Reaction mixture was stirred for 16 hours at room temperature, extracted with Et_2O and washed with 5% $NaHCO_3$-solution and water. Purification with flash silica gel column eluting with 20% EtOAc in petroleum ether afforded **2d** as a yellow oil in 51% yield; ^1H NMR ($CDCl_3$): δ 7.8–7.1 (6 H, m, aromatic), 6.5 (1 H, qq, ClC*H*Me), 3.9 (1 H, q, C*H*Me), 3.9 (3 H, s, CH_3O), 1.7 (3 H, dd, CH_3) 1.6 (3 H, dd, CH_3C); HR-MS data not available.

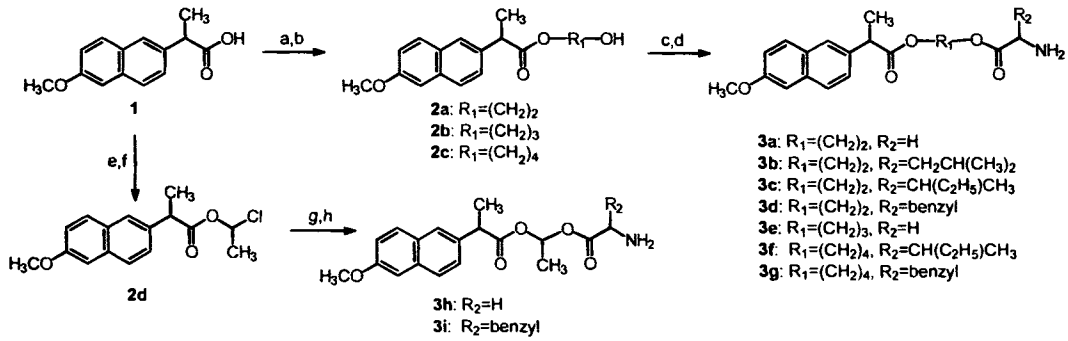
2-(glycyloxy)ethyl 2-(6-methoxy-2-naphthyl)propanoate (3a)

A mixture of *2a*, (4.2 mmol), BOC-glycine (4.2 mmol), DMAP (0.2 mmol) and DCC (5.5 mmol) in dry CH_2Cl_2 (60 mL) was stirred at 60°C for 24 h. The precipitated dicyclohexylurea was filtered off, the filtrate was evaporated. The BOC-protected **3a** was purified by flash silica gel column chromatography eluting with 20% EtOAc in petroleum ether and treated with TFA:CH_2Cl_2 (1:1, 14 mL) at 25°C for 3 h followed by concentration under reduced pressure. The free base was generated by dissolving the residue in a 5% aqueous solution of $NaHCO_3$ (2 × 25 mL) and extracting the free amine using ethyl acetate (EtOAc) (2 × 50 mL). The organic layer was dried over anhydrous $CaSO_4$, filtered and evaporated in vacuo. The free base was dissolved in Et_2O and treated with saturated Et_2O-HCl to give a HCl salt, which was recrystallized from EtOAc to give **3a** in 78% yield; mp 113–4°C; ^1H NMR ($CDCl_3$/CD_3OD 8:2, 400 MHz) δ 7.71–7.12 (6 H, m, aromatic), 4.30 (4 H, m, OCH_2CH_2O), 3.90 (3 H, s, CH_3O), 3.87 (1 H, q, C*H*Me), 3.66 (2 H, bs, CH_2NH_2), 1.57 (3 H, d, CH_3C); HR-MS data not available.

Compounds **3b-3e** were prepared as described for **3a** from appropriate naproxen hydroxyalkyl ester and BOC-protected amino acid.

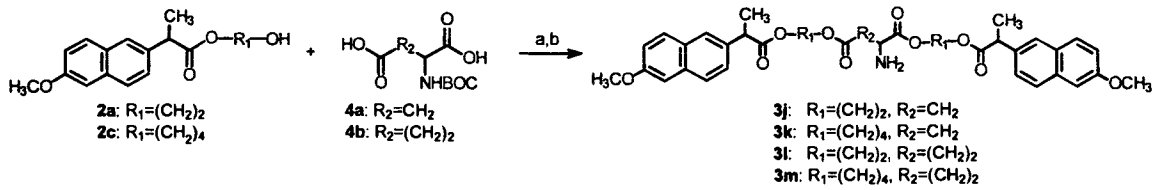
2-(L-leucyloxy)ethyl 2-(6-methoxy-2-naphthyl)propanoate (3b)

Obtained as HCl salt in 56% yield; mp 96–7°C; ^1H NMR ($CDCl_3$/CD_3OD 8:2, 400 MHz) δ 7.72–7.12 (6 H, m, aromatic), 4.41 (2 H, m, OCH_2CH_2O), 4.39/4.33* (2 H, m, OCH_2CH_2O), 3.90 (1 H, q, C*H*Me), 3.88 (3 H, s, CH_3O), 3.88 (1 H, t, C*H*NH_2),



[a] Reagents: (a) NaOH; (b) Br-R_1-OH, DMF; (c) BOC-NH-CHR_2-CO_2H, DCC, DMAP, CH_2Cl_2; (d) TFA/CH_2Cl_2; (e) $SOCl_2$, toluene; (f) CH_3CHO, $ZnCl_2$; (g) BOC-NH-CHR_2-CO_2Na, KI, DMF; (h) HCl/dioxane

Scheme 1.



[a] Reagents: (a) DCC, DMAP, EtOAc; (b) HCl/dioxane or HCl/EtOAc

Scheme 2.

1.71 (1 H, m, C*H*(Me)$_2$,) 1.65 (2 H, m, CH_2CH), 1.57 (3 H, d, CH_3C), 0.89 (3 H, d, CH_3C), 0.88 (3 H, d, CH_3C) (*due to the chiral center, the two CH_2 protons are not equivalent); HR-MS: Calculated mass for $C_{22}H_{29}NO_5$: 387.205. Measured mass: 387.207.

*2-(L-*isoleucyloxy)ethyl 2-(6-methoxy-2-naphthyl)propanoate (3c)

Obtained as HCl salt in 90% yield; mp 99–100°C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.52* (2 H bs, NH_2), 7.72–7.14 (6 H, m, aromatic), 4.49/4.30** (2 H m, OCH_2CH_2O), 4.33 (2 H, m, OCH_2CH_2O), 3.91 (3 H, s, CH_3O), 3.88 (1 H, q, C*H*Me), 3.84 (1 H, d, C*H*NH_2), 1.90 (1 H, m, C*H*CH_3), 1.58 (3 H, d, CH_3C), 1.37/1.20* (2 H, m, CH_2CH_3), 0.88 (3 H, d, CHCH_3), 0.84 (3 H, t, CH_3C) (*free base, **due to the chiral center, the two CH_2 protons are not equivalent); HR-MS: Calculated mass for $C_{22}H_{29}NO_5$: 387.205. Measured mass: 387.201.

*2-(L-*phenylalanyloxy)ethyl 2-(6-methoxy-2-naphthyl)propanoate (3d)

Obtained as HCl salt in 28% yield; mp 187–8°C; ^{1}H NMR ($CDCl_3$/CD_3OD 8:2, 400 MHz) δ 7.67–7.05 (11 H, m, aromatic), 4.39/4.29* (2 H, m, OCH_2CH_2O), 4.26 (2 H, m, OCH_2-CH_2O), 4.09 (1 H, t, C*H*NH_2), 3.88 (3 H, s, CH_3O), 3.88 (1 H, q, C*H*Me), 3.03 (2 H, m, CH_2Ar), 1.58 (3 H, d, CH_3C) (*due to the chiral center, the two CH_2 protons are not equivalent); HR-MS: Calculated mass for $C_{25}H_{27}NO_5$: 421.189. Measured mass: 421.200

3-(glycyloxy)propyl 2-(6-methoxy-2-naphthyl)propanoate (3e)

Obtained as waxy oil of free amine in 72% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (2 H, NH_2, bs), 7.65–7.07 (6 H, m, aromatic), 4.11 (2 H, m, $-CH_2O$), 4.07 (2 H, m, OCH_2-), 3.85 (3 H, s, CH_3O), 3.81 (1 H, q, C*H*Me), 3.73 (2 H, bs), 1.86 (2 H, m, $-CH_2-$), 1.52 (3 H, d, CH_3C); HR-MS: Calculated mass for $C_{19}H_{23}NO_5$: 345.158. Measured mass: 345.165.

*4-(L-*isoleucyloxy)butyl 2-(6-methoxy-2-naphthyl)propanoate (3f)

Obtained as HCl salt in 69% yield; mp 72–3°C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.82* (2 H, bs, NH_2), 7.69–7.10 (6 H, m, aromatic), 4.12 (2 H, m, $-CH_2O$), 4.07 (2 H, m, OCH_2-), 3.96 (1 H, d, C*H*NH_2), 3.90 (1 H, s, CH_3,O), 3.84 (3 H, s, C*H*Me), 2.15 (2 H, bs), 1.62 (4 H, m, $-CH_2CH_2-$), 1.56 (3 H, d, CH_3C), 1.41/1.46** (2 H, m), 1.06 (3 H, d, CH_3C), 0,91 (3 H, t, CH_3C) (*free base, **due to the chiral center, the two CH_2 protons are not equivalent); HR-MS: Calculated mass for $C_{24}H_{33}NO_5$: 415.236. Measured mass: 415.222.

*4-(L-*phenylalanyloxy)butyl 2-(6-methoxy-2-naphthyl)propanoate (3g)

Obtained as a free amine which was purified by flash silica gel column chromatography eluting with 20% MeOH in EtOAc to afford 3g as a waxy oil in 41% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70–7.10 (11 H, m, aromatic), 4.06 (2 H, m, $-CH_2O$), 4.01 (2 H, m, OCH_2-), 3.90 (3 H, s, CH_3O), 3.84 (1 H, q, C*H*Me), 3.68 (1 H, dd, C*H*NH_2), 3.01/2.83* (2 H, dd, CH_2Ar), 1.58 (3 H, d, CH_3C), 1.54 (4 H, m, $-CH_2CH_2-$) (*due to the chiral center, the two CH_2 protons are not equivalent); HR-MS: Calculated mass for $C_{27}H_{31}NO_5$: 449.220. Measured mass: 449.225.

1-(glycyloxy)ethyl 2-(6-methoxy-2-naphthyl)propanoate (3h)

The sodium salt of BOC-glycine was dissolved in 10 mL of DMF and allowed to react with **2d** (4.5 mmol) and potassium iodide (0.80 g, 5.3 mmol). The mixture was stirred at room temperature for 24 h and the DMF was removed *in vacuo*. The residue was taken up in chloroform and washed with 5% $NaHCO_3$-solution and then twice with saturated aqueous NaCl-solution, dried over $MgSO_4$ and evaporated. The residue was chromatographed (flash silica gel, 1:2 EtOAc: petroleum ether) to give BOC-protected 1-(glycyloxy)ethyl ester of naproxen (0.93 mmol, 21%) as a viscous oil. The BOC-protected ester (400 mg) was dissolved in dioxane (5 mL) and 4 N HCl/dioxane (4 mL) was added and solution was stirred at room temperature for 4 h followed by concentration under reduced pressure. The residue was recrystallized from dioxane to give the HCl salt of **3h** in 21% yield; mp 149–50°C; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.74–7.13 (6 H, m, aromatic), 6.95/6.98* (1 H, q, C*H*Me), 3.91 (1 H, q, C*H*Me), 3.90 (3 H, s, CH_3O), 3.74/3.67 (2 H, d, CH_2NH_2), 1.55 (3 H, d, CH_3C), 1.51/1.42 (3 H, d, CH_3) (*two diastereomers, ratio 1:1); HR-MS: Calculated mass for $C_{18}H_{21}NO_5$: 331.142. Measured mass: 331.135.

*1-(L-*phenylalanyloxy)ethyl 2-(6-methoxy-2-naphthyl)propanoate (3i)

Synthesized analogously to 3h. The residue was recrystallized from THF to give the HCl salt of **3i** in 27% yield; mp

176°C; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.74–7.08 (11 H, m, aromatic), 6.95/6.91 (1 H, m, OCH(CH_3)O), 4.22/4.15 (1 H, dd, C*H*NH_2), 3.90/3.89 (1 H, q, C*H*Me), 3.87/3.86 (3 H, s, CH_3O), 3.06/2.86 (2 H, m, CH_2Ar), 1.57/1.54 (3 H, d, CH_3C), 1.43/1.40 (3 H, d, OCH(CH_3)O) (*two diastereomers ratio 7:3); HR-MS: Calculated mass for $C_{25}H_{27}NO_5$: 421.189. Measured mass: 421.181.

Bis[2-(6-methoxy-2-naphthyl)propanoyloxyethyl] L-aspartate (3j)

A mixture of **2a** (2.7 mmol), N-BOC-L-aspartic acid (1.4 mmol), DMAP (4.1 mmol) and DCC (4.1 mmol) in dry EtOAc (50 mL) was stirred at room temperature for 24 h. The precipitated dicyclohexylurea was filtered off, and the solvent was evaporated to afford a solid residue, which was purified with flash silica gel chromatography to yield BOC-protected **3j**, that was dissolved the in 4 mL of dioxane and treated with 3 mL 4 M HCl/dioxane for 8 h. Concentration under reduced pressure yield **3j** as an oil in 47% yield which changed solid foam when drying in vacuum; ^{1}H NMR ($CDCl_3/CD_3OD$ 8:2, 400 MHz) δ 7.70–7.13 (12 H, m, aromatic), 4.20–4.40 (8 H, m, OC.H_2-CH_2O), 3.91 (6 H, s, CH_3O), 3.87 (2 H, q, C*H*Me), 3.76 (1 H, m, C*H*NH_2), 2.93 (2 H, qd, CHCH_2COO−), 1.54–1.58 (6 H, dd, CH_3C); HR-MS data not available.

Bis[2-(6-methoxy-2-naphthyl)propanoyloxybutyl] L-aspartate (3k)

Prepared by the same procedure as described for the synthesis of **3j**. The HCl salt was converted to free amine, which was purified with flash chromatography using 50 g of silica gel (1:20 methanol/EtOAc) giving a yellow oil in 28% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.69–7.10 (12 H, m, aromatic), 4.01–4.10 (4 H, m, OCH_2CH_2), 3.90 (6 H, s, CH_3O), 3.84 (2 H, q, C*H*Me), 3.61 (1 H, q, C*H*NH_2), 2.65 (2 H, qd, CHCH_2COO−), 1.57–1.68 (4 H, m, OCH_2CH_2), 1.57 (6 H, dd, CH_3C); HR-MS data not available.

Bis[2-(6-methoxy-2-naphthyl)propanoyloxyethyl] L-glutamate (3l)

Purified by the same procedure as described for the 3k giving free amine as an oil in 41% yield; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71–7.11 (12 H, m, aromatic), 4.63–4.20 (8 H, m, OCH_2CH_2O), 3.91 (6 H, s, CH_3O), 3.87 (2 H, q, C*H*Me), 3.75 (1 H, bs, C*H*NH_2), 2.13 (2 H, m, CH_2CH_2COO), 1.95–1.85 (2 H, m, CHCH_2CH_2COO), 1.59/1.58 (6 H, dd, CH_3C); HR-MS data not available.

Bis[2-(6-methoxy-2-naphthyl)propanoyloxybutyl] L-glutamate (3m).

Purified by the same procedure as described for the **3k** giving free amine as an oil in 48% yield; ^{1}H NMR($CDCl_3$, 400 MHz) δ 7.70–7.12 (12 H, m, aromatic), 4.12–3.99 (8 H, m, OCH_2CH_2), 3.91 (6 H, s, CH_3O), 3.84 (2 H, q, C*H*Me), 3.55 (1 *H*, t, $CHNH_2$), 2.40–2.00 (2 H, m, $CHCH_2CH_2$COO), 2.38 (2 H, m, CH_2CH_2COO), 1.69–1.57 (8 H, m, OCH_2CH_2), 1.57 (6 H, dd, CH_3C); HR-MS data not available.

Aqueous Solubility

The solubility of naproxen and its aminoacyloxyalkyl prodrugs (**3a-i**) was determined in phosphate buffer (0.16 M) at pH 5.0 and 7.4 at room temperature. The aqueous solubility of **3j-m** was not determined. Excess amounts of each compound were added to 4 mL of buffer, the mixtures were vortexed either for 60 min (pH 5.0) or for 10 min (pH 7.4), filtered (Millipore 0.45 μm), and analyzed by the HPLC method described earlier (method 1). The pH of the mixtures was checked while vortexing and adjusted if necessary.

Apparent Partition Coefficients

The apparent partition coefficients (P_{app}) of naproxen and its aminoacyloxyalkyl prodrugs (**3a-i**) were determined at room temperature in a 1-octanol-phosphate buffer system at pH 5.0 and 7.4. The apparent partition coefficients of **3j-m** were not determined. A known concentration of prodrug in phosphate buffer was shaken either for 30 min (pH 5.0) or 5 min (pH 7.4), with a suitable volume of the 1-octanol. After shaking, the phases were separated by centrifugation at 14,000 rpm for 3 min. The concentrations of the compounds in the buffer phase before and after partitioning was determined by the HPLC analytical method described earlier (method 1).

Hydrolysis in Aqueous Solution

The rates of chemical hydrolysis of aminoacyloxyalkyl prodrug derivatives (**3a-i**) were studied in aqueous phosphate buffer solution of pH 7.4 and pH 5.0 (0.16 M, ionic strength 0.5) at 37°C. The naproxenoxyalkyl diesters of glutamic and aspartic acids (**3j-m**) were tested only in buffer solution of pH 5.0. Solutions of prodrugs were preparated by dissolving an appropriate amount of compound in 10 mL the preheated buffer. After vortexing the solutions for 5 min, the solutions were placed in a thermostatically controlled water bath at 37°C. At appropriate intervals, samples were taken and analyzed for remaining prodrug by the HPLC method described earlier (method 1).

Hydrolysis in Human Serum

The rates of enzymatic hydrolysis for naproxen prodrugs (**3a-m**) were studied in human serum (Institute of Public Health, University of Kuopio) diluted to 80% with 0.16 M phosphate buffer of pH 7.4 at 37°C. The reactions were initiated by dissolving an appropriate amount of prodrug in phosphate buffer, and prewarmed human serum was added. The solutions were kept in a water bath at 37°C, and at suitable intervals 0.5 mL samples of serum/buffer mixture were withdrawn and added to 1.0 mL of ethanol in order to precipitate protein from the serum. After immediate mixing and centrifugation for 10 min at 14,000 rpm, the resulting clear supernatant was analyzed for remaining prodrug and released naproxen by the HPLC method described earlier (method 1). Pseudo-first-order half-times ($t_{1/2}$) for the hydrolysis of prodrugs were calculated from the linear slopes of plots of the logarithm of remaining prodrugs against time. The pseudo-first-order times, at which 50% of total parent compound had been formed ($f_{50\%}$), were determined from the linear slopes of the logarithm of unformed parent compound (log (parent $compound_{max}$-parent $compound_t$)) over time (13).

Table I. Apparent Partition Coefficient (log P_{app}, mean ± SD; n = 2–3) and Aqueous Solubility (mean ± SD; n = 2–4) of Naproxen and Its Various Aminoacyloxyalkyl Prodrugs

	log P_{app}[a]		Aqueous solubility (mM)	
Compound	pH 7.4	pH 5.0	pH 7.4	pH 5.0
Naproxen	0.30 ± 0.03	2.38 ± 0.02	101.9 ± 1.3	0.40 ± 0.04
3a	2.80 ± 0.04	0.67 ± 0.01	4.73 ± 1.06	10.38 ± 0.75
3b	3.30 ± 0.07	2.16 ± 0.02	0.15 ± 0.01	0.31 ± 0.04
3c	3.37 ± 0.01	2.13 ± 0.02	0.15 ± 0.01	1.69 ± 0.32
3d	2.93 ± 0.01	2.30 ± 0.00	0.03 ± 0.00	0.18 ± 0.01
3e	2.25 ± 0.05	0.99 ± 0.04	3.24 ± 0.05	5.87 ± 1.04
3f	2.56 ± 0.07	2.72 ± 0.04	0.03 ± 0.00	1.07 ± 0.02
3g	2.90 ± 0.10	2.91 ± 0.06	0.004 ± 0.001	0.10 ± 0.04
3h	—[b]	1.37 ± 0.00	—[b]	3.66 ± 0.01
3i	—[b]	3.04 ± 0.00	—[b]	0.32 ± 0.02

[a] P_{app} is an apparent partition coefficient between 1-octanol and phosphate buffer (pH 7.4 and 5.0) at room temperature.
[b] Values were not determined due to poor aqueous stability of prodrugs.

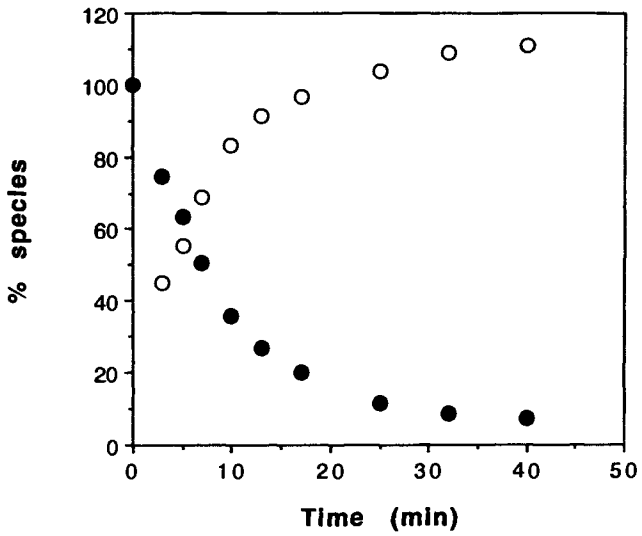


Fig. 1. Time courses for naproxen 2-(L-isoleucyloxy)ethyl ester (3c)(●) and naproxen (○) during hydrolysis of the prodrug in 80% human serum (pH 7.4) at 37°C.

In Vitro Skin Permeation

The permeation studies were carried out with the previously described method (11). Samples of postmortem human skin were used as the model membranes in Franz-type diffusion cell (PermeGear, Inc., Riegel, PA, USA). The dermal side of the skin was exposed to the receptor medium (0.05 M isotonic phosphate buffer solution of pH 5.0) which was stirred magnetically and kept at a constant temperature of 37°C with a water bath throughout the study. The selected compounds (50 mM) were applied as solutions or suspensions in 0.05 M phosphate buffer of pH 5.0. At appropriate time intervals samples were taken from the receptor phase and replaced with fresh buffer. The drug concentrations were assayed by HPLC as described earlier (method 2). The steady-state fluxes for naproxen and its prodrugs **3a**, **3b**, **3c**, **3e**, **3f** and **3k** were determined by plotting the cumulative amounts (in nmol) of the parent drugs, intermediates and intact prodrugs that were measured in the receptor phase against time, and then dividing the slopes of the steady-state positions by the surface area of the diffusion cell (0.71 cm^2). A one-factor analysis of variance (ANOVA factorial) was used to test the statistical significance of differences between naproxen and prodrugs. Significance in the differences in the means was tested using Fisher's protected least significance difference (PLSD) at the 95% confidence.

Table II. Rate of Hydrolysis of Naproxen Aminoacyloxyalkyl Prodrugs **3a-i** and Naproxenoxyalkyl Diesters of Glutamic and Aspartic Acids **3j-m** in Buffered Solutions (pH 7.4 and 5.0), and in 80% Human Serum (pH 7.4) at 37°C

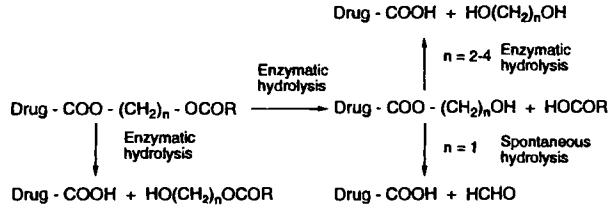
Compound	$t_{1/2}$ (h) phosphate buffer pH 7.4	$t_{1/2}$ (d) phosphate buffer pH 5.0	$t_{1/2}$ (min) 80% human serum	$f_{50\%}$ (min)[a] 80% human serum
3a	2.6	9.7	19	26
3b	3.2	3.5	10	10
3c	13	27	8	10
3d	3.7	2.2	9	9
3e	4.2	4.8	13	16
3f	81	148	5	6
3g	23	3.0	13	9
3h	0.5	0.2	4	13
3i	0.9	0.3	9	9
3j	—[b]	0.9	116	114
3k	—[b]	1.7	47	44
3l	—[b]	0.5	32	67
3m	—[b]	—[b]	46	37

[a] $f_{50\%}$ is the time by which 50% of total naproxen is formed.
[b] Not determined.

RESULTS AND DISCUSSION

Aqueous Solubility and Apparent Partition Coefficient

The aqueous solubility and the apparent partition coefficients (log P_{app}) of **3a-i** at pH 7.4 and pH 5.0 are shown in Table I. In contrast to naproxen, the prodrugs are more soluble in acidic than in neutral aqueous solutions, due to the ionizable primary amine in the promoiety. Therefore, all the prodrugs possessed lower aqueous solubility, compared to naproxen at pH 7.4, and they are clearly more lipophilic than naproxen as illustrated by the log P_{app} values. At pH 5.0 most prodrugs showed an increase in aqueous solubility compared to naproxen and maintained a lipophilicity comparable to naproxen. Previous studies have suggested a balance between the lipid and water solubilities of drug are needed for enhanced dermal drug delivery, due to biphasic nature of the skin (14–16).



Scheme 3.

Hydrolysis in Aqueous Solutions

The chemical degradation of each naproxen prodrug (**3a-m**) followed pseudo-first-order kinetics at pH 5.0 and 7.4 (**3j-m** were not determined at pH 7.4) and the degradation half-lives of the prodrugs are shown in Table II. The chemical stability of aminoacyloxyalkyl prodrugs (**3a-m**) was substantially greater at pH 5.0 than at pH 7.4. Prodrugs having an isoleucine promoiety (**3c** and **3f**) were most stable towards chemical degradation, most probably due to increased branching of the acyl portion. As in the examples of **3c** and **3f**, or **3d** and **3g**, an increase in alkyl chain length between ester groups increased the chemical stability of the prodrugs. Branching and/or decreasing of the carbon chain between ester groups (**3h** and **3i**) resulted in the most chemically labile prodrugs. The prodrugs **3j-l** were all very unstable, even at pH 5.0.

Enzymatic Hydrolysis

Human serum or plasma is commonly used as a medium to determine the hydrolysis of ester prodrugs for dermal delivery (11,17,18). In the present study, the rates of enzymatic hydrolysis of the naproxen prodrugs (**3a-m**) were determined in 80% human serum (pH 7.4) at 37°C to predict the susceptibility of prodrugs to undergo bioconversion by esterases in the skin. The hydrolysis of each prodrug derivative (**3a-m**) followed pseudo-first-order kinetics, and the prodrugs hydrolyzed quantitatively to naproxen (Fig. 1). The half-lives ($t_{1/2}$) of prodrugs **3a-i** ranged from 4 to 19 min, and half-lives for formation of naproxen ($f_{50\%}$) ranged from 6 to 26 min (Table II). The prodrugs **3j-m** were more stable against enzymatic hydrolysis, having half-lives ranging from 37 to 114 min.

Acyloxymethyl derivatives has been reported as double prodrugs because of their two-step cleavage mechanism (Scheme III) (19,20). The first step, rate determining one, is enzymatic hydrolysis of the terminal ester group with the formation of an unstable hydroxymethyl ester, which spontaneously dissociates to the parent drug. As reported earlier (11), naproxen hydroxyethyl, -propyl and -butyl esters (**2a-c**), are stable in a buffered solution but hydrolyze to naproxen in human serum. However, a similar order of magnitude of $t_{1/2}$ and $f_{50\%}$ values indicate the formation of naproxen takes place at the same rate as the loss of the prodrugs in human serum. Therefore, the hydrolysis of prodrugs **3a-i** may primarily occur enzymatically by attack on the carbonyl of the parent drug rather than the carbonyl of the promoiety (Scheme III).

In Vitro Skin Permeation

Excised postmortem human skin was used to assess skin permeabilities of naproxen and its representative aminoacyloxyalkyl prodrugs **3a-c**, **3e** and **3f**. The naproxenoxyalkyl diester of aspartic acid (**3k**) was also tested. Isotonic phosphate buffer (0.05 M, pH 5.0) in the receiving compartment was used, since prodrugs show better chemical stability at pH 5.0 than at pH 7.4. Suspensions of prodrugs in phosphate buffer were applied in order to keep a constant driving force for diffusion and to provide the maximum flux attainable.

Representative plots of the cumulative amounts (in nmol of naproxen) of total naproxen, intermediate or intact prodrug, through human skin from phosphate buffer (pH 5.0), divided by the surface area of the diffusion cell and time are shown in Fig. 2. The steady-state fluxes (J_{ss}) obtained from the slopes of the linear portions of these plots are given in Table III.

The diffusion experiments showed the skin permeation of naproxen can be significantly improved via aminoacyloxyalkyl prodrugs. The prodrugs **3a** and **3c** resulted in a 3-fold higher flux when compared to naproxen itself at pH 5.0. Moreover, it is important to point out that naproxen showed an 8-fold greater flux across the skin at pH 5.0 (1.8 ± 0.1 nmol/cm^2 · h) than at pH 7.4 (0.23 ± 0.03 nmol/cm^2 · h). The increased flux is due to decreased ionization and an increase in the partition coefficient of naproxen at pH 5.0, compared to pH 7.4. Comparisons of fluxes and solubilities verify the most permeable prodrugs are ones that combine an adequate aqueous solubility

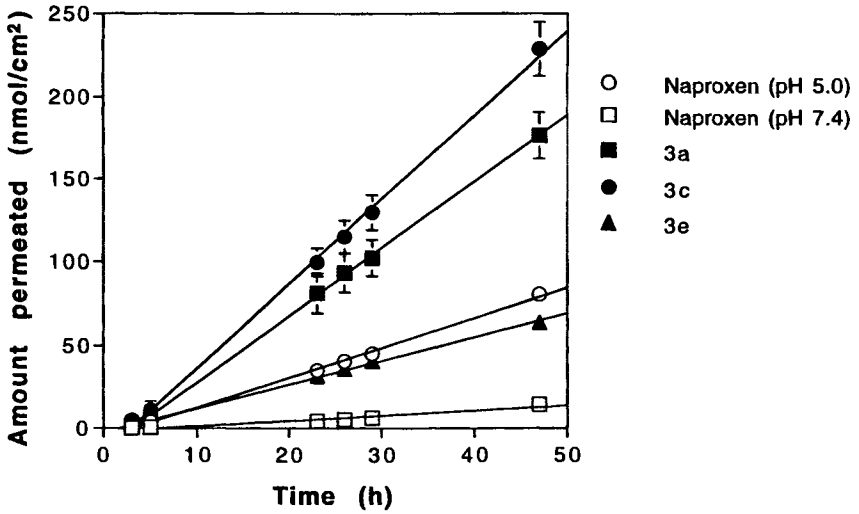


Fig. 2. Permeation profiles (mean ± SE, n = 3–10) for naproxen (○) and prodrugs **3a** (■), **3c** (●), and **3e** (▲) through postmortem human skin from 0.05 M phosphate buffer (pH 5.0) vehicle and for naproxen (□) from a buffered solution at pH 7.4. The data represent the sum of naproxen, the intermediate and its prodrug in the case of prodrug application.

Table III. Steady-State Fluxes (mean ± SE, n = 3–10) for Delivery of Total Naproxen Species Through Excised Human Skin In Vitro from Isotonic Phosphate Buffer (0.05 M, pH 5.0) at 37°C

Compound	Flux (nmol/cm^2·h)
Naproxen[a]	1.8 ± 0.1
3a	4.0 ± 0.4[b]
3b	1.6 ± 0.0
3c	5.1 ± 0.4[b]
3e	1.5 ± 0.1
3f	1.8 ± 0.3
3k	0.05 ± 0.01[b]

[a] The steady-state flux for naproxen from isotonic phosphate buffer of pH 7.4 (0.05 M) was 0.23 ± 0.03 nmol/cm^2·h.
[b] Significantly different from the flux value for naproxen ($p < 0.05$ by ANOVA, Fisher's PLSD test).

and lipophilicity over the parent drug. Prodrug **3k** resulted in a much lower flux than naproxen which is most probably due to a high partition coefficient, that is associated with a very poor aqueous solubility (data not shown).

In conclusion, the present study shows the permeation of naproxen through human skin can be improved by using aminoacyloxyalkyl prodrugs of naproxen. The ionizable prodrugs combine the desirable properties of water solubilities and lipophilicities for skin permeation.

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