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Soft β-adrenergic agonists for the topical treatment of psoriasis†

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Summary — The soft-drug **1** (R = Me, Et) and pro-soft-drug **3** have been prepared as models of topical anti-psoriatic β-adrenergic agonists. The chemical hydrolysis of **3** proceeded via the acid **18** with a maximum stability at apparent pH ~ 4.0. In the presence of PLCE, the required metabolism of **3** to the soft-drug **1** (R = Et) was achieved, which slowly degraded to the dihydroxy acid **2**. Soft-drug **1** (R = Et) was poorly transported across a silicone membrane, whereas the pro-soft-drug **3** was more efficient and the rate increased over the donor apparent pH range 3–8. Soft-drug **1** (R = Et) was a full β-agonist on the guinea-pig tracheal preparation, whereas the pro-soft-drug **3** produced only slowly developing responses at high concentrations (>10 μM).

pro-drug / soft-drug / transport / psoriasis / β-adrenergic agonist

Introduction

Psoriasis [1] is characterised by epidermal proliferation and inflammation resulting in the appearance of elevated erythematous plaques. Available treatments are ameliorative rather than curative; many are only moderately effective or are associated with undesirable levels of toxicity, and no ideal regimen exists. With topical treatments, problems due to percutaneous absorption, particularly following long-term treatment, limit the dose of the drug that may be safely applied to the skin. These deficiencies warrant the development of more effective topical antipsoriatic drugs.

A defect in the cyclic nucleotide system in the epidermis, reflected by a decrease in the c-AMP/c-GMP ratio, has been proposed to play a central role in the pathogenesis of psoriasis [2, 3]. This decreased ratio is thought to be attributable to both a decrease in the rate of c-AMP formation (from intracellular ATP catalysed by adenylate cyclase) and an increased rate of degradation of c-AMP (to AMP, catalysed by cyclic nucleotide phosphodiesterase (c-AMP-PDE)) in psoriatic skin when compared with normal skin [4]. Approaches to increase the c-AMP/c-GMP ratio may have a role in the treatment of psoriasis. This could be achieved by administration of c-AMP [5] or its lipophilic dibutyl prodrug derivative [6], but both are short-lived and quickly hydrolyse to AMP. Alternatively, the metabolism of c-AMP could be modified either by the stimulation of adenylate cyclase or by the inhibition of c-AMP-PDE [7]. When the epidermal cell surface receptors are stimulated by β-adrenoceptor agonists [8] (for example, isoprenaline [9] or salbutamol [10, 11]), adenylate cyclase is stimulated [10] which increases the c-AMP/c-GMP ratio [5, 12]. A topical application of 0.1% isoprenaline sulphate has been reported to decrease scaliness and cause remission of psoriasis [9]. In contrast, β-adrenoceptor antagonists, for example, propranolol, produces psoriasiform-lesions in guinea pigs [13], and in latent psoriasis, they greatly increase epidermal proliferation [14]. Inhibitors of c-AMP-PDE, such as papaverine and Ro 20-1724, have also been shown to be beneficial in psoriasis [15].

For a β-adrenoceptor agonist to be used for the treatment of psoriasis, it is a pre-requisite that activity be restricted to the percutaneous layers of the skin, and that no untoward cardiovascular effects occur as a consequence of systemic absorption [16]. As the drug enters the blood stream, it needs to be metabolised into an inactive moiety. β-Adrenoceptor activity can be terminated either by loss of the basic center at nitrogen (for example, the *O*-acyl prodrugs of β-adrenoceptor antagonists [17] may undergo an

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O-to-N acyl rearrangement to give inactive amide derivatives) or by a dramatic polarity change in the *N*-substitution. Here, the second approach has been evaluated and the ester soft-drugs **1** (R = Me, Et) have been synthesised for topical delivery [18]. The ester groups of **1** (R = Me, Et) should undergo hydrolysis, catalysed by esterases in the blood, to give the inactive carboxylate metabolite **2**, thereby minimising cardiovascular side-effects.

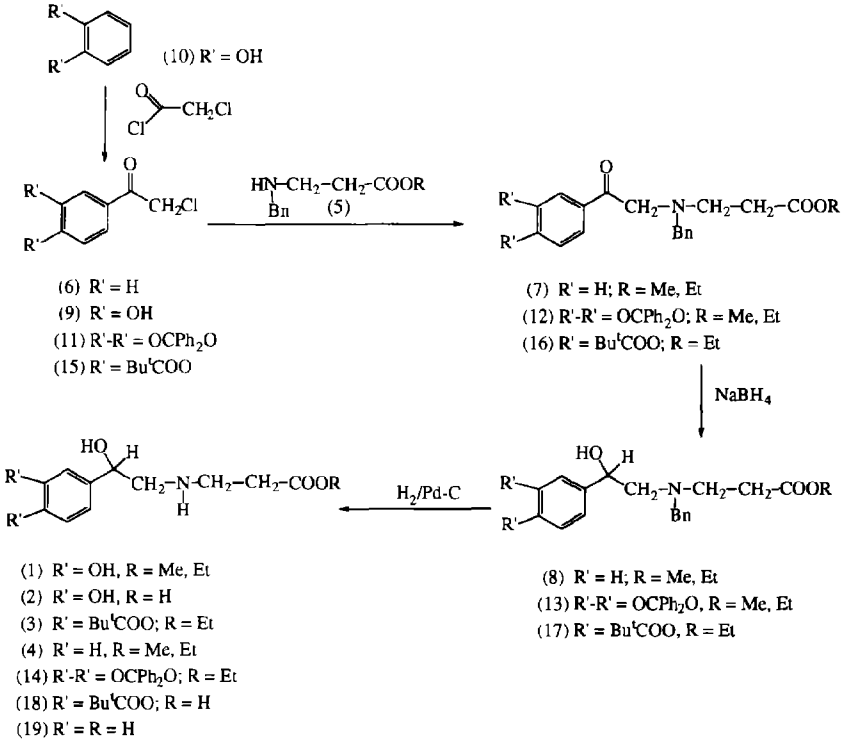
However, soft-drug **1** (R = Et) is hydrophilic and its usefulness may be limited by both poor bioavailability and the susceptibility of the catechol hydroxy groups to undergo oxidation and metabolism. A pro-soft-drug [19, 20] was therefore considered. For example, the lipophilic dipivaloyl prodrug, dipivefrin, has led to improved ocular delivery of epinephrine and decreased cardiac side-effects [21–23]. Here, the lipophilic dipivaloyl pro-soft-drug **3** has been synthesised. After penetrating the epidermal barrier, this pro-soft-drug is required to undergo a controlled in vivo bioactivation catalysed by esterases in the skin to give the active soft-drug **1** (R = Et). After eliciting a pharmacological response, soft-drug **1** (R = Et) needs to be metabolised to the inactive dihydroxy acid **2**. Prior to incorporation of the catechol group, which is important for β-adrenergic activity, the model phenyl analogues **4** were evaluated.

Chemistry

Synthesis

The general route for the preparation of **1** (R = Me, Et), **3** and **4** (R = Me, Et) is shown in scheme 1. First, the preparation of the phenyl analogues **4** (R = Me, Et) is discussed. Alkyl *N*-benzyl-3-aminopropionates **5** were prepared from the reaction of methyl and ethyl 3-bromopropionate with benzylamine at 40 °C. Treatment of **5** (R = Me, Et) with α-chloroacetophenone (**6**) in the presence of sodium iodide and triethylamine afforded alkyl *N*-(benzoylmethyl)-*N*-benzyl-3-aminopropionates **7** (R = Me, Et). These compounds were unstable. However, the keto group of **7** can readily be reduced in situ with sodium borohydride to yield the corresponding alcohols **8** (R = Me, Et) which were more stable. The *N*-benzyl group of **8** (R = Me, Et) was then removed by hydrogenation catalysed by 10% Pd-C to give the alkyl *N*-(2'-hydroxy-2'-phenylethyl)-3-aminopropionates **4** (R = Et, Me).

The synthesis of the soft-drugs **1** (R = Me, Et) first required the protection of the catechol ring, and the diphenylmethylene acetal group was employed. 3,4-Dihydroxy-α-chloroacetophenone **9**, prepared by the Friedel–Crafts acylation reaction of catechol **10** with chloroacetyl chloride [24], was heated with dichlorodiphenylmethane at 170–180 °C to give **11**. This was



Scheme 1. The general route for the preparation of **1** (R = Me, Et), **3** and **4** (R = Me, Et).

then coupled with **5** (R = Me, Et) to give **12** (R = Me, Et), the keto groups of which were reduced with sodium borohydride to give the corresponding alcohols **13** (R= Me, Et). In the presence of one atmosphere of hydrogen with 10% Pd-C, compounds **13** (R= Me, Et) in methanol or ethanol respectively, lost only the *N*-benzyl group to give alkyl *N*-[2'-(3",4"-diphenylmethylenedioxyphenyl)-2'-hydroxyethyl]-3-aminopropionates **14** (R = Me, Et). However, when the hydrogen pressure was increased to 60 psi, after 18 h both the diphenylmethylene and *N*-benzyl groups were removed from **13** (R = Et) to give compound **1** (R = Et). The catechol product was very sensitive to oxygen, but the rate of oxidation could be decreased by preparation of the hydrochloride salt.

The synthesis of the dipivaloyl pro-soft-drug **3** was analogous to that described above, except that 3,4-dihydroxy-α-chloroacetophenone **9** was treated with two equivalents of pivaloyl chloride in the presence of triethylamine to give 3,4-dipivaloyloxy-α-chloroacetophenone **15**. Compound **15** was then coupled with ethyl *N*-benzyl-3-aminopropionate (**5**, R = Et) to give **16** in a yield of 70%, which was then reduced to the corresponding alcohol **17** with sodium borohydride. The *N*-benzyl group of **17** was removed by hydrogenation at one atmosphere catalysed by 10% Pd-C to give the pro-soft-drug **3**.

Chemical and esterase-catalysed hydrolyses

Reversed-phase HPLC methods for the assay of soft-drug **1** (R = Et), pro-soft-drug **3**, phenyl analogue **4** (R = Et) and their hydrolysis products are summarised in table I. Despite manipulation of the mobile phase with ion-pairing agents, the pro-soft-drug **3** and its hydrolysis products, dipivaloyl acid (**18**), soft-drug **1** (R = Et) and dihydroxy acid **2** could not be monitored simultaneously due to large differences in their polarities. Therefore, a two-stage analysis by HPLC was employed for these compounds. The mobile phase consisting of 30% acetonitrile in water with 0.1% v/v tetrabutylammonium hydroxide at apparent pH 3.0 was used to separate pro-soft-drug **3** (t_R 9.4 min) and dipivaloyl acid **18** (t_R 5.4 min) from **1** (R = Et) and **2**. A mobile phase comprising 4% acetonitrile in water with 0.1% v/v diethylamine at apparent pH 3.0 was then used to separate **1** (R = Et) from dihydroxy acid **2**.

Table I. HPLC solvent systems and retention times for **1** (R = Et), **3** and **4** (R = Et) and their hydrolysis products using a Hypersil-ODS reversed-phase column.

Mobile phase, apparent pH 3.0	*Compound*	*Retention time (t_R) (min)*
4% CH_3CN in water with 0.1% diethylamine	**1** (R = Et)	8.8
	2	2.4
30% CH_3CN in water with 0.1% tetrabutylammonium hydroxide	**3**	9.4
	18	5.4
15% CH_3CN in water with 0.1% diethylamine	**4** (R = Et)	8.8
	19	1.6

The hydrolyses of **1** (R = Et) and **4** (R = Et) can only proceed by cleavage of the ethyl ester. In contrast, the hydrolysis of **3** can occur either at the ethyl or pivaloyl esters. By HPLC, compound **3** had a retention time of 9.4 min and the first formed product had a retention time of 5.4 min, the identity of which was investigated. A solution of **3** in phosphate/citrate buffer containing 10% acetonitrile (apparent pH 8.0) at 50 °C was monitored by HPLC. After 80% reaction, the solution was extracted successively with hexane, diethyl ether, chloroform and ethyl acetate. By HPLC and ^{1}H-NMR spectroscopy, the hexane extract showed the presence of only **3**, whereas the chloroform extract contained the majority of the first formed product. The ^{1}H-NMR spectrum of the chloroform extract showed the absence of an ethyl group and the presence of a pivaloyl ester, consistent with the formation of the dipivaloyl acid **18**. Further evidence was sought by monitoring the hydrolysis of **3** (peaks included δ_H 1.15 (3H, t, J_{HH} = 7.15 Hz) and 4.05 (2H, q, J_{HH} = 7.1 Hz) for the ethyl, and δ_H 1.25 (9H, s) and 1.26 (9H, s) for the pivaloyl esters) by ^{1}H-NMR spectroscopy in deuterated potassium phosphate buffer (apparent pD 8.57) containing 20% acetonitrile at 37 °C. The hydrolysis proceeded with the formation of ethanol (δ_H 1.08 (3H, t, J_{HH} = 7.1 Hz) and 3.54 (2H, q, J_{HH} = 7.1 Hz)), and the dipivaloyl acid **18** (peaks included δ_H 1.26 (9H, s) and 1.27 (9H, s) for the pivaloyl esters). The dipivaloyl acid **18** showed slow hydrolysis of either the 3- or 4-pivaloyl groups to give the monopivaloyl compounds, with the formation of potassium pivaloate (δ_H 1.01 (s, 9H)). A small amount (< 5%) of the aminochrome oxidation product (δ_H 5.45–5.60 (m) and 6.00–6.40 (m)) was also observed [25].

Similarly, the hydrolysis of the soft-drug **1** (R = Et) (δ 1.14 (t, J_{HH} = 7.15 Hz, 3H) and 4.07 (q, J_{HH} = 7.15 Hz, 2H) for the ethyl ester) to the dihydroxy acid **2** was monitored by ^{1}H-NMR spectroscopy at 37 °C by the formation of ethanol (δ_H 1.08 (t, J_{HH} = 7.1 Hz, 3H) and 3.54 (q, J_{HH} = 7.1 Hz, 2H)) with a rate constant of 5.0648 x 10^{-2} h^{-1} (half-life 13.7 h).

The chemical hydrolyses of the hydrochloride salt of **1** (R = Et), the pro-soft-drug **3** and the phenyl analogue **4** (R = Et) in phosphate/citrate buffer containing 10% acetonitrile were monitored by HPLC over the apparent pH range 2.0 to 10.0 at 50 °C. At

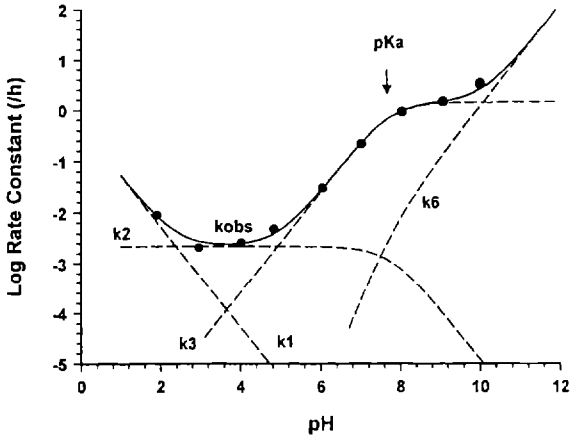


Fig 1. pH-rate profile for the hydrolysis of soft-drug **1** (R = Et). The line represents the theoretical pH rate-profile calculated from equation [1]. The contributions of the component rate constants, k_1, k_2, k_3 and k_6 are given by the dashed lines.

each apparent pH and for each compound, first order kinetics was observed ($r^2 \gg 0.97$). The apparent pH rate profile for **1** (R = Et) is shown in figure 1, and similar plots were obtained for compounds **3** and **4** (R = Et). Each showed a U-shaped profile at moderate apparent pH values but a discontinuity, typical of the involvement of an ionisation process, at higher values. Compounds **1** (R = Et), **3** and **4** (R = Et) were most stable at apparent pH values of 3.7, 4.0 and 4.1, with half-lives of 295, 560 and 438 h, respectively.

The PLCE-catalysed hydrolysis of the hydrochloride salt of soft-drug **1** (R = Et) followed a first-order model. The half-lives of **1** (R = Et) (164.0 μM) in phosphate/citrate buffer (10 mL) at pH 7.4 and 37 °C without enzyme and with 31 and 253 U of PLCE were 5.28, 4.84 and 0.59 h respectively.

The hydrolyses of the hydrochloride salt of pro-soft-drug **3** (80.0 μM, 10 mL) at pH 7.4 at 37 °C were monitored by HPLC in the presence of 0, 5.06, 6.29 and 12.58 U of PLCE. The hydrolyses were first-order and the half-lives were 19.31, 0.275, 0.14 and 0.071 h ($r^2 > 0.99$), respectively.

Pharmacology

Permeation studies

Initial permeation experiments of the soft-drug **1** (R = Et) through a Silastic membrane mounted in Franz cells were performed with 10% propylene glycol in water at apparent pH 6.5 in the donor compartment and at apparent pH 3.0 in the receiving compartment. The transport of **1** (R = Et) was linear up to 3.0 h with a flux (J) of 2.13 x 10^{-3} μmol/cm²/h, after which time the rate of permeation decreased. In contrast, **1** (R = Et) was not transported when the apparent pH of the donor compartment was 3.0 because the protonated soft-drug had little affinity for the membrane. In these experiments, the solution in the donor compartment turned dark which is attributable to oxidation of the catechol ring. Further, the soft-drug **1** (R = Et) is hydrophilic, a factor which will limit the rate of transport through the Silastic membrane. Attention was then focused on the transport of pro-soft-drug **3**, which is more lipophilic and much more stable to oxidation than the soft-drug **1** (R = Et).

Permeation results of **3** with the donor compartment at apparent pH 7.0 and a receiver compartment containing 10% propylene glycol in water (apparent pH 6.5) showed an initial lag phase followed by non-linear transport with a steadily falling rate. The drug in the receiving fluid slowly darkened due to slow hydrolysis of the pivaloyl esters followed by oxidation of the catechol ring. The pro-soft-drug **3** was not stable at apparent pH 6.5, therefore the apparent pH of the receiving fluid was decreased to 3.0, where the compound showed good stability. The permeation studies of **3** (10.55 μM) at apparent pH values of 3.0–8.0 (buffered 10% propylene glycol in water) in the donor compartment and at apparent pH 3.0 in the receiving compartment (10% propylene glycol in water) showed that steady-state fluxes were obtained. The cumulative transport and fluxes of **3** at different apparent pH values are shown in figure 2 and the mean flux (J), permeability coefficient (K_p) and lag time (t_L) are summarised in table II.

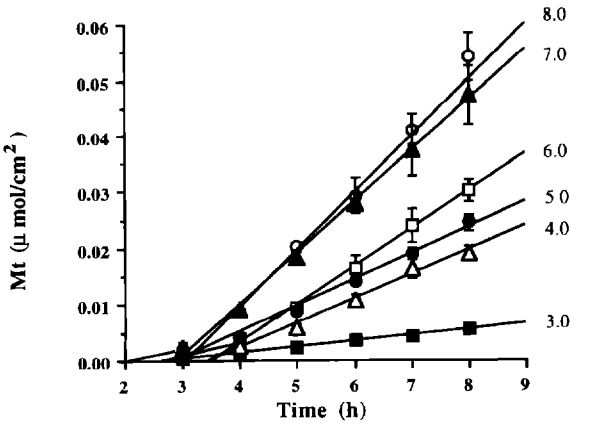


Fig 2. Transport of **3** from 10% aqueous propylene glycol at various pH values (buffered pH 3.0–8.0) through Silastic membrane.

Table II. Silastic membrane transport data of **3** at buffered apparent pH 3.0–8.0 in 10% propylene glycol in water in the donor compartment.

pH	*Percentage free base*	*Flux (J) (μmol/cm²/h) (± sd)*	*Permeability coefficient (K_p) (cm/h) (± sd)*	*Lag time (t_L) (h) (± sd)*
3.0	0.004	1.007×10^{-3} ($\pm 3.83 \times 10^{-4}$)	9.542×10^{-5} ($\pm 3.63 \times 10^{-5}$)	2.56 (± 0.20)
4.0	0.04	4.317×10^{-3} ($\pm 4.07 \times 10^{-4}$)	4.092×10^{-4} ($\pm 3.86 \times 10^{-5}$)	3.14 (± 0.05)
5.0	0.37	5.437×10^{-3} ($\pm 4.76 \times 10^{-5}$)	5.154×10^{-4} ($\pm 4.51 \times 10^{-6}$)	1.80 (± 0.2)
6.0	3.6	6.740×10^{-3} ($\pm 4.53 \times 10^{-4}$)	6.389×10^{-4} ($\pm 4.30 \times 10^{-5}$)	3.18 (± 0.03)
7.0	27.2	9.108×10^{-3} ($\pm 1.11 \times 10^{-3}$)	8.633×10^{-4} ($\pm 1.05 \times 10^{-4}$)	2.92 (± 0.19)
8.0	78.9	1.057×10^{-2} ($\pm 9.72 \times 10^{-4}$)	1.002×10^{-3} ($\pm 9.20 \times 10^{-5}$)	3.08 (± 0.07)

The flux of drug across the membrane (J) is the rate of transport (dM/dt) per unit area of membrane and is given by $J = (dM/dt)/A = DPC_d/h$ where D denotes the diffusion coefficient of the drug in the membrane, of thickness h, P is the partition coefficient between donor and membrane, and C_d is the concentration of drug in the donor solution. The permeability coefficient is $K_p = DP/h = J/C_d$. The lag time is the time taken for steady-state transport to be reached and depends upon diffusion coefficient and membrane thickness such that $t_L = h^2/6D$.

*Effect of soft-drug **1** (R = Et), pro-soft-drug **3** and dihydroxy acid **2** on β-adrenoceptors*

Figure 3 shows the effect of the soft-drug **1** (R = Et), isoprenaline and the dihydroxy acid **2** on pre-contracted guinea-pig trachea. The soft-drug was a full agonist and produced a maximum response (1.34 ± 0.22 g, $n = 6$) that was not significantly different from that produced by isoprenaline (1.33 ± 0.01 g, $n = 6$). The soft-drug **1** was marginally less potent (ED_{50} 4.07 (± 0.49) x 10^{-7} M, $n = 6$) compared with isoprenaline (ED_{50} 2.68 (± 0.27) x 10^{-7} M, $n = 6$), $P < 0.05$, but the dihydroxy acid was markedly less potent than isoprenaline (ED_{50} 2.95 (± 0.9) x 10^{-5} M, $n = 6$), $P < 0.01$. The pro-soft-drug **3** had little activity on the pre-contracted guinea-pig trachea except at high concentrations (> 10 μM) when it produced a very slowly developing response.

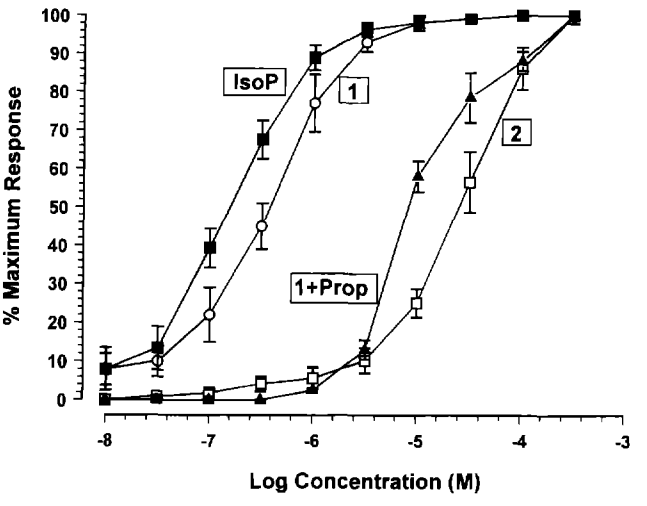


Fig 3. Dose–response curves for the soft-drug (**O**; **1**, R = Et) and dihydroxy acid (❑ ; **2**) compared to the agonist isoprenaline (■ ; **IsoP**) and to the effect of the antagonist propranolol [▲ ; **1 + Prop**] on the soft-drug. (ED_{50} values are quoted in the text.)

Results and discussion

For chemical hydrolysis at 50 °C, compounds **1** (R = Et), **3** and **4** (R = Et) were most stable at apparent pH

values of 3.7, 4.0 and 4.1, with half-lives of 295, 560 and 438 h, respectively. The overall degradation rate (k_{obs}) for the hydrolysis of an ester which possesses a basic group is given by [26]:

$$k_{obs} = \alpha(k_1[H_3O^+] + k_2[H_2O] + k_3[HO^-]) + (1 - \alpha)(k_4[H_3O^+] + k_5[H_2O] + k_6[HO^-]) \quad [1]$$

where k_1, k_2 and k_3 are the second order rate constants for the individual reactions of the protonated base involving proton, solvent and hydroxide catalysis respectively, and k_4, k_5 and k_6 are the corresponding constants for the reactions involving the free base. The fraction protonated (α) is dependent upon the pK_a of the base and pH of the solution:

$$\alpha = \frac{[H_3O^+]}{K_a + [H_3O^+]}$$

To obtain the individual rate constants for the hydrolysis of **1** (R = Et), **3** and **4** (R = Et), the kinetic data were fitted to equation [1] by non-linear regression using programs NONREG [27] and FIGP (Biosoft Ltd). Preliminary analysis showed that the coefficients corresponding to proton- (k_4) and solvent- (k_5) catalysed hydrolysis of the free-base were insignificant contributors to the overall degradation. These parameters were eliminated from the model ($k_4 = k_5 = 0$) and the calculations were repeated. Iteration was rapid and yielded the rate constants and pK_a values recorded in table III. Small differences are apparent between the corresponding rate constants for each compound. However, the overall trends are comparable and demonstrate the relative importance of the individual components of the reaction. Degradation at acidic pH mainly involves the protonated base undergoing proton- (k_1) and hydroxide- (k_3) catalysed hydrolysis, the latter being very fast because the protonated ammonium salt can assist hydrolysis of the ethyl ester via intramolecular hydrogen bonding. These two processes vastly exceed any solvent-mediated degradation. As the pH increases, the concentration of the free base grows and hydroxide catalysis (k_6) becomes dominant while proton- (k_4) and solvent- (k_5) catalysed degradation are insignificant. The pK_a values obtained are in good agreement with those determined by direct titration (table III) and indicate that satisfactory convergence was achieved. Moreover, estimation of the pK_a of **4** using either the ΔpK_a method (CH_2COOEt, 1.3; CH_2OH, 1.2; CH_2Ph, 0.8) or the Taft equation (pK_a = 10.59–3.23$\Sigma(\sigma^*)$; CH_2COOEt, 0.424; CH_2OH, 0.248; CH_2Ph, 0.108) gives values of 7.9 and 8.07, respectively [28]. Although the pK_a values are somewhat low, this is not unexpected [17, 29] and may reflect the effect of hydrogen bonding interactions between the basic centre and polar substituents. Values are well distant from that expected (pK_a 9.34) from ionisation involving the catechol hydroxy centres [30] so that little interaction is anticipated. To further confirm the integrity of the iteration, the apparent pH at which the minimum degradation rate is observed (pH_{min}) of **1** (R = Et), **3** and **4** (R = Et) is in agreement with that obtained by inspection of the plots; for example, see figure 1 for **1** (R = Et).

Table III. Individual rate constants (L mol^{-1} h^{-1}), pK_a values and apparent pH at which the minimum degradation rate is observed (pH_{min}) calculated by non-linear regression for the hydrolysis of **1** (R = Et), **3** and **4** (R = Et).

Parameter	*1 (R = Et)*	*3*	*4 (R = Et)*
k_1	0.529	0.793	0.735 (0.079)
k_2	0.00219	0.00111	0.00134 (0.00037)
k_3	2 608 500	1 365 600	1 354 900 (13 290)
k_6	11 910	14 920	15,780 (10)
pK_a	7.74	7.40	7.39
pK_a by titration	7.40	7.43	7.76
pH_{min}	3.8	4.0	4.1

Estimates are based upon nine data points and values in parentheses quoted for **4** (R = Et) are standard errors.

The hydrolysis of **3**, analogous to that observed for **1** and **4** (R = Et), proceeded by hydrolysis of the ethyl esters, to give the dipivaloyl acid **18**. Subsequently, **18** showed slow hydrolysis of either the 3- or 4-pivaloyl groups to give the monopivaloyl compounds, in agreement with that observed for dipivefrin [31]. The mono(pivaloyl) intermediates could not be detected because of their interconversion by acyl group migration [32] and their fast hydrolysis [33]. The second pivaloyl group hydrolyses rapidly to give the dihydroxy acid **2**, because of the ability of the *o*-hydroxyl group in the monopivaloyl compounds to protonate the leaving group. The hydrolysis of catechol monoacetate is 500 and 700 times faster than the hydrolyses of catechol diacetate and phenyl acetate, respectively, which is attributed to the inductive and resonance effects of the *o*-hydroxy group and intramolecular hydrogen bonding [34]. The kinetic data followed a first-order model and the rate constant for the hydrolysis of **3** to the dipivaloyl acid **18** at 37 °C and apparent pH 7.4 is 7.477 x 10^{-3} h^{-1} (half-life 92.0 h). Hydrolysis of **18** to the dihydroxy acid **2** was significantly slower, and after 300 h, the reaction mixture comprised of 9% **3**, together with 23% of **18** and 68% of **2**.

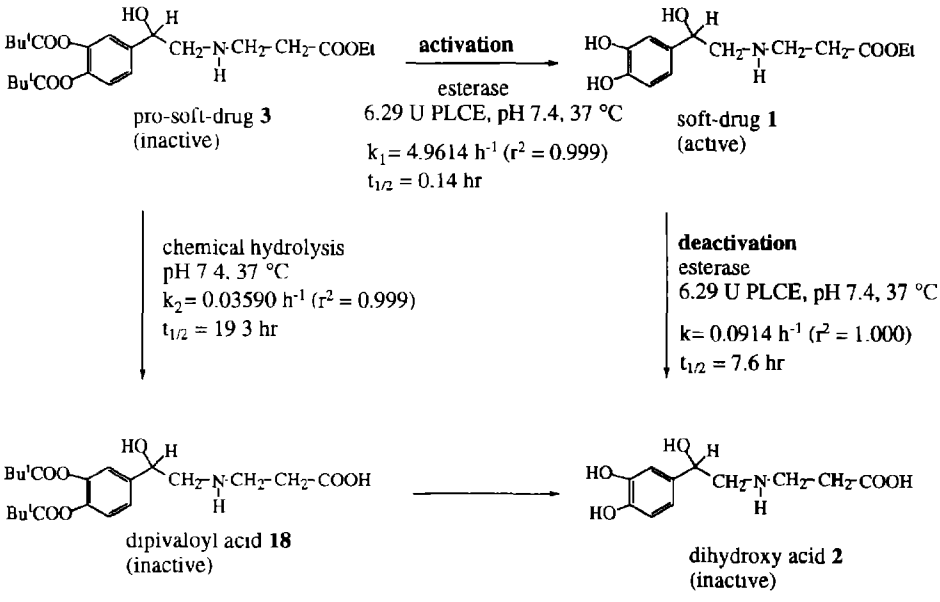
Enzyme-catalysed reactions generally follow the Michaelis–Menten kinetics; the integrated form of this model is shown in equation [2] where S_0 is the initial substrate concentration, S_t represents its value at time t, K_m is the Michaelis-Menten constant and V_{max} denotes the maximum reaction rate.

$$\ln (S_t) + \frac{S_t}{K_m} = \ln (S_0) + \frac{S_0}{K_m} - \frac{V_{max}}{K_m} \cdot t \qquad [2]$$

This model approximates to the zero-order case when $S_0 \gg K_m$ and to the first-order case when $K_m \gg S_0$. In the latter case, the apparent first-order rate constant is the quotient V_{max}/K_m. The PLCE-catalysed hydrolysis of the hydrochloride salt of soft-drug **1** (R = Et) followed a first-order model. The half-lives of **1** (R = Et) (164.0 μM) in phosphate/citrate buffer (10 mL) at pH 7.4 and 37 °C without enzyme and with 31 and 253 U of PLCE were 5.28, 4.84 and 0.59 h, respectively.

To meet our design constraints, the pro-soft-drug **3** is required to undergo hydrolysis to the soft-drug **1** (R = Et) after penetrating the epidermal barrier. Subsequent to exerting a pharmacological response, metabolism of the active entity **1** (R = Et) to the inactive dihydroxy acid **2** is the next requirement. For this strategy to be satisfied, k_1 (the rate of hydrolysis of **3** to **1** (R = Et)) must be greater than k_2 (the rate of hydrolysis of **3** into **18**) such that the dominant pathway follows (**3** → **1** (R = Et) → **2**; $k_1 \gg k_2$) which is not consistent with that observed for the chemical hydrolysis (scheme 2). The esterase-catalysed hydrolysis of the pro-soft-drug **3** was very rapid (half-life of 0.14 h with 6.29 U of PLCE), and in contrast to the chemical hydrolysis, which gave the dipivaloyl acid **18**, the more lipophilic pivaloyl esters are cleaved to give the soft-drug **1** (R = Et) as required. For the experiment with 6.29U PLCE, soft-drug **1** (R = Et) subsequently hydrolysed slowly to the dihydroxy acid **2** with a half-life of 7.6 h (r^2 = 1.000). The PLCE-catalysed hydrolysis of the more lipophilic pivaloyl ester is ~ 50 times faster than the ethyl ester. The chemical and esterase-catalysed hydrolyses of the pro-soft-drug **3** is summarised in scheme 2, with k_1 being high in the PLCE-catalysed hydrolysis and k_2 dominant for the chemical hydrolysis. There is thus a good prospect that pro-soft-drug **3** has the appropriate kinetic features to enable it to be evaluated as a drug for the treatment of psoriasis.

The therapeutic response of a drug depends on its release from the topical vehicle, penetration through the skin barriers and production of the desired pharmacological response [35, 36]. Skin penetration can be tested in vitro using a diffusion cell equipped with animal, human or silicone membrane and this procedure can give a good indication of the penetration potential of the drug. The largely lipoidal nature of the stratum corneum means that permeation studies can



Scheme 2. Activation and deactivation of β-adrenergic agonists for the topical treatment of psoriasis. The pro-soft-drug **3** undergoes esterase-catalysed hydrolysis at the pivaloyl groups to yield the soft-drug **1**, which is deactivated by further hydrolysis to yield the dihydroxy acid **2**.

be modelled by the use of simple lipid membranes [37]. The Silastic membrane is a useful initial model for excised human skin [38] and was used in this study.

Soft-drug **1** (R = Et) was poorly transported through the membrane because of its hydrophilicity and oxidation of the catechol ring. The pro-soft-drug **3** has an increased penetration profile due to the lipophilic pivaloyl groups and its greater stability towards oxidation. The observed fluxes of **3** at different apparent pH values are shown in figure 2, and the mean flux (J), permeability coefficient (K_p) and lag time (t_L) are summarised in table II, showing that transport is enhanced at higher apparent pH values. A plot of flux against apparent pH was linear (J = 1.814×10^{-3} pH $- 3.781 \times 10^{-3}$, $r^2 = 0.977$ by linear regression). According to the simple form of the pH-partition hypothesis, only unionised molecules pass across lipid membranes in significant amounts [39]. Partitioning into the membrane is favoured by the undissociated form of the drug, the percentage of which increases with increasing pH for basic drugs such as **3**. Using a pK_a value of 7.43 for **3**, the percentage of free base over the apparent pH range 3.0 to 8.0 is given in table II. A plot of flux as a function of the fraction of drug in the unionised form suggests that the ionised form does not penetrate the membrane, although it does not show the expected linear relationship. However, at apparent pH values higher than 6.0, the drug begins to degrade at measurable rates. Therefore apparent pH 6.0 is optimal for its formulation where there is a fine balance between flux and the stability of the drug.

Stimulation of β-adrenoceptors leads to the activation of the membrane-bound enzyme, adenylate cyclase, which catalyses conversion of ATP to c-AMP [40]. The spirally cut guinea-pig tracheal preparation, first described by Constantine [41], has been widely used for the pharmacological evaluation of β-agonism [42]. A modification of this preparation was used in the present study. The soft-drug **1** (R = Et) was a full agonist and produced a maximum response similar to that achieved with the potent β-adrenoceptor agonist isoprenaline (fig 3). Furthermore, the β-adrenoceptor antagonist propranolol (1 μM) caused a competitive antagonism of the responses to the soft-drug **1** (R = Et) characterised by a parallel displacement of the dose–response curve to the right with no change in the maximum response (fig 3). These results demonstrate that the soft-drug **1** (R = Et) has functional activity at β-adrenoceptors. The dihydroxy acid **2** also caused a dose-dependent relaxation of the trachea but was significantly less potent than either isoprenaline or the soft-drug **1** (R = Et) (fig 3). Thus the maximum response of **2** was attained at a concentration of 300 μM whereas with **1** (R = Et) the maximum response was achieved at a concentration of 10 μM. The pro-soft-drug **3** had little activity on tracheal preparation but at high concentrations (> 10 μM) it produced a slowly developing relaxation. These findings are consistent with the in vitro hydrolysis studies which show that the dipivaloyl groups are more readily hydrolysed than the ethyl ester to produce the β-agonist soft-drug **1** (R = Et).

Conclusion

In summary, pro-soft-drug **3** is a membrane-permeable compound, that gives, by esterase-catalysed hydrolysis, the active β-adrenergic agonist **1**. The agonist is deactivated by esterase-catalysed hydrolysis to give inactive **2**. Lipophilic compounds of type **3** are likely to be of interest in the topical treatment of psoriasis as they can be passively transported through the upper layers of the skin, where they will be metabolised to the active β-adrenergic agonist giving a therapeutic effect by increasing the c-AMP/c-GMP ratio. Subsequent controlled degradation of the agonist (by esterases in the blood) to an inactive derivative would minimise any cardiovascular side effects.

Experimental protocols

Syntheses

General procedures

^{1}H-NMR (250 MHz) and ^{13}C-NMR (250 MHz) spectra were recorded on a Bruker AC-250 MHz spectrometer with TMS as the internal standard. The solvent used was $CDCl_3$ unless otherwise stated. MS were recorded at the Department of Chemistry, University of Swansea, UK (EPSRC service) on a Micromass 12 instrument. IR spectra were recorded on a Perkin-Elmer 1310 or Unicam Mattson 2020 or 3000 FTIR spectrometer. The IR spectra were recorded as thin films unless otherwise stated. Mps were measured on an Electrothermal digital apparatus, Reichert-Jung of Cambridge Instruments and are not corrected. TLC was performed using DC-Plastikfolien Kieselgel 60 F_{254} containing a fluorescent indicator. Spots were visualised under 254 nm UV light and/or with the aid of iodine. Silica gel C 60-H (40–60 μm) was used in flash chromatography. Elemental analyses were recorded at Butterworth Laboratories Ltd, Middlesex. Analyses are indicated by the symbols of the elements and were within ±0.4% of theoretical values.

*Methyl N-benzyl-3-aminopropionate **5** (R = Me)*

A solution of methyl 3-bromopropionate (5.0 g, 30 mmol), benzylamine (6.42 g, 60 mmol) and THF (40 mL) was stirred at 40 °C for 1 h. Diethyl ether was added and benzylammonium hydrochloride was removed by filtration. The filtrate was evaporated and the oil was purified by flash chromatography eluting with ethyl acetate (R_f 0.38) to give **5** (R = Me). Yield: 3.78 g (65%). IR (cm^{-1}): 3325 (N-H), 1735 (C=O), 1454, 1439 (C=C). ^{1}H-NMR: δ 1.68 (s, 1H, -N*H*-), 2.52 (t, J_{HH} = 6.5 Hz, 2H, -CH_2COO-), 2.88 (t, J_{HH} = 6.5 Hz, 2H, -NCH_2CH$_2$-), 3.66

(s, 3H, -OCH_3), 3.79 (s, 2H, -CH_2Ph), 7.21–7.30 (m, 5H, *Ph*). ^{13}C-NMR: δ 34.5 (-CH_2COO-), 44.4 (-NCH_2CH$_2$-), 51.5 (-OCH_3), 53.7 (-CH_2Ph), 126.9, 128.0, 128.3 (aromatic CH), 140.1 (aromatic C), 173.1 (C=O).

Ethyl N-benzyl-3-aminopropionate 5 (R = Et)
Compound **5** (R = Et) was prepared using a method similar to that described for the methyl analogue **5** (R = Me) from ethyl 3-bromopropionate and benzylamine. The product **5** (R = Et) was isolated as a viscous colourless oil. Yield: 77%. ^{1}H-NMR: δ 1.25 (t , J_{HH} = 7.1 Hz, 3H, -CH_3), 1.72 (s, 1H, -N*H*-), 2.52 (t, J_{HH} = 6.4 Hz, 2H, -CH_2COO-), 2.90 (t, J_{HH} = 6.4 Hz, 2H, -NCH_2CH$_2$-), 3.80 (s, 2H, -CH_2Ph), 4.12 (q, J_{HH} = 7.1 Hz, 2H, -COOCH_2-), 7.2–7.4 (m, 5H, *Ph*). ^{13}C-NMR: δ 14.2 (-CH_3), 34.8 (-CH_2-COO-), 44.5 (-NCH_2CH$_2$-), 53.8 (-CH_2Ph), 60.4 (-COOCH_2-), 126.9, 128.1, 128.4 (aromatic CH), 140.2 (aromatic C), 172.8 (C=O).

3,4-Diphenylmethylenedioxy-α-chloroacetophenone 11
A mixture of 3,4-dihydroxy-α-chloroacetophenone [24] (1.6 g, 8.43 mmol) and α,α-dichlorodiphenylmethane (2.0 g, 8.43 mmol) was stirred and heated to 170–180 °C. After 15 min, the reaction mixture was cooled and extracted with hexane. The product **11** was isolated by flash chromatography eluting with ethyl acetate/hexane (1:1) (R_f 0.68), as a colourless solid. Yield: 2.4 g (81%). Mp 91.0–92.5 °C. IR (KBr, cm^{-1}): 1697 (C=O), 1616, 1498 (C=C). ^{1}H-NMR: δ 4.61 (s, 2H, -CH_2Cl), 6.94 (d, J_{ortho} = 8.1 Hz, 1H, H_5-aromatic), 7.35–7.45 (m, 6H, aromatic), 7.50–7.60 (m, 6H, aromatic). ^{13}C-NMR: δ 45.6 (-CH_2Cl), 125.0, 126.1, 128.3, 128.8, 129.4 (aromatic CH), 108.2, 108.4, 139.3, 147.4, 152.3 (aromatic C), 193.0 (C=O).

3,4-Dipivaloyloxy-α-chloroacetophenone 15
Pivaloyl chloride (6.98 g, 57.9 mmol) was added dropwise over 10 min to a stirred solution of 3,4-dihydroxy-α-chloroacetophenone [24] (5.4 g, 28.9 mmol) and triethylamine (5.86 g, 57.9 mmol) in THF (50 mL) at 0 °C. Triethylammonium hydrochloride was removed by filtration and the filtrate was evaporated under vacuum to give a solid. Compound **15** was purified by flash chromatography eluting with ethyl acetate/hexane (1:4), (R_f 0.32). Yield: 9.0 g (88%). Mp 62.5 °C. ^{1}H-NMR: δ 1.36 (s, 9H, *Bu^t*COO), 1.37 (s, 9H, *Bu^t*COO), 4.68 (s, 2H, -COCH_2Cl), 7.28 (d, J_{ortho} = 8.5 Hz, 1H, H_5-aromatic), 7.74 (d, J_{meta} = 2.1 Hz, 1H, H_2-aromatic), 7.83 (dd, J_{meta} = 2.1, J_{ortho} = 8.5 Hz, 1H, H_6-aromatic). ^{13}C-NMR: δ 27.1 (-C*Me$_3$*), 27.2 (-C*Me$_3$*), 39.2 (-*C*Me$_3$), 39.3 (-*C*Me$_3$), 45.7 (-COCH_2Cl), 123.9, 124.0, 126.7 (aromatic CH), 132.3, 143.1, 147.4 (aromatic C), 175.3 (Bu^t*C*=O), 175.6 (Bu^t*C*=O), 189.3 (-*C*OCH$_2$Cl). $C_{18}H_{23}O_5{}^{35}Cl{\cdot}NH_4^+$ 372.15778. MS (CI, NH_3): 372.1578. Anal $C_{18}H_{23}O_5Cl$ (C, H).

Ethyl N-(benzoylmethyl)-N-benzyl-3-aminopropionate 7 (R = Et)
A solution of α-chloroacetophenone (0.5 g, 3.23 mmol) in DMF (10 mL) was added dropwise over 15 min to a stirred solution of **5** (R = Et) (1.0 g, 4.85 mmol), triethylamine (0.33 g, 3.23 mmol) and sodium iodide (0.049 g, 0.32 mmol) in DMF (10 mL) at 0 °C. After 3 h, the reaction mixture was filtered and the filtrate was concentrated under vacuum. The residue was isolated by flash chromatography eluting with ethyl acetate/hexane (1:5) (R_f 0.25), to give **7** (R = Et) as a colourless oil. Yield: 0.75 g (71.5%). ^{1}H-NMR: δ 1.12 (t, J_{HH} = 7.1 Hz, 3H, -CH_3), 2.51 (t, J_{HH} = 6.5 Hz, 2H, -CH_2COO-), 3.08 (t, J_{HH} = 6.5 Hz, 2H, -NCH_2CH$_2$-), 3.82 (s, 2H, NCH_2Ph), 3.92 (s, 2H, -COCH_2N-), 4.11 (q, J_{HH} = 7.1 Hz, -COOCH_2-), 7.2–7.6 (m, 8H, aromatic), 7.8–7.9 (m, 2H, aromatic). ^{13}C-NMR: δ 14.2 (-CH_3), 33.2 (-CH_2COO-), 50.0 (-NCH_2CH$_2$-), 58.0 (-NCH_2Ph), 59.4 (-COCH_2N-), 60.3 (-COOCH_2), 127.1, 128.2, 128.2, 128.3, 129.0, 133.0 (aromatic CH), 135.9, 138.5 (aromatic C), 172.4 (C=O ester), 196.0 (C=O keto).

Ethyl N-[(3',4'-diphenylmethylenedioxy)benzoylmethyl]-N-benzyl-3-aminopropionate 12 (R = Et)
Compound **12** (R = Et) was prepared as described for **7** (R = Et) from **11** and **5** (R = Et). Yield: 85.5%. IR (cm^{-1}): 1730 (C=O ester), 1680 (C=O keto), 1493, 1445 (C=C). ^{1}H-NMR: δ 1.15 (t, J_{HH} = 7.1 Hz, 3H, -CH_3), 2.50 (t, J_{HH} = 7.0 Hz, 2H, -CH_2COO-), 3.03 (t, J_{HH} = 7.0 Hz, 2H, -NCH_2CH$_2$-), 3.78 (s, 2H, -NCH_2Ph), 3.81 (s, 2H, -COCH_2N-), 4.02 (q, J_{HH} = 7.1 Hz, 2H, -COOCH_2-), 6.84 (d, J_{ortho} = 8.0 Hz, 1H, H_5-aromatic), 7.2–7.6 (m, 17H, aromatic). ^{13}C-NMR: δ 14.1 (-CH_3), 33.2 (-CH_2COO-), 50.1 (-NCH_2CH$_2$-), 58.1 (-N-CH_2Ph), 59.4 (-COCH_2N-), 60.3 (-COOCH_2-), 117.9 (Ph$_2$C-), 107.8, 108.2, 124.5, 126.1, 127.2, 128.2, 128.3, 129.1, 129.3, 130.7, 138.5, 139.5, 147.5, 151.2 (aromatic), 172.4 (-*C*OO-), 196.5 (-*C*OCH$_2$N-).

Methyl N-[(3',4'-diphenylmethylenedioxy)benzoylmethyl]-N-benzyl-3-aminopropionate 12 (R = Me)
Compound **12** (R = Me) was prepared as described for **7** (R = Et) from **11** and **5** (R = Me). Yield: 83%. IR (cm^{-1}): 1731 (-COO-), 1678 (C=O), 1495, 1448 (C=C). ^{1}H-NMR: δ 2.52 (t, J_{HH} = 7.0 Hz, 2H, -CH_2COO-), 3.10 (t, J_{HH} = 7.0 Hz, 2H, -NCH_2CH$_2$-), 3.78 (s, 2H, -NCH_2Ph), 3.80 (s, 2H, -COCH_2N-), 3.62 (s, 3H, -COOCH_3), 6.86 (d, J_{ortho} = 8.0 Hz, 1H, H_5-aromatic), 7.2–7.8 (m, 17H, aromatic). ^{13}C-NMR: δ 33.2 (-CH_2COO-), 50.2 (-NCH_2CH$_2$-), 51.8 (-COOCH_3), 58.1 (-NCH_2Ph), 59.4 (-COCH_2N-), 117.9 (Ph$_2$C-), 107.8, 108.2, 124.5, 126.1, 127.2, 128.2, 128.3, 129.1, 129.3, 130.7, 138.5, 139.5, 147.5, 151.2 (aromatic), 172.4 (-*C*OO-), 196.5 (-*C*OCH$_2$N-).

Ethyl N-[(3',4'-dipivaloyloxy)benzoylmethyl]-N-benzyl-3-aminopropionate 16
Compound **16** was prepared as described for **7** (R = Et) from **15** and **5** (R = Et). Yield: 3.13 g (70%). IR (cm^{-1}): 1763 (C=O ester), 1734 (C=O ester), 1689 (C=O keto), 1604, 1481 (C=C). ^{1}H-NMR: δ 1.21 (t, J_{HH} = 7.1 Hz, 3H, -CH_3), 1.34 (s, 9H, *Bu^t*COO), 1.36 (s, 9H, *Bu^t*COO), 2.52 (t, J_{HH} = 7.0 Hz, 2H, -CH_2COO-), 3.05 (t, J_{HH} = 7.0 Hz, 2H, -NCH_2CH$_2$-), 3.80 (s, 2H, -NCH_2Ph), 3.87 (s, 2H, -COCH_2N-), 4.07 (q, J_{HH} = 7.1 Hz, 2H, -COOCH$_2$-), 7.17 (d, J_{ortho} = 8.4 Hz, 1H, H_5-aromatic), 7.20–7.39 (m, 5H, Ph-), 7.69 (d, J_{meta} = 1.9 Hz, 1H, H_2-aromatic), 7.75 (dd, J_{ortho} = 8.4, J_{meta} = 2.0 Hz, 1H, H_6-aromatic). ^{13}C-NMR: δ 14.1 (-CH$_3$), 27.1 (-C*Me$_3$*), 27.2 (-C*Me$_3$*), 33.2 (-CH$_2$COO), 39.15 (-*C*Me$_3$), 39.25 (-*C*Me$_3$), 50.2 (-NCH$_2$CH$_2$-), 58.2 (-NCH$_2$Ph), 59.6 (-COCH$_2$N-), 60.4 (-COOCH$_2$-), 123.4, 123.6, 126.5, 127.3, 128.4, 129.1 (aromatic CH), 134.1, 138.4, 142.6, 146.7 (aromatic C), 172.5 (CH$_2$*C*OO), 175.4 (*Bu^t*COO), 175.6 (Bu^t*C*OO), 196.6 (-*C*OCH$_2$N-).

Ethyl N-(2'-hydroxy-2'-phenylethyl)-N-benzyl-3-aminopropionate 8 (R = Et)
Sodium borohydride (1.80 g, 46.0 mmol) was added in small portions over 15 min to a stirred solution of **7** (R = Et) (6.0 g, 18.4 mmol) in ethanol (80 mL). After 1 h at room temperature, the reaction mixture was evaporated under vacuum. Water was added and the mixture was extracted with dichloromethane. The organic layer was dried (molecular sieves) and evaporated under vaccum. Compound **8** (R = Et) was isolated by flash chromatography eluting with ethyl acetate (R_f 0.60) to give a colourless oil. Yield: 4.50 g (74.5%). IR (cm^{-1}): 3471 (OH),

1730 (COO), 1452 (C=C). ^{1}H-NMR: δ 1.23 (t, J_{HH} = 7.1 Hz, -CH_3), 2.40–2.59 (m, 3H, -C(OH)HCH_AH$_B$NCH$_2$CH_2-), 2.64 (dd, J_{gem} = 13.0, J_{HH} = 3.9 Hz, 1H, -C(OH)CH$_A$$H_B$N-), 2.76 (dt, J_{gem} = 13.1, J_{HH} = 6.5 Hz, 1H, -NCH_AH$_B$CH$_2$-), 3.05 (dt, J_{gem} = 13.2, J_{HH} = 7.4 Hz, 1H, -NCH$_A$$H_BCH_2$-), 3.50 (d, J_{gem} = 13.5 Hz, 1H, -NCH_AH$_B$Ph), 3.86 (d, J_{gem} = 13.5 Hz, 1H, -NCH$_A$$H_B$Ph), 4.07 (dq, J_{gem} = 10.8, J_{HH} = 7.1 Hz, 1H, -COOCH_AH$_B$-), 4.17 (dq, J_{gem} = 10.8, J_{HH} = 7.1 Hz, 1H, -COOCH$_A$$H_B$-), 4.67 (dd, J_{HH} = 9.7, J_{HH} = 3.8 Hz, 1H, -C(OH)H-), 7.18–7.35 (m, 10H, aromatic) (by integration -OH is between 3.70 and 4.0). ^{13}C-NMR: δ 14.1 (-CH_3), 32.8 (-CH_2COO-), 49.6 (-NCH_2CH$_2$), 58.6 (-NCH_2Ph), 60.6 (-COOCH_2-), 62.8 (-HOCHCH_2N-), 69.9 (-C(OH)H), 125.8, 126.1, 127.3, 128.2, 128.4, 129.0, 129.3 (aromatic CH), 138.2 142.0 (aromatic C), 172.5 (C=O).

Methyl N-[2'-(3",4"-diphenylmethylenedioxyphenyl)-2'-hydroxyethyl]-N-benzyl-3-aminopropionate ***13*** *(R = Me)*

Compound **13** (R = Me) was prepared as described for **8** (R = Et) by the sodium borohydride reduction of **12** (R = Me) in methanol (50 mL). Yield: 3.91 g (78%). ^{1}H-NMR: δ 2.47–2.65 (m, 4H, -C(OH)CH_2NCH$_2$CH_2-), 2.78 (dt, J_{gem} = 13.0, J_{HH} = 6.5 Hz, 1H, -NCH_AH$_B$CH$_2$-), 3.06 (dt, J_{gem} = 13.2, J_{HH} = 7.5 Hz, 1H, -NCH$_A$$H_BCH_2$-), 3.52 (d, J_{gem} = 13.5 Hz, 1H, -NCH_AH$_B$Ph), 3.69 (s, 3H, -OCH_3), 3.87 (d, J_{gem} = 13.6 Hz, 1H, -NCH$_A$$H_B$Ph), 3.92 (broad s, 1H, -OH), 4.61 (dd, J_{HH} = 4.5, J_{HH} = 8.9 Hz, 1H, -C(OH)H-), 6.78 (dd, J_{ortho} = 8.0, J_{meta} = 1.4 Hz, 1H, H_6-aromatic), 6.84 (d, J_{ortho} = 7.9 Hz, 1H, H_5-aromatic), 6.93 (d, J_{meta} = 1.3 Hz, 1H, H_2-aromatic), 7.25–7.43 (m, 11H, aromatic) and 7.57–7.64 (m, 4H, aromatic). ^{13}C-NMR: δ 32.6 (-CH_2COO-), 49.5 (-NCH_2CH$_2$-), 51.7 (-OCH_3), 58.6 (-NCH_2Ph), 62.7 (-C(OH)CH_2N-), 69.8 (-C(OH)H), 106.6, 108.0, 116.6, 119.4, 126.3, 127.4, 128.2, 128.4, 128.96, 129.0, 135.9, 138.1, 140.3, 146.5, 147.2 (aromatic), 172.9 (C=O).

Ethyl N-[2'-(3",4"-diphenylmethylenedioxyphenyl)-2'-hydroxyethyl]-N-benzyl-3-aminopropionate ***13*** *(R = Et)*

Compound **13** (R = Et) was prepared as described for **8** (R = Me) by the reduction of **12** (R = Et) with sodium borohydride in ethanol. Yield: 79%. IR (cm^{-1}): 3466 (broad, -OH), 1728 (-COO-), 1494, 1448 (C=C). ^{1}H-NMR: δ 1.24 (t, J_{HH} = 7.1 Hz, 3H, -CH_3), 2.44–2.61 (m, 4H, -C(OH)CH_2NCH$_2$CH_2-), 2.75 (dt, J_{gem} = 13.0, J_{HH} = 6.3, 1H, -NCH_AH$_B$CH$_2$-), 3.05 (dt, J_{gem} = 13.2, J_{HH} = 7.5 Hz, 1H, -NCH$_A$$H_BCH_2$-), 3.50 (d, J_{gem} = 13.5 Hz, 1H, -NCH_AH$_B$Ph), 3.85 (d, J_{gem} = 13.5 Hz, 1H, -NCH$_A$$H_B$Ph), 3.90 (broad s, 1H, -OH), 4.10 (dq, J_{gem} = 10.85, J_{HH} = 7.1 Hz, 1H, -COOCH_AH$_B$-), 4.15 (dq, J_{gem} = 10.85, J_{HH} = 7.15 Hz, 1H, -COOCH$_A$$H_B$-), 4.57 (dd, J_{HH} = 4.5, J_{HH} = 8.9 Hz, 1H, -C(OH)H-), 6.73 (dd, J_{ortho} = 8.0, J_{meta} = 1.4 Hz, 1H, H_6-aromatic), 6.79 (d, J_{ortho} = 7.9 Hz, 1H, H_5-aromatic), 6.87 (d, J_{meta} = 1.3 Hz, 1H, H_2-aromatic), 7.25–7.4 (m, 11H, Ph), 7.52– 7.58 (m, 4H, Ph). ^{13}C-NMR: δ 14.1 (-CH_3), 32.7 (-CH_2COO-), 49.5 (-NCH_2CH$_2$-), 58.5 (-NCH_2Ph), 60.6 (-COOCH_2-), 62.6 (-C(OH)CH_2N), 69.7 (-C(OH)H), 116.6 (Ph$_2$$C$-), 106.5, 108.0, 119.3, 126.2, 127.3, 128.1, 128.4, 128.9 (aromatic CH), 135.8, 138.1, 140.2, 146.4, 147.2 (aromatic C), 172.5 (C=O).

Ethyl N-[2'-(3",4"-dipivaloyloxyphenyl)-2'-hydroxyethyl]-N-benzyl-3-aminopropionate ***17***

Compound **17** was prepared as described for **8** (R = Me) by the reduction of **16** with sodium borohydride in ethanol. Yield: 1.41 g (45%). IR (neat, cm^{-1}): 3462 (OH), 1759 (C=O, Bu^t ester), 1732 (C=O, ethyl ester), 1504, 1479 (C=C). ^{1}H-NMR: δ 1.24 (t, J_{HH} = 7.1 Hz, 3H, -CH_3), 1.32 (s, 9H, *Bu^t*COO), 1.33 (s, 9H, *Bu^t*COO), 2.45–2.55 (m, 3H, -C(OH)CH_AH$_B$N-, -CH_2COO), 2.67 (dd, J_{gem} = 13.0, J_{HH} = 3.2 Hz, 1H, -C(OH)-CH$_A$$H_B$N-), 2.78 (dt, J_{gem} = 13.2, J_{HH} = 6.1 Hz, 1H, -NCH_A-H$_B$CH$_2$-), 3.05 (dt, J_{gem} = 13.2, J_{HH} = 7.2 Hz, 1H, -NCH$_A$$H_B$-CH$_2$-), 3.53 (d, J_{gem} = 13.5 Hz, 1H, -NCH_AH$_B$Ph), 3.86 (d, J_{gem} = 13.5 Hz, 1H, -NCH$_A$$H_B$Ph), 4.10 (dq, J_{gem} = 10.8, J_{HH} = 7.1 Hz, 1H, -COOCH_AH$_B$-), 4.17 (dq, J_{gem} = 10.8, J_{HH} = 7.18 Hz, 1H, -COOCH$_A$$H_B$-), 4.66 (dd, 1H, J_{HH} = 3.2, J_{HH} = 10.1 Hz, -C(OH)H-), 7.05 (d, J_{ortho} = 8.3 Hz, 1H, H_5-aromatic), 7.08 (d, J_{meta} = 1.8 Hz, 1H, H_2-aromatic), 7.15 (dd, J_{ortho} = 8.3, J_{meta} = 1.9 Hz, 1H, H_6-aromatic), 7.21–7.35 (m, 5H, Ph) (OH not observed). ^{13}C-NMR: δ 14.1 (-CH_3), 27.2 (-CMe_3), 32.8 (-CH_2COO), 39.0 (-CMe_3), 49.5 (-NCH_2CH$_2$-), 58.7 (-NCH_2Ph), 60.7 (-COOCH_2-), 62.7 (-C(OH)CH_2N-), 69.2 (-C(OH)CH$_2$N-), 120.7, 123.0, 123.5, 127.4, 128.4, 129.0 (aromatic CH), 138.0, 140.5, 141.6, 142.5 (aromatic C), 172.5 (CH$_2$$COO$), 175.7 (Bu^t$COO$), 175.9 (Bu^t$COO$).

Ethyl N-(2'-hydroxy-2'-phenylethyl)-3-aminopropionate ***4*** *(R = Et)*

A solution of **8** (R = Et) (100 mg, 0.305 mmol) in ethanol (15 mL) was subjected to an atmosphere of hydrogen in the presence of 10% palladium on charcoal (20 mg) at room temperature and pressure. After 40 min the catalyst was removed by filtration through celite and the filtrate was evaporated. The residue was recrystallised from hexane to give **4** (R = Et). Yield: 20 mg (27%). Mp 75–77 °C. IR (KBr, cm^{-1}): 3428 (broad -OH), 3300 (-NH-), 1723 (C=O). ^{1}H-NMR: δ 1.26 (t, J_{HH} = 7.1 Hz, 3H, -CH_3), 2.3–2.6 (broad s, 2H, -OH, -NH-), 2.51 (t, J_{HH} = 6.3 Hz, 2H, -CH_2COO-), 2.70 (dd, J_{gem} = 12.2, J_{HH} = 9.2 Hz, 1H, -C(OH)CH_AH$_B$N-), 2.85–3.03 (m, 3H, -C(OH)CH$_A$$H_BNCH_2$-), 4.15 (q, J_{HH} = 7.1 Hz, 2H, -COOCH_2), 4.70 (dd, J_{HH} = 9.0, J_{HH} = 3.5 Hz, 1H, -C(OH)H-], 7.25–7.4 (m, 5H, aromatic). ^{13}C NMR: δ 14.1 (-CH_3), 34.7 (-CH_2COO-), 44.5 (-NCH_2CH$_2$-), 56.8 (-C(OH)CH_2N-], 60.5 (-COOCH_2-), 71.5 (-C(OH)-), 125.7, 127.4, 128.3 (aromatic CH), 142.3 (aromatic C), 172.6 (C=O). Mass spectrum (CI, NH_3): calculated mass for [$C_{13}H_{20}NO_3$ (M + H$^+$)] 238.1443. Observed accurate mass 238.1443 (M + H$^+$). Anal $C_{13}H_{19}NO_3$ (C, H, N).

Methyl N-(2'-hydroxy-2'-phenylethyl)-3-aminopropionate ***4*** *(R = Me)*

Compound **7** (R = Me) was prepared from α-chloroacetophenone **6** and **5** (R = Me) using the method described for **7** (R = Et). Yield: 3.05 g (65.0%). The keto group of **7** (R = Me) was reduced with sodium borohydride as described for **7** (R = Et), to give **8** (R = Me) as an oil. Yield: 2.27 g (75.0%).

A solution of **8** (R = Me) (2.27 g, 7.26 mmol) in methanol (20 mL) was exposed to hydrogen in the presence of 10% Pd/C (0.23 g) at room temperature and pressure. After 40 min, the catalyst was removed by filtration through celite and the filtrate was evaporated to dryness. The residue was crystallised from hexane to give **4** (R = Me). Yield 1.15 g (70%). ^{1}H-NMR (DMSO-d_6): δ 2.44 (t, J_{HH} = 6.6 Hz, 2H, -CH_2COO-), 2.50 (s, 1H, -NH-), 2.60 (d, J_{HH} = 6.0 Hz, 2H, -C(OH)CH_2N-), 2.77 (t, J_{HH} = 6.6 Hz, 2H, -NCH_2CH$_2$-), 3.1–3.6 (broad s, 1H, -OH), 3.58 (s, 3H, -OCH_3), 4.59 (t, J_{HH} = 6.2 Hz, 1H, -C(OH)H-), 7.22–7.35 (m, 5H, aromatic). ^{13}C NMR (DMSO-d_6): δ 34.6 (-CH_2COO-), 44.8 (-NCH_2CH$_2$-), 51.4 (-OCH_3), 57.6 (-C(OH)-CH_2N-), 71.6 (-C(OH)-), 126.1, 127.0, 128.1 (aromatic CH), 144.8 (aromatic C), 172.9 (C=O).

Ethyl N-[2'-(3",4"-diphenylmethylenedioxyphenyl)-2'-hydroxyethyl]-3-aminopropionate ***14***

Compound **14** was prepared using a method similar to that described for **8** (R = Et), by hydrogenation of **13** in ethyl acetate at 30 psi hydrogen pressure for 48 h, followed by flash chromatography (10% ethanol in chloroform, R_f 0.42) and recrystallisation from ethyl acetate/hexane. ^{1}H-NMR: δ 1.24

(t, J_{HH} = 7.1 Hz, 3H, -CH_3), 2.2–2.69 (broad s, 2H, -OH, -NH-), 2.49 (t, J_{HH} = 6.4 Hz, 2H, -CH_2COO-), 2.65 (dd, J_{gem} = 12.2, J_{HH} = 9.1 Hz, 1H, -C(OH)C$H_A$$H_B$N-), 2.84 (dd, J_{gem} = 12.2, J_{HH} = 3.6 Hz, 1H, -C(OH)CH$_A$$H_B$N-), 2.8–3.0 (m, 2H, -NC$H_2CH_2$-), 4.12 (q, J_{HH} = 7.1 Hz, 2H, -COOCH_2-), 4.60 (dd, J_{HH} = 3.6, J_{HH} = 9.1 Hz, 1H, -C(OH)H-), 6.75–6.85 (m, 2H, aromatic), 6.93 (s, 1H, aromatic), 7.3–7.45 (m, 6H, aromatic), 7.5–7.65 (m, 4H, aromatic). ^{13}C NMR: δ 14.1 (-CH_3), 34.7 (-CH_2COO-), 44.5 (-N-CH_2CH$_2$-), 56.8 (-C(OH)CH_2N-), 60.5 (COOCH_2-), 71.4 (-C(OH)-), 116.5 (Ph$_2$$C$-), 106.4, 108.1, 119.2, 126.2, 128.1, 129.0, 136.3, 140.2, 146.5, 147.3 (aromatic), 172.5 (C=O).

*Ethyl N-[2'-(3",4"-dihydroxyphenyl)-2'-hydroxyethyl]-3-aminopropionate **1** (R = Et)*

A solution of **13** (R = Et) (0.30 g, 0.573 mmol) in ethanol (25 mL) was hydrogenated at room temperature under 60 psi hydrogen atmosphere in the presence of 10% Pd/C catalyst (30 mg). After 18 h, the catalyst was removed by filtration through celite and the filtrate was evaporated. The residue was washed with toluene to remove diphenylmethane. The residue was evaporated, the product was dissolved in ethanol and dry ether, and dry HCl gas was passed through the solution. The precipitated hydrochloride salt of **1** (R = Et) was collected by filtration. The compound was stored under argon and was protected from light. Yield: 0.143 g (92%). Mp 156.8 °C. IR (KBr, cm^{-1}): 3404 (broad, -N$^+$H$_2$-), 3315 (broad, -OH), 1731 (C=O). ^{1}H-NMR (CD$_3$OD): δ 1.23 (t, J_{HH} = 7.1 Hz, 3H, -CH_3), 2.52 (t, J_{HH} = 6.6 Hz, 2H, -CH_2COO-), 2.69 (dd, J_{gem} = 12.0, J_{HH} = 4.8 Hz, 1H, -C(OH)C$H_A$$H_B$N-), 2.78 (dd, J_{HH} = 12.0, J_{HH} = 8.3 Hz, 1H, -C(OH)C$H_A$$H_B$N-), 2.87 (t, J_{HH} = 6.6 Hz, 2H, -N-CH_2CH$_2$-), 4.11 (q, J_{HH} = 7.1 Hz, 2H, -COOCH_2-), 4.60 (dd, J_{HH} = 8.3, J_{HH} = 4.8 Hz, 1H, -C(OH)H), 6.67 (dd, J_{ortho} = 8.1, J_{meta} = 1.9 Hz, 1H, H_6-aromatic), 6.73 (d, J_{ortho} = 8.0 Hz, 1H, H_5-aromatic), 6.80 (d, J_{meta} = 1.8 Hz, 1H, H_2-aromatic). ^{13}C NMR (CD$_3$OD): δ 14.5 (-CH_3), 34.9 (-CH_2COO-), 45.6 (-NCH_2CH$_2$-), 57.7 (-C(OH)CH_2N-), 61.6 (-COOCH_2-), 73.2 (-C(OH)-), 114.2, 116.2, 118.6 (aromatic CH), 135.8, 146.0, 146.4 (aromatic C), 174.0 (C=O). Mass spectrum (FAB): calculated mass for cation [$C_{13}H_{20}NO_5$ (M$^+$ – Cl)] 270.13415. Observed accurate mass 270.13415 (M$^+$ – Cl). Anal $C_{13}H_{20}ClNO_5$ (C, H, N).

*Methyl N-[2'-(3",4"-dihydroxyphenyl)-2'-hydroxyethyl]-3-aminopropionate **1** (R = Me)*

A solution of **13** (R = Me) (3.91 g, 7.68 mmol) in methanol (50 mL) was hydrogenated at room temperature under 60 psi hydrogen atmosphere in the presence of 10% Pd/C catalyst (0.39 g) for 12 h. The catalyst was removed by filtration through celite and the filtrate evaporated under vacuum to dryness. The residue was washed with toluene to remove diphenylmethane. The residue was evaporated under vacuum to give **1** (R = Me) which was stored in the dark under argon. Yield: 1.82 g (93%). ^{1}H-NMR (CD$_3$OD): δ 2.75 (broad, 2H, -CH_2COO-), 3.04 (broad, 2H, -C(OH)CH_2N), 3.22 (broad, 2H, -NCH_2CH$_2$-), 3.71 (s, 3H, -OCH_3), 4.76 (broad, 1H, -C(OH)H), 6.66–6.94 (m, 3H, aromatic). ^{13}C NMR (CD$_3$OD): δ 31.0 (-CH_2COO-), 44.4 (-NCH_2CH$_2$), 50.0 (-OCH_3), 54.4 (-C(OH)-CH_2N-), 79.5 (-C(OH)-), 114.7, 116.6, 119.7 (aromatic CH), 129.3, 147.0, 147.2 (aromatic C), 172.4 (C=O).

*Ethyl N-[2'-(3",4"-dipivaloyloxyphenyl)-2'-hydroxyethyl]-3-aminopropionate **3***

A solution of **17** (0.80 g, 1.55 mmol) in ethanol (50 mL) was subjected to hydrogenation in the presence of 10% Pd/C catalyst (80 mg) at room temperature and pressure. After 40 min, the catalyst was removed by filtration through celite and the filtrate was evaporated to dryness. The residue was extracted into ether, which was evaporated and recrystallised from hexane to give **3**. Yield: 0.67 g (89%). IR (KBr, cm^{-1}): 3506 (OH), 1759 (COOBu^t), 1734 (COOC$_2$H$_5$), 1504, 1481 (C=C). ^{1}H-NMR: δ 1.26 (t, J_{HH} = 7.13 Hz, 3H, -CH_3), 1.33 (s, 9H, Bu^tCOO), 1.34 (s, 9H, Bu^tCOO), 2.49 (t, J_{HH} = 6.41 Hz, 2H, -CH_2COO-), 2.66 (dd, J_{gem} = 12.2, J_{HH} = 9.1 Hz, 1H, -C(OH)-C$H_A$$H_B$N-), 2.84–3.00 (m, 3H, -C(OH)CH$_A$$H_B$N-, -NC$H_2CH_2$-), 4.14 (q, J_{HH} = 7.1 Hz, 2H, -COOCH_2-), 4.67 (dd, J_{HH} = 9.1, J_{HH} = 3.4 Hz, 1H, -C(OH)H-), 7.08 (d, J_{ortho} = 8.3 Hz, 1H, H_5-aromatic), 7.15 (d, J_{meta} = 1.7 Hz, 1H, H_2-aromatic), 7.19 (dd, J_{ortho} = 8.3, J_{meta} = 1.7 Hz, 1H, H_6-aromatic) (by integration OH and NH lie between 2.2–3.4 as very broad peaks). ^{13}C-NMR: δ 14.1 (-CH_3), 27.1 (-CMe_3), 34.6 (-CH_2COO), 38.6 (-CMe_3), 44.5 (-NCH_2CH$_2$-), 56.7 (-C(OH)CH_2N-), 60.4 (-COOCH_2-), 70.6 (-C(OH)-), 120.6, 123.1, 123.4 (aromatic CH), 141.2, 141.5, 142.3 (aromatic C), 172.5 (CH$_2$COO), 175.7 (Bu^{t}COO), 175.8 (Bu^{t}COO). Mass spectrum (CI, NH$_3$): calculated mass for $C_{23}H_{36}NO_7$ 438.2492 (M + H$^+$). Observed accurate mass 438.2492 (M + H$^+$). Anal $C_{23}H_{35}NO_7$ (C, H, N).

*N-[2'-(3",4"-Dihydroxyphenyl)-2'-hydroxyethyl]-3-aminopropionic acid **2***

A solution of **1** (R = Et) (0.1 g, 0.327 mmol) in water (25 mL) containing concentrated HCl (0.5 mL) was stirred at room temperature for 2 days. Water was removed under vacuum to give the hydrochloride salt of **2** (0.085 g, 0.306 mmol, 94%). ^{1}H-NMR (D$_2$O): δ 2.91 (t, J_{HH} = 6.6 Hz, 2H, CH_2COO), 3.22–3.42 (m, 2H, -C(OH)CH_2N-), 3.42–3.52 (m, 2H, -NCH_2CH$_2$-), 4.97 (dd, J_{HH} = 8.3, J_{HH} = 4.9 Hz, 1H, -C(OH)H-), 6.91 (dd, J_{ortho} = 8.3, J_{meta} = 2.0 Hz, 1H, H_6-aromatic), 6.99 (d, J_{ortho} = 8.3 Hz, 1H, H_5-aromatic), 7.0 (d, J_{meta} = 2.0 Hz, 1H, H_2-aromatic). ^{13}C-NMR (D$_2$O): δ 31.0 (-CH_2COO-), 44.3 (-NCH_2CH$_2$-), 54.3 (-C(OH)CH_2N-), 69.45 (-C(OH)-), 115.0, 117.5, 119.8 (aromatic CH), 133.3, 145.4, 145.5 (aromatic C), 175.5 (C=O).

Determination of pK_a values

Measurements of pH were undertaken with a Philips CD 660 digital pH meter (three decimal digit display) fitted with a Gallenkamp combined glass electrode. Ethyl *N*-(2'-hydroxy-2'-phenylethyl)-3-aminopropionate (**4**, R = Et) (19.5 mg) in water (10 mL) was titrated with 0.1 M HCl. The hydrochloride salt of ethyl *N*-[2'-(3",4"-dipivaloyloxyphenyl)-2'-hydroxyethyl]-3-aminopropionate (**3**) (180.8 mg) in water (22 mL) was titrated with 0.1 M KOH. All end-points were determined potentiometrically and the data were analysed according to equation [3] [43].

$$pK_a = pH + \log\left(\frac{a - [H_3O^+] + [HO^-]}{b + [H_3O^+] - [HO^-]}\right) \quad [3]$$

where a and b are the stoichiometric concentrations of protonated and free base at various points on the titration curve at the measured pH. The pK_a values are given in table III.

High-performance liquid chromatography

HPLC grade solvents were used and other chemicals were either Analar or reagent grade. HPLC analyses were performed using a system comprising an Altex 100A dual reciprocating, solvent-metering pump delivering mobile phases to a stainless-steel column (10 cm x 4.6 mm) packed with 5 μm Hypersil-ODS (Shandon, UK) reversed-phase material. Samples were

introduced through a Rheodyne 7120 injection valve fitted with 20 µL loop. Pye Unicam LC variable UV wavelength detector equipped with an 8 µL flow cell was used, with a sensitivity setting between 0.08–1.28 AUFS. A Gallenkamp Euroscribe chart recorder was used operating at a chart speed of 15 cm h^{-1}. A Kerry laboratory sonicator was employed to aid dissolution or solvent degassing. The mobile phases were degassed by vacuum filtration or sonication prior to HPLC analyses.

The soft β-adrenoceptor agonists and their analogues were analysed with the mobile phases summarised in table I. For each mobile phase, the apparent pH was adjusted to 3.0 with phosphoric acid. A flow rate of 1 mL min^{-1} was employed and the column eluant was usually measured spectrophotometrically at 210 nm. The HPLC methods were validated in terms of linearity of calibration plots of peak height with respect to analyte concentration ($r^2 > 0.99$).

Chemical hydrolyses

The phosphate/citrate buffers at pH 2.0 to 10.0 were McIlvaine's buffer [44]. The final pH values were adjusted by the addition phosphoric acid. Chemical hydrolyses of **1** (R = Et), the hydrochloride salt of **3**, and **4** (R = Et) were conducted over a pH range 2.0 to 10.0 in phosphate-citrate buffer at 50 °C. Compounds **1** and **4** (R = Et) required the addition of 10% acetonitrile to aid solubility. Chemical hydrolysis of the hydrochloride salt of the soft-drug **1** (R = Et) and the pro-soft-drug **3** were also completed at pH 7.4 and 37 °C to allow comparison with the enzyme-catalysed hydrolyses.

Samples (0.5 mL) were withdrawn at appropriate time intervals during the experiment and 20 µL were analysed by HPLC. Concentrations of ester were calculated from a calibration line prepared from standards chromatographed under the same conditions.

Esterase-catalysed hydrolyses

Porcine liver carboxyesterase (PLCE) was obtained (Sigma) as a suspension in 3.2 M ammonium sulphate at pH 8.0. Each milligram of protein was equivalent to 230 units, where each unit (U) hydrolysed 1 µmol of ethyl butyrate per minute at pH 8.0 and 25 °C. The esterase solution containing 11.0 mg of protein was diluted with water, prior to use. The reaction mixtures were maintained under argon and protected from light to minimise oxidation of the catechol group. A solution of the soft-drug **1** (R = Et) (164.0 µM) in phosphate/citrate buffer (pH 7.4, 10 mL) was stirred at 37 °C. The reaction was initiated by the addition of PLCE (i) 31 U and (ii) 253 U which was previously incubated at 37 °C. The hydrolyses were monitored by HPLC. A solution of the pro-soft-drug **3** (80.0 µM) in phosphate-citrate buffer (pH 7.4, 10 mL) was maintained at 37 °C. PLCE was added (i) 5.06 U, (ii) 6.29 U and (iii) 12.58 U to the reaction mixture. In all experiments, 0.5 mL samples were withdrawn at appropriate time intervals and analysed by HPLC (20 µL sample injected). The concentration of ester was calculated from a calibration line prepared from standards assayed under the same conditions. Experiments were performed in duplicate, together with controls in the absence of PLCE.

*Chemical hydrolysis studies of pro-soft-drug **3** and soft-drug **1** (R = Et) by ^{1}H-NMR spectroscopy*

Potassium dihydrogen phosphate (100 mg) was dissolved in D_2O (10 mL) and evaporated to dryness under high vacuum at 50 °C. This was repeated three times, D_2O was added and the pH adjusted to pH 8.17 (pD 8.57 at 22 °C [45]) with potassium deuteroxide to give a 0.1 M buffer. A solution of pro-soft-drug **3** (5 mM) in potassium phosphate buffer (0.1 M, D_2O, pD 8.57)/CD_3CN (8:2, v/v) was monitored over 300 h by ^{1}H-NMR spectroscopy at 37 °C. A solution of soft-drug **1** (R = Et) (5 mM) in potassium phosphate buffer (0.1 M, D_2O, pD 8.57)/CD_3CN (9:1, v/v) was monitored over 30 h by ^{1}H-NMR spectroscopy at 37 °C.

*Chemical hydrolysis studies of pro-soft-drug **3** and soft-drug **1** (R = Et) by HPLC*

The solution of pro-soft-drug **3** (R = Et) (105.5 µM) in phosphate/citrate buffer (pH 8.0) containing 10% acetonitrile at 50 °C was monitored with time by HPLC (table I). When 80% of the triester had reacted, the mixture was cooled and extracted successively with hexane (2 x 20 mL), diethyl ether (2 x 20 mL), chloroform (2 x 20 mL) and ethyl acetate (2 x 20 mL). Each extract was analysed by HPLC and ^{1}H-NMR spectroscopy. The chemical hydrolysis of soft-drug **1** (R = Et) (163.5 µM) in phosphate/citrate buffer (pH 7.4) containing 10% acetonitrile at 37 °C was monitored by HPLC.

Permeation studies

The silicone rubber membrane (dimethyl polysiloxane, Silastic Medical grade 500-3, Dow Corning, 0.010 inch thick) was rinsed in distilled water to remove surface deposits and cut into rectangular pieces, prior to mounting onto Franz-type diffusion cells. The membrane was mounted between the two halves of the diffusion cell, comprising upper donor and lower receptor compartments, with a surface area of approximately 2.0 cm^2. The sampling port of the receptor compartment (20–30 mL) was sealed between sampling and the donor compartment was sealed with a perspex lid to minimise evaporation. The solution in the receiving compartment was stirred. The receptor cell was maintained at 37 °C which gave a donor compartment (and skin surface) temperature of 32 ± 1 °C.

Non-buffered and buffered 10% v/v propylene glycol in water were used as test vehicles for the donor and receptor compartments. The buffered 10% v/v propylene glycol solutions (apparent pH 3.0–8.0) of constant ionic strength were prepared from the phosphate/citrate (McIlvaine) buffers, the pH being adjusted with sodium hydroxide or phosphoric acid. A solution of the drug in ethanol was added to the membrane surface of the donor phase. The pro-soft-drug **3** (500 mg) was dissolved in ethanol (10 mL) to make a stock solution of 50 mg/mL. The stock solution (100 µL) was added over the membrane (10.55 µM). After 5 min, during which time the ethanol had evaporated, 0.5 mL of phosphate/citrate buffer in 10% propylene glycol at apparent pH 3.0–8.0 was added over the membrane.

The receptor compartment was filled with the chosen vehicle (20–30 mL), which had been maintained at 37 °C and degassed by sonication. The membrane was allowed to equilibrate with the receptor solution for 30 min. Samples from the receptor compartment (1 mL) were removed every hour and analysed by HPLC. After withdrawal of each sample, the receptor fluid was replenished with an equivalent volume of the drug-free vehicle. All permeation studies were performed in triplicate and were protected from light and air (argon).

The concentration of the permeant in the receptor compartment at each sampling point was determined by HPLC (table I). The amount of drug penetrating the membrane per unit area was calculated from the volume of the receptor compartment and the area of the membrane available for diffusion, which varied slightly for each cell. The cumulative

amount of drug penetrated per unit area was calculated according to equation [4] which allows for both sampling losses and dilutions.

$$M_t(n) = V_r \bullet C_n + V_s \bullet \sum_{m=1}^{n-1} C_m \quad [4]$$

Where $M_t(n)$ is the current, cumulative mass of drug transported across the membrane at time t, C_n represents the current concentration in the receiver medium and ΣC_m denotes the summed total of the previous measured concentrations (m = 1 to (n – 1)); V_r is the volume of the receiver medium and V_s corresponds to the volume of sample removed for analysis. A plot of the cumulative amount penetrated per unit area as a function of time yielded the steady-state flux (slope) (J) and the lag time (t_L) (table II).

*Interaction of soft-drug **1** (R = Et), pro-soft-drug **3** and dihydroxy acid **2** with β-adrenoceptors*

The trachea was dissected from a guinea pig (300–400 g) and opened into a sheet by making a longitudinal incision opposite to the smooth muscle. The tracheal preparation was made by a modification of the method of Constantine [41]. Alternate cuts were made through the tissue and the strip so formed was mounted in an organ bath maintained at 37 °C and bathed in physiological salt solution (PSS) of the following composition (mM): NaCl 118.5, KCl 4.75, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4 \bullet 7H_2O$ 1.17 and dextrose 11.6. The tissues were gassed with 95% O_2/5% CO_2. The tissue was mounted under a resting tension of 2 g and all contractions were recorded via Pioden isometric transducers by means of UF1 transducers from Ormed, preamplified to a BBC flatbed recorder and a MacLab system. Tissues were pre-contracted with acetylcholine (10 μM) and the effect of the experimental compounds and isoprenaline were determined as relaxations of the pre-contracted tissues. In experiments with propranolol, this compound was added to the PSS and preparations were pre-incubated for 30 min prior to the addition of an agonist.

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