was evaporated to dryness under reduced pressure. The residue was taken up in anhydrous EtOH, and Et_2O was added to the cloud point. On prolonged cooling, the solution deposited crystals, which were collected on a filter, washed with anhydrous Et_2O, and dried under reduced pressure. Two additional recrystallizations provided 0.090 g (59%) of material: mp 270–272 °C dec; MS, m/e 265 (M^+ - HCl); $[\alpha]^{26.7}_{578}$ -85.2° (c 0.55, MeOH); NMR (0.005 g in 0.4 mL of Me_2SO-d_6) δ 8.76 (s, 1 H, O*H*, exchanged with D_2O). Anal. ($C_{18}H_{20}ClNO$) C, H, N.

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Registry No. 5, 111635-19-9; 5·HCl, 111635-21-3; 9, 55592-66-0; 10, 40169-97-9; 11, 40169-98-0; 12·HCl, 83207-97-0; 13, 88247-20-5; 13·HBr, 83247-89-6; 14, 111635-20-2; 14·HCl, 111635-22-4.

(Acyloxy)alkyl Carbamates as Novel Bioreversible Prodrugs for Amines: Increased Permeation through Biological Membranes

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(Acyloxy)alkyl carbamates of the type R_1R_2N–CO–O–CHR_3–OCO–R_4 are described as novel bioreversible prodrugs for primary and secondary amines. These were prepared either by a one-step reaction involving nucleophilic attack on *p*-nitrophenyl α-(acyloxy)alkyl carbonates with displacement of *p*-nitrophenol or by reaction of α-haloalkyl carbamates with silver or mercury salts of carboxylic acids. Enzymatic hydrolysis of the ester bond in these ester carbamates leads to a cascade reaction resulting in rapid regeneration of the parent amine. Permeability measurements of such nonionic derivatives of atenolol, betaxolol, pindolol, propranolol, and timolol through fuzzy rat skin and rabbit cornea mounted on diffusion cells show that derivatization of the hydrophilic β-blockers results in several-fold increase in permeation through these biological membranes. However, prodrug modification of the lipophilic β-blockers leads to little advantage in permeability characteristics.

Chemical modification of drugs into labile derivatives (prodrugs[1]) with improved physicochemical properties that enable better transport through biological barriers is a useful approach for improving drug delivery. Such transformation is practiced more often on ionizable molecules such as carboxylic acids[2] and amines in order to modify their ionization at physiological pH and to render desirable partition and solubility properties. Amino functional drugs have been converted[3] in the past to amides,[4] enamines,[5] Schiff bases,[6] oxazolidines,[7] Mannich bases,[8] hydroxymethyl derivatives,[9] *N*-(acyloxy)alkyl derivatives,[10] and carbamates[11] for these purposes. A necessary requirement of this approach is the capability of the prodrug to revert to the parent drug in the body by enzymatic or chemical action, quantitatively, and at a desirable rate. Many of the above amino derivatives are relatively stable to enzymatic hydrolysis and have to depend on acid or base catalysis for reconversion to the parent amines. In addition to this lack of adequate control in regeneration, some of the above methods are limited in their application only to amines with specific features such as low basicity, or with structural requirements such as mono- or trisubstitution on the nitrogen, or the presence of another neighboring functional group (such as hydroxyl).

We chose to study carbamylation as a means of prodrug modification since no bioreversible, chemically stable, and generally applicable prodrug strategy exists for primary and secondary amines. A priori carbamates have the following advantages: (a) carbamylated amines do not ionize and hence are more compatible with organic and lipoidal systems, (b) the modification is applicable to primary and secondary amines essentially irrespective of basicity, (c) there is potential for chemical selectivity in the presence of competing functionalities such as hydroxyl, and (d) they are chemically stable. However, success with a carbamate as a latentiating group requires that the prodrug by hydrolyzed in the body to an alcohol and a carbamic acid. The latter is unstable under physiologic pH and decomposes to the parent amine and carbon dioxide. Though several carbamate prodrugs with simple alcohol leaving groups in the carbamate moiety have been described in literature,[11] the approach has not often met

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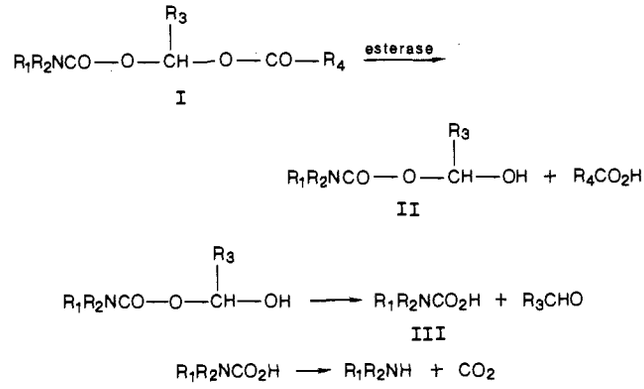
with success, probably because these derivatives were relatively resistant to enzymatic hydrolysis. There does not appear to be a carbamate specific enzyme in mammals.[12] Cholinesterases are known to hydrolyze carbamates,[13] but as the enzyme is reversibly inhibited in the process, the rates are too slow.[14] Although chymotrypsin can undergo carbamylation,[15] this reaction generally does not occur with carbamates. Attempts to facilitate faster hydrolysis of carbamates through chemical manipulation involving neighboring group participation[16] or activation of the leaving group by electron-withdrawing substituents[12,17] have also been described in literature. But these approaches also had very limited success from a prodrug standpoint.

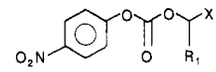
Our results[18] in enzymatically triggering the regeneration of amines from carbamate prodrugs are reported here. Esterases are ubiquitous in most animals including humans, and several types are present in the blood, liver, and other tissues. We anticipated that the desirable properties of a carbamate and the widely utilized hydrolytic suceptibility of esters could be combined in novel *N*-[(acyloxy)alkoxy]carbonyl derivatives of structure I. Ester-



ase-mediated hydrolysis of this modified double ester would lead to a hemiacetal II, which in an aqueous environment would spontaneously decompose to regenerate the parent amine through the intermediacy of carbamic acid III. The eventual side products of the reaction are a carboxylic acid, an aldehyde (or ketone, and carbon dioxide.

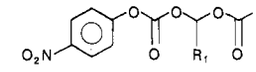
There is a great deal of interest in percutaneous transport of β-blockers for hypertension and ocular delivery for glaucoma. Direct application of many of these drugs cause severe irritation, presumably as a result of alkalinity produced by the strongly basic amines in an aqueous environment. With a view to overcome this problem and to improve the transdermal penetration, we applied this novel prodrug concept to several β-blockers.

Chemistry. The prodrug group was introduced in a one-step reaction involving nucleophilic attack of the primary or secondary amine on *p*-nitrophenyl acetoxyethyl carbonate (**2a**). The reagent **2a** was prepared in two steps.



(1)

a. R_1 = H, X = Cl
b. R_1 = H, X = I
c. R_1 = Me, X = Cl
d. R_1 = Me, X = I



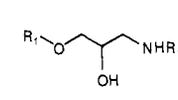
(2)

a. R_1 = R_2 = Me
b. R_1 = Me, R_2 = t-Bu
c. R_1 = H, R_2 = Me

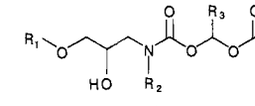
α-Chloroethyl *p*-nitrophenyl carbonate (**1c**) was obtained by the reaction of *p*-nitrophenol with α-chloroethyl chloroformate in the presence of pyridine. Substitution for chloride in the α-chloroethyl carbonate was achieved by reacting it with mercuric acetate in acetic acid. Pivaloxyethyl *p*-nitrophenyl carbonate (**2b**) was prepared similarly with mercuric pivalate in refluxing dichloromethane.

A convenient procedure to obtain acetoxymethyl *p*-nitrophenyl carbonate (**2c**) was to react iodomethyl *p*-nitrophenyl carbonate (**1b**), obtainable from the corresponding chloro compound **1a** by the Finkelstein reaction, with the silver acetate in refluxing benzene. We have found that this reaction also works well with the silver salts of hexanoic, octadecanoic, and nicotinic acids. But this procedure was unsatisfactory for the preparation of (acyloxy)ethyl carbonates such as **2a** because of the tendency of the iodoethyl carbonate **1d** to undergo ready eliminative decomposition.

β-Blockers with *N*-isopropyl substituents, viz., atenolol (**3a**), pindolol (**3b**), propanolol (**3c**), and betaxolol (**3d**), reacted readily with the reagent **2a** to give the corresponding *N*-(acetoxyethoxy)carbonyl derivatives **4a–d**, respectively. The corresponding timolol derivative **4f**,



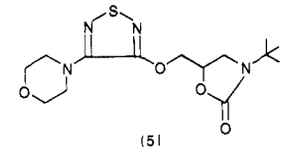
(3)



(4)

a. (atenolol) R_1 = 4-carbamoylmethylphenyl, R_2 = $CHMe_2$, R_3 = Me
b. (pindolol) R_1 = 4-indolyl, R_2 = $CHMe_2$, R_3 = Me
c. (propranolol) R_1 = 1-naphthyl, R_2 = $CHMe_2$, R_3 = Me
d. (betaxalol) R_1 = 4-(cyclopropylmethoxyethyl)phenyl, R_2 = $CHMe_2$, R_3 = Me
e. (timolol) R_1 = 3-(4-morpholino-1,2,5-thiadiazolyl), R_2 = CMe_3, R_3 = H
f. (timolol) R_1 = 3-(4-morpholino-1,2,5-thiadiazolyl), R_2 = CMe_3, R_3 = Me

however, was unstable. Though its presence could be detected in the reaction mixture by TLC and NMR analyses, it could not be isolated in a pure form. The only pure isolable product in the reaction was the corresponding oxazolidone[19] **5**, presumably formed from **4f** by attack of



(5)

the secondary hydroxyl on the carbamate carbonyl with the elimination of acetaldehyde and acetic acid. The instability of **4f** compared to the corresponding derivatives of atenolol, pindolol, propranolol, and betaxolol is attributed to the steric crowding caused by the *N*-*tert*-butyl group. In an attempt to trap the carbamate as the *O*-acetyl derivative, a reaction mixture containing the carbamate, oxazolidone, and unreacted timolol was acetylated with acetyl chloride and pyridine. The *O*-acetyl derivative of the carbamate **4f** was detected by NMR and TLC analyses,

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Table I. Hydrolysis Half-Lives of Selected Prodrugs at 37 °C

prodrug	medium	$t_{1/2}$, min
4e	rat plasma	3
4e	dog plasma	9.7
4e	pH 7.4 phosphate buffer	1410
4c	rat plasma	3
4c	pH 7.4 phosphate buffer	2670

but even this compound decomposed on standing overnight in chloroform solution at room temperature. Reaction of timolol with α-chloroethyl chloroformate also gave the oxazolidone **5**.

However, timolol did form a stable *N*-(acetoxymethoxy)carbonyl derivative **4e**, which, lacking a methyl substituent on the carbamate ester side chain, experiences less steric crowding. This was prepared by reacting timolol with chloromethyl chloroformate followed by substitution of chlorine with silver or mercuric acetate. This two-step sequence using α-chloroethyl chloroformate and mercuric acetate was also employed to obtain the betaxolol derivative **4d** and the propranolol derivative **4c**, but the halide-exchange step in the latter case was complicated by mercuration of the naphthalene ring. Reaction of α-chloroethyl chloroformate with pindolol gave products arising from N- and C-acylation of the indole nucleus in addition to acylation of the secondary nitrogen. The two-step sequence was also applicable to the primary amino function in β-(3,4-dimethoxyphenyl)ethylamine.

Evidence for N- rather than O-substitution in these derivatives of β-hydroxy amines is provided by their mode of formation resulting from the higher nucleophilicity of nitrogen and the better stability of *N*-acyl derivatives.[20] Their insolubility in dilute aqueous acids is made use of in the workup procedure to remove any unreacted amines. Two distinct peaks are present in the IR spectra of these compounds, one at 1740–1750 cm^{-1} assigned to ester carbonyl and the other at 1700–1720 cm^{-1} characteristic of carbamate carbonyl.[21] In the ^1H NMR spectra, the isopropyl groups showed a downfield shift of 0.15–0.2 ppm on derivatization and appeared as overlapping doublets due to the conformational restriction of rotation about the C–N bond of the amide.[22] The *tert*-butyl group in timolol moved 0.3 ppm downfield on carbamylation. Characteristically absent was any acylation shift[23] of the proton α to the secondary hydroxyl group.

Biological Results

In order for these modified carbamates to be useful as prodrugs, they should revert rapidly and quantitatively to the parent amines in the body. The hydrolysis half-lives[25] of the *N*-(acetoxymethoxy)carbonyl derivative of timolol (**4e**) and the *N*-(acetoxyethoxy)carbonyl derivative of propranolol (**4c**) were measured in rat and dog plasma and in pH 7.4 phosphate buffer (see Table I). Although the enzyme-catalyzed hydrolytic reconversion in plasma was very rapid and complete, the hydrolysis of **4e** in phosphate buffer was accompanied by formation of significant quantities of the oxazolidone **5**. No oxazolidone was detected in the buffer hydrolysis of **4c**. We found in a separate experiment that the oxazolidone **5** was completely resistant to enzymatic cleavage to timolol in rat plasma.

Table II. Diffusion of β-Blockers and Prodrugs through Fuzzy Rat Skin

compound	% diffused at 48 h ± SD	mean apparent permeability, nmol/cm^2 per h
atenolol	0.13 ± 0.03	0.36
4a	0.49 ± 0.08	2.04
pindolol	0.03 ± 0.02	0.15
4b	0.35 ± 0.06	2.27
propranolol	2.4 ± 0.59	11.1
4c	0.45 ± 0.15	3.12
timolol	18.14 ± 0.79	199
4e	0.53 ± 0.13	5.3

Table III. Permeation of β-Blockers and Prodrugs through Isolated Albino Rabbit Cornea

compound	permeability, nmol/cm^2 per h
pindolol	5.58
4b	45.36
propranolol	18.9
4c	15.36
betaxolol	14.64
4d	21.96
timolol	7.26
timolol + formaldehyde	7.38
timolol	12.60[a]
4e	39.3
4e	10.32[a]

[a] Denuded (epithelium-free) cornea.

To assess the effect of reversibly masking the amino function on transport through biological membranes, a study of the permeation of these β-blockers and their prodrugs through dermal and corneal membranes was undertaken. Freshly excised fuzzy rat skin mounted on a diffusion cell[26] was chosen as a model for skin permeability studies. Examination of Table II reveals that prodrug modification of hydrophilic[27] atenolol and pindolol resulted in a 5- to 15-fold increase in permeation through fuzzy rat skin whereas in lipophilic timolol and propanolol the modification led to a decrease in permeability. The transport of the prodrugs through the skin was accompanied by complete reconversion to the parent amine. No detectable level of the prodrug was found in the receptor side of the diffusion cell.

As a result of β-adrenergic activity[28] or alkalinity produced by the free base form of the amine, some β-blockers show local skin irritation. It was anticipated that as the carbamates do not undergo protonation under physiologic pH and the amino function necessary for β-adrenergic action is blocked, such prodrugs would not cause local irritation. Timolol, which is a moderate irritant,[26] on occlusive application causes well-defined erythema and

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swelling in which fluid accumulation in the subcutaneous tissue raises the exposed area to approximately 1 mm. These symptoms were not shown by the prodrug **4e**.

The transcorneal penetration rates of the β-blockers and their prodrugs determined with isolated albino rabbit corneas mounted on a diffusion cell[29] are shown in Table III. Increasing the lipophilicity by prodrug modification resulted in greatly increased permeability for pindolol and timolol (8 and 5 times, respectively), but the effect was less significant on the more lipophilic betaxolol and propranolol. The various layers of the cornea, the more lipid like epithelial and endothelial layers and the more polar stroma, contribute differently as barriers to drug penetration, depending on the physicochemical properties of the penetrating agent. The transport of timolol, for example, through denuded albino rabbit cornea (epithelium-free) was faster than that of the prodrug **4e** although in the intact cornea, **4e** moved 5.4 times faster than timolol. In all cases, the transport of the prodrugs through cornea was accompanied by reconversion to the parent drug. Buffer hydrolysis in the donor compartment was 1–2%. Addition of 1 equiv of formaldehyde or acetaldehyde to timolol did not produce any change in the permeation rate.

In conclusion, the *N*-[(acyloxy)alkoxy]carbonyl derivatives have been shown to be potentially useful biolabile prodrugs for amino functional drugs. This novel prodrug moiety combines the desirable chemical properties of a carbamate and the susceptibility to undergo enzymatic regeneration of the parent amine by ester hydrolysis. The chemical stability of such carbamate esters has been shown[25] to depend on the hydrolytic stability of the ester function and has been found to be high.[30] The possibility for variation in the structure of the carboxylic acid group should allow modulation of water/lipid solubility properties and enzymatic/chemical hydrolytic susceptibility.

Experimental Section

Chemistry. The following spectrometers were used: IR, Nicolet 5DX or Beckman Acculab 4; ^{1}H NMR, Varian T-60; MS, Riber R_{10}-10 interfaced with a PDP-8A computer. Chemical shifts are reported in parts per million relative to tetramethylsilane internal reference. Column chromatography was performed on silica gel 60 (E. Merck). Precoated silica gel 60 F254 (E. Merck) or Analtech GHLF plates were used for thin-layer chromatography.

α-Acetoxyethyl *p*-Nitrophenyl Carbonate (2a). To an ice-cold reaction mixture of 1.39 g (10 mmol) of *p*-nitrophenol and 0.8 g (10 mmol) of pyridine in 50 mL of chloroform was added 1.7 g (11 mmol) of α-chloroethyl chloroformate.[24] The cooling bath was removed after $^1/_2$ h, and the reaction mixture was stirred at room temperature for 16 h. After being washed successively with water, 0.5% aqueous sodium hydroxide, and water, the chloroform layer was dried (Na_2SO_4) and evaporated to a thick light yellow oil 2.3 g (93%), which was practically pure α-chloroethyl *p*-nitrophenyl carbonate (**1c**). It was crystallized from hexane: mp 69–70 °C; IR (KBr) 1778 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.93 (d, 3 H, CHMe), 6.53 (q, 1 H, CHCl), 7.40 (d, 2 H, aromatic), 8.26 (d, 2 H, aromatic). Anal. ($C_9H_8NO_5Cl$) C, H, N, Cl.

To a solution of 1 g (4 mmol) of the above chloroethyl carbonate in 25 mL of acetic acid was added 1.5 g (4.7 mmol) of mercuric acetate, and the mixture was stirred at room temperature for 22 h. The acetic acid was evaporated off in vacuum at room temperature, and the residue taken in ether was washed free of acid. Evaporation of ether gave 1.1 g of a thick oil. Chromatography of this on silica gel with chloroform–ethyl acetate (95:5) gave 0.82 g (74.5%) of the pure **2a** as an oil: IR (film) 1745 (C=O), 1616, 1594, 1527, 1492, 1349, 1266, 1214, 1109, 1076, 858 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.62 (d, 3 H, CHMe), 2.13 (s, 3 H, OAc), 6.86 (q, 1 H, CHOAc), 7.41 (d, 2 H, aromatic), 8.28 (d, 2 H, aromatic). Anal. ($C_{11}H_{11}NO_7$) C, H, N.

Method A. 4-[2-Hydroxy-3-[*N*-[(1-acetoxyethyl)carbonyl]-*N*-isopropylamino]propoxy]indole (4b). A solution of 1 g (4 mmol) of pindolol and 1 g (4 mmol) of **2a** in 5 mL of dry hexamethylphosphoramide was stirred at room temperature. When the reaction was practically complete (TLC), the reaction mixture was diluted with water to about 100 mL and extracted with ether. The ether extract was washed repeatedly with ice-cold 0.5% aqueous sodium hydroxide and water, dried (Na_2SO_4), and evaporated to furnish a residue weighing 1.15 g. Chromatography on silica gel using ethyl acetate–chloroform (5:95) for elution gave 0.8 g (52.6%) of the pure **4b** as a glass: IR (KBr) 3410, 1745, 1701 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20 and 1.25 (d, 3 H each, isopropyl), 1.51 (d, 3 H, CHMe), 2.05 (s, 3 H, OAc), 3.5 (br d, 2 H, NCH_2), 4.13 (m, 4 H, HCOH, CHN, and OCH_2), 6.55 (m, 2 H, aromatic), 6.81 (q, 1 H, MeCH), 7.0 (m, 3 H, aromatic) 8.31 (br, 1 H, aromatic NH); MS, *m/e* 378 (M^+), 275, 202; (M^+) calcd for $C_{19}H_{26}N_2O_6$ 378.1789, obsd 378.1794.

1-[*N*-[(1-Acetoxyethoxy)carbonyl]-*N*-isopropylamino]-3-(1-naphthyloxy)-2-propanol (4c) was obtained as an oil after column chromatographic purification in 61% yield by method A with 1.5 g (5 mmol) of propranolol hydrochloride and 1.36 g (5 mmol) of Proton Sponge (Aldrich): IR (film) 3475, 1748, 1705 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20 and 1.24 (d, 3 H each, $CHMe_2$), 1.49 (d, 3 H, $CHMe)_3$, 2.05 (s, 3 H, OAc), 3.51 (d, 2 H, NCH_2), 3.9–4.5 (m, 4 H, HCOH, CHN, and OCH_2), 6.80 (q, 1 H, MeCH), 6.6–8.3 (m, 7 H, aromatic); MS, *m/e* 389 (M^+), 286, 202; (M^+) calcd for $C_{21}H_{27}NO_6$ 389.1836, obsd 389.1827.

4-[2-Hydroxy-3-[*N*-[(1-acetoxyethoxy)carbonyl]-*N*-isopropylamino]propoxy]benzeneacetamide (4a) was obtained as an oil in 32% yield after chromatographic purification by method A: IR (film) 3476, 1747, 1700, 1682 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.21 (d, 6 H, $CHMe_2$), 1.48 (d, 3 H, CHMe), 2.03 (s, 3 H, OAc), 3.40 (d, 2 H, NCH_2), 3.46 (s, 2 H, $NCOCH_2$), 3.8–4.3 (m, 5 H, OCH_2, OH, HCO, and NCH), 5.7 and 6.16 (br s, 1 H each, $CONH_2$), 6.8 (q, 1 H, MeCH), 6.83 (s, 2 H, aromatic), 7.13 (d, 2 H, aromatic). Anal. ($C_{19}H_{28}N_2O_7$) C, H, N.

Method B. 3-[3-[*N*-[(Acetoxymethoxy)carbonyl]-*N*-*tert*-butylamino]-2-hydroxypropoxy]-4-morpholino-1,2,5-thiadiazole (4e). A solution of 3.6 g (11.4 mmol) of timolol in 100 mL of chloroform was cooled in an ice bath, and 2.5 g (11.6 mmol) of Proton Sponge was added to it, followed by the dropwise addition of 1.5 g (11.6 mmol) of chloromethyl chloroformate. After being stirred at room temperature for 16 h, the reaction mixture was washed (1 N HCl and water), dried (Na_2SO_4), and evaporated to furnish 4.34 g (91%) of the corresponding chloromethyl carbamate, which was practically pure by TLC: IR (film) 3400–3450, 1730, 1538 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9 H, CMe_3), 2.96 (s, 1 H, OH exchangeable with D_2O), 3.3–4.0 (m, br, 10 H, morpholino H and NCH_2), 4.20 (m, 1 H, HCO), 4.5 (m, 2 H, OCH_2), 5.76 (s, 2 H, OCH_2Cl).

The above chloromethyl carbamate (4.3 g, 10.3 mmol) was dissolved in 100 mL of acetic acid and stirred with 5 g (30 mmol) of silver acetate. After 4.5 h, the reaction mixture was filtered, and the filtrate was evaporated. The residue was dissolved in chloroform and washed with water. Evaporation of chloroform gave 4.6 g of the crude product which was purified by preparative liquid chromatography to furnish 2.1 g (50%) of pure **4e** as an oil: IR (film) 3480, 1763, 1726, cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9 H, CMe_3), 2.08 (s, 3 H, OAc), 3.6–3.8 (m, 10 H, morpholino H and NCH_2), 4.2 (m, 1 H, HCO), 4.43 (d, 2 H, OCH_2), 5.73 (s, 2 H, OCH_2O); MS, *m/e* 432 (M^+), 433 ($M + 1)^+$, 377, 287, 246. Anal. ($C_{17}H_{28}H_4O_7S$) C, H, N, S.

1-[*N*-[(1-Acetoxyethoxy)carbonyl]-*N*-isopropylamino]-3-[*p*-(cyclopropylmethoxyethyl)phenoxy]-2-propanol (4d). Method B. Reaction of 150 mg (0.43 mmol) of betaxolol hydrochloride, 188 mg (0.87 mmol) of 1,8-bis(dimethylamino)-naphthalene, and 74 mg (0.5 mmol) of α-chloroethyl chloroformate in chloroform gave the corresponding α-chloroethyl carbamate: IR (film) ν 3446, 2935, 2869, 1715, 1512, 1245, 1097 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.05–0.75 (m, 4 H), 0.9–1.05 (m, 1 H), 1.21 (d, 6 H), 1.8

(29) Edelhauser, H. F.; Hoffert, J. R.; Fromm, P. O. *Invest. Ophthalmol.* **1965**, *4*, 290. O'Brien, W. J.; Edelhauser, H. F.; *Invest, Ophthalmol. Visual Sci.* **1977**, *16*, 1093; Schownwald, R. D.; Ward, R. L. *J. Pharm. Sci.* **1978**, *67*, 786.

(30) The shelf life in aqueous solution has been estimated to be 3–5 years at optimal conditions (when stored at 4 °C at pH 4).

(d, 3 H), 2.06 (s, 3 H), 2.80 (t, 2 H), 3.25 (d, 2 H), 3.4–3.7 (m, 3 H), 3.8–4.7 (m, 4 H), 6.58 (q, 1 H), 6.78 (s, 2 H), 7.11 (d, 2 H). Without purification, this compound was stirred with mercuric acetate (200 mg) in acetic acid (10 mL) for 8 h. The acetic acid was distilled off in vacuum, and the organic compound was extracted into chloroform. The chloroform extract after washing and drying was evaporated. Chromatography of the residue on silica gel furnished pure **4d** as an oil (140 mg): IR (film) ν 3458, 1758, 1712, 1512, 1244, 1081, 1011, 943 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05–0.75 (m, 4 H), 0.9–1.05 (m, 1 H), 1.21 (d, 6 H), 1.5 (d, 3 H), 2.06 (s, 3 H), 2.81 (t, 3 H), 3.26 (d, 2 H), 3.4–3.8 (m, 3 H), 3.8–4.4 (m, 4 H), 6.78 (d, 2 H), 6.81 (q, 1 H), 7.08 (d, 2 H); MS, m/e 437; (M^+) calcd for $C_{23}H_{35}NO_7$ 437.2411, obsd 437.2412.

***N*-[(1-Acetoxyethoxy)carbonyl]-β-(3,4-dimethoxyphenyl)ethylamine** prepared by method B, with mercuric acetate, has the following spectral properties: IR (film) 3345, 1742, 1723 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (d, 3 H, CHMe), 2.06 (s, 3 H, OAc), 2.76 (t, 2 H, $ArCH_2$), 3.38 (t, 2 H, $NHCH_2$), 3.9 (s, 6 H, OMe), 5.06 (t, br, 1 H, NH), 6.6–7.0 (m, 4 H, aromatic and MeCH); MS, m/e 311 (M^+), 207, 164, 151. Anal. ($C_{15}H_{21}NO_6$) C, H, N.

Analytical Methods. A high-pressure liquid chromatography method was used for the analysis of the β-blockers and their prodrugs. The chromatographic analysis was performed on a component system consisting of a Waters Associates Model 6000 A solvent delivery system, a Rheodyne injector, a Kratos Spectraflo 773 variable-wavelength detector, and a Waters data module. A 20-μL injection of each sample and a standard of similar concentration was made on the HPLC, which was equipped with a 10-cm Spheri-5 RP-18 column (Brownlee Labs) and a similar 4-cm guard column at ambient temperature. The mobile phase was 45%–70% CH_3CN containing 0.1% acetic acid and 0.1% sodium lauryl sulfate. The flow rate was 2 mL/min. The detector was set at 295 nm for propranolol and timolol, at 275 nm for atenolol, and at 220 nm for pindolol. The concentration was calculated from the areas of the sample and standard peak and the concentration of the standards by using an external standard procedure.

Plasma Hydrolysis. One milliliter of plasma maintained at 37 °C was added to 0.1–0.2 mg of prodrug dissolved in a small drop of DMSO. The plasma solution was placed in a thermostated water bath at 37 °C. The disappearance of the prodrug and/or appearance of the parent amine was followed by withdrawing samples at various time points and subjecting them to HPLC analysis by direct injection. Buffer hydrolysis was conducted similarly with pH 7.4 Sorensen's buffer instead of plasma.

In Vitro Diffusion Cell Model for Skin Permeability. Fuzzy rats, 8 weeks old and weighing an average of 250 g, were used to study in vitro dermal permeation of timolol. The animals were obtained from Animal Services, Skin and Cancer Hospital, Temple University, Philadelphia, PA. The diffusion cells consisted of a Plexiglas receptor chamber equipped with a sidearm to allow receptor phase sampling, a Teflon lid, and a Teflon-coated stirring bar (Kersco Engineering Consultants, Palo Alto, CA). The receptor fluid was 45 mL of buffer solution consisting of 0.15 M NaCl, 0.47 mM NaH_2PO_4, 0.8 mM Na_2HPO_4, and 22 ppm of gentamicin sulfate, adjusted to pH 7.0 with 0.067 M NaH_2PO_4 and/or 0.067 M Na_2HPO_4. Rats were sacrificed by injection of a euthanasia solution, and the whole ventral and dorsal skin was removed. A section of skin was stretched over the lower opening of the Teflon lid and secured with a rubber gasket. The lid was placed firmly on the lower chamber of the diffusion cell and held in place with retaining bolts. The opening in the lid left an 8-cm^2 area exposed on the epidermal side through which permeation occurred. Ethanolic solution of the test substances were applied by droppers and were spread evenly over the skin surface with smooth glass rods. The cells were occluded, placed in a 32 °C incubator, and stirred at 150 rpm. Periodically 0.2-mL samples were taken over a 48-h period, and an equal volume of fresh buffer solution was added to the cell. The samples were stored frozen until assayed at the end of the experiment. The experiments were done in triplicate. The following are the amounts applied to the skin surface: timolol, 130 mg; **4e**, 107 mg; atenolol, 53.3 mg; **4a**, 84.5 mg; pindolol, 49.6 mg; **4b**, 61.7; propranolol, 46.0 mg; **4c**, 69.1 mg. Mean apparent permeability is expressed as the amount permeated per unit area per hour.

Skin Irritation Study. Fuzzy rats (Temple University), 150–200 g, were used to study the dermal irritancy. Test preparations (50 mg) were applied to circular gause pads, 1 mm thick and 16 mm in diameter, and affixed to the animals' dorsal surface with occlusive adhesive film (Adhesive Plaster for Patch Test, Kanebo, Ltd., Osaka, Japan). The occlusive dressings were removed after 3 or 7 days. Treated skin areas were then evaluated according to a modified Draize scoring method, and the irritation index was evaluated for each test site. The first or "primary irritation index" (PII) was an average value reflecting irritation both immediately after dressing removal and 72 h later. The "secondary irritation index" (SII) was determined 7 days after dressing removal. The maximum possible PII or SII was 8, with a total possible score of 4 for erythema and a total possible score of 4 for edema. A PII or SII less than or equal to 2 indicated a mild irritant, a PII or SII greater than 2 but less than or equal to 6 indicated a moderate irritant, and a PII greater than 6 indicated a severe irritant.

Timolol (neat) had a PII of 2 ± 1.5 (n = 9) for 3-day application and 3 ± 2 (n = 5) for 7-day application. The SII was 0.5 ± 1 (n = 9) for 3-day application and 2 ± 2 (n = 5) for 7-day application. The prodrug **4e** (neat) had a PII of 0.1 ± 0.1 (n = 3) for 3-day application and no irritation for 7-day application. Atenolol, **4a**, and propranolol did not show any irritation in this model.

Measurement of Corneal Penetration. Ablino rabbits (~2.5 kg) were sacrificed, and the eyes were enucleated with their lids and conjuctival sacs attached. After excess tissue was removed, the cornea was tied to a corneal holder[29] at the conjuctiva with a silk thread, and the scleral ring was pressed into the corneal holder to expose the intact cornea with surface area of 1.1 cm^2. The mounted cornea was rinsed thrice with a bicarbonate–Ringer's solution (NaCl, 111.5 mM; KCl, 4.82 mM, $CaCl_2$, 1.04 mM; $MgCl_2$, 0.75 mM, NaH_2PO_4, 0.86 mM; $NaHCO_3$, 29.2 mM; and glucose, 5.01 mM) and placed in a lucite block corneal perfusion chamber.[29] The bicarbonate–Ringer's solution (6.5 mL) was added to the endothelial side, and a solution or suspension containing the test compound (6.5 mg) in the Ringer's solution (6.5 mL) was placed in the epithelial side of the perfusion chamber. The block system and the bathing solutions were maintained at a constant temperature of 37 °C. Constant mixing and a pH of 7.6 were maintained in the bathing fluid by a steady stream of 5% CO_2–95% air through siphon air lift ports entering both sides of the diffusion chamber. Serial samples (0.2 mL) were removed from each reservoir at 1 h and then half hourly for 4 h, and the concentration of the samples were analyzed by HPLC by establishing a standard for each compound.

Registry No. **1c**, 101623-69-2; **2a**, 101623-68-1; **3a**, 29122-68-7; **3b**, 13523-86-9; **3c**·HCl, 318-98-9; **3d**·HCl, 63659-19-8; **3d**(α-chloroethyl carbamate), 111823-09-7; **3e**, 26839-75-8; **3e** (chloromethyl carbamate), 111823-08-6; **4a**, 101623-75-0; **4b**, 101623-74-9; **4c**, 99106-37-3; **4d**, 101623-77-2; **4e**, 99106-28-2; $ClCO_2CH(Cl)Me$, 50893-53-3; $ClCO_2CH_2Cl$, 22128-62-7; $MeCH(OAc)OCONH(CH_2)_2$-3,4-$(OMe)_2C_6H_3$, 99106-33-9; $NH_2(CH_2)_2$-3,4-$(OMe)_2C_6H_3$, 120-20-7; $ClCO_2Et$, 541-41-3.