Development of a Highly Cardioselective Ultra Short-Acting β-Blocker, ONO-1101

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A novel, highly cardioselective ultra short-acting β-blocker, ONO-1101, has been developed for application in the emergency treatment of tachycardia and better control of heart rate in surgery. This agent is approximately nine times more potent in β-blocking activity *in vivo* and eight times more cardioselective *in vitro* than esmolol. This β-blocking drug has a short duration of activity, enabling rapid recovery after cessation of administration if side effects occur. It can be used safely in patients suffering from acute heart disease and represents a major therapeutic advance in the treatment of heart disease.

Keywords β-blocker; ultra short-acting; cardioselective; emergency treatment; heart disease; ONO-1101; controllable therapy

β-Blockers, of which propranolol is representative, are widely used by oral administration for the treatment of hypertension and arrhythmia. β-Blocker drugs exert their effects by competitively inhibiting the binding of catecholamines to β-adrenergic receptors. For emergency treatment of tachycardia and better control of heart rate in surgery, either intravenous bolus injection or infusion is usually used in order to achieve a therapeutic level rapidly. However, because of the long-lasting effect of currently available β-blockers, the unexpected emergence of side effects, especially acute cardiac failure by β_1-blocking activity and bronchospasm by β_2-blocking activity, poses a significant problem in their usage.[1] Therefore, there is a need for a β-blocker with a short period of action which can be rapidly removed if side effects occur, *i.e.* an ultra short-acting β-blocker.[2] It is important that such an agent would allow not only controllable induction of β-blockade but also rapid recovery if adverse effects occur. It is difficult to achieve these actions with the currently available long-acting agents.

The use of an ultra short-acting β-blocker in patients with acute ischemic heart disease, where prolonged exposure to β-blockade would remove essential sympathetic support, leading to heart failure,[3] is a novel concept. Modification of a β-blocker with substituents which would be readily metabolized to functional groups known to produce inactivity against the β_1 receptor should be a means of providing controlled and titratable therapy for post-myocardial-infraction patients.

Esmolol, which is now under clinical use, is the first ultra short-acting β-blocker developed by American Critical Care.[4] It exhibits fair cardioselectivity ($\beta_1/\beta_2 = 32$) with a rapid onset of infusion. The ultra short-duration of esmolol is due to the rapid hydrolysis of its alkyl ester link, mediated primarily *via* an esterase which is located in the red blood cell cytosol. With respect to the mechanism of its ultra short-duration of action,[4] it was believed that hydrolysis of esmolol and similar esters produces compounds whose carboxylate anion dramatically increases the polarity of the aryl system present in β-blockers (Chart 1). It is possible that such an alteration would render the hydrolyzed molecules unacceptable to the β-adrenergic receptor and that these compounds would, therefore, be inactive or only very weakly active as β-adrenergic blocking agents.

Due to the low activity of esmolol compared to conventional β-blockers (1/50—1/100 compared to propranolol), large amounts of this agent are required to achieve a therapeutic level. Therefore, we have searched for more potent ultra short-acting β-blockers with higher cardioselectivity. This has culminated in the development of ONO-1101. In this paper we describe the chemical synthesis of ONO-1101, its related compounds and structure–activity relationships.

Chemistry The synthesis of ONO-1101 and its related compounds proceeded as shown in Chart 2. Treatment of appropriate 3-(4-hydroxyphenyl)propionic acid (**1**) with 1 eq of potassium hydroxide in ethanol at 23 °C afforded the

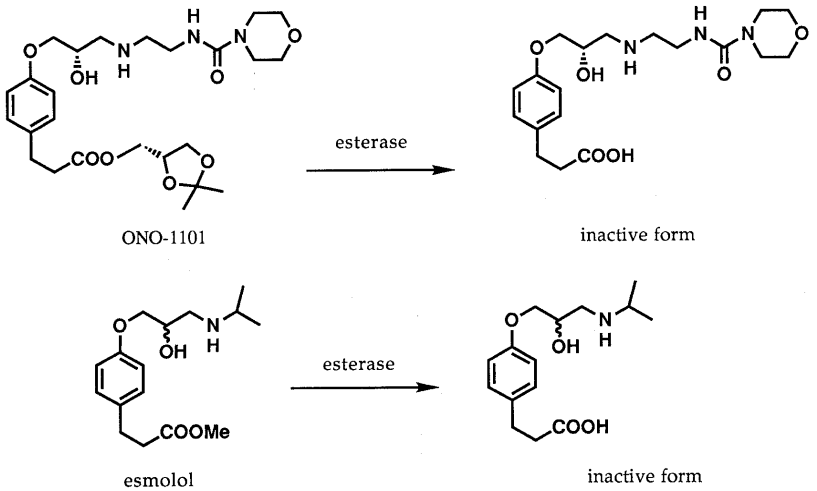


Chart 1. Structures of ONO-1101, Esmolol, and Their Inactive Forms

potassium carboxylate **2** in 96% yield, which precipitated immediately from the reaction medium. After filtration and drying, **2** was allowed to react with the alkyl tosylate, [(*S*)-(+)-2,2-dimethyl-1,3-dioxolan-4-ylmethyl *p*-toluenesulfonate (**3**) in the case of ONO-1101] or alkyl halide in anhydrous dimethylsulfoxide at 100 °C to give the ester **4** in good yield. The possible byproduct, phenylether, was not detected by thin-layer chromatographic (TLC) analysis. Alternatively, esterification with methanol, ethanol, or 2-propanol was effected by refluxing the subject mixture in the desired alcohol in the presence of a catalytic amount of concentrated H_2SO_4 while removing water with a Soxhlet extractor charged with 3A molecular sieves.[5] The phenolic ester **4** was alkylated with excess epibromohydrin or (2*S*)-(+)-glycidyl tosylate (**5**) in dimethylformamide (DMF) using potassium carbonate. The resulting epoxide **6** was then opened by an equivalent amount of the appropriate alkylamine in 2-propanol at room temperature to afford the desired final product **8**. As expected,[6] nucleophilic attack at the secondary carbon atom was exclusively observed, as determined by TLC and nuclear magnetic resonance (NMR) analysis of the crude reaction products. When methanol was utilized as a solvent instead of 2-propanol in the ring-opening reaction with the alkyl amine, total transesterification to the methyl ester occurred.

The biologically potent β-blockers possessing a triple bond in the molecule were synthesized starting with 4-hydroxyphenylpropiolic acid (**11**) in the same way as shown in Chart 3. This substance was prepared by three different methods. As shown in Chart 3, method A, 4-tetrahydropyranyloxybenzaldehyde (**9**) was allowed to react with the ylide derived from (bromomethyl)-triphenylphosphonium bromide[7] and excess potassium *tert*-butoxide in tetrahydrofuran (THF) at −78 °C to give the phenylacetylene **10** in 74% yield. The resulting phenylacetylene was treated sequentially with *n*-butyllithium in THF at −78 °C and carbon dioxide to produce 4-hydroxyphenylpropiolic acid (**11**) after acidic workup in good yield. The second method (Chart 3, method B) started with 4-methoxybenzaldehyde (**12**). Quantitative dibromoolefination[8] of this aldehyde **12** was effected by treatment with triphenylphosphine and carbon tetrabromide in dichloromethane. After deprotection of the phenolic hydroxyl group with boron tribromide, treatment with 3 eq of *n*-butyllithium in THF at −78 °C followed by addition of carbon dioxide produced 4-hydroxyphenylpropiolic acid (**11**) in 82% yield. The third procedure (Chart 4) was suitable for a large scale production. 4-Hydroxy-3-methoxycinnamic acid (**15**) was esterified with methanol in the presence of a catalytic amount of concentrated H_2SO_4 and then acetylated with acetic anhydride in pyridine to yield the ester **17** quantitatively. Addition of bromine to olefin **17** readily gave **18**. Treatment of the dibromide **18** with potassium hydroxide in a mixture of 2-propanol and ethanol

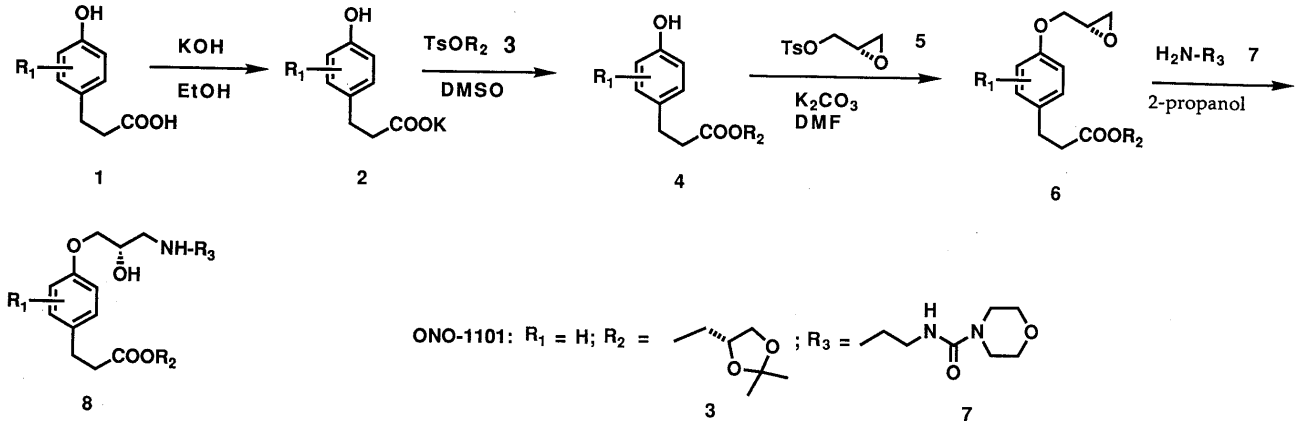


Chart 2. Synthesis of ONO-1101 and Its Related Compounds

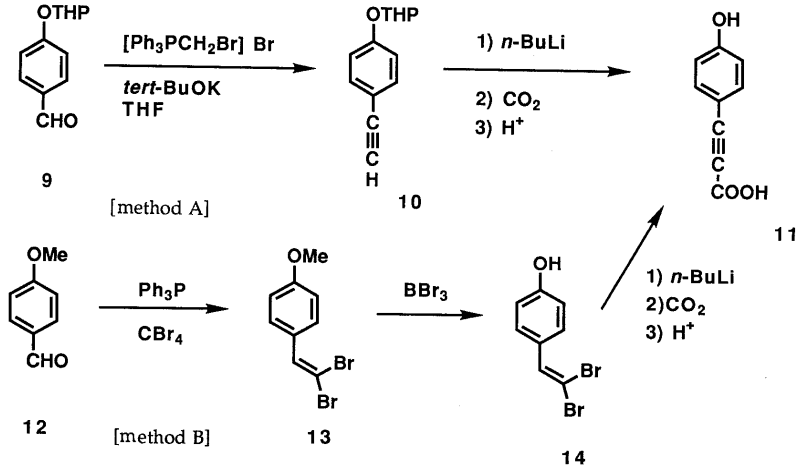


Chart 3. Synthesis of 3-(4-Hydroxyphenyl)propiolic Acid

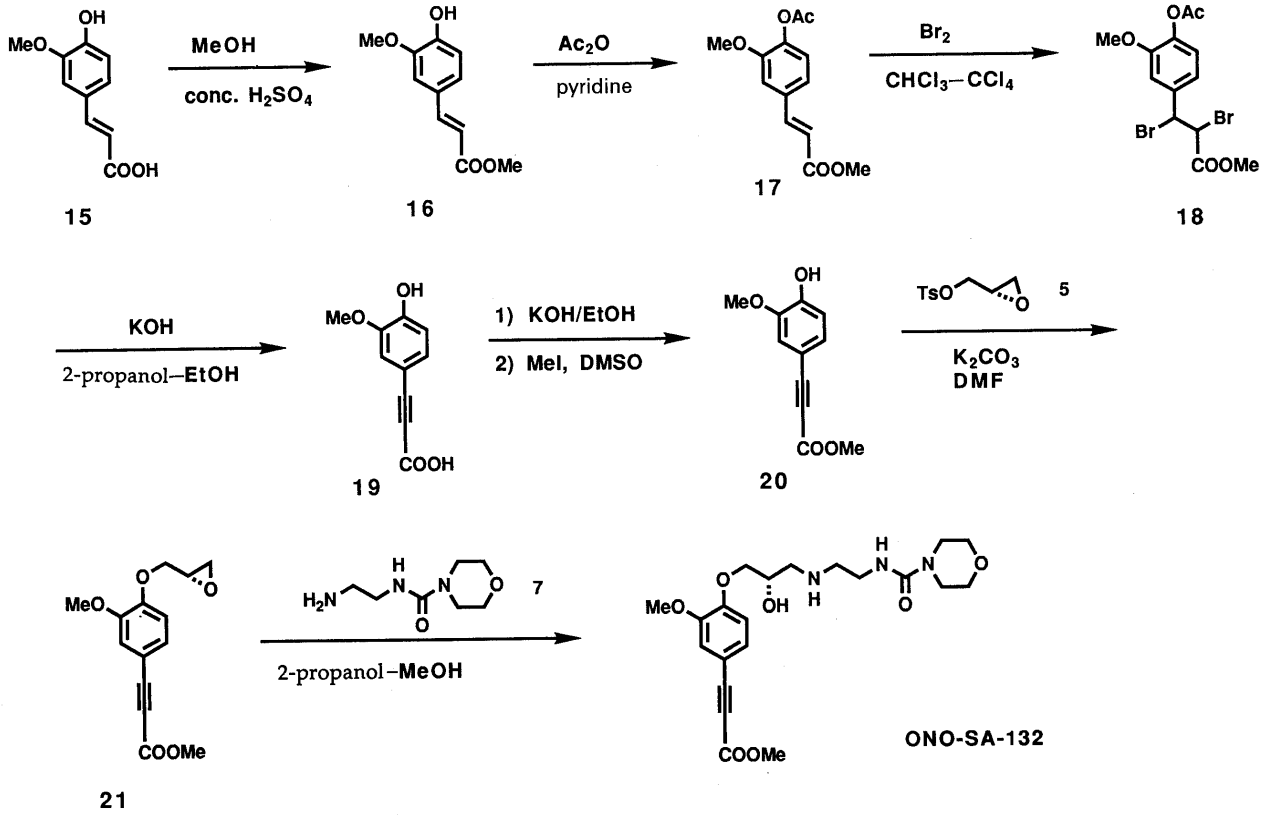


Chart 4. Synthesis of ONO-SA-132 [Method C]

at 100 °C afforded 4-hydroxy-3-methoxyphenylpropiolic acid (**19**) in 65% yield from **17**. Conversion of **19** to SA-132 was carried out by the same procedure as described for ONO-1101.

Biological Results and Discussion

β-Blocking activity and duration of action were determined *in vivo* using anesthetized dogs. Cardioselectivity (β_1/β_2-receptor activity) was assessed *in vitro* with guinea pig right atria and tracheal strips mounted in tissue baths containing oxygenated Krebs physiological salt solution at 37 °C.

ONO-1101 and esmolol were intravenously administered *in vivo* at a rate of 10 and 100 μg/kg/min, respectively. As shown in Fig. 1 (ordinate: percent inhibition of heart rate response to isoproterenol; abscissa: time after initiation of infusion of β-blockers), in both compounds, steady-state levels were rapidly achieved (10 min post infusion start) and maintained thereafter during the infusion. Approximately the same inhibition was observed in infusions of 10 μg/kg/min ONO-1101 and 100 μg/kg/min esmolol. After termination of infusion, the inhibitory effect decreased rapidly, and 50% recovery from the blockade occurred in about 10 min; complete recovery was observed about 30 min after cessation of the infusion. ONO-1101 has a rapid onset (10 min) and offset of action (20—30 min), and is thus acting as an ultra short-acting β-blocker.

Modification of the ester group of esmolol did not afford superior compounds to esmolol in β-blocking activity, duration of action, or cardioselectivity (Table I). At the next stage, the aminoalcohol moiety was subsequently modified. Replacing the isopropyl group with the

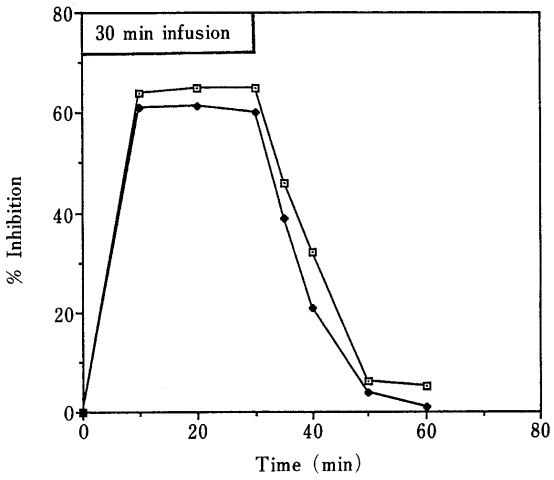


Fig. 1. Inhibitions of Isoproterenol Induced Tachycardia by ONO-1101 and Esmolol (30 min Infusion) in Anesthetized Dogs

Ordinate: percent inhibition of heart rate response to isoproterenol; abscissa: time after initiation of infusion of β-blockers. ⊡, esmolol (100 μg/kg/min); ◆, ONO-1101 (10 μg/kg/min).

2,2-dimethylethylurea function (SA-032 and SA-048, Table II) increased the β-blocking activity (30 and 10 times more potent than esmolol, respectively); however, the cardioselectivities of SA-032 ($\beta_1/\beta_2 = 1.2$) and SA-048 ($\beta_1/\beta_2 = 1.6$) were about 20—25 fold less than that of esmolol ($\beta_1/\beta_2 = 33$). This lack of cardioselectivity was undesirable as the contraction of trachea (β_2-blocking activity) may cause serious complications in clinical use. Removal of the dimethyl group in SA-048 led to a ten-fold decrease in the β-blocking activity (SA-050, data and structure not

TABLE I. Biological Activities of Esmolol Type Analogs

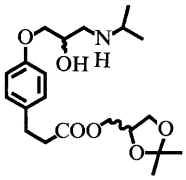
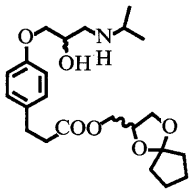
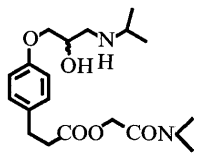
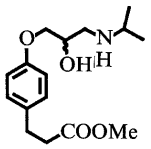
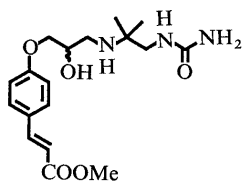
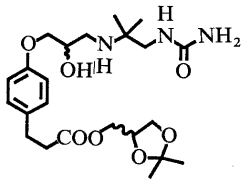
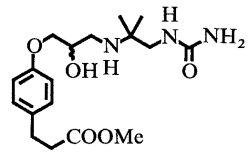
Compound	Structure	Activity Esmolol = 1	Duration $t_{1/2}$ (min)	Cardio-selectivity (β_1/β_2)
SA-001		0.9	5	11
SA-009		0.9	6	—
SA-015		0.3	6	—
Esmolol		1.0	9	33

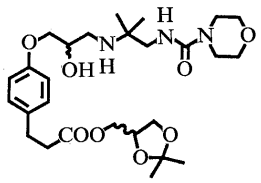
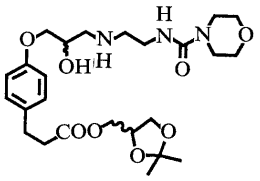
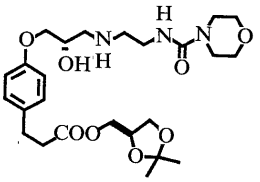
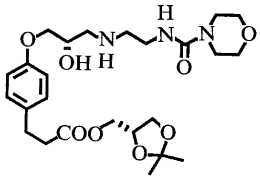
TABLE II. Biological Activities of Urea Type Analogs

Compound	Structure	Activity Esmolol = 1	Duration $t_{1/2}$ (min)	Cardio-selectivity (β_1/β_2)
SA-098		30	360	—
SA-048		10	9	1.6
SA-032		35	13	1.2

shown).

Replacement of the urea function with morpholinocarbonylamino moiety (Table III) yielded higher cardioselectivity. SA-064 ($\beta_1/\beta_2 = 7.4$), which had the dimethyl group next to the amino group, was two times more potent than SA-062 ($\beta_1/\beta_2 = 37$) which had no dimethyl group. However, this increase in the β-blocking activity was accompanied by a decrease in its cardioselectivity. The cardioselectivity of

TABLE III. Biological Activities of Morpholino Type Analogs

Compound	Structure	Activity Esmolol = 1	Duration $t_{1/2}$ (min)	Cardio-selectivity (β_1/β_2)
SA-064		10	15	7.4
SA-062		5	10	37
SA-109		9	12	30
ONO-1101		9	9	255

SA-062 approached that of esmolol. All four isomers of SA-062 were synthesized and tested. The two isomers having a hydroxyl group in *R*-configuration were almost inactive. SA-109, which has *S*-configurated hydroxyl and *R*-configurated ester functions, had 9 times more potent β-blocking activity than esmolol. However, its duration of action was somewhat longer ($t_{1/2} = 12$ min) and cardioselectivity was almost the same as that of esmolol ($\beta_1/\beta_2 = 30$, Table III). ONO-1101, which has *S*-configurated hydroxyl and *S*-configurated ester functions, provided the optimum performance of the compounds tested: 9 times more potent in the β-blocking activity and 8 times more potent in the cardioselectivity ($\beta_1/\beta_2 = 255$, Table V) compared with esmolol. It seems that the high cardioselectivity of ONO-1101 is due to the stereochemistry of its ester and hydroxyl functions. Substituents (*e.g.*, MeO, CN, halogen, NO_2, or allyl group) were introduced into the phenyl ring at the *ortho* position to the oxygen atom of the ether linkage without significant influence on β-blocking activity, duration of action, or cardioselectivity.

Finally, a double bond or triple bond was introduced between the phenyl ring and the ester function (Tables II and IV). With respect to the alkyl chain between the phenyl ring and the ester function, it was known that the ethylene chain here is the best for the short-duration of action.[4] The olefinic analog (SA-098) was about 30 times more potent in β-blocking activity than esmolol, but it was not short-acting: 30 min after the termination of infusion, the same β-blocking activity was maintained compared with its steady state during infusion. The acetylene SA-129 showed 10-fold higher activity in β-blocking effect than esmolol with

TABLE IV. Biological Activities of Morpholino Type Analogs Containing the Triple Bond

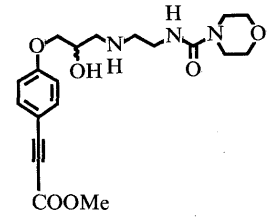
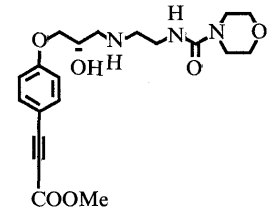
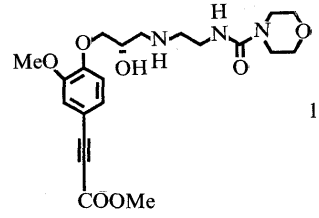
Compound	Structure	Activity Esmolol=1	Duration $t_{1/2}$ (min)	Cardio-selectivity (β_1/β_2)
SA-113		5	10	19
SA-129		10	10	7
SA-132		16	9	14

TABLE V. *In Vitro* Cardioselectivity of Ultra Short-Acting β-Blockers

Drug	Atria		Trachea		Cardioselectivity (β_1/β_2)
	n	pA_2	*n*	pA_2	
ONO-1101	14	6.59	9	4.18	255
ONO-SA-132	15	6.75	12	5.62	14
Esmolol	15	6.11	14	4.59	33
Propranolol	12	8.60	12	8.68	0.68

the same duration of action and lower cardioselectivity ($\beta_1/\beta_2=7$). Further, by introduction of a methoxy group into the phenyl ring, SA-132 was obtained which exhibited about a 1.6-fold increase in β-blocking activity and 2-fold increase in cardioselectivity ($\beta_1/\beta_2=14$, Table V) over SA-129.

The LD_{50} values (mg/kg, i.v.) for acute toxicity (mice) of ONO-1101, SA-132, and esmolol were 290, 91, and 83, respectively. ONO-1101 showed the lowest toxicity of the three compounds.

According to the above results, ONO-1101 may be administered safely to patients suffering from heart disease, and it is also expected to give the controllable and titratable therapy necessary for the emergency treatment of tachycardia and better control of heart rate during surgery. Full pharmacological data will be published in due course.

Experimental

^{1}H- and ^{13}C-NMR spectra were taken on a Varian VXR500S, VXR200S, or JEOL FX90Q FT spectrometer in $CDCl_3$ or CD_3OD. Chemical shifts are reported as parts per million relative to tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR 1760X spectrometer. Mass spectra (MS) were obtained on a JEOL JMS-DX303HF (for electron impact (EI)- and exact MS) mass spectrometer. For TLC analysis throughout this work, Merck TLC plates (Kiesel gel $60F_{254}$, pre-coated, layer thickness 0.25 mm) were used. Column chromatography was carried out on silica gel (YMC gel, particle size 70/230 mesh, Mallinckrodt CC-7 gel, or Wako gel C200). Microanalyses were performed by the Material Analysis Center of the Institute of Scientific and Industrial Research, Osaka University. Unless otherwise specified, all reactions were carried out under an atmosphere of argon. THF was distilled from the sodium benzophenone ketyl under argon. CH_2Cl_2 was distilled from calcium hydride.

2,2-Dimethyl-1,3-dioxolan-4*S*-ylmethyl 3-(4-Hydroxyphenyl)propionate (4) To a solution of potassium hydroxide (1.95 g, 34.9 mmol) in EtOH (60 ml) was added dropwise at room temperature a solution of 3-(4-hydroxyphenyl)propionic acid (**1**) (5.80 g, 34.9 mmol) in EtOH (30 ml). White solid immediately precipitated. The mixture was stirred at room temperature for 2 h, and then the potassium salt **2** was collected by filtration, dried under vacuum (96% yield). The salt was treated with (*S*)-(+)-2,2-dimethyl-1,3-dioxolan-4-yl methyl *p*-toluene sulfonate (**3**) (8.33 g, 29.1 mmol) in anhydrous dimethyl sulfoxide (DMSO) at 100 °C for 1 h. The mixture was diluted with aqueous $NaHCO_3$ solution (500 ml), and the product was extracted with ether. The combined ethereal extracts were washed with aqueous $NaHCO_3$ solution to remove the starting material followed by brine, dried on $MgSO_4$, and concentrated *in vacuo* to leave the crude ester **4** (7.91 g, 98% yield), which was pure enough to be used for the next reaction without purification. TLC (*n*-hexane–AcOEt 1:1) *Rf* 0.751. ^{1}H-NMR ($CDCl_3$) δ: 7.03 (2H, d, J=9 Hz, Ph), 6.76 (2H, d, J=9 Hz, Ph), 4.23 (1H, m, $COOCH_2C\underline{H}$–O), 4.18—4.00 (3H, m, $COOC\underline{H}_2$ and O–CH–C$\underline{H}$(β)–O–), 3.70 (1H, dd, J=10, 6 Hz, O–CH–C$\underline{H}$(α)–O–), 2.90 (2H, t, J=7 Hz, Ph–CH_2), 2.62 (2H, t, J=7 Hz, CH_2COO), 1.42 and 1.38 (3H each, s each, Me). MS *m/z*: 280 (M^+), 265 (M^+−Me), 222 (M^+−2-propanol).

2,2-Dimethyl-1,3-dioxolan-4*S*-ylmethyl 3-[4-{2(*S*),3-Epoxypropoxy}-phenyl]propionate (6) A mixture of 2,2-dimethyl-1,3-dioxolan-4*S*-ylmethyl 3-(4-hydroxyphenyl)propionate (**4**) (1.23 g, 4.38 mmol), (2*S*)-(+)-glycidyl tosylate (**5**) (1.00 g, 4.38 mmol), anhydrous K_2CO_3 (1.21 g, 8.76 mmol) and anhydrous DMF (10 ml) was stirred at 70 °C for 15 h. The mixture was diluted with AcOEt (60 ml), and the resulting solution was washed with water and brine successively, dried on $MgSO_4$, and concentrated *in vacuo*. Column chromatography on silica gel (YMC gel, 30 g, CH_2Cl_2–AcOEt 95:5) gave the desired epoxide **6** (1.18 g, 80% yield). TLC (CH_2Cl_2–AcOEt 9:1) *Rf* 0.791. ^{1}H-NMR ($CDCl_3$) δ: 7.10 (2H, d, J=9 Hz, Ph), 6.82 (2H, d, J=9 Hz, Ph), 4.38—3.90 (6H, m, PhO–CH_2, $COOC\underline{H}_2$–C$\underline{H}$–, and CH(β)–O), 3.70 (1H, dd, J=10, 6 Hz, CH(α)–O), 3.35 (1H, m, $CH_2C\underline{H}$–O in epoxide), 2.90 (3H, m, PhCH_2 and CH–O in epoxide), 2.76 (1H, dd, J=6, 3 Hz, CH–O in epoxide), 2.63 (2H, t, J=7 Hz, CH_2COO), 1.41 and 1.38 (3H each, s each, Me). MS *m/z*: 336 (M^+), 321 (M^+−Me), 273 (M^+−2-propanol).

2,2-Dimethyl-1,3-dioxolan-4*S*-ylmethyl 3-[4-{3-{2-(Morpholinocarbonyl-amino)ethylamino}-2*S*-hydroxypropoxy}phenyl]propionate (ONO-1101) A mixture of the above epoxide **6** (9.34 g, 27.7 mmol), *N*-(2-aminoethyl)-morpholinocarboxamide (**7**) (4.80 g, 27.7 mmol) and 2-propanol (30 ml) was stirred at room temperature for 15 h. After concentration *in vacuo*, the residue was column chromatographed on silica gel (Mallinckrodt CC-7 gel, 150 g, AcOEt–MeOH 4:1) to afford the pure final compound, ONO-1101 (9.76 g, 68% yield). mp (HCl salt) 125.4 °C. TLC (AcOEt–MeOH) *Rf* 0.163. ^{1}H-NMR ($CDCl_3$) δ: 7.11 (2H, d, J=9 Hz, Ph), 6.83 (2H, d, J=9 Hz, Ph), 5.05 (t-like, 1H, NHCO), 4.28 (1H, quintet, J=9 Hz, $COOCH_2C\underline{H}$–O–), 4.15 (1H, dd, J=11, 5 Hz, COOCH), 4.08 (1H, dd, J=11, 6 Hz, COOCH), 4.06 (1H, m, C$\underline{H}$–OH), 4.03 (1H, dd, J=9, 6 Hz, CH(β)–O), 3.96 (1H, dd, J=9, 4 Hz, PhOCH), 3.94 (1H, dd, J=9, 6 Hz, PhOCH), 3.68 (1H, dd, J=9, 6 Hz, CH(α)–O), 3.66 (4H, t, J=5 Hz, $(CH_2)_2O$), 3.37 (2H, q, J=6 Hz, $C\underline{H}_2NHCO$), 3.33 (4H, t, J=5 Hz, $N(CH_2)_2$), 2.90 (2H, t, J=7 Hz, PhCH_2), 2.85 (1H, dd, J=12, 4 Hz, C$\underline{H}$NH), 2.82 (2H, t, J=6 Hz, $NHC\underline{H}_2$), 2.78 (1H, dd, J=12, 7 Hz, C$\underline{H}$NH), 2.64 (2H, t, J=7 Hz, CH_2COO), 1.42 (s, 3H, CH_3(α)), 1.36 (s, 3H, CH_3(β)). ^{13}C-NMR ($CDCl_3$, for convenience, the carbon adjacent to the oxygen in the morphorine group is read as C_1 and the methyl group in the α-configuration in oxolan group is C_{21}). δ: 172.56 (C_{15}), 157.96 (C_3), 157.04 (C_9), 132.94 (C_{12}), 129.28 (C_{10}), 114.55 (C_{11}), 109.79 (C_{19}), 73.55 (C_{17}), 70.45 (C_8), 68.63 (C_7), 66.47 (C_1), 66.31 (C_{18}), 64.71 (C_{16}), 51.44 (C_5), 49.23 (C_6), 43.96 (C_2), 40.42 (C_4), 35.90 (C_{14}), 30.02 (C_{13}), 26.70 (C_{21}), 25.40 (C_{20}). IR *v*: 3323, 1736, 1613, 1543, 1614, 1383, 1288, 1115, 1068 cm^{-1}. MS *m/z*: 407, 324, 143, 125, 99. *Anal.* Calcd for $C_{25}H_{39}N_3O_8$ HCl: C, 54.98; H, 7.38; N, 7.69. Found: C, 54.95; H, 7.32;

N, 7.81.

***N*-(2-Aminoethyl)morphorinocarboxamide (7)** To a solution of carbonyldiimidazole[9] (153.9 g, 0.95 mol) in $CHCl_3$ (700 ml, distilled from CaH_2) was added a solution of morpholine (82.6 g, 0.95 mol) in distilled $CHCl_3$ (400 ml) at room temperature in a period of 1.5 h. The temperature raised from 20 to 26 °C. The mixture was stirred further for 30 min at room temperature. To a solution of ethylenediamine (253 ml, 3.8 mol) in distilled $CHCl_3$ (700 ml) was added at room temperature the above $CHCl_3$ solution over a period of 1.5 h. The resulting mixture was stirred at room temperature for 15 h. After concentration *in vacuo*, the residue was column chromatographed on silica gel (Wako gel C-200, 2 kg × 2, $CHCl_3$–MeOH 7 : 3 with 4% of triethylamine) to afford the pure desired product **7** (106.6 g, 65% yield). TLC ($CHCl_3$–MeOH–Et_3N 7 : 3 : 2) *Rf* 0.190. ^{1}H-NMR ($CDCl_3$) δ: 5.32 (1H, br, NHCO), 3.66 (4H, m, $(CH_2)_2O$), 3.55—3.21 (6H, m, CH_2NHCO and $(CH_2)_2N$), 2.88 (2H, t, NH_2CH_2). MS *m/z*: 174 (M^+), 157, 144, 131, 114.

4-Tetrahydropyranyloxyphenylacetylene (10). Method A To a suspension of (bromomethyl)triphenylphosphonium bromide (21.3 g, 48.8 mmol) in anhydrous THF (200 ml) cooled to −78 °C was added potassium *tert*-butoxide (10.9 g, 97.6 mmol) portionwise. The mixture was stirred at that temperature for 30 min. To this ylide solution was added a solution of 4-tetrahydropyranyloxybenzaldehyde (**9**) (3.35 g, 16.3 mmol) in anhydrous THF (80 ml) over a period of 20 min. The resulting mixture was stirred at −78 °C for 2 h, and then warmed up to room temperature, being stirred for 2 h at room temperature. After dilution with water (200 ml), the product was extracted with ether. The combined ethereal extracts were washed with water and brine, successively, dried on $MgSO_4$, and concentrated *in vacuo*. Column chromatography on silica gel (YMC gel, 300 g, CH_2Cl_2–*n*-hexane 2 : 3) afforded the desired crystalline phenylacetylene **10** (2.42 g, 74% yield). TLC (AcOEt–*n*-hexane 1 : 2) *Rf* 0.871. ^{1}H-NMR ($CDCl_3$) δ: 7.17 (2H, d, J=9 Hz, Ph), 6.93 (2H, d, J=9 Hz, Ph), 5.39 (1H, m, O–CH–O), 4.00—3.43 (2H, m, –O–CH_2), 2.97 (1H, s, C≡CH), 2.04—1.23 (6H, m, CH_2). MS *m/z*: 202, 185, 174, 119, 89, 85. IR (KBr) *v*: 3289, 2945, 2106, 1605, 1506 cm^{-1}.

4-Hydroxyphenylpropiolic Acid (11) To a solution of 4-tetrahydropyranyloxyphenylacetylene (**10**) (2.42 g, 12.0 mmol) in anhydrous THF (150 ml) was added *n*-butyllithium (1.59 M, 9.04 ml, 14.4 mmol) dropwise. The mixture was stirred at −78 °C for 10 min. Carbon dioxide gas was introduced into the reaction mixture at −78 °C for 15 min. The mixture was warmed up to room temperature, and stirring was continued for 40 min. 1 N NaOH (100 ml) was added, and the solution was washed with ether. The aqueous layer was acidified with 1 N HCl and extracted with ether. This ethereal extract was washed with brine, dried on $MgSO_4$, and concentrated *in vacuo*. The residue was treated with *p*-toluenesulfonic acid (50 mg) in MeOH (30 ml) at room temperature for 3 h. After concentration *in vacuo*, the residue was dissolved in 2 N NaOH (20 ml), and the solution was washed with ether. The aqueous layer was acidified with 2 N HCl, and then the product was extracted with AcOEt. The combined AcOEt layers were washed with water and brine, successively, dried on $MgSO_4$, and concentrated *in vacuo* to give 4-hydroxyphenylpropiolic acid (**11**) (1.67 g, 85% yield). The desired product was pure enough to be used for the next reaction without purification. TLC (CH_2Cl_2–THF–AcOH 20 : 2 : 1) *Rf* 0.242. ^{1}H-NMR ($CDCl_3$ + CD_3OD) δ: 7.40 (2H, d, J=9 Hz, Ph), 6.77 (2H, d, J=9 Hz, Ph). MS *m/z*: 162 (M^+), 145, 134, 118. IR *v*: 3327, 2214, 1687 cm^{-1}.

Method B To a cooled (ice-water) solution of carbon tetrabromide (33.1 g, 100 mmol) in anhydrous CH_2Cl_2 (100 ml) was added triphenylphosphine (52.4 g, 200 mmol) portionwise over a period of 15 min. The mixture was stirred at 0 °C for 10 min. A solution of 4-methoxybenzaldehyde (**12**) (6.80 g, 50 mmol) in anhydrous CH_2Cl_2 (20 ml) was added to the above solution over a period of 15 min. The resulting mixture was stirred in an ice-water bath for 15 min. Water (100 ml) was added, and the product was extracted with CH_2Cl_2. The combined extracts were dried on $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (YMC gel, 150 g, CH_2Cl_2 followed by AcOEt–*n*-hexane 1 : 100) to give 1,1-dibromo-2-(4-methoxyphenyl)ethylene (**13**) (15.0 g, quantitative). TLC (AcOEt–*n*-hexane 1 : 2) *Rf* 0.612. To a solution of the above product (15.0 g, 50 mmol) in anhydrous CH_2Cl_2 (300 ml) at −78 °C was added dropwise a solution of boron tribromide (25 g, 100 mmol) in anhydrous CH_2Cl_2 (50 ml). The mixture was stirred at room temperature for 9 h, and then poured into ice-water (100 ml). The product was extracted with ether. The ethereal extracts were washed with water and brine, successively, dried on $MgSO_4$, and concentrated *in vacuo*. The residue was column chromatographed on silica gel (YMC gel, 300 g, *n*-hexane–AcOEt 10 : 1) to give 1,1-dibromo-2-(4-hydroxyphenyl)ethylene (**14**) (11.3 g, 81% yield). TLC (AcOEt–*n*-hexane 1 : 2) *Rf* 0.701. ^{1}H-NMR ($CDCl_3$) δ: 7.45 (2H, d, J=9 Hz, Ph), 7.40 (1H, s, olefinic), 6.80 (2H, d, J=9 Hz, Ph). MS *m/z*: 278 (M^+), 197 (M^+ − HBr). To a solution of 1,1-dibromo-2-(4-hydroxyphenyl)ethylene (5.22 g, 17.6 mmol) in anhydrous THF (50 ml) was added *n*-butyllithium (1.58 M, 37.6 ml, 58.0 mmol) dropwise at −78 °C. The mixture was warmed up to room temperature, and then stirred for 20 min. After cooling the mixture again to −78 °C, carbon dioxide was introduced into the mixture for 30 min. The mixture was warmed up to room temperature and stirred for another 45 min. After the mixture was poured into cold 1 N HCl (50 ml), the product was extracted with ether. The combined ethereal extracts were extracted with 2 N NaOH, and then the aqueous layer was acidified with 2 N HCl, followed by extraction with ether to give the desired 4-hydroxyphenylpropiolic acid (2.62 g, 82% yield).

4-Hydroxy-3-methoxyphenylpropiolic Acid (19). Method C To a solution of methyl 4-acetoxy-3-methoxycinnamate (**17**) (3.00 g, 12.0 mmol) in a 1 : 1 mixture of $CHCl_3$ and CCl_4 (50 ml) was added dropwise 1 M solution of bromine in $CHCl_3$ (12 ml, 12 mmol) at 4 °C. The mixture was stirred at 4 °C for 30 min, at room temperature for 30 min, and at 40 °C for 30 min. The mixture was poured into saturated aqueous $Na_2S_2O_3$ (50 ml), and the solution turned colorless. The product was extracted with AcOEt. The combined AcOEt layers were washed with water and brine in succession, dried on $MgSO_4$, and concentrated *in vacuo*. The residue (dibromide) **18** was treated with potassium hydroxide (13.4 g, powdered, 240 mmol) in a mixture of 2-propanol (100 ml) and EtOH (100 ml) at 110 °C for 15 h. After cooling to room temperature, the mixture was concentrated *in vacuo*, and the residue was dissolved in water (100 ml). The neutral materials were removed by extraction with ether, and the aqueous layer was acidified with conc. H_2SO_4. The product was extracted with AcOEt, and the combined extracts were washed with aqueous saturated $Na_2S_2O_3$, water and brine, successively, dried on $MgSO_4$, and concentrated *in vacuo*. The crude acetylenic acid was recrystallized from benzene to give pure 4-methoxy-3-hydroxypropiolic acid (**19**) (1.49 g, 65% yield). TLC (CH_2Cl_2–THF–AcOH 20 : 2 : 1) *Rf* 0.254. ^{1}H-NMR ($CDCl_3$) δ: 7.13 (1H, dd, J=10, 2.5 Hz, Ph), 7.10 (1H, d, J=2.5 Hz, Ph), 6.81 (1H, d, J=10 Hz, Ph), 3.81 (3H, s, OMe). IR ($CHCl_3$) *v*: 3200, 2210, 1687 cm^{-1}. MS *m/z*: 192 (M^+), 177 (M^+ − Me), 148 (M^+ − COOH).

Methyl 4-Hydroxy-3-methoxyphenylpropiolate (20) By the same way as described above, 3-methoxy-3-hydroxypropiolic acid (**19**) (0.94 g, 4.89 mmol) was converted to the methyl ester (0.94 g, 95% yield), using potassium hydroxide (0.27 g, 4.89 mmol) in EtOH (10 ml) followed by treatment with methyl iodide (3.00 ml, 48.9 mmol) in DMSO at 40 °C. TLC (CH_2Cl_2–AcOEt 95 : 5) *Rf* 0.880. ^{1}H-NMR ($CDCl_3$) δ: 7.20 (1H, d, J=2.5 Hz, Ph), 7.05 (1H, dd, J=10, 2.5 Hz, Ph), 6.83 (1H, d, J=10 Hz, Ph), 3.83 (3H, s, PhOMe), 3.79 (3H, s, COOMe). MS *m/z*: 206 (M^+), 175 (M^+ − OMe), 148 (M^+ − COOMe).

Methyl 4-{2(*S*),3-Epoxypropoxy}-3-methoxyphenylpropiolate (21) Methyl 4-hydroxy-3-methoxyphenylpropiolate (**20**) (0.940 g, 4.56 mmol) was treated with (2*S*)-(+)-glycidyl tosylate (**5**) (0.93 g, 4.10 mmol) in the presence of potassium carbonate (1.25 g, 9.12 mmol) in DMF (15 ml) at 70 °C for 1 h. The same workup was carried out as described before. Column chromatography on silica gel (YMC gel, 30 g, CH_2Cl_2–AcOEt 97 : 3) afforded the titled epoxide **21** (0.966 g, 82% yield). TLC (CH_2Cl_2–AcOEt 95 : 5) *Rf* 0.765. ^{1}H-NMR ($CDCl_3$) δ: 7.20 (1H, dd, J=10, 2.5 Hz, Ph), 7.06 (1H, d, J=2.5 Hz, Ph), 6.84 (1H, d, J=10 Hz, Ph), 4.30 (1H, dd, J=10, 4 Hz, PhOCH), 4.02 (1H, dd, J=10, 5 Hz, PhOCH), 3.82 (3H, s, COOMe), 3.76 (3H, s, PhOMe), 3.39 (1H, m, CH–O), 2.90 (1H, t, J=5 Hz, CH(α)–O), 2.72 (1H, dd, J=5, 3 Hz, CH(β)–O). MS *m/z*: 262 (M^+), 231 (M^+ − OMe).

Methyl 3-Methoxy-4-[3-{2-(morpholinocarbonylamino)ethylamino}-2*S*-hydroxypropoxy]phenylpropiolate (ONO-SA-132) A mixture of the above epoxide **21** (0.330 g, 1.25 mmol), *N*-(2-aminoethyl)morpholinocarboxamide (**7**) (0.21 g, 1.25 mmol), 2-propanol (1.0 ml), and MeOH (1.0 ml) was stirred at 35 °C for 15 h. After concentration *in vacuo*, the residue was column chromatographed on silica gel (Mallinckrodt CC-7, 15 g, AcOEt–MeOH 7 : 3) to afford the oily desired product. To a solution of this oil in CH_2Cl_2 (2 ml) was added aqueous saturated oxalic acid until the solution was acidic. Ether (5 ml) was added with stirring, and immediately a white solid appeared. The solvent was removed by decantation, and the solid was washed with ether by decantation. Drying with a vacuum pump gave the salt of the titled compound, SA-132. mp 117 °C. TLC (AcOEt–MeOH 1 : 1) *Rf* 0.105. ^{1}H-NMR (CD_3OD) δ: 7.22 (1H, dd, J=10, 2.5 Hz, Ph), 7.20 (1H, d, J=2.5 Hz, Ph), 7.02 (1H, d, J=10 Hz, Ph), 4.25 (1H, m, CH–OH), 4.10 (2H, d, J=5 Hz, $PhOCH_2$), 3.85 (3H, s, COOMe), 3.80 (3H, s, PhOMe), 3.60 (4H, t, J=5 Hz, $(CH_2)_2O$),

3.45 (2H, t-like, CH_2NHCO), 3.35—3.16 (8H, m, $C\underline{H}_2NHC\underline{H}_2$ and $N(CH_2)_2$). MS *m/z*: 436 ($M^+ + 1$). IR (KBr) *v*: 3369, 2953, 2213, 1708, 1602 cm^{-1}. Exact MS Calcd for $C_{21}H_{30}N_3O_7$: 436.2084. Found: 436.2101.

SA-001 mp 119 °C. TLC (CH_2Cl_2–MeOH 7:3) *Rf* 0.25. ^{1}H-NMR ($CDCl_3$) δ: 7.03 (2H, d, J=9 Hz, Ph), 6.75 (2H, d, J=9 Hz, Ph), 4.25 (1H, m, $CO_2CH_2C\underline{H}$), 4.15—3.80 (6H, m, $C\underline{H}$–OH, $PhOCH_2$–, CO_2CH_2, and CH–O), 3.70 (1H, dd, J=7, 5 Hz, CH–O), 3.50—3.00 (3H, m, $C\underline{H}_2NHC\underline{H}$), 2.85 (2H, t, J=7 Hz, $PhCH_2$), 2.60 (2H, t, J=7 Hz, CH_2CO_2), 1.42 (3H, s, Me), 1.38 (3H, s, Me), 1.28 (6H, d, J=5 Hz, Me×2). MS *m/z*: 396 ($M^+ + 1$), 380 ($M^+ - Me$). IR ($CHCl_3$) *v*: 3300, 2950, 2850, 1720, 1600 cm^{-1}. *Anal.* Calcd for $C_{22}H_{34}NO_8$ (oxalic acid salt): C, 59.96; H, 7.78; N, 3.18. Found: C, 58.98; H, 7.40; N, 3.22.

SA-009 mp 100 °C. TLC (CH_2Cl_2–MeOH 7:3) *Rf* 0.26. ^{1}H-NMR ($CDCl_3$) δ: 7.05 (2H, d, J=9 Hz, Ph), 6.75 (2H, d, J=9 Hz, Ph), 4.20 (1H, m, $CO_2CH_2C\underline{H}$), 4.16—3.80 (6H, m, $C\underline{H}$–OH, $PhOCH_2$–, CO_2CH_2, and CH–O), 3.65 (1H, dd, J=7, 5 Hz, CH–O), 3.55—3.00 (3H, m, $C\underline{H}_2NHC\underline{H}$), 2.85 (2H, t, J=7 Hz, $PhCH_2$), 2.60 (2H, t, J=7 Hz, CH_2CO_2), 1.90—1.60 (8H, m, methylene in cyclopentane ring), 1.34 (6H, d, J=5 Hz, Me×2). MS *m/z*: 422 ($M^+ + 1$), 406 ($M^+ - Me$). IR ($CHCl_3$) *v*: 3600, 2950, 2850, 1731, 1600 cm^{-1}. *Anal.* Calcd for $C_{24}H_{36}NO_8$ (oxalic acid salt): C, 61.76; H, 7.78; N, 3.00. Found: C, 60.76; H, 7.18; N, 3.01.

SA-015 mp 154 °C. TLC ($CHCl_3$–MeOH 4:1), *Rf* 0.24. ^{1}H-NMR ($CDCl_3$) δ: 7.06 (2H, d, J=9 Hz, Ph), 6.76 (2H, d, J=9 Hz, Ph), 4.70 (2H, s, $COOCH_2CO$), 4.30 (1H, m, $C\underline{H}$–OH), 4.06—3.80 (2H, m, $PhOCH_2$), 3.50—3.00 (3H, m, $C\underline{H}_2NHC\underline{H}$), 2.96 (6H, s, NMe), 2.83 (2H, t, J=7 Hz, $PhCH_2$), 2.64 (2H, t, J=7 Hz, CH_2CO_2), 1.31 (6H, d, J=5 Hz, Me×2). MS *m/z*: 367 (M^+). IR ($CHCl_3$) *v*: 3600, 2920, 2850, 1730, 1640 cm^{-1}. *Anal.* Calcd for $C_{20}H_{31}N_2O_7$ (oxalic acid salt): C, 56.17; H, 7.31; N, 6.55. Found: C, 57.01; H, 7.31; N, 6.44.

SA-098 mp 207 °C. TLC (CH_2Cl_2–MeOH 7:3) *Rf* 0.12. ^{1}H-NMR ($CDCl_3$) δ: 7.64 (1H, d, J=15 Hz, olefinic), 7.56 (2H, d, J=9 Hz, Ph), 7.02 (2H, d, J=9 Hz, Ph), 6.40 (1H, d, J=15 Hz, olefinic), 4.12 (1H, m, CH–OH), 4.07 (2H, m, $PhOCH_2$), 3.78 (3H, s, COOMe), 3.50—3.00 (4H, m, $C\underline{H}_2NH$×2), 1.36 (6H, s, Me×2). MS *m/z*: 292, 171. IR (KBr) *v*: 3452, 3351, 2947, 1713, 1679, 1635, 1603 cm^{-1}. *Anal.* Calcd for $C_{19}H_{28}N_3O_7$ (oxalic acid salt): C, 55.58; H, 6.88; N, 10.24. Found: C, 55.61; H, 6.79; N, 10.28.

SA-048 TLC (CH_2Cl_2–MeOH 3:1) *Rf* 0.33. ^{1}H-NMR ($CDCl_3$) δ: 7.14 (2H, d, J=9 Hz, Ph), 6.88 (2H, d, J=9 Hz, Ph), 4.30—4.14 (2H, m, $CO_2CH_2C\underline{H}$, $COOC\underline{H}$), 4.06 (2H, m, CH–OH, CH–O), 4.04—3.97 (3H, m, $PhOCH_2$, COOCH), 3.58 (1H, dd, J=9.6 Hz, CH–O), 3.44—3.24 (3H, m, $C\underline{H}_2NHCO$, CHNH), 3.11 (1H, dd, J=12, 9 Hz, CH–O), 2.87 (2H, t, J=7.5 Hz, $PhCH_2$), 2.62 (2H, t, J=7.5 Hz, CH_2COO), 1.36—1.32 (12H, m, Me×4). MS *m/z*: 292, 171. IR (KBr) *v*: 3452, 3351, 2947, 1713, 1679, 1635, 1603 cm^{-1}. Exact MS Calcd for $C_{23}H_{38}N_3O_7$: 468.2709. Found: 468.2696.

SA-064 Oil. TLC (MeOH) *Rf* 0.43. ^{1}H-NMR ($CDCl_3$) δ: 7.12 (2H, d, J=9 Hz, Ph), 6.83 (2H, d, J=9 Hz, Ph), 5.05 (1H, t-like, NHCO), 4.28 (1H, quintet, J=5 Hz, $COOCH_2C\underline{H}$–O–), 4.15 (1H, dd, J=10, 5 Hz, COOCH), 4.09 (1H, dd, J=10, 8 Hz, COOCH), 4.04 (1H, m, $C\underline{H}$–OH), 4.03 (1H, dd, J=9, 6 Hz, CH(β)–O), 3.96 (1H, dd, J=9, 4 Hz, PhOCH), 3.93 (1H, dd, J=9, 6 Hz, $PhOC\underline{H}$), 3.69 (1H, dd, J=9, 6 Hz, CH(α)–O), 3.67 (4H, t, J=5 Hz, $(CH_2)_2O$), 3.37 (2H, q, J=6 Hz, $C\underline{H}_2NHCO$), 3.33 (4H, t, J=5 Hz, $N(CH_2)_2$), 2.90 (2H, t, J=7 Hz, $PhCH_2$), 2.81 (1H, dd, J=12, 4 Hz, $C\underline{H}NH$), 2.78 (1H, dd, J=12, 7 Hz, $C\underline{H}NH$), 2.64 (2H, t, J=7 Hz, CH_2COO), 1.40—1.29 (12H, m, Me×4). MS *m/z*: 537, 522, 451. IR (KBr) *v*: 3351, 3318, 2984, 1737, 1618, 1544 cm^{-1}. Exact MS Calcd for $C_{27}N_{44}N_3O_8$: 538.3137. Found: 538.3142.

SA-032 mp 175 °C. TLC (AcOEt–MeOH 1:1) *Rf* 0.41. ^{1}H-NMR ($CDCl_3$, free amine) δ: 7.10 (2H, d, J=9 Hz, Ph), 6.80 (2H, d, J=9 Hz, Ph), 5.50 (1H, t-like, NHCO), 4.76 (2H, br, $CONH_2$), 4.10—3.90 (3H, m, CH–OH, $PhOCH_2$), 3.65 (3H, s, COOMe), 3.37 (2H, m, $C\underline{H}_2NH$), 2.90 (2H, t, J=7 Hz, $PhCH_2$), 2.85—2.78 (2H, m, $C\underline{H}_2NH$), 2.64 (2H, t, J=7.5 Hz, CH_2COO), 1.05 (6H, s, Me×2). MS *m/z*: 368 (M^+), 351 ($M^+ - H_2O$). IR (film, free amine) *v*: 3300, 2950, 1740, 1650, 1600 cm^{-1}. *Anal.* Calcd for $C_{19}H_{30}N_3O_7$ (free amine): C, 55.31; H, 7.33; N, 10.19. Found: C, 54.76; H, 7.29; N, 10.11.

SA-062 TLC (MeOH) *Rf* 0.263. ^{1}H-NMR (CD_3OD) δ: 7.10 (2H, d, J=9 Hz, Ph), 6.81 (2H, d, J=9 Hz, Ph), 5.00 (1H, t-like, $N\underline{H}CO$), 4.30—3.90 (7H, m, $COOCH_2C\underline{H}$–O–, $COOCH_2$, $C\underline{H}$–OH, CH(β)–O, $PhOCH_2$), 3.68—3.60 (5H, m, CH(α)–O, $(CH_2)_2O$), 3.37 (2H, m, $C\underline{H}_2NHCO$), 3.33 (4H, t, J=5 Hz, $N(CH_2)_2$), 2.91 (2H, t, J=7.5 Hz, $PhC\underline{H}_2$), 2.80 (1H, dd, J=12, 4 Hz, $C\underline{H}NH$), 2.82 (2H, t, J=6 Hz, $NHC\underline{H}_2$), 2.78 (1H, dd, J=12, 7 Hz, $C\underline{H}NH$), 2.64 (2H, t, J=7.5 Hz, CH_2COO), 1.42 (3H, s, $CH_3(\alpha)$), 1.36 (3H, s, $CH_3(\beta)$). MS *m/z*: 442, 407. IR (KBr) *v*: 3349, 2986, 1737, 1619 cm^{-1}. *Anal.* Calcd for $C_{26}H_{40}N_3O_{10}$ (oxalic acid salt): C, 56.29; H, 7.27; N, 7.58. Found: C, 56.31; H, 7.21; N, 7.55.

SA-109 mp 128 °C. TLC (CH_2Cl_2–MeOH 4:1) *Rf* 0.156. ^{1}H-NMR (CD_3OD) δ: 7.12 (2H, d, J=9 Hz, Ph), 6.87 (2H, d, J=9 Hz, Ph), 5.03 (1H, t-like, $N\underline{H}CO$), 4.30—3.90 (7H, m, $COOCH_2C\underline{H}$–O–, $COOCH_2$, $C\underline{H}$–OH, CH(β)–O, $PhOCH_2$), 3.68—3.60 (5H, m, CH(α)–O, $(CH_2)_2O$), 3.40—3.30 (6H, m, $C\underline{H}_2NHCO$, $N(CH_2)_2$), 3.19 (2H, t, J=5 Hz, $PhCH_2$), 2.82 (2H, t, J=7.5 Hz, $NHC\underline{H}_2$), 2.80 (1H, dd, J=12, 4 Hz, $C\underline{H}NH$), 2.78 (1H, dd, J=12, 7 Hz, $C\underline{H}NH$), 2.62 (2H, t, J=7.5 Hz, CH_2COO), 1.42 (3H, s, $CH_3(\alpha)$), 1.36 (3H, s, $CH_3(\beta)$). MS *m/z*: 407, 329. IR (KBr) *v*: 3350, 2986, 1737, 1625 cm^{-1}. *Anal.* Calcd for $C_{26}H_{40}N_3O_{10}$ (oxalic acid salt): C, 56.29; H, 7.27; N, 7.58. Found: C, 56.27; H, 7.22; N, 7.60.

SA-113 TLC (CH_2Cl_2–MeOH 4:1) *Rf* 0.110. ^{1}H-NMR (CD_3OD) δ: 7.53 (2H, d, J=9 Hz, Ph), 7.02 (2H, d, J=9 Hz, Ph), 4.27 (1H, m, COOCH), 4.06 (2H, m, COOCH, CH–OH), 3.78 (3H, s, COOMe), 3.64 (4H, t, J=5 Hz, $(CH_2)_2O$), 3.49 (2H, m, CH_2NH), 3.40—3.25 (6H, m, $C\underline{H}_2NHCO$, $N(CH_2)_2$), 3.18 (2H, m, $NHCH_2$). MS *m/z*: 318, 330. IR (KBr) *v*: 3339, 2955, 1709, 1604 cm^{-1}. Exact MS Calcd for $C_{21}H_{28}N_3O_8$: 450.1866. Found: 450.1860.

SA-129 TLC (AcOEt–MeOH 1:1) *Rf* 0.145. ^{1}H-NMR (CD_3OD) δ: 7.55 (2H, d, J=9 Hz, Ph), 7.00 (2H, d, J=9 Hz, Ph), 4.27 (1H , m, COOCH), 4.06 (2H, m, COOCH, CH–OH), 3.78 (3H, s, COOMe), 3.64 (4H, t, J=5 Hz, $(CH_2)_2O$), 3.49 (2H, m, CH_2NH), 3.40—3.25 (6H, m, $C\underline{H}_2NHCO$, $N(CH_2)_2$), 3.15 (2H, m, $NHCH_2$). MS *m/z*: 406 ($M^+ - 1$). IR (KBr) *v*: 3371, 2927, 1708, 1605 cm^{-1}. *Anal.* Calcd for $C_{21}H_{28}N_3O_8$ (oxalic acid salt): C, 55.97; H, 6.27; N, 9.33. Found: C, 55.90; H, 6.29; N, 9.22.

***In Vivo* Studies** Both the *in vivo* and *in vitro* experiments were conducted according to the reported literature.[4,10] β-Blocking activity and its duration were determined *in vivo* with anesthetized dogs instrumented for measurement of heart rate with a cardio tachometer (Nippon Kohden RJG-4128) triggered electronically by a phasic aortic blood pressure signal. Mongrel dogs of either sex were anesthetized with sodium barbital (300 mg/kg, i.p.) and ventilated with room air. A femoral artery, femoral vein and brachial vein were cannulated for measurement of arterial blood pressure and administration of drugs, respectively. The degree of β-blockade was assessed by inhibition of tachycardia induced by intravenous administration of isoproterenol (0.1 μg/kg, i.v.) at 10 min intervals prior to, at various times during, and following termination of 30 min intravenous infusion of β-blockers. Each β-blocker was infused at appropriate rates for each compound for analysis of ED_{50} for % inhibition of tachycardia. The percent inhibition of the isoproterenol-induced tachycardia was calculated during and following infusion of β-blockers using the following formula: $(1 - A/B) \times 100$, where B refers to the degree of tachycardia before, and A to that during or after the infusion of β-blockers, respectively.

***In Vitro* Studies** Respective β_1- and β_2-blocking activities were determined in guinea pig right atria and trachea preparations.[4,10] The right atria and trachea were removed from stunned animals (guinea pig) and mounted in 20 ml constant temperature baths (37 °C) in a Krebs–Henseleit solution. The solution was aerated with 95% O_2/5% CO_2 to oxygenate the tissue. Tissues were connected to a force transducer, and resting tension adjusted to 0.5 g for each tissue. Concentration–response curves to isoproterenol were constructed before, and 60 min following addition of β-blockers to the bath. Each tissue was exposed to only one concentration of each compound. In the right atria and trachea experiments isoproterenol responses were expressed as a percentage of maximum change in rate or tension, and the concentration required for 50% of the maximum response was measured in the absence or presence of blocking agent. Concentration ratios and the pA_2 values were calculated by the method of Arunlakshana and Schild (Table V).

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