Synthesis and Biological Activity of Novel Carbacyclins Having Bicyclic Substituents on the ω-Chain

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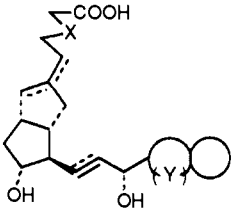
A number of carbacyclins having bicyclic substituents on the ω-chain have been synthesized and tested for antiplatelet aggregation activity in vitro (against collagen-induced aggregation of rat platelet), for reduction of systemic blood pressure in vivo (ability to reduce the blood pressure in anesthetized rat by iv injection), and for cytoprotective activity (protection against ethanol-induced rat gastric lesion). The most effective compound for each activity was [3a*S*-[2*E*,3aα,4α(3*R*),5β,6aα]]-5-[hexahydro-5-hydroxy-4-[3-hydroxy-3-(2-indanyl)-1-propynyl]-2(1*H*)-pentalenylidene]pentanoic acid (compound **11a**), while some 1,4-benzodioxan analogues had selectivity for organ-protective activity, and indan analogues showed selectivity in their antiaggregation activity.

Prostacyclin (PGI_2) is the most potent natural inhibitor of platelet aggregation known.[1] Its anti-gastric ulcer activity has been considered for clinical use because of its lower diarrheogenic activity than that of the other prostaglandins such as the PGE series.[2,3] However, due to the inherent chemical instability of PGI_2 under acidic conditions, many efforts have been made to improve its stability. Carbacyclin analogues, in which the ether oxygen of PGI_2 is replaced by a methylene group, have given chemically stable compounds with high potency.[4-6]

In addition to chemical stability, biological stability to impede metabolic inactivation has been desired in prostacyclin analogues. Prostaglandins are susceptible to facile metabolism such as oxidation of the 15-hydroxyl, as well as ω-oxidation in ω-chain and β-oxidation in α-chain.[7-9] In a few synthetic prostaglandins, a bulky ring system was introduced at the end of a ω-chain such as phenoxy[10] or cyclopentane[5] group to prevent oxidation of the 15-hydroxy group and to avoid ω-oxidation, and these attempts appeared to be successful. On the basis of these findings, we tried to explore a few bulky bicyclo ring moieties, such as 1,4-benzodioxan (Ia) and indan (Ib), as ω-chain substituents. The introduction of an oxygen atom on α-chain at C-3 position was also attempted to prevent the enzymatic β-oxidation of the chain. Preliminary biological studies with these compounds suggested that those with indan substituents are rather selective to platelet inactivation and those with 1,4-benzodioxan substituents are effective against ethanol-induced experimental gastric lesions.



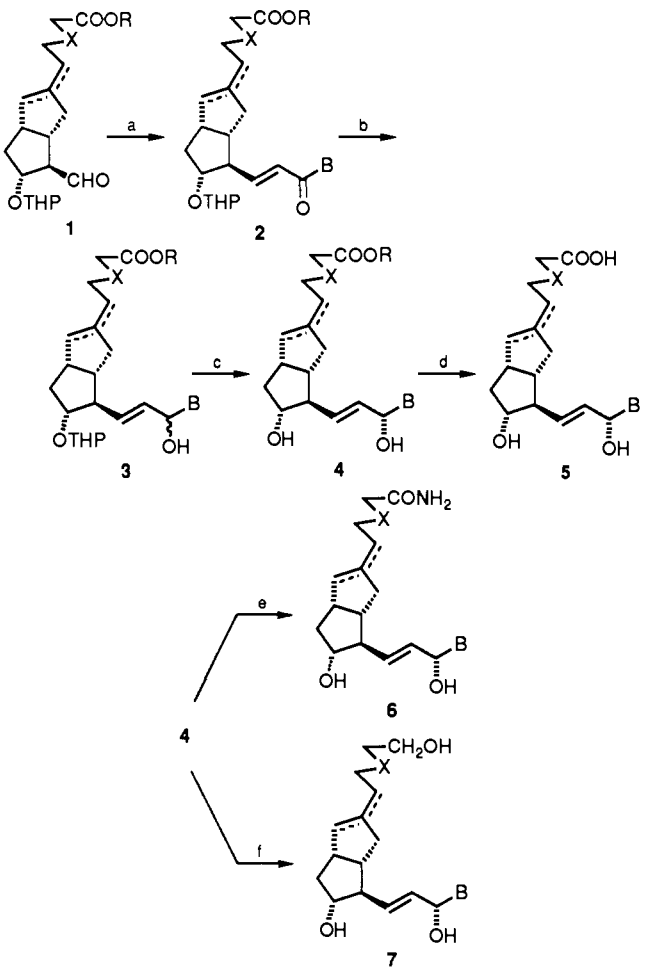
X = O or CH_2
Y = O (Ia) or CH_2 (Ib)

I

Chemistry

There are several synthetic routes to reach carbacyclin.[11-13] In this study, aldehydes 1 have been formed from Corey's lactone according to Shibasaki et al., and the introduction of ω-chains was achieved by Horner–Emmons reaction with appropriate β-keto phosphonate.[14] The assignment of α and β stereochemistry of 15-hydroxy compounds was carried out by comparison of their R_f values and their biological activities: All of the tested 15α-isomers with biological activities gave lower R_f values than the corresponding β-isomers except for only two compounds, **5v** and **11a**. It is assumed that the isomer with

Scheme I[a]



R = CH_3 or $C(CH_3)_3$
X = CH_2 or O

[a] Reagents: (a) NaH or KH, $(H_3CO)_2P(O)CH_2C(O)B$, THF; (b) $NaBH_4$, CH_3OH; (c) AcOH–H_2O–THF (3:1:1), then separation; (d) 10% aqueous NaOH, CH_3OH, or 7% KOH/CH_3OH; (e) 28% aqueous NH_3, CH_3OH; (f) Red-Al, Et_2O.

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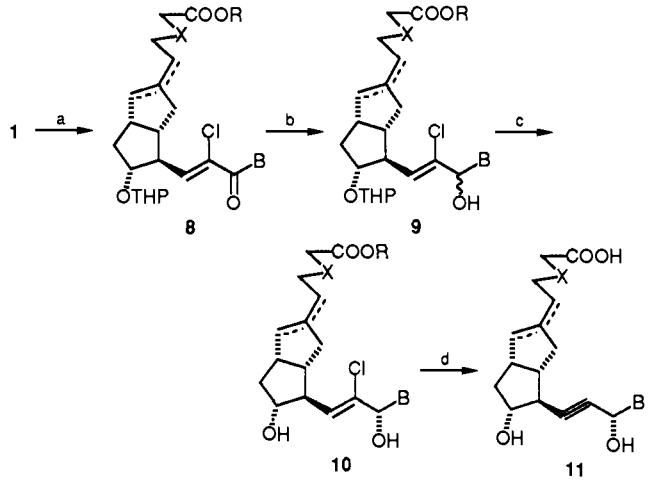
Scheme II[a]



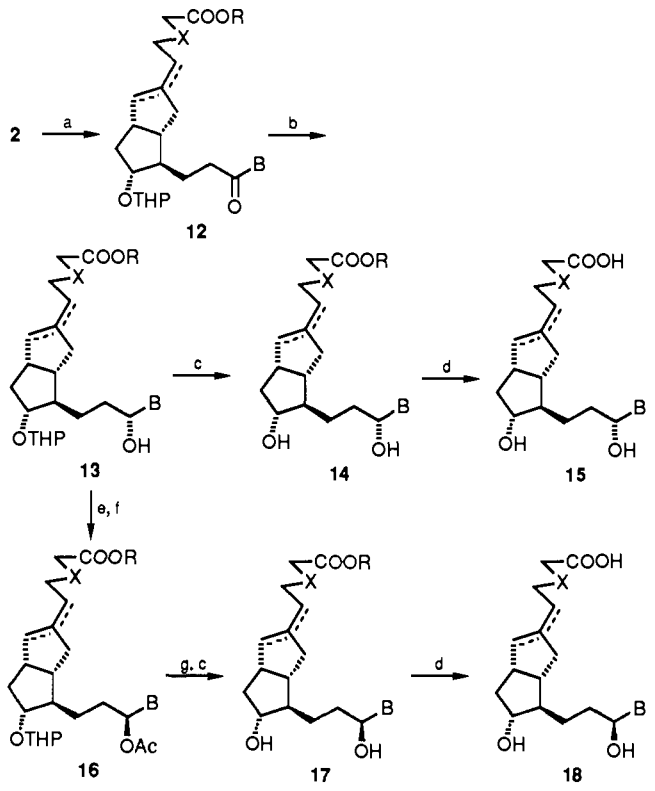
[a] Reagents: (a) NaH, $(H_3CO)_2P(O)CHClC(O)B$, DME; (b) $NaBH_4$, CH_3OH; (c) AcOH–H_2O–THF (3:1:1), then separation; (d) *t*-BuOK, DMSO–THF.

biological activity has the 15α-configuration.

(a) Synthesis of Carbacyclin Analogues—Method A (Scheme I). Aldehydes 1 [X = CH_2 (carbacyclin), X = O (3-oxacarbacyclin), X = O (3-oxaisocarbacyclin] were prepared according to the methods reported by Shibasaki et al.[14-17] The aldehydes 1 were condensed with sodium or potassium salts of β-keto phosphonate to give the enones **2**. Reduction of **2** with sodium borohydride furnished a mixture of allylic alcohols **3**. Deblocking of the tetrahydropyranyl ether of **3** with acetic acid and aqueous THF gave the diols **4** and the corresponding epimeric alcohols, which were readily separated by silica gel column. Hydrolysis of **4** with 10% aqueous NaOH and methanol or 7% KOH in methanol afforded the carbacyclin analogues **5**. Furthermore, the esters **4** were converted to the corresponding amides **6** by aminolysis with 28% aqueous NH_3 and methanol, or into alcohols **7** by reduction with Red-Al.

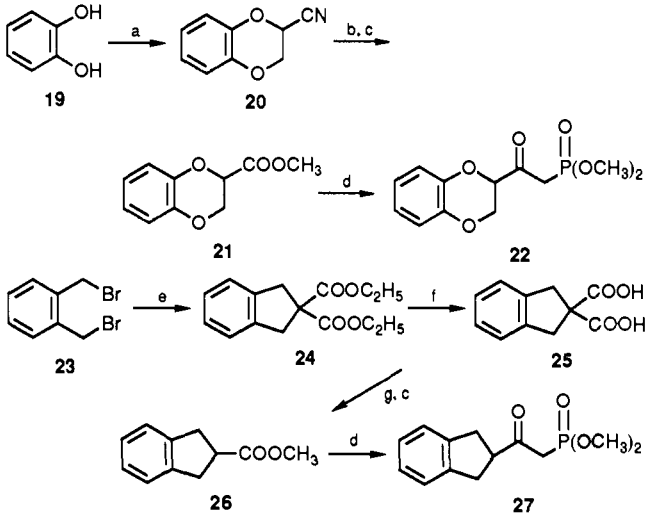
(b) Synthesis of 13,14-Dehydrocarbacyclin Analogues—Method B (Scheme II). The chlorinated β-keto phosphonates were prepared by reaction of β-keto phosphonates anion with *N*-chlorosuccinimide. The aldehydes **1** were condensed with sodium salts of β-keto phosphonate to give a mixture of *cis*- and *trans*-α-chloroenones **8**. Reduction of **8** with sodium borohydride gave a mixture of allylic alcohols **9**, followed by deblocking of the tetrahydropyranyl ether of **9** with acetic acid and aqueous THF to provide the diols **10** and the corresponding epimers of **10**, which were separated chromatographically. Dehydrochlorination of **10** with potassium

Scheme III[a]



[a] Reagents: (a) methyl benzoate–$Cr(CO)_3$, H_2 (70 kg/cm^2), acetone; (b) $NaBH_4$, CH_3OH; (c) AcOH–H_2O–THF (3:1:1); (d) 10% aqueous NaOH, CH_3OH; (e) MsCl, Py, CH_2Cl_2; (f) cesium acetate, 18-crown-6, toluene; (g) K_2CO_3, CH_3OH.

Scheme IV[a]



[a] Reagents: (a) $BrCH_2CH_2BrCN$, K_2CO_3, acetone; (b) concentrated H_2SO_4, AcOH, H_2O; (c) concentrated H_2SO_4, CH_3OH; (d) $LiCH_2P(O)(OCH_3)_2$, THF; (e) $NaOC_2H_5$, $CH_2(COOC_2H_5)_2$, Et_2O; (f) aqueous KOH; (g) 200 °C.

tert-butoxide in DMSO–THF proceeded smoothly and took place simultaneously with cleavage of the ester to give 13,14-dehydrocarbacyclin analogues **11**.[18]

(c) Synthesis of 13,14-Dihydrocarbacyclins Analogues—Method C (Scheme III). The enones **2**, prepared by method A, were reduced by Shibasaki hydrogenation to give the ketones **12**.[19] Reduction of **12** with

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sodium borohydride gave selectively the corresponding single isomers of the alcohols **13**. In order to determine the stereochemistry of the hydroxy group, the inversion of 15-hydroxy group of **13** was examined. The hydroxy group of **13** was inverted according to the methods reported by Ikegami et al.[20] After mesylation of the alcohols **13**, the treatment with cesium acetate in the presence of 18-crown-6 yielded the corresponding acetates **16**. Transesterification of **16** with potassium carbonate and methanol, followed by deblocking of the tetrahydropyranyl ether with acetic acid and aqueous THF, provided the diols **17**. Each of the **17** analogues was confirmed to be an isomer of **14** by HPLC analysis by using a chiral stationary phase after derivatizing them into the corresponding dibenzoates.[21] The alcohols **14** and **17** were then transformed to 13,14-dihydrocarbacyclin analogues by hydrolysis with 10% aqueous NaOH and methanol.

(d) Preparation of β-Keto Phosphonate (Scheme IV). The β-keto phosphonate for introduction of the ω-chain was obtained by condensation of the corresponding ester with the lithium salt of dimethyl methylphosphonate. For example, synthesis of 2-(methoxycarbonyl)-1,4-benzodioxan (**21**),[22,23] a precursor of β-keto phosphonate, was as follows: O-Alkylation of a catechol **19** with α,β-dibromopropionitrile in the presence of potassium carbonate provided 2-cyano-1,4-benzodioxan (**20**). Hydrolysis of **20** under acidic conditions followed by esterification with catalytic concentrated H_2SO_4 in methanol led to formation of 2-(methoxycarbonyl)-1,4-benzodioxan (**21**). 2-(Methoxycarbonyl)indan (**26**) was synthesized by the method of Carlson:[24] Reaction of α,α′-dibromo-*o*-xylene (**23**) with diethyl malonate anion furnished the diester **24**. Hydrolysis of **24** with aqueous KOH provided dicarboxylic acid **25**. Decarbonylation of **25**, followed by esterification with catalytic concentrated H_2SO_4 in methanol, led to 2-(methoxycarbonyl)indan (**26**). The resulting ester **21** or **26** was condensed with the lithium salt of dimethyl methylphosphonate to yield the corresponding β-keto phosphonate **22** or **27**, respectively.

Structures, synthetic routes, and physical properties of compounds are given in Table I.

Biological Results and Discussion

These compounds were evaluated in the following three pharmacological activities: (1) Data of organ-protective activities in ethanol-induced gastric lesions in the rat are expressed as the doses that inhibit 50% of lesion of control (ED_{50} po). (2) The dose that reduces mean blood pressure by 15 mmHg (peak effect) in rat iv (MBP_{15} is expressed as hypotensive activity). (3) Antiaggregation activities are expressed as IC_{50} for the doses that inhibit rat platelet aggregation induced by collagen by 50% of that of the control. These results are shown in Table II. The drop in blood pressure is considered to be an unfavorable effect for clinical use; therefore, the ratios of relative potency (organ protection/hypotension) of the compounds to PGE_2 and the ratio (antiaggregation/hypotension) to PGI_2 were calculated and are summarized in Tables III and IV. Considering their intrinsic potency and the ratio, compounds **5b** and **5e** (1,4-benzodioxan analogue) and compound **11a** (indan analogue) show satisfactory activity against ethanol-induced lesions and, therefore, were considered to be promising candidates for further studies. In the case of antiaggregation activity, compounds **5h** and **5s** (indan analogues) gave the favorable ratio. Compound **11a** was about half as active as naturally occurring PGI_2. This potency, however, was rather prominent compared to the other PGI_2 analogues which have been already reported. Figure 1 shows the ex vivo antiaggregation effects of compounds **5h**, **5s**, and **11a** and PGI_2Na at 15, 30, and 60 min after oral administration. Compounds **5h**, **5s**, and **11a** at 2 mg/kg orally inhibited platelet aggregation induced by collagen ex vivo, whereas PGI_2Na administered as the same route and dose has no inhibition.

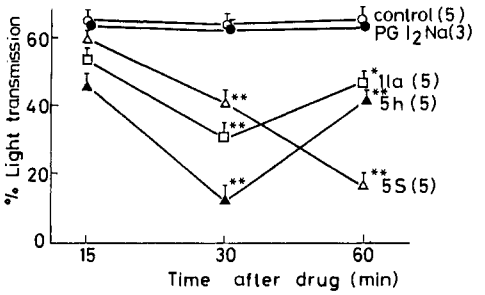

Figure 1. Inhibitory effects of compounds **5h**, **5s**, and **11a** and PGI_2Na on collagen-induced (5 μg/mL) platelet aggregation in conscious rats ex vivo. Each plot shows the mean ± SEM of the change in the light transmission as a measure of the aggregation, and the numbers in parentheses represent the number of experiments. Asterisks [(*) $P < 0.05$; (**) $P < 0.01$] indicate the statistical significance (*t* test) of the difference from the control (vehicle-treated group).

In conclusion, these carbacyclin analogues having bicyclic substituents on the ω-chain preserve the PGI_2-like activity, and furthermore, the metabolic stability seems to be improved because some of these compounds were orally effective. It is noteworthy that the carbacyclins with indan side chains tend to increase antiaggregation activity and those with 1,4-benzodioxan side chains tend toward organ-protective activity. The above findings give the possibility that the introduction of various bicyclic substituents on the ω-chain may lead to other selective PGI_2 analogues.

Experimental Section

Analysis. Melting points were recorded on a Yamato MP-21. ^1H NMR spectra were recorded on a Hitachi R-90H (90-MHz) spectrometer. Chemical shifts were expressed in parts per million relative to internal tetramethylsilane ($\delta = 0$) or chloroform ($\delta = 7.26$). IR spectra were obtained with a Hitachi 270-30 spectrophotometer. Mass spectra and high-resolution mass spectra were recorded with a Hitachi M-80B mass spectrometer. The purity of all tested compounds was determined by a high-performance liquid chromatography (Jasco UVIDEC-100-III), using a TSK-80TM (4.6 i.d. × 150) and a UV detector (280 nm) equipped with a chromatointegrator (Hitachi D-2000), and 0.02 M acetate buffer–CH_3CN (55:45) as a solvent, and a flow rate of 1 mL/min. High-performance liquid chromatography for separation of 15α- and 15β-isomers was carried out by using a TSK-GEL Enantio P1 column with *n*-hexane–dichloroethane–ethanol (70:29:1).

Thin-layer chromatography (TLC) was carried out on 0.25-mm precoated silica gel plates (E. Merck; 60F-254) by using UV light and/or 7% phosphomolybdic acid in ethanol and heat as developing agent. Preparative layer chromatography (PLC) was performed on 0.25, 0.5, or 2 mm × 20 × 20 cm precoated silica gel plates (E. Merck; 60F-254). Column choromatography was conducted by using silica gel (Fuji Devison BW-200, 150–325 mesh).

Solvents. Ether and THF were distilled over sodium–benzophenone ketyl under Ar atmosphere. CH_2Cl_2, toluene, triethylamine, pyridine, and DMSO were distilled over CaH_2 under Ar atmosphere.

Method A. **[3a*S*-[2*E*,3aα,4α(1*E*),5β,6aα]]-[2-[Hexa-**

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hydro-5-(tetrahydropyranyloxy)-4-[3-(2-indanyl)-3-oxo-1-propenyl]-2(1*H*)-pentalenylidene]ethoxy]acetic Acid *tert*-Butyl Ester (2S). To a stirred suspension of 23 mg (0.57 mmol) of 60% sodium hydride dispersion in 0.6 mL of THF cooled to 0 °C under Ar atmosphere was added dropwise a solution of 210 mg (0.76 mmol) of (2-indanoyl)methyl dimethylphosphonate in 2.4 mL of THF. After the solution was stirred for 20 min, a solution of 150 mg (0.38 mmol) of aldehyde 1 (X = O, 3-oxacarbacyclin) in 4.0 mL of THF was added and the mixture was stirred for an additional 20 min at room temperature. The reaction was then quenched with saturated aqueous NH_4Cl. Ether was added to obtain the ether extract, which was washed with brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude enone, which was chromatographed on 6.0 g of silica gel. Elution with ether–*n*-hexane (1:1) gave 123 mg (61% yield) of enone as a colorless oil: IR (neat) 2932, 2866, 1740, 1689, 1665, 1623 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 (s, 9 H), 1.02–1.80 (m, 6 H), 1.93–2.70 (m, 9 H), 3.00–3.27 (d, 4 H), 3.27–4.11 (m, 4 H), 3.91 (s, 2 H), 4.00, 4.80 (d, 2 H), 4.43–4.68 (m, 1 H), 5.46 (br t, 1 H), 6.10–6.39 (dd, 1 H), 6.60–7.10 (m, 1 H), 7.11 (s, 4 H); MS *m/z* 536 ($M^{\bullet+}$).

[3a*S*-[2*E*,3aα,4α(1*E*,3*RS*),5β,6aα]]-[2-[Hexahydro-5-(tetrahydropyranyloxy)-4-[3-(2-indanyl)-3-hydroxy-1-propenyl]-2(1*H*)-pentalenylidene]ethoxy]acetic Acid *tert*-Butyl Ester (3S). A solution of 110 mg (0.206 mmol) of enone in 4.0 mL of MeOH was treated with 12 mg (0.206 mmol) of sodium borohydride at 0 °C under Ar atmosphere and stirred for 15 min. After acetone was added at 0 °C and the mixture stirred for 20 min at room temperature, it was neutralized with saturated aqueous NH_4Cl. The solvent was removed under reduced pressure, and then the aqueous solution was extracted with ether. The ether extract was washed with brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude allylic alcohol, which was chromatographed on 3.0 g of silica gel. Elution with ether–*n*-hexane (2:1) gave 100 mg (91% yield) of allylic alcohol as a colorless oil: IR (neat) 3450, 2926, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 (s, 9 H), 1.10–2.68 (m, 17 H), 2.68–3.18 (m, 4 H), 3.29–4.22 (m, 4 H), 3.91 (s, 2 H), 4.00–4.08 (d, 2 H), 4.61 (br s, 1 H), 5.30–5.70 (br t + m, 3 H), 7.10 (s, 4 H); MS *m/z* 538 ($M^{\bullet+}$).

[3a*S*-[2*E*,3aα,4α(1*E*,3*R*),5β,6aα]]-[2-[Hexahydro-5-hydroxy-4-[3-(2-indanyl)-3-hydroxy-1-propenyl]-2(1*H*)-pentalenylidene]ethoxy]acetic Acid *tert*-Butyl Ester (4S). A solution of 100 mg (0.186 mmol) of the allylic alcohol was treated with 5.0 mL of mixture (AcOH–H_2O–THF = 3:1:1). After stirring for 12 h at 45 °C, the reaction mixture was concentrated under reduced pressure. Following distillation with toluene azeotropically, the crude diol was chromatographed on 4.0 g of silica gel. Elution with ethyl acetate–*n*-hexane (2:1) gave 46 mg (54.5% yield) of α-isomer at C-15 from the more polar fraction as a colorless oil [IR (neat) 3400, 2926, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (s, 9 H), 1.55–2.54 (m, 10 H), 2.55–3.29 (m, 6 H), 3.45–4.10 (m, 2 H), 3.90 (s, 2 H), 3.99, 4.05 (d, 2 H), 5.27–5.56 (br t + m, 3 H), 7.10 (s, 4 H); MS *m/z* 454 ($M^{\bullet+}$)] and 25 mg (29.7% yield) of β-isomer at C-15 from the less polar fraction as a colorless oil [IR (neat) 3400, 2926, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (s, 9 H), 1.56–2.77 (m, 10 H), 1.90 (br s, 2 H), 2.78–3.28 (m, 4 H), 3.47–4.18 (m, 2 H), 3.91 (s, 2 H), 3.98, 4.06 (d, 2 H), 5.25–5.65 (br t + m, 3 H), 7.10 (s, 4 H); MS *m/z* 454 ($M^{\bullet+}$)].

[3a*S*-[2*E*,3aα,4α(1*E*,3*R*),5β,6aα]]-[2-[Hexahydro-5-hydroxy-4-[3-(2-indanyl)-3-hydroxy-1-propenyl]-2(1*H*)-pentalenylidene]ethoxy]acetic Acid (5S). The 7% KOH–MeOH solution (2.0 mL) was added to 40 mg (0.088 mmol) of diol (15-) at 0 °C and stirred for 12 h. The mixture was adjusted to pH 7.0 with 10% aqueous HCl at 0 °C, and solvent was removed under reduced pressure. Brine was added to the resulting aqueous solution. The pH was adjusted to 3–4 with 10% aqueous HCl at 0 °C and extracted with ethyl acetate. Extract was washed with H_2O and brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude acid, which was chromatographed on 1.0 g silica gel. Elution with THF–MeOH (4:1) gave 25 mg (71% yield) of acid as colorless crystals: mp 52–53 °C; IR (KBr) 3400, 2944, 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60–2.55 (m, 10 H), 2.56–3.23 (m, 4 H), 3.50–4.15 (d + m, 4 H), 4.03 (s, 2 H), 4.21 (br s, 3 H), 5.30–5.65 (br t + m, 3 H), 7.10 (s, 4 H); MS *m/z* 398 ($M^{\bullet+}$); HR-MS ($M^{\bullet+}$) calcd for $C_{24}H_{30}O_5$ 398.2091, found 398.2150. Purity by HPLC: 99.2%.

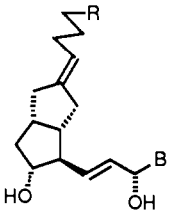
[3a*S*-[2*E*,3aα,4α(1*E*,3*R*),5β,6aα]]-5-[Hexahydro-5-hydroxy-4-[3-(2-indanyl)-3-hydroxy-1-propenyl]-2(1*H*)-pentalenylidene]pentanamide (6a). A solution of 30 mg (0.0732 mmol) of ester (4h) in 2.8 mL of MeOH was treated with 2.8 mL of 28% aqueous NH_3 at 0 °C. After stirring for 18 h, the reaction mixture was extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine and was dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude amide, which was chromatographed on 1.0 g of silica gel. Elution with ethyl acetate–MeOH (3:1) gave 18 mg (63% yield) of amide as colorless crystals: mp 85–88 °C; IR (KBr) 3424, 2926, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85–2.60 (m, 15 H), 2.60–3.20 (m, 5 H), 3.25–4.20 (m, 4 H), 5.20 (br t, 1 H), 5.50 (m, 2 H), 5.80–6.35 (m, 2 H), 7.10 (s, 4 H); MS *m/z* 396 ($M^{\bullet+}$); HR-MS ($M^{\bullet+}$) calcd for $C_{25}H_{33}O_3N$ 395.2459, found 395.2423. Purity by HPLC: 99.5%.

[3a*S*-[2*E*,3aα,4α(1*E*,3*R*),5β,6aα]]-1-Hydroxy-5-[hexahydro-5-hydroxy-4-[3-(2-indanyl)-3-hydroxy-1-propenyl]-2(1*H*)-pentalenylidene]pentane (7a). To a solution of 60 mg (0.147 mmol) of ester (4h) in 2.4 mL of ether was added dropwise 0.06 mL (0.147 mmol) of 3.4 M Red-Al at 0 °C under Ar atmosphere. After 30 min at room temperature, the reaction was quenched with brine. The mixture was extracted with ethyl acetate. Ethyl acetate extract was washed with brine and dried over $MgSO_4$. Filtration and evaporation of solvent under reduced pressure provided crude alcohol, which was chromatographed on 1.0 g of silica gel. Elution with ethyl acetate–MeOH (4:1) gave 40 mg (72% yield) of alcohol as a colorless oil: IR (neat) 3370, 2926 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90–2.65 (m, 15 H), 2.65–3.40 (m, 8 H), 3.60 (t, 3 H), 4.00 (m, 1 H), 5.25 (br t, 1 H), 5.50 (m, 2 H), 7.15 (s, 4 H); MS *m/z* 382 ($M^{\bullet+}$); HR-MS ($M^{\bullet+}$) calcd for $C_{25}H_{34}O_3$ 382.2506, found 382.2484. Purity by HPLC: 98.9%.

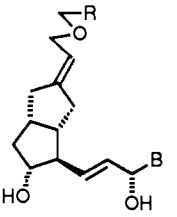
Method B. Preparation of Chloro(2-indanoyl)methyl Dimethylphosphonate. To a stirred suspension of 94 mg (2.36 mmol) of 60% sodium hydride dispersion in 1.6 mL of DME cooled to 0 °C under Ar atmosphere was added dropwise a solution of 630 mg (2.36 mmol) of (2-indanoyl)ethyl dimethylphosphonate in 11.0 mL of DME. After stirring for 30 min, 315 mg (2.36 mmol) of *N*-chlorosuccinimide was added to the suspension. After stirring for 1 h, the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with ether, and the ether extract was washed with water and brine and dried over $MgSO_5$. Filtration and evaporation of the solvent under reduced pressure provided crude product, which was chromatographed on 18.0 g of silica gel. Elution with ethyl acetate–*n*-hexane (1:1) gave 351 mg (49% yield) of chlorinated β-keto phosphonate as pale yellow crystals: mp 57–58 °C; IR (KBr) 2944, 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.14, 3.27 (d, 4 H), 3.34–3.65 (m, 1 H), 3.73 (s, 3 H), 3.90 (s, 3 H), 4.63, 4.93 (d, 1 H), 7.11 (s, 4 H); MS *m/z* 303 ($M^{\bullet+}$).

[3a*S*-[2*E*,3aα,4α(1*E*),5β,6aα]]-5-[Hexahydro-5-(tetrahydropyranyloxy)-4-[2-chloro-3-oxo-3-(2-indanyl)-1-propenyl]-2(1*H*)-pentalenylidene]pentanoic Acid Methyl Ester and [3a*S*-[2*E*,3aα,4α(1*Z*),5β,6aα]]-5-[Hexahydro-5-(tetrahydroypyranyloxy)-4-[2-chloro-3-oxo-3-(2-indanyl)-1-propenyl]-2(1*H*)-pentalenylidene]pentanoic Acid Methyl Ester (8a). To a stirred suspension of 8 mg (0.187 mmol) of 60% sodium hydride dispersion in 0.1 mL of DME cooled to 0 °C under Ar atmosphere was added dropwise a solution of 114 mg (0.374 mmol) of chloro(2-indanoyl)methyl dimethylphosphonate in 1.2 mL of DME. After the solution was stirred at room temperature for 20 min, the mixture was cooled to 0 °C and a solution of 51 mg (0.142 mmol) of aldehyde (X = CH_2, carbacyclin) in 1.2 mL of DME was added and stirred for 1 h at 0 °C, 2 h at room temperature, and 4 h at 50 °C. The reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with ether, and the ether extract was washed with brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude α-chloro enone, which was chromatographed on 2.0 g of silica gel. Elution with ether–*n*-hexane (1:4) gave 22 mg (29% yield) of trans isomer from the polar fraction as the colorless oil [IR (neat) 2932, 1734, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15–2.77 (m, 21 H), 3.05–4.35 (m, 4 H), 3.20, 3.35 (d, 4 H), 3.65 (s, 3 H), 4.68 (br s, 1 H), 5.21 (br t, 1 H), 6.06, 6.22 (d, 1 H), 7.20 (s, 4 H); MS *m/z* 527 ($M^{\bullet+}$)] and 38 mg (51% yield) of cis isomer from the more polar fraction as a colorless oil [IR

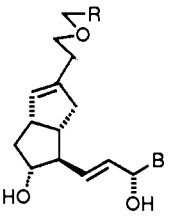
Table I. Structures, Synthetic Routes, and Physical Properties of Carbacyclins, 3-Oxacarbacyclins, and 3-Oxaisocarbacyclins



carbacyclin



3-oxacarbacyclin



3-oxaisocarbacyclin

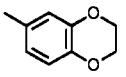
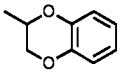
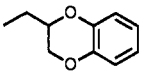
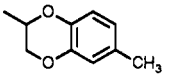
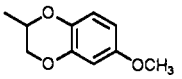
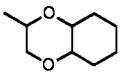
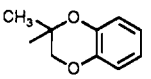
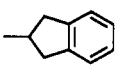
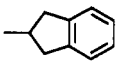
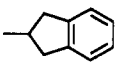
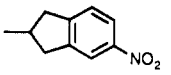
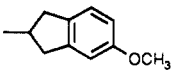
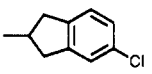
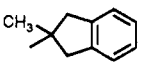
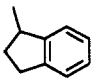
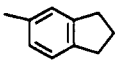
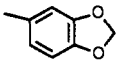
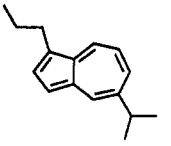
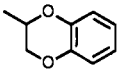
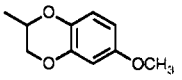
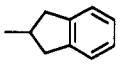
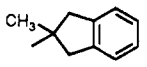
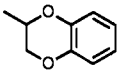
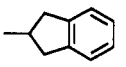
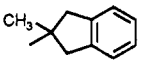
compd	R	B	method	mp, °C	yield,[a] %	formula	M$^{\bullet+}$ calcd	M$^{\bullet+}$ found ER (MU)	t_R, min[b]		purity, %
carbacyclins											
5a	COOH		A	oil	4.2	$C_{24}H_{30}O_6$	414.2040	414.2078 (+3.8)	6.49		99.0
5b	COOH		A	oil	19.2	$C_{24}H_{30}O_6$	414.2040	414.2015 (−2.5)	9.45[c] 9.88	54.3 45.3	99.6
5c	COOH		A	oil	3.4	$C_{25}H_{32}O_6$	428.2205	428.2197 (+0.8)	10.49[c] 12.27	49.4 49.4	98.8
5d	COOH		A	oil	25.7	$C_{25}H_{32}O_6$	428.2197	428.2208 (+1.1)	12.92		98.5
5e	COOH		A	oil	5.5	$C_{25}H_{32}O_7$	444.2146	444.2130 (−1.6)	9.08		99.3
5f	COOH		A	oil	23.1	$C_{24}H_{36}O_6$	420.2510	420.2503 (−0.7)	4.95[c] 5.35	38.0 60.9	98.9
5g	COOH		A	oil	38.3	$C_{25}H_{32}O_6$	428.2197	428.2191 (−0.6)	11.48[c] 12.26	19.9 79.5	99.4
5h	COOH		A	63–64	25.2	$C_{25}H_{32}O_4$	396.2299	396.2261 (−3.8)	5.20		99.6
6a	$CONH_2$		A	87–88	26.6	$C_{25}H_{33}O_3N$	395.2459	395.2423 (−3.6)	7.46		99.5
7a	CH_2OH		A	oil	30.5	$C_{25}H_{34}O_3$	382.2506	382.2484 (−2.2)	15.65		98.9
5i	COOH		A	oil	25.9	$C_{25}H_{31}O_6N$	441.2150	441.2141 (−0.9)	11.87		99.2
5j	COOH		A	oil	29.8	$C_{26}H_{34}O_5$	426.2403	426.2400 (−0.3)	11.49		99.0
5k	COOH		A	oil	27.9	$C_{25}H_{31}O_4Cl$	430.1909	430.1920 (+1.1)	18.21		99.0
5l	COOH		A	oil	43.5	$C_{26}H_{34}O_4$	410.2456	410.2429 (−2.7)	18.50		99.1
5m	COOH		A	oil	26.8	$C_{25}H_{32}O_4$	396.2299	396.2270 (−2.9)	11.76[c] 12.25	74.1 24.7	98.8
5n	COOH		A	oil	29.6	$C_{25}H_{32}O_4$	396.2299	396.2270 (−2.9)	17.11		98.7
5o	COOH		A	oil	25.9	$C_{23}H_{28}O_6$	400.1884	400.1877 (−0.7)	6.85		99.4
5p	COOH		A	oil	25.9	$C_{31}H_{40}O_4$	476.2924	476.2909 (−1.5)	9.72		99.0
			B	oil	17.7	$C_{25}H_{30}O_4$	394.2142	394.2186 (+4.4)	12.82		99.3
			(11a) 13,14-dehydro-**5h**								
			C	oil	56.2	$C_{25}H_{34}O_4$	398.2455	398.2470 (+1.5)	15.85		99.5
			(15a) 13,14-dihydro-**5h**								

Table I (Continued)

compd	R	B	method	mp, °C	yield,[a] %	formula	M•+ calcd	M•+ found ER (MU)	t_R, min[b]		purity, %
3-oxacarbacyclins											
5q	COOH		A	106–107	18.9	$C_{23}H_{28}O_7$	416.1832	416.1818 (−1.4)	3.95[c] 4.11	63.3 36.2	99.5
5r	COOH		A	52–53	14.4	$C_{24}H_{30}O_8$	446.1938	446.1922 (−1.6)	3.92		98.9
5s	COOH		A	52–53	21.0	$C_{24}H_{30}O_5$	398.2091	398.2140 (+4.9)	5.27		99.2
5t	COOH		A	59–60	32.6	$C_{25}H_{32}O_5$	412.2248	412.2239 (−0.9)	7.01		99.0
3-oxaisocarbacyclins											
5u	COOH		A	62–63	16.9	$C_{23}H_{28}O_7$	416.1832	416.1859 (+2.7)	4.13[c] 4.25	66.1 33.0	99.1
5v	COOH		A	oil	26.4	$C_{24}H_{30}O_5$	398.2091	398.2093 (+0.2)	4.77		99.4
5w	COOH		A	116–117	37.9	$C_{25}H_{32}O_5$	412.2248	412.2239 (−0.9)	6.90		99.5

[a] Overall yield from aldehyde 1. [b] See Experimental Section. [c] Enantiomers of C-16 position were separated under this HPLC condition.

Table II. Biological Effects of Carbacyclin Analogues

compd	organ-protective act. ED_{50},[a] μg/kg, po	hypotensive act. MBP_{15},[b] μg/kg, iv	antiaggregation act. IC_{50},[c] ng/mL
5a	>300	>100	>1000
5b	4.5	2.0 ± 0.1	323.6 ± 10.1
5c	>300	44.0 ± 5.2	323.6 ± 5.8
5d	110.0	13.0 ± 2.0	647.1 ± 20.4
5e	2.1	3.5 ± 1.0	355.1 ± 10.2
5f	256.3	13.5 ± 5.0	>1000
5g	14.2	3.6 ± 0.3	221.3 ± 8.5
5h	56.2	4.3 ± 0.2	20.4 ± 1.6
6a	112.9	97.7 ± 5.0	53.7 ± 2.4
7a	53.6	>100	>1000
5i	140.0	8.5 ± 0.7	150.0 ± 10.3
5j	14.3	6.0 ± 0.4	70.5 ± 5.0
5k	>300	>100	407.3 ± 19.5
5l	45.2	5.3 ± 0.5	30.2 ± 1.8
5m	NE[d]	>100	>1000
5n	NE	>100	>1000
5o	NE	>100	>1000
5p	NE	>100	>1000
11a	1.7	1.3 ± 0.1	8.2 ± 0.9
15a	NE	35.0 ± 5.0	76.4 ± 6.3
5q	112.7	2.5 ± 0.3	582.2 ± 21.5
5r	NE	6.2 ± 0.4	>1000
5s	67.5	3.8 ± 0.7	15.3 ± 0.8
5t	39.7	7.6 ± 1.0	117.8 ± 11.1
5u	8.5	1.7 ± 0.2	53.1 ± 3.2
5v	27.4	2.2 ± 0.3	56.7 ± 2.3
5w	6.0	2.0 ± 0.1	69.2 ± 3.1
PGE_2	34.1	1.2 ± 0.2	NE
PGI_2 Na salt	NE	0.3 ± 0.1	3.4 ± 0.1

[a] The dose that inhibits EtOH-induced gastric lesions by 50%. ED_{50} was calculated by linear regression from three dose groups of eight animals each. [b] The dose that lowers the mean blood pressure by 15 mmHg (peak effect). [c] The concentration that inhibits collagen-induced aggregation by 50%. MBP_{15} and IC_{50} were mean ± SEM and were calculated by linear regression from three dose groups of five or six animals. [d] NE represents no effect.

Table III. Relative Potency and Ratio of Compounds[a]

compd	relative potency: organ protection	relative potency: hypotension	ratio organ protection, hypotension
5b	7.6	0.6	12.7
5e	16.2	0.3	47.6
11a	20.1	0.9	21.8
PGE_2	1.0	1.0	1.0

[a] These values are calculated from Table II.

Table IV. Relative Potency and Ratio of Compounds[a]

compd	relative potency: antiaggregation	relative potency: hypotension	ratio antiaggregation, hypotension
5h	0.17	0.07	2.4
11a	0.42	0.23	1.8
5s	0.23	0.08	2.8
PGI_2Na	1.00	1.00	1.0

[a] These values are calculated from Table II.

(neat) 2923, 1737, 1689 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10–2.84 (m, 21 H), 3.11–4.38 (m, 4 H), 3.20, 3.34 (d, 4 H), 3.63 (s, 3 H), 4.50–4.71 (m, 1 H), 5.38 (br t, 1 H), 6.91, 7.10 (d, 1 H), 7.20 (s, 4 H); MS m/z 527 ($M^{\bullet+}$)].

[3a*S*-[2*E*,3aα,4α(1*Z*,3*RS*),5β,6aα]]-5-[Hexahydro-5-(tetrahydropyranyloxy)-4-[2-chloro-3-hydroxy-3-(2-indanyl)-1-propenyl]-2(1*H*)-pentalenylidene]pentanoic Acid Methyl Ester (9a). A solution of 220 mg (0.418 mmol) of enone in 2.2 mL of MeOH was treated with 20 mg (0.418 mmol) of sodium borohydride at 0 °C under Ar atomosphere and stirred for 15 min. After acetone was added at 0 °C and stirred for 30 min at room temperature, the mixture was neutralized with saturated aqueous NH_4Cl. The solvent was removed under reduced pressure, and then the aqueous solution was extracted with ether. The ether extract was washed with brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude allylic alcohol, which was chromatographed on 6.0 g of silica gel. Elution with ether–*n*-hexane (2:1) gave 180 mg (82% yield) of allylic alcohol as a colorless oil: IR (neat) 3418, 2932, 1731 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00–2.63 (m, 21 H), 2.64–3.20 (m + d, 5 H), 3.20–4.21 (m, 5 H), 3.63 (s, 3 H), 4.63 (br s, 1 H), 5.25 (br t, 1 H), 5.61, 5.75 (d, 1 H), 7.10 (s, 4 H); MS m/z 528 ($M^{\bullet+}$).

[3a*S*-[2*E*,3aα,4α(1*Z*,3*RS*),5β,6aα]]-5-[Hexahydro-5-hydroxy-4-[2-chloro-3-hydroxy-3-(2-indanyl)-1-propenyl]-2-(1*H*)-pentalenylidene]pentanoic Acid Methyl Ester and [3a*S*-[2*E*,3aα,4α(1*RS*,3*S*),5β,6aα]]-5-[Hexahydro-5-hydroxy-4-[2-chloro-3-hydroxy-3-(2-indanyl)-1-propenyl]-2-(1*H*)-pentalenylidene]pentanoic Acid Methyl Ester (10a). A solution of 240 mg (0.454 mmol) of allylic alcohol was treated with 10.0 mL of mixture (AcOH–H_2O–THF = 3:1:1). After stirring for 8 h at 45 °C, the reaction mixture was concentrated under reduced pressure. After distillation with toluene azeotropically, the crude diol was chromatographed on 10.0 g of silica gel. Elution with ethyl acetate–*n*-hexane (1:1) gave 71 mg (35% yield) of α-isomer at C-15 from the less polar fraction as a colorless oil [IR (neat) 3394, 2932, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.01–2.71 (m, 17 H), 2.72–3.14 (m, 5 H), 3.44–4.29 (m, 2 H), 3.63 (s, 3 H), 5.24 (br t, 1 H), 5.60, 5.78 (d, 1 H), 7.12 (s, 4 H); MS m/z 444 ($M^{\bullet+}$)] and 104 mg (52% yield) of β-isomer at C-15 from the more polar fraction as the colorless oil [IR (neat) 3370, 2932, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00–2.61 (m, 17 H), 2.61–3.38 (m, 5 H), 3.39–4.30 (m, 2 H), 3.65 (s, 3 H), 5.29 (br t, 1 H), 5.51, 5.69 (d, 1 H), 7.10 (s, 4 H); MS m/z 444 ($M^{\bullet+}$).

[3a*S*-[2*E*,3aα,4α(3*R*),5β,6aα]]-5-[Hexahydro-5-hydroxy-4-[3-hydroxy-3-(2-indanyl)-1-propynyl]-2(1*H*)-pentalenylidene]pentanoic Acid (11a). A solution of 30 mg (0.0675 mmol) of diol of 2.0 mL of DMSO and 1.0 mL THF was mixed with 38 mg (0.337 mmol) of potassium *tert*-butoxide. After stirring for 10 h at room temperature, the reaction mixture was adjusted to pH 4.0 with 10% aqueous HCl at 0 °C and was extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure gave crude acid, which was purified by preparative TLC with ethyl acetate as the eluant to obtain 21 mg (79% yield) of acid as a colorless oil: IR (neat) 3376, 2932, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30–2.70 (m, 15 H), 2.90 (m, 5 H), 3.50–4.10 (m, 1 H), 4.20–4.41 (d, 1 H), 4.93–5.40 (br s + br t, 5 H), 7.08 (s, 4 H); MS m/z 394 ($M^{\bullet+}$); HR-MS ($M^{\bullet+}$) calcd for $C_{25}H_{30}O_4$ 394.2142, found 394.2186. Purity by HPLC: 99.3%.

Method C. [3a*S*-[2*E*,3aα,4α(3*R*),5β,6aα]]-5-[Hexahydro-5-(tetrahydropyranyloxy)-4-[3-oxo-3-(2-indanyl)-1-propanyl]-2(1*H*)-pentalenylidene]pentanoic Acid Methyl Ester (12a). To a solution of 500 mg (1.02 mmol) of enone **2h** in 10.0 mL of acetone (Ar bubbling) was added 60 mg (0.204 mmol) of (methyl benzoate)chromium tricarbonyl was added. This mixture was transfered to an autoclave and stirred for 12 h at 120 °C under 70 kg/cm H_2 pressure. The reaction mixture was cooled to room temperature and removed from the autoclave. The mixture was stirred for 1 h at room temperature, and the solvent was removed under reduced pressure. The residue was chromatographed on 14.0 g of silica gel. Elution with ether–*n*-hexane (1:4) gave 423 mg (84% yield) of ketone as a colorless oil: IR (neat) 2950, 1740, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00–2.49 (m, 22 H), 2.51–2.71 (t + m, 3 H), 3.12, 3.20 (d, 4 H), 3.27–4.01 (m, 4 H), 3.65 (s, 3 H), 4.58 (br s, 1 H), 5.17 (br t, 1 H), 7.15 (s, 4 H); MS m/z 494 ($M^{\bullet+}$).

[3a*S*-[2*E*,3aα,4α(3*R*),5β,6aα]]-5-[Hexahydro-5-(tetrahydropyranyloxy)-4-[3-hydroxy-3-(2-indanyl)-1-propanyl]-2(1*H*)-pentalenylidene]pentanoic Acid Methyl Ester (13a). A solution of 300 mg (0.607 mmol) of ketone in 6.0 mL of MeOH was treated with 28 mg (0.607 mmol) of sodium borohydride at 0 °C under Ar atmosphere and stirred for 20 min. After acetone was added at 0 °C and the mixture stirred for 30 min at room temperature, the mixture was neutralized with saturated aqueous NH_4Cl. The solvent was removed under pressure, and then the aqueous solution was extracted with ether. The ether extract was washed with brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude alcohol, which was chromatographed on 10.0 g of silica gel. Elution with ether–*n*-hexane (1:1) gave 300 mg (100% yield) of alcohol as a colorless oil: IR (neat) 3454, 2926, 1731 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78–2.68 (m, 27 H), 2.69–3.23 (m, 4 H), 3.23–4.07 (m, 4 H), 3.63 (s, 3 H), 4.60 (br s, 1 H), 5.17 (br t, 1 H), 7.12 (s, 4 H); MS m/z 496 ($M^{\bullet+}$).

[3a*S*-[2*E*,3aα,4α(3*R*),5β,6aα]]-5-[Hexahydro-5-hydroxy-4-[3-hydroxy-3-(2-indanyl)-1-propanyl]-2(1*H*)-pentalenylidene]pentanoic Acid Methyl Ester (14a). A solution of 300 mg (0.609 mmol) of alcohol was treated with 7.5 mL of mixture (AcOH–H_2O–THF = 3:1:1). After stirring for 12 h at 45 °C, the reaction mixture was concentrated under reduced pressure. After distillation with toluene azeotropically, the crude diol was chromatographed on 10.0 g of silica gel. Elution with ethyl acetate–*n*-hexane (1:1) gave 242 mg (97% yield) as a colorless oil: IR (neat) 3370, 2926, 1731 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00–2.77 (m, 22 H), 2.78–3.12 (m, 4 H), 3.29–3.83 (m, 2 H), 3.63 (s, 3 H), 5.16 (br t, 1 H), 7.11 (s, 4 H); MS m/z 412 ($M^{\bullet+}$).

[3a*S*-[2*E*,3aα,4α(3*R*),5β,6aα]]-5-[Hexahydro-5-hydroxy-4-[3-hydroxy-3-(2-indanyl)-1-propanyl]-2(1*H*)-pentalenylidene]pentanoic Acid (15a). To a solution of 100 mg (0.243 mmol) of diol in 4.0 mL of MeOH was added 2.0 mL of 10% aqueous NaOH at 0 °C and the mixture stirred for 10 h. The mixture was adjusted to pH 7.0 with 10% aqueous HCl at 0 °C, and the solvent was removed under reduced pressure. Brine was then added to the aqueous solution. The pH was adjusted to 3–4 with 10% aqueous HCl at 0 °C, and the reaction mixture was extracted with water and brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude acid, which was chromatographed on 3.0 g of silica gel. Elution with ethyl acetate–MeOH (15:1) gave 90 mg (94% yield) as a colorless oil: IR (neat) 3460, 2926, 1737 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00–2.66 (m, 20 H), 2.67–3.12 (m, 4 H), 3.49–3.79 (m, 2 H), 5.18 (br t, 1 H), 5.58 (br s, 3 H), 7.11 (s, 4 H); MS m/z 398 ($M^{\bullet+}$); HR-MS ($M^{\bullet+}$) calcd for $C_{25}H_{34}O_4$ 398.2455, found 398.2470. Purity by HPLC: 99.5%.

[3a*S*-[2*E*,3aα,4α(3*S*),5β,6aα]]-5-[Hexahydro-5-(tetrahydropyranyloxy)-4-[3-acetoxy-3-(2-indanyl)-1-propanyl]-2(1*H*)-pentalenylidene]pentanoic Acid Methyl Ester (16a). To a solution of 100 mg (0.202 mmol) of alcohol in 3.0 mL of CH_2Cl_2 were added 0.06 mL (0.404 mmol) of triethylamine and 0.03 mL (0.404 mmol) of methanesulfonyl chloride under Ar atmosphere, and the mixture was stirred for 1 h. The reaction was quenched with water. The mixture was extracted with ether, and the ether extract was washed with brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude mesylate, which was chromatographed on 3.0 g of silica gel. Elution with ether–*n*-hexane gave 110 mg (95% yield) of mesylate as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.21–2.54 (m, 20 H), 2.68–3.27 (m, 4 H), 2.89 (s, 3 H), 3.28–4.00 (m, 3 H), 3.65 (s, 3 H), 4.58 (br s, 1 H), 5.73–5.94 (m, 1 H), 5.17 (br t, 1 H), 7.13 (s, 4 H). To a solution of 100 mg (0.174 mmol) of mesylate in 5.0 mL of toluene were added 23 mg (0.087 mmol) 18-crown-6 and 200 mg (1.04 mmol) of cesium acetate under Ar atmosphere. The solution was stirred at reflux for 1 h and cooled to room temperature, and 100 mg (0.52 mmol) of cesium acetate was then added. The resulting solution was stirred at reflux for 2 h. The reaction was quenched with water at room temperature. The mixture was extracted with ether, and the ether extract was washed with water and brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude acetate, which was chromatographed on 3.0 g of silica gel. Elution with ether–*n*-hexane (1:1) gave 75 mg (81% yield) of acetate as a colorless oil: IR (neat) 2932, 1734 cm^{-1}; ^1H NMR

($CDCl_3$) δ 1.20–2.53 (m, 20 H), 2.01 (s, 3 H), 2.54–3.13 (m, 4 H), 3.30–4.02 (m, 3 H), 4.50–4.70 (m, 1 H), 4.88–5.30 (br t + m, 2 H), 7.11 (s, 4 H); MS *m/z* 538 ($M^{\cdot+}$).

[3a*S*-[2*E*,3aα,4α(3*S*),5β,6aα]]-5-[Hexahydro-5-hydroxy-4-[3-hydroxy-3-(2-indanyl)-1-propanyl]-2(1*H*)-pentalenylidene]pentanoic Acid Methyl Ester (17a). To a solution of 55 mg (0.102 mmol) of acetate in 1.0 mL of MeOH was added 54 mg (0.400 mmol) of K_2CO_3 under Ar atmosphere, and the mixture was stirred for 1 h at room temperature and 3 h at 45 °C. The reaction mixture was cooled to −25 °C, mixed with 10% aqueous HCl in one portion, and at once neutralized with saturated aqueous $NaHCO_3$. The solvent was removed under reduced pressure, and then the aqueous solution was extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine and dried over $MgSO_4$. Filtration and evaporation of solvent under reduced pressure provided crude alcohol, which was chromatographed on 1.5 g of silica gel. Elution with ether–*n*-hexane (2:1) gave 50 mg (99% yield) of alcohol as a colorless oil: IR (neat) 3440, 2920, 1731 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00–3.22 (m, 25 H), 2.68–3.23 (m, 4 H), 3.24–4.03 (m, 3 H), 3.64 (s, 3 H), 4.60 (br s, 1 H), 5.66 (br t, 1 H), 7.13 (s, 4 H); MS *m/z* 496 ($M^{\cdot+}$). A solution of 150 mg (0.302 mmol) of alcohol was treated with 5.0 mL of mixture (AcOH–H_2O–THF = 3:1:1). After stirring for 10 h at 45 °C, the reaction mixture was concentrated under reduced pressure. After distillation with toluene azeotropically, the crude diol was chromatographed on 4.5 g of silica gel. Elution with ethyl acetate–*n*-hexane (1:1) gave 112 mg (90% yield) of diol as a colorless oil: IR (neat) 3328, 2920, 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00–2.67 (m, 19 H), 2.68–3.26 (m, 6 H), 3.40–3.82 (m, 2 H), 3.64 (s, 3 H), 5.18 (br t, 1 H), 7.11 (s, 4 H); MS *m/z* 412 ($M^{\cdot+}$).

[3a*S*-[2*E*,3aα,4α(3*S*),5β,6aα]]-5-[Hexahydro-5-hydroxy-4-[3-hydroxy-3-(2-indanyl)-1-propanyl]-2(1*H*)-pentalenylidene]pentanoic Acid (18a). To a solution of 80 mg (0.194 mmol) of ester in 4.0 mL of MeOH was added 2.0 mL of 10% aqueous NaOH at 0 °C, and the mixture was stirred for 10 h. The mixture was adjusted to pH 7.0 with 10% aqueous HCl at 0 °C, solvent was removed under reduced pressure, and brine was added to the resulting aqueous solution. The pH was adjusted to 3–4 with 10% aqueous HCl at 0 °C, and the solution was treated ethyl acetate. The ethyl acetate extract was washed with water and brine and dried over $MgSO_4$. Filtration and evaporation under reduced pressure provided crude acid, which was choromatpgraphed on 2.5 g of silica gel. Elution with ethyl acetate–MeOH (15:1) gave 70 mg (92% yield) of acid as a colorless oil: IR (neat) 3370, 2920, 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.04–2.65 (m, 20 H), 2.66–3.26 (m, 4 H), 3.40–3.90 (m, 2 H), 4.90 (br s, 3 H), 5.20 (br t, 1 H), 7.14 (s, 4 H); MS *m/z* 398 ($M^{\cdot+}$); HR-MS ($M^{\cdot+}$) calcd for $C_{25}H_{34}O_4$ 398.2455, found 398.2454. Purity by HPLC: 99.0%.

2-Cyano-1,4-benzodioxan (20). To a solution of 32.0 g (290 mmol) of catechol in 25 mL of acetate were added 61.6 g (290 mmol) of α,β-dibromopropionitrile (freshly prepared by bromination of acrylonitrile in $CHCl_3$ at room temperature) and 84.6 g (609 mmol) of K_2CO_3, and the mixture was stirred for 12 h at reflux under Ar atmosphere. The mixture was allowed to cool. The solids were then filtered, and the filtrate was evaporated under reduced pressure. The residue was dissolved in ether, washed with 10% aqueous NaOH, water, and brine, and dried over $MgSO_4$. Filtration and evaporation under reduced pressure provided crude nitrile, which was isolated by distillation (137 °C/10 mmHg). A total of 39.4 g (85% yield) of nitrile was obtained as colorless crystals; MS *m/z* 161 ($M^{\cdot+}$).

2-(Methoxycarbonyl)-1,4-benzodioxan (21). 2-Cyano-1,4-benzodioxan (28.1 g, 175 mmol) was treated with mixture (56.3 mL of AcOH, 56.3 mL of water, and 18.8 mL of concentrated H_2SO_4). After stirring for 15 h at 110 °C, the mixture was cooled to room temperature and poured into ice–water. The resulting solid was filtered, washed with water, and dried in a vacuum desiccator to give 30.7 g (98% yield) of colorless crystals; MS *m/z* 180 ($M^{\cdot+}$). To a solution of 30.7 g (171 mmol) of acid in 300 mL of MeOH was added 6 drops of concentrated H_2SO_4, and the mixture was stirred at reflux for 1 h. The mixture was cooled to room temperature and was evaporated under reduced pressure. The residue was dissolved in ether, washed with saturated aqueous $NaHCO_3$, water, and brine, and dried over $MgSO_4$. Filtration and evaporation under reduced pressure provided crude ester, which was isolated by distillation (125 °C/5 mmHg). A total of 31.9 g (97% yield) of ester was obtained as colorless crystals; MS *m/z* 194 ($M^{\cdot+}$).

Dimethyl [(2-Benzodioxanoyl)methyl]phosphonate (22). A solution of 2.7 mL (24.7 mmol) of dimethyl methylphosphonate in 2.7 mL of THF at −78 °C was treated over a 10-min period with 16.5 mL (24.7 mmol) of 1.50 M *n*-butyllithium in *n*-hexane. The resulting suspension was stirred for 30 min at −78 °C and then treated with a solution of 4.0 g (20.6 mmol) of ester in 30.0 mL of THF and stirred for 1.5 h. The reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with ethyl acetate, and the ethyl acetate extract was washed with brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude product, which was chromatographed with ethyl acetate on 15.0 g of silica gel. Elution with ethyl acetate gave 4.0 g (68% yield) of β-keto phosphonate as a colorless oil: IR (neat) 2944, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.16–3.55 (2 d, 2 H), 3.66–3.80 (2 d, 6 H), 4.27–4.34 (2 d, 2 H), 4.70 (t, 1 H), 6.70–7.04 (m, 4 H); MS *m/z* 286 ($M^{\cdot+}$ − 1).

2,2-Indandicarboxylic Acid (25). In a 3-L, three-necked flask fitted with a magnetic stirrer, 270 g of absolute ethanol and 19.0 g (0.82 mol) of sodium metal which had been cut into small pieces were placed. After the solution was complete, 750 mL of anhydrous ether was added, followed by 63.0 g (1.12 mol) of diethyl malonate. To this solution was added, as quickly as possible, 105.0 g (390 mmol) of α,α′-dibromo-*o*-xylene in 750 mL of ether. The reaction mixture was stirred under reflux for 5 h. The precipitate was allowed to settle, and the solution was filtered. The solvent was removed, and the residue was refluxed with 500 mL of water and 90.0 g of KOH for 16 h. At the end of that time, the solution was cooled and acidified with 30% (v/v) HCl. The white diacid was collected and dried in a vacuum desiccator.

2-(Methoxycarbonyl)indan (26). The diacid obtained from the previous reaction was placed in a covered dish with aluminum foil and heated to 200 °C on a hot plate. The liquefied compound was held at 200 °C for 20 min and then was allowed to cool. The product was recrystallized from hexane, affording 57.0 g (94% yield from dibromo-*o*-xylene) of the monoacid as white plates. To a solution of 20.9 g (102 mmol) of monoacid in 200 mL of MeOH was added 5 drops of concentrated H_2SO_4 and the mixture was stirred at reflux for 1 h. The mixture was cooled to room temperature and was evaporated under reduced pressure. The residue was dissolved in ether, washed with saturated aqueous $NaHCO_3$, water, and brine, and dried over $MgSO_4$. Filtration and evaporation under reduced pressure provided crude ester, which was isolated by distillation (127 °C/12 mmHg). A total of 15.3 g (94% yield) of ester was obtained as a colorless oil; MS *m/z* 176 ($M^{\cdot+}$).

Dimethyl [(2-Indanoyl)methyl]phosphonate (27). A solution of 4.0 mL (36.9 mmol) of dimethyl methylphosphonate in 4.0 mL of THF at −78 °C was treated over a 10-min period with 23.1 mL (36.9 mmol) of 1.60 M *n*-butyllithium in *n*-hexane. The resulting suspension was stirred for 30 min at −78 °C and then treated with a solution of 5.0 g (28.4 mmol) of ester in 40.0 mL of THF and stirred for 1.5 h. The reaction was quenched with saturated NH_4Cl. The mixture was extracted with ethyl acetate, and the ethyl acetate extract was washed with brine and dried over $MgSO_4$. Filtration and evaporation of the solvent under reduced pressure provided crude β-keto phosphonate, which was chromatographed on 20.0 g of silica gel. Elution with ethyl acetate gave 4.80 g (63% yield) of β-keto phosphonate as a colorless oil: IR (neat) 2932, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.07, 3.31 (d, 2 H), 3.14, 3.22 (d, 4 H), 3.70 (s, 3 H), 3.82 (s, 3 H), 7.12 (s, 4 H); MS *m/z* 269 ($M^{\cdot+}$).

Organ-Protective Activity in Vivo. Male Sprague-Dawley rats (200–300 g) were deprived of food for 24 h before the experiments. Gastric lesions were produced by giving 1 mL of 100% ethanol/rat, and animals were killed 1 h later. The stomach was removed and fixed by formalin. Subsequently, the stomach was incised along the greater curvature and examined for lesions. Drugs were given po in a volume of 2 mL/kg of body weight at 30 min before ethanol administration.

Hypotensive Activity in Vivo. Male Wistar rats (200–300 g) were anesthetized with urethane. Blood pressure was measured by a transducer connected to the catheter inserted into the femoral artery. The test compound was injected via the femoral vein. The hypotensive activity was expressed as the dose that lowered the

mean blood pressure by 15 mmHg (peak effect).

Antiaggregation Activity in Vitro. Male Wistar rats (250–350 g) under ether anesthesia were used. Blood sample was taken from the abdominal aorta into a plastic syringe containing 0.1 volume of 3.13% sodium citrate solution. Platelet aggregation was measured by using an aggregometer (Chrono-Log Corp., Havertown, PA) at 37 °C under stirring. Aggregation was initiated by adding 5 μL of collagen (final concentration 5 μg/mL).

Antiaggregation Activity ex Vivo. Male Wistar rats (150–200 g) were orally given a test drug solubilized in 1.3% $NaHCO_3$ with 5% EtOH or the vehicle alone as a control. At the scheduled time after oral administration of the drug, the blood which was obtained by the same manner as in vitro was centrifuged at 250*g* for 7 min at room temperature. The supernatant fraction was used as platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was obtained from the supernatant fraction of the residual blood by centrifugation at 1300*g* for 10 min. A 495-μL volume of PRP was placed in a cuvette and incubated at 37 °C for 3 min. After incubation, platelet aggregation was initiated by adding 5 μL of collagen (final concentration 5 μg/mL). Changes in the light transmission in the cuvette after addition of collagen were recorded.

Alterations in the Stereochemistry of the κ-Selective Opioid Agonist U50,488 Result in High-Affinity σ Ligands

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The synthesis and in vitro σ receptor activity of the two diastereomers of U50,488 [(±)-**2**], namely, (1*R*,2*S*)-(+)-*cis*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(+)-**1**] and (1*S*,2*R*)-(−)-*cis*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(−)-**1**], are described. (+)-1 and (−)-**1** were synthesized from (±)-*trans*-*N*-methyl-2-aminocyclohexanol [(±)-**3**]. Pyridinium chlorochromate (PCC) oxidation of the *N*-*t*-Boc-protected derivative of (±)-**3** afforded (±)-2-[*N*-[(*tert*-butyloxy)carbonyl]-*N*-methylamino]cyclohexanone [(±)-**5**]. The sequence of enamine formation with pyrrolidine, catalytic reduction, N-deprotection, and optical resolution afforded (1*R*,2*S*)-(−)-*cis*-2-pyrrolidinyl-*N*-methylcyclohexylamine [(−)-**10**] and (1*S*,2*R*)-(+)-*cis*-2-pyrrolidinyl-*N*-methylcyclohexylamine [(+)-**10**]. The optical purity (>99.5%) of (−)-**10** and (+)-**10** was determined by HPLC analysis of the diastereomeric ureas formed by reaction with optically pure (*R*)-α-methylbenzyl isocyanate. The absolute configuration of (−)-**10** and (+)-**10** was determined by single-crystal X-ray diffractometry of the bis-(*R*)-mandelate salt. Condensation of optically pure (−)-**10** and (+)-**10** with 3,4-dichlorophenylacetic acid furnished (+)-**1** and (−)-**1**, respectively. Compounds (+)-**1**, (−)-**1**, (−)-**2**, and (+)-**2** were compared for their binding affinities at κ opioid, σ, D_2-dopamine, and phencyclidine (PCP) receptors in competitive binding assays using [^{3}H]bremazocine ([^{3}H]BREM) or [^{3}H]U69,593, [^{3}H]-(+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [[^{3}H]-(+)-3-PPP], or [^{3}H]-1,3-di(*o*-tolyl)guanidine ([^{3}H]DTG), [^{3}H]-(−)-sulpiride [[^{3}H]-(−)-SULP], and [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP), respectively. In the systems examined, (−)-**2** exhibited the highest affinity for κ receptors, with a K_i of 44 ± 8 nM. However, (−)-**2** also showed moderate affinity for σ receptors, with a K_i of 594 ± 3 nM [[^{3}H]-(+)-3-PPP]. The (1*R*,2*R*)-(+)-enantiomer, (+)-**2**, had low affinity for both κ and σ receptors, exhibiting K_i values of 1298 ± 49 nM at κ ([^{3}H]BREM) and 1270 ± 168 nM at σ [[^{3}H]-(+)-3-PPP]. In contrast, the chiral cis compounds (+)-**1** and (−)-**1** showed high affinity for σ receptors and negligible affinity for κ opioid receptors in the [^{3}H]BREM assay. Compound (−)-**1** exhibited a K_i of 81 ± 13 nM at σ receptors [[^{3}H]-(+)-3-PPP] and 250 ± 8 nM ([^{3}H]DTG). The corresponding values for (+)-**1** were 221 ± 36 nM [[^{3}H]-(+)-3-PPP] and 118 ± 7 nM ([^{3}H]DTG). Compounds (−)-**2** and (+)-**2** lacked affinity for D_2-dopamine receptors. Compounds (+)-1 and (−)-**1** bound only weakly to D_2-dopamine receptors, displaying K_i values of 14039 ± 1429 nM and 3762 ± 829 nM, respectively. All of the compounds lacked affinity for PCP receptors.

Psychotomimetic compounds have been shown to interact with σ and phencyclidine (PCP) receptors among others in the central nervous system. σ receptors are nonopioid, nondopaminergic receptors that bind antipsychotic drugs such as haloperidol and the (+)-enantiomer of opiate benzomorphans such as pentazocine and cyclazocine.[1-5] These receptors have been implicated in regulation of neurotransmitter release,[6] smooth muscle contraction,[6,7] control of motor behavior,[8,9] and modulation of phosphoinositide turnover.[10] However, many of the physiological functions of σ receptors remain to be elucidated. PCP receptors bind both PCP-related compounds and (+)-benzomorphans.[1] In addition to its psychotomimetic actions, the PCP receptor has recently been associated with anticonvulsant and neuroprotective activity.

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