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An Azido-Functionalized Isocarbacyclin Analogue Acting as an Efficient Photoaffinity Probe for a Prostacyclin Receptor

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Key Words: Prostacyclin; Isocarbacyclin; Photoaffinity probe; Prostacyclin receptor; Mastocytoma P-815 cell

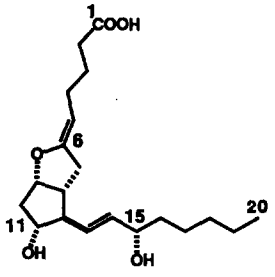
Abstract: *A stable prostacyclin analogue, (15S)-18c, having an azidophenyl group as a photoaffinity labeling functionality has been synthesized. This compound has a sufficiently high affinity to the prostacyclin receptor protein in mastocytoma P-815 cells, exhibiting an IC_{50} value of 3 nM for the replacement of iloprost bound to the receptor protein. A photoaffinity probe compound, [^{3}H]-(15S)-18c, is obtainable by reduction of the ketone 16c with [^{3}H]$NaBH_4$ –$CeCl_3$ followed by alkaline hydrolysis of the methyl ester.*

INTRODUCTION

Prostaglandins (PGs) are recognized as physiologically important compounds which are involved in vital defense processes such as inflammation, tissue repair, and immune response.[1] The diverse biological activities exhibited by PGs are thought to emerge via a series of signal communications initiated by their binding to the corresponding specific receptor proteins in cell membranes. Accordingly characterization of such receptor proteins has become one of the major objectives in PG science.[2]

Prostacyclin (PGI_2) (**1**) is a particularly potent vasodilator and inhibitor of platelet aggregation.[3] This compound activates adenylate cyclase in platelets,[4a,b] vascular smooth muscles,[4c] NCB-20 cells,[4d] mastocytoma P-815 cells,[4e] etc. However, there has been little progress in the study of the structures of the PGI_2 receptor

proteins because of their low concentration in cell membranes and the lack of suitable antagonists making it difficult to solubilize a receptor protein to the homogeneous state without loss of the binding activity.[5] We have been intrigued by the use of the photoaffinity labeling method[7] as another valuable tool for the characterization of the receptor protein. Described herein is the synthesis of an azido-functionalized photoaffinity probe compound, with high affinity to the PGI_2 receptor protein, based on the structural modification of isocarbacyclin (**2**),[8] a chemically stable PGI_2 analogue.[9]



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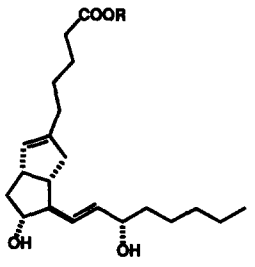


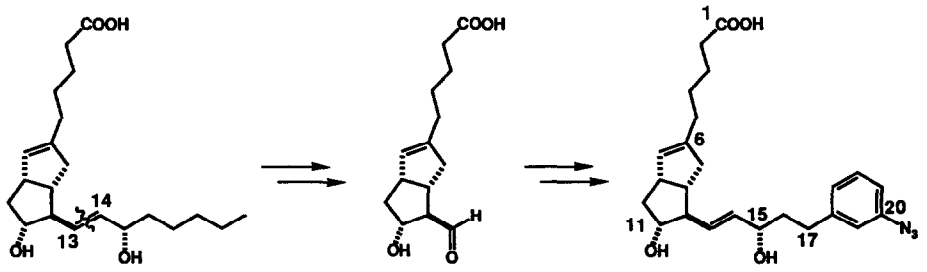
2, R = H
3, R = CH_3

RESULTS

Synthesis

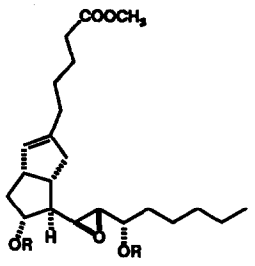
We assumed that the prostacyclin receptor protein recognizes the terminal carboxylic acid, sp^2-hybridized C(6) atom, chiral cyclopentane ring, and the 11- and 15-hydroxyl groups of **1** and its analogues. Accordingly we set up an azidophenyl derivative, given in Scheme 1, as an initial synthetic target. This compound has a photoreactive azido function[7] at the terminal of the ω side-chain comprising the zigzag-oriented twenty carbons including three in the aromatic ring. We also intended to investigate the influence of the ω side-chain length and the position of azido function on the aromatic ring to the affinity for the receptor protein.

Since isocarbacyclin (**2**) is sufficiently supplied by efficient chemical syntheses based on a three-component coupling PG synthesis,[10,11] the selective oxidative cleavage of the C(13)—C(14) double bond of **2** leading to an aldehyde and subsequent reconstruction of an ω side-chain having an azidophenyl moiety would be straightforward as shown in Scheme 1. Thus the C(13)—C(14) double bond of isocarbacyclin methyl ester (**3**)

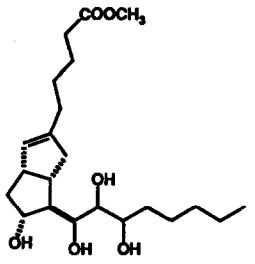


Scheme 1. Basic Plan for the Incorporation of an Azidophenyl Function

was selectively epoxidized by Sharpless epoxidation[12] giving monoepoxide **4** in 93% yield, whose 11- and 15-hydroxyl groups were acetylated under standard conditions to give **5** in 96% yield. Epoxy ring opening with CH_3COOH–H_2O at 100 °C[13] and subsequent deacetylation with aqueous K_2CO_3 gave tetraol **6** in 92% as a mixture of two diastereomers, which underwent oxidative cleavage with $NaIO_4$ to give aldehyde **7** in 96% yield.



4, R = H
5, R = $COCH_3$



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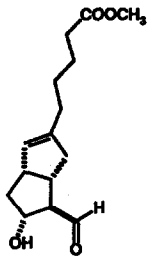

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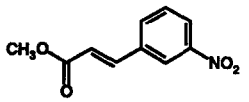
Three kinds of Horner-Emmons reagents were prepared as follows. First, the olefinic double bond and nitro group of methyl 3-nitrocinnamate (**8**)[14] were reduced in 95% yield by hydrogenation in the presence of 5% Pd on charcoal as catalyst, and the amino group of the resulting **9** was tritylated with trityl bromide in pyridine to give **10** in 83% yield. Condensation of ester **10** with the anion of dimethyl methylphosphonate (43%) followed by in situ detritylation of the resulting β-keto phosphonate **11a** with aqueous acetic acid and conversion of the amino group to azido group by Sandmeyer reaction (91%) furnished the *m*-azidophenyl derivative **12a**. The Horner-Emmons reagent **12b** having an azido group at para position of the phenyl ring was prepared via **11b**[15] in a similar manner. The Horner-Emmons reagent **12c** having extra two carbons was also prepared from methyl (2*E*,4*E*)-5-(3-nitrophenyl)-2,4-pentadienoate (**13**) with similar synthetic operations as those for **12a**.



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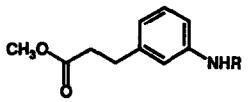
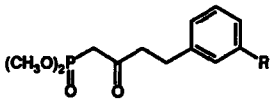
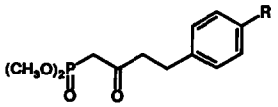


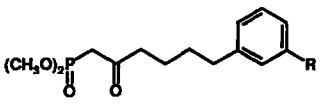
9, R = H
10, R = Tr



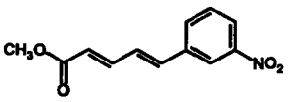
11a, R = NHTr
12a, R = N_3



11b, R = NHTr
12b, R = N_3



11c, R = NHTr
12c, R = N_3



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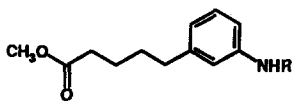
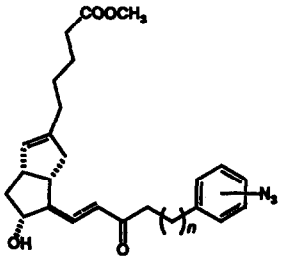
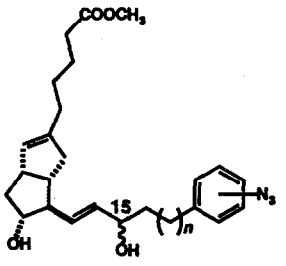


14, R = H
15, R = Tr

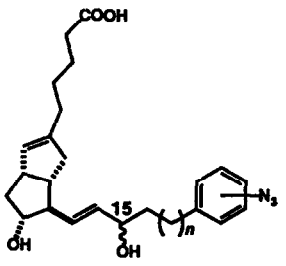
Condensation of aldehyde **7** with **12a** gave the enone **16a** in 89% yield, which was reduced to **17a** with $NaBH_4$–$CeCl_3$ at 0 °C in 98% yield.[16] Under these reducing conditions, the C(15) carbonyl was selectively reduced leaving the azidophenyl moiety intact.[17] Alkaline hydrolysis of ester **17a** gave **18a** as a 1:1 mixture of 15-epimers in 98% yield. In a like manner, carboxylic acid **18b** was prepared as a 1:1 epimeric mixture in three steps with 90% overall yield starting from **7** and **12b**. The enone **16c**, prepared in 92% yield by condensation of **7** and **12c**, was converted to **17c** as a mixture of C(15) stereoisomers. These isomeric alcohols were separated by silica gel chromatography to yield (15*S*)-**17c** as a more polar material and (15*R*)-**17c** as a less polar one in 38 and 38% yields, respectively. Finally, alkaline hydrolysis of ester (15*S*)- and (15*R*)-**17c** gave the (15*S*)- and (15*R*)-**18c**, respectively, in quantitative yields.



16a, n = 1, *m*-N_3
16b, n = 1, *p*-N_3
16c, n = 3, *m*-N_3

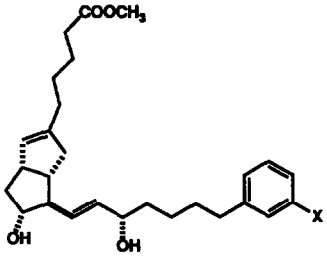


17a, n = 1, *m*-N_3
17b, n = 1, *p*-N_3
17c, n = 3, *m*-N_3

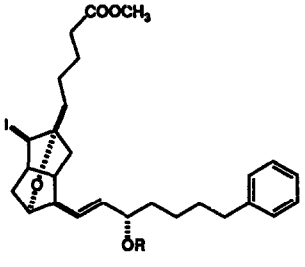


18a, n = 1, *m*-N_3
18b, n = 1, *p*-N_3
18c, n = 3, *m*-N_3

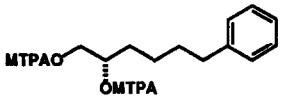
The C(15) absolute configurations of the more polar and less polar **17c** were definitively determined to be *S* and *R*, respectively, by chemical correlation. Thus an aminophenyl derivative **19** derived from the more polar **17c** by hydrogenation was deaminated by successive treatment with $NaNO_2$ and H_3PO_2 to give **20**.[18] Selective masking of the double bond in the five-membered ring of **20** was achieved by the formation of the tricyclic compound **21**[19] upon the treatment with I_2 in aqueous CH_3OH. Subsequent esterification of the hydroxyl group in **21** with (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA chloride) gave the MTPA ester **22**. Then, oxidative cleavage of the C(13)—C(14) double bond by successive treatment with OsO_4 and $NaIO_4$, quick reduction of an aldehydic product with $NaBH_4$, and final MTPA esterification provided an optically active bisMTPA ester (*S*)-**23**. Independently, the stereo-defined bisMTPA ester, (*S*)-**23**, was obtained from an



19, X = NH_2
20, X = H

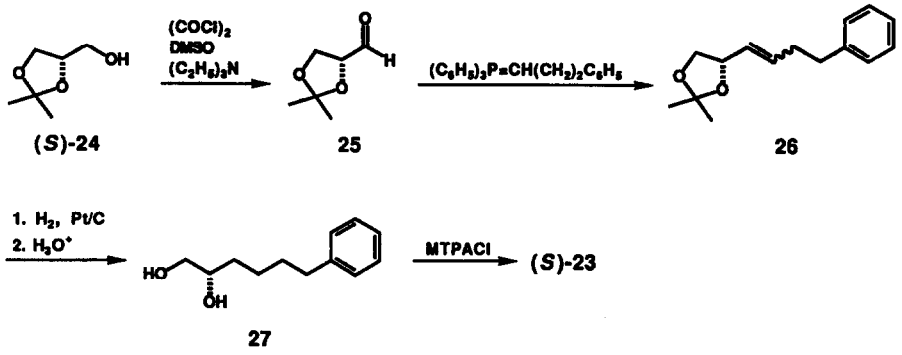


21, R = H
22, R = MTPA



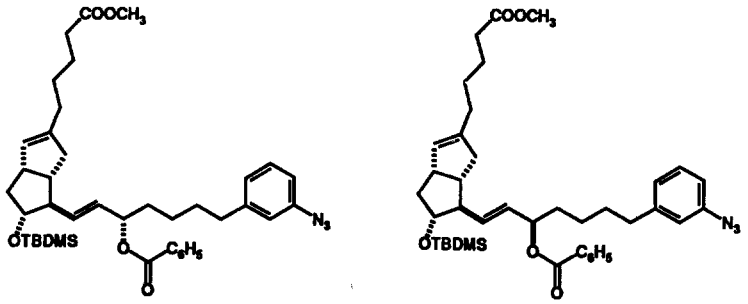
(*S*)-**23**

optically pure glycerol derivative (*S*)-**24** as shown in Scheme 2. The bisMTPA ester (*S*)-**23** derived from the more polar **17c** was identical with the authentic (*S*)-**23** as judged by their ^{1}H NMR spectra.



Scheme 2. Preparation of Stereo-defined (*S*)-23.

The exciton chirality method[20] was also applied to deduce the absolute stereochemistry at C(15) of the acyclic allylic alcohol **17c**. Thus two kinds of monobenzoates, **28** and **29**, which correlate to the more polar and less polar **17c**, respectively, were prepared from **16c** by successive silylation of the hydroxyl group, reduction of the C(15) carbonyl followed by separation of C(15) epimers, and benzoylation. These monobenzoates, **28** and **29**, exhibited Cotton effects of $\Delta\varepsilon$ +1.5 and –0.6, respectively. Although the observed $\Delta\varepsilon$ values are small, the positive and negative signs of the Cotton effects are consistent with those reported for the *S* and *R* absolute stereochemistries, respectively.[20]



TBDMS = $Si(CH_3)_2$-t-C_4H_9

Binding Assay

The binding assay of the azidophenyl derivatives, **18a**—**c**, for the PGI_2 receptor protein in mastocytoma P-815 cells[4e] was performed using C(15) tritium labeled iloprost ([^{3}H]iloprost) (**30**) as a radioligand. The binding affinity was estimated by the degree of dissociation of **30** from the cell membrane against the concentration of the added azidophenyl derivatives. As shown in Figure 1, we found that the compound **18a** has higher affinity than **18b** to the receptor protein and that (15*S*)-**18c**, which has two more carbons in its ω side-chain, has the highest affinity among the azidophenyl derivatives and exhibited an IC_{50} value of 3 nM. As

expected, (15*R*)-**18c** has a hundred times lower affinity than (15*S*)-**18c** does, suggesting that the PGI_2 receptor protein strongly recognizes the difference in the C(15) absolute stereochemistries (Figure 2).

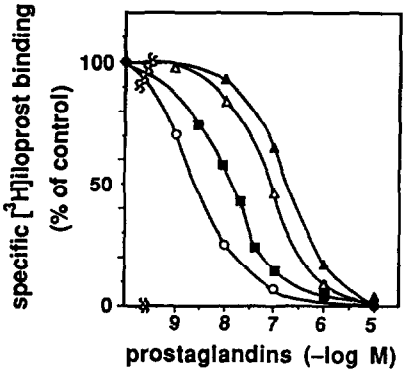


Figure 1. Displacement of [^{3}H]iloprost (**30**) by azidophenyl derivatives. Displacement curves were generated by using 20 nM of **30** and various concentrations of **18a** (△), **18b** (▲), (15*S*)-**18c** (○), or iloprost (■). All values were corrected for nonspecific binding and represent means of triplicate determinations.

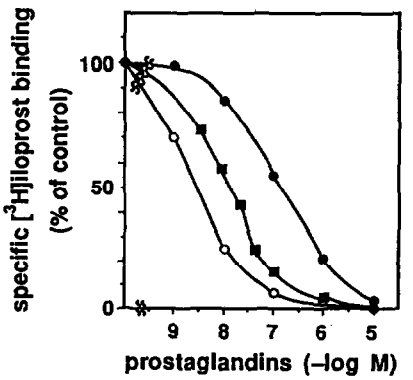
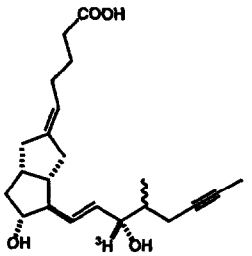


Figure 2. Displacement of [^{3}H]iloprost (**30**) by azidophenyl derivatives. Displacement curves were generated by using 20 nM of **30** and various concentrations of (15*S*)-**18c** (○), (15*R*)-**18c** (●), or iloprost (■). All values were corrected for nonspecific binding and represent means of triplicate determinations.



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Biological Activities

The biological activity of the azidophenyl derivative (15*S*)-**18c** was examined to determine whether this compound could be involved in signal transduction processes as an agonist. First, we investigated the effect of (15*S*)-**18c** on the stimulation of adenylate cyclase in the mastocytoma P-815 membrane fraction using iloprost as a standard compound. As shown in Figure 3, the azidophenyl derivative (15*S*)-**18c** actually dose-dependently stimulated adenylate cyclase in the presence of guanosine triphosphate (GTP). The half-maximal concentration for the stimulation was 50 nM. This value was higher than that of iloprost, but the potency for the activation of the adenylate cyclase convinced us that (15*S*)-**18c** has considerable agonist character for the PGI_2 receptor. In addition, the effect on the the inhibition of platelet aggregation was tested for the rabbit platelet using isocarbacyclin (**2**) as a standard compound, indicating that the IC_{50} values of (15*S*)-**18c** and **2** were 29.39 and 6.01 ng/mL, respectively.[21] Thus, it was found that (15*S*)-**18c** has about one-fifth the activity of **2** for the inhibition of the rabbit platelet aggregation.

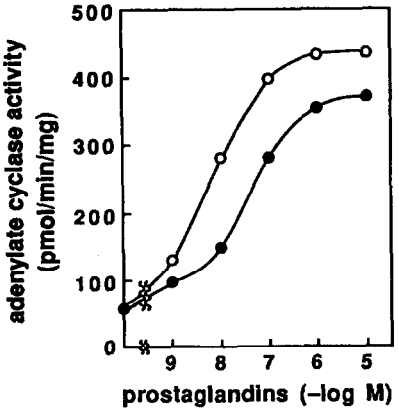
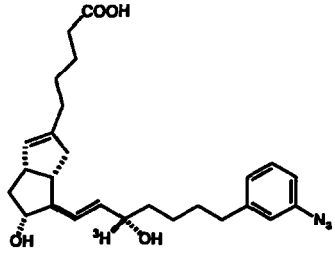


Figure 3. Effects of iloprost and the azidophenyl derivative (15*S*)-**18c** on adenylate cyclase activity. The samples contained the indicated concentrations of iloprost (○) or (15*S*)-**18c** (●) with 1 μM of GTP. The samples were assayed for adenylate cyclase activity as described under experimental section.

Tritium Labeling

Based on the results of the binding assays and biological tests described above, we selected (15*S*)-**18c** as a candidate for the probe compound for the photoaffinity labeling experiment.[22] The radioisotope labeling of (15*S*)-**18c** at the 15-position with tritium was achieved by the use of [^{3}H]$NaBH_4$ (60 Ci/mmol) in the reduction of the C(15) carbonyl of enone **16c** giving a tritium labeled compound [^{3}H]-**17c** as a 1:1 epimeric mixture with specific activity of ca. 15 Ci/mmol. This operation was done after confirmation of reproducibility by several cold runs using a tiny amount of **16c**.[23] Separation of the 15*S*-epimer from [^{3}H]-**17c** with reversed-phase HPLC followed by ester hydrolysis gave the desired [^{3}H]-(15*S*)-**18c**.

The details of the successful characterization of the PGI_2 receptor protein by photoreaction using this tritium labeled compound will be reported separately.[22]



[^{3}H]-(15*S*)-**18c**

EXPERIMENTAL SECTION

General. Chemical shifts of ^1H NMR spectra are reported relative to tetramethylsilane (δ 0) or chloroform (δ 7.26). Chemical shifts of ^{13}C NMR spectra are reported relative to tetramethylsilane (δ 0) or chloroform-*d* (δ 77.1). The abbreviations s, d, t, q, m, and br signify singlet, doublet, triplet, quartet, multiplet, and broad, respectively.

R_f values on TLC were recorded on E. Merck precoated (0.25 mm) silica gel 60 F_{254} plates. The plates were sprayed with a solution of 2% *p*-anisaldehyde in 5% ethanolic sulfuric acid or 5% phosphomolybdic acid in ethanol and then heated until the spots became clearly visible. CD spectra were obtained with a JASCO J-500E spectrometer. The high-performance liquid chromatography (HPLC) for the preparation of [^3H]-(15*S*)-**17c** was carried out on a Waters liquid chromatograph model 510 equipped with a Waters LC spectrophotometer model 481 using a Cosmosil 5C_{18} column (4.6 x 150 mm) (Nacalai Tesque): solvent, 100:100:0.04 acetonitrile/water/acetic acid; flow rate, 1.0 mL/min; detection, UV (215 nm) and radioactivity. Radioactivity in the eluates was determined by a Packard Tri-Carb 2200CA liquid scintillation analyzer.

Commercial *n*-butyllithium hexane solution (Nacalai Tesque) was used directly from the bottle after titration.[24] A lithium bis(trimethylsilyl)amide THF solution (Aldrich) was used directly from the bottle without titration. The solution of *tert*-butyl hydroperoxide (TBHP) in toluene was prepared according to the Sharpless's method[12]. L-(+)-Diisopropyl tartrate (DIPT) (Aldrich) was used directly from the bottle. Five percent palladium on charcoal and 5% platinum on charcoal were purchased from Nippon Engelhard Co. (*S*)-(+)-2,2-Dimethyl-1,3-dioxolane-4-methanol ((*S*)-**24**) (Aldrich) and (±)-2,2-dimethyl-1,3-dioxolane-4-methanol ((±)-**24**) (Nacalai Tesque) were used without purification. A part of isocarbacyclin methyl ester (**3**) was donated from Teijin Co. Methyl 3-nitrocinnamate (**8**) (mp, 123—124 °C, lit.[14] 123—124 °C) was prepared by refluxing a solution of 3-nitrocinnamic acid in CH_3OH with catalytic amount of H_2SO_4 followed by a standard workup and isolation procedure. Methyl (*E*)-4-(diethoxyphosphoryl)-2-butenoate was prepared according to the literature.[25a] (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((*S*)-MTPA chloride) was prepared from (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (Aldrich) according to the procedure reported in the literature.[26] [^3H]Iloprost (**30**) and iloprost were purchased from Amersham. The membrane fraction of mastocytoma P-815 cells was prepared as previously reported.[4e]

13,14-Epoxy-13,14-dihydroisocarbacyclin Methyl Ester (4). In a 20-mL Schlenk tube were placed crashed molecular sieves 3A (100 mg) and CH_2Cl_2 (1.0 mL). To this L-(+)-DIPT (16.5 μL, 0.078 mmol) was added at room temperature and the resulting mixture was cooled to –10 °C. Ti(O-*i*-C_3H_7)$_4$ (18.5 μL, 0.062 mmol) was added to this mixture at the same temperature and addition of TBHP (30% in toluene, 0.14 mL, 0.467 mmol) at –20 °C was followed after 15 min. After the mixture was stirred for 10 min, a solution of isocarbacyclin methyl ester (**3**) (113.5 mg, 0.311 mmol) in CH_2Cl_2 (1.3 mL) was added at –20 °C. The resulting mixture was stirred at –20 °C in a CryoCool-controlled bath for 6.5 h and then ethyl acetate (3 mL) and water (2 mL) were successively added. The organic layer was separated and aqueous layer was extracted with ethyl acetate (1 mL x 2). The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated. The residual oil was chromatographed on SiO_2 (4 g) using a 2:1 to 1:1 mixture of hexane and ethyl acetate as eluent to give **4** (110.0 mg, 93%) as a colorless oil. TLC R_f 0.31 (1:1 hexane/ethyl acetate); IR (thin film) 3400, 1740, 1438, 907 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 0.89 (t, 3, J = 6.9 Hz, CH_3), 1.1—1.8 (m, 12, 6 CH_2), 1.9—2.6 (m, 10, 4

CH_2 and 2 CH), 2.91 (d, 1, J = 2.5 Hz, CHO), 3.01 (d, 1, J = 8.4 Hz, allylic CH), 3.07 (d, 1, J = 5.9 Hz, CHO), 3.66 (s, 3, OCH_3), 3.6—3.8 (br, 1, CHO), 3.88 (dd, 1, J = 8.9, 15.8 Hz, CHO), 5.28 (s, 1, vinyl); ^{13}C NMR ($CDCl_3$, 22.5 MHz) δ 12.9, 22.5, 24.5, 24.7, 26.9, 30.3, 31.7, 33.7, 34.0, 40.1, 40.8, 41.1, 45.6, 51.4, 54.7, 57.6, 59.5, 70.2, 74.5, 127.7, 141.3, 174.1; MS, *m/z* 380 (M^+), 362 ($M^+ - H_2O$); HRMS, *m/z* calcd for $C_{22}H_{36}O_5$ (M^+) 380.2563, found 380.2570.

11,15-*O*-Bisacetyl-13,14-epoxy-13,14-dihydroisocarbacyclin Methyl Ester (5). In a 10-mL test tube was placed a solution of **4** (84.6 mg, 0.222 mmol) in CH_2Cl_2 (2.0 mL) at room temperature. To this solution pyridine (0.05 mL, 0.62 mmol), acetic anhydride (0.05 mL, 0.53 mmol), and 4-(dimethylamino)-pyridine (5.0 mg, 0.041 mmol) were successively added. The resulting mixture was stirred at 21 °C for 48 h and then poured into saturated NH_4Cl aqueous solution (2 mL). The separated organic layer was successively washed with saturated NH_4Cl aqueous solution (2 mL x 3), 20% $CuSO_4$ aqueous solution (2 mL), water (2 mL), and brine (2 mL). The resulting solution was dried over Na_2SO_4, filtered, and concentrated to give an oil, which was chromatographed on SiO_2 (3 g) using a 20:1 mixture of hexane and ethyl acetate as eluent to give **5** (99.0 mg, 96%) as a colorless oil. TLC R_f 0.74 (1:1 hexane/ethyl acetate); IR (thin film) 1739, 1437, 1372, 1242 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 0.88 (t, 3, J = 6.4 Hz, CH_3), 1.1—1.7 (m, 13, 6 CH_2 and CH), 2.03 (s, 3, $CH_3C(O)$), 2.06 (s, 3, $CH_3C(O)$), 2.0—2.2 (m, 4, 2 CH_2), 2.3—2.6 (m, 5, 2 CH_2 and CH), 2.75 (dd, 1, J = 2.0, 5.4 Hz, CHO), 2.91 (dd, 1, J = 2.0, 6.9 Hz, CHO), 3.0—3.2 (br, 1, allylic CH), 3.67 (s, 3, OCH_3), 4.12 (dd, 1, J = 7.4, 14.3 Hz, CHO), 4.69 (dd, 1, J = 6.2, 12.1 Hz, CHO), 5.24 (s, 1, vinyl); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 13.9, 20.9, 21.0, 22.4, 24.5 (2 C), 27.0, 30.3, 31.2, 31.5, 33.8, 36.9, 40.7, 41.5, 46.2, 51.3, 52.8, 56.8, 57.8, 72.7, 75.8, 127.5, 141.6, 170.2, 170.5, 173.9; MS, *m/z* 464 (M^+); HRMS, *m/z* calcd for $C_{26}H_{40}O_7$ (M^+) 464.2774, found 464.2766.

13,14-Dihydroxy-13,14-dihydroisocarbacyclin Methyl Ester (6). Bisacetyl epoxide **5** (112.1 mg, 0.24 mmol) was dissolved in a 9:1 mixture of acetic acid and water (1.0 mL), and the mixture was stirred at 100 °C for 100 min. After being cooled to room temperature, the mixture was diluted with ethyl acetate (2 mL) and poured into saturated $NaHCO_3$ aqueous solution (2 mL). $NaHCO_3$ was added to this mixture until getting saturated aqueous phase. The resulting mixture was extracted with ethyl acetate (1 mL x 3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a yellow oil (120.3 mg). To a solution of this yellow oil (100.5 mg) in CH_3OH (1 mL), 20% K_2CO_3 aqueous solution (0.1 mL) was added and the resulting mixture was stirred at room temperature for 1 h followed by addition of ethyl acetate (2 mL) and water (1 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (1 mL x 2). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a colorless oil, which was chromatographed on SiO_2 (20 g) using 1:1, 1:2, and 1:3 mixtures of hexane and ethyl acetate to give **6** as a mixture of two diastereomers. The less polar **6** (13.5 mg, 17%) as a colorless powder. TLC R_f 0.38 (1:2 hexane/ethyl acetate); IR ($CHCl_3$) 3410, 1727, 1439 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.89 (t, 3, J = 6.7 Hz, CH_3), 1.2—1.7 (m, 12, 6 CH_2), 1.7—1.9 (m, 2, CH_2), 2.0—2.1 (m, 3, CH_2 and CH), 2.2—2.4 (m, 3, CH_2 and CH), 2.44 (dd, 1, J = 8.9, 16.5 Hz, CH), 2.67 (dd, 1, J = 8.6, 17.1 Hz, CH), 2.7—2.8 (br, 1, CH), 2.9—3.0 (m, 1, allylic CH in ring), 3.6—3.7 (m, 1, CHO), 3.67 (s, 3, OCH_3), 3.72 (dt, 1, J = 2.8, 8.3 Hz, CHO), 3.98 (br s, 1, CHO), 4.15 (dd, 1, J = 8.7, 17.1 Hz, CHO), 5.30 (br s, 1, vinyl); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 14.1, 22.7, 24.5, 25.2, 27.0, 30.4, 32.0, 33.9,

34.1, 40.1, 40.6, 40.9, 45.5, 51.6, 53.7, 71.0, 71.4, 73.5, 76.5, 128.7, 141.2, 174.7; MS, *m/z* 398 (M^+); HRMS, *m/z* calcd for $C_{22}H_{38}O_6$ (M^+) 398.2669, found 398.2678. The more polar **6** (59.8 mg, 75%) as a colorless powder. TLC R_f 0.22 (1:2 hexane/ethyl acetate); IR ($CHCl_3$) 3412, 1729, 1438 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.89 (t, 3, J = 6.7 Hz, CH_3), 1.2—1.8 (m, 13, 6 CH_2 and CH), 1.8—1.9 (m, 1, CH), 2.05 (t, 2, J = 7.2 Hz, CH_2), 2.2—2.3 (m, 2, CH_2), 2.32 (t, 2, J = 7.3 Hz, $CH_2C(O)$), 2.5—2.6 (m, 2, 2 CH), 2.9—3.0 (m, 1, allylic CH in ring), 3.0—3.6 (br, 4, 4 OH), 3.45 (d, 1, J = 7.6 Hz, CHO), 3.67 (s, 3, OCH_3), 3.77 (br s, 1, CHO), 3.92 (dd, 1, J = 9.2, 16.2 Hz, CHO), 4.01 (br s, 1, CHO), 5.30 (br s, 1, vinyl); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 14.1, 22.7, 24.6, 25.9, 27.1, 30.5, 31.9, 33.9, 37.5, 41.1, 42.4, 46.1, 51.6, 55.9, 69.6, 70.5, 73.7, 74.8, 127.3, 141.9, 174.6; MS, *m/z* 398 (M^+); HRMS, *m/z* calcd for $C_{22}H_{38}O_6$ (M^+) 398.2669, found 398.2666.

Methyl (1*R*,5*S*,6*S*,7*R*)-6-Formyl-7-hydroxybicyclo[3.3.0]oct-2-ene-3-pentanoate (7). To a solution of **6** (28.0 mg, 0.070 mmol) in THF–ether (2:1) (1.5 mL), $NaIO_4$ (145.0 mg, 0.68 mmol) and water (0.5 mL) were successively added. The resulting mixture was vigorously stirred at room temperature for 1 h followed by addition of ethyl acetate (0.5 mL) and water (0.5 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (0.5 mL x 2). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give crude **7** (17.9 mg, 96%) as a colorless oil. TLC R_f 0.38 (1:1 hexane/ethyl acetate); IR ($CHCl_3$) 3474, 1727, 1438 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.4—1.7 (m, 4, 2 CH_2), 1.9—2.2 (m, 4, 2 CH_2), 2.33 (t, 2, J = 7.2 Hz, $CH_2C(O)$), 2.4—2.7 (m, 3, CH_2 and CH), 2.86 (ddd, 1, J = 2.0, 8.4, 16.8 Hz, CHC(O)), 3.1—3.2 (br, 1, allylic CH), 3.67 (s, 3, OCH_3), 4.3—4.4 (br, 1, CHO), 5.35 (br s, 1, vinyl), 9.79 (d, 1, J = 2.0 Hz, C(O)H); MS, *m/z* 266 (M^+), 235 ($M^+ - OCH_3$); HRMS, *m/z* calcd for $C_{15}H_{22}O_4$ (M^+) 266.1518, found 266.1514. This unstable compound was subjected to Horner-Emmons reaction without purification.

Methyl 3-(3-Aminophenyl)propionate (9). In a 50-mL round-bottomed flask was placed a solution of methyl 3-nitrocinnamate (**8**)[14] (1.626 g, 7.85 mmol) in a 1:1 mixture of CH_3OH and benzene (30 mL). To this solution 5% Pd on charcoal (80 mg) was added and the resulting mixture was stirred at 25 °C for 24 h under H_2 atmosphere. Pd on charcoal was removed by filtration and the filtrate was concentrated under reduced pressure to give a black oil, which was chromatographed on SiO_2 (6 g) using a 5:1 mixture of hexane and ethyl acetate to give **9** (1.334 g, 95%) as a yellow oil. TLC R_f 0.19 (5:1 hexane/ethyl acetate); IR (thin film) 3452, 3370, 1732, 1622, 1606, 1493, 1460, 1437 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 2.60 (t, 2, J = 7.9 Hz, CH_2), 2.86 (t, 2, J = 7.9 Hz, CH_2), 3.4—3.8 (br, 2, NH_2), 3.67 (s, 3, OCH_3), 6.52—6.55 (m, 2, aromatic), 6.59 (d, 1, J = 7.9 Hz, aromatic), 7.70 (dt, 1, J = 7.9, 7.9 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 30.9, 35.6, 51.5, 113.0, 115.0, 118.3, 129.4, 141.7, 146.6, 173.4; MS, *m/z* 180 ($M^+ + H$). Anal. Calcd for $C_{10}H_{13}O_2N$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.98; H, 7.28; N, 7.69.

Methyl 3-(3-(Tritylamino)phenyl)propionate (10). In a 20-mL round-bottomed flask was placed a solution of **9** (1.80 g, 10.0 mmol) and trityl bromide (3.24 mg, 10.0 mmol) in pyridine (7.0 mL). This was stirred at room temperature for 48 h. After being diluted with ethyl acetate (15 mL), the resulting mixture was successively washed with saturated NH_4Cl aqueous solution (10 mL x 10), 20% $CuSO_4$ aqueous solution (10 mL x 2), and brine (10 mL). This solution was dried over Na_2SO_4, filtered, and concentrated under reduced

pressure to give an oil, which was chromatographed on SiO_2 (25 g) using 10:1, 5:1, and 3:1 mixtures of hexane/ethyl acetate to give **10** (3.49 g, 83%) as a colorless foam. TLC R_f 0.38 (5:1 hexane/ethyl acetate); IR ($CHCl_3$) 3432, 1731, 1605, 1487, 1446 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 2.31 (t, 2, J = 8.2 Hz, CH_2), 2.64 (t, 2, J = 8.2 Hz, CH_2), 3.62 (s, 3, OCH_3), 4.9—5.1 (br, 1, NH), 6.14 (d, 1, J = 2.0 Hz, aromatic), 6.21 (d, 1, J = 7.9 Hz, aromatic), 6.39 (d, 1, J = 7.4 Hz, aromatic), 6.83 (t, 1, J = 7.9 Hz, aromatic), 7.2—7.4 (m, 15, trityl); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 31.0, 35.6, 51.6, 71.5, 114.2, 116.0, 117.4, 126.9, 128.0, 128.3, 129.3, 140.5, 145.5, 146.5, 173.5; MS, *m/z* 421 (M^+), 390 ($M^+ - OCH_3$); HRMS, *m/z* calcd for $C_{29}H_{27}O_2N$ (M^+) 421.2042, found 421.2041.

1-(Dimethoxyphosphoryl)-4-(3-(tritylamino)phenyl)-2-butanone (11a). In a 100-mL round-bottomed flask was placed a solution of dimethyl methylphosphonate (736.0 mg, 5.93 mmol) in THF (18 mL) at –78 °C. To this a solution of *n*-butyllithium in hexane (1.57 M, 3.8 mL, 5.97 mmol) was added at –78 °C over 20 min to give white suspension. This was transferred to a 100-mL round-bottomed flask containing a solution of **10** (500.0 mg, 1.19 mmol) in THF (12 mL) kept at –78 °C over 20 min. The resulting mixture was further stirred at –78 °C for 1 h followed by pouring into saturated NH_4Cl aqueous solution (15 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (10 mL x 2). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The residual material was chromatographed on SiO_2 (26 g) using a 1:1 to 1:2 mixture of hexane and ethyl acetate to give **11a** (265.4 mg, 43%). TLC R_f 0.20 (1:2 hexane/ethyl acetate); IR ($CHCl_3$) 3428, 1715, 1605, 1488, 1447 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 2.61 (s, 4, 2 CH_2), 2.95 (d, 2, $J_{P\text{-}H}$ = 22.3 Hz, CH_2), 3.73 (d, 6, $J_{P\text{-}H}$ = 11.4 Hz, 2 OCH_3), 4.9—5.1 (br, 1, NH), 6.14 (s, 1, aromatic), 6.18 (dd, 1, J = 7.7, 1.7 Hz, aromatic), 6.37 (d, 1, J = 7.4 Hz, aromatic), 6.81 (t, 1, J = 7.7 Hz, aromatic), 7.2—7.4 (m, 15, trityl); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 29.4, 41.2 (d, J = 127.0 Hz), 45.3, 52.8 (d, J = 6.2 Hz), 71.2, 113.9, 115.8, 117.2, 126.6, 127.7, 128.1, 129.0, 140.2, 145.2, 146.2, 201.0 (d, J = 6.2 Hz); MS, *m/z* 513 (M^+); HRMS, *m/z* calcd for $C_{31}H_{32}O_4NP$ (M^+) 513.2069, found 513.2062.

4-(3-Azidophenyl)-1-(dimethoxyphosphoryl)-2-butanone (12a). In a 10-mL round-bottomed flask was placed a solution of **11a** (265.4 mg, 0.52 mmol) in THF (2 mL). To this a 9:1 mixture of acetic acid and water (1.5 mL) was added. The resulting mixture was stirred for 2 h and cooled to 0 °C. To this $NaNO_2$ (72 mg, 1.0 mmol) was added and the resulting mixture was stirred for 5 min. To this mixture NaN_3 (84 mg, 1.3 mmol) was added. After the mixture was stirred at 0 °C for 10 min, saturated $NaHCO_3$ aqueous solution (4 mL) was added. $NaHCO_3$ powder was added to this mixture until getting saturated aqueous phase. The resulting mixture was extracted with ethyl acetate (1 mL x 3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on SiO_2 (12 g) using 1:2, 1:8, and 1:16 mixtures of hexane and ethyl acetate to give **12a** (140.0 mg, 91%) as a yellow oil. TLC R_f 0.28 (ethyl acetate); IR (thin film) 2110, 1715, 1605, 1585 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 2.8—3.0 (m, 4, 2 CH_2), 3.08 (d, 2, $J_{P\text{-}H}$ = 22.8 Hz, CH_2), 3.76 (d, 6, $J_{P\text{-}H}$ = 11.4 Hz, 2 OCH_3), 6.85 (s, 1, aromatic), 6.86 (d, 1, J = 7.4 Hz, aromatic), 6.97 (d, 1, J = 7.4 Hz, aromatic), 7.26 (t, 1, J = 7.4 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 28.9, 41.4 (d, J = 128.3 Hz), 44.9, 52.9 (d, J = 6.2 Hz), 116.7, 118.9, 124.9, 129.7, 139.9, 142.6, 200.4 (d, J = 6.2 Hz); MS, *m/z* 297 (M^+); HRMS, *m/z* calcd for $C_{12}H_{16}O_4N_3P$ (M^+) 297.0878, found 297.0883.

4-(4-Azidophenyl)-1-(dimethoxyphosphoryl)-2-butanone (12b). Prepared by using **11b**[15] (250.0 mg, 0.49 mmol) with similar operations as those for **12a** in 85% yield as a yellow oil. TLC R_f 0.33 (ethyl acetate); IR (thin film) 2100, 1716, 1508 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 2.6—3.0 (m, 4, 2 CH_2), 3.07 (d, 2, J_{P-H} = 22.8 Hz, CH_2), 3.75 (d, 6, J_{P-H} = 11.4 Hz, 2 OCH_3), 6.94 (d, 2, J = 8.4 Hz, aromatic), 7.18 (d, 2, J = 8.4 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 28.7, 41.5 (d, J = 128.3 Hz), 45.4, 53.0 (d, J = 6.3 Hz), 119.1, 129.8, 137.4, 138.0, 200.7 (d, J = 6.2 Hz); MS, *m/z* 297 (M^+), 269 ($M^+ - N_2$); HRMS, *m/z* calcd for $C_{12}H_{16}O_4NP$ ($M^+ - N_2$) 269.0817, found 269.0821.

Methyl (2*E*,4*E*)-5-(3-Nitrophenyl)-2,4-pentadienoate (13). In a 50-mL Schlenk tube was placed a solution of methyl (*E*)-4-(diethoxyphosphoryl)-2-butenoate[25a] (3.0 g, 12.7 mmol) in THF (10 mL) at –78 °C. To this a solution of $LiN[Si(CH_3)_3]_2$ in THF[25b] (1.0 M, 13.0 mL, 13.0 mmol) was added at –78 °C. After the mixture was stirred for 2 h, 3-nitrobenzaldehyde (1.44 g, 9.52 mmol) was added and the resulting mixture was stirred at –45 °C for 10 min and then at 23 °C for 18 h. To this saturated NH_4Cl aqueous solution (5 mL) were added and concentrated to give a brown oil, which was diluted with $CHCl_3$ (20 mL). After addition of saturated NH_4Cl aqueous solution (40 mL), the organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (15 mL x 3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give an oil, which was chromatographed on SiO_2 (50 g) using 1:1, 1:2, 1:5, and 1:8 mixtures of hexane and $CHCl_3$ to give a semi-purified **13** (2.7 g) as a yellow powder. This material was used for the synthesis of **14** without further purification. The pure compound was obtained after recrystallization from hexane/$CHCl_3$. mp, 147—148 °C; TLC R_f 0.71 (2:1 hexane/ethyl acetate, twice elution); IR ($CHCl_3$) 1708, 1631, 1613, 1531, 1437, 1352 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 3.79 (s, 3, OCH_3), 6.10 (d, 1, J = 15.3 Hz, C(2)H), 6.9—7.1 (m, 2, C(4)H and C(5)H), 7.45 (dd, 1, J = 15.8, 8.9 Hz, C(3)H), 7.53 (t, 1, J = 7.9 Hz, aromatic), 7.75 (d, 1, J = 7.9 Hz, aromatic), 8.1—8.2 (m, 1, aromatic), 8.32 (t, 1, J = 2.0 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 51.7, 121.5, 123.0, 123.3, 129.0, 129.8, 132.8, 137.3, 137.7, 143.5, 148.7, 167.0; MS, *m/z* 233 (M^+), 202 ($M^+ - OCH_3$). Anal. Calcd for $C_{12}H_{11}O_4N$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.79; H, 4.56; N, 5.99.

Methyl 5-(3-Aminophenyl)pentanoate (14). Prepared by using crude **13** (2.7 g) with similar operations as those for **9** in 54% yield (based on 3-nitrobenzaldehyde) as a yellow oil. TLC R_f 0.56 (1:1 hexane/ethyl acetate); IR (thin film) 3454, 3370, 1732, 1622, 1606 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.5—1.7 (m, 4, 2 CH_2), 2.32 (t, 2, J = 6.9 Hz, CH_2), 2.53 (t, 2, J = 6.9 Hz, CH_2), 3.65 (s, 3, OCH_3), 3.2—3.9 (br, 2, NH_2), 6.50 (br s, 2, aromatic), 6.58 (d, 1, J = 7.4 Hz, aromatic), 7.05 (t, 1, J = 8.2 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.5, 30.6, 33.9, 35.4, 51.4, 112.6, 115.1, 118.6, 129.1, 143.3, 146.4, 174.0; MS, *m/z* 208 ($M^+ + H$). Anal. Calcd for $C_{12}H_{17}O_2N$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.50; H, 8.30; N, 6.86.

Methyl 5-(3-(Tritylamino)phenyl)pentanoate (15). Prepared by using **14** (223.6 mg, 1.08 mmol) with similar operations as those for **10** in 62% yield as yellow crystals. mp 135—136 °C; TLC R_f 0.56 (2:1 hexane/ethyl acetate); IR ($CHCl_3$) 3432, 1731, 1604, 1486, 1446 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.2—1.5 (m, 4, 2 CH_2), 2.20 (t, 2, J = 7.2 Hz, CH_2), 2.31 (t, 2, J = 7.2 Hz, CH_2), 3.64 (s, 3, OCH_3), 4.96 (s, 1, NH), 6.11 (s, 1, aromatic), 6.20 (dt, 1, J = 1.2, 7.9 Hz, aromatic), 6.36 (d, 1, J = 7.4 Hz, aromatic),

6.81 (t, 1, J = 7.9 Hz, aromatic), 7.1—7.4 (m, 15, trityl); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.3, 30.5, 33.9, 35.4, 51.4, 71.4, 113.7, 116.2, 117.5, 126.7, 127.9, 128.0, 129.2, 142.0, 145.5, 146.2, 174.1; MS, *m/z* 449 (M^+), 418 ($M^+ - OCH_3$). Anal. Calcd for $C_{31}H_{31}O_2N$: C, 82.82; H, 6.95; N, 3.12. Found: C, 82.72; H, 6.87; N, 3.13.

1-(Dimethoxyphosphoryl)-6-(3-(tritylamino)phenyl)-2-hexanone (11c). Prepared by using **15** (502.0 mg, 1.12 mmol) with similar operations as those for **11a** in 39% yield as a colorless foam. TLC R_f 0.21 (1:2 hexane/ethyl acetate); IR ($CHCl_3$) 3430, 1714, 1604, 1486, 1447 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.2—1.5 (m, 4, 2 CH_2), 2.31 (t, 2, J = 6.9 Hz, CH_2), 2.51 (t, 2, J = 6.9 Hz, CH_2), 3.03 (d, 2, J_{P-H} = 22.8 Hz, CH_2), 3.76 (d, 6, J_{P-H} = 11.4 Hz, 2 OCH_3), 4.9—5.0 (br, 1, NH), 6.13 (s, 1, aromatic), 6.19 (dd, 1, J = 1.5, 7.9 Hz, aromatic), 6.35 (d, 1, J = 7.2 Hz, aromatic), 6.80 (t, 1, J = 7.7 Hz, aromatic), 7.1—7.4 (m, 15, trityl); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 22.8, 30.2, 35.5, 41.2 (d, J = 128.0 Hz), 43.9, 53.0 (d, J = 6.2 Hz), 71.3, 113.7, 116.2, 117.5, 126.7, 127.8, 128.0, 129.2, 141.9, 145.4, 146.2, 201.9 (d, J = 6.3 Hz); MS, *m/z* 541 (M^+); HRMS, *m/z* calcd for $C_{33}H_{36}O_4NP$ (M^+) 541.2382, found 541.2377.

6-(3-Azidophenyl)-1-(dimethoxyphosphoryl)-2-hexanone (12c). Prepared by using **11c** (235.4 mg, 0.44 mmol) with similar operations as those for **12a** in 91% yield as a yellow oil. TLC R_f 0.31 (ethyl acetate); IR ($CHCl_3$) 2110, 1716 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.5—1.7 (m, 4, 2 CH_2), 2.5—2.7 (m, 4, benzylic CH_2 and $CH_2C(O)$), 3.07 (d, 2, J_{P-H} = 22.8 Hz, CH_2P), 3.78 (d, 6, J_{P-H} = 10.9 Hz, 2 OCH_3), 6.8—6.9 (m, 2, aromatic), 6.94 (d, 1, J = 7.4 Hz, aromatic), 7.24 (t, 1, J = 7.7 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 22.7, 30.2, 35.3, 41.2 (d, J = 128.3 Hz), 43.6, 52.9 (d, J = 7.4 Hz), 116.3, 118.8, 124.9, 129.5, 139.7, 144.0, 201.4 (d, J = 6.3 H); MS, *m/z* 325 (M^+); HRMS, *m/z* calcd for $C_{14}H_{20}O_4N_3P$ (M^+) 325.1191, found 325.1208.

17-(3-Azidophenyl)-15-dehydro-18,19,20-trinorisocarbacyclin Methyl Ester (16a). In a 10-mL test tube was placed a solution of crude **7** prepared from **6** (17.0 mg, 0.043 mmol) in DME–toluene (7:1) (1.0 mL). In another 10-mL test tube was placed a solution of **12a** (26.0 mg, 0.087 mmol) in DME–toluene (7:1) (1.0 mL). To this solution NaH (50% oil dispersion, 4.2 mg, 0.088 mmol) was added at room temperature followed by stirring for 15 min. To the resulting suspension, a solution of crude **7** in DME–toluene prepared above was added. The resulting mixture was stirred for 10 min and followed by addition of ethyl acetate (1 mL) and saturated NH_4Cl aqueous solution (1 mL). The organic layer was separated and the aqueous phase was extracted with ethyl acetate (0.5 mL x 3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on SiO_2 (2 g) using a 3:1 mixture of hexane and ethyl acetate to give **16a** (16.7 mg, 89%) as a yellow oil. TLC R_f 0.54 (1:1 hexane/ethyl acetate); IR ($CHCl_3$) 3474, 2110, 1730, 1669, 1625 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.2—1.7 (m, 5, 2 CH_2 and CH), 1.9—2.2 (m, 4, 2 CH_2), 2.2—2.5 (m, 5, $CH_2C(O)$, CH_2, and CH), 2.8—3.0 (m, 4, 2 CH_2), 3.0—3.1 (br, 1, allylic CH in ring), 3.67 (s, 3, OCH_3), 3.91 (dd, 1, J = 8.9, 16.3 Hz, CHO), 5.30 (br s, 1, vinyl in ring), 6.20 (dd, 1, J = 1.0, 15.3 Hz, vinyl in chain), 6.75 (dd, 1, J = 8.4, 15.8 Hz, vinyl in chain), 6.85 (s, 1, aromatic), 6.86 (dd, 1, J = 1.0, 6.4 Hz, aromatic), 6.99 (d, 1, J = 7.4 Hz, aromatic), 7.26 (t, 1, J = 8.1 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.7, 27.2, 29.8, 30.5, 33.9, 39.8, 40.3, 41.7, 42.3, 46.0, 51.6, 58.1, 77.2,

116.8, 119.2, 125.2, 128.2, 129.9, 131.0, 140.1, 141.5, 143.3, 148.3, 174.2, 198.9; MS, *m/z* 437 (M^+), 419 ($M^+ - H_2O$), 409 ($M^+ - N_2$); HRMS, *m/z* calcd for $C_{25}H_{31}O_4N_3$ (M^+) 437.2315, found 437.2311.

A Mixture of 17-(3-Azidophenyl)-18,19,20-trinorisocarbacyclin Methyl Ester and its 15-Epimer (17a). In a 10-mL test tube, was placed a solution of **16a** (14.8 mg, 0.034 mmol) in CH_3OH (0.6 mL). To this $CeCl_3 \cdot 7H_2O$ (15.0 mg, 0.040 mmol) was added at room temperature. After the mixture was cooled to 0 °C, $NaBH_4$ (2.0 mg, 0.053 mmol) was added and the resulting mixture was stirred for 5 min. Water (1 mL) and ethyl acetate (1 mL) were successively added to the mixture. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (0.5 mL x 3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give **17a** (14.5 mg, 98%, 1:1 mixture of stereoisomers) as a yellow oil. TLC R_f 0.32 and 0.26 (1:1 hexane/ethyl acetate); IR ($CHCl_3$) 3418, 2110, 1730, 1604 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.2—2.1 (m, 11, 5 CH_2 and CH), 2.1—2.4 (m, 3, CH_2 and CH), 2.31 (t, 2, J = 7.4 Hz, $CH_2C(O)$), 2.5—2.8 (m, 2, benzylic CH_2), 2.9—3.1 (m, 1, allylic CH), 3.66 (s, 3, OCH_3), 3.7—3.8 (m, 1, CHO), 4.0—4.2 (m, 1, CHO), 5.28 (br s, 1, vinyl in ring), 5.5—5.7 (m, 2, vinyl in chain), 6.86 (s, 2, aromatic), 6.98 (d, 1, J = 7.4 Hz, aromatic), 7.26 (t, 1, J = 7.4 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.7, 27.2, 27.3, 30.6, 31.8, 33.9, 38.4, 38.6, 39.6, 39.7, 39.8, 39.9, 44.3, 44.4, 45.6, 45.7, 51.6, 57.9, 58.2, 71.7, 72.3, 77.2, 77.3, 116.6, 119.1, 125.2, 128.4, 129.8, 132.8, 133.8, 134.8, 135.0, 141.4, 141.5, 144.1, 174.3; MS, *m/z* 421 ($M^+ - H_2O$); HRMS, *m/z* calcd for $C_{25}H_{31}O_3N_3$ ($M^+ - H_2O$) 421.2366, found 421.2380.

A Mixture of 17-(3-Azidophenyl)-18,19,20-trinorisocarbacyclin and its 15-Epimer (18a). In a 10-mL test tube was placed a solution of **17a** (11.3 mg, 0.026 mmol) in CH_3OH (0.6 mL). To this solution an aqueous solution of LiOH (3 N, 0.1 mL) was added. The resulting mixture was stirred at room temperature for 14 h. To this solution ethyl acetate (1 mL) and water (1 mL) were added after addition of $NaHSO_4$ to adjust pH of 4. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (0.5 mL x 3). The combined organic extracts were washed with brine (1.5 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give **18a** (10.7 mg, 98%, 1:1 mixture of stereoisomers) as a yellow oil. TLC R_f 0.47 and 0.36 (1:4 hexane/ethyl acetate); IR ($CHCl_3$) 3394, 2110, 1711, 1604 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.1—2.1 (m, 11, 5 CH_2 and CH), 2.2—2.5 (m, 5, 2 CH_2 and CH), 2.6—2.8 (m, 2, benzylic CH_2), 2.9—3.1 (br, 1, allylic CH), 3.6—3.8 (m, 1, CHO), 4.0—4.2 (m, 1, CHO), 4.2—5.2 (br, 2, 2 OH), 5.29 (br s, 1, vinyl in ring), 5.4—5.7 (m, 2, vinyl in chain), 6.8—6.9 (m, 2, aromatic), 6.98 (d, 1, J = 7.4 Hz, aromatic), 7.26 (t, 1, J = 7.4 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.3, 24.4, 26.9, 27.0, 30.3, 30.4, 31.7, 31.8, 33.8, 33.9, 38.2, 38.4, 39.1, 39.4, 39.5, 39.7, 44.3, 44.4, 45.5, 45.6, 57.8, 58.0, 71.6, 72.6, 77.2, 77.3, 116.6, 119.1, 125.2, 128.7, 128.9, 129.8, 132.6, 134.1, 134.7, 135.2, 141.1, 141.3, 144.0, 144.1, 178.7; MS, *m/z* 407 ($M^+ - H_2O$); HRMS, *m/z* calcd for $C_{24}H_{29}O_3N_3$ ($M^+ - H_2O$) 407.2209, found 407.2203.

17-(4-Azidophenyl)-15-dehydro-18,19,20-trinorisocarbacyclin Methyl Ester (16b). Prepared by using **6** (25.5 mg, 0.064 mmol) and **12b** (47.9 mg, 0.161 mmol) with similar operations as those for **16a** in 92% yield as a yellow oil. TLC R_f 0.39 (1:1 hexane/ethyl acetate); IR ($CHCl_3$) 3474, 2108, 1730, 1507 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.2—1.6 (m, 5, 2 CH_2 and CH), 1.9—2.2 (m, 4, 2 CH_2), 2.2—2.5

(m, 5, $CH_2C(O)$, CH_2, and CH), 2.8—3.0 (m, 4, 2 CH_2), 3.0—3.1 (br, 1, allylic CH in ring), 3.67 (s, 3, OCH_3), 3.90 (dd, 1, J = 7.4, 14.8 Hz, CHO), 5.30 (br s, 1, vinyl in ring), 6.19 (d, 1, J = 15.8 Hz, vinyl in chain), 6.74 (dd, 1, J = 8.4, 15.8 Hz, vinyl in chain), 6.94 (d, 2, J = 8.4 Hz, aromatic), 7.19 (d, 2, J = 8.4 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.6, 27.1, 29.3, 30.5, 33.9, 39.8, 40.3, 41.9, 44.2, 46.0, 51.6, 58.1, 77.2, 119.1, 128.1, 129.9, 131.0, 137.8, 138.0, 141.5, 148.2, 174.2, 199.1; MS, *m/z* 437 (M^+), 419 ($M^+ - H_2O$), 409 ($M^+ - N_2$); HRMS, *m/z* calcd for $C_{25}H_{31}O_4N_3$ (M^+) 437.2315, found 437.2297.

A Mixture of 17-(4-Azidophenyl)-18,19,20-trinorisocarbacyclin Methyl Ester and its 15-Epimer (17b). Prepared by using **16b** (25.8 mg, 0.059 mmol) with similar operations as those for **17a** in 99% yield (1:1 mixture of stereoisomers) as a yellow oil. TLC R_f 0.45 and 0.34 (1:2 hexane/ethyl acetate); IR ($CHCl_3$) 2108, 1730, 1605, 1506 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.2—2.1 (m, 11, 5 CH_2 and CH), 2.1—2.4 (m, 3, CH_2 and CH), 2.31 (t, 2, J = 7.4 Hz, $CH_2C(O)$), 2.5—2.8 (m, 2, benzylic CH_2), 2.9—3.1 (m, 1, allylic CH), 3.65 (s, 3, OCH_3), 3.7—3.8 (m, 1, CHO), 4.0—4.2 (m, 1, CHO), 5.27 (br s, 1, vinyl in ring), 5.4—5.7 (m, 2, vinyl in chain), 6.95 (d, 2, J = 8.4 Hz, aromatic), 7.19 (d, 2, J = 8.4 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.6, 24.7, 27.1, 27.2, 30.6, 31.3, 33.9, 38.6, 38.8, 39.5, 39.7, 39.8, 44.2, 44.3, 45.5, 45.6, 51.6, 57.9, 58.1, 71.6, 72.4, 77.1, 77.2, 119.0, 128.4, 129.9, 132.6, 133.9, 134.8, 135.1, 138.8, 141.3, 141.4, 174.3; MS, *m/z* 421 ($M^+ - H_2O$), 411 ($M^+ - N_2$); HRMS, *m/z* calcd for $C_{25}H_{31}O_3N_3$ ($M^+ - H_2O$) 421.2366, found 421.2371.

A Mixture of 17-(4-Azidophenyl)-18,19,20-trinorisocarbacyclin and its 15-Epimer (18b). Prepared by using **17b** (18.6 mg, 0.042 mmol) with similar operations as those for **18a** in 99% yield (1:1 mixture of stereoisomers) as a yellow oil. TLC R_f 0.23 and 0.18 (1:4 hexane/ethyl acetate); IR ($CHCl_3$) 3416, 2108, 1712, 1605, 1506 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.2—2.1 (m, 11, 5 CH_2 and CH), 2.1—2.5 (m, 5, $CH_2C(O)$, CH_2, and CH), 2.5—2.8 (m, 2, benzylic CH_2), 2.9—3.1 (m, 1, allylic CH), 3.6—3.8 (m, 1, CHO), 4.0—4.2 (m, 1, CHO), 4.2—6.0 (br, 2, 2 OH), 5.29 (br s, 1, vinyl in ring), 5.4—5.7 (m, 2, vinyl in chain), 6.95 (d, 2, J = 8.1 Hz, aromatic), 7.18 (d, 2, J = 8.1 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.3, 24.4, 26.9, 27.0, 30.3, 30.4, 31.3, 33.9, 34.0, 38.4, 38.5, 39.1, 39.3, 39.4, 39.6, 44.1, 44.2, 45.5, 57.6, 57.9, 71.5, 71.6, 77.1, 77.2, 119.0, 128.7, 128.8, 129.8, 132.5, 134.1, 134.7, 135.2, 138.7, 138.8, 141.0, 141.3, 178.8; MS, *m/z* 407 ($M^+ - H_2O$); HRMS, *m/z* calcd for $C_{24}H_{29}O_3N_3$ ($M^+ - H_2O$) 407.2209, found 407.2202.

19-(3-Azidophenyl)-15-dehydro-20-norisocarbacyclin Methyl Ester (16c). Prepared by using **6** (45.0 mg, 0.11 mmol) and **12c** (54.0 mg, 0.17 mmol) with similar operations as those for **16a** in 92% yield as a yellow oil. TLC R_f 0.48 (1:1 hexane/ethyl acetate); IR ($CHCl_3$) 2210, 1731, 1605 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.3—1.7 (m, 9, 4 CH_2 and CH), 2.0—2.2 (m, 4, 2 CH_2), 2.3—2.5 (m, 5, $CH_2C(O)$, CH_2, and CH), 2.6—2.7 (m, 4, benzylic CH_2 and $CH_2C(O)$), 3.0—3.1 (br, 1, allylic CH in ring), 3.69 (s, 3, OCH_3), 3.91 (dd, 1, J = 7.0, 14.0 Hz, CHO), 5.36 (s, 1, vinyl in ring), 6.23 (d, 1, J = 15.9 Hz, vinyl in chain), 6.75 (dd, 1, J = 15.8, 8.4 Hz, vinyl in chain), 6.83 (s, 1, aromatic), 6.85 (d, 1, J = 7.9 Hz, aromatic), 6.96 (d, 1, J = 7.4 Hz, aromatic), 7.26 (t, 1, J = 7.7 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 23.6, 24.6, 27.1, 30.5, 30.8, 33.8, 35.5, 39.8, 40.1, 40.3, 44.2, 46.0, 51.5, 58.0, 77.1, 116.4, 118.9, 125.1, 128.1,

129.6, 130.9, 139.9, 141.4, 144.3, 147.7, 174.1, 200.1; MS, *m/z* 465 (M^+), 447 ($M^+ - H_2O$), 437 ($M^+ - N_2$); HRMS, *m/z* calcd for $C_{27}H_{35}O_4N_3$ (M^+) 465.2628, found 465.2622.

19-(3-Azidophenyl)-20-norisocarbacyclin Methyl Ester ((15*S*)-17c) and its 15-Epimer ((15*R*)-17c). In a 10-mL test tube was placed a solution of **16c** (47.2 mg, 0.10 mmol) in CH_3OH (0.8 mL). To this $CeCl_3 \cdot 7H_2O$ (39.9 mg, 0.107 mmol) was added at room temperature. After the mixture was cooled to 0 °C, $NaBH_4$ (4.0 mg, 0.106 mmol) was added and followed by stirring for 3 min. To this water (0.5 mL) and ethyl acetate (1 mL) were successively added. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (0.5 mL x 3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on SiO_2 (2.5 g) using 4:1, 3:1, 2:1, and 1:1 mixtures of hexane and ethyl acetate to give (15*S*)-**17c** (18.0 mg, 38%) and (15*R*)-**17c** (18.0 mg, 38%) as yellow oils, respectively. (15*S*)-**17c**: TLC R_f 0.28 (1:2 hexane/ethyl acetate); IR ($CHCl_3$) 2210, 1730, 1604 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.2—1.8 (m, 13, 5 CH_2, CH, and 2 OH), 1.8—2.1 (m, 4, 2 CH_2), 2.1—2.4 (m, 5, 2 CH_2 and CH), 2.61 (t, 2, J = 7.7 Hz, benzylic CH_2), 2.9—3.1 (br, 1, allylic CH), 3.67 (s, 3, OCH_3), 3.77 (dd, 1, J = 8.9, 17.8 Hz, CHO), 4.08 (dd, 1, J = 5.7, 11.4 Hz, CHO), 5.28 (s, 1, vinyl in ring), 5.5—5.6 (m, 2, vinyl in chain), 6.83 (s, 1, aromatic), 6.85 (d, 1, J = 8.9 Hz, aromatic), 6.95 (d, 1, J = 7.4 Hz, aromatic), 7.26 (t, 1, J = 7.4 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.7, 25.2, 27.2, 30.6, 31.2, 34.0, 35.8, 37.0, 39.6, 39.8, 44.3, 45.7, 51.6, 58.2, 73.1, 77.3, 116.5, 119.0, 125.2, 128.4, 130.0, 133.4, 135.3, 139.9, 141.4, 144.7, 174.3; MS, *m/z* 449 ($M^+ - H_2O$), 439 ($M^+ - N_2$); HRMS, *m/z* calcd for $C_{27}H_{35}O_3N_3$ ($M^+ - H_2O$) 449.2679, found 449.2695. (15*R*)-**17c**: TLC R_f 0.41 (1:2 hexane/ethyl acetate); IR ($CHCl_3$) 2210, 1730, 1604 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.2—1.8 (m, 13, 5 CH_2, CH, and 2 OH), 1.8—2.1 (m, 4, 2 CH_2), 2.2—2.5 (m, 5, 2 CH_2 and CH), 2.61 (t, 2, J = 7.7 Hz, benzylic CH_2), 2.9—3.1 (br, 1, allylic CH), 3.67 (s, 3, OCH_3), 3.76 (ddd, 1, J = 6.9, 13.8, 13.8 Hz, CHO), 4.10 (dd, 1, J = 5.4, 10.8 Hz, CHO), 5.29 (s, 1, vinyl in ring), 5.4—5.6 (m, 2, vinyl in chain), 6.83 (s, 1, aromatic), 6.85 (d, 1, J = 7.9 Hz, aromatic), 6.95 (d, 1, J = 7.9 Hz, aromatic), 7.26 (t, 1, J = 7.9 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.7, 25.2, 27.3, 30.6, 31.2, 34.0, 35.8, 37.2, 39.8, 39.9, 44.4, 45.7, 51.6, 58.0, 72.7, 77.4, 116.4, 119.0, 125.2, 128.4, 129.7, 132.5, 135.1, 139.9, 141.6, 144.7, 174.3; MS, *m/z* 449 ($M^+ - H_2O$), 439 ($M^+ - N_2$); HRMS, *m/z* calcd for $C_{27}H_{35}O_3N_3$ ($M^+ - H_2O$) 449.2679, found 449.2667.

19-(3-Azidophenyl)-20-norisocarbacyclin ((15*S*)-18c). Prepared by using (15*S*)-**17c** (12.8 mg, 0.027 mmol) with similar operations as those for **18a** in 98% yield as a yellow oil. TLC R_f 0.28 (1:4 hexane/ethyl acetate); IR ($CHCl_3$) 3390, 2110, 1710, 1605, 1588 cm^{-1}; ^1H NMR (CD_3OD, 270 MHz) δ 1.2—1.7 (m, 13, 5 CH_2, CH, and 2 OH), 1.8—2.2 (m, 4, 2 CH_2), 2.2—2.4 (m, 5, 2 CH_2 and CH), 2.66 (t, 2, J = 7.4 Hz, benzylic CH_2), 2.9—3.1 (br, 1, allylic CH), 3.73 (ddd, 1, J = 6.9, 13.8, 13.8 Hz, CHO), 4.03 (dd, 1, J = 6.4, 12.8 Hz, CHO), 5.33 (s, 1, vinyl in ring), 5.4—5.6 (m, 2, vinyl in chain), 6.91 (br s, 2, aromatic), 7.04 (d, 1, J = 7.4 Hz, aromatic), 7.32 (t, 1, J = 7.4 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.2, 25.2, 26.8, 30.2, 31.1, 33.9, 35.7, 36.6, 38.9, 39.3, 44.2, 45.4, 57.9, 73.3, 77.2, 116.3, 118.9, 125.1, 128.9, 129.6, 133.6, 135.4, 139.8, 141.0, 144.6, 178.5; MS, *m/z* 435 ($M^+ - H_2O$), 425 ($M^+ - N_2$); HRMS, *m/z* calcd for $C_{26}H_{33}O_3N_3$ ($M^+ - H_2O$) 435.2522, found 435.2511.

19-(3-Azidophenyl)-15-*epi*-20-norisocarbacyclin ((15*R*)-18c). Prepared by using (15*R*)-**17c** (16.6 mg, 0.036 mmol) with similar operations as those for **18a** in 96% yield as a yellow oil. TLC R_f 0.35 (1:4 hexane/ethyl acetate); IR ($CHCl_3$) 3398, 2110, 1711, 1605, 1586 cm^{-1}; ^1H NMR (CD_3OD, 270 MHz) δ 1.2—1.7 (m, 13, 5 CH_2, CH, and 2 OH), 1.8—2.2 (m, 4, 2 CH_2), 2.2—2.5 (m, 3, CH_2 and CH), 2.33 (t, 2, J = 7.4 Hz, $CH_2C(O)$), 2.66 (t, 2, J = 7.4 Hz, benzylic CH_2), 2.9—3.1 (br, 1, allylic CH), 3.6—3.8 (m, 1, CHO), 4.05 (dd, 1, J = 5.4, 10.8 Hz, CHO), 5.34 (s, 1, vinyl in ring), 5.5—5.7 (m, 2, vinyl in chain), 6.91 (br s, 2, aromatic), 7.03 (d, 1, J = 7.9 Hz, aromatic), 7.31 (dt, 1, J = 1.8, 7.9 Hz, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.4, 25.2, 27.0, 30.5, 31.2, 33.9, 35.8, 37.0, 39.5, 39.7, 44.4, 45.6, 57.8, 72.6, 77.2, 116.4, 119.0, 125.2, 128.6, 129.7, 132.4, 135.0, 139.9, 141.4, 144.7, 178.8; MS, *m/z* 435 ($M^+ - H_2O$), 425 ($M^+ - N_2$); HRMS, *m/z* calcd for $C_{26}H_{33}O_3N_3$ ($M^+ - H_2O$) 435.2522, found 435.2528.

Determination of the C(15) stereochemistries of 17c.

Degradation of the more polar 17c to (S)-23: A mixture of more polar **17c** (2.0 mg, 0.0043 mmol) and 5% Pd on charcoal (1 mg) in CH_3OH (0.5 mL) was stirred under hydrogen at room temperature for 10 min. Removal of the catalyst by filtration followed by evaporation of the solvent gave a yellow oil, which was subjected to preparative TLC using a 1:4 mixture of hexane and ethyl acetate to give an amine **19** (1.2 mg, 63%). **19**: TLC R_f 0.27 (1:4 hexane/ethyl acetate); ^1H NMR ($CDCl_3$, 500 MHz) δ 1.2—1.7 (m, 8, 4 CH_2), 1.9—2.1 (m, 6, 3 CH_2), 2.1—2.3 (m, 5, 2 CH_2 and CH), 2.41 (dd, 1, J = 8.6, 16.3 Hz, CH), 2.52 (t, 2, J = 7.6 Hz, benzylic CH_2), 2.9—3.0 (br, 1, allylic CH in ring), 3.4—3.7 (br, 2, NH_2), 3.67 (s, 3, OCH_3), 3.76 (dd, 1, J = 7.0, 14.0 Hz, CHO), 4.09 (dd, 1, J = 6.1, 12.2 Hz, CHO), 5.29 (br s, 1, vinyl in ring), 5.5—5.6 (m, 2, vinyl), 6.50 (d, 1, J = 7.3 Hz, aromatic), 6.51 (s, 1, aromatic), 6.58 (d, 1, J = 7.6 Hz, aromatic), 7.06 (t, 1, J = 7.0 Hz, aromatic). A 13,14-dihydro derivative of **19** (TLC R_f 0.38 (1:4 hexane/ethyl acetate)) was obtained as a byproduct in 15% yield under these conditions. A solution of aminophenyl derivative **19** (2.0 mg, 0.005 mmol) in acetic acid–water (9:1) (0.3 mL) was stirred with $NaNO_2$ (3.0 mg, 0.04 mmol) for 10 min at 0 °C. An aqueous solution of H_3PO_2 (50%, 0.3 mL) was added to this mixture at 0 °C.[18] After 10 min, ethyl acetate (1 mL) and saturated $NaHCO_3$ aqueous solution were successively added. Extraction with ethyl acetate and chromatography (SiO_2, 1 g, eluent: 2:1 hexane/ethyl acetate) gave **20** (0.8 mg, 42%). **20**: TLC R_f 0.24 (1:1 hexane/ethyl acetate); ^1H NMR ($CDCl_3$, 500 MHz) δ 1.2—1.7 (m, 10, 5 CH_2), 1.8—2.1 (m, 5, 2 CH_2 and CH), 2.2—2.4 (m, 3, CH_2 and CH), 2.31 (t, 2, J = 7.3 Hz, $CH_2C(O)$), 2.61 (t, 2, J = 7.6 Hz, benzylic CH_2), 2.9—3.1 (br, 1, allylic CH in ring), 3.67 (s, 3, OCH_3), 3.75 (dd, 1, J = 7.0, 16.5 Hz, CHO), 4.07 (dd, 1, J = 6.4, 12.8 Hz, CHO), 5.28 (br s, 1, vinyl in ring), 5.5—5.6 (m, 2, vinyl in chain), 7.1—7.3 (m, 5, aromatic). A solution of I_2 (50 mg, 0.20 mmol) in benzene (0.2 mL) was added to a solution of **20** (14.1 mg, 0.033 mmol) in CH_3OH–water (3:1) (0.5 mL) and the resulting mixture was stirred at room temperature for 5 min. After dilution with ethyl acetate (1 mL), 10% $Na_2S_2O_3$ aqueous solution (1 mL) was added to this mixture. Extraction with ethyl acetate (0.5 mL x 2) followed by chromatography (SiO_2, 2 g, eluent: 6:1 hexane/ethyl acetate) gave **21**[19] (14.0 mg, 77%) as a yellow oil. **21**: TLC R_f 0.49 (1:1 hexane/ethyl acetate); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.2—1.8 (m, 15, 7 CH_2 and a proton of CH_2), 2.33 (t, 2, J = 7.5 Hz, $CH_2C(O)$), 2.5—2.6 (m, 1, CH), 2.61 (t, 2, J = 7.5 Hz, benzylic CH_2), 2.73 (dd, 1, J = 6.8, 6.8 Hz, CH), 2.86 (dd, 1, J = 7.3, 12.2 Hz, a proton of CH_2), 3.0—3.1 (br, 1, CH), 3.67 (s, 3, OCH_3), 4.01 (dd, 1, J = 6.3, 12.4 Hz, CHO), 4.18 (d, 1, J = 2.1 Hz, CHI), 4.29 (s, 1, CHO), 5.3—5.5 (m, 2, vinyl), 7.1—7.4 (m, 5, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 23.7, 25.0, 25.3, 31.3, 34.1, 35.6, 35.9, 37.2, 39.3, 43.0, 43.2, 43.5, 48.2, 51.6, 55.5, 72.8, 81.6, 88.2,

125.8, 128.3, 128.4, 131.7, 133.7, 142.4, 174.2. An alcohol **21** (14.0 mg, 0.025 mmol) was stirred with (*S*)-MTPA chloride (13.0 mg, 0.051 mmol) and pyridine (10 μL, 0.12 mmol) in CH_2Cl_2 (0.7 mL) at 20 °C for 3.5 h. After aqueous workup, **22** (18.2 mg, 95%) was obtained as a colorless oil by chromatography on SiO_2 (1.5 g) using 80:1, 40:1, 10:1, and 5:1 mixtures of hexane and ethyl acetate. **22**: TLC R_f 0.51 (2:1 hexane/ethyl acetate); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.2—1.8 (m, 15, 7 CH_2 and a proton of CH_2), 2.33 (t, 2, J = 7.4 Hz, $CH_2C(O)$), 2.58 (t, 2, J = 7.7 Hz, benzylic CH_2), 2.6—2.7 (m, 2, 2 CH), 2.85 (dd, 1, J = 7.4, 12.4 Hz, a proton of CH_2), 3.00 (dd, 1, J = 6.2, 6.2 Hz, CH), 3.48 (d, 3, J = 1.0 Hz, OCH_3), 3.67 (s, 3, OCH_3), 4.16 (d, 1, J = 2.0 Hz, CHI), 4.20 (s, 1, CHO), 5.2—5.5 (m, 3, CHO and vinyl), 7.1—7.5 (m, 10, aromatic). A solution of monoMTPA ester **22** (18.2 mg, 0.024 mmol) in pyridine (0.5 mL) was stirred with OsO_4 (12.1 mg, 0.048 mmol). After being stirred at 22 °C for 17 h and then at 75 °C for 9 h, an aqueous solution of $NaHSO_3$ (22 mg, 0.21 mmol) was added at 22 °C and the resulting mixture was stirred for 10 min. Aqueous workup followed by extraction with ethyl acetate and chromatography (SiO_2, 1.5 g, eluent: 1:1 to 1:3 hexane/ethyl acetate) gave a brown oil (12.2 mg, TLC R_f 0.21 and 0.12 (1:2 hexane/ethyl acetate)), which was further stirred with $NaIO_4$ (40 mg, 0.19 mmol) in ether–THF–water (1:2:1) (1 mL) at 22 °C for 1.5 h. Aqueous workup followed by extraction with ethyl acetate (0.5 mL x 2) gave an aldehydic compound as a colorless oil, which was immediately stirred with $NaBH_4$ (5 mg, 0.13 mmol) in CH_3OH (0.5 mL). After aqueous workup, the resulting alcoholic product was stirred with (*S*)-MTPA chloride (30 mg, 0.12 mmol) and pyridine (20 μL, 0.25 mmol) in CH_2Cl_2 (0.7 mL) at 21 °C for 10 min. Aqueous workup followed by purification by the combination of chromatography (SiO_2, 1 g, eluent: 5:1 hexane/ethyl acetate) and preparative TLC (eluent: 2:1 hexane/ethyl acetate) gave (*S*)-**23** (1.5 mg, 10% based on **22**) and a monoMTPA ester as the counterpart (1.7 mg, 12% based on **22**) as colorless oils, respectively. (*S*)-**23**: TLC R_f 0.66 (2:1 hexane/ethyl acetate); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.2—1.8 (m, 6, 3 CH_2), 2.55 (t, 2, J = 7.6 Hz, benzylic CH_2), 3.38, 3.43 (s each, 6, 2 OCH_3), 4.25 (dd, 1, J = 5.0, 12.4 Hz, a proton of $C(1)H_2$), 4.52 (dd, 1, J = 2.8, 12.2 Hz, a proton of $C(1)H_2$), 5.2—5.3 (m, 1, C(2)H), 7.0—7.5 (m, 15, 3 C_6H_5). The monoMTPA ester as a counterpart of (*S*)-**23**: TLC R_f 0.46 (2:1 hexane/ethyl acetate); IR (thin film) 1745, 1453 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.3—1.5 (m, 2, CH_2), 1.5—1.8 (m, 6, 2 CH_2 and a proton of CH_2 x 2), 1.93 (d, 1, J = 13.1 Hz, a proton of CH_2), 2.13 (t, 2, J = 7.5 Hz, $CH_2C(O)$), 2.36 (dd, 1, J = 7.6, 7.6 Hz, CH), 2.56 (t, 1, J = 6.0, 6.0 Hz, CH), 2.84 (dd, 1, J = 7.3, 12.2 Hz, a proton of CH_2), 3.06 (t, 1, J = 6.4, 6.4 Hz, CH), 3.53 (s, 3, OCH_3), 3.67 (s, 3, OCH_3), 3.96 (dd, 1, J = 8.5, 11.3 Hz, a proton of CH_2O), 4.07 (dd, 1, J = 7.3, 11.3 Hz, a proton of CH_2O), 4.16 (d, 1, J = 2.1 Hz, CHI), 4.34 (s, 1, CHO), 7.3—7.6 (m, 5, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) (DEPT) δ 23.5 (CH_2), 25.1 (CH_2), 33.9 (CH_2), 35.4 (CH_2), 37.7 (CH), 42.1 (CH), 42.7 (CH_2), 43.5 (CH_2), 48.2 (CH), 51.5 (OCH_3), 52.3 (CH), 55.4 (OCH_3), 66.7 (CH_2), 78.9 (CH), 84.5 (C, q, J = 27.0 Hz), 88.4 (C), 123.2 (CF_3, q, J = 288.0 Hz), 127.1 (CH), 128.5 (CH), 129.7 (CH), 131.9 (C), 166.5 (C(O)), 174.1 (C(O)); MS, *m/z* 610 (M^+), 579 ($M^+ - OCH_3$), 483 ($M^+ - I$); HRMS, *m/z* calcd for $C_{25}H_{30}O_6F_3I$ (M^+) 610.1038, found 610.1064. This monoMTPA ester (34.8 mg, 0.057 mmol) was stirred with tri-*n*-butyltin hydride (0.1 mL, 0.37 mmol) and di-*tert*-butyl peroxide (2.5 mg) in toluene (0.3 mL) at 85 °C for 3 h. After being cooled to room temperature, the mixture was subjected to chromatography on SiO_2 (5 g) using 100:1, 4:1, and 3:1 mixtures of hexane and ethyl acetate to yield the corresponding deiodination product (23.8 mg, 86%) as a colorless oil. TLC R_f 0.43 (2:1 hexane/ethyl acetate); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.2—1.4 (m, 2, CH_2), 1.4—1.8 (m, 10, 5 CH_2), 2.30 (t, 2, J = 7.5 Hz, $CH_2C(O)$), 2.33 (dd, 1, J = 7.9, 7.9 Hz, CH), 2.44 (dd, 1, J = 5.8, 5.8 Hz, CH), 2.63 (dd, 1, J = 6.1, 9.4 Hz, CH), 3.53 (s, 3, OCH_3), 3.66 (s, 3, OCH_3), 3.98 (dd, 1, J = 8.5, 11.3 Hz, a proton of CH_2O), 4.09 (dd, 1,

J = 7.3, 11.3 Hz, a proton of CH_2O), 4.25 (s, 1, CHO), 7.3—7.6 (m, 5, aromatic); ^{13}C NMR ($CDCl_3$, 67.5 MHz) (DEPT) δ 24.0 (CH_2), 25.5 (CH_2), 34.0 (CH_2), 35.2 (CH), 36.3 (CH_2), 38.8 (CH), 39.1 (CH_2), 46.4 (CH_2), 46.5 (CH_2), 51.5 (OCH_3), 52.4 (CH), 55.4 (OCH_3), 67.3 (CH_2), 78.8 (CH), 84.6 (C, q, J = 28.7 Hz), 84.9 (C), 123.2 (CF_3, q, J = 289.0 Hz), 127.2 (CH), 128.4 (CH), 129.7 (CH), 132.1 (C), 166.6 (C(O)), 174.2 (C(O)); MS, *m/z* 484 (M^+), 453 ($M^+ - OCH_3$). The 9-oxatricyclo[3.3.1.0^{3,7}]nonane structure of this compound was confirmed by the observation of a new methylene group at δ 39.1 in the ^{13}C NMR DEPT spectrum, turning back to prove that the compound **21** possesses the same tricyclic structure.

Identification of (S)-23 derived from the more polar 17c with the stereo-defined (S)-23 derived from (S)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol ((S)-24): A solution of dimethylsulfoxide (0.5 mL, 7.1 mmol) in CH_2Cl_2 (3 mL) was slowly added to a solution of $(COCl)_2$ (0.45 mL, 5.0 mmol) in CH_2Cl_2 (15 mL) at –78 °C. After the mixture was stirred at –78 °C for 15 min, a solution of (*S*)-**24** (300 mg, 2.3 mmol) in CH_2Cl_2 (1 mL) was added. The resulting mixture was further stirred –78 °C for 10 min and then at –45 °C for 60 min. Triethylamine (3.0 mL, 21.5 mmol) was added to this mixture and the resulting mixture was slowly warmed to 0 °C. After the mixture was stirred for 20 min, saturated NH_4Cl aqueous solution (20 mL) was added.[27] Extraction with CH_2Cl_2 (10 mL x 2) followed by chromatography (SiO_2, 20 g, eluent: 2:1 hexane/ethyl acetate) gave a crude (*R*)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (**25**)[28] (115.6 mg). **25**: TLC R_f 0.31 (1:1 hexane/ethyl acetate); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.38, 1.45 (s each, 6, 2 CH_3), 3.5—4.4 (m, 3, CH_2O and CHO), 9.73 (d, 1, J = 2.0 Hz, C(O)H). A solution of this crude aldehyde **25** (115.6 mg) in THF (2 mL) was added to a mixture of 3-phenylpropylphosphonium bromide (460.0 mg, 1.0 mmol, prepared from equimolar amounts of triphenylphosphine and 1-bromo-3-phenylpropane) and *n*-butyllithium (1.57 M, 0.64 mL, 1.0 mmol) in THF–toluene (5:3) (8 mL) at 23 °C. The whole mixture was stirred for 10 min and followed by addition of saturated NH_4Cl aqueous solution (5 mL). Extraction with ethyl acetate (5 mL x 2) and chromatography (SiO_2, 5 g, eluent: 30:1 hexane/ethyl acetate) yielded the condensation product **26** (7.7 mg). **26**: TLC R_f 0.68 (2:1 hexane/ethyl acetate); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.36, 1.39 (s each, 6, 2 CH_3), 2.3—2.8 (m, 4, 2 CH_2), 3.34 (dd, 1, J = 7.9, 7.9 Hz, a proton of CH_2O), 3.76 (dd, 1, J = 6.2, 7.9 Hz, a proton of CH_2O), 4.70 (ddd, 1, J = 7.3, 7.3, 7.3 Hz, CHO), 5.42 (dd, 1, J = 9.4, 9.4 Hz, vinyl), 5.64 (dt, 1, J = 10.9, 7.9 Hz, vinyl), 7.1—7.4 (m, 5, aromatic). A mixture of crude **26** (7.7 mg) and 5% Pt on charcoal (3 mg) in CH_3OH (1.5 mL) was stirred under hydrogen at 21 °C for 2 h. Removal of catalyst by filtration followed by evaporation of the solvent gave an oil. This material was further stirred with 1 N HCl (0.5 mL) in THF (1 mL) at 20 °C for 5 h and addition of saturated $NaHCO_3$ aqueous solution (2 mL) was followed. Extraction with ethyl acetate (1 mL x 3) and concentration under reduced presure gave (*S*)-6-phenylhexan-1,2-diol (**27**)[29] (5.5 mg). **27**: TLC R_f 0.13 (2:1 hexane/ethyl acetate); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.3—1.7 (m, 6, 3 CH_2), 1.7—2.2 (br, 2, 2 OH), 2.62 (t, 2, J = 7.5 Hz, benzylic CH_2), 3.42 (dd, 1, J = 8.0, 10.9 Hz, a proton of C(1)H_2), 3.64 (dd, 1, J = 3.0, 10.9 Hz, a proton of C(1)H_2), 3.6—3.7 (m, 1, C(2)H), 7.1—7.4 (m, 5, aromatic). A mixture of crude diol **27** (5.5 mg), (*S*)-MTPA chloride (50 mg, 0.20 mmol), and pyridine (60 μL, 0.74 mmol) in CH_2Cl_2 (0.5 mL) was stirred at 20 °C for 12 h. The aqueous workup followed by chromatography on SiO_2 (2 g) with a 40:1 mixture of hexane and ethyl acetate as eluent gave bisMTPA ester (*S*)-**23** (13.7 mg). (*S*)-**23**: TLC R_f 0.66 (2:1 hexane/ethyl acetate); IR (thin film) 1752, 1603, 1496, 1453 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.2—1.8 (m, 6, 3 CH_2), 2.55 (t, 2, J = 7.5 Hz, benzylic CH_2), 3.38, 3.43 (s each, 6, 2 OCH_3), 4.25 (dd, 1, J = 5.0, 12.4 Hz, a proton of C(1)H_2), 4.52 (dd, 1, J = 3.0, 12.2 Hz, a proton of C(1)H_2), 5.2—5.3 (m, 1, C(2)H), 7.0—7.5 (m, 15, 3 C_6H_5); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 24.6, 30.1, 31.0, 35.6, 55.4, 55.5, 65.8, 73.8, 84.7 (q,

J = 28 Hz), 123.2 (q, J = 288 Hz, CF_3), 125.9, 127.3, 127.4, 128.4, 128.5, 129.7, 129.8, 131.9, 132.0, 142.0, 166.2, 166.4; MS, *m/z* 626 (M^+), 557 ($M^+ - CF_3$); HRMS, *m/z* calcd for $C_{31}H_{32}O_6F_3$ ($M^+ - CF_3$) 557.2151, found 557.2143. (*S*)-**23** can readily be differentiated from the corresponding diastereoisomer (*R*)-**23** by the chemical shifts of their C(1) protons. The chemical shifts of the C(1) protons in (*R*)-**23** were assigned to be δ 4.28 and 4.60 by comparison of the ^1H NMR spectrum of (*S*)-**23** with a diastereomeric mixture derived from (±)-**24** by similar operations as those conducted in the synthesis of (*S*)-**23**. A diastereomeric mixture of (*S*)-**23** and (*R*)-**23**: TLC R_f 0.66 (2:1 hexane/ethyl acetate); ^1H NMR ($CDCl_3$, 500 MHz) δ 1.2—1.8 (m, 12, 3 CH_2 x 2), 2.50 (t, 2, J = 7.6 Hz, benzylic CH_2), 2.55 (t, 2, J = 6.9 Hz, benzylic CH_2), 3.38, 3.39, 3.43, 3.47 (s each, 12, 2 OCH_3 x 2), 4.25 (dd, 1, J = 5.0, 12.4 Hz, a proton of C(1)H_2), 4.28 (dd, 1, J = 6.4, 12.2 Hz, a proton of C(1)H_2), 4.52 (dd, 1, J = 3.1, 12.2 Hz, a proton of C(1)H_2), 4.60 (dd, 1, J = 3.1, 12.2 Hz, a proton of C(1)H_2), 5.2—5.3 (m, 2, C(2)H x 2), 7.0—7.5 (m, 30, 3 C_6H_5 x 2). Thus the ^1H NMR spectrum of (*S*)-**23** derived from the more polar **17c** was identical with that of the stereo-defined (*S*)-**23** derived from (*S*)-**24**. The relative polarity of (15*S*)-**17c** and (15*R*)-**17c** is consistent with that reported for isocarbacyclin methyl ester (**3**) (more polar) and its 15-epimer (less polar).[8]

Derivatization of **16c** *to 19-(3-Azidophenyl)-15-O-benzoyl-11-O-(tert-butyldimethylsilyl)-20-noriso-carbacyclin Methyl Ester (28) and its 15-Epimer (29):* In a 10-mL round-bottomed flask was placed a solution of **16c** (12.0 mg, 0.026 mmol) in CH_2Cl_2 (1 mL) at room temperature. Imidazole (11.4 mg, 0.17 mmol) and *tert*-butyldimethylsilyl chloride (25.2 mg, 0.17 mmol) were successively added to this solution. The resulting mixture was stirred at room temperature for 12 h and poured into water (1 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (1 mL x 2). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated to give a colorless oil, which was chromatographed on SiO_2 (1 g) using a 25:1 mixture of hexane and ethyl acetate to give the corresponding 11-*O*-silylated derivative (11.6 mg, 77%): TLC R_f 0.80 (1:1 hexane/ethyl acetate); ^1H NMR ($CDCl_3$, 270 MHz) δ –0.03, 0.00 (s each, 6, $Si(CH_3)_2$), 0.83 (s, 9, Si-*t*-C_4H_9), 1.2—1.7 (m, 10, 5 CH_2), 1.8—2.5 (m, 8, 4 CH_2), 2.5—2.6 (m, 4, benzylic CH_2 and 2 CH), 2.9—3.1 (br, 1, allylic CH in ring), 3.67 (s, 3, OCH_3), 3.82 (dd, 1, J = 2.5, 9.4 Hz, CHO), 5.26 (s, 1, vinyl in ring), 6.12 (d, 1, J = 15.8 Hz, vinyl), 6.72 (dd, 1, J = 8.4, 15.8 Hz, vinyl), 6.83 (s, 1, aromatic), 6.84 (d, 1, J = 8.4 Hz, aromatic), 6.94 (d, 1, J = 7.4 Hz, aromatic), 7.25 (t, 1, J = 7.4 Hz, aromatic). This material was dissolved in a solution of $CeCl_3 \cdot 7H_2O$ in CH_3OH (0.018 M, 1.1 mL, 0.02 mmol). $NaBH_4$ (1.0 mg, 0.03 mmol) was added to this solution at 0 °C. The resulting mixture was stirred at 0 °C for 3 min and followed by addition of ethyl acetate (1.5 ml) and water (0.5 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (1 mL x 2). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated to give a yellow oil, which was subjected to preparative TLC using a 5:1 mixture of hexane and ethyl acetate as eluent (twice elution) to give two 15-epimeric alcohols having different R_f values on TLC. The less polar alcohol (5.9 mg, 51%): TLC R_f 0.43 (5:1 hexane/ethyl acetate, twice elution); ^1H NMR (C_6D_6, 270 MHz) δ 0.13, 0.15 (s each, 6, $Si(CH_3)_2$), 1.04 (s, 9, Si-*t*-C_4H_9), 1.2—1.6 (m, 11, 5 CH_2 and CH), 1.9—2.3 (m, 6, 3 CH_2), 2.14 (t, 2, J = 7.4 Hz, CH_2), 2.41 (t, 2, J = 7.4 Hz, benzylic CH_2), 2.4—2.5 (m, 1, CH), 2.9—3.0 (m, 1, allylic CH in ring), 3.39 (s, 3, OCH_3), 3.73 (dd, 1, J = 2.5, 9.4 Hz, CHO), 3.9—4.0 (br, 1, CHO), 5.30 (br s, 1, vinyl), 5.5—5.7 (m, 2, vinyl), 6.70 (d, 1, J = 7.9 Hz, aromatic), 6.78 (d, 1, J = 7.9 Hz, aromatic), 6.80 (s, 1, aromatic), 6.99 (t, 1, J = 7.9 Hz, aromatic). The more polar alcohol (3.0 mg, 26%): TLC R_f 0.35 (5:1 hexane/ethyl acetate, twice elution); ^1H NMR (C_6D_6, 270 MHz) δ 0.13, 0.15 (s each, 6, $Si(CH_3)_2$), 1.04 (s, 9, Si-*t*-C_4H_9), 1.2—1.6 (m, 11, 5 CH_2 and CH), 1.9—2.3 (m, 6, 3 CH_2), 2.14 (t, 2, J = 7.4 Hz, CH_2), 2.41

(t, 2, J = 7.4 Hz, benzylic CH_2), 2.4—2.5 (m, 1, CH), 2.9—3.0 (m, 1, allylic CH in ring), 3.39 (s, 3, OCH_3), 3.73 (dd, 1, J = 2.5, 9.4 Hz, CHO), 4.0—4.1 (br, 1, CHO), 5.31 (br s, 1, vinyl in ring), 5.5—5.7 (m, 2, vinyl), 6.70 (d, 1, J = 7.7 Hz, aromatic), 6.78 (d, 1, J = 7.9 Hz, aromatic), 6.81 (s, 1, aromatic), 6.99 (t, 1, J = 7.9 Hz, aromatic). The less polar 11-*O*-silylated alcohol obtained above was correlated to the more polar **17c** by desilylation upon treatment with $(n\text{-}C_4H_9)_4NF$ in THF. The more polar 11-*O*-silylated alcohol was also correlated to the less polar **17c** by a similar treatment. After such correlation, the 11-*O*-silylated less polar alcohol (3 mg, 0.005 mmol) was again dissolved in CH_2Cl_2 (0.2 mL) and pyridine (1 μL, 12.4 μmol) and benzoyl chloride (1 μL, 8.6 μmol) were successively added. The resulting mixture was stirred at room temperature for 12 h and followed by submission to preparative TLC using a 2:1 mixture of hexane and ethyl acetate as eluent to give **28** (2.8 mg, 78%) as a colorless oil. **28**: TLC R_f 0.63 (5:1 hexane/ethyl acetate); ^1H NMR ($CDCl_3$, 270 MHz) δ 0.00, 0.01 (s each, 6, $Si(CH_3)_2$), 0.85 (s, 9, Si-*t*-C_4H_9), 1.2—2.2 (m, 14, 7 CH_2), 2.2—2.5 (m, 4, CH_2 and 2 CH), 2.32 (t, 2, J = 7.4 Hz, $CH_2C(O)$), 2.60 (t, 2, J = 7.7 Hz, benzylic CH_2), 2.9—3.1, (br, 1, allylic CH in ring), 3.66 (s, 3, OCH_3), 3.72 (dd, 1, J = 6.9, 13.8 Hz, CHO), 5.24 (br s, 1, vinyl in ring), 5.5—5.7 (m, 3, CHO and vinyl in chain), 6.81 (s, 1, aromatic), 6.83 (d, 1, J = 7.4 Hz, aromatic), 6.92 (t, 2, J = 7.4 Hz, aromatic), 6.93 (d, 1, J = 7.9 Hz, aromatic), 7.43 (t, 2, J = 7.4 Hz, aromatic), 7.54 (d, 1, J = 7.4 Hz, aromatic), 8.03 (d, 2, J = 7.4 Hz, aromatic); CD (cyclohexane) λ_{max} 221 nm (Δε +1.5). The corresponding 15-epimer **29** was also derived in a similar manner from the more polar 11-*O*-silylated alcohol (1.5 mg, 0.003 mmol) in 57% yield. **29**: TLC R_f 0.63 (5:1 hexane/ethyl acetate); ^1H NMR ($CDCl_3$, 270 MHz) δ 0.00, 0.07 (s each, 6, $Si(CH_3)_2$), 0.84 (s, 9, Si-*t*-C_4H_9), 1.2—2.2 (m, 14, 7 CH_2), 2.2—2.5 (m, 4, CH_2 and 2 CH), 2.30 (t, 2, J = 7.4 Hz, $CH_2C(O)$), 2.59 (t, 2, J = 7.4 Hz, benzylic CH_2), 2.9—3.0, (br, 1, allylic CH in ring), 3.65 (s, 3, OCH_3), 3.67 (dd, 1, J = 6.9, 13.8 Hz, CHO), 5.24 (br s, 1, vinyl in ring), 5.5—5.7 (m, 3, CHO and vinyl in chain), 6.81 (s, 1, aromatic), 6.82 (d, 1, J = 7.4 Hz, aromatic), 6.93 (d, 1, J = 7.4 Hz, aromatic), 7.22 (t, 1, J = 8.4 Hz, aromatic), 7.43 (t, 2, J = 7.2 Hz, aromatic), 7.55 (d, 1, J = 6.9 Hz, aromatic), 8.04 (d, 2, J = 7.4 Hz, aromatic); CD (cyclohexane) λ_{max} 222 nm (Δε –0.6).

Binding Assay of Azidophenyl Derivatives 18a—c to the PGI_2 Receptor Protein in Mastocytoma P-815 Cells. The membrane fraction of mastocytoma P-815 cells (200 μg) was incubated with [^{3}H]iloprost (**30**) (20 nM, 0.028 μCi) in the presence of various concentrations of **18a** at 30 °C for 60 min in K_3PO_4 buffer containing 1 mM of ethylenediaminetetraacetic acid (EDTA) and 10 mM of $MgCl_2$ (100 μL, pH 6.0). Incubation was terminated by rapid filtration onto a Whatman GF/C glass filter, which was washed with the same buffer (2 mL x 4). Radioactivity associated with the glass filter was measured by scintillation counting to determine the total binding. Nonspecific binding was determined in the presence of iloprost (10 μM) under the same conditions as used for the total binding measurement. Specific binding was determined by subtracting nonspecific binding from the total binding. Data represent the means of triplicate determinations. Data for compounds **18b**, (15*R*)-**18c**, and (15*S*)-**18c** were obtained in the similar manner described above.

Adenylate Cyclase Assay in Mastocytoma P-815 Cell Membrane Fraction. The mixture comprising the membrane fraction of mastocytoma P-815 cells (20 μg of protein), EDTA (1 mM), $MgCl_2$ (10 mM), dithiothreitol (1 mM), 3-isobutyl-1-methylxanthine (1 mM), and ATP (1 mM) in Hepes–NaOH (50 mM, pH 8.0, 100 μL) was prepared. Reactions were started by addition of the serial concentration of (15*S*)-**18c** and allowed to proceed for 10 min at 37 °C and then terminated by addition of an aqueous solution of trichloroacetic

acid (10%, 100 μL). The cAMP formed during the reaction was measured by radioimmunoassaying with an Amersham cAMP[^{125}I] assay system. Data for iloprost was obtained in the similar manner described above.

[15-$^3H_1$]-19-(3-Azidophenyl)-20-norisocarbacyclin Methyl Ester ([^{3}H]-(15*S*)-17c). The crude product of the reduction of **16c** with [^{3}H]$NaBH_4$ (60 Ci/mmol)/$CeCl_3 \cdot 7H_2O$ obtained from Amersham was subjected to reversed-phase HPLC (for conditions, see General). The retention times for each epimer were 21.6 and 27.4 min for [^{3}H]-(15*S*)-**17c** and [^{3}H]-(15*R*)-**17c**, respectively.

[15-$^3H_1$]-19-(3-Azidophenyl)-20-norisocarbacyclin ([^{3}H]-(15*S*)-18c). To a solution of [^{3}H]-(15*S*)-**17c** (66.7 μCi, 4.4 nmol) in CH_3OH (2 mL), aqueous solution of NaOH (5 N, 0.12 mL) was added at 25 °C. The resulting mixture was stirred at 25 °C for 4 h and followed by addition of $NaHSO_4$ (10 mg) to be pH 3. To this mixture were added water (4 mL) and ethyl acetate (3 mL). The organic layer was separated and an aqueous layer was extracted with ethyl acetate (1 mL x 2). The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated to give [^{3}H]-(15*S*)-**18c** (66.4 μCi, 99.6%). This material was used in biochemical experiments without further purification.

REFERENCES AND NOTES

1. Nelson, N. A.; Kelly, R. C.; Johnson, R. A. *Chem. Eng. News* **1982**, *60*, 30; Vane, J. R. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 741; Samuelsson, B. *ibid.* **1983**, *22*, 805; Bergström, S. *ibid.* **1983**, *22*, 858. For recent advances, see *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*, Vol. 11—21; Raven: New York, 1983—1991.
2. Robertson, R. P. *Prostaglandins* **1986**, *31*, 395; Halushka, P. V.; Mais, D. E.; Mayeux, P. R.; Morinelli, T. A. *Ann. Rev. Pharm. Tox.* **1989**, *10*, 213. The molecular structure of a thromboxane A_2 receptor protein was recently elucidated. See, Hirata, M.; Hayashi, Y.; Ushikubi, F.; Yokota, Y.; Kageyama, R.; Nakanishi, S.; Narumiya, S. *Nature* **1991**, *349*, 617.
3. Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. *Nature* **1976**, *263*, 663; Kadowitz, P. J.; Chapnick, B. M.; Feigen, L. P.; Hyman, A. L.; Nelson, P. K.; Spannhake, E. W. *J. Appl. Physiol.* **1978**, *45*, 408.
4. (a) Gorman, R. R.; Bunting, S.; Miller, O. V. *Prostaglandins* **1977**, *13*, 377. (b) Tateson, J. E.; Moncada, S.; Vane J. R. *ibid.* **1977**, *13*, 389. (c) MacDermot, J.; Barnes, P. J. *Eur. J. Pharmacol.* **1980**, *67*, 419. (d) Blair, I. A.; Hensby, C. N.; MacDermot, J. *Br. J. Pharmacol.* **1980**, *69*, 519. (e) Hashimoto, H.; Negishi, M.; Ichikawa, A. *Prostaglandins* **1990**, *40*, 491.
5. Reviews: Oliva, D.; Nicosia, S. *Pharmacol. Res. Commun.* **1987**, *19*, 735; Tsai, A.-l.; Wu, K. K. *Eicosanoids* **1989**, *2*, 131. Although it had been reported that PGI_2 receptor protein was purified by the SDS-PAGE method after solubilization with Triton X-100 as the PGE_1/PGI_2 receptor protein,[6a] such results were claimed to be nonreproducible on reexamination using [^{3}H]iloprost (**30**), a stable PGI_2 agonist, for binding studies.[6b] The molecular weight of the PGI_2 receptor protein has been estimated by radiation inactivation of lyophilized membranes[6c] but the homogeneity of a receptor protein can not be judged by this method.[6d]

6. (a) Dutta-Roy, A. K.; Sinha, A. K. *J. Biol. Chem.* **1987**, *262*, 12685. (b) Tsai, A.-l.; Hsu, M.-J.; Vijjeswarapu, H.; Wu, K. K. *J. Biol. Chem.* **1989**, *264*, 61. (c) Leigh, P. J.; Cramp, W. A.; MacDermot, J. *J. Biol. Chem.* **1984**, *259*, 12431. (d) Jung, C. Y. In *Receptor Biochemistry and Methodology*; Venter, J. C.; Harrison, L. C., Eds.; Alan R. Riss: New York, 1984; Vol. 3, Chapter 8.
7. For the photoaffinity labeling method, see reviews: Chowdhry, V.; Westheimer, F. H. *Ann. Rev. Biochem.* **1979**, *48*, 293; Schuster, D. I.; Probst, W. C.; Ehrlich, G. K.; Singh, G. *Photochem. Photobiol.* **1989**, *49*, 785.
8. Shibasaki, M.; Torisawa, Y.; Ikegami, S. *Tetrahedron Lett.* **1983**, *24*, 3493.
9. A part of this paper was presented at the 33rd Symposium on the Chemistry of Natural Products, Osaka, Japan, October 1991; paper 691 and the 64th Annual Meeting of the Japanese Biochemical Society, Tokyo, Japan, October 1991; paper 2825.
10. (a) Suzuki, M.; Koyano, H.; Noyori, R. *J. Org. Chem.* **1987**, *52*, 5583. (b) Suzuki, M.; Morita, Y.; Koyano, H.; Koga, M.; Noyori, R. *Tetrahedron* **1990**, *46*, 4809. (c) Noyori, R. *Chem. Br.* **1989**, 883. (d) Noyori, R.; Suzuki, M. *Chemtracts—Org. Chem.* **1990**, *3*, 173. See also: (e) Bannai, K.; Tanaka, T.; Okamura, N.; Hazato, A.; Sugiura, S.; Manabe, K.; Tomimori, K.; Kato, Y.; Kurozumi, S.; Noyori, R. *Tetrahedron* **1990**, *46*, 6689. (f) Tanaka, T.; Bannai, K.; Hazato, A.; Koga, M.; Kurozumi, S.; Kato, Y. *Tetrahedron* **1991**, *47*, 1861.
11. For other methods for the synthesis of **2**: (a) Shibasaki, M.; Fukasawa, H.; Ikegami, S. *Tetrahedron Lett.* **1983**, *24*, 3497. (b) Sodeoka, M.; Shibasaki, M. *Chem. Lett.* **1984**, 579. (c) Torisawa, Y.; Okabe, H.; Shibasaki, M.; Ikegami, S. *ibid.* **1984**, 1069. (d) Torisawa, Y.; Okabe, H.; Ikegami, S. *J. Chem. Soc., Chem. Commun.* **1984**, 1602. (e) Ogawa, Y.; Shibasaki, M. *Tetrahedron Lett.* **1984**, *25*, 1067. (f) Mase, T.; Sodeoka, M.; Shibasaki, M. *ibid.* **1984**, *25*, 5087. (g) Koyama, K.; Kojima, K. *Chem. Pharm. Bull.* **1984**, *32*, 2866. (h) Nagao, Y.; Nakamura, T.; Kume, M.; Ochiai, M.; Fuji, K.; Fujita, E. *J. Chem. Soc., Chem. Commun.* **1987**, 269. (i) Hashimoto, S.; Shinoda, T.; Shimada, Y.; Honda, T.; Ikegami, S. *Tetrahedron Lett.* **1987**, *28*, 637. (j) Hashimoto, S.; Shinoda, T.; Ikegami, S. *J. Chem. Soc., Chem. Commun.* **1988**, 1137. (k) Hashimoto, S.; Kase, S.; Shinoda, T.; Ikegami, S. *Chem. Lett.* **1989**, 1063. (l) Hemmerle, H.; Gais, H.-J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 349. (m) Sodeoka, M.; Ogawa, Y.; Mase, T.; Shibasaki, M. *Chem. Pharm. Bull.* **1989**, *37*, 586. (n) Mandai, T.; Matsumoto, S.; Kohama, M.; Kawada, M.; Tsuji, J.; Saito, S.; Moriwake, T. *J. Org. Chem.* **1990**, *55*, 5671. (o) Bund, J.; Gais, H.-J.; Erdelmeier, I. *J. Am. Chem. Soc.* **1991**, *113*, 1442.
12. Hill, J. G.; Sharpless, K. B.; Exon, C. M.; Regenye, R. *Org. Synth.* **1985**, *63*, 66.
13. Roush, W. R.; Brown, R. J.; DiMare, M. *J. Org. Chem.* **1983**, *48*, 5083.
14. *CRC Handbook of Chemistry and Physics*, 70th ed.; Weast, R. C., Ed.; CRC: Boca Ratom, FL, 1989.
15. The β-keto phosphonate **11b** was used in the synthesis of a photoaffinity probe compound for the study of $PGF_{2\alpha}$ receptors. See: Kawada, K.; Dolence, E. K.; Morita, H.; Kometani, T.; Watt, D. S.; Balapure, A.; Fitz, T. A.; Orlicky, D. J.; Gerschenson, L. E. *J. Med. Chem.* **1989**, *32*, 256.
16. Luche, J.-L.; Rodriguez-Hahn, L.; Crabbé, P. *J. Chem. Soc., Chem. Commun.* **1978**, 601; Luche, J.-L. *J. Am. Chem. Soc.* **1978**, *100*, 2226.
17. Azidobenzene is known to be reduced to aniline with $NaBH_4$ in 2-propanol at higher temperatures: Smith, P. A. S.; Hall, J. H.; Kan, R. O. *J. Am. Chem. Soc.* **1962**, *84*, 485. The incorporation of a

tritium atom into the PG skeleton has been realized by this controlled reduction near the end of the synthesis.

18. Kornblum, N. *Org. Synth., Coll. Vol. 3* **1955**, 295.
19. The 9-oxatricyclo[3.3.1.0^{3,7}]nonane structure was determined after oxidative cleavage of the C(13)—C(14) double bond (see experimental section).
20. Gonnella, N. C.; Nakanishi, K.; Martin, V. S.; Sharpless, K. B. *J. Am. Chem. Soc.* **1982**, *104*, 3775.
21. The inhibitory effects were tested at the Teijin Institute for Biomedical Research, Tokyo, Japan.
22. Ito, S.; Hashimoto, H.; Negishi, M.; Suzuki, M.; Koyano, H.; Noyori, R.; Ichikawa, A. submitted for publication.
23. The tritium incorporation was carried out at Amersham International plc in England.
24. Kofron, W. G.; Baclawski, L. M. *J. Org. Chem.* **1976**, *41*, 1879.
25. (a) Sato, K.; Mizuno, S.; Hirayama, M. *J. Org. Chem.* **1967**, *32*, 177. (b) Roush, W. R. *J. Am. Chem. Soc.* **1980**, *102*, 1390.
26. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
27. Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.
28. Marco, J. L.; Rodríguez, B. *Tetrahedron Lett.* **1988**, *29*, 1997.
29. Blasius, E.; Rausch, R. A.; Andreetti, G. D.; Rebizant, J. *Chem. Ber.* **1984**, *117*, 1113.

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