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A NEW SYNTHESIS OF (+)-6a-CARBAPROSTAGLANDIN I_2 EMPLOYING YEAST REDUCTION OF A β-KETO ESTER DERIVED FROM *cis*-BICYCLO[3.3.0]OCTANE-3,7-DIONE AS THE KEY-STEP†

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Abstract — (+)-6a-Carbaprostaglandin I_2 (carbacyclin), a stable mimic of prostaglandin I_2 (prostacyclin), was synthesized by utilizing the kinetic resolution of (±)-2-ethoxycarbonyl-7,7-ethylenedioxybicyclo[3.3.0]octan-3-one in the course of its yeast reduction as the simple and key resolution step.

Prostaglandin I_2 1 (PGI_2, prostacyclin) is a labile metabolite of arachidonic acid, which shows potent antithrombotic and vasodilatory properties.[1] Because of the instability of PGI_2, a more stable analogue was thought to be a useful therapeutic agent. 6a-Carbaprostaglandin I_2 2 (carba-PGI_2; trivial names -- carbacyclin, carbaprostacyclin and 9(0)-methanoprostacyclin) is such an analogue of 1 and has been shown to possess a very similar biological properties to PGI_2.[2] Although there reported a number of syntheses of (±)-2,[3~8] only three syntheses of (+)-carba-PGI_2 2 have been reported to date by workers at Upjohn[9,11] and Ono.[10] Two out of the three syntheses started from "Corey lactone" or a related advanced intermediate in PG synthesis,[10,11] while Morton and Brokaw employed a resolved cyclobutanone as their starting material.[9]

We became interested in developing a new and simpler synthesis of (+)-carba-PGI_2 2 employing a biochemical process. (±)-2-Ethoxycarbonyl-7,7-ethylenedioxybicyclo[3.3.0]-octan-3-one 4 or the corresponding Me ester is a popular intermediate in the synthesis of (±)-carba-PGI_2 2.[3~5,8] No one, however, used this (±)-β-keto ester 4 in a synthesis of (+)-2 presumably because of the difficulty encountered in resolving (±)-4. It occurred to us that a kinetic resolution of (±)-4 in the course of its reduction with baker's yeast would provide a new chiral intermediate useful in the synthesis of (+)-2 (Fig. 1). Enantioselective reduction of β-keto esters is a well-known process,[12] and (±)-4 is readily available from *cis*-bicyclo[3.3.0]octane-3,7-dione 3[5] or from 5-norbornen-2-one.[8]

As shown in Fig. 2, our synthesis started from 3, which was prepared efficiently from dimethyl 3-oxoglutarate and glyoxal according to Bertz *et al.*[14] To prepare monoacetal 6, the diketone 3 was first converted quantitatively to bisacetal 5 in the usual manner. Subsequent partial hydrolysis of 5 with *p*-TsOH in aq acetone furnished 6[5] in 65 % yield. Recovered 3 and 5 were recycled to give an additional amount of 6 raising the total yield

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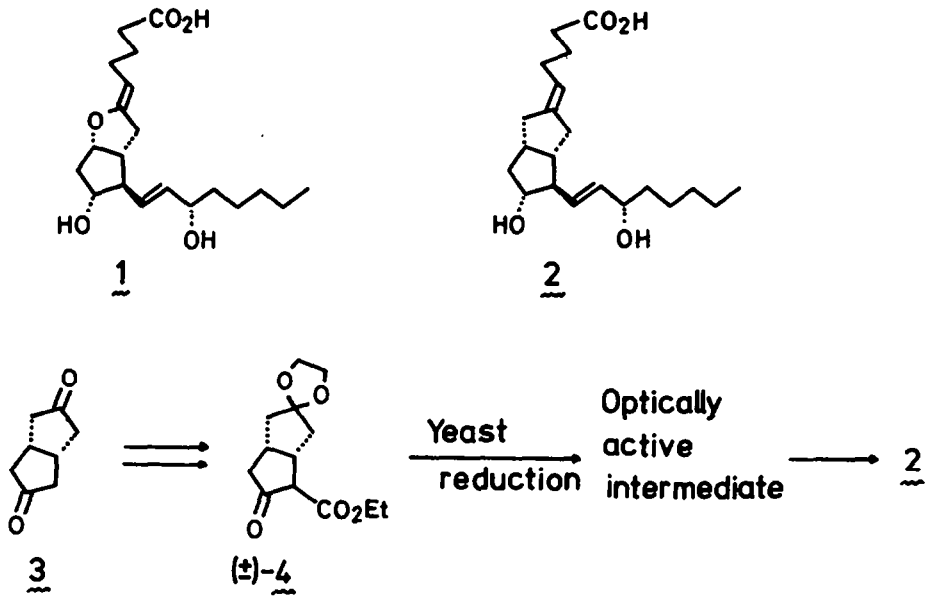

Fig. 1. Synthetic plan for (+)-carba-PGI_2

to ≥ 80% from **3**. Pure monoacetal **6** was stable at room temp in the presence of K_2CO_3, although it was reported to be unstable due to its tendency to disproportionate.[14] Ethoxycarbonylation of **6** with NaH and $CO(OEt)_2$ afforded the desired β-keto ester (±)-**4** in 91 % yield.

Addition of (±)-**4** to a briskly fermenting suspension of baker's yeast in tap water containing sucrose[15] was found to effect hydrolytic removal of the acetal group. To circumvent this deacetalization, (±)-**4** was stirred at 30° with fermenting baker's yeast in 0.1 M phosphate buffer (pH 7) containing sucrose for 24 h. The recovered organic material was chromatographed over SiO_2 to give recovered (+)-**4** (36 % yield), $[\alpha]_D^{22}$ +15.6° ($CHCl_3$), and reduced β-hydroxy ester (+)-**7** (26 % yield), $[\alpha]_D^{22}$ +3.9° ($CHCl_3$). Since both of the two products were optically active, the yeast reduction was enantioselective at least to some extent. The enantiomeric purity of (+)-**7** was estimated to be 98.0 % e.e. by the HPLC analysis of its (S)-α-methoxy-α-trifluoromethylphenylacetate (MTPA ester).[16] The reduced product (+)-**7** was identical with one of the diastereomers obtained by reduction of (±)-**4** with $NaBH_4$ on the basis of IR, NMR and TLC comparison.

The next task was the determination of the absolute stereochemistries of the two products (+)-**4** and (+)-**7**. First, the relative configuration of the OH and CO_2Et groups of (+)-**7** was assigned as *cis*, since in its ^1H NMR spectrum a 1H signal at δ 4.50 due to CHOH exhibited the $W_{1/2}$ of 10 Hz. Baumann *et al.* reported the $W_{1/2}$ of two vicinal protons of a cyclopentane derivatives to be 11 Hz in the case of *cis*-protons, while it was 17.5 Hz in the case of *trans*-protons.[17] As to the absolute steric course of the yeast reduction, ethyl 2-oxocyclopentanecarboxylate is known to give the corresponding (S)-β-hydroxy ester.[12] Therefore (+)-**7** must be either **8** or **9**. Of these two alternatives, **9** possesses the ester group in more crowded concave side, and therefore would epimerize to give a more stable isomer **10**. Actually when (+)-**7** was treated with a trace amount of NaOH in EtOH, it was converted to an isomer, (-)-**10**, $[\alpha]_D^{23}$ -25.6° ($CHCl_3$). This was identical with the major product of $NaBH_4$ reduction of (±)-**4** on the basis of IR, NMR and TLC comparison. Thus (+)-**7** should be represented by the stereoformula **9**.

Our deduction as above was based on the assumption that baker's yeast generally produces (S)-alcohols. To make our stereochemical assignment more solid, it seemed desirable to convert our product(s) into a compound of known absolute configuration. A

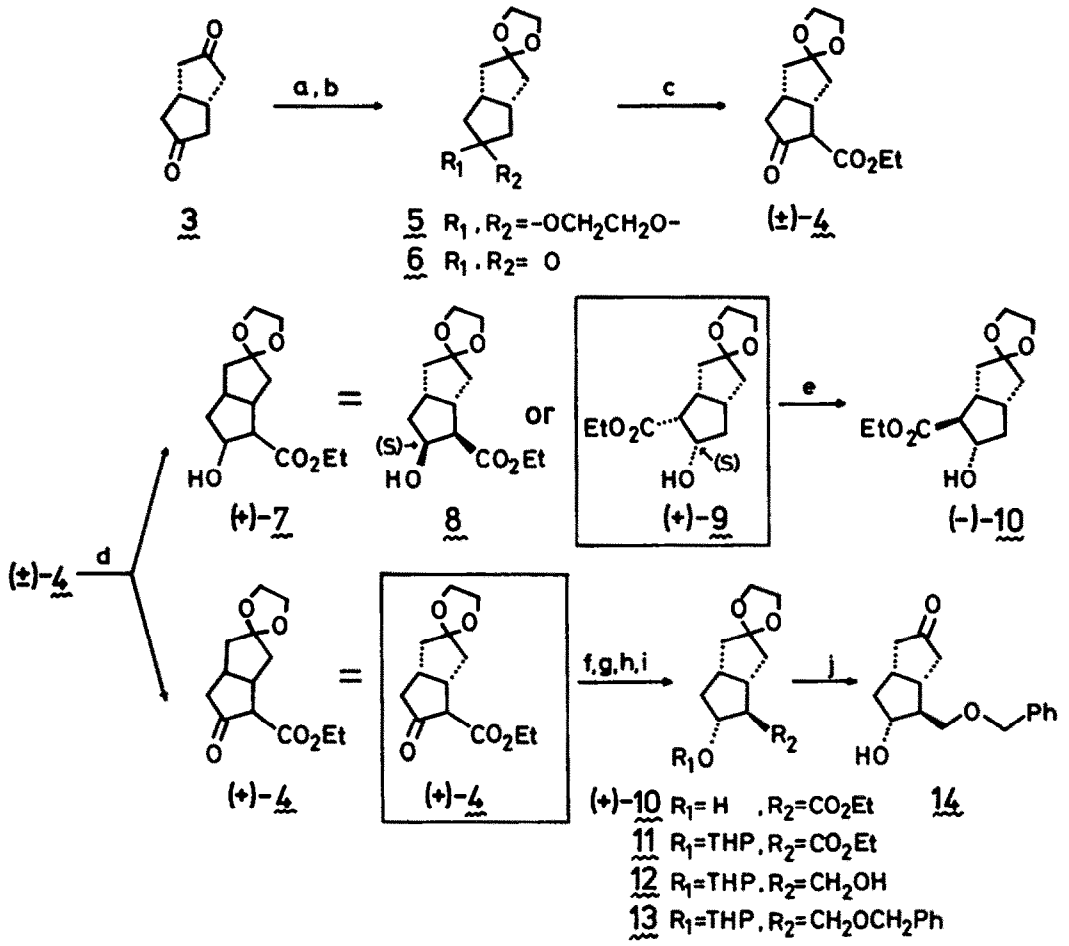

Fig. 2. Elucidation of the steric course of the yeast reduction of (±)-**4**.

a) $HOCH_2CH_2OH$, p-TsOH, C_6H_6; b) 0.05 eq p-TsOH, acetone-H_2O (3:1), r.t. 1.5 h; c) 3 eq NaH, 4 eq $CO(OEt)_2$, C_6H_6, Δ, 4 h; d) baker's yeast, sucrose, 0.1 M phosphate buffer (pH 7), 30°, 24 h; e) NaOH, EtOH, r.t.; f) $NaBH_4$, EtOH, -15°; g) DHP, PPTS, CH_2Cl_2; h) LAH, ether; i) NaH, $PhCH_2Cl$, THF; j) AcOH-H_2O-THF (3:1:1), 45°.

compound served for this purpose was **14**. This was an intermediate in a synthesis of (+)-**2** by Ono group, and its CD data were described in detail.[10] If our stereochemical deduction was correct, the recovered (+)-**4** should lead to **14**. Reduction of (+)-**4** with $NaBH_4$ in EtOH gave (+)-**10**, $[\alpha]_D^{23}$ +16.1° ($CHCl_3$), as the major product in 76 % yield. The enantiomeric purity of (+)-**10** was determined to be 61.8 % e.e. by the HPLC analysis of the corresponding (S)-MTPA ester.[16] Conventional protection of the OH group of (+)-**10** as a THP ether afforded **11**, which was reduced with LAH to give **12** in 98 % yield from (+)-**10**. Benzylation of **12** to **13** was followed by deprotection of the THP and acetal protective groups to give **14** in 66.4 % yield from **12**. Thus obtained **14**, $[\alpha]_D^{21}$ +8.9° ($CHCl_3$), showed two negative extrema at 290 ($[\theta]= -5.33\times10^2$) and 296 ($[\theta]= -5.40\times10^2$) nm in its CD spectrum measured as its MeOH soln. These CD data were in agreement with the data reported for authentic **14**.[10] This concluded the elucidation of the steric course of the yeast reduction of (±)-**4**. We were frustrated to know the low enantiomeric purity of (+)-**4** (61.8 % e.e.) which would lead to (+)-**2**.

Table 1. Screening of the various strains of yeast for the reductive kinetic resolution of (±)-4.[a]

	Yeast	Reaction time (h)	Recovery (+)-4 (%)	$[\alpha]_D$ ($CHCl_3$)	Optical purity[b] (%)
1.	baker's yeast	24	36	+15.6°	61.8
2.	Pichia terricola KI 0117	20	16	+14.9°	59
3.	Saccharomyces cerevisiae NCYC 240	50	40	+13.2°	52
4.	Saccharomyces carlsbergensis IFO 0565	68	44	+12.6°	50
5.	Saccharomyces uvarum IFO 1225	167	20	+18.3°	73
6.	Saccharomyces bailii KI 0116	5	33	+23.7°	94
7.	Saccharomyces bailii IFO 1611	46	28	+17.5°	70
8.	Saccharomyces bailii IFO 1801	48	31	+16.6°	66

a) A procedure for reduction with baker's yeast is described in Experimental. Reduction of (±)-4 (0.5 g) was carried out with wet yeast (13~27 g) in 0.1 M phosphate buffer (pH 7, 100 ml) containing glucose at 37° in the cases of 2 and 6 and at 30° in other cases.[15,18]

b) Since (+)-4 of 61.8 % e.e. showed an $[\alpha]_D$ value of +15.6°, the optical purity was calculated as follows: Optical purity (%) = ($[\alpha]_D$ observed/25.2°)x100.

To circumvent the difficulty in securing (+)-4 of high e.e., we first lengthened the period of yeast reduction so as to achieve more complete kinetic resolution. Our attempts were not so rewarding after all. Reduction of (±)-4 with baker's yeast for 3 to 6 days enhanced the optical purity of (+)-4 to some extent (75~79 % e.e.). The recovery yield of (+)-4, however, decreased (17~22 %) owing to hydrolytic deacetalization during the reduction. The second and successful attempt was to test various strains of yeast other than baker's yeast so that we could find a more useful strain to produce (+)-4 of high e.e. The result of our screening experiment is shown in Table 1. In every case so far examined, (+)-9 was obtained as the reduction product and (+)-4 was recovered. To our pleasure, a thermophilic yeast Saccharomyces bailii KI 0116[15,18] was found to give (+)-4 in as high optical purity as 94 % in a short reaction time of 5 h.

The reduction of (±)-4 was then executed in a preparative scale. Action of Saccharomyces bailii KI 0116 on (±)-4 (5 g) in 0.1 M phosphate buffer (pH 7) containing glucose was allowed to continue for 5 h at 37°. The recovered organic material was purified to give (+)-4 in 40 % yield together with 38 % yield of (+)-9. After reducing (+)-4 with $NaBH_4$, the resulting (+)-10 was shown to be of 93.6 % e.e. as determined by the HPLC analysis of its (R)-MTPA ester. The enantiomeric purity of (+)-9 was also determined by the HPLC analysis of its (R)-MTPA ester, and found to be 99.2 % e.e. The unwanted (+)-9 could be recycled: CrO_3 oxidation of (+)-9 was followed by saponification and decarboxylation to give back 6, the precursor of (±)-4. Saccharomyces bailii KI 0116 could be used repeatedly. The yeast was collected by centrifugation at the end of the fermentation. In one occasion, the yeast was used three times for reduction of (±)-4 in 10 g-scale each, and the resulting (+)-4 was of 92.0 % e.e. in average.

After finishing the screening of the yeast strain, we then turned our attention to the screening of substrates for this kinetic resolution. Modification of the ester and/or acetal moiety of (±)-**4** provided three new substrates, (±)-**15**, (±)-**16** and (±)-**17**. The result of our screening of the substrates is shown in Table 2. As can be seen from it, (±)-**4** was confirmed to be the best choice.

Table 2. Screening of the substrates for the reductive kinetic resolution of (±)-**4**[a)]

	Substrate X	R	Reaction time (h)	Recovery of the keto ester (%)	Enantiomeric purity of the recovered keto ester (%e.e.)[b)]
1. (±)-**4**	$-OCH_2CH_2O-$	Et	5	40	93.6
2. (±)-**15**	$-OCH_2CH_2O-$	Me	46	26	78.0
3. (±)-**16**	$-OCH_2\overset{Me\ Me}{\overset{\backslash\ /}{C}}CH_2O-$	Et	19	9	41.0
4. (±)-**17**	$-OCH_2\overset{Me\ Me}{\overset{\backslash\ /}{C}}CH_2O-$	Me	24	29	52.4

a) Reduction was carried out with Saccharomyces bailii KI 0116.

b) The recovered β-keto ester was reduced with $NaBH_4$ to the corresponding β-hydroxy ester, and its (R)-MTPA ester was analyzed by HPLC to estimate the enantiomeric purity.

With a sufficient amount of (+)-**4**, we started the synthesis of (+)-carba-PGI_2 **2** and its (5Z)-isomer **25** as shown in Fig. 3. Our synthesis followed the route previously developed by others.[3~5,10] The keto ester (+)-**4** (92~94 % e.e.) was converted to **12** in three steps[(i) $NaBH_4$ reduction, (ii) protection of the OH group, and (iii) LAH reduction] in 74 % yield. Oxidation of **12** with $CrO_3 \cdot C_5H_5N \cdot HCl$ (PCC) in the presence of NaOAc in CH_2Cl_2 gave aldehyde **18**. One of the side-chains was then attached to **18** by the Horner-Wadsworth-Emmons reaction[19,20] to give **19** in 66 % yield from **12**. Reduction of **19** with $NaBH_4$ was followed by deprotection under acid condition to give a mixture of epimers at C-15 (PG numbering). These two were separable by SiO_2 chromatography giving the desired and more polar isomer **20**[9] in 47 % yield from **19**. The less polar isomer **21**[9] was obtained in 34 % yield from **19**. The two OH groups of **20** were protected as THP ethers to give **22**[11] in 93 % yield. Another side-chain was attached to **22** by the Wittig reaction to give a mixture of geometrical isomers at C-5 (PG numbering). The isomers were separated by SiO_2 chromatography to give the desired more polar (5E)-isomer **23**[11] (48 % yield) and the less polar (5Z)-isomer **24**[11] (28 % yield). Deprotection of **23** under acid condition furnished (+)-carba-PGI_2 **2**, m.p. 58.5~60.0°, $[\alpha]_D^{26}$ +88.8° (MeOH), in 79 % yield. Similarly, deprotection of **24**, gave (5Z)-(+)-carba-PGI_2 **25**, m.p. 105~106°, $[\alpha]_D^{22}$ +40.4° (MeOH), in 53 % yield. The physical and spectral data of **2** and **25** were in accord with those reported earlier.[9~11]

In conclusion, we synthesized (+)-carba-PGI_2 **2** from the readily available bicyclic diketone **3** in 2.0 % overall yield in 14 steps. (5Z)-Carba-PGI_2 **25** was obtained in 0.8 % overall yield in 14 steps from **3**. Other recorded syntheses of (+)-**2** required over 20 steps to reach the goal.[9~11] The use of a biochemical reaction enabled us to complete an efficient synthesis of (+)-**2** by decreasing the number of the necessary synthetic steps.

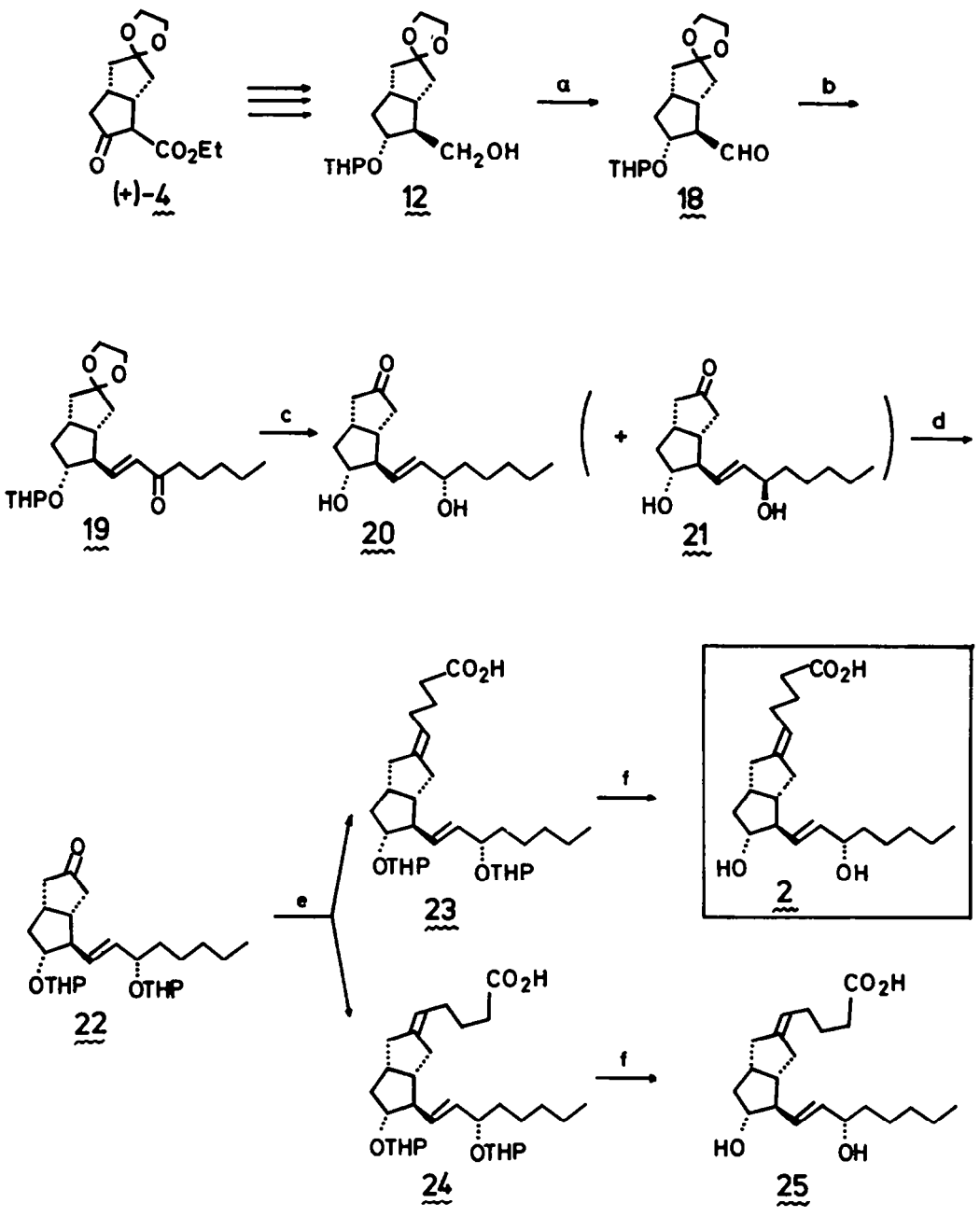

Fig. 3. Synthesis of carba-PGI_2 2 and its (5Z)-isomer 25.

a) PCC, NaOAc, CH_2Cl_2; b) $(MeO)_2P(O)CH_2COC_5H_{11}$, NaH, THF; b) (i) $NaBH_4$, MeOH, (ii) AcOH-H_2O-THF (3:1:1), 45°, (iii) SiO_2 chromatog.; d) DHP, PPTS, CH_2Cl_2; e) (i) $Ph_3P{=}CH(CH_2)_3CO_2Na$, DMSO, 45°, 22 h, (ii) SiO_2 chromatog.; f) AcOH-H_2O-THF (3:1:1), 45°.

EXPERIMENTAL

All m.ps were uncorrected. IR spectra were measured as films for oils or as KBr discs for solids on a Jasco IRA-102 spectrometer. NMR spectra were recorded with TMS as an internal standard and $CDCl_3$ as a solvent at 60 MHz on a Hitachi R-24A spectrometer or at 100 MHz on a JEOL JNM FX-100 spectrometer. Optical rotations were measured with $CHCl_3$ as a solvent on a Jasco DIP 140 polarimeter unless otherwise stated. CD spectra were measured on a Jasco J-500A spectrometer. Merck Kieselgel 60 (Art 7734, 70~230 mesh) or Fuji Davison BW-820 MH was used for SiO_2 column chromatography unless otherwise stated. TLC analyses were performed on a Merck Kieselgel 60 F-254 (0.25 mm, Art 5715).

Reduction of (±)-**4** with baker's yeast. (1S,5R)-2-Ethoxycarbonyl-7,7-ethylenedioxybicyclo[3.3.0]octan-3-one (+)-**4** and (1S,5R,6R,7S)-6-ethoxycarbonyl-3,3-ethylenedioxy-7-hydroxybicyclo[3.3.0]octane (+)- **9**. Dry baker's yeast (7.0 g, Oriental Yeast Co., Ltd.,) was dispersed to 0.1 M phosphate buffer (pH 7, 100 ml) containing sucrose (15 g) at 30°. The flask was shaken at 30° for 30 min, when brisk fermentation took place. An emulsion of (±)-**4** (505 mg, 2.0 mmol) in 0.2 % Triton X-100 soln (15 ml) was added to the fermentation mixture and the shaking culture was continued at 30°. Sucrose (10 g) was added to the mixture after 8 h and the fermentation was continued for further 16 h. The total fermentation period was 24 h. The fermentation broth was mixed with a small amount of ether and Celite, and filtered through Celite. The filtrate was saturated with NaCl and extracted with EtOAc (100 ml x 3). The combined EtOAc soln was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over SiO_2 (8 g). The fraction earlier eluted with n-hexane-ether (4:1) gave 183 mg (36 %) of (+)-**4**, $[\alpha]_D^{22}$ +15.6° (c=1.48); νmax 1765 (s), 1735 (s), 1665 (s), 1625 (m), 1335 (m), 1285 (s), 1240 (s), 1180 (s), 1115 (s), 1050 (s), 1025 (s), 955 (m) cm^{-1}; δ 1.26 (3H, t, J=7 Hz), 1.5~3.6 (9H, m), 3.87 (4H, s), 4.16 (2H, q, J=7 Hz); TLC (n-hexane-ether=1:4): Rf 0.66. The fraction later eluted with n-hexane-ether (4:1) gave 134 mg (26 %) of (+)-**9**, $[\alpha]_D^{22}$ +3.7° (c=1.48); νmax 3550 (w), 1720 (s), 1335 (s), 1250 (m), 1190 (s), 1120 (s), 1040 (m), 1030 (s), 985 (m) cm^{-1}; δ 1.27 (3H, t, J=7 Hz), 1.70~3.10 (9H, m), 3.69 (1H, br.s), 3.90 (4H, s), 4.20 (2H, q, J=7 Hz), 4.50 (1H, m, $W_{1/2}$=10 Hz); TLC (n-hexane-ether=1:4): Rf 0.45. A small amount of (+)-**9** was converted to the corresponding (S)-MTPA ester in the conventional manner, which was analyzed by HPLC. HPLC (Column, NUCLEOSIL®50-5, 25 cm x 4.6 mm; Solvent, n-hexane-THF (8:1), 1.25 ml/min; Detected at 254 nm) Rt 27.8 min (99.0 %), 30.2 min (1.0 %). Therefore the optical purity of **9** was 98.0 % e.e.

(1S,5R,6S,7S)-6-Ethoxycarbonyl-3,3-ethylenedioxy-7-hydroxybicyclo[3.3.0]octane (-)-**10**. To a soln of (+)-**9** (265 mg, 1.0 mmol; prepared from (±)-**4** with baker's yeast) in dry EtOH (3 ml) was added NaOH (8 mg). The mixture was stirred for 2 h at room temp. Then the mixture was diluted with brine, neutralized with N-HCl, and extracted with EtOAc. The EtOAc soln was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over SiO_2 (6 g). Elution with n-hexane-ether (4:1~1:1) gave 183 mg (72 %) of (-)-**10**, $[\alpha]_D^{23}$ -25.6° (c=1.89); νmax 3500 (m), 1730 (s), 1330 (m), 1260 (m), 1180 (s), 1135 (m), 1105 (s), 1030 (s), 950 (m) cm^{-1}; δ 1.24 (3H, t, J=7 Hz), 1.4~2.8 (9H, m), 2.98 (1H, br.s), 3.84 (4H, s), 4.23 (2H, q, J=7 Hz), ~4.55 (1H, m); TLC (n-hexane-ether=1:4): Rf 0.33.

(1R,5S,6R,7R)-6-Ethoxycarbonyl-3,3-ethylenedioxy-7-hydroxybicyclo[3.3.0]octane (+)-**10**. A soln of (+)-**4** (0.79 g, 3.1 mmol; prepared from (±)-**4** with baker's yeast) in EtOH (10 ml) was stirred at -15°. To this was added $NaBH_4$ (80 mg, 2.1 mmol) portionwise over 30 min at that temp. The mixture was stirred for 1 h at that temp. Then EtOH was removed in vacuo from the reaction mixture, and the residue was diluted with water. The mixture was extracted twice with EtOAc. The EtOAc soln was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over SiO_2 (20 g). Elution with n-hexane-ether (4:1~1:1) gave 0.61 g (76 %) of (+)-**10**, $[\alpha]_D^{22}$ +16.1° (c=2.90); TLC (n-hexane-ether=1:4): Rf 0.33. Its IR and NMR spectra were identical with those of (-)-**10**. A small amount of (+)-**10** was converted to the corresponding (S)-MTPA ester in the conventional manner, which was analyzed by HPLC. HPLC (Column, NUCLEOSIL®50-5, 25 cm x 4.6 mm; Solvent, n-hexane-THF (10:1), 1.05 ml/min; Detected at 254 nm) Rt 17.2 min (19.1 %), 19.0 min (80.9 %). Therefore the optical purity of (+)-**10** was 61.8 % e.e.

(1R,5S,6R,7R)-6-Ethoxycarbonyl-3,3-ethylenedioxy-7-tetrahydropyranyloxybicyclo[3.3.0]octane **11**. A soln of (+)-**10** (530 mg, 2.1 mmol), dihydropyran (268 mg, 3.2 mmol), PPTS (52 mg, 0.2 mmol) in dry CH_2Cl_2 (6 ml) was stirred for 3 h at room temp. Then the mixture was washed with sat $NaHCO_3$ soln. The aq layer was extracted with CH_2Cl_2. The combined CH_2Cl_2 soln was washed with brine, dried (Na_2SO_4), and concentrated in vacuo to give 0.74 g of crude **11**, νmax 1735 (s), 1265 (m), 1205 (m), 1130 (s), 1040 (s), 980 (m), 875 (m) cm^{-1}. This was employed in the next step without further purification.

(1R,5S,6S,7R)-3,3-Ethylenedioxy-6-hydroxymethyl-7-tetrahydropyranyloxybicyclo[3.3.0]octane **12**. To a stirred and ice-cooled suspension of LAH (118 mg, 3.1 mmol) in dry ether (10 ml) was added dropwise over 1 h a soln of crude **11** (0.74 g) in dry ether (3 ml). The mixture was stirred for 30 min at room temp. Then excess LAH was destroyed by the successive addition of water (0.1 ml), 10 % NaOH aq soln (0.3 ml) and water (0.1 ml) to the stirred and ice-cooled mixture. The stirring was continued for 1 h. The mixture was filtered through Celite and the filtrate was dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over SiO_2 (10 g). Elution with n-hexane-EtOAc (3:1) gave 607 mg (98 % from (+)-**10**) of **12**, νmax 3470 (m), 1325 (m), 1260 (m), 1200 (m), 1120 (s), 1095 (s), 1085 (s), 1025 (s) cm^{-1}; δ 1.1~2.7 (10H, m), 3.2~4.2 (5H, m), 3.90 (4H, s), 4.65 (1H, m).

(1R,5S,6S,7R)-6-Benzyloxymethyl-3,3-ethylenedioxy-7-tetrahydropyranyloxybicyclo[3.3.0]octane **13**. To a suspension of NaH (100 mg, 60 % dispersed in a mineral oil, 2.5 mmol) in dry THF (2 ml) was added dropwise a soln of **12** (479 mg, 1.6 mmol) in dry THF (2 ml) at room temp, and the mixture was stirred and heated under reflux for 1 h under Ar. Then a soln of benzyl chloride (275 mg, 2.2 mmol) in dry THF (1 ml) was added dropwise to the stirred and heated mixture at reflux temp. The mixture was stirred and heated under reflux for 8 h after the addition of benzyl chloride. After cooling, the mixture was poured into water and extracted with EtOAc. The EtOAc soln was washed with water and brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed over SiO_2 (10 g). Elution with n-hexane-ether (4:1) gave 499 mg (80 %) of **13**, νmax 1350 (m), 1320 (m), 1255 (m), 1200 (m), 1120 (s), 1075 (m), 1020 (s), 980 (m), 735 (m), 695 (m) cm^{-1}; δ 1.0~2.7

(15H, m), 3.2~4.2 (5H, m), 3.90 (4H, s), 4.53 (2H, s), 4.70 (1H, m), 7.38 (5H, s).

(1R,5S,6S,7R)-6-Benzyloxymethyl-7-hydroxybicyclo[3.3.0]octan-3-one **14**. **13** (499 mg, 1.3 mmol) was dissolved in AcOH-water-THF (3:1:1, 6 ml), and the soln was stirred for 5 h at 45°. After cooling, the mixture was diluted with water and extracted twice with EtOAc. The EtOAc soln was washed with water, sat $NaHCO_3$ soln and brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed over SiO_2 (10 g). Elution with n-hexane-ether (2:1) gave 277 mg (83 %) of **14**, $[\alpha]_D^{21}$ +8.9° (c=1.68); CD (c=3.04x10^{-3}, MeOH) [θ] (nm) 0 (245), -5.33x10^2 (290), -5.40x10^2 (296), -3.69x10^2 (sh, 307), -1.12x10^2 (sh, 318), 0 (330) [lit.[10] CD (c=7.07x10^{-3}, MeOH) [θ] (nm) 0 (245), -6.40x10^2 (289, 297), -4.34x10^2 (sh, 307), -0.88x10^2 (sh, 319), 0 (330)]. Its IR and NMR spectra were identical with those of **14** reported previously.[10]

Reduction of (±)-**4** in a preparative scale employing Saccharomyces bailii KI 0116. (1S,5R)-2-Ethoxycarbonyl-7,7-ethylenedioxybicyclo[3.3.0]octan-3-one (+)-**4** and (1S,5R,6R,7S)-6-ethoxycarbonyl-3,3-ethylenedioxy-7-hydroxybicyclo[3.3.0]octane (+)-**9**. The yeast was pre-cultivated in four Sakaguchi flasks containing 100 ml each of the culture medium containing malt extract (2 g), peptone (0.1 g) and glucose (2 g) for 2 days at 37°. This pre-cultivated suspension of the yeast (200 ml) was added to 1.8 l of the same medium in a 5 l-flask. Two 5 l-flasks were shaken at 37° on a gyrorotary shaker for 2 days. Then the yeast-cells were collected by centrifugation (3000 rpm, 10 min). The cells were added to two 5 l-flasks containing 2 l each of the culture media containing malt extract (20 g), peptone (2 g), glucose (20 g) and NH_4NO_3 (10 g). The flasks were shaken at 37° on a gyrorotary shaker. After 1 day, malt extract (20 g) was added to each of the flasks. The flasks were shaken at 37° for another day. Then the yeast-cells were collected by centrifugation, added to a new medium and shaken for another day. This procedure was repeated six times to give ca 108 g of the wet cells of Saccharomyces bailii KI 0116. This was dispersed to 0.1 M phosphate buffer (pH 7, 1 l) containing glucose (200 g) at 37°. After the flask was shaken at 37° for 40 min, an emulsion of (±)-**4** (5.00 g, 20 mmol) in 0.2 % Triton X-100 soln (150 ml) was added to the fermentation mixture and the mixture was shaken at 37° for 5 h. Then the yeast-cells were removed by centrifugation. The supernatant was saturated with NaCl and extracted with EtOAc (500 ml x3). The combined EtOAc soln was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over SiO_2 (75 g). The fraction earlier eluted with n-hexane-ether (4:1) gave 2.01 g (40 %) of (+)-**4**, n_D^{21} 1.4927; $[\alpha]_D^{21}$ +23.9° (c=2.55). Its IR and NMR spectra were identical with those of earlier described (+)-**4**. (Found: C, 61.23; H, 7.10. Calc for $C_{13}H_{18}O_5$: C, 61.40; H, 7.14 %). The fraction later eluted with n-hexane-ether (4:1) gave 1.90 g (38 %) of (+)-**9**, n_D^{21} 1.4850; $[\alpha]_D^{21}$ +4.1° (c=1.87). Its IR and NMR spectra were identical with those of earlier described (+)-**9**. (Found: C, 60.57; H, 7.90. Calc for $C_{13}H_{20}O_5$: C, 60.92; H, 7.87 %). A small amount of this (+)-**9** was converted to the corresponding (R)-MTPA ester in the conventional manner, which was analyzed by HPLC under the same condition as described for (+)-**9**-(S)-MTPA ester: Rt 27.4 min (0.4 %), 29.1 min (99.6 %). Therefore the optical purity of this (+)-**9** was 99.2 % e.e.

(1R,5S,6R,7R)-6-Ethoxycarbonyl-3,3-ethylenedioxy-7-hydroxybicyclo[3.3.0]octane (+)-**10**. In the same manner as described for (+)-**4** (baker's yeast origin), (+)-**4** (S. bailii KI 0116 origin) was converted to (+)-**10**, n_D^{24} 1.4833; $[\alpha]_D^{21}$ +26.1° (c=1.84). Its IR and NMR spectra were identical with those of (-)-**10**. (Found: C, 60.51; H, 7.86. Calc for $C_{13}H_{20}O_5$: C, 60.92; H, 7.87 %). A small amount of (+)-**10** was converted to the corresponding (R)-MTPA ester in the conventional manner, which was analyzed by HPLC under the same condition as described for (+)-**10**(baker's yeast origin)-(S)-MTPA ester: Rt 21.9 min (96.8 %), 25.1 min (3.2 %). Therefore the optical purity of (+)-**10** was 93.6 % e.e.

Reduction of (±)-**4** in a large scale employing Saccharomyces bailii KI 0116 The yeast was cultivated using four 5 l-flasks in the same manner as described above. Ca 160 g of the wet cells were used for the first reduction [(±)-**4** (8.00 g), glucose (240 g), 0.1 M phosphate buffer (pH 7, 1.6 l), at 37°, 6 h]. After the reaction, the yeast-cells were collected by centrifugation. The recovered wet cells (ca 109 g) were added to four 5 l-flasks containing 2 l each of the culture medium containing malt extract (20 g), peptone (2 g), glucose (20 g) and NH_4NO_3 (10 g). The flasks were shaken at 37° on a gyrorotary shaker. The re-cultivation procedure (collection of yeast-cells by centrifugation, addition to a new medium, and shaking at 37° on a gyrorotary shaker) was repeated five times to give ca 207 g of wet cells. This was used for the second reduction [(±)-**4** (10.00 g), glucose (300 g), 0.1 M phosphate buffer (pH 7, 2 l), at 37°, 5 h]. After the reaction the yeast-cells were collected by centrifugation. The recovered wet cells (ca 189 g) were added a new medium. The re-cultivation procedure as described above was repeated twice to give ca 253 g of wet cells. This was used for the third reduction [(±)-**4** (10.43 g), glucose (320 g), 0.1 M phosphate buffer (pH 7, 2 l), at 37°, 6 h]. The each supernatant after removing the yeast-cells was worked up in the same manner as described for the preparative-scale reduction. The combined residue was chromatographed over SiO_2 to give 11.81 g (42 %) of (+)-**4** [$[\alpha]_D^{21}$ +23.5° (c=2.35)] and 11.87 g (41 %) of (+)-**9** [$[\alpha]_D^{21}$ +4.0° (c=1.78)]. In the same manner as described previously, (+)-**4** was converted to (+)-**10** [$[\alpha]_D^{22}$ +24.8° (c=1.89)]. A small amount of (+)-**10** was converted to the corresponding (R)-MTPA ester in the conventional manner, which was analyzed by HPLC under the same condition as described previously: Rt 20.5 min (96.0 %), 23.8 min (4.0 %). Therefore the optical purity of this (+)-**10** was 92.0 % e.e.

(1R,5S,6S,7R)-3,3-Ethylenedioxy-6-hydroxymethyl-7-tetrahydropyranyloxybicyclo[3.3.0]octane **12**. In the same manner as described previously, (+)-**10** was converted to **12**, n_D^{22} 1.4945; $[\alpha]_D^{22}$ -15.9° (c=1.62). Its IR and NMR spectra were identical with those described previously in this paper. (Found: C, 64.69; H, 8.78. Calc for $C_{16}H_{26}O_5$: C, 64.40; H, 8.78 %).

(1R,5S,6R,7R)-3,3-Ethylenedioxy-6-formyl-7-tetrahydropyranyloxybicyclo[3.3.0]octane **18**. To a mixture of **12** (3.04 g, 10.2 mmol) and NaOAc (0.34 g, 4.0 mmol) in dry CH_2Cl_2 (45 ml) was added PCC (4.40 g, 20.4 mmol) at room temp and the mixture was stirred for 3 h at room temp. Then the CH_2Cl_2 layer was decanted, and the residue was washed with ether. The combined organic layer was passed through a short column of Florisil, and concentrated in vacuo to give 2.67 g of crude **18**, vmax 2730 (w), 1730 (s), 1330 (m), 1260 (m), 1205 (m), 1130 (s), 1080 (s), 1030 (s), 980 (m) cm^{-1}. This was employed in the next step without further purification.

(1R,5S,6R,7R,1'E)-3,3-Ethylenedioxy-6-(3'-oxo-1'-octenyl)-7-tetrahydropyranyloxybicyclo[3.3.0]octane **19.** To a stirred suspension of NaH (380 mg, 60 % dispersed in a mineral oil, 9.5 mmol) in dry THF (50 ml) was added dropwise over 10 min a soln of dimethyl 2-oxoheptylphosphonate (2.20 g, 9.9 mmol) in dry THF (6 ml) at room temp under Ar. The mixture was stirred for 30 min at room temp, then to the mixture a soln of crude **18** (2.67 g) in dry THF (6 ml) was added dropwise. The mixture was stirred for 30 min at room temp. The mixture was passed through a pad of SiO_2, and the filtrate was concentrated in vacuo. The residue was chromatographed over SiO_2 (70 g). Elution with n-hexane-EtOAc (9:1) gave 2.62 g (66 % from **12**) of **19**, n_D^{24} 1.4833; $[\alpha]_D^{22}$ +12.4° (c=1.51); νmax 1695 (m), 1670 (m), 1630 (m), 1260 (m), 1200 (m), 1115 (s), 1080 (m), 1025 (s), 985 (m) cm^{-1}; δ 0.88 (3H, deformed t, J=5 Hz), 1.0~2.9 (23H, m), 3.4~4.2 (3H, m), 3.89 (4H, s), 4.62 (1H, m), 6.17 (1H, dd, J=15 and 3 Hz), 6.5~7.2 (1H, m). (Found: C, 69.91; H, 9.10. Calc for $C_{23}H_{26}O_5$: C, 70.37; H, 9.24 %).

(1R,5S,6R,7R,1'E,3'S)-7-Hydroxy-6-(3'-hydroxy-1'-octenyl)bicyclo[3.3.0]octan-3-one **20** and its (1'E,3'R)-isomer **21.** A soln of **19** (3.34 g, 8.5 mmol) in MeOH (50 ml) was stirred and cooled at -15°. $NaBH_4$ (0.16 g, 4.2 mmol) was added portionwise to the stirred mixture at -15°. The mixture was stirred for 30 min at that temp. Then the mixture was concentrated in vacuo to remove MeOH. The residue was diluted with brine, and the mixture was extracted twice with EtOAc. The EtOAc soln was concentrated in vacuo. The residue was dissolved in AcOH-water-THF (3:1:1, 50 ml), and the soln was stirred for 3 h at 45°. After cooling, the mixture was diluted with brine and extracted with EtOAc. The EtOAc soln was washed with water, sat $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 (Merck Art. 9385, 230-400 mesh, 180 g). The fraction first eluted with CH_2Cl_2-acetone (4:1) gave 0.66 g of **21**, $[\alpha]_D^{24}$ -23.9° (c=1.58). TLC (CH_2Cl_2-acetone=7:3): Rf 0.44. Its IR and NMR spectra were identical with those of **21** reported previously.[9] Further elution with CH_2Cl_2-acetone (4:1) gave a mixture of **20** and **21** (0.50 g). The finally eluted fraction with CH_2Cl_2-acetone (4:1) gave 0.71 g of **20**, $[\alpha]_D^{23}$ -8.2° (c=1.53); TLC (CH_2Cl_2-acetone=7:3): Rf 0.37. Its IR and NMR spectra were identical with those of **20** reported previously.[9] Further purification of the mixture of **20** and **21** by SiO_2 column chromatography gave 0.35 g of **20** and 0.11 g of **21**. The combined yield of **20** was 47 % and that of **21** was 34 %, respectively.

(1R,5S,6R,7R,1'E,3'S)-7,3'-Bis(tetrahydropyranyloxy)-6-(1'-octenyl)bicyclo[3.3.0]octan-3-one **22.** A soln of **20** (698 mg, 2.62 mmol), dihydropyran (611 mg, 7.87 mmol), PPTS (132 mg, 0.53 mmol) in dry CH_2Cl_2 (15 ml) was stirred for 3 h at room temp. Then the mixture was washed with sat $NaHCO_3$ soln and brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed over SiO_2 (18 g). Elution with n-hexane-EtOAc (9:1~4:1) gave 1059 mg (93 %) of **22**, $[\alpha]_D^{24}$ -29.7° (c=1.68). Its IR and NMR spectra were identical with those of **22** reported previously.[11]

(5E)-Carba-PGI$_2$ bis(tetrahydropyranyl)ether **23** and (5Z)-carba-PGI$_2$ bis(tetrahydropyranyl)ether **24.** A soln of $NaCH_2SOCH_3$ (28.5 mmol) was prepared from NaH (1.14 g, 60 % dispersed in a mineral oil, 28.5 mmol) and dry DMSO (14.8 ml). To a stirred soln of (4-carboxybutyl)triphenylphosphonium bromide (6.30 g, 14.2 mmol) in dry DMSO (15 ml) was added the soln of $NaCH_2SOCH_3$ described above at such a rate to maitain the soln at 25° under Ar. The mixture was stirred for 30 min at room temp to yield the red soln of the ylide. To this ylide soln was added a soln of **22** (617 mg, 1.42 mmol) in dry DMSO (1.5 ml), and the mixture was stirred for 22 h at 45° under Ar. After cooling, the mixture was quenched by the addition of water, neutralized with AcOH and extracted three times with ether. The extract was washed with water (x5) and brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed over SiO_2 (30 g). Elution with n-hexane-EtOAc (3:1) gave 730 mg of a mixture of **23** and **24**. This mixture was further chromatographed over SiO_2 (Merck Art. 9385, 230-400 mesh, 45 g). The fraction first eluted with n-hexane-EtOAc (17:3) gave 155 mg of **24**, νmax 1740 (m), 1715 (s), 1265 (m), 1205 (m), 1130 (m), 1080 (m), 1040 (s), 1025 (m), 980 (s) cm^{-1}; δ 0.7~2.8 (38H, m), 3.2~4.2 (6H, m), 4.70 (2H, m), 5.0~5.7 (3H, m), 8.75 (1H, br.s); TLC (n-hexane-EtOAc=2:1): Rf 0.40. Further elution with n-hexane-EtOAc (17:3) gave a mixture of **23** and **24** (214 mg). The finally eluted fraction with n-hexane-EtOAc (17:3) gave 280 mg of **23**, νmax 1745 (m), 1715 (s), 1265 (m), 1205 (m), 1130 (s), 1080 (s), 1040 (s), 1025 (s), 980 (s) cm^{-1}; δ 0.6~2.7 (38H, m), 3.2~4.2 (6H, m), 4.70 (2H, m), 5.0~5.7 (3H, m), 8.75 (1H, br.s); TLC (n-hexane-EtOAc=2:1): Rf 0.33. Further purification of the mixture of **23** and **24** by SiO_2 column chromatography gave 73 mg of **23** and 51 mg of **24**. The combined yield of **23** was 48 % and that of **24** was 28 %, respectively.

(5E)-Carba-PGI$_2$ **2.** **23** (409 mg, 0.79 mmol) was dissolved in AcOH-water-THF (3:1:1, 16 ml), and the soln was stirred for 3 h at 45°. After cooling, the mixture was diluted with brine and extracted with EtOAc. The EtOAc soln was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was diluted with benzene, and the soln was concentrated in vacuo to remove any remaining acetic acid. The residue was chromatographed over SiO_2 (15 g). Elution with n-hexane-EtOAc (1:1) gave 219 mg (79 %) of **2** as an oil, which solidified on standing. Recrystallization from ether-n-hexane gave pure **2** as a white powder, m.p. 58.5~60.0°; $[\alpha]_D^{26}$ +88.8° (c=0.525, MeOH) [lit.[9] m.p. 62.4~63.3°; $[\alpha]_D$ +90° (c=0.810, MeOH); lit[10] m.p. 64.5~66.5°; $[\alpha]_D^{20}$ +92.2° (c=0.515, MeOH); lit[11] m.p. 61~62.5°; $[\alpha]_D$ +91° (c=0.964, MeOH)]; νmax 3500 (m), 3370 (m), 3150 (m), 2750 (w), 2640 (w), 2550 (w), 1725 (s), 1675 (s), 1455 (m), 1430 (m), 1380 (w), 1345 (m), 1300 (m), 1265 (m), 1250 (m), 1170 (m), 1130 (m), 1080 (m), 995 (m), 975 (s), 905 (w), 875 (w) cm^{-1}; δ (100 MHz) 0.90 (3H, t, J=5 Hz), 1.0~2.7 (23H, m), 3.70 (1H, m), 4.06 (1H, m), 4.43 (3H, br.s), 5.22 (1H, m), 5.50 (2H, m). Its IR and NMR spectra were identical with those of **2** reported previously.[9~11] TLC (n-hexane-EtOAc-AcOH=25:25:1, double development): Rf 0.45. (Found: C, 72.05; H, 9.66. Calc for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78 %).

(5Z)-Carba-PGI$_2$ **25.** In the same manner as described for **23**, **24** (117 mg, 0.23 mmol) was converted to 42 mg (53 %) of **25**. Recrystallization from acetone-n-hexane gave pure **25** as a white microcrystalline material, m.p. 105~106°; $[\alpha]_D^{22}$ +40.4° (c=0.525, MeOH) [lit.[9] m.p. 107.5~108.8°; $[\alpha]_D$ +39° (c=0.866, MeOH); lit[10] m.p. 113~114°; $[\alpha]_D^{20}$ +40.1° (c=0.535, MeOH)]; νmax 3500 (m), 3400 (m), 2640 (w), 1720 (sh), 1695 (s), 1455 (m), 1395 (m), 1350 (m), 1320 (m), 1290 (m), 1240 (w), 1200 (m), 1175 (m), 1130 (w), 1090 (m), 1070 (m), 1015 (m), 995 (m), 975 (s), 860 (m) cm^{-1}; δ (100 MHz) 0.90 (3H, t, J=7 Hz),

1.0~2.7 (23H, m), 3.63 (1H, m), 4.02 (1H, m), 4.46 (3H, br.s), 5.21 (1H, m), 5.50 (2H, m). Its IR and NMR spectra were identical with those of **25** reported previously.[9,10] TLC (n-hexane-EtOAc-AcOH=25:25:1, double development): Rf 0.51. (Found: C, 71.97; H, 9.86. Calc for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78 %).

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