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Practical, Asymmetric Synthesis of the Cyclohexyl C_{28}-C_{34} Fragment of the Immunosuppressant FK-506 *via* (*S*)-(-)-3-Cyclohexenecarboxylic Acid

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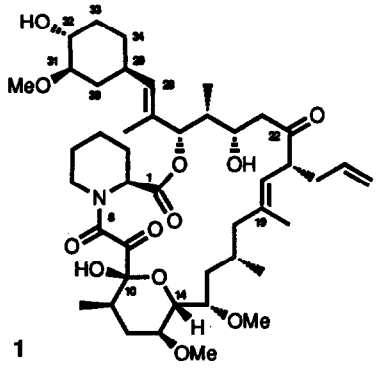
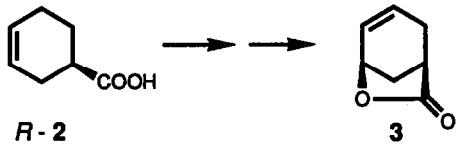
Abstract: The asymmetric synthesis of the cyclohexyl fragment of FK-506 is reported. This new synthesis (five steps, 30% overall yield) starts, for the first time, from (S)-(-)-3-cyclohexenecarboxylic acid (S-2) instead of the commonly used R form (acid R-2) and utilizes an epimerization reaction. The overall yield is improved to 35% by recycling recovered starting product (hydroxy ester 7c) from the epimerization step.

The unique immunosuppressive properties of the 21-membered macrolactam FK-506 (**1**),[1] isolated from *Streptomyces tsukubaensis* (no.9993),[2] prompted a great deal of work in the total synthesis of this compound[3] and in the synthesis of its fragments.[4] The C_{28}-C_{34} fragment has been envisioned as an important building block in the total synthesis of **1**[3] and several non-racemic, multistep syntheses of this segment have been reported.[5] Acyclic chiral compounds,[5a,f,j] racemic cyclic derivatives,[5g,h] or D-(-) quinic acid[5i] were variously used as starting materials. Several of these syntheses utilize (*R*)-(+)-3-cyclohexenecarboxylic acid (*R*-**2**) as the chiral building block; for example, after iodolactonization and base-induced dehydrohalogenation, *R*-**2** is transformed into lactone **3**.[5b-e]



1

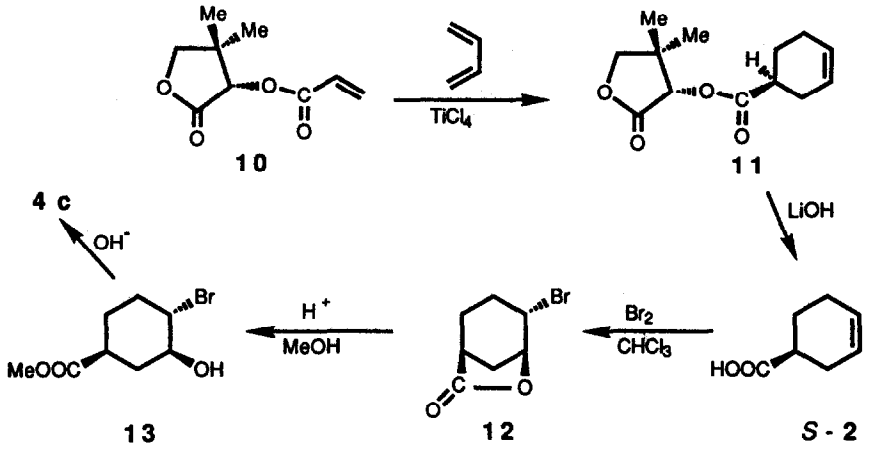


R-**2** **3**

Possible alternative approaches to the synthesis of C_{28}-C_{34} fragment of **1** could utilize *cis* epoxides of type **4a-c** and *trans* epoxides of type **5a-c**, easily obtained from acid **2**[6] (Schemes 1 and 2). Unfortunately, the

direct methanolysis of *trans* epoxides **5a-c**, prepared from *R*-**2** afforded only minor amounts (<5%) of the desired regioisomers of type **6a-c** exhibiting the correct regio- and stereochemistry for the natural C_{28}-C_{34} fragment of **1**. On the other hand, the methanolysis of *cis* epoxides **4a-c** [prepared from (*S*)-(-)-3-cyclohexenecarboxylic acid (*S*-**2**)] afforded high yields of the regioisomers **7a-c** structurally corresponding to **6a-c**, but epimeric at the carbon α to the **R** group. Our new synthesis of the C_{28}-C_{34} fragment of **1** is based on the preparation of compounds of type **7**, followed by an epimerization reaction at the carbon α to the **R** group (see Scheme 2). In our synthetic scheme the acid *S*-**2** is utilized for the first time unlike the other syntheses[5b-e] of the C_{28}-C_{34} fragment of **1**, which use the *R* form (acid *R*-**2**).

SCHEME 1

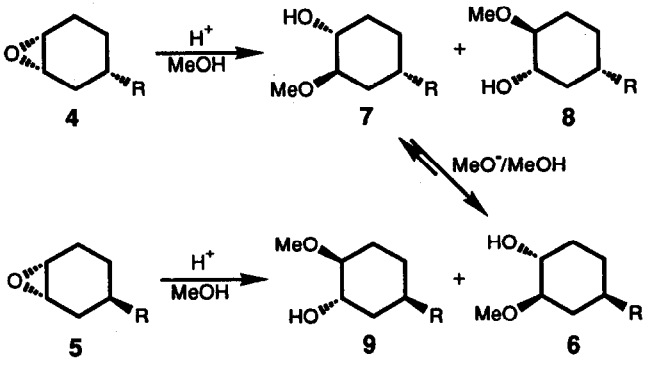


The acid *S*-**2** necessary to our synthesis was achieved (see Scheme 1) starting from the $TiCl_4$-catalyzed asymmetric Diels-Alder reaction of 1,3-butadiene with the acrylic ester of the commercially available (*R*)-(-) pantolactone (**10**)[7] (Fluka) to give ester **11** (82% de) which after four crystallizations from hexane was shown to be diastereoisomerically pure.[7] After removal (LiOH, THF/H_2O)[7] of the chiral auxiliary, **11** afforded crude *S*-**2**. The acid *S*-**2** was converted into the *cis* epoxide **4c**, in a modification of the same reaction sequence realized for the preparation of the corresponding racemic compounds.[8] Accordingly, treatment of a dilute solution of *S*-**2** in $CHCl_3$ with 1M Br_2 in $CHCl_3$ in the presence of NEt_3 at 0°C yielded the bromolactone **12** (80% yield). The reaction of **12** with MeOH in the presence of 0.5% (v/v) H_2SO_4[8] at refluxing temperature gave the bromohydrin **13** (95% yield), which, by base treatment, was transformed into the *cis* epoxide **4c** (85% yield) (Scheme 1). The optical homogenity (100%) of the bromolactone **12** was checked by 200 MHz ^1H NMR analysis in the presence of Pirkle's chiral solvating agent,[9] as previously reported for the corresponding iodolactone.[10]

The acid methanolysis of the epoxy ester **4c** afforded almost quantitatively an 8 : 2 mixture (GC) of the two regioisomeric products **7c** and **8c**[6] from which **7c** was obtained pure by flash chromatography (70% yield calculated on the epoxide) (Scheme 2).

The transformation of **7c** to **6c** could be achieved by epimerizing the carbon α to the carboxylic ester group, keeping in mind that in the most stable conformation of **7c**[6] the hydroxy and the methoxy groups are equatorial and the methoxycarbonyl group is axial, whereas in the most stable conformation of **6c** all the groups are equatorial. Therefore, due to the conformational energy[11] of the methoxycarbonyl group, the **7c** ⇌ **6 c** equilibrium should be largely in favor of **6c**.

SCHEME 2



a : R = CH_2OBn; **b** : R = CH_2OH; **c** : R = COOMe

The **7c** ⇌ **6c** base-catalyzed equilibration was carried out by refluxing (140 h) **7c** in a 0.5 N MeONa solution in anhydrous MeOH affording an 81 : 19 mixture of **6c** and **7c** which was subjected to flash chromatography to give **6c** (65% yield), and the starting **7c** (17% yield) which can be recycled. Ester **6c** was quantitatively reduced ($LiAlH_4$ in anhydrous Et_2O) to the alcohol **6b**. Both ester **6c** and alcohol **6b** can be further elaborated to more complex FK-506 fragments as previously reported in the literature (see, for example, ref. 5g for **6c** and ref. 5a for **6b**) (Scheme 2).

In summary, our asymmetric synthesis of **6c** and **6b** as key intermediates of the C_{28}-C_{34} fragment of **1** appears efficient and convenient with respect to the others previously reported.[5] It can be accomplished from the acid *S*-**2** in five easy steps in 30% overall yield. The yield is improved to 35% by recycling the recovered starting compound **7c** from the epimerization step. In this synthesis the stereogenicity of the natural C_{28}-C_{34} fragment of **1** is introduced at the level of the carboxylic acid **2** through an asymmetric Diels-Alder reaction, the acid *S*-**2** being used, contrary to the other previously described syntheses[5b-e] which utilize the acid *R*-**2**.

Experimental

Melting point were determined on a Kofler apparatus and are uncorrected. ^{1}H NMR spectra were determined with a Bruker AC-200 spectrometer on $CDCl_3$ solutions. Routine IR spectra were taken on paraffin oil mulls with a Perkin-Elmer Infracord Model 137. GC analyses were performed on a Perkin-Elmer 8420 apparatus (FI detector) with a 30 m x 0.25 mm (i.d.) x 0.25 μm (film thickness) DB-WAX fused silica capillary column: column 200°C, injector and detector 250°C, nitrogen flow 2 ml/min. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. 230-400 mesh Silica gel (Merck) was used for flash chromatography. Petroleum ether refers to the fraction with bp 40-70°C. All compounds gave satisfactory microanalysis results (C,H ± 0.3% of the calculated value).

(*S*)-(-)-3-Cyclohexenecarboxylic Acid (*S*-2) was prepared following a previously described procedure:[7] $[\alpha]_D^{20}$ = -91.0°, *c* = 1.1, CH_3OH; lit.:[7] $[\alpha]_D^{22}$ = -95.0°, *c* = 7.0, CH_3OH.

(1*S*,4*S*,5*S*) 4-Bromo-6-oxabicyclo[3.2.1]octan-7-one (12). In a modification of a described procedure,[8] a 1M solution of Br_2 in $CHCl_3$ (30 ml) was slowly added at 0°C to a solution of acid *S*-2 (3.02 g, 0.024 mol) in $CHCl_3$ (600 ml) in the presence of an equimolar amount of NEt_3 (3.33 ml). The workup[8] yielded the crude solid lactone **12** which was recrystallized from petroleum ether to give pure **12** (3.94 g, 80% yield) as a solid, mp 135-136°C; $[\alpha]_D^{20}$ = -26.4°, *c* = 1.0, $CHCl_3$; IR ν_{max} 1780 cm^{-1} (C=O); ^{1}H NMR δ 4.81 (t, J=5.0 Hz,1H), 4.41 (t, J=4.4 Hz,1H), 2.66 (d,J=12.2 Hz,1H), 2.08-2.58 (m, 4H), 1.80-2.08 (m,2H).

^{1}H NMR Analysis of (±)-12 and (-)-12 in the Presence of Pirkle's Chiral Solvating Agent.[9,10] (*R*)-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol (60.6 mg) was mixed with (±)-**12** or (-)-**12** (15 mg) in $CHCl_3$ (0.35 ml). Signals due to C*H*Br of (±)-**12** were observed as completely separated triplets at δ 4.19 and 4.10. In the case of (-)-**12**, only one triplet was observed at δ 4.19 indicating for this compound a 100% ee.

(1*S*,3*S*,4*S*) Methyl 4-bromo-3-hydroxy-1-cyclohexenecarboxylate (13). In accordance with the procedure described for the racemic compound,[8] a solution of **12** (3.9 g, 0.019 mol) in MeOH (140 ml) containing 0.5% (v/v) H_2SO_4 was refluxed for 3 h. The workup[8] yielded pure bromohydrin **13** (4.27 g, 95% yield) as a solid, mp 124-125°C; $[\alpha]_D^{20}$ = +31.3°, *c* = 0.6, $CHCl_3$; IR ν_{max} 3450 (OH) and 1720 cm^{-1} (C=O); ^{1}H NMR δ 3.81-4.05 (m,2H), 3.69 (s,3H), 2.37-2.57 (m,3H), 1.75-2.10 (m,2H), 1.42-1.70 (m,2H).

(1*S*,3*S*,4*R*) Methyl 3,4-epoxy-1-cyclohexanecarboxylate (4c). Bromohydrin **13** (2.00 g, 8.43 mmol) in 2-propanol (40 ml) was titrated to a phenolphthalein end-point over 1 h at room temperature with a 1N aqueous NaOH solution (8.5 ml), as described for the corresponding racemic compound.[6,8] The workup[6,8] yielded pure **4c** (1.12 g, 85% yield) as a liquid, $[\alpha]_D^{20}$ = -68.0°, *c* = 0.1, $CHCl_3$; IR ν_{max} 1735 cm^{-1} (C=O); ^{1}H NMR[6] δ 3.67 (s,3H), 3.17 (unresolved singlet, 2H), 2.04-2.34 (m,3H), 1.55-1.86 (m,4H).

Methanolysis of epoxide 4c. A solution of epoxide **4c** (1.50 g, 9.61 mmol) in 0.2 N methanolic H_2SO_4 (150 ml) was stirred at room temperature for 1h, as previously described for the corresponding racemic compound.[6] The workup[6] yielded a liquid residue (1.80 g) which contained an 8 : 2 mixture of **7c** and **8c**

(GC). Flash chromatography of this residue on silica gel (4 cm x 20 cm column; hexane/AcOEt 65:35) gave pure **7c** (1.27 g, 70% yield calculated on the epoxide) and **8c** (0.325 g, 18% yield).

(1*S*,3*R*,4*R*) Methyl 4-hydroxy-3-methoxy-1-cyclohexanecarboxylate (7c), a liquid, $[\alpha]_D^{20}$ = -27.0°, *c* = 0.42, $CHCl_3$; IR ν_{max} 3450 (OH) and 1720 cm^{-1} (C=O); ^1H NMR[6] δ 3.70 (s,3H), 3.56 (m,1H), 3.41 (s,3H), 3.25 (m,1H), 2.72 (m,1H), 2.31 (m,1H), 1.72-2.05 (m,2H), 1.32-1.66 (m,3H).

(1*S*,3*S*,4*S*) Methyl 3-hydroxy-4-methoxy-1-cyclohexanecarboxylate (8c), a liquid; IR ν_{max} 3450 (OH) and 1720 cm^{-1} (C=O); ^1H NMR[6] δ 3.68 (s,3H), 3.47 (m,1H), 3.41 (s,3H), 2.98 (m,1H), 1.95-2.49 (m,4H), 1.11-1.62 (m,3H). In this case, the $[\alpha]_D^{20}$ was not calculated because, even after repeated purification attempts by TLC, **8c** turned out to be contaminated with a 10-15% of an unknown product (GC).

Epimerization of 7c. A solution of ester **7c** (2.0 g, 10.6 mmol) in 0.5 M MeONa in anhydrous MeOH (30 ml) was refluxed 140 h in a sealed tube. After cooling to room temperature, the reaction mixture was concentrated at reduced pressure, then acidified with 10% aqueous HCl. Extraction with ether and evaporation of the washed (saturated aqueous NaCl solution) ether extracts afforded a liquid residue (1.99 g) consisting of a 81:19 mixture of **6c** and **7c** (GC). Flash chromatography of this residue on silica gel (5 cm x 20 cm column; hexane/AcOEt 6:4) afforded pure starting ester **7c** (0.34 g, 17% yield) and **(1*R*,3*R*,4*R*) methyl 4-hydroxy-3-methoxy-1-cyclohexanecarboxylate (6c)** (1.30 g, 65% yield), as a liquid, $[\alpha]_D^{20}$ = -70.8°, *c* = 0.1, $CHCl_3$ (lit.:[5g] $[\alpha]_D^{23}$ = -70.1°, *c* = 2.0, $CHCl_3$); IR ν_{max} 3450 (OH) and 1720 cm^{-1} (C=O); ^1H NMR δ 3.69 (s,3H), 3.41 (s,3H), 3.38-3.45 (m,1H), 2.96-3.06 (m,1H), 2.30-2.42 (m,2H), 1.95-2.10 (m,2H), 1.20-1.52 (m,3H).

(1*R*,3*R*,4*R*) 4-Hydroxy-3-methoxy-1-cyclohexanemethanol (6b). A stirred solution of **6c** (0.282 g, 1.50 mmol) in anhydrous ether (50 ml) was treated with $LiAlH_4$ (0.10 g) and the resulting suspension was stirred at room temperature for 2 h. The usual workup afforded pure **6b** (0.24 g) as a liquid, $[\alpha]_D^{20}$ = -57.3°, *c* = 0.30, $CHCl_3$ (lit.:[5a] $[\alpha]_D^{23}$ = -57.0°, *c* = 0.30, $CHCl_3$); IR ν_{max} 3450 cm^{-1}(OH) ; ^1H NMR[2b] δ 3.52 (dd, J=6.1 and 3.6 Hz, 1H), 3.44 (m,1H), 3.41 (s,3H), 3.01 (ddd, J= 11.2, 8.8 and 4.4 Hz,1H), 2.22 (m,1H), 2.07 (m,1H), 1.41-1.76 (m,3H), 1.57 (m,1H), 1.34 (quartet of doublets, J= 11.6 and 3.6 Hz, 1H), 1.07 (quartet of doublets, J= 11.7 and 3.5 Hz, 1H), 0.88 (q, J=11.7 Hz, 1H).

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