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Efficient Total Synthesis of AI-77-B, A Gastroprotective Substance from *Bacillus pumilus* AI-77[1,2]

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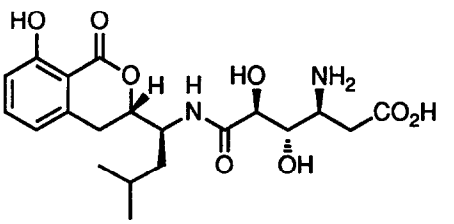
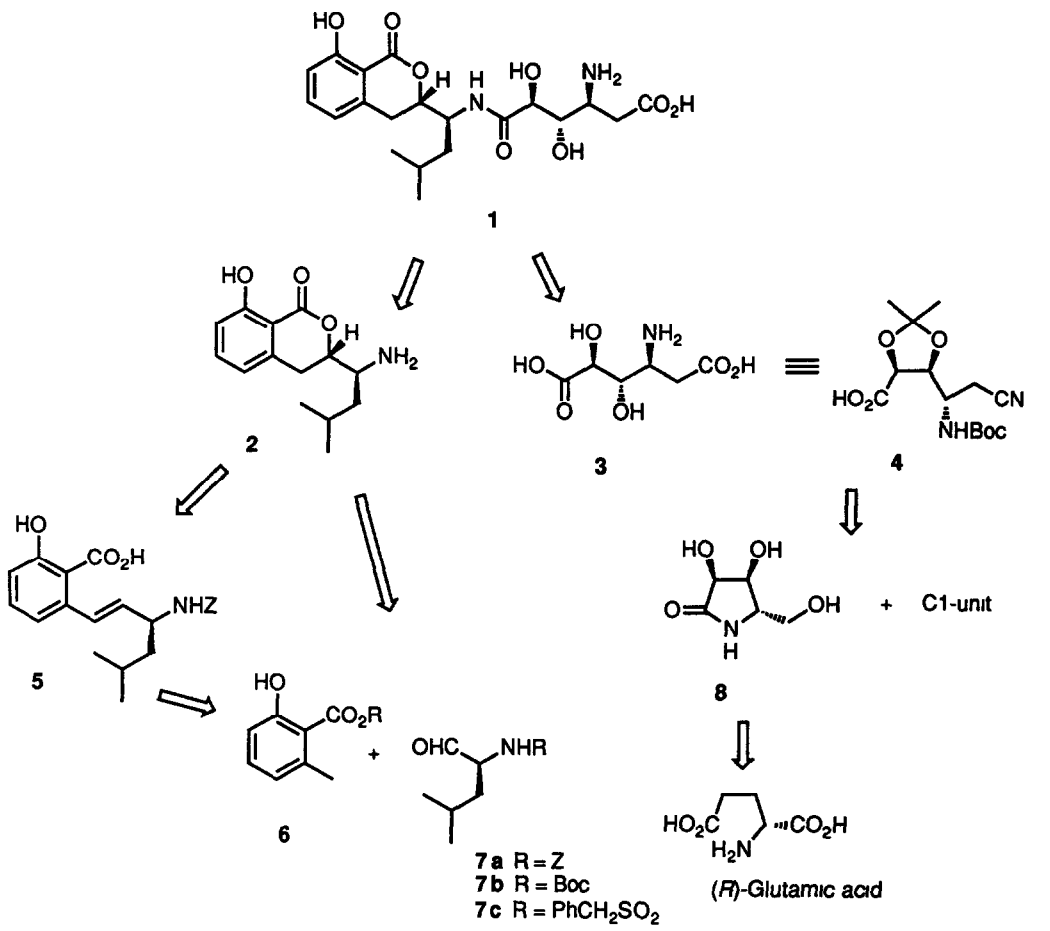
Abstract First total synthesis of AI-77-B (1), a gastroprotective substance from Bacillus pumilus AI-77, was achieved in a stereoselective and convergent manner In this synthesis, the dihydroisocoumarin part 2 was constructed in one step through 1,2-addition of the benzylic anion 17b to Boc-L-leucinal 7b The hydroxy amino acid 4 was elaborated from (R)-glutamic acid in a highly stereoselective manner Condensation of 2•HCl and 4, intramolecular Pinner reaction, followed by mild hydrolysis afforded AI-77-B (1)

AI-77's[3,4] were isolated as the dihydroisocoumarin antibiotics with characteristic fluorecense from a cultured broth of *Bacillus pumilus* AI-77 in 1982 The stereostructure of the major product AI-77-B (**1**) was determined by means of X-ray crystallographic analysis together with chemical degradation studies and revealed to consist of a hydroxy amino acid and a dihydroisocoumarin with the side chain having the stereostructure of (S)-leucine as shown below The absolute stereochemistries of **1** at five asymmetric centers have been also determined to bear (S)-configurations. In the pharmacological studies,[5] **1** has been found to exhibit unique antiulcerogenicity action against stress ulcer in rats without anticholinergic, antihistaminergic, and central suppressive effects This medicinally interesting molecule has led the efforts aimed at total synthesis by a few groups including us.[2,6]



AI-77-B (1)

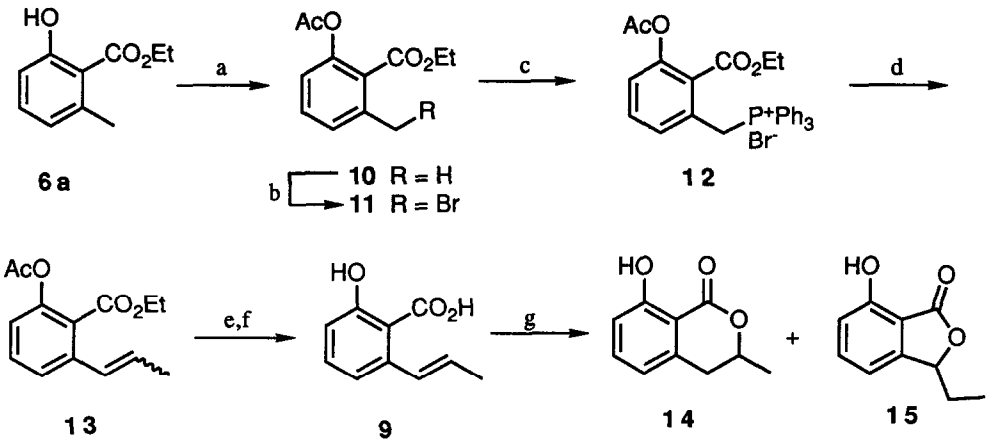
In connection with our interest in the syntheses of amino sugars and amino acids with biologically interesting activity,[7] we have undertaken the first total synthesis[8] of AI-77-B (1) in a stereoselective and convergent manner.



Scheme 1

Scheme 1 outlines the key features of our synthetic sequence involving the two fragments, the dihydroisocoumarin **2** and the hydroxy amino acid **3** The dihydroisocoumarin **2** can be derived from **5** by acid-mediated intramolecular cyclization of a carboxylic acid group on a neighboring double bond which process is the 6-end-Trig cyclization and favors on Baldwin rule [9] The requisite **5** can be obtained from alkyl 6-methyl salicylate **6** and the α-amino aldehyde **7** Alternatively, **2** would be derived through 1,2-addition to the N-protected leucinal **7** with a suitable benzylic anion equivalent of **6** The hydroxy amino acid **3** is equivalent to the protective derivative **4** which is dissected the lactam **8** having the cis-diol function and C1-unit. The lactam **8** would be derived from commercially available (R)-glutamic acid by the formation of γ-lactam ring followed by the stereoselective introduction of the cis-diol function On the basis of this retrosynthetic analysis, we have succeeded the stereoselective synthesis of AI-77-B (**1**) as described below

Scheme 2



(a) $(CH_3CO)_2O$, pyridine, room temperature, 3 h, quant (b) N-bromosuccinimde, benzoyl peroxide, CCl_4, reflux 4 h, 93% (c) Ph_3P, EtOAc, reflux, 17 h, 90% (d) CH_3CHO, Et_3N DMSO, 120°C, 21 h, 99% (e) p-toluenesulfinic acid, toluene, reflux, 6 5 h, quant (f) NaOH, EtOH, reflux, 5 h, 96% (g) see Table I

Table I Acid-mediated Cyclization

entry	acid (equiv)	solvent	reaction conditions	isolated yield (%) 14	15
1	d-CSA[a](5)	CF_3CO_2H	rt, 24 h	9	9
2	d-CSA (5)	PhF	reflux, 22 h	10	9
3	CH_3SO_3H		rt, 18 h	36	43
4	TMSOTf[b](2)	CH_2Cl_2	rt, 23 h	35	43

(a) d-CSA d-camphor-10-sulfonic acid (b) TMSOTf trimethylsilyl trifluoromethanesulfonate

Preparation of the Dihydroisocoumarin Fragment 2

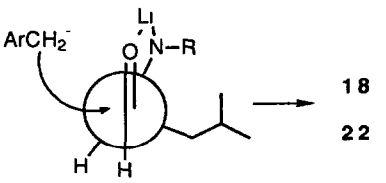
First, we investigated the 6-endo-Trig process by use of **9** as a model compound which was derived in 6 steps from ethyl 6-methylsalicylate (**6a**) The starting material **6a** was prepared by slight modification of the Hauser's method[10] in 53% overall yield and subjected to derivatization in 4 steps as follows (1) acetylation of **6a** with acetic anhydride and pyridine, (2) benzylic bromination with N-bromosuccinimide in carbon tetrachloride, (3) reaction of the resulting bromide **11** with triphenylphosphine, and (4) Wittig reaction of the phosphonium salt **12** with acetaldehyde in the presence of triethylamine in dimethyl sulfoxide The ethyl salicylate **13** thus obtained was found to be a mixture of Z- and E-isomers with a ratio of 43 57 The geometrically pure (E)-isomer **9** was obtained by isomerization of **13** with p-toluenesulfinic acid[11] in refluxing benzene and saponification of the two ester functions of **13** with sodium hydroxide followed by recrystallization The results of the acid-mediated cyclization of the carboxylic acid **9** are briefly summarized in Table I The desired 6-endo-Trig cyclization of **9** proceeded in acidic media to give melein[12](**14**), although the competing 5-exo-Trig cyclization also favored on Baldwin's rule took place as anticipated to produce the phthalide **15** After several experiments, we discovered that use of a large amount of

methanesulfonic acid or slight excess of trimethylsilyl trifluoromethanesulfonate in methylene chloride was effective in this reaction However, we did not pursue this approach since 5-exo-Trig cyclization was major pathway

Alternatively, we examined one-step construction of the dihydroisocoumarin ring system from the alkyl 6-methylsalicylate and (S)-leucinal derivative, which process contained diastereoselective addition followed by spontaneous lactone formation The requisite derivatives **16b**, **17a**, and **17d** for generating the benzylic anion or its synthetic equivalent were routinely derived from **6a** as shown Scheme 3 The results on reaction of the α-aminoaldehyde **7b**[13] with **16b**, **17a**, and **17d** are summarized in Table II Initially, we took the Barbier type reaction (entry 1-3) using the bromide **16b** In the coupling reaction of **7b** and **16b**, the use of magnesium in THF gave the desired dihydroisocoumarin **18a** as a mixture of chromatographically separable diastereomers in poor yield owing to undesired side reaction Ultrasonic agitation for accelating the reaction was ineffective Replacement of magnesium with samarium diiodide[14] enhanced the reaction rate but resulted in low yield and no diastereoselectivity The stereochemistry of the major product **18a** was unambiguously confirmed to be the desired (S,S)-isomer by conversion to **2**•HCl and its comparison with a sample derived from natural AI-77-B as follows , (1) deprotection of the methyl ether and Boc functions by reaction with boron tribromide in methylene chloride, and (2) conversion of the resulting hydrobromide to the corresponding hydrochloride **2**•HCl which was identical with naturally derived one by TLC, mp, specific rotation, IR, ^{1}H-NMR, and ^{13}C-NMR spectra

Next, we investigated the benzylic anion **17b** for this reaction [15] Deprotonation of **17a** was carried out by reaction with a mixture of LDA and TMEDA at -74°C for 0 5 h in THF Complete deprotonation at the benzylic position was proved by quantitative production of the silyl derivative **17c** by trapping of the corresponding anion **17b** with chlorotrimethylsilane and triethylamine Thus, the anion **17b** generated as above reacted with a slight excess of **7b** at -75°C, affording a mixture of the diastereomers **18b** and **19b** in a combined yield of 33% with no diastereoselectivity (entry 4) Their stereochemical assignments were again made by conversion to the corresponding hydrochlorides **2**•HCl and **20**•HCl and their comparisons with naturally derived **2**•HCl In the case of an excess of LDA (entry 5), improved diastereoselectivity up to 81 19 was observed, though the yield together with the recovery of **6a** still remained unsatisfactory In this reaction, the α-aminoaldehyde was seemed to be deprotonated with an excess of base to generate the N-lithium derivative which rather than the NH carbamate served as internal ligand for chelation-control as shown in Scheme 4 In contrast to the lithio reagent **17b**, the benzyl titanium reagent prepared by addition of titanium tetrachloride to the above experiment gave the dihydroisocoumarins (**18b** and **19b**) in moderate yield but reversed diastereoselectivity (entry 7)

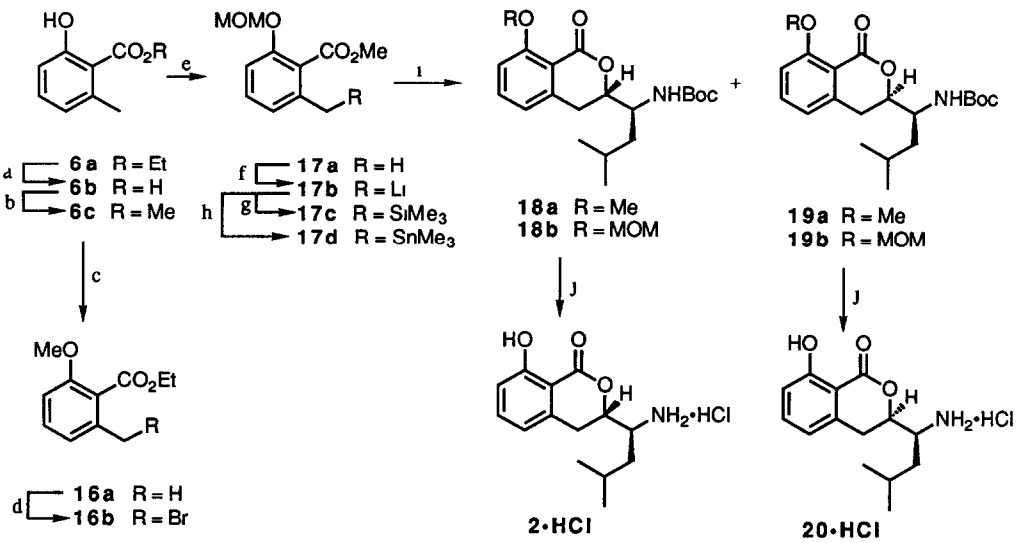
Finally, we investigated N-benzylsulfonyl-(S)-leucinal bearing the easily removable NH proton with the hope that complete conversion of the sulfonamide to the N-lithium sulfonamide might lead to improved stereoselectivity through the chelation pathway [16] Thus, the leucinal **7c**, prepared from methyl leucinate hydrochloride in 2 steps, was converted to the N-lithio derivative **21b** with n-butyl lithium prior to addition reaction and treated with the benzylic anion **17b** at -75°C to afford the dihydroisocoumarin **22** as a nearly single isomer in 30%



21a R = Boc
21b R = $PhCH_2SO_2$-

Scheme 4

Scheme 3



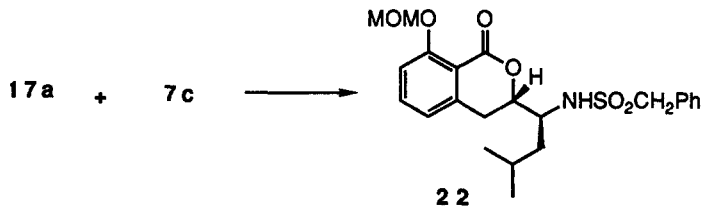
(a) 1N NaOH, EtOH, reflux, 5h, 95% (b) MeOH, H_2SO_4, reflux, 27h, 90% (c) MeI (3 eq), K_2CO_3 (1 2 Mol eq), acetone, reflux, 25h, 100% (d) NBS, benzoyl peroxide, CCl_4, reflux, 95% (e) CH_3OCH_2Cl (2eq), iPr_2NEt (3eq), CH_2Cl_2, 0°C, 1h, rt, 3h, 97% (f) LDA (1 2 eq), TMEDA (0 83 eq), THF, -74°C, 0 5h (g) Then, Me_3SiCl (2 5 eq), Et_3N (0 25 eq), -74°C, 43 5h, 97% (h) Then, Me_3SnCl (1 5 eq), THF, -78°C, 2h, 82% (i) see, Table II (j) BBr_3 (8 1 eq), CH_2Cl_2, -75°C, 1h, rt, 18h

Table II One-step construction of the dihydroisocoumarin

entry	salicylate	leucinal	reaction conditions	isolated yield (%)	ratio of 18 : 19
1	**16b**	**7b**(0 5eq)	Mg, THF, 0°, 7 5h	14	76 24
2	**16b**	**7b**(0 5eq)	Mg, THF, 0°, 4h[a]	25	71 29
3	**16b**	**7b**(0 5eq)	SmI_2, THF, rt, 2min	21	50 50
4	**17a**	**7b**(1 4eq)	LDA(1 4eq), TMEDA THF, -75°~-73°	33	57 43
5	**17a**	**7b**(1 4eq)	LDA(2 6eq), TMEDA THF, -75°~-73°	32 (61)[b]	81 19
6	**17a**	**7b**(1 4eq)	LiTMP, TMEDA	29	63 37
7	**17a**	**7b**(1 4eq)	LDA(2 8eq), TMEDA then $TiCl_4$(2eq)	58	26 74
8	**17d**	**7b**(1 4eq)	BuLi, THF	41	50 50
9	**17d**	**7b**(1 4eq)	BuLi, TMEDA THF	53	52 48

(a) The reaction was carried out under sonochemical conditions (b) The parenthesis number is yield based on consumed starting material **17a**

yield The stereochemistry was tentatively assigned by analogy to the above experiment Unfortunately, we could not find the reaction conditions for deprotection of the N-benzylsulfonyl group



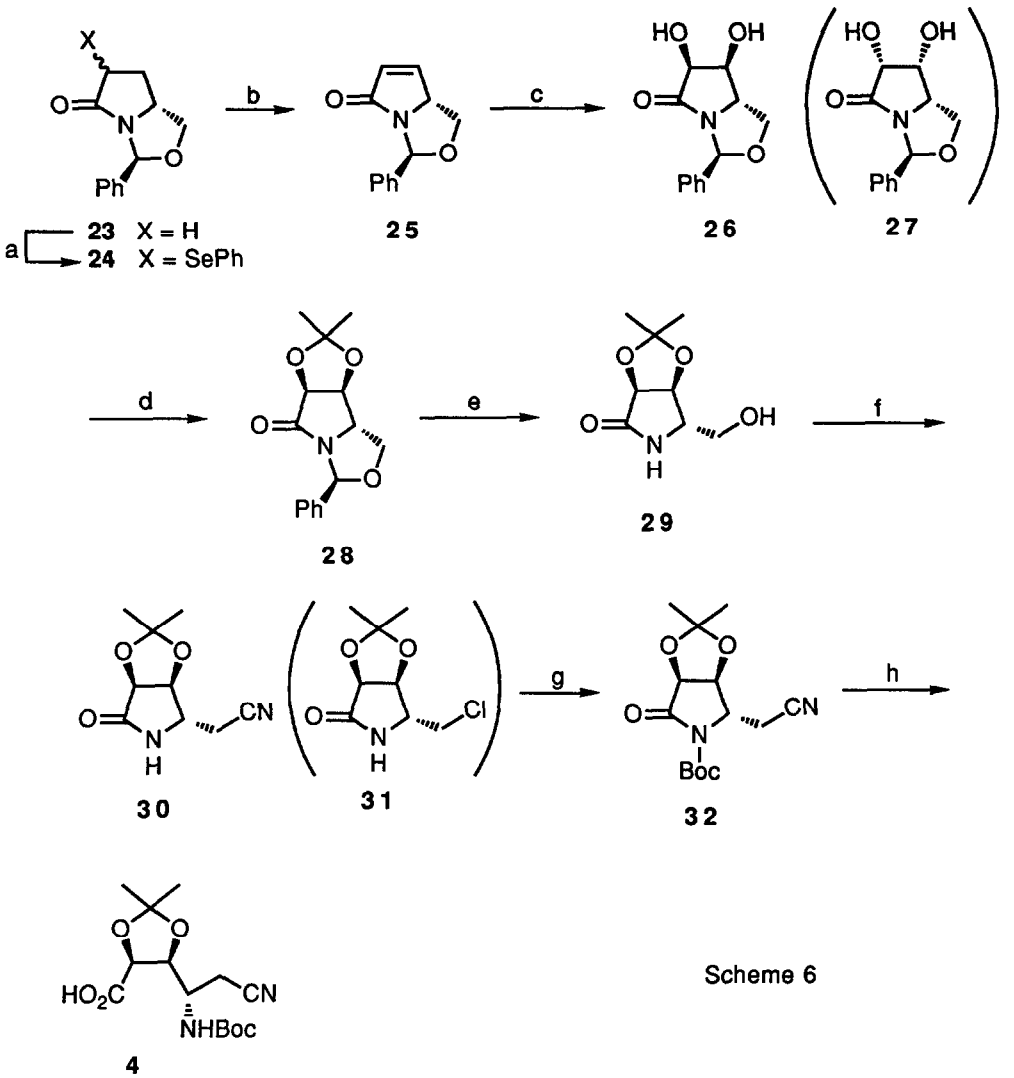
Scheme 5

Preparation of the Hydroxy Amino Acid Fragment 4

We chose the bicyclic lactam **23** suitable as a starting material which was prepared from (R)-glutamic acid in 4 steps according to the method[17] of the Squibb group for the (S)-enantiomer Introduction of the double bond to the lactam **23** was performed by a sequence of selenylation and deselenoxylation The lactam **23** was treated with LDA at -70°C and then phenylselenyl bromide generated in situ from diphenyl diselenide in THF Deselenoxylation of the resulting selenide **24** was examined by 2 ways as follows (A) reaction of 30% hydrogen peroxide in the presence of pyridine and (B) oxidation with ozone in CH_2Cl_2 The latter procedure was somewhat effective and gave the α,β-unsaturated lactam **25** in 75% yield The unsaturated lactam **25** was, as anticipated, labile owing to the possible ring tension Stereoselective introduction of the diol function to the lactam **25** was achieved by catalytic osmylation together with N-methyl morpholine N-oxide (NMMO) as cooxidant in aqueous acetone The desired lactam **26** was obtained as a major isomer in 65% yield with stereoselectivity of 98 4 1 6 after chromatographic separation of the minor isomer **27** Stereochemistry of the lactam **26** was tentatively assigned on the basis of osmylation from the less hindered convex face In this reaction, the benzylidene acetal of **25** serves as the N,O-protecting group as well as face differentiating one and leads high diastereoselectivity The lactam **26** has the required continuous three asymmetric centers for **4** Protection of the diol function of **26** with 2,2-dimethoxypropane and pyridinium p-toluenesulfonate (PPTS) furnished the acetonide **28** in vertually quantitative yield Removal of the benzylidene acetal of **28** was unexpectedly difficult Hydrogenolysis using palladium on carbon or reductive cleavage with sodium metal in liquid ammonia was ineffective Fortunately, by use of hydrazine hydrate as a hydrogen source on the transfer hydrogenation using 5% palladium on carbon,[18] the desired lactam **29** was obtained after treatment at reflux for 30 min in 95% yield One carbon homologation was carried out by the method[19] developed by us Thus, reaction of the alcohol **29** with tri-n-butyl phosphine, carbon tetrachloride, potassium cyanide, and 18-crown-6 in acetonitrile sluggishly proceeded at room temperature to give the intermediate chloride **31** instead of the nitrile **30** The elevated temperature, however, afforded the desired nitrile **30** with full carbon skeleton of the hydroxy amino acid **3** in 71% yield Mild hydrolytic cleavage of the lactam ring was accomplished after attachment of the Boc function at nitrogen, affording the hydroxy amino acid **4** in 70% yield

Construction of AI-77-B (1)

With the required dihydroisocoumarin and hydroxy amino acid fragments in hand, we have accomplished the construction of AI-77-B (**1**) Condensation of the hydroxy amino acid **4** and dihydroisocoumarin **2**•HCl with diethyl phosphorocyanidate (DEPC, $(C_2H_5O)_2P(O)CN$)[20] and



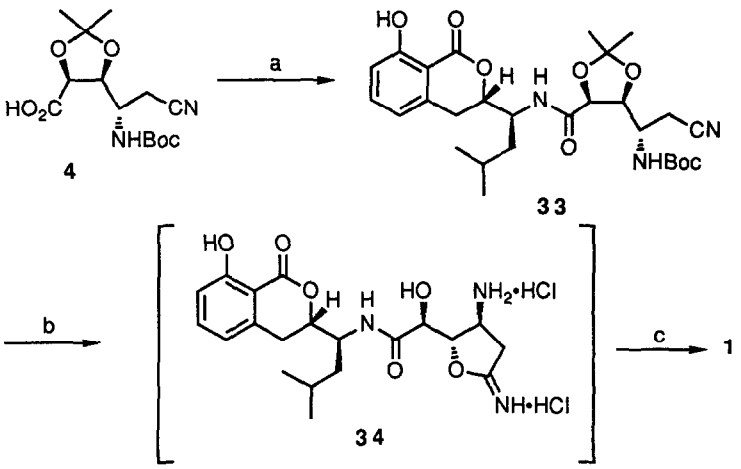
Scheme 6

(a) LDA (1 3 equiv), THF, -70°C, 0 5 h, then PhSeBr (1 2 equiv), THF, -70°C, 15 min (b) ozone, CH_2Cl_2, -74°C, 2 h, pyridine, -74°C→room temperature, 75% from **23** (c) OsO_4 (0 1 equiv), NMMO (1 4 equiv), aqueous acetone, room temperature, 15 h, 65% (d) 2,2-dimethoxypropane (excess), PPTS (cat), acetone, room temperature, 10 h, 98% (e) 5% Pd-C, $NH_2NH_2 \cdot H_2O$, MeOH, 95% (f) KCN (2 equiv), 18-crown-6 (0 1equiv), Bu_3P (1 1 equiv), CCl_4 (1 1 equiv), CH_3CN, 30-40°C, 1 h, then 70-80°C, 2 h, 71% (g) $(Boc)_2O$ (1 12 equiv), DMAP (cat), CH_3CN, room temperature, 1 h, 92% (h) LiOH, 70% aqueous THF, room temperature, 30 min, 70%

triethylamine was slightly sluggish and after additional reagents afforded the amide **33** with full carbon skeleton of **1** Hydrolysis of the nitrile function to the carboxylic one was difficult problem owing to coexistence of the internal amide bond, which was solved by use of the intramolecular Pinner reaction The amide **33** was treated with 5% hydrogen chloride in MeOH in the presence of trimethyl orthoformate at 5°C and the resulting imino ester **34** was hydrolyzed by addition of water at room temperature to produce the γ-lactone derivative The final transformation of the γ-lactone to **1** in the presence of the δ-lactone function was

performed by careful addition of 0 1N aqueous sodium hydroxide maintaining at pH 9 0 in aqueous MeOH After neutralization with 0 1N hydrochloric acid, the crude **1** was purified on Amberlite XAD-2 followed by Sephadex G-10 , yielding the pure material which was identical with the natural product in all respects

Scheme 8



(a) **2**•HCl , DEPC (1 25 equiv), Et_3N (3 2 equiv), DMF, 0°C, 3 h, room temperature, 20 h, then an additional DEPC (0 45 equiv), an additional Et_3N (1 47 equiv), room temperature, 11 h, 70% (b) 5% HCl-MeOH, trimethyl orthoformate (excess), 5°C, 44 5 h (c) H_2O, 12 h, 0 1 N NaOH (pH 9), aqueous MeOH, room temperature, 3 h, 0 1 N HCl (pH 6 5), 76% from **33**

In summary, we have achieved the first total synthesis of AI-77-B (**1**), the unique 3,4-dihydroisocoumarin antibiotic, in a stereoselective and convergent manner which involved (1) one-step construction of the 3,4-dihydroisocoumarin **18b** with moderate stereoselectivity, (2) highly diastereoselective osmylation of the bicyclic α,β–unsaturated lactam **25**, and (3) mild conversion of the nitrile **33** to **1** using the intramolecular Pinner reaction This synthesis will provide an easy entry to many other congeners required for pharmacological evaluation

Experimental

Melting points are uncorrected IR spectra were recorded on a JASCO IRA-2 spectrometer NMR spectra were recorded on JEOL FX-100 or GSX-400 spectrometers in $CDCl_3$ using tetramethylsilane as an internal standard EI and FAB mass spectra were obtained with a JEOL DX-300 spectrometer Optical rotations were determined on a JASCO DIP-140 automatic polarimeter Analytical TLC was performed on a silica gel plate (E Merck Art 5715) Normal column chromatography was carried out with silica gel BW-820MH (Fuji Davison Co) and flash chromatography was performed with silica gel BW-200 (Fuji Davison Co)

Ethyl 6-methylsalicylate (6a). Prepared according to the slightly modified method of Hauser's one[10] as follows to a stirred solution of ethyl acetoacetate (220 ml, 1 75 mol) and freshly prepared sodium ethoxide (50 mmol) in EtOH (450 ml) at 0°C was added dropwise crotonaldehyde (143 ml, 1 73 mol) over 30

min and the reaction mixture was allowed to warm to ambient temperature. After being stirred for 50 h, the reaction mixture was recooled to ice-bath temperature, saturated with hydrogen chloride, allowed to warm again to ambient temperature, and stirred for 40 h Removal of the volatile gave the crude ethyl 6-methyl-2-oxo-3-cyclohexenecarboxylate as a brown oil which was directly used for the next reaction without further purification

The above material was added dropwise to a stirred mixture of cupric chloride (322 g, 2 4 mol) and lithium chloride (73 g, 1 7 mol) in dimethylformamide (400 ml) at 90°C over 30min and the reaction mixture was kept at this temperature for 2 5 h Then, the reaction mixture was poured into ice (400 g) and the precipitate was formed After removal of the precipitate by filtration, the filtrate was extracted 5 times with EtOAc-PhH (2 1, 500 ml) The organic layer was washed with water (400 ml x 5) and saturated brine (300 ml x 2), dried over Na_2SO_4, and concentrated in vacuo to leave 265 g of the crude ethyl 6-methylsalicylate as a brown oil Distillation of the residue (bp 95-100°C at 0 1 mmHg) gave the pure material (165 5 g, 53 % from crotonaldehyde) as colorless crystals, which were recrystallized from n-hexane mp 44-45°C (lit [10] mp 42°C from aqueous MeOH), IR υ_{max} (KBr disc) 3400, 1660, 1605, 1250 cm^{-1}, ^1H NMR δ 1 43 (3H, t, J = 7Hz), 2 55 (3H, s), 4 45 (2H, q, J = 7Hz), 6 73 (1H, d, J = 8Hz), 6 87 (1H, d, J = 8Hz), 7 35 (1H, t, J = 8Hz), 11 83(1H, s, disappeared with D_2O)

Ethyl O-acetyl-6-methylsalicylate (10). Ethyl 6-methylsalicylate (**6a**) (18 g, 0 1 mol) was dissolved in pyridine (50 ml) and acetic anhydride (20 ml, 0 2 mol) was added at room temperature After being stirred for 3 h, the reaction mixture was diluted with EtOAc (500 ml), washed with 10% hydrochloric acid (until lower layer became pH 2), water (100 ml x 2), saturated aqueous $NaHCO_3$ (100 ml), and saturated brine (100 ml), and dried over Na_2SO_4 Filtration followed by evaporation of the filtrate gave the acetate **10** (22 9 g, quant) as colorless crystals which were recrystallized from n-hexane mp 36 5-37°C, IR υ_{max} (KBr disc) 1770, 1720, 1600, 1265cm^{-1}, ^1H NMR δ 1 17 (3H, t, J = 7Hz), 2 07 (3H, s), 2 23 (3H, s), 4 23 (2H, q, J = 7Hz), 6 87 (1H, d, J = 8Hz), 7 01 (1H, d, J = 8Hz), 7 23 (1H, t, J = 8Hz) Anal calcd for $C_{12}H_{14}O_4$ C, 64 85, H, 6 35 Found C, 64 59, H, 6 40

Ethyl O-acetyl-6-bromomethylsalicylate (11) A mixture of the acetate **10** (555 mg, 2 5 mmol), N-bromosuccinimde (490 mg, 2 75 mmol), and benzoyl peroxide (60 mg, 0 25 mmol) in carbon tetrachloride was heated at reflux for 4 h After cooling, the reaction mixture was filtered and the filtrate was condensed in vacuo to give the crude product (793 mg) as a yellow oil containing the bromide **11** (690 mg, 92%) and a small amount of starting material (43 mg, 7 7%) along with a trace of the corresponding dibromide (judged by ^1H NMR) The crude bromide **11** IR υ_{max} ($CHCl_3$) 2980, 1770, 1725, 1372, 1285, 1195 cm^{-1}, ^1H NMR δ 1 37 (3H, t, J = 7Hz), 2 26 (3H, s), 4 40 (2H, q, J = 7Hz), 4 67 (1H, s), 7 03-7 56 (3H, m) The crude material was directly used for the next reaction

3-Acetoxy-2-ethoxycarbonylbenzyltriphenylphosphonium bromide (12) A mixture of the bromide **11** (13 4 g, 45 mmol) and triphenylphosphine (14 2 g, 54 mmol) in EtOAc (200 ml) was heated at reflux for 17 h during which time the precipitate came out of the solution The reaction mixture was filtered through sintered glass filter and washed with EtOAc to give the phosphonium salt (22 8 g, 90%) as colorless crystals which were recrystallized from EtOH-EtOAc mp 194-196°C, IR υ_{max} (KBr disc) 1765, 1720, 1440, 1110 cm^{-1}, ^1H NMR δ 1 13 (2H, d, J = 15Hz), 2 23 (3H, s), 3 96 (2H, q, J = 7Hz), 5 83 (2H, d, J = 15Hz), 7 0-8 1(18H, m) Anal calcd for $C_{30}H_{28}BrO_4P$ C, 63 95, H, 5 01 Found C, 64 16, H, 4 82

Ethyl (E)-O-acetyl-6-(1-propenyl)salicylate (13) The phosphonium salts **12** (563 mg, 1 mmol) was dissolved in DMSO (5 ml) and triethylamine (180 µl, 1 2 mmol) and acetaldehyde (150 µl, 2 7 mmol) was added The reaction mixture was heated to 120°C for 21 h, then allowed to cool to ambient

temperature, poured into ice-water (25 ml), and extracted three times with ether (each 50 ml). The organic layer was washed with water (50 ml) and saturated brine (50 ml), dried over Na_2SO_4, filtered, and condensed in vacuo to afford the crude product as an oil Chromatographic purification of the residue (EtOAc-hexane = 1.9) gave the title compound (245mg, 99%) as a mixture of geometric isomers with a ratio of 43 57 (Z E) judged by GLC analysis on 2% SE-30 (2 m). The mixture (100 mg, 0 4 mmol) was dissolved in dioxane (1 ml) and p-toluenesulfinic acid (6 mg, 0 04 mmol) was added The reaction mixture was heated at reflux for 6 5 h and diluted with EtOAc (40 ml) The whole was washed with saturated aqueous $NaHCO_3$ (8 ml x 2) and saturated brine (20 ml x 2), dried over Na_2SO_4, and concentrated in vacuo to afford the (E)-isomer **13** (100 mg, 100%) with 94% geometric purity IR υ_{max} (neat) 1770, 1725, 1270, 1200 cm^{-1}, ^1H NMR δ 1 36 (3H, t, J = 7Hz), 1 86 (3H, dd, J = 7Hz, 2Hz), 2.26 (3H, s), 4 36 (2H, q, J = 7Hz), 6 16 (1H, dq, J = 17Hz, 7Hz), 6 50 (1H, dq, J = 17Hz, 2Hz), 6 90-7 04 (1H, m), 7 16-7 30 (2H, m)

6-(1-Propenyl)salicylic acid (9) A mixture of the ester **13** (1 37 g, 5.5 mmol) and 1N NaOH (16 ml, 16 mmol) in EtOH (30 ml) was heated at reflux for 5 h After removal of the solvent, the residue was acidified to pH 2 with 10% hydrochloric acid, diluted with water (10 ml), and extracted three times with EtOAc (each 20 ml) The organic layer was washed with water (25 ml x 2) and saturated brine (25 ml), dried over Na_2SO_4, and concentrated in vacuo to give the salicylic acid **9** (941 mg, 96%) as colorless crystals mp 145 5-146°C, IR υ_{max} (KBr disc) 3500-2500, 1640, 1600, 1435 cm^{-1}, ^1H NMR ($CDCl_3$-DMSO-d_6) δ 1 86 (3H, dd, J = 7Hz, 2Hz), 5 66-6 33 (1H, m), 6 82-7 06 (3H, m), 7 43 (1H, t, J = 8Hz), 8 33-9.5 (2H, br s, disappeared with D_2O) High mass calcd for $C_{10}H_{10}O_3$ 178 0629 Found 178 0616

Melein (14) (i) With d-camphor-10-sulfonic acid---The salicylic acid **9** (70 mg, 0.39 mmol) was dissolved in trifluoroacetic acid (1 ml), and d-camphor-10-sulfonic acid (460 mg, 2 mmol) was added at room temperature After being stirred for 23 h, the reaction mixture was concentrated in vacuo, neutralized with saturated aqueous $NaHCO_3$ (1ml), and extracted with CH_2Cl_2 (20 ml x 2) The organic layer was washed with water (5 ml) and saturated brine (5 ml), dried over Na_2SO_4, and removed in vacuo to give the product (68 mg) as a crude oil The residue was purified on silica gel plate (Merck Art 5744) using EtOAc-hexane (1 4) to afford melein (**14**) (19 mg, 27%) and the phthalide **15** (18mg, 26%) **14** mp 43-44°C (from Et_2O-n-hexane) (lit [12] (R)-isomer mp 38°C from CH_2Cl_2-n-hexane), IR υ_{max} (solution in $CHCl_3$) 3150, 1675, 1620, 1460, 1120 cm^{-1}, ^1H NMR δ 1 54 (3H, d, J = 7Hz), 2 92 (2H, d, J = 7Hz), 4 56-4 88 (1H, m), 6 72, 6 88 (2H, d, J = 8Hz), 7 40 (1H, t, J = 8Hz), 11 02 (1H, s, disappeared with D_2O) High mass calcd for $C_{10}H_{10}O_3$ 178 0628 Found 178 0618 **15** mp 71-72 5°C, IR υ_{max} (solution in $CHCl_3$) 3400, 1730 cm^{-1}, ^1H NMR δ 1 04 (3H, t, J = 7Hz), 1 60-2 30(2H, m), 5 53 (1H, dd, J = 7Hz, 4Hz), 6 90, 6 92 (2H, d, J = 8Hz) 7 56 (1H, t, J = 8Hz), 7 80 (1H, br s, disappeared with D_2O) High mass calcd for $C_{10}H_{10}O_3$ 178 0629 Found 178 0633

(ii) With trimethylsilyl trifluoromethanesulfonate (TMSOTf)---To a stirred solution of the salicylic acid **9** (40 mg, 0 22 mmol) in CH_2Cl_2 at room temperature was added a 0 4 M solution of TMSOTf in CH_2Cl_2 (1 1 ml, 0 44 mmol) After being stirred for 23 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (5 ml) and extracted with CH_2Cl_2 (30 ml x 2) The organic layer was washed with water (20 ml) and saturated brine (20 ml), dried over Na_2SO_4, and removed in vacuo to give a yellow oil (35 mg) which was purified as above to afford **14** (14 mg, 35%) and **15** (17 mg, 43%)

Ethyl 2-methoxy-6-methylbenzoate (16a) Ethyl 6-methylsalicylate (**6a**) (9 01 g, 50 mmol) was dissolved in acetone (70 ml) and potassium carbonate (8 29 g, 60 mmol) and iodomethane (9 3 ml, 150 mmol) were added The reaction mixture was heated at reflux for 25 h After cooling, the reaction mixture

was filtered and the filtrate was concentrated in vacuo. The residue was diluted with EtOAc (200 ml), washed with water (50 ml) and saturated brine (50 ml), dried over Na_2SO_4, and concentrated in vacuo to give the title compound **16a** (9 68 g, 100%) as a pale yellow oil. IR υ_{max} (neat) 1730, 1580, 1470 cm^{-1}, ^1H NMR δ 1 3 (3H, t, J = 7Hz), 2 3 (3H, s), 3 82(3H, s), 4 43 (2H, q), 6 76-6 92 (2H, m), 7 33 (1H, t, J = 8Hz)

Ethyl 3-O-methyl-6-bromomethylsalicylate (16b) A mixture of the ethyl 6-methylsalicylate **16a** (4 86 g, 25 mmol), N-bromosuccinimde (4 89 g, 27 5 mmol), and benzoyl peroxide (0 16 g, 0 65 mmol) in carbon tetrachloride (60 ml) was heated at reflux for 8 h. After cooling, the reaction mixture was filtered and the filtrate was condensed in vacuo The residue was diluted with EtOAc (200 ml), washed with saturated aqueous $NaHCO_3$ (100 ml), water (100 ml), and saturated brine (100 ml), and dried over Na_2SO_4 Evaporation of the solvent followed by chromatographic purification of the residue (BW-200, 200 g, n-hexane-EtOAc = 10 1) gave the bromide **16b** (6 33 g, 93%) as a slightly yellow oil IR υ_{max} (neat) 1770, 1725, 1372, 1285 cm^{-1}, ^1H NMR δ 1 37 (3H, t, J = 7Hz), 2 26 (3H, s), 4 40 (2H, q, J = 7Hz), 4 67 (2H, s), 7 03-7 56 (3H,m)

6-Methylsalicylic acid (6b) Prepared by hydrolysis of **6a** with 1N NaOH (2 9 equiv) and EtOH (5 5 ml/mmol) under reflux in 95% yield Recrystallization from EtOAc-n-hexane gave the pure material as colorless needles mp 170-172°C, IR υ_{max} (KBr disc) 3400-2500, 1640, 1440 cm^{-1}, ^1H NMR δ 2 56 (3H, s), 6 63, 6 73 (2H, d, J = 8Hz), 7 20 (1H, t, J = 8Hz), 8 33-8 83 (2H, br, disappeared with D_2O) Anal calcd for $C_8H_8O_3$ C, 63 15, H, 5 30. Found C, 62 97, H, 5 14

Methyl 6-methylsalicylate (6c) The salicylic acid **6b** (18 24 g, 0 12 mol) was dissolved in MeOH (100 ml) and concentrated sulfuric acid (5 ml) was added The reaction mixture was heated at reflux for 27 h After removal of the solvent, the residue was taken up in EtOAc (200 ml), washed with saturated aqueous $NaHCO_3$ (50 ml x 2) and water (50 ml), and dried over $MgSO_4$ Evaporation of the organic layer gave the ester **6c** (18 g, 90%) as colorless crystals which were recrystallized from n-hexane mp 32°C, IR υ_{max} (KBr disc) 1660, 1440 cm^{-1}, ^1H NMR δ 2 63 (3H, s), 3 10 (3H, s), 6 90, 7 10 (2H, d, J = 8Hz), 7 53 (1H, t, J = 8Hz), 11 42 (1H, s, disappeared with D_2O) Anal calcd for $C_9H_{10}O_3$ C, 65 05, H, 6 07 Found C, 64 58, H, 5 97

Methyl 2-O-methoxymethyl-6-methylsalicylate (17a) To a stirred solution of methyl 6-methylsalicylate (11 63 g, 70 mmol) in CH_2Cl_2 (100 ml) at ice-bath temperature was added dropwise diisopropylethylamine (36 6 ml, 210 mmol) and chloromethyl methyl ether (10 6 ml, 140 mmol) The reaction mixture was stirred at the same temperature for 35 h After addition of 10% aqueous citric acid, the whole was extracted twice with CH_2Cl_2 (200 ml) The organic layer was washed with water (200 ml x 2) and saturated brine (100 ml), dried over Na_2SO_4, and concentrated in vacuo to leave the title compound **17a** as a brown oil (15 35 g, 97%) Distillation (bp 114°C at 1 mmHg) gave the pure material (14 2 g, 90%) as a colorless oil IR υ_{max} (neat) 1720, 1580, 1460 cm^{-1}, ^1H NMR δ 2 26 (3H, s), 3 43 (3H, s), 3 86 (3H,s), 5 13 (2H, s), 6 79 (1H, d, J = 8Hz), 6 92 (1H, d, J = 8Hz), 7 23 (1H, t, J = 8Hz) High mass calcd for $C_{11}H_{14}O_4$ 210 0892 Found 210 0895

Methyl 2-O-methoxymethyl-6-trimethylsilylmethylsalicylate (17c) To a stirred solution of diisopropylamine (0 5 ml, 3 5 mmol) and TMEDA (0 38 ml, 2 5 mmol) in THF (4 ml) was added n-BuLi (1 59 M solution in n-hexane, 2 2 ml, 3 5 mmol) at -74°C under argon After the mixture was stirred at -74°C for 0 5 h, the methyl salicylate **17a** (630 mg, 3 mmol) in THF (2ml) was added and the mixture was kept at this condition for 1 h with stirring A mixture of chlorotrimethylsilane (0 95 ml, 7 5 mmol) and triethylamine (0 1 ml, 0 75 mmol) in THF (2 ml) was added dropwise After being stirred at this temperature for 4 5 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (10 ml) and water (10 ml) and allowed to

warm to ambient temperature The whole was extracted twice with ether (100 ml) and the organic layer was washed with saturated brine (30 ml), dried over Na_2SO_4, and concentrated in vacuo to give a yellow oil Purification of the residue by bulb-to-bulb distillation gave the silylated product **17c** (818 mg, 97%) as a colorless oil bp 95-105°C at 1 mmHg, IR υ_{max} (neat) 1730, 1580, 1260 cm^{-1}, ^1H NMR δ 0 (9H, s), 2 10 (2H, s), 3 46 (3H, s), 3 89 (3H, s), 5 13 (2H, s), 6 56, 6 69 (2H, d, J = 8Hz), 7 17 (1H, t, J = 8Hz) High mass calcd for $C_{14}H_{22}O_4Si$ 282 1287 Found 282 1256

Methyl 2-O-methoxymethyl-6-trimethylstannylmethylsalicylate (17d). To a stirred solution of LDA (3 5 mmol) and TMEDA (2 5 mmol) in THF (4 ml), prepared as in the preparation of **17c**, was added dropwise the methyl salicylate **17a** (630 mg, 3 mmol) in THF (4 ml) at -75°C and the reaction mixture was kept at the same conditions with stirring A solution of trimethylstannyl chloride (725 mg, 3 6 mmol) in THF (4 ml) was added dropwise After being stirred at -78°C for 2 h and at room temperature for 2 h, the reaction mixture was concentrated in vacuo Chromatographic purification of the residue followed by bulb-to-bulb distillation gave the stannane **17d** (916 mg, 82%) as a colorless oil bp 130-135°C at 0 4 mmHg, IR υ_{max} (neat) 1720, 1600, 1460 cm^{-1}, ^1H NMR δ 0 03 (9H, s), 2 2(2H, s), 3 33 (3H, s), 3 73 (3H,s), 5 03 (2H, s), 6 56 (1H, t, J = 8Hz) High mass calcd for $C_{14}H_{22}O_4Sn$ 374 0539 Found 374 0539

tert-Butoxycarbonyl-(S)-leucinal (7b) Prepared by our method[13] and purified by bulb-to-bulb distillation bp 85°C at 0 5 mmHg, $[\alpha]_D^{21}$+17 55° (c 0 99, CH_2Cl_2)

(3S)-3-[(1S)-N-tert-Butoxycarbonylamino-3-methylbutyl]-8-methoxymethyloxy-3,4-dihydroxyisocoumarin (18b). LDA (1 4 mmol) was prepared by the addition of n-BuLi (1 45 M solution in n-hexane, 1 ml, 1 4 mmol) to a solution of diisopropylamine (0 2 ml, 1 4 mmol) and TMEDA (0 2 ml, 1 4 mmol) in THF (9 ml) at 0°C and allowed to cool to -75°C A solution of the methyl salicylate **17a** (210 mg, 1 mmol) in THF (1 ml) was added dropwise via cannula to this solution and the mixture was stirred at -75°C for 1 hr Then a solution of tert-butoxycarbonyl-(S)-leucinal (301 mg, 1 4 mmol) in THF (1 ml) was added dropwise via cannula and the resulting solution was kept at the same conditions for 3 h with stirring After being quenched with saturated aqueous ammonium chloride (5 ml), the mixture was allowed to warm to ambient temperature, diluted with water (5 ml), and extracted with ether (50 ml x 2) The organic layer was washed with saturated brine (20 ml), dried over Na_2SO_4, filtered, and concentrated in vacuo to leave a yellow oil (367 mg) The crude material was chromatographed on silica gel (15 g) using EtOAc-n-hexane (1 5) to give the crude product (190 mg) as a mixture of diastereomers Further purification on silica gel plate (Merck Art 5744) using EtOAc-n-hexane (1 2) gave the desired (S,S)-isomer **18b** (73 mg, 19%) along with the (S,R)-isomer **19b** (56 mg, 14%) **18b** $[\alpha]_D^{25}$-92 8° (c 1 13, CH_2Cl_2), IR υ_{max} (neat) 3350, 1732, 1715, 1705, 1520, 1507, 1254, 1156, 1049, 1022 cm^{-1}, ^1H NMR δ 0 96 (6H, d, J = 6Hz), 1 48 (9H, s), 1 28-1 76 (3H, m), 2 68-3 28 (2H, m), 3 56 (3H, s), 3 80-4 08 (1H, m), 4 80-4 92 (1H, m), 5 34 (2H, dd, J = 8Hz, 7Hz), 6 92 (1H, d, J = 8Hz), 7 16 (1H, d, J = 8Hz), 7 48 (1H, t, J = 8Hz) High mass calcd for $C_{21}H_{31}NO_6$ 393 2151 Found 393 2139 **19b** IR υ_{max} (neat) 3360, 1732, 1717, 1700, 1522, 1509, 1266, 1238, 1157, 1042 cm^{-1}, ^1H NMR δ 0 96 (6H, d, J = 6Hz), 1 43 (9H, s), 1 23-2 13 (3H, m), 2 8-3 13 (2H, m), 3 5 (3H, s), 3 83-4 13 (1H, m), 5 23 (2H, s), 6 8 (1H, d, J = 8Hz), 7 07 (1H, d, J = 8Hz), 7 36 (1H, t, J = 8Hz) High mass calcd for $C_{21}H_{31}NO_6$ 393 2151 Found 393 2066

(3S)-3-[(1S)-Amino-3-methylbutyl]-8-hydroxy-3,4-dihydroisocoumarin hydrochloride (2•HCl) i) From **18a**---The dihydroisocoumarin **18a** (116 mg, 0 32 mmol) was dissolved in CH_2Cl_2 (50 ml) and cooled to -75°C Boron tribromide (0 25 ml, 2 6 mmol) in CH_2Cl_2 (5 ml) was added dropwise and the reaction mixture was kept at this temperature, allowed to warm to ambient temperature, and stirred for 18 h The reaction mixture was extracted three times with water (25 ml) and the

aqueous layer was condensed in vacuo The residue was treated with saturated aqueous $NaHCO_3$ (10 ml) and extracted twice with $CHCl_3$ (50 ml) The organic layer was dried over Na_2SO_4, filtered, concentrated in vacuo to leave a light brown oil (61 mg) which was treated with 10% HCl-MeOH (1 ml) to form the hydrochloride (63 mg, 69%) as light brown crystals The crystals were recrystallized from EtOH-Et_2O mp 206-207°C (lit [5] mp 210°C from water), $[\alpha]_D^{22}$-47 42° (c 0 11, MeOH) (authentic sample $[\alpha]_D^{22}$-47 45°(c 0 11, MeOH)), IR υ_{max} (KBr disc) 1680 cm^{-1}, ^1H NMR (DMSO-d_6) δ 0 92 (6H,d, J = 6Hz), 1 48-1 92 (3H,m), 2 48-2 56 (2H, m), 3 12-3 42 (1H,m), 4 76-4 96(1H, m), 6 84, 6 90 (2H, d, J = 8Hz), 7 42 (1H, t, J = 8Hz), 8 42 (3H, br, disappeared with D_2O) Anal calcd for $C_{14}H_{19}NO_3$•HCl C, 58 84, H, 7 05, N, 4 90 Found C, 58 77, H, 6 93, N, 4 95

ii) From **18b**---The protected **18b** (60 mg, 0 15 mmol) was dissolved in 10% hydrogen chloride-MeOH at room temperature and the mixture was stirred for 4 h Removal of the volatiles gave the title compound **2**•HCl as pale yellow crystals which were recrystallized from EtOH-Et_2O This compound was identical with the authentic sample

(3R)-3-[(1S)-Amino-3-methylbutyl]-8-hydroxy-3.4-dihydroisocoumarin hydrochloride (20•HCl) This compound was prepared as in preparation of **2•HCl** from **18a** in 63% yield and recrystallized from EtOH-Et_2O mp 202-203°C, $[\alpha]_D^{22}$+34 89°(c 0 1, MeOH), IR υ_{max} (KBr disc) 1680 cm^{-1}, ^1H NMR (DMSO-d_6) δ 0 80 (3H, d, J = 6Hz), 0 92 (3H, d, J = 6Hz), 1 28-1 80 (3H, m), 2 44-2 52 (m), 3 08-3 52 (m), 4 96-5 10 (1H, m), 6 88-6 96 (2H, m), 7 56 (1H, t, J = 8Hz), 8 70 (3H, broad, disappeared with D_2O), 10 76 (1H, broad, disappeared with D_2O)

Methyl N-benzylsulfonyl-(S)-leucinate Methyl (S)-leucinate hydrochloride (1 82g, 10 mmol) was suspended in CH_2Cl_2 and cooled in an ice-bath Then, benzylsulfonylchloride (2 1g, 11 mmol) followed by triethylamine (1 6ml, 20 mmol) was added and the reaction mixture was stirred at ice-bath temperature for 1 h and at ambient temperature for 18 h After filtration of the resulting salts, the filtrate was washed twice with water (20ml), dried over Na_2SO_4, filtered, and concentrated in vacuo to give the title compound (2 78g, 94%) as a colorless oil after chromatographic purification (BW-200, 100g, EtOAc-n-hexane = 1 4) IR υ_{max} (neat) 3250, 2950, 1740, 1330 cm^{-1} ^1H NMR δ 0 89 (6H, d, J = 6Hz), 1 33-1 76 (3H, m), 3 66 (3H, s), 3 66-4 00 (1H, m), 4 25 (2H, s), 4 94 (1H, broad d, J = 9Hz), 7 33 (5H, s) High mass calcd for $C_{14}H_{21}NO_4S$ 299 1191 Found 299 1172

N-Benzylsulfonyl-(S)-leucinal(7c) Prepared by reduction of methyl N-benzylsulfonyl-(S)-leucinate in CH_2Cl_2 with diisobutylaluminum hydride in hexane in 86% yield **7c** colorless oil, $[\alpha]_D^{25}$+14 35°(c 4 5, CH_2Cl_2), IR υ_{max} (neat) 3250, 2950, 1730, 1320 cm^{-1}, ^1H NMR δ 0 89 (6H, d, J = 6Hz), 1 40-1 78 (3H, m), 3 53-3 86 (1H, m), 4 26 (2H, s), 5 50 (2H, d, J = 9Hz), 7 33 (5H, s), 9 40 (1H, s)

(3S)-3-[(1S)-N-Benzylsulfonylamino-3-methylbutyl]-8-methoxy-3,4-dihydroxy-isocoumarin (22) N-Benzylsulfonyl-S-leucinal (**7c**) (27mg, 0 1 mmol) was dissolved in THF (1 ml), cooled to -75°C and treated with n-BuLi (67μl, 0 1 mmol) at -75°C for 20 min and at -20°C for 5min Then, the benzyl lithium **17b** (0 1 mmol), prepared as in the preparation of **17c**, in THF at -75°C was added dropwise via cannula to the above N-lithium aldehyde After being stirred for 3 h at the same conditions, the reaction mixture was quenched with saturated aqueous ammonium chloride (2 ml) and water (5 ml) The whole was extracted twice with ether (each 30 ml) The organic layer was dried over Na_2SO_4 and concentrated in vacuo The residue was purified on a preparative thin layer chromatography (Merck Art 5744, EtOAc-n-hexane = 1 2) to give the title compound **22** (14 mg, 30%) as a pale yellow oil IR υ_{max} (neat) 3350, 1720, 1320 cm^{-1} ^1H NMR δ 0 90-0 96 (6H, m), 1 28-1 80 (3H, m), 2 36 (2H, m), 3 46 (3H, s),

4 28-4 44 (3H,m), 5 18 (2H, s), 5 24-5 40 (1H, m), 6 96 (1H, d, J = 8Hz), 7 08 (1H, d, J =8Hz), 7 32 (1H, t, J = 8Hz), 7 44 (5H, s) FAB mass for $C_{23}H_{29}NO_6S$ (glycerin) m/z (M+1) = 448.

(R)-Pyroglutaminol To a stirred suspension of (R)-glutamic acid (38 4 g, 0.261 mol) in EtOH (375 ml) at 0°C was added dropwise thionyl chloride (45 ml, 0 617 mol) The reaction mixture was allowed to warm at ambient temperature, stirred for 1 h, and then heated to reflux for 30 min After cooling, the mixture was concentrated in vacuo, dissolved in a small amount of EtOH, neutralized with saturated aqueous potassium carbonate, and extracted three times with chloroform-ether (1·1, 250 ml) The organic layer was removed in vacuo to leave the crude diethyl glutamate as an oil which was heated to 150°C at 15 mmHg for 1 5 h Ethyl pyroglutamate (32 8 g) thus obtained was used for the next reduction without further purification

The crude ethyl pyroglutamate was dissolved in EtOH (300 ml), cooled to 0°C, and treated with sodium borohydride (7 9 g, 0 21 mol) After being stirred at room temperature for 2 h, the reaction mixture was cooled in an ice-MeOH bath, acidified with concentrated hydrochloric acid, concentrated in vacuo, and filtered to leave the title compound (33 g) as a crude oil Chromatographic purification (BW-200, 350 g, EtOAc-EtOH = 4 1) gave the (R)-pyroglutaminol (23 7 g, 79%) as colorless crystals mp 70-73°C (lit [21] mp 71-73°C), IR υ_{max} (neat) 3300, 1660, 1420, 1280 cm^{-1}

(2S,5R)-2-Phenyl-1-aza-3-oxabicyclo[3.3.0]octan-8-one (23) A mixture of (R)-pyroglutaminol (22 g, 0 191 mol), benzaldehyde (23 5 g), and p-toluenesulfonic acid (0 45 g) in toluene (140 ml) was heated to reflux with vigorous stirring and water formed was azeotropically removed by using a Dean- Stark water separator with molecular sieves 4 A (20 g) After cooling, the reaction mixture was washed with 5% $NaHCO_3$ (50 ml x 2), saturated aqueous sodium sulfite (50 ml x 2), water (50 ml x 2), and saturated brine (50 ml x 1), dried over Na_2SO_4, filtered, and concentrated in vacuo The resulting brown oil was purified by distillation (150°C at 0 1 mmHg) to give the bicyclic lactam **23** (20 g, 72%) as a yellow oil $[\alpha]_D^{22}$-252 2° (c 1, $CHCl_3$) (lit [17] (S)-enantiomer $[\alpha]_D^{22}$+269 6° (c 1, $CHCl_3$)) This material was identical with the reported (S)-enantiomer by IR, ^1H NMR, and ^{13}C NMR spectra

(2S,5R)-2-Phenyl-1-aza-3-oxabicyclo[3.3.0]oct-6-en-8-one (25) To a stirred solution of LDA (14 mmol, prepared by treatment of diisopropylamine (2 0 ml, 14 3 mmol) in THF (40 ml) with 1 6 M butyllithium (9 0 ml) in n-hexane) at -70°C under argon was added dropwise a solution of the benzylidene acetal **23** (2 28 g, 11 2 mmol) in THF (4 ml) and the solution was stirred for 30 min To the resulting enolate was rapidly added a solution of phenylselenyl bromide (freshly prepared by treatment of diphenyldiselenide (2 1 g, 6 7 mmol) in THF (4 ml) with bromine (0 35 ml, 6 6 mmol) at 0°C under argon) in THF After 15min, the reaction mixture was poured into a mixture of 0 5 N HCl (50 ml) and ether-pentane (1 1, 50 ml) The organic layer was separated, washed with water (50 ml x 1), saturated aqueous $NaHCO_3$ (50 ml x 1), and saturated brine (50 ml x 1), and dried over Na_2SO_4 Filtration and evaporation gave the selenide **24** (4 15 g) as a yellow oil which was used for the next step without purification

A solution of the above crude selenide **24** (4 14 g) in CH_2Cl_2 (50 ml) was cooled to -74°C and ozonized (O_2, 2 Kg/cm^3, 60 V 1 hr→80 V 1 hr) until the colorless solution turned to pale blue in color For removal of excess ozone, argon gas was bubbled through the solution After addition of pyridine (1 9 ml), the reaction mixture was allowed to warm to ambient temperature and poured into a mixture of 7% aqueous $NaHCO_3$ (30 ml) and CH_2Cl_2 (50 ml) The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (30 ml x 3) The combined organic layer was washed with 10% HCl (30 ml x 1) and saturated brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give the α,β-unsaturated lactam **25** (3 4 g) as a brown oil This crude material was purified by chromatography (silica gel, EtOAc-n-hexane = 1 1) to

yield the α,β–unsaturated lactam **25** (1 69 g, 75% from **23**) as a pale yellow solid: mp 85-86°C, $[\alpha]_D^{22}$-215 6° (c 1 05, $CHCl_3$), IR υ_{max} (neat) 2850, 1705, 1490, 1445, 1330, 1225 cm^{-1}, ^1H NMR δ 3 38 (1H, t, J = 8Hz), 4.22 (1H, t, J = 8Hz), 4 58 (1H, dt, J = 3Hz, 8Hz), 6 13 (1H, dd, J = 3Hz, 6Hz), 6.18 (1H, s), 7 24 (1H, dd, J = 3Hz, 6Hz), 7 40 (5H, m), ^{13}C NMR δ 61 135, 65 113, 87 344, 126 132, 128 355, 128 940, 129 583, 138 651, 148 07, 176 912 High mass calcd for $C_{12}H_{11}NO_2$· 201 0789 Found 201 0794

(2S,5R,6S,7S)-2-Phenyl-6,7-dihydroxy-1-aza-3-oxabicyclo[3.3.0]octan-8-one (26) To a stirred solution of the α,β–unsaturated lactam **25** (637 mg, 3 1 mmol) in water and acetone (1 8, 27 ml) was added N-methylmorphline N-oxide (742 mg, 6 3 mmol) and then 0 1M OsO_4 in t-BuOH (3 1 ml, 0 31 mmol) under cooling by a water bath After being stirred at room temperature for 15 hr, the reaction mixture was poured into saturated aqueous $NaHSO_3$ (30 ml) with ice-cooling and vigorously stirred for 15 min The whole was extracted with EtOAc (50 ml x 4) The organic layer was washed with saturated brine (100 ml x 1), dried over Na_2SO_4, and concentrated in vacuo to give the cis-diol **26** (964 mg) as a pale yellow oil The material was purified by chromatography (silica gel, EtOAc-n-hexane = 9 1) to afford the cis-diol **26** (482 mg, 65%) as a white solid mp 164-166°C, $[\alpha]_D^{25}$-221° (c 1 14, MeOH), IR υ_{max} (KBr disc) 3280, 2900, 1680, 1410, 1350, 1100, 1030, 920 cm^{-1}, ^1H NMR ($CDCl_3$-DMSO-d_6) δ 3 72 (1H, dd, J = 6Hz, 8Hz), 4 00-4 50 (4H, m), 4 60 (1H, br, disappeared with D_2O), 5 72 (1H, br, disappeared with D_2O), 6 28(1H, s), 7 40 (5H, m), ^{13}C NMR ($CDCl_3$-DMSO-d_6) δ 64 674, 69 402, 71 498, 74 471, 86 801, 126 035, 128 375, 128 618, 137 927, 175 114 Anal calcd for $C_{12}H_{13}NO_4$ C, 61.27, H 5 57, N, 5 95 Found C, 60 81, H, 5 55, N, 5 80

(2S,5R,6S,7S)-2-Phenyl-6,7-(isopropylidene)dioxy-1-aza-3-oxabicyclo[3.3.0]octan-8-one (28) To a stirred solution of the diol **26** (1 1 g, 4 6 mmol) in acetone (15 ml) at room temperature was added 2,2-dimethoxypropane (3 ml, 24 6 mmol) and PPTS (10 mg, 0 04 mmol) and the reaction mixture was stirred for 10 h. After removal of the solvent, the crude mixture was purified by chromatography (silica gel, EtOAc-n-hexane = 1 1) to give the acetonide **28** (1 26 g, 98%) as a colorless oil IR υ_{max} (neat) 3400, 1720, 1380, 1220, 1100 cm^{-1}, ^1H NMR δ 1 40 (3H, s), 1 53 (3H, s), 3 42 (1H, dd, J = 8Hz, 10Hz), 4 0-5 0 (4H, m), 6 32 (1H, s), 7 40 (5H, br s) Anal calcd for $C_{15}H_{17}NO_4$ C, 65 44, H, 6 22, N, 5 09 Found C, 65 43, H, 6 35, N, 4 98

(3S,4S,5S)-3,4-(Isopropylidene)dioxy-2-hydroxymethylpyrrolidin-1-one (29) To a stirred solution of the acetonide **28** (865 mg, 3 mmol) in MeOH (60 ml) at room temperature was added 5% Pd-C (875 mg) and 100% N_2H_4•H_2O (3 ml) The suspension was heated at reflux with vigorously stirring for 30 min The catalyst was filtered and washed with MeOH The combined filtrates were evaporated in vacuo and the residue was purified by chromatography (silica gel, EtOAc-EtOH = 5 1) to give the lactam alcohol **29** (558 mg, 95%) as a white solid mp 141-142°C, $[\alpha]_D^{24}$+46 7° (c 1 03, MeOH), IR υ_{max} (neat) 3300, 1705, 1385, 1275, 1085 cm^{-1}, ^1H NMR δ 1 37 (3H, s), 1 45 (3H, s), 3 70 (3H, m), 4 50 (1H, br s), 4 62 (2H, m), 7 70 (1H, br s) Anal calcd for $C_8H_{13}NO_4$ C, 51 33, H, 7 00, N, 7 48 Found C, 51 12, H, 7 14, N, 7 42

(3S,4S,5S)-3,4-(Isopropylidene)dioxy-2-cyanomethyl-pyrrolidin-1-one (30) To a stirred suspension of potassium cyanide (130 mg, 2 mmol), the lactam alcohol **29** (187 mg, 1 mmol), and 18-crown-6 (26 mg, 0 1 mmol) in CH_3CN under argon at 0-5°C was added dropwise a solution of carbon tetrachloride (169 mg, 1 1 mmol) in CH_3CN (1 ml) The reaction mixture was stirred at 30-40°C for 15 h The additional carbon tetrachloride (85 mg, 0 55 mmol) was added to the mixture, and the solution was stirred at 70-80°C for 2 h After cooling, the solvent was removed in vacuo and the crude product was

purified by chromatography (silica gel, 40 g, EtOAc-EtOH = 10 1, then EtOAc-EtOH = 4 1) to afford the cyanide **30** (139 mg, 95%) as a pale brown oil IR υ_{max} (neat) 3250, 2940, 1720, 1380, 1220, 1100, 760 cm^{-1}, ^1H NMR δ 1 37 (3H, s), 1 40 (3H, s), 2 63 (2H, d, J = 5Hz, 3.97 (1H, m), 4 50-4 75 (2H, m), 7 57 (1H, br s) This material was crystallized as colorless needles mp 207-208°C, $[\alpha]_D^{26}$+42° (c 0 84, MeOH) Anal calcd for $C_9H_{12}N_2O_3$ C,55 08, H, 6 18, N, 14 28 Found C, 55,05, H, 6 20, N, 14 04

(3S,4S,5S)-N-(tert-Butoxycarbonyl)-3,4-(isopropylidene)dioxy-2-cyanomethyl-pyrrolidin-1-one (32) To a stirred solution of the cyanide **30** (54 mg, 0 27 mmol) and DMAP (3 mg, 0 02 mmol) in CH_3CN (3 ml) was added a solution of di-tert-butyl dicarbonate (68 mg, 0 31 mmol) in CH_3CN (2 ml) After being stirred at room temperature for 1h, the mixture was diluted with EtOAc, washed with 10% aqueous citric acid (10 ml x 1), water (10 ml x 1), and saturated brine (10 ml x 1), and dried over Na_2SO_4 The solvent was removed in vacuo and the residue was purified by chromatography (silica gel, 4 g, CH_2Cl_2-EtOAc = 9 1) to give the lactam **32** (75 mg, 92%) as a colorless oil IR υ_{max} (neat) 2900, 2350, 1800, 1765, 1730, 1380, 1310, 1180 cm^{-1}, ^1H NMR δ 1 34 (3H, s), 1 40 (3H, s), 1 49 (9H, s), 2 84 (2H, d, J = 6Hz), 3 84-4 55 (2H, m), 4 81 (1H,d, J = 6Hz) High mass calcd for $C_{14}H_{20}N_2O_5$ 296 1372 Found 296 1398

(2S,3S,4S)-2,3-(isopropylidene)dioxy-4-(N-tert-butoxycarbonyl)amino-5-cyano-pentanoic acid (4). To a stirred solution of the lactam **32** (75 mg, 0 25 mmol) in THF-H_2O (7 3, 10 ml) at ice-bath temperature was added a solution of $LiOH{\bullet}H_2O$ (32 mg, 0 75 mmol) in water (0 7 ml) After 30 min, the solvent was removed and the residue was diluted with water (4 ml) The solution was acidified with 10% aqueous citric acid and extracted four times with ether (20 ml) The organic layer was washed with saturated brine (10 ml x 1), dried over Na_2SO_4, and concentrated in vacuo to give the hydroxy amino acid **4** (80mg, 70%) as a white solid IR υ_{max} (neat) 3700-2500 (br), 2350, 1720 (br), 1530cm^{-1}, ^1H NMR δ 1 38 (3H, s), 1 43 (9H, s), 1 57 (3H, s), 2 80 (2H, m), 4 0 (1H, br s), 4 57(1H, t, J = 7 3Hz), 4 71 (1H, d, J = 6 6Hz), 5 31 (1H, br d, J = 8 1Hz), 6 20-7 30 (1H, br) Anal calcd for $C_{14}H_{22}N_2O_6$ C, 53 48, H, 7 07, N, 8 91 Found C, 53 43, H, 6 95, N, 8 65

The protected AI-77-B 33 To a stirred solution of the dihydroisocoumarin **2**•HCl (20 mg, 0 07 mmol) and the hydroxy amino acid **4** (18 mg, 0 058 mmol) in DMF (0 4 ml) with an ice-bath were added DEPC (11 μl, 0 074 mmol) and Et_3N (26 μl, 18 mmol) under argon After 3h, the solution was allowed to warm to ambient temperature and stirred at room temperature overnight Then a solution of additional DEPC (4 μl, 0 02 mmol) in DMF (0 2 ml) was added to the mixture at room temperature After 1 h, additional Et_3N (12 μl, 0 08 mmol) was added to the solution The mixture was stirred for 11 h at room temperature The reaction was diluted with PhH-EtOAc (2 1, 45 ml), washed with 10% aqueous citric acid (10 ml x 1), and dried over Na_2SO_4 The solvent was removed in vacuo to give the crude product **33** (59 mg) as a pale yellow oil The residue was purified on preparative TLC ($CHCl_3$-EtOAc = 2 1) to afford the protected AI-77-B **33** (22 mg,70%) as an oil IR υ_{max} (solution in $CHCl_3$) 2900, 1710 (s), 1665, 1620, 1470, 1200, 1160, 1110, 920 cm^{-1}, ^1H NMR δ 0 97 (3H, d, J = 2 9Hz), 0 99 (3H, d, J = 3Hz), 1 38 (3H, s), 1 45 (3H, s), 1 77 (1h, m), 2 82 (2H, dd, J = 3Hz, 16 4Hz), 3 0 (1H, dd, J = 12 8Hz, 16 5Hz), 3 83 (1H, br s), 4 28 (1H, m), 4 59 (2H, d, J = 2 5Hz), 4 63 (1H, dt, J = 11 7Hz, 1 9Hz), 5 35 (1H, br s), 6 69 (1H, d, J = 7 3Hz), 6 87 (2H, m), 7 41 (1H, t J = 7Hz, 8 4Hz)

AI-77-B (1) The nitrile **33** (5 4 mg, 0 01 mmol) was dissolved in 5% hydrogen chloride in MeOH (2 ml) under argon with ice-MeOH bath and trimethyl orthoformate (0 05 ml, excess) was added The mixture was stirred for 44 5 h at 5°C Water (3 ml) was added to the solution After being stirred at ambient temperature for 12 h, the solution was diluted with 50% aqueous MeOH (2 ml) and 0 1N NaOH was added

dropwise maintaining at pH 9 with stirring After 3 h, the pH was adjusted to 6 5 with 0 1N hydrochloric acid The mixture was charged on an Amberlite XAD-2 column (10 ml in water) The column was washed with 20% aqueous MeOH (100 ml) and eluted with 80% aqueous MeOH Fractions containing AI-77-B were combined and then evaporated The residue was dissolved in water and purified by column chromatography (Sephadex G-10, 9 ml, H_2O) to afford AI-77-B (**1**) (3 2 mg, 76%) $[\alpha]_D^{22}$-72 2° (c 0 07, MeOH) (authentic sample $[\alpha]_D^{22}$-78 2° (c 0 08, MeOH)) The synthetic material was identical with the natural one by TLC, ^1H NMR and FAB mass spectral comparisons

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