HETEROCYCLES, Vol. 58, 2002, pp. 471-504, Received, 8th April, 2002

TOTAL SYNTHESIS AND ABSOLUTE CONFIGURATION OF RADIOSUMIN, A STRONG TRYPSIN INHIBITOR FROM THE BLUE-GREEN ALGA *PLECTONEMA RADIOSUM*

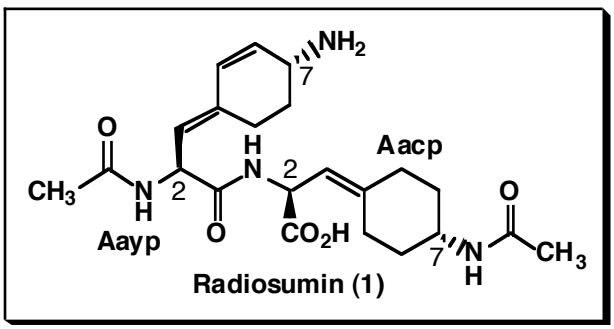
Hirohide Noguchi,† Toyohiko Aoyama,* and Takayuki Shioiri‡*

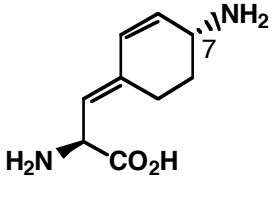
Graduate School of Pharmaceutical Sciences, Nagoya City University
Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

Dedicated to Prof. A. I. Meyers on the occasion of his 70th birthday.

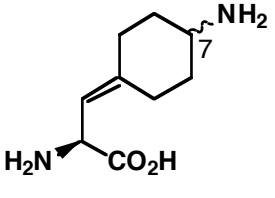
Abstract-Radiosumin (**1**), a strong trypsin inhibitory dipeptide isolated from the freshwater blue-green alga *Plectonema radiosum* (NIES-515), was synthesized for the first time by use of the hetero Diels-Alder reaction, the Horner-Wadsworth-Emmons reaction, the Corey-Winter reaction, regioselective hydrogenation, and reduction with zinc and formic acid as key steps, which unambiguously determined the absolute configuration of the structurally unique and biologically intriguing aquatic natural product (**1**).

Radiosumin was isolated by Murakami and co-workers from the freshwater blue-green alga *Plectonema radiosum* (NIES-515).[1] Its structure was elucidated to be the dipeptide composed of two unusual amino acids: (2*S*,7*R*)-Aayp (2-amino-3-(4-amino-2-cyclohexen-1-ylidene)propionic acid) and (2*S*,7*aR* or *S*)-Aacp (2-amino-3-(4-amino-2-cyclohexylidene)propionic acid). Radiosumin was revealed to be a protease inhibitor, which inhibited trypsin with an IC_{50} of 0.14 μg/mL, plasmin with an IC_{50} of 6.2 μg/mL, but did not inhibit chymotrypsin, elastase, or papain at 200 μg/mL.[1] We have been quite interested in the synthesis of aquatic natural products having unusual amino acids,[2] and the structural curiosity as well as its interesting biological activities stimulated us to synthesize radiosumin.[3]





Aayp



Aacp

Figure 1

We now describe the first total synthesis of radiosumin which clearly identified the configuration at C-7 position of Aacp to be (*aS*) and culminated in the determination of the absolute stereostructure of this interesting molecule to be **1**, as shown in Figure 1.

Before the construction of the whole molecule of radiosumin (**1**), two components, Aayp and Aacp, had to be synthesized by a stereodefined manner. Since these two amino acids have (*S*)-configuration at each C-2 position, use of proteinogenic (*S*)-α-amino acids as starting materials would be feasible. Furthermore, since the absolute configuration at the C-7 position of Aacp remained to be determined, both (*aR*)- and (*aS*)-isomers should be prepared in a convenient way. According to these considerations, we thought that the two (*S*)-alanine synthons (**2**) [4a] or (**3**) would react with the carbonyl compounds (**4** or **5**) or the anion (**6**) to give Aayp or Aacp, as shown in Figure 2.

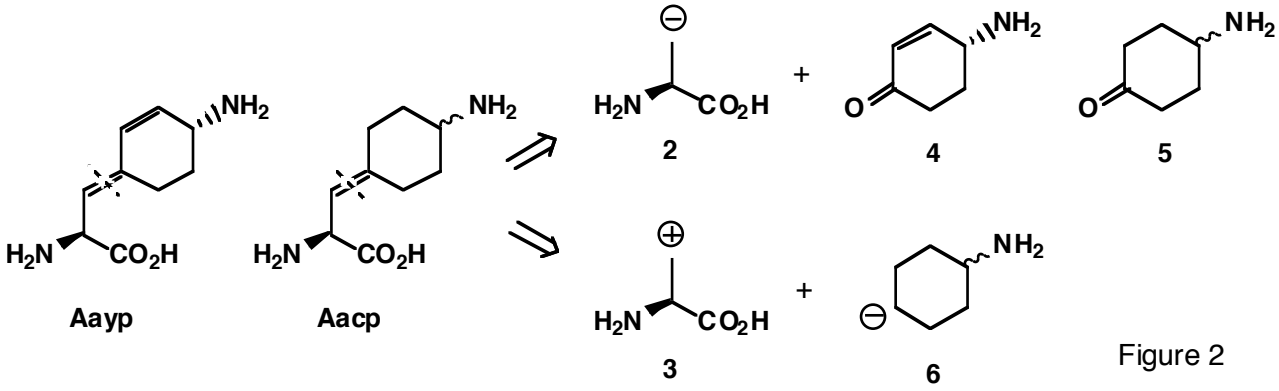
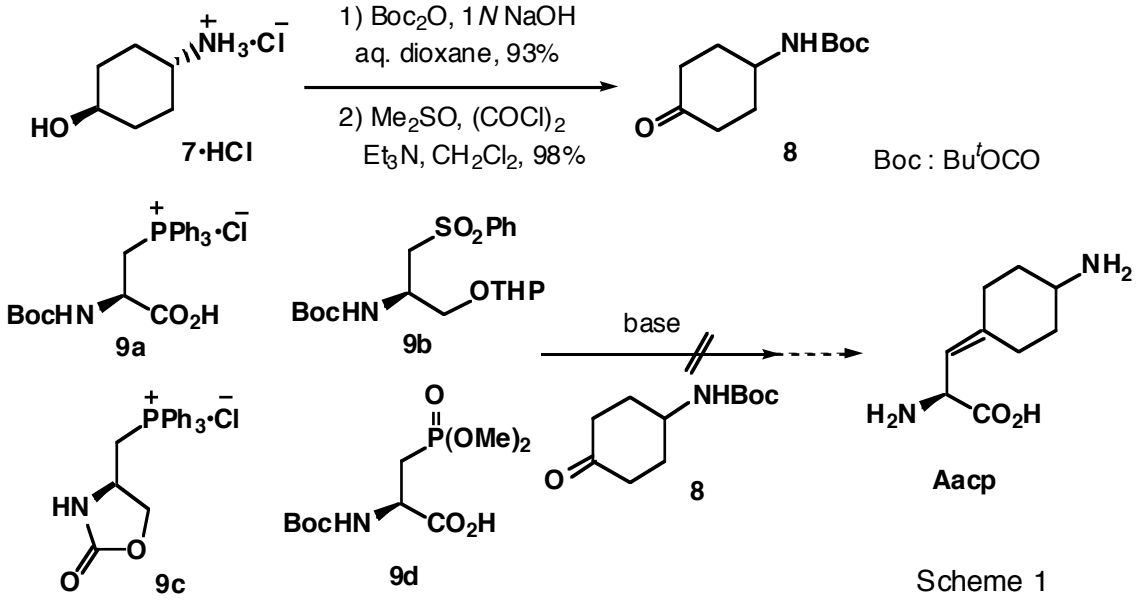


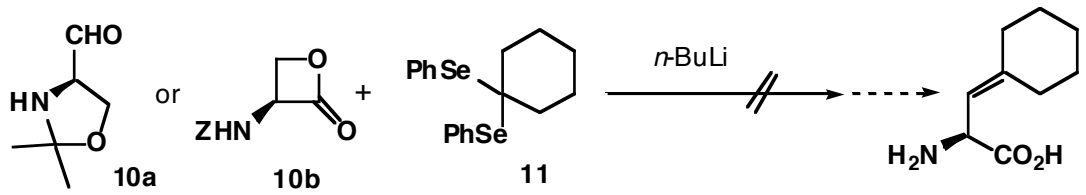
Figure 2

First attempt of the synthesis of Aacp started from 4-*tert*-butyloxycarbonylaminocyclohexanone (**8**), easily prepared from 4-aminocyclohexanol hydrochloride (**7•HCl**), as shown in Scheme 1. However, condensation of **8** with **9a-d**,[4b-e] equivalents of the alanine anion synthon (**2**), mostly resulted in the recovery of the starting materials without the formation of the desired products.



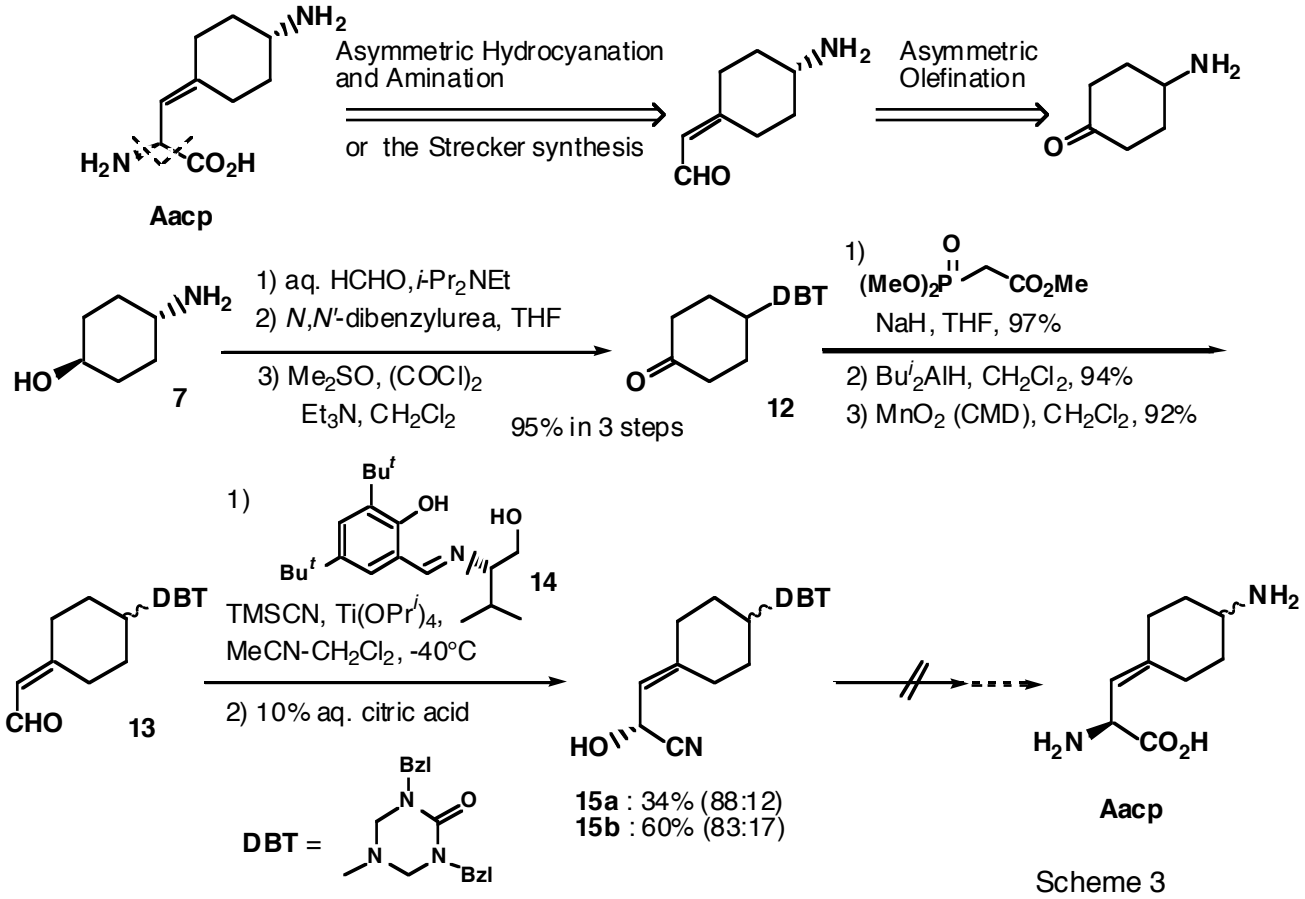
Scheme 1

In another attempt, the alanine cation equivalent (**10a**)[5a,b] or (**10b**) [5c] corresponding to **3** was allowed to react with the lithium salt from the diselenoacetal (**11**),[6] but the reaction took a complicated course. Thus the direct use of (*S*)-α-amino acids proved to be fruitless (Scheme 2).



Scheme 2

Next, we designed a synthetic route using an asymmetric olefination[7] followed by the asymmetric hydrocyanation then amination or the Strecker synthesis. Thus, *trans*-4-aminocyclohexanol (**7**) was first converted to the DBT-ketone (**12**),[8] which was transformed to the α,β-unsaturated DBT-aldehyde (**13**) by the Horner-Wadsworth-Emmons reaction, reduction with diisobutylaluminum hydride, followed by oxidation with chemical manganese dioxide (CMD),[9] as shown in Scheme 3. The racemic DBT-aldehyde (**13**) thus obtained underwent the asymmetric catalytic hydrocyanation by use of Oguni's protocol.[10] The desired cyanohydrin was obtained in 94% yield as a diastereoisomeric mixture of **15a** and **15b** with enantiomeric ratio of ca. 8 : 1 by use of the Schiff base (**14**) as a chiral catalyst. The enantiomeric ratio of each diastereomer was determined by ^{1}H NMR spectra of the (*S*)-MTPA esters of **15a** and **15b**. However, conversion of the cyanohydrins (**15a**, **b**) to the corresponding amino compounds could not be brought about. The attempted Strecker synthesis[11] by use of the aldehyde (**13**) was also unsuccessful.



Scheme 3

Figure 3 shows the alternative approaches, routes A and B, to Aacp. The route A might enable construction of the stereogenic centers at both the C-2 and C-7 positions by isomerization of the double bond when

asymmetric hydrazination is carried out.[12] In the route B, the stereogenic center at the C-2 position might be stereoselectively introduced.

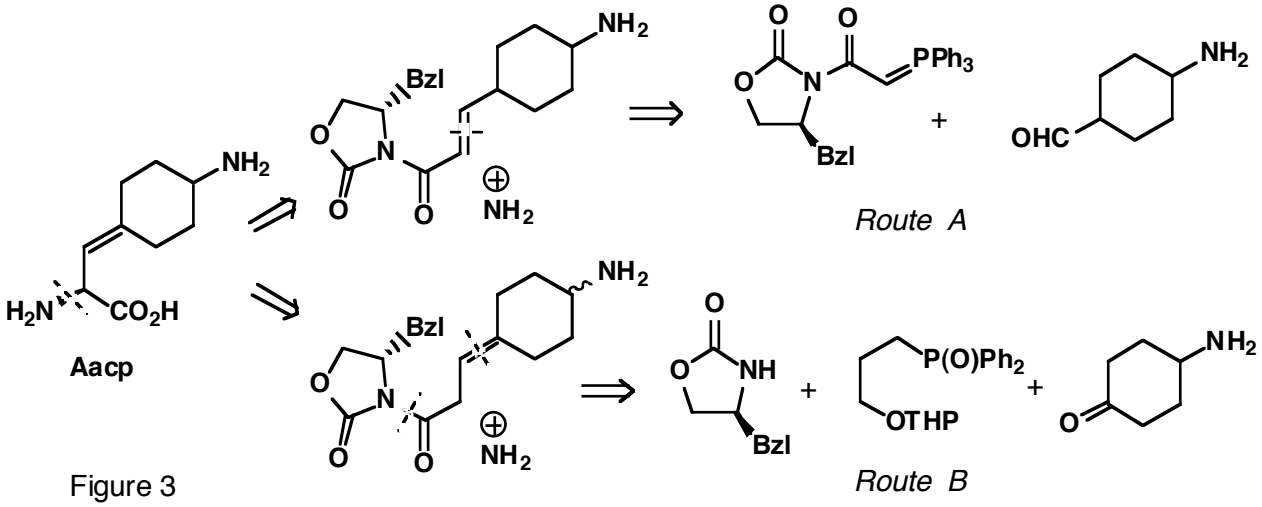
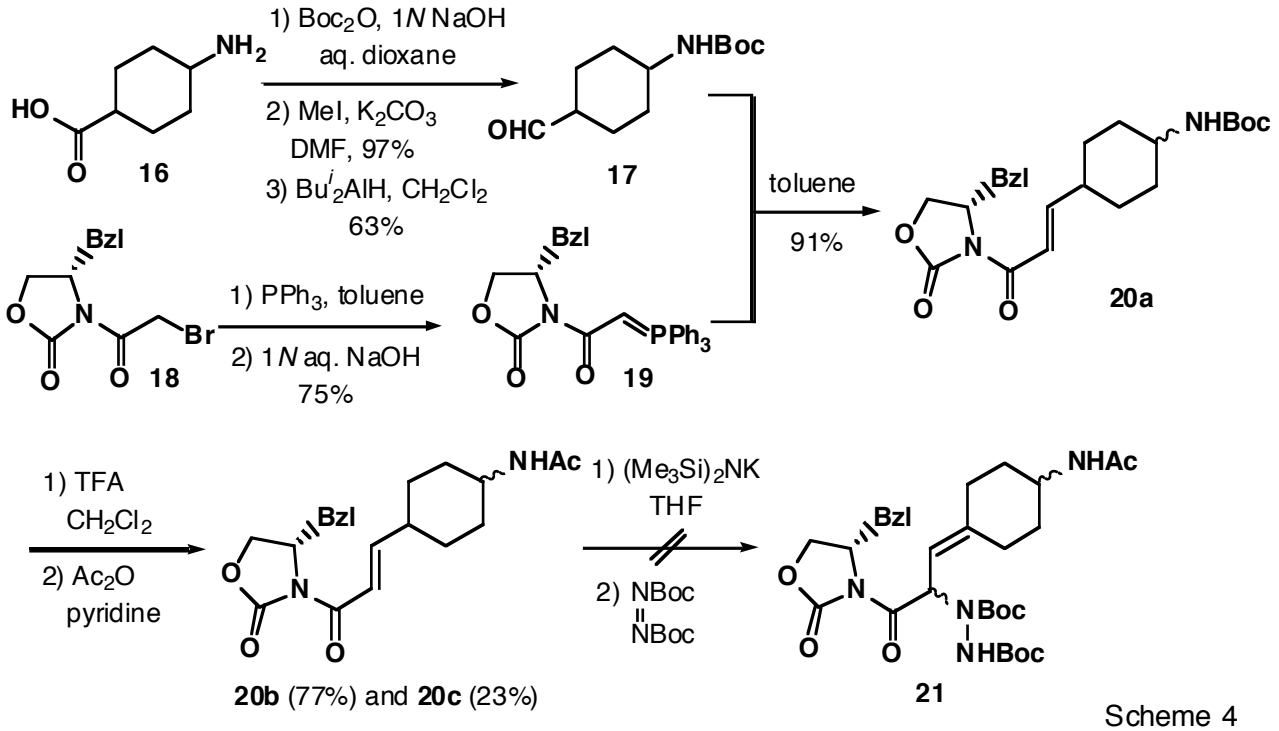


Figure 3

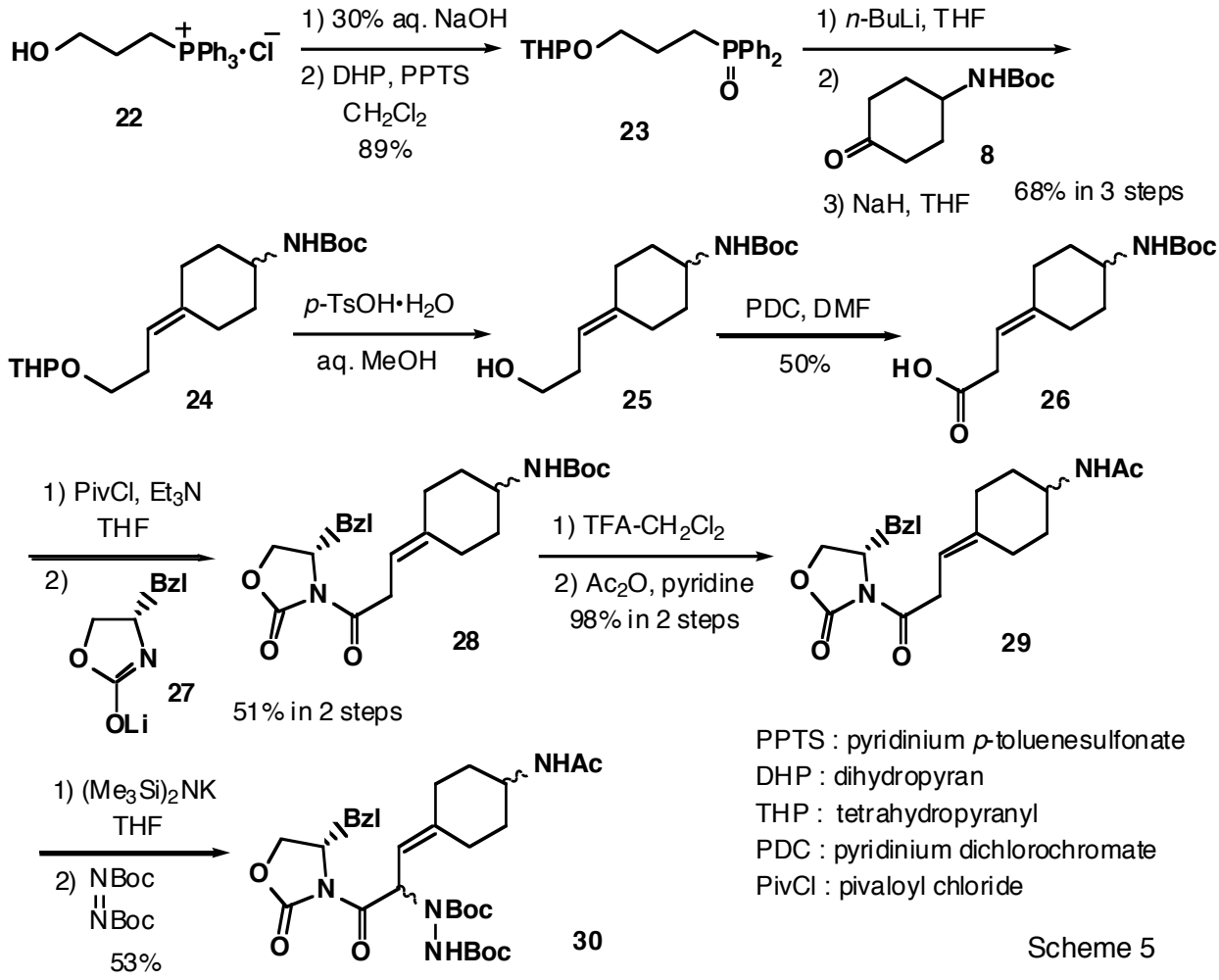
In the route A, the aldehyde (**17**) was first prepared from the amino acid (**16**) by amine protection, methyl esterification, and then reduction with diisobutylaluminum hydride, as shown in Scheme 4. The aldehyde (**17**) reacted with the keto phosphorane (**19**) prepared from the bromide (**18**) to give the coupling product (**20a**) in 91% yield. Boc protected **20a** was transformed to the *N*-acetyl compounds which were separated to furnish **20b** (the less polar isomer) in 77% yield and **20c** (the more polar isomer) in 23% yield. Unfortunately, however, addition of di-*tert*-butyl azodicarboxylate to **20b** under basic conditions failed to give the desired Aacp derivative (**21**).



Scheme 4

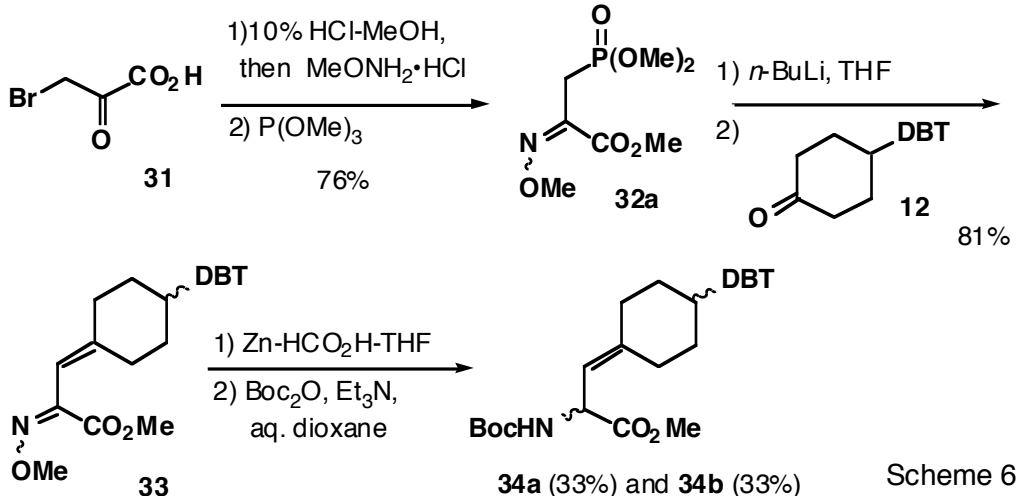
To realize the route B, the β,γ-unsaturated carboxylic acid (**26**) was prepared from the phosphonium salt (**22**) and the ketone (**8**) *via* **23**-**25**, as shown in Scheme 5. Conversion of **26** to the Evans amide (**28**) with the enolate (**27**) followed by replacement of the Boc group with the acetyl moiety afforded the *N*-acetyl Evans

amide (**29**). Addition of di-*tert*-butyl azodicarboxylate to **29** proceeded to give the desired hydrazino derivative (**30**). However, separation of the diastereomers in **30** failed and removal of the chiral auxiliary from **30** was found to be difficult without epimerization at the C-2 stereogenic center. Thus this route again had to be abandoned.



Scheme 5

Next, we investigated the synthesis of racemic β,γ-unsaturated trisubstituted α-amino acids according to the method of Elder and co-workers[13] followed by optical resolution, as shown in Scheme 6. The Elder's phosphonate (**32a**), obtained from bromopyruvic acid (**31**) by oximation and then Arbuzov reaction, was

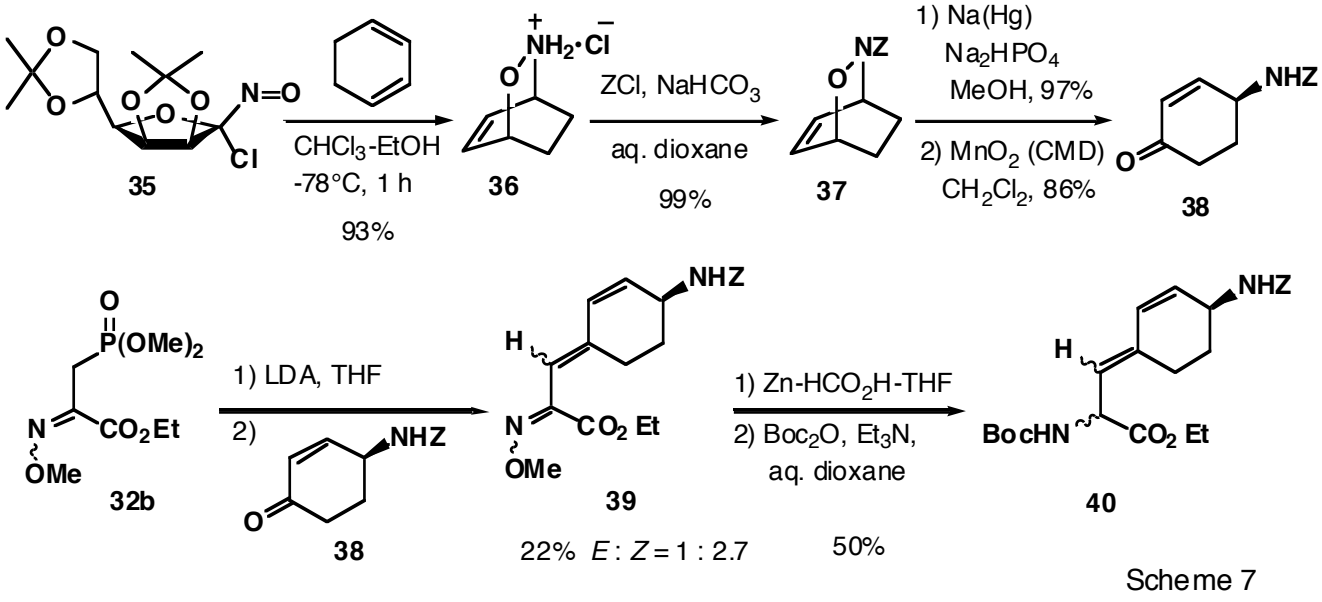


34a (33%) and **34b** (33%)

Scheme 6

lithiated and reacted with the DBT-ketone (**12**) to give the Horner-Wadsworth-Emmons product (**33**). Attempted kinetically controlled optical resolution by AD-mix[14] failed to give the optically active product whereas the asymmetric reduction of the oxime[15] resulted in the formation of an uncharacterized product. Reduction of **33** with zinc and formic acid[13] followed by treatment with Boc_2O afforded **34a** and **34b**, the protected forms of Aacp, in almost equal ratio. After their chromatographic separation, attempted optical resolution of these isomers and their derivatives was unfortunately unsuccessful.

Alternatively, the oxazine (**36**) was prepared from the hetero Diels-Alder reaction of 1,3-cyclohexadiene and the α-nitroso-α-chloro sugar derivative (**35**) according to the literature,[16] shown in Scheme 7. Treatment of **36** with benzyloxycarbonyl chloride (ZCl), reductive cleavage of the N-O bond,[17] followed by oxidation with CMD[9] gave **38**. Coupling of **38** with the Elder's phosphonate (**32b**) was attempted under reaction conditions analogous to the coupling of the methyl ester (**32a**) with the ketone (**12**), giving the ethyl ester (**39**) in lower yield as a mixture of (*E*)- and (*Z*)-isomers in preference of the undesired *Z* isomer. Although the reduction of the oxime function in **39** followed by amine protection with Boc_2O afforded the desired Aayp derivative (**40**), it was revealed to be impossible to separate each isomer in pure state.



Scheme 7

Based on these numerous unsuccessful attempts, we designed the alternative route that first introduced the C-7 amino function in a stereoselective manner, as shown in Figure 4. As an electrophile which would react with the Elder's phosphonate (**32a**), we employed optically active aminocyclitol (**41**), which could be prepared from **42**,[18] the hetero Diels-Alder adduct from the nitroso sugar derivative (**35**) and the diene (**43**) analogously as the conversion of **35** to **36**. The acetonide group in the cyclitol (**41**) was expected to function as followed: (1) protection of the double bond, (2) increase of stereoselectivity and chemical yield during the Horner-Wadsworth-Emmons reaction, and (3) easiness of separation of diastereomers. After preparation of (7*R*)-Aayp, selective reduction of the disubstituted double bond would give (7*aS*)-Aacp. The synthetic works according to this retrosynthetic consideration culminated in the successful synthesis of radiosumin and the determination of its absolute configuration.

Thus, the asymmetric hetero Diels-Alder reaction[16] of the α-chloro-α-nitroso compound (**35**) and *cis*-dihydrocatechol (**43**)[18,19] afforded the adduct (**42**), whose amino group was protected with Boc_2O to give

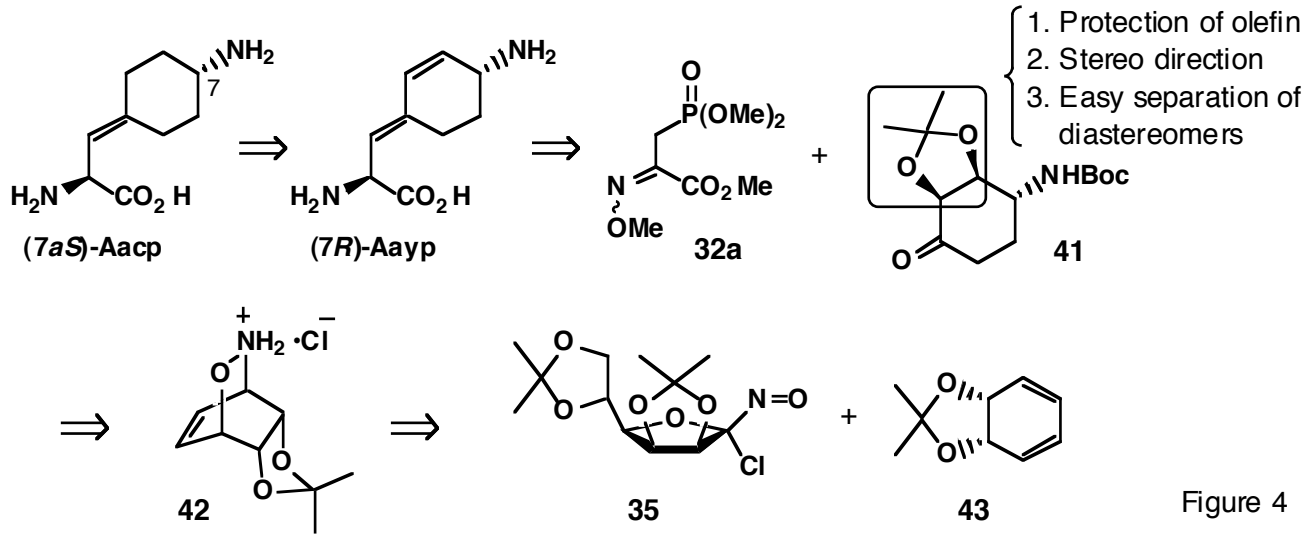
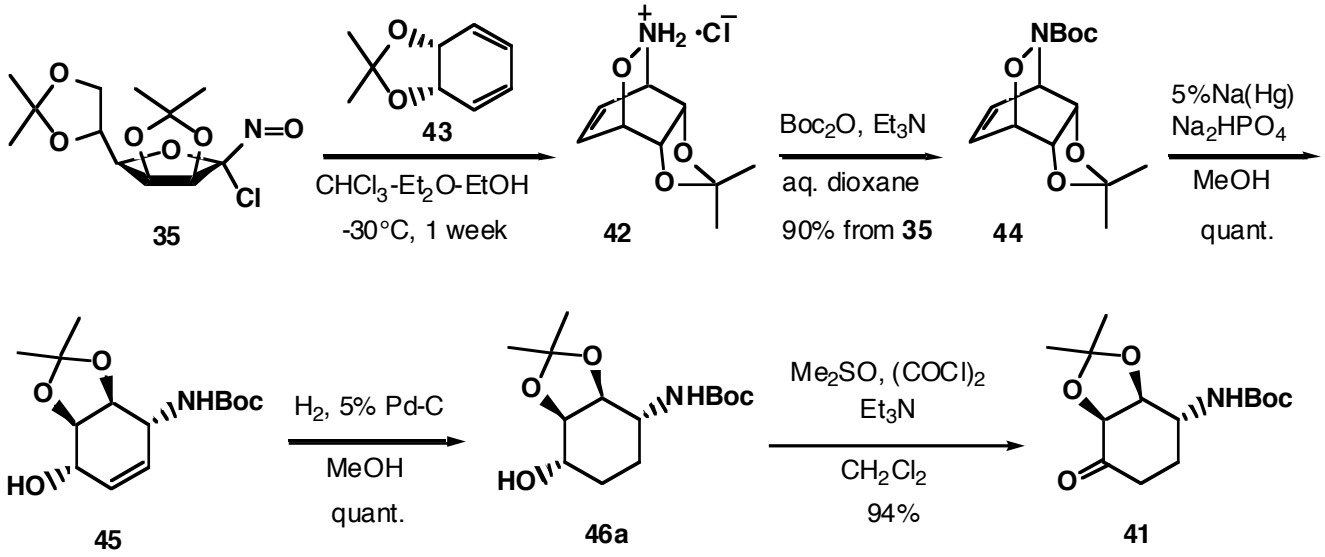


Figure 4

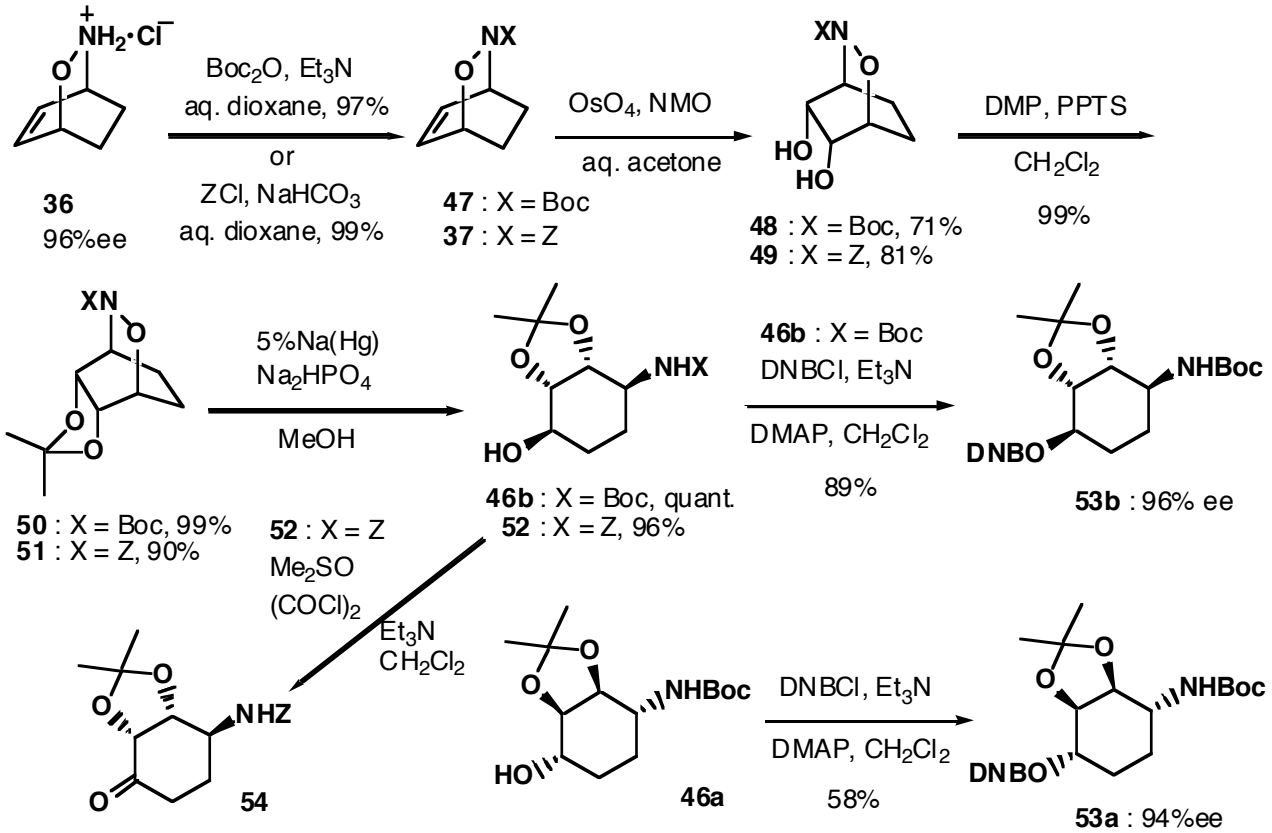
the Boc-adduct (**44**) in 90% yield in 2 steps, as shown in Scheme 8. The oxazine ring of **44** was reductively cleaved with sodium amalgam in a buffered solution[17] to give the cyclohexenol (**45**). Catalytic hydrogenation of **45** over Pd/C followed by the Swern oxidation of the resulting cyclohexanol derivative (**46a**) afforded the desired aminocyclitol (**41**) in excellent yield.



Scheme 8

Analogously, **46b** antipodal to **46a** was prepared from the Diels-Alder adduct (**36**), as shown in Scheme 9. The successive Boc protection, dihydroxylation with osmium, acetalization followed by amalgam reduction afforded **46b**. The both cyclohexanols (**46a**) and (**46b**) were respectively converted to the corresponding 3,5-dinitrobenzoates (**53a**) and (**53b**) to determine the enantiomeric excess of each isomer. Comparison of the both compounds on chiral HPLC revealed their enantiomeric excess to be 94% for **53a** and 96% for **53b**. The corresponding benzyloxycarbonyl derivative (**52**) was similarly prepared from **37**, and converted to the *Z*-ketone (**54**).

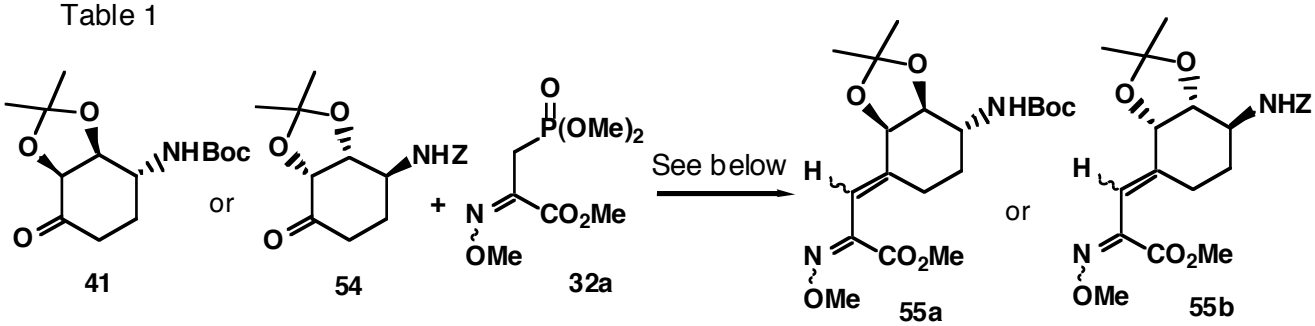
Utilizing the aminocyclitols (**41**) and (**54**), the Horner-Wadsworth-Emmons reaction with the Elder's phosphonates (**32a**) was investigated under various reaction conditions, as summarized in Table 1. The use of butyllithium in 1,2-dimethoxyethane (DME) gave the best result, and the product (**55a**) was obtained in



NMO : *N*-methylmorpholine *N*-oxide; DMP : 2,2-dimethoxypropane;
DNB : 3,5-dinitrobenzoyl ; DMAP : 4-(*N*,*N*-dimethylamino)pyridine

Scheme 9

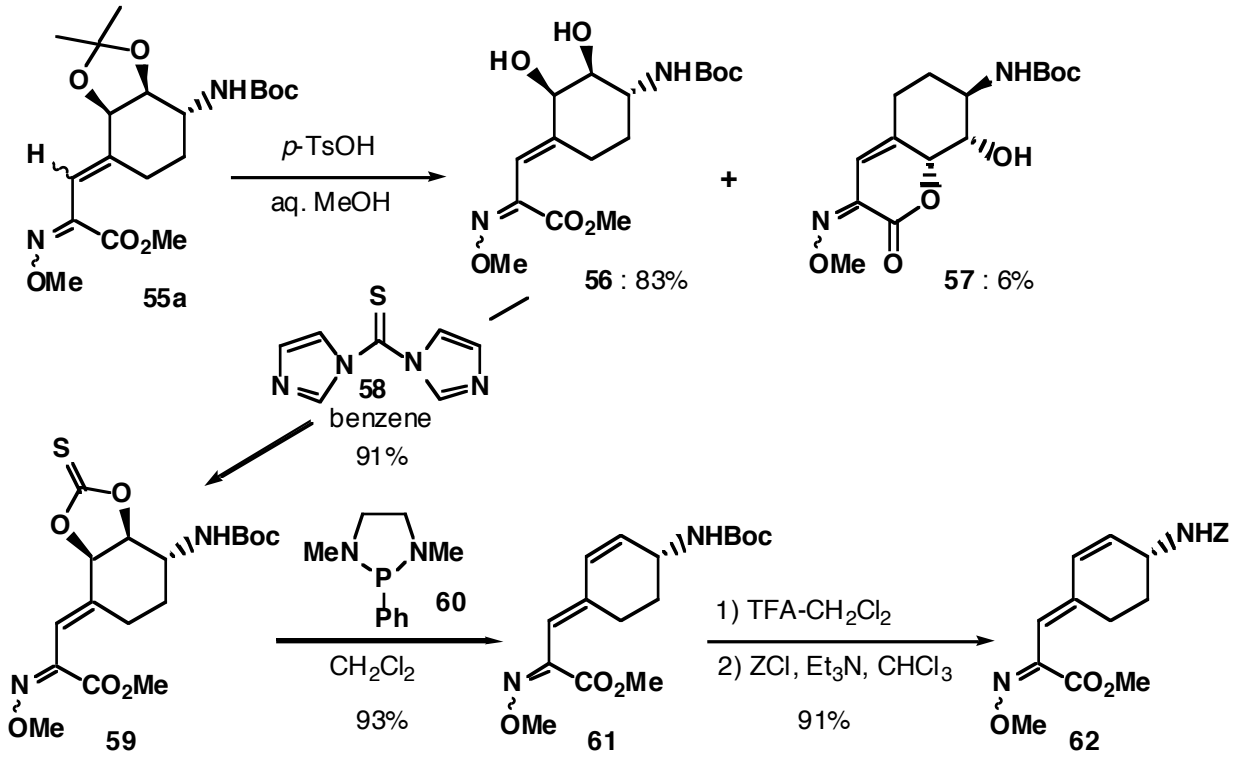
Table 1



entry	ketone	base	solvent	temp.	time (h)	yield (%)	*E* : *Z*
1	**54**	*n*-BuLi	THF	-78°C to rt	20	58	86 : 14
2	**41**	*n*-BuLi	THF	-78°C to rt	20	88	82 : 18
3	**54**	LDA	THF	-78°C to -20°C	18	66	83 : 17
4	**41**	LDA	THF	-78°C to rt	15	90	78 : 22
5	**54**	LiCl-DBU	MeCN	rt	3 days	5	----
6	**41**	*n*-BuLi	THF-HMPA	-78°C to rt	15	71	87 : 13
7	**41**	*n*-BuLi	DME	-78°C to rt	15	86	94 : 6
8	**41**	*n*-BuLi	DME-HMPA	-78°C to rt	15	64	75 : 25

preference of the *E*-isomer (94 : 6) in 86% yield (see entry 7 in Table 1).

The compound (**55a**) underwent the acidic removal of the acetonide function to give the diol (**56**) together with a small amount of the lactone (**57**), whose separation was easily achieved on a silica gel column. Treatment of the diol (**56**) with 1,1'-thiocarbonyldiimidazole (**58**) gave the thionocarbonate (**59**), which smoothly gave the diene (**61**) by the action of the phospholidine (**60**) according to the Corey-Winter protocol.[20] Replacement of the Boc function of **61** with the benzyloxycarbonyl one afforded the Aayp equivalent (**62**), as shown in Scheme 10.

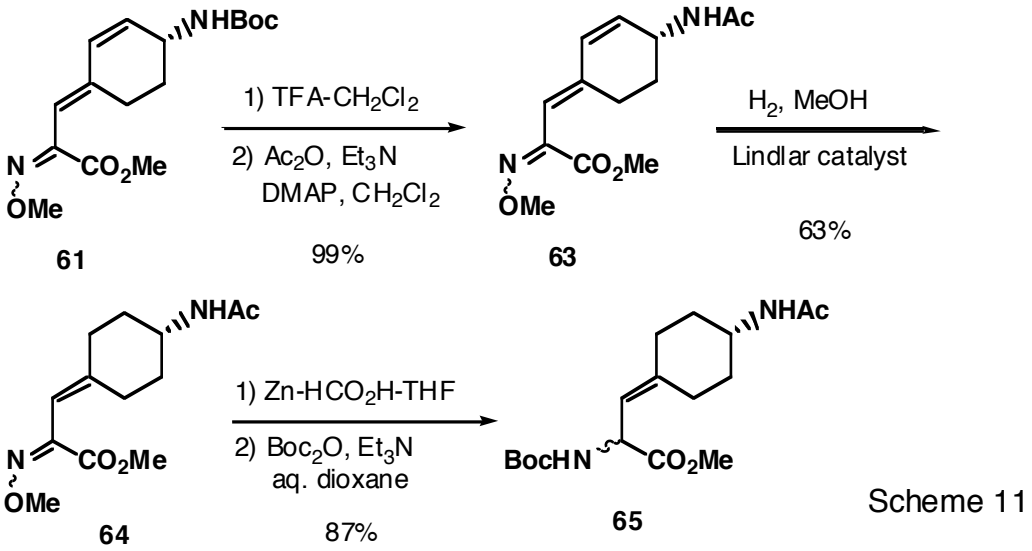


Scheme 10

Toward the synthesis of the (7*aS*)-Aacp derivative (**65**), the common intermediate (**61**) was first transformed to its *N*-acetyl derivative (**63**), whose double bond in the ring was selectively hydrogenated by use of the Lindlar catalyst to give the cyclohexane derivative (**64**).[21] Reduction of the oxime ether group with zinc in formic acid and then treatment with Boc_2O afforded the protected form (**65**) of (7*aS*)-Aacp as a mixture of diastereoisomers at the C-2 position, shown in Scheme 11.

With the (7*R*)-Aayp equivalent (**62**) and (7*aS*)-Aacp derivative (**65**) in hand, we synthesized radiosumin (**1**) as summarized in Scheme 12. After each protective group of **62** and **65** was removed, coupling by use of diethyl phosphorocyanidate (DEPC, $(EtO)_2P(O)CN$)[22] efficiently afforded the dipeptide as a mixture of diastereoisomers (**66a**) and (**66b**) in a ratio of 55:45, which were separated by precipitation with chloroform and ether. The dipeptides (**66a**) and (**66b**) thus obtained were respectively reduced with zinc in formic acid, and the *N*-acetylation of the reduction products afforded the protected forms (**67a-d**). The diastereomeric ratio of **67a** and **67b** was 55:45 while that of **67c** and **67d** was 44:56.

On the other hand, natural radiosumin (**1**) was converted to the protected form (**67**) by treatment with benzyloxycarbonyl chloride and then trimethylsilyldiazomethane in methanol.[23]



Scheme 11

Comparison of the product on HPLC clearly revealed that **67a** was identical with **67** derived from natural radiosumin. In addition, the synthetic **67a** was identified with the naturally derived **67** by spectral comparisons as well as the optical rotation.

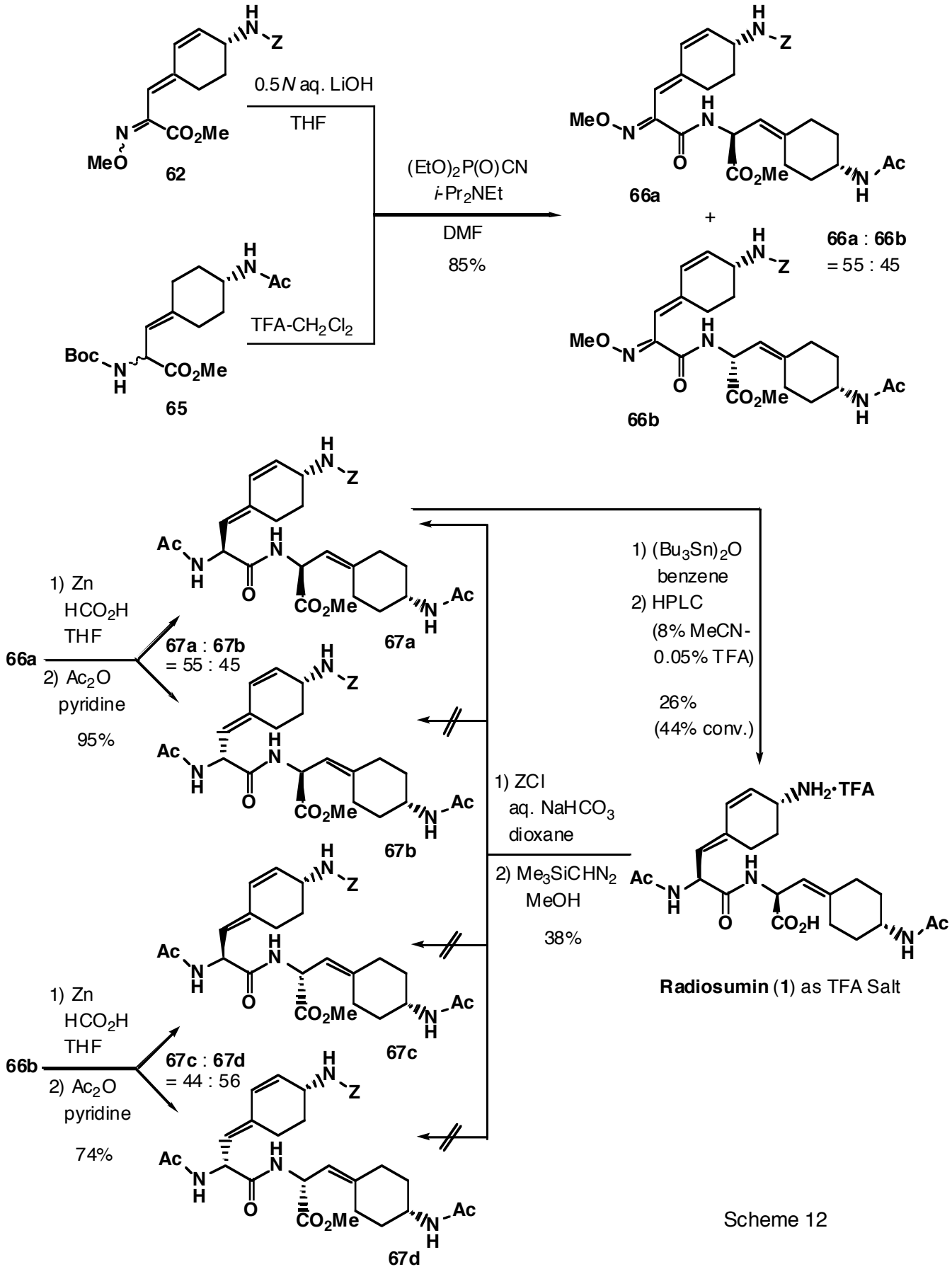
Thus the absolute configuration of the C-7 position in the Aacp unit of radiosumin (**1**) was determined clearly and proved to be the (*aS*)-configuration hence the absolute stereostructure of radiosumin was determined as shown in the structure (**1**).

Finally, conversion of the Z-radiosumin methyl ester (**67a**) to radiosumin (**1**) was achieved by use of bis(tri-*n*-butyltin)oxide in refluxing benzene[24] under neutral conditions, giving radiosumin (**1**) together with the corresponding methyl ester and the starting material (**67a**). The synthetic radiosumin was identified with the natural one by comparison of their ^{1}H NMR spectra and HPLC behavior. Radiosumin thus obtained was actually its trifluoroacetic acid (TFA) salt because 8% aqueous acetonitrile-0.05% TFA was used as an eluent for purification of the crude product on HPLC. The synthetic sample showed $[\alpha]_D^{20}$+74.4° (c 0.1, H_2O) while $[\alpha]_D^{20}$+96° (c 0.77, H_2O) was reported for the natural sample.[1] This discrepancy may be attributed to the content of TFA attached to radiosumin because the natural sample showed $[\alpha]_D^{20}$+79.2° (c 0.1, H_2O) after retreatment with 8% aq. MeCN-0.05% TFA on HPLC.

Thus, we have succeeded in the first total synthesis of radiosumin (**1**) which determined the unidentified stereogenic center to be (*aS*) and hence the whole absolute stereostructure. The method employed here will be useful for the synthesis of radiosumin B,[25] isolated recently, and other biologically interesting molecules.

EXPERIMENTAL

General: All melting and boiling points are uncorrected. Distillation was carried out by a Kugelrohr apparatus. IR spectra were measured with a SHIMADZU FTIR-8100 spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL EX-270 or GSX-400 spectrometer in $CDCl_3$ with tetramethylsilane or $CHCl_3$ as an internal standard, unless otherwise stated. MS spectra were obtained on a JEOL DX-300 spectrometer. Optical rotations were measured on a JASCO DIP-140 or DIP-1000 automatic polarimeter. Silica gel BW-820MH, BW-200, or BW-300 (purchased from Fuji Davison Co. Ltd.) and Silica gel 60-C_{18} (purchased from NACALAI TESQUE, INC) were used for column chromatography. Analytical TLC was carried out


0.5N aq. LiOH
THF
62
65
(EtO)2P(O)CN
i-Pr2NEt
DMF
85%
66a
66b
66a : 66b
= 55 : 45
TFA-CH2Cl2
1) Zn
HCO2H
THF
2) Ac2O
pyridine
95%
67a : 67b
= 55 : 45
67a
67b
1) (Bu3Sn)2O
benzene
2) HPLC
(8% MeCN-
0.05% TFA)
26%
(44% conv.)
1) ZCl
aq. NaHCO3
dioxane
2) Me3SiCHN2
MeOH
38%
Radiosumin (1) as TFA Salt
1) Zn
HCO2H
THF
2) Ac2O
pyridine
74%
67c : 67d
= 44 : 56
67c
67d

Scheme 12

on a silica gel plate (Merck Art. 5715). THF and DME were dried by distillation from benzophenone ketyl. CH_2Cl_2, MeCN, Et_3N, DBU, and HMPA were dried by distillation from CaH_2. Benzene and toluene were dried by distillation from $LiAlH_4$. MeOH and EtOH were dried by distillation from magnesium alcoholate.

4-*tert*-Butoxycarbonylaminocyclohexanone (8). A solution of *trans*-4-aminocyclohexanol hydrochloride (**7**•HCl, 25.66 g, 169.19 mM) in H_2O-dioxane (170 mL-510 mL) was neutralized with 1*N* aq. NaOH (170 mL, 170 mmol), and Boc_2O (40.62 g, 186.11 mmol) was added at 0°C. After being stirred at ambient temperature for 2 h, the mixture was quenched with 1M $KHSO_4$. The whole was extracted with $CHCl_3$ (x 2) and washed with brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo* to give a white solid. The crude solid was recrystallized from $CHCl_3$-hexane to give *trans*-4-*t*-butoxycarbonylaminocyclohexanol (33.75 g, 93%) as colorless needles, mp 146-148°C. IR ν_{max} (KBr): 4000-3000, 3847, 1684, 1534, 1456, 1387, 1366, 1320, 1273, 1254, 1231, 1183, 1071, 1042, 1026, 1005, 968, 953, 907, 891, 862, 801, 783, 764, 743 cm^{-1}. ^1H NMR δ 1.06-1.28 (m, 2H), 1.29-1.55 (m, 3H), 1.43 (s, 9H), 1.98 (m, 4H), 3.40 (br, 1H), 3.60 (m, 1H), 4.34 (br, 1H). Anal. Calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.18; H, 9.78; N, 6.38.

To a stirred solution of $(COCl)_2$ (8.05 mL, 92.23 mmol) in CH_2Cl_2 (400 mL) was added dropwise DMSO (7.85 mL, 110.5 mmol) at -78°C under argon and the mixture was stirred for 30 min. A solution of the above alcohol (13.22 g, 61.4 mmol) in CH_2Cl_2 (100 mL) was added and the mixture was stirred for 30 min. After addition of Et_3N (42.8 mL, 307 mmol), the whole was warmed to rt and stirred for 1 h. The mixture was quenched with H_2O, and extracted with CH_2Cl_2 (x 2). The extracts were washed with H_2O and saturated brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was recrystallized from $CHCl_3$-hexane to give **8** (12.86 g, 98%) as a white solid, mp 97-98°C. IR ν_{max} (KBr): 3364, 1719, 1680, 1526, 1474, 1458, 1448, 1389, 1373, 1364, 1314, 1260, 1234, 1167, 1105, 1051, 1032, 1001, 941, 882, 760 cm^{-1}. ^1H NMR δ 1.45 (s, 9H), 1.70 (br, 2H), 2.24 (m, 2H), 2.42 (m, 4H), 3.92 (br, 1H). Anal. Calcd for $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57. Found: C, 61.90; H, 9.05; N, 6.57.

4-(1,3-Dibenzylhexahydro-2-oxo-1,3,5-triazin-5-yl)cyclohexanone (12). A mixture of *trans*-4-aminocyclohexanol (**7**) (6.15 g, 53.375 mmol), formalin (53.4 mL), and *i*-Pr_2NEt (9.3 mL, 53.375 mmol) was stirred at ambient temperature for 10 min. The mixture was diluted with toluene and concentrated *in vacuo*. This work-up was repeated three times. The residue was dried under reduced pressure (2.0 mmHg) at rt for 5 h, dissolved in THF (267 mL), and then *N*, *N*'-dibenzylurea (12.81 g, 53.375 mmol) was added. After being refluxed for 3 h, the mixture was concentrated *in vacuo*. The residue was extracted with EtOAc (x 2), and washed with H_2O (x 3). The extracts were dried over Na_2SO_4, then concentrated *in vacuo*. The residual oil was purified by silica gel column chromatography (BW-200, 250 g, acetone:hexane=2:3) to give *trans*-4-(1,3-dibenzylhexahydro-2-oxo-1,3,5-triazin-5-yl)cyclohexanol (20.18 g, 100%) as a colorless prism, mp 187-189°C. IR ν_{max} ($CHCl_3$): 3418, 1622, 1504, 1455, 1358, 1298, 1250, 1215, 1161, 1140, 1123, 1065, 1028, 1019, 972, 951, 804, 758, 704, 669 cm^{-1}. ^1H NMR δ 0.94 (m, 4H), 1.35 (m, 3H), 1.75 (br d, 2H, J=10.6 Hz), 2.42 (m, 1H), 3.40 (m, 1H), 4.18 (s, 4H), 4.53 (s, 4H), 7.33 (m, 10H). Anal. Calcd for $C_{23}H_{29}N_3O_2 \cdot {}^3/_4H_2O$: C, 70.29; H, 7.82; N, 10.69. Found: C, 69.90; H, 7.49; N, 10.59.

The above alcohol (3.50 g, 9.235 mmol) was oxidized as described for 4-*t*-butoxycarbonylamino-cyclohexanone (**8**) to give crude **12**, which was purified by silica gel column chromatography (BW-200, 200 g, EtOAc:hexane=2:5 to 1:2) to furnish **12** (3.295 g, 95%) as a white waxy solid, mp 79-82°C. IR ν_{max} ($CHCl_3$): 1717, 1640, 1497, 1455, 1429, 1358, 1330, 1296, 1291, 1258, 1188, 1148, 1132, 1075, 1028, 1013, 981, 953, 939, 887, 805, 750, 706, 668 cm^{-1}. ^1H NMR δ 1.35 (m, 2H), 1.50 (br, 2H), 1.95 (ddd, 2H, J=15.2, 9.9, 5.3 Hz), 2.15 (m, 2H), 2.87 (ddd, 1H, J=11.8, 8.3, 3.3 Hz), 4.23 (s, 4H), 4.55 (s, 4H), 7.35 (m, 10H). Anal. Calcd for $C_{23}H_{27}N_3O_2$: C, 73.18; H, 7.21; N, 11.13. Found: C, 72.93; H, 7.29; N, 10.93.

4-(1,3-Dibenzylhexahydro-2-oxo-1,3,5-triazin-5-yl)cyclohexylidenacetaldehyde (13). To a suspension of NaH (60% oil dispersion, 131 mg, 3.282 mmol) in THF (10.0 mL) was added dropwise a solution of trimethyl phosphonoacetate (531 μL, 3.282 mmol) in THF (5.0 mL) at 0° C under argon, and the mixture was stirred for 30 min. A solution of **12** (1.125 g, 2.984 mmol) in THF (15.0 mL) was added to the mixture. After being stirred at rt for 1 h, the mixture was quenched with 10% aq. citric acid. The whole mixture was extracted with EtOAc twice, and the extracts were washed with H_2O and brine, dried over Na_2SO_4, then concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (BW-200, 300 g, EtOAc:hexane=3:2 to 2:1) to give methyl 4-DBT-cyclohexylidenacetate (1.282 g, 97%) as a white waxy solid, mp 52-54°C. IR ν_{max} (neat): 1715, 1640, 1497, 1455, 1296, 1244, 1196, 1163, 1146, 1076, 1028, 941, 862, 808, 749, 704 cm^{-1}. ^1H NMR δ 1.0-1.2 (br, 2H), 1.3-1.5 (br, 2H), 1.7-1.9 (m, 2H), 2.0-2.2 (br d, 1H, J=14.2 Hz), 2.67 (m, 1H), 3.39 (br d, 1H, J=15.2Hz), 3.65 (s, 3H), 4.20 (s, 4H), 4.54 (s, 4H), 5.52 (s, 1H), 7.34 (m, 10H). Anal. Calcd for $C_{26}H_{31}N_3O_3$: C, 72.03; H, 7.21; N, 9.69. Found: C, 72.25; H, 7.33; N, 9.55.

To a solution of the above ester (1.937 g, 4.37 mmol) in CH_2Cl_2 (20 mL) was added diisobutylaluminum hydride (1.5 M in toluene, 8.7 mL, 13.12 mmol) at -78°C under argon. After being stirred at this temperature for 3 h, the mixture was quenched with MeOH, followed by the addition of 1M $KHSO_4$. The whole mixture was extracted with CH_2Cl_2 (x 2), and the extracts were washed with sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 200 g, EtOAc) to give the allyl alcohol (1.705 g, 94%) as a white solid, mp 109-111°C. IR ν_{max} ($CHCl_3$): 3700-3100, 3015, 2940, 1628, 1504, 1454, 1441, 1356, 1298, 1252, 1217, 1150, 1132, 1075, 1012, 943, 701, 668 cm^{-1}. ^1H NMR δ 0.8-1.1 (br, 2H), 1.3-1.6 (m, 4H), 1.74 (br, 1H), 2.02 (br d, 1H, J=10.9 Hz), 2.35 (br d, 1H, J=13.2 Hz), 2.61 (m, 1H), 4.05 (d, 2H, J=7.33Hz), 4.19 (s, 4H), 4.53 (ABq, 4H, J=15.2 Hz), 5.28 (t, 1H, J=7.3 Hz), 7.33 (m, 10H). Anal. Calcd for $C_{25}H_{31}N_3O_2 \cdot {}^1/_4H_2O$: C, 73.23; H, 7.74; N, 10.25. Found: C, 73.57; H, 7.77; N, 10.12.

To a solution of the above alcohol (1.605 g, 3.867 mmol) in CH_2Cl_2 (40 mL) was added CMD (6.72 g, 77.34 mmol). After being stirred at ambient temperature for 8 h, the mixture was filtered through the pad of celite. The filtrate was concentrated *in vacuo* to give a yellow oily residue. The crude oil was purified by silica gel column chromatography (BW-820MH, 150 g, EtOAc:hexane= 7:3) to give **13** (1.467 g, 92%) as a white solid, mp 132-134°C. IR ν_{max} ($CHCl_3$): 3019, 1672, 1630, 1499, 1455, 1354, 1298, 1252, 1217, 1184, 1115, 1076, 1012, 982, 943, 887, 808, 706, 668 cm^{-1}. ^1H NMR δ 1.1 (m, 2H), 1.4 (m, 2H), 1.90 (dt, 2H,

J=10.7, 2.3 Hz), 2.17 (br d, 1H, J=13.9 Hz), 2.68 (m, 1H), 2.94 (br d, 1H, J=13.9 Hz), 4.20 (s, 4H), 4.54 (ABq, 4H, J=16.86 Hz), 5.72 (d, 1H, J=7.9 Hz), 7.35 (m, 10H), 9.88 (d, 1H, J=8.3 Hz). Anal. Calcd for $C_{25}H_{29}N_3O_2$: C, 74.41; H, 7.24; N, 10.41. Found: C, 74.13; H, 7.28; N, 10.36.

2-Hydroxy-3-[4-(1,3-Dibenzylhexahydro-2-oxo-1,3,5-triazin-5-yl)cyclohexylidene]propionitrile (15). To a solution of **14** (33 mg, 0.11 mmol) in CH_2Cl_2 (0.5 mL) was added $Ti(OPr^i)_4$ (28 μL, 0.1 mmol) at 0°C under argon, and the mixture was stirred for 1 h, then cooled to -78°C. A solution of **13** (207 mg, 0.5 mmol) in CH_2Cl_2-MeCN (2.0 mL-0.8 mL) was added dropwise to the mixture over 5 min period, followed by the addition of TMSCN (143 μL, 1.15 mmol) at -78°C. After being stirred at -50°C for 3 days and at -40°C for 5 days, the mixture was quenched with sat. aq. $NaHCO_3$. The whole mixture was extracted with EtOAc twice and washed with brine. The extracts were combined and dried over Na_2SO_4 and concentrated *in vacuo* to give a yellow oil. The crude oil was treated with 10% aq. citric acid-MeOH (1.0 mL-3.0 mL) at ambient temperature for 1 h. The whole mixture was extracted with EtOAc (x 2), and washed with sat. aq. $NaHCO_3$ and brine. The extracts were combined and dried over Na_2SO_4, then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 20g, EtOAc:hexane=3:1 to 4:1) to give **15a** (less polar diastreomer, 75 mg, 34%) and **15b** (polar diastereomer, 133 mg, 60%), respectively. The enantiomeric ratio of each diastereomer was determined by ^1H NMR spectroscopy after conversion to their (*S*)-MTPA esters.

Compound (15a): a white solid, mp 128-130°C. IR ν_{max} ($CHCl_3$): 3140, 2247, 1640, 1504, 1455, 1439, 1356, 1344, 1298, 1260, 1215, 1154, 1132, 1121, 1076, 1028, 1007, 943, 933, 910, 839, 810 cm^{-1}. ^1H NMR δ 0.93 (m, 2H), 1.26 (br, 2H), 1.59 (br t, 1H, J=12.9 Hz), 1.63 (br t, 1H, J=11.5 Hz), 2.02 (br d, 1H, J=12.9 Hz), 2.25 (br d, 1H, J=14.2 Hz), 2.59 (m, 1H), 3.7 (br, 1H), 4.18 (s, 4H), 4.52 (ABq, 4H, J=16.5 Hz), 5.00 (d, 1H, J=8.6 Hz), 5.27 (d, 1H, J=8.6 Hz), 7.33 (m, 10H). Anal. Calcd for $C_{26}H_{30}N_4O_2$: C, 72.53; H, 7.02; N, 13.01. Found: C, 72.36; H, 7.09; N, 12.97. **(*S*)-MTPA ester of 15a**: a viscous oil. IR ν_{max} ($CHCl_3$): 2361, 2342, 1759, 1632, 1501, 1455, 1354, 1298, 1217, 1188, 1173, 1123, 1109, 1080, 1013, 945, 918, 704, 667 cm^{-1}. ^1H NMR δ 1.02 (m, 2H), 1.35 (m, 2H), 1.5-1.9 (m, 2H), 2.08 (br d, 1H, J=13.9 Hz), 2.08 (br d, 1H, J=14.2 Hz), 2.62 (m, 1H), 3.52 and 3.55 (d, J=1.0 Hz and br s, 3H), 4.18 (s, 4H), 4.53 (ABq, 4H, J=15.5 Hz), 5.22 and 5.31 (d, 0.12H, J=8.9 Hz and d, 0.88H, J=8.9 Hz), 6.06 and 6.08 (d, J=8.9 Hz and d, J=8.9 Hz; 1H), 7.33 (m, 10H), 7.44 (m, 5H).

Compound (15b): a white solid, mp 104-106°C. IR ν_{max} ($CHCl_3$): 3250, 2253, 1619, 1509, 1454, 1437, 1360, 1352, 1298, 1259, 1215, 1146, 1134, 1103, 1076, 1030, 1013, 939, 908, 833 cm^{-1}. ^1H NMR δ 0.92 (m, 2H), 1.26 (br, 2H), 1.55 (br t, 1H, J=11.2 Hz), 1.72 (br t, 1H, J=11.2 Hz), 1.99 (br d, 1H, J=13.9 Hz), 2.24 (br d, 1H, J=13.9 Hz), 2.57 (m, 1H), 4.16 (s, 4H), 4.3 (br, 1H), 4.50 (ABq, 4H, J=18.2 Hz), 4.98 (d, 1H, J=8.3 Hz), 5.24 (d, 1H, J=8.3 Hz), 7.32 (m, 10H). **(*S*)-MTPA ester of 15b**: a viscous oil. IR ν_{max} ($CHCl_3$): 2361, 2343, 1759, 1636, 1499, 1455, 1354, 1298, 1236, 1186, 1173, 1123, 1107, 1080, 1015, 945, 930, 806, 706, 668 cm^{-1}. ^1H NMR δ 0.90 (m, 2H), 1.36 (m, 2H), 1.5-1.9 (m, 2H), 2.07 (br d, 1H, J=15.8 Hz), 2.36 (br d, 1H, J=14.2 Hz), 2.59 (m, 1H), 3.48 and 3.54 (d, J=1.0 Hz and br s, 3H), 4.17 (s, 4H), 4.53 (ABq, 4H, J=15.5 Hz), 5.19 and 5.30 (d, 0.17H, J=9.2 Hz and d, 0.83H, J=9.2 Hz), 6.09 and 6.10 (d, J=9.2 Hz and d, J=9.2 Hz; 1H), 7.3 (m, 10H), 7.4 (m, 5H).

4-*t*-Butoxyaminocyclohexanaldehyde (17). To a solution of 4-aminocyclohexanecarboxylic acid (**16**) (8.407 g, 56 mmol) in H_2O-dioxane (90 mL-280 mL) was added 1*N* aq. NaOH (56 mL, 56 mmol) and Boc_2O (13.4 g, 61.6 mmol) at 0°C. After being stirred at rt for 3 h, the mixture was concentrated *in vacuo*. 1M $KHSO_4$ was added to the mixture, which was extracted with EtOAc(x 2). The extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a white powder. The crude acid was used for the next step without further purification.

The above acid was dissolved in DMF (200 mL) and K_2CO_3 (8.17 g, 59 mmol), and MeI (3.68 mL, 59 mmol) were added at ambient temperature. After being stirred at rt for 12 h, the mixture was diluted with EtOAc. The whole mixture was extracted with EtOAc (x 2). The extracts were washed with H_2O and brine, dried over Na_2SO_4, then concentrated *in vacuo*. The residual oil was purified by silica gel column chromatography (BW-200, 300 g, EtOAc:hexane=1:4) to give methyl 4-*t*-butoxycarbonylamino-cyclohexanecarboxylate (13.89 g, 97%) as a viscous oil. IR ν_{max} (neat): 3400, 1732, 1701, 1518, 1366, 1248, 1171, 1043, 1026 cm^{-1}. ^1H NMR δ 1.44 (s, 9H), 1.4-2.0 (m, 8H), 2.47 (m, 1H), 3.6-3.7 (m, 1H), 3.68 (s, 3H), 4.58 (br, 1H). Anal. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.55; H, 8.94; N, 5.46.

To a solution of methyl 4-*t*-butoxycarbonylaminocyclohexanecarboxylate (1.515 g, 5.895 mmol) in CH_2Cl_2 (60 mL) was added diisobutylaluminum hydride (1.5M solution in toluene, 4.3 mL, 6.484 mmol) at -78°C under argon. After being stirred at -78°C for 2.5 h, diisobutylaluminum hydride (0.4 mL, 0.6 mmol) was added again to the mixture, which was stirred at this temperature for 1 h. The whole mixture was quenched with 1M $KHSO_4$,warmed to rt, and extracted with CH_2Cl_2 (x 2). The extracts were washed with brine, dried over Na_2SO_4, then concentrated *in vacuo* to give an oily residue. Purification of the residue by silica gel column chromatography (BW-200, 100 g, EtOAc:hexane=1:4) afforded **17** (845 mg, 63%) as a colorless viscous oil. IR ν_{max} ($CHCl_3$): 3343, 1721, 1686, 1520, 1450, 1391, 1368, 1246, 1040, 1026, 877 cm^{-1}. ^1H NMR δ 1.2-1.6 (m, 2H), 1.44 (s, 9H), 1.6-1.9 (m, 4H), 1.9-2.1 (m, 2H), 2.38 (br, 1H), 3.69 (br, 1H), 4.45 (br, 1H), 9.67 (s, 1H).

3-((4*S*)-4-Phenylmethyl-2-oxazolidinoyl)carbonylmethylenetriphenylphosphorane (19). To a solution of (4*S*)-3-(2-bromoacetyl)-4-phenylmethyl-2-oxazolidinone (**18**) (2.365 g, 8.842 mmol) in toluene (30 mL) was added Ph_3P (2.551 g, 9.746 mmol). After being stirred at ambient temperature for 5 days, the mixture was filtered and the precipitates were washed with Et_2O. The precipitates were dissolved in H_2O and added to 1*N* aq. NaOH. The mixture was triturated from Et_2O-1*N* aq. NaOH to give crude **19** (3.18 g, 75%) as a pale brown powder, a part of which was purified by silica gel column chromatography (BW-200, EtOAc:hexane=3:2) to give **19** (50% from (4*S*)-3-(2-bromoacetyl)-4-phenylmethyl-2-oxazolidinone) as a white powder, which was used for analyses, mp 187-188°C. $[\alpha]_D^{23}$ +44.1° (c=1.0, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3019, 1751, 1582, 1570, 1439, 1367, 1107 cm^{-1}. ^1H NMR δ 2.84 (dd, 1H, J=13.2, 9.2 Hz), 3.28 (dd, 1H, J=13.2, 3.6 Hz), 4.02 (dd, 1H, J=8.9, 3.0 Hz), 4.10 (m, 1H), 4.78 (m, 2H), 7.2-7.7 (m, 20H). Anal. Calcd for $C_{30}H_{26}NO_3P$: C, 75.14; H, 5.47; N, 2.92. Found: C, 74.92; H, 5.55; N, 3.08.

(4*S*)-3-[*trans*-4-*t*-Butoxycarbonylaminocyclohexyl]propenoyl]-4-phenylmethyl-2-oxazolidinone (20a) and (4*S*)-3-[*cis*-4-*t*-Butoxycarbonylaminocyclohexyl]propenoyl]-4-phenylmethyl-2-oxazolidinone (20a'). A mixture of **17** (6.103 g, 26.885 mmol) and **19** (12.878 g, 26.885 mmol) in toluene (150 mL) was refluxed for 30 h. After cool, the mixture was concentrated *in vacuo* to give a yellow oily residue. The residual oil was purified by silica gel column chromatography (BW-200, 150 g, Et_2O:hexane=1:3 to 1:1) to give **20a** (10.462 g, 91%) and its isomer (**20a'**) (120 mg, 1%), respectively.

Compound (20a): a less polar geometric mixture of the cyclohexane ring, a white solid, mp 121-123°C. IR ν_{max} ($CHCl_3$): 3340, 1779, 1707, 1690, 1632, 1499, 1354, 1169, 1047, 1028, 928, 669 cm^{-1}. ^1H NMR δ 1.1-1.6 (m, 2H), 1.45 (s, 9H), 1.6-1.8 (m, 4H), 1.8-2.5 (m, 3H), 2.79 (dd, 1H, J=13.2, 9.6 Hz), 3.35 (dd, 1H, J=13.5, 3.2 Hz), 3.73 (br, 1H), 4.20 (m, 2H), 4.40 and 4.60 (br and br, 1H), 7.05-7.38 (m, 7H). Anal. Calcd for $C_{24}H_{32}N_2O_5$: C, 67.27; H, 7.53; N, 6.54. Found: C, 67.03; H, 7.57; N, 6.36.

Compound (20a'): a more polar geometric mixture of the cyclohexane ring, a pale brown solid, mp 115-120°C. IR ν_{max} ($CHCl_3$): 3339, 1784, 1713, 1674, 1609, 1499, 1454, 1366, 1246, 926, 702 cm^{-1}. ^1H NMR δ 1.1-1.4 (m, 1H), 1.44 (s, 9H), 1.4-1.6 (m, 3H), 1.65 (m, 3H), 2.05 (br, 1H), 2.27 (br, 1H), 2.66 (dd, 1H, J=13.9, 8.9Hz), 3.14 (dd, 1H, J=13.9, 5.6Hz), 3.70 (br, 1H), 3.95-4.24 (m, 2H), 4.44 (m, 1H), 4.56 (br, 1H), 5.96 and 5.99 (d, J=15.8 and d, J=16.2 Hz; 1H), 6.50 and 6.57 (dd, J=19.8, 7.6 Hz, and dd, J=18.8, 6.3 Hz; 1H), 7.2-7.4 (m, 5H).

(4*S*)-3-[*trans*-3-(*trans*-4-Acetaminocyclohexyl)propenoyl]-4-phenylmethyl-2-oxazolidinone (20b) and (4*S*)-3-[*trans*-3-(*cis*-4-Acetaminocyclohexyl)propenoyl]-4-phenylmethyl-2-oxazolidinone (20c). To a solution of **20** (831 mg, 1.94 mmol) in CH_2Cl_2 (4.85 mL) was added TFA (1.75 mL) at ambient temperature. After being stirred at rt for 6 h, the mixture was concentrated *in vacuo* to give a pale yellow oil. Toluene was added to the mixture, which was concentrated *in vacuo*. This work-up was repeated three times to complete removal of the excess of TFA. The crude amine TFA salt was dissolved in pyridine (3 mL) and treated with Ac_2O (275 μL, 2.91 mmol). After being stirred at rt for 10 h, the mixture was added to an ice-cooled 1*N* aq. HCl. The whole was extracted with EtOAc (x 2), and the extracts were washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, then concentrated *in vacuo*. The residual oil was purified by silica gel column chromatography (BW-200, 80 g, EtOAc to EtOAc:MeOH=35:1 to 50:3) to give **20b** (555 mg, 77%) and **20c** (165 mg, 23%).

Compound (20b): a less polar geometric isomer of the cyclohexane ring, a white amorphous powder, mp 47-54°C. $[\alpha]_D^{23}$ +44.8° (c=1.0, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3430, 1779, 1732, 1682, 1634, 1520, 1455, 1354, 1111, 1007, 930, 853 cm^{-1}. ^1H NMR δ 1.5-1.8 (m, 7H), 1.98 (s, 3H), 2.0-2.1 (br, 1H), 2.45 (m, 1H), 2.80 (dd, 1H, J=13.2, 9.6 Hz), 3.36 (dd, 1H, J=13.2, 3.3 Hz), 4.25 (br, 1H), 4.22 (m, 2H), 4.74 (ddd, 1H, J=13.2, 6.9, 3.3 Hz), 5.48 (br, 1H), 7.1-7.4 (m, 7H). Anal. Calcd for $C_{21}H_{26}N_2O_4$: C, 68.09; H, 7.07; N, 7.56. Found: C, 67.59; H, 7.34; N, 7.46.

Compound (20c): a more polar geometric isomer of the cyclohexane ring, a white powder, mp 152-155°C. $[\alpha]_D^{23}$ +53.3° (c=1.0, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3440, 1777, 1700, 1683, 1632, 1518, 1453, 1354, 1107, 1007, 930, 861 cm^{-1}. ^1H NMR δ 1.16 (dt, 1H, J=12.5, 3.3 Hz), 1.20 (dt, 1H, J=11.9, 3.0 Hz), 1.35 (br t, 1H, J=13.2 Hz), 1.40 (br t, 1H, J=13.2 Hz), 1.88 (br d, 1H, J=13.2 Hz), 1.96 (s, 3H), 2.07 (br

d, 2H, J=13.2 Hz), 2.22 (m, 1H), 2.79 (dd, 1H, J=13.2, 9.6 Hz), 3.34 (dd, 1H, J=13.2, 3.3 Hz), 3.76 (m, 1H), 4.20 (m, 2H), 4.73 (ddd, 1H, J=13.2, 6.9, 3.3 Hz), 5.30 (br d, 1H, J=8.3 Hz), 7.09 (dd, 1H, J=15.5, 6.9 Hz), 7.2-7.4 (m, 6H). Anal. Calcd for $C_{21}H_{26}N_2O_4$: C, 68.09; H, 7.07; N, 7.56. Found: C, 67.96; H, 7.14; N, 7.55.

Diphenyl-3-tetrahydropyranyloxypropylphospine oxide (23). 3-Hydroxypropyltriphenylphosphonium chloride (**22**) (12.0 g, 33.66 mmol) was treated with 30% aq. NaOH (50 mL) at 100°C for 4 h. After cooling, the mixture was extracted with CH_2Cl_2 three times. The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a pale yellow viscous oil. The crude oil was used for the next step without further purification. The above oil was dissolved in CH_2Cl_2 (110 mL), then 2,3-dihydropyran (3.7 mL, 40.39 mmol) and pyridinium *p*-toluenesulfonate (846 mg, 3.37 mmol) was added to the mixture at rt. After being stirred at rt for 20 h, the mixture was washed with H_2O and brine. The CH_2Cl_2 layer was dried over Na_2SO_4, and concentrated *in vacuo*. The residual oil was purified by silica gel column chromatography (BW-200, 200 g, $CHCl_3$:MeOH=30:1) to give **23** (10.285 g, 89%) as a white waxy solid, mp 98-100°C. IR ν_{max} ($CHCl_3$): 1439, 1176, 1120, 1028 cm^{-1}. ^1H NMR δ 1.5-2.0 (m, 8H), 2.3-2.4 (m, 2H), 3.4-3.5 (m, 2H), 3.7-3.8 (m, 2H), 4.50 (brs, 1H), 7.4-7.5 (m, 6H), 7.7-7.8 (m, 4H). Anal. Calcd for $C_{20}H_{25}O_3P$: C, 69.75; H, 7.32. Found: C, 69.66; H, 7.32.

3-(4-*t*-Butoxycarbonylaminocyclohexylidene)-1-tetrahydropyranyloxypropane (24). To a solution of **23** (722 mg, 2.1 mmol) in THF (8.0 mL) was added *n*-BuLi (1.63 M solution in hexane, 1.35 mL, 2.2 mmol) at -78°C under argon. After 10 min, a solution of **8** (213 mg, 1.0 mmol) in THF (2.0 mL) was added and the mixture was stirred at –78°C for 10 min, warmed to rt, and stirred for 30 min. Sat. aq. NH_4Cl was added to this mixture and the mixture was extracted with EtOAc (x 2). The extracts were washed with H_2O and brine, dried over Na_2SO_4, and then concentrated *in vacuo*. The crude residual oil was purified by silica gel column chromatography (BW-200, 40 g, EtOAc:hexane=4:1 to EtOAc to EtOAc:MeOH=10:1) to give the adduct (327 mg, 59%) as a white solid, mp 72-76°C. IR ν_{max} ($CHCl_3$): 3700-3100, 3340, 1700, 1505, 1439, 1366, 1167 cm^{-1}. Anal. Calcd for $C_{31}H_{44}NO_6P \cdot 1/4H_2O$: C, 66.23; H, 7.98; N, 2.49. Found: C, 66.12; H, 8.02; N, 2.56.

The above adduct (2.436 g, 4.327 mmol) in THF (45 mL) was added NaH (60% oil dispersion, 525 mg, 13,12 mmol) at ambient temperature under argon. After being refluxed for 1.5 h, the mixture was quenched with sat. aq. $NaHCO_3$. The whole mixture was extracted with EtOAc (x 2). The extracts were washed with brine, dried over Na_2SO_4, and then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 80 g, Et_2O:hexane=2:5) to give **24** (1.013 g, 68%) as a white waxy solid, mp 47-48°C. IR ν_{max} ($CHCl_3$): 3445, 1705, 1502, 1368, 1315, 1169, 1028 cm^{-1}. ^1H NMR δ 1.1-1.3 (m, 3H), 1.44 (s, 9H), 1.4-2.7 (m, 10H), 2.30 (br q, 2H, J=7.3 Hz), 2.53 (br d, 1H, J=5.5 Hz), 3.37 (m, 1H), 3.4-3.7 (m, 3H), 3.8-3.9 (m, 1H), 4.47 (br, 1H), 4.59 (t, 1H, J=3.0 Hz), 5.15 (t, 1H, J=7.3 Hz).

3-(4-*t*-Butoxycarbonylaminocyclohexylidene)-1-propanol (25). To a solution of the tetrahydropyranyl ether (**24**) (998 mg, 2.944 mmol) in 90% aq. MeOH (30 mL) was added *p*-TsOH·H_2O

(112 mg, 0.589 mmol) at rt. After being stirred at rt for 12 h, the mixture was neutralized with Et_3N (82 mL, 0.589 mmol), then concentrated *in vacuo*. The residue was extracted with EtOAc (x 2), and the extracts were washed with H_2O and brine, dried over Na_2SO_4, and then concentrated *in vacuo* to give a colorless oil. The oil was purified by silica gel column chromatography (BW-200, 80 g, EtOAc:hexane=1:1) to give a white waxy solid **25**, mp 97-99°C. IR ν_{max} ($CHCl_3$): 3700-3100, 3445, 1700, 1505, 1368, 1316, 1169, 1045, 880 cm^{-1}. ^1H NMR δ 1.1-1.3 (m, 2H), 1.45 (s, 9H), 1.62 (br, 1H), 1.8-2.1 (m, 3H), 2.1-2.3 (m, 2H), 2.27 (br q, 2H), 2.54 (br d, 1H), 3.61 (br t, 3H, J=7.5 Hz), 4.42 (br, 1H), 5.13 (t, 1H, J=7.5 Hz). Anal. Calcd for $C_{14}H_{25}NO_3$: C, 65.85; H, 9.87; N, 5.49. Found C, 65.68; H, 9.74; N, 5.49.

3-(4-*t*-Butoxycarbonylaminocyclohexylidene)propionic Acid (26). To a solution of the above alcohol (**25**) (44 mg, 0.171 mmol) in DMF (2.0 mL) was added PDC (322 mg, 0.855 mmol) at rt. The mixture was stirred at rt for 2 h, and then 1*N* aq. NaOH was added. The mixture was washed with Et_2O and the aqueous layer was acidified with 1M $KHSO_4$. The aqueous layer was extracted with EtOAc (x 2), and the extracts were washed with H_2O and brine, and then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, EtOAc:hexane=2:5) to give **26** (23 mg, 50%) as a white solid, mp 102-104°C. IR ν_{max} ($CHCl_3$): 3700-3200, 3355, 1709, 1651, 1505, 1368, 1166, 1047, 926, 880, 853 cm^{-1}. ^1H NMR δ 1.1-1.4 (m, 2H), 1.45 (s, 9H), 1.8-2.4 (m, 5H), 2.47 (br d, 1H), 3.08 (br d, 2H, J=6.9 Hz), 3.61 (br, 1H), 4.44 (br, 1H), 5.30 (t, 1H, J=7.3 Hz), 5.4-5.8 (br, 1H). Anal. Calcd for $C_{14}H_{23}NO_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.18; H, 8.59; N, 5.19.

(4*S*)-3-[1-Oxo-3-(4-(*t*-butoxycarbonylamino)cyclohexylidene)propyl]-4-phenylmethyl-2-oxazolidinone (28). To a solution of the acid (**26**) (184 mg, 0.684 mmol) and Et_3N (124 μL, 0.889 mmol) in THF (6 mL) was added pivaloyl chloride (93 μL, 0.752 mmol) at -78°C under argon. After being stirred at this temperature for 15 min, the mixture was warmed to rt and stirred for 45 min. The lithiated oxazolidinone (**27**) (prepared as follows; to a solution of (4*S*)-benzyl-2-oxazolidinone (436 mg, 2.463 mmol) in THF (8 mL) was added *n*-BuLi (1.61 M solution in hexane, 1.53 mL, 2.467 mmol) at -78°C under argon, and the mixture was stirred for 15 min) was added to the mixture at -78°C by cannula. After being stirred at -78°C for 15 min, the mixture was warmed to rt during 2 h. The mixture was quenched with 1M $KHSO_4$ and concentrated *in vacuo*. The residue was extracted with CH_2Cl_2 (x 3), and the extracts were washed with sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and then concentrated *in vacuo* to give an oily residue. The residue was purified by silica gel column chromatography (BW-200, 50 g, EtOAc:hexane=12:5 to acetone:hexane=3:2 to 2:1) to give **28** (150 mg, 51%) as a colorless oil. IR ν_{max} (neat): 3370, 1777, 1713, 1684, 1505, 1391, 1367, 1316, 1171, 1105, 1048, 881, 758 cm^{-1}. ^1H NMR δ 1.1-1.4 (m, 2H), 1.44 (s, 9H), 1.9-2.1 (m, 2H), 2.1-2.5 (m, 3H), 2.5-2.6 (m, 1H), 2.79 (dd, 1H, J=13.2, 9.6 Hz), 3.27 (dd, 1H, J=13.2, 3.3 Hz), 3.67 (m, 3H), 4.20 (m, 2H), 4.53 (br, 1H), 4.66 (m, 1H), 5.39 (t, 1H, J=6.9 Hz), 7.18-7.36 (m, 5H).

(4*S*)-3-[1-Oxo-3-(4-acetamidocyclohexylidene)propyl]-4-phenylmethyl-2-oxazolidinone (29). To a solution of **28** (141 mg, 0.329 mmol) in CH_2Cl_2 (876 μL) was added trilfluoroacetic acid (315

μL) at rt. After being stirred at rt for 9 h, the mixture was concentrated *in vacuo* to give an oily residue. Toluene was added to the residual oil and the mixture was concentrated *in vacuo*. This work-up was repeated three times. The residue was used for the next step without further purification. The above amine TFA salt was treated with pyridine-acetic anhydride (2.0 mL-47 μL) at rt for 12 h. The mixture was added to the ice-cooled 1*N* aq. HCl, and extracted with EtOAc (x 2). The extracts were washed with sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 20 g, acetone:hexane=5:6) to give **29** (120 mg, 98%) as a white solid. IR ν_{max} ($CHCl_3$): 3440, 1779, 1703, 1659, 1538, 1445, 1389, 1309, 1211, 1111, 1047, 986 cm^{-1}. ^1H NMR δ 1.1-1.4 (m, 2H), 1.9-2.1 (m, 2H), 1.96 (s, 3H), 2.1-2.4 (m, 3H), 2.57 (m, 1H), 2.80 (dd, 1H, J=13.5, 9.3 Hz), 3.27 (dd, 1H, J=13.5, 3.3 Hz), 3.68 (m, 2H), 3.95 (m, 1H), 4.21 (m, 2H), 4.67 (ddd, 1H, J=9.3, 3.6, 3.3 Hz), 5.40 (t, 1H, J=6.9 Hz), 5.81 (br d, 1H, J=7.9 Hz), 7.2-7.4 (m, 5H).

(4*S*)-3-[1-Oxo-2-(*N*,*N*'-bis-*t*-butoxycarbonylhydrazino)-3-(4-acetamidocyclohexylidene)-propyl]-4-phenylmethyl-2-oxazolidinone (30). To a solution of **29** (54 mg, 0.164 mmol) in THF (3.0 mL) was added KHMDS (0.5 M solution in toluene, 613 μL, 0.307 mmol) at -78°C under argon. After being stirred at this temperature for 30 min, the mixture was added a precooled (-78°C) solution of di-*tert*-butyl azodicarboxylate (40 mg, 0.175 mmol) in CH_2Cl_2 (1.0 mL). After 3 min, the mixture was quenched with AcOH (100 μL), and a phosphate buffer (pH=7) was added to this mixture and then the mixture was warmed to rt. The whole mixture was extracted with EtOAc (x 2), and the extracts were washed with sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 20 g, acetone:hexane=5:6) to give **30** (46 mg, 53%) as a colorless oil. IR ν_{max} ($CHCl_3$): 3401, 1784, 1740, 1701, 1659, 1510, 1392, 1370, 1242, 1157, 1109, 1051, 855 cm^{-1}. ^1H NMR δ 1.1-1.4 (br, 2H), 1.43 and 1.45 (s and s; 9H), 1.468 and 1.474 (s and s; 9H), 1.92 and 1.93 (s and s; 3H), 1.8-2.3 (br, 5H), 2.65-2.9 (m, 2H), 3.1-3.4 (br, 1H), 3.96 (br, 1H), 4.17 (m, 2H), 4.5-4.8 (m, 1H), 5.06 (br, 1H), 5.40 (br, 1H), 6.49 (m, 2H), 7.1-7.4 (m, 5H).

Methyl 3-Bromo-2-methoxyiminopropionate. A mixture of bromopyruvic acid (**31**) (13.09 g, 78.38 mmol) in 10% HCl-MeOH (80 mL) was stirred at ambient temperature for 15 h. $MeONH_2 \cdot HCl$ (9.82 g, 117.58 mmol) was added to the mixture and the whole was stirred for 6 h, then concentrated *in vacuo*. The residue was extracted with Et_2O (x 2) and washed with H_2O and brine. The extracts were dried over Na_2SO_4, and then concentrated *in vacuo*. The residual crude oil was distilled under reduced pressure (bp 94-96°C /19 mmHg) to give methyl 2-methoxyimino-3-bromopropionate[13] (12.43 g, 76%) as a colorless oil.

Ethyl 3-Bromo-2-methoxyiminopropionate. To a solution of ethyl 3-bromopyruvate (1.95 g, 10 mmol) in EtOH (30 mL) was added $MeONH_2 \cdot HCl$ (1.25 g, 15 mmol) and the mixture was stirred for 3 h, and then concentrated *in vacuo*. The residue was extracted with Et_2O (x 2) and washed with H_2O and brine. The extracts were dried over Na_2SO_4, and then concentrated *in vacuo*. The crude oil was distilled under reduced pressure (bp 80-85°C /4 mmHg), to give ethyl 2-methoxyimino-3-bromopropionate[13] (2.06 g, 92%) as a colorless oil. IR ν_{max} (neat): 1722, 1597, 1375, 1333, 1177, 1049, 855 cm^{-1}. ^1H NMR δ 1.35 (t, 3H,

J=7.3 Hz), 4.16 (s, 1H), 4.17 (s, 3H), 4.20 (s, 1H), 4.33 (q, 2H, J=7.3 Hz).

Trimethyl Phosphono-2-methoxyiminopropionate (32a). A mixture of methyl methoxyimino-3-bromopropionate (6.80 g, 32.38 mmol) and $P(OMe)_3$ (5.73 mL, 48.57 mmol) was refluxed for 48 h. After cooling, the mixture was concentrated *in vacuo*. The crude oil was distilled under reduced pressure (119-121°C/0.8 mmHg) to give **32a** (8.01 g, quant.) as a colorless oil. IR ν_{max} (neat): 1728, 1609, 1443, 1345, 1264, 1210, 1171, 1040, 845, 776 cm^{-1}. [lit.,[13] IR ν_{max} (film): 1720, 1270, 1210, 1170, 1040, 850, 780 cm^{-1}]. ^1H NMR δ 3.34 (d, 2H, J=23.4 Hz), 3.75 (d, 6H, J=11.2 Hz), 3.89 (s, 3H), 4.13 (s, 3H). [lit.,[13] ^1H NMR (250 MHz, $CDCl_3$) δ 3.34 (d, 2H, J=23.5 Hz), 3.75 (d, 6H, J=11.2 Hz), 3.89 (s, 3H), 4.13 (s, 3H)].

Ethyl Dimethylphosphono-2-methoxyiminopropionate (32b). Ethyl 3-bromo-2-methoxy-iminopropionate (1.70 g, 7.603 mmol) was treated as described for **32a** to give **32b**[30] (1.96 g, quant.) as a colorless oil, bp 120°C/1.5 mmHg. IR ν_{max} (neat): 1721, 1607, 1466, 1333, 1266, 1179, 1040, 936, 857, 775 cm^{-1}.

Methyl 2-Methoxyimino-3-[4-(1,3-dibenzylhexahydro-2-oxo-1,3,5-triazin-5-yl)cyclo-hexylidene]propionate (33). To a solution of **32a** (7.61 g, 31.83 mmol) in THF (100 mL) was added dropwise *n*-BuLi (1.61 M in hexane solution, 19.8 mL, 31.83 mmol) over 15 min period at -78°C under argon. The mixture was stirred for 1 h, then a solution of **12** (10.0 g, 26.53 mmol) in THF (30 mL) was added to the mixture over 20 min period. After being stirred at -78°C for 2 h, the mixture was warmed to rt during 2 h, and then stirred at rt for 12 h. The whole was quenched with sat. aq. NH_4Cl and extracted with EtOAc (x 2). The extracts were washed successively with 10% aq. citric acid, sat. aq. $NaHCO_3$, and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 200 g, acetone:hexane=1:2) to give **33** (10.53 g, 81%) as a pale yellow oil, which was used for the next step. IR ν_{max} ($CHCl_3$): 1732, 1689, 1499, 1455, 1298, 1254, 1202, 1148, 1015, 914, 706 cm^{-1}. ^1H NMR δ 0.8-1.2 (m, 2H), 1.3-1.7 (m, 3H), 1.7-2.0 (m, 2H), 2.05-2.1 (m, 1H), 2.57 (m, 1H), 3.82 (s, 3H), 4.01 (s, 3H), 4.18 (s, 4H), 4.52 (s, 4H), 5.67 (s, 1H), 7.21-7.36 (s, 10H).

Methyl 2-*t*-Butoxycarbonylamino-3-[4-(1,3-dibenzylhexahydro-2-oxo-1,3,5-triazin-5-yl)cyclohexylidene]propionate (34). To a solution of **33** (443 mg, 0.904 mmol) in THF (10 mL) were added Zn dust (296 mg, 45.204 mmol) and HCO_2H (3.4 mL, 90.405 mmol) at ambient temperature. After being stirred at rt for 1.5 h, the mixture was filtered through a pad of celite, then the filtrate was concentrated *in vacuo* to give a yellow oil. Toluene was added to the crude oil and the mixture was concentrated *in vacuo*. This work-up was repeated three times to complete removal of the excess of HCO_2H. The crude amine formic acid salt was dissolved in H_2O-dioxane (1.0 mL-3.0 mL) and neutralized with Et_3N (189 μL, 1.356 mmol). Boc_2O (296 mg, 1.356 mmol) was added to the whole at ambient temperature and stirred for 12 h. The mixture was quenched with 1M $KHSO_4$, and extracted with EtOAc (x 2). The extracts were washed with sat. aq. $NaHCO_3$ and brine, then dried over Na_2SO_4. The mixture was concentrated *in vacuo*, and the

residue was purified by silica gel column chromatography (BW-200, 80 g, EtOAc:hexane=2:1) to give **34a** (less polar diastereomer, 170 mg, 33%) and **34b** (polar diastereomer, 169 mg, 33%), respectively.

Compound (34a): a white amorphous powder, mp 48-52°C. IR ν_{max} ($CHCl_3$): 3445, 1744, 1709, 1632, 1501, 1454, 1298, 1252, 1161, 1049, 1028, 943, 928 cm^{-1}. ^1H NMR δ 0.8-1.1 (m, 2H), 1.43 (s, 9H), 1.3-1.5 (m, 2H), 1.5-2.1 (m, 4H), 2.4-2.7 (m, 2H), 3.71 (s, 3H), 4.18 (s, 4H), 4.53 (ABq, 4H, J=15.2 Hz), 4.89 (brs, 2H), 5.02 (br, 1H), 7.24-7.35 (m, 10H). Anal. Calcd for $C_{32}H_{43}N_4O_5$: C, 68.30; H, 7.52; N, 9.96. Found: C, 67.96; H, 7.38; N, 9.79.

Compound (34b): a white amorphous powder, mp 48-56°C. IR ν_{max} ($CHCl_3$): 3443, 1746, 1705, 1634, 1505, 1455, 1298, 1252, 1163, 1049, 1028, 943, 929 cm^{-1}. ^1H NMR δ 0.8-1.05 (m, 2H), 1.44 (s, 9H), 1.3-1.5 (m, 2H), 1.55-1.8 (m, 2H), 1.98 (m, 1H), 2.47 (br d, 1H, J=14.2 Hz), 2.62 (m, 1H), 3.66 (s, 3H), 4.19 (s, 4H), 4.53 (ABq, 4H, J=15.2 Hz), 4.88 (br s, 2H), 5.06 (br, 1H), 7.23-7.35 (m, 10H). Anal. Calcd for $C_{32}H_{42}N_4O_5$: C, 68.30; H, 7.52; N, 9.96. Found: C, 67.94; H, 7.39; N, 9.97.

(1*R*,4*S*)-3-Aza-2-oxabicyclo[2.2.2]oct-5-ene Hydrochloride (36). To a solution of **35** (9.2 g, 29.897 mmol) in $CHCl_3$ (85 mL) was added a solution of 1,3-cyclohexadiene (5.7 mL, 59.794 mmol) in EtOH (28 mL) at -78°C over 30 min period. After being stirred at this temperature for 4 h, the mixture was quenched with 1*N* aq. HCl. The whole was washed with $CHCl_3$ (x 3), and the H_2O layer was concentrated *in vacuo*. The residue was recrystallized from hot EtOH to give **36** (3.648 g, 93%) as colorless prisms, mp 134-136°C (decomp) [lit.,[16c] mp 135°C (decomp)]. $[\alpha]_D^{25}$ +25.9° (c=1.0, MeOH) [lit.,[16b] $[\alpha]_D^{20}$ +24.0° (c=5.0, MeOH)]. IR ν_{max} (KBr) 3600-3300, 3050-2000, 1560, 1452, 1435, 1390, 1363, 1315, 1277, 1210, 1163, 1120, 1065, 1026, 990, 941, 922, 860, 821, 802, 777, 656 cm^{-1}. [lit.,[16c] IR ν_{max} (KBr) 3600-3300, 3025-2330, 1540, 1452, 1415, 1381, 1359, 1310, 1280, 1265, 1220, 1164, 1120, 1080, 1058, 1022, 1005, 988, 959, 940, 920, 856, 810, 798, 773, 661, 650 cm^{-1}]. ^1H NMR (TMS/CD_3OD) δ 1.5-1.7 (m, 2H), 2.1-2.4 (m, 2H), 4.56 (br, 1H), 4.98 (m, 1H), 6.65 (ddd, 1H, J=8.2, 6.3, 1.7 Hz), 6.93 (ddd, 1H, J=8.2, 5.9, 1.7 Hz). [lit.,[16c] ^1H NMR (200 MHz, D_2O) δ 1.60 (m, 2H), 2.16 (m, 1H), 2.25 (m, 1H), 4.60 (ddd, 1H, J=6.3, 3.5, 1.5 Hz), 5.01 (ddd, 1H, J=5.8, 3.8, 1.5 Hz), 6.63 (ddd, 1H, J=8.4, 6.3, 1.5 Hz), 6.90 (ddd, 1H, J=8.4, 5.8, 1.5 Hz)].

(1*R*,4*S*)-3-*t*-Benzyloxycarbonyl-3-aza-2-oxabicyclo[2,2,2]oct-5-ene (37). To a solution of **36** (9.4 g, 63.69 mmol) in H_2O-dioxane (60 mL-180 mL) were added $NaHCO_3$ (12.85 g, 152.85 mmol) and ZCl (11.0 mL, 76.43 mmol) at 0°C. After being stirred at 0°C for 1.5 h, the mixture was added to Et_2O. The whole mixture was extracted with Et_2O (x 2) and washed with H_2O and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo* to give oily residue. The crude oil was purified by silica gel column chromatography (BW-200, 300 g, EtOAc:hexane=2:7) to give **37** (15.45 g, 99%) as a white solid, mp 46-47°C. $[\alpha]_D^{25}$ +7.3° (c=3.3, $CHCl_3$). IR ν_{max} (neat): 2970, 2940, 2987, 2867, 1716, 1497, 1454, 1395, 1372, 1292, 1266, 1233, 1215, 1167, 1109, 1076, 1051, 959, 899, 894, 830, 753, 698 cm^{-1}. ^1H NMR δ 1.3-1.6 (m, 2H), 2.0-2.3 (m, 2H), 4.77 (br, 1H), 4.82 (br, 1H), 5.17 (ABq, 2H, J=12.2 Hz), 6.55 (m, 2H), 7.34 (m, 5H). Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.62; H,

6.26; N, 5.69.

4*S*-Benzyloxycarbonylamino-2-cyclohexenone (38). To a mixture of **37** (1.227 g, 5.008 mmol) in dry MeOH (100 mL) were added Na_2HPO_4 (3.55 g, 25.041 mmol) and freshly crushed 5% Na(Hg) (18.4 g, 15 times weight of **37**) at -10°C under argon. After being stirred at -10°C for 4 h, THF-Et_2O (200 mL-200 mL) was added to the mixture and the mixture was stirred at ambient temperature for 10 min. The whole was decanted to a silica gel short column and eluted with THF. The eluate was concentrated *in vacuo* to give a pale yellow oil, which was extracted with EtOAc (x 2), and washed with H_2O and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo* to give an oily residue. The crude oil was purified by silica gel column chromatography (BW-200, 100 g, EtOAc:hexane=1:1) to give (4*S*)-benzyloxycarbonylamino-2-cyclohexenol (1.2 g, 97%) as a colorless oil. $[\alpha]_D^{25}$ -38.9° (c=1.3, $CHCl_3$). IR ν_{max} (neat): 3320, 1694, 1530, 1456, 1406, 1306, 1250, 1124, 1065, 1028, 990, 949, 839, 741, 698 cm^{-1}. ^1H NMR δ 1.58 (br, 1H), 1.6-1.8 (m, 2H), 1.8-2.0 (m, 2H), 4.18 (br, 2H), 4.80 (br, 1H), 5.11 (s, 2H), 5.75 (dd, 1H, J=10.2, 2.3 Hz), 5.88 (br d, 1H, J=8.9 Hz), 7.36 (s, 5H). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 67.76; H, 7.05; N, 5.51.

To a solution of the above alcohol (953 mg, 3.86 mmol) in CH_2Cl_2 (40 mL) was added CMD (6.7 g, 77.17 mmol) at ambient temperature. After being stirred at rt for 12 h, the mixture was filtered through a pad of celite. The filtrate was concentrated *in vacuo* to give a pale yellow oil. The residual oil was purified by silica gel column chromatography (BW-200, 80 g, EtOAc:hexane=1:2) to give **38** (815 mg, 86%) as a white solid, mp 91-93°C. $[\alpha]_D^{25}$ -109.0° (c=1.1, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3339, 1710, 1684, 1510, 1455, 1418, 1300, 1217, 1055, 1026, 930, 876, 849, 777, 669 cm^{-1}. ^1H NMR δ 1.8-2.0 (ddd, 1H, J=22.4, 12.2, 5.0 Hz), 2.3-2.4 (m, 1H), 2.4-2.6 (m, 2H), 4.58 (br, 1H), 5.12 (br s, 3H), 5.99 (dd, 1H, J=10.2, 2.0 Hz), 6.81 (br d, 1H, J=10.2 Hz), 7.35 (s, 5H). Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.41; H, 6.23; N, 5.71.

Ethyl 2-Methoxyimino-3-(4*S*-benzyloxycarbonylamino-2-cyclohexenylidene)propionate (39). To a solution of LDA (prepared from (*i*-Pr)$_2$NH (350 μL, 2.5 mmol) and *n*-BuLi (1.61 M solution in hexane, 1.55 mL, 2.5 mmol) in THF (10.0 mL)) was added a solution of **32b** (633 mg, 2.5 mmol) in THF (2.0 mL) at -78°C under argon. A solution of **38** (245 mg, 1.0 mmol) in THF (2.0 mL) was added to the mixture at -78°C, and the mixture was stirred at this temperature for 1 week. The whole mixture was quenched with sat. aq. NH_4Cl, and extracted with EtOAc (x 2). The extracts were washed with H_2O and brine and combined. The whole was dried over Na_2SO_4 and concentrated *in vacuo* to give a dark brown oil, which was purified by silica gel column chromatography (BW-200, 30 g, EtOAc:hexane=2:5) to give **39** (80 mg, 22%; *E*:*Z*=1:2.7 mixture) as a colorless oil. IR ν_{max} (neat): 3441, 1732, 1698, 1630, 1525, 1456, 1371, 1156, 1053, 1024, 930, 845, 738, 698 cm^{-1}. ^1H NMR δ 1.34 and 1.35 (t, J=7.3 Hz and t, J=7.3 Hz; 3H), 1.5-1.7 (m, 1H), 2.0-2.2 (m, 1H), 2.26 and 2.51 (br t, J=4.9 Hz and br t, J=6.3 Hz), 4.05 and 4.07 (s and s; 3H), 4.33 and 4.32 (q, J=7.3 Hz and q, J=7.3 Hz), 4.40 (br m, 1H), 5.76 (br d, 1H, J=8.6 Hz), 5.11 (s, 2H), 5.79-5.91 (m, 2H), 5.86 (br s, 0.73H), 5.99 (br s, 0.27H), 6.09 (dd, J=9.6, 1.7 Hz, 0.73Hz), 6.24 (dd, J=9.9, 2.0 Hz, 0.27H), 7.37 (s, 5H).

Ethyl 2-(*t*-Butoxycarbonylamino)-3-(4*S*-benzyloxycarbonylamino-2-cyclohexenylidene)-propionate (40). To a solution of **39** (80 mg, 0.215 mmol) in THF (2.0 mL) were added Zn dust (703 mg, 10.752 mmol) and HCO_2H (811 μL, 21.51 mmol) at ambient temperature. After being stirred at rt for 1 h, the mixture was filtered through the pad of celite. The filtrate was concentrated *in vacuo*. Toluene was added to the residue and concentrated *in vacuo* to give an oily residue. This work-up was repeated three times to complete removal of the excess of HCO_2H. The crude residue was used for the next step without further purification. The crude amine formic acid salt was dissolved in H_2O-dioxane (0.3 mL-1.0 mL) and neutralized with Et_3N (45 mL, 0.323 mmol). Boc_2O (70 mg, 0.323 mmol) was added to the mixture, which was stirred at ambient temperature for 20 h. The mixture was quenched with 1M $KHSO_4$, and extracted with EtOAc (x 2). The extracts were washed with sat. aq. $NaHCO_3$ and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo*. The residual oil was purified by silica gel column chromatography (BW-200, 20g, EtOAc:hexane=1:2) to give **40** (48 mg, 50%) as a colorless oil. ^1H NMR δ 1.24 (t, 3H, J=7.3 Hz), 1.43 (s, 9H), 1.5-1.7 (br m, 1H), 2.0-2.1 (br m, 1H), 2.34 (br m, 2H), 4.17 (q, 2H, J=7.3 Hz), 4.3-4.4 and 4.8-4.9 (br and br; 1H), 5.11 (br s, 2H), 4.9-5.1 (br m, 3H), 5.74 and 6.0-6.1 (m and m; 1H), 5.84 and 5.86 (d, J=10.2 Hz and d, J=10.2 Hz; 1H), 6.65 (br d, 1H, J=10.2 Hz), 7.35 (s, 5H).

(1*S*,4*R*,7*S*,8*S*)-7,8-*O*-Isopropylidenedioxy-3-*t*-butoxycarbonyl-3-aza-2-oxabicyclo-[2.2.2]oct-5-ene (44). To a solution of **35** (22.53 g, 73.26 mmol) in Et_2O-CH_2Cl_2-EtOH (200 mL-110 mL-8 mL) was added dropwise a solution of **43** (11.14 g, 73.26 mmol) in 22 mL of Et_2O at -40°C over 30 min period. After being stirred at -30°C for 1 week, the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 300 g, EtOAc:hexane=2:3 to MeOH:EtOAc=2:5) to give an oily **42**, which was used for the next reaction without further purification.
The above crude **42** was treated with Boc_2O (19.2 g, 87.91 mmol), Et_3N (12.3 mL, 87.91 mmol) in dioxane-H_2O (210 mL-70 mL). The mixture was stirred at ambient temperature for 10 h, then quenched with 1M $KHSO_4$. The whole was extracted with EtOAc (x 2), and washed with sat. aq. $NaHCO_3$ and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo*. The white solid was purified by silica gel column chromatography (BW-200, 300 g, Et_2O:hexane=1:2) to give **44** (18.65 g, 90% in 2 steps) as a white solid, mp 118-121°C. $[\alpha]_D^{24}$ +24.7° (c=1.0, $CHCl_3$). IR ν_{max} ($CHCl_3$): 1713, 1458, 1385, 1372, 1330, 1252, 1211, 1074, 1022, 994, 885, 870 cm^{-1}. ^1H NMR δ 1.31 (s, 3H), 1.32 (s, 3H), 1.47 (s, 9H), 4.53 (m, 2H), 4.88 (m, 1H), 4.99 (m, 1H), 6.44 (m, 2H). Anal. Calcd for $C_{14}H_{21}NO_5$: C, 59.35; H, 7.47; N, 4.94. Found: C, 59.33; H, 7.49; N, 4.74.

(1*S*,2*R*,3*S*,4*R*)-2,3-*O*-Isopropylidenedioxy-4-*t*-butoxycarbonylamino-5-cyclohexenol (45). To a mixture of **44** (2.567 g, 9.071 mmol) in dry MeOH (180 mL) were added Na_2HPO_4 (6.44 g, 45.35 mmol) and freshly crushed 5% Na(Hg) (38.50 g, 15 times weight of **40**) at -10°C under argon. After being stirred at -10°C for 18 h, THF-Et_2O (200 mL-200 mL) was added to the mixture and the mixture was stirred at ambient temperature for 10 min. The whole was decanted to a silica gel short column and eluted with THF. The eluate was concentrated *in vacuo* to give a pale yellow oil, which was extracted with EtOAc (x 2)

and washed with H_2O and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo* to give an oily residue. The crude oil was purified by silica gel column chromatography (BW-200, 200 g, Et_2O:hexane=2:1) to give **45** (2.667 g, quant.) as a white solid, mp 121-122°C. $[\alpha]_D^{25}$ -44.2° (c=1.1, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3441, 3386, 1698, 1514, 1456, 1370, 1254, 1163, 1061, 876 cm^{-1}. ^1H NMR δ 1.35 (s, 3H), 1.45 (s, 12H), 2.54 (br, 1H), 4.02 (m, 1H), 4.21 (m, 3H), 5.00 (br, 1H), 5.80 (ddd, 1H, J=9.9, 3.3, 1.3 Hz), 6.10 (ddd, 1H, J=9.9, 2.6, 2.3 Hz). Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.70; H, 8.11; N, 4.93.

(1*S*,2*R*,3*S*,4*R*)-2,3-*O*-Isopropylidenedioxy-4-*t*-butoxycarbonylaminocyclohexanol (46a). A mixture of **45** (17.44 g, 61.19 mmol), 5% Pd/C (1.75 g) in MeOH (1.22 L) was stirred at ambient temperature for 2 h under H_2. The mixture was filtered through the pad of celite, and the precipitates were washed with MeOH. The filtrate and washed solution were combined and concentrated *in vacuo* to give a colorless oil. The crude oil was purified by silica gel column chromatography (BW-200, 300 g, EtOAc:hexane=1:1) to give **46a** (17.79 g, quant.) as a hygroscopic oil. $[\alpha]_D^{25}$ -7.1° (c=1.1, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3441, 3385, 1705, 1505, 1456, 1370, 1244, 1165, 1022, 994, 874 cm^{-1}. ^1H NMR δ 1.36 (s, 3H), 1.45 (s, 9H), 1.52 (s, 3H), 1.5-1.7 (m, 2H), 1.8-2.0 (m, 2H), 2.12 (br, 1H), 3.81 (m, 1H), 4.03 (m, 3H), 4.68 (br d, 1H, J=7.9 Hz). Anal. Calcd for $C_{14}H_{25}NO_5$: C, 58.52; H, 8.77; N, 4.87. Found: C, 58.22; H, 8.87; N, 4.86.

(2*S*,3*S*,4*R*)-2,3-*O*-Isopropylidenedioxy-4-*t*-butoxycarbonylaminocyclohexanone (41). The alcohol (**46a**) (5.834 g, 18.951 mmol) was oxidized as described for the oxidation of *trans*-4-*t*-butoxycarbonylaminocyclohexanol to give crude **41**, which was purified by silica gel column chromatography (BW-200, 300 g, EtOAc:hexane=2:3, then acetone:hexane=1:3) to give **41** (5.07 g, 94%) as a hygroscopic viscous oil. $[\alpha]_D^{28}$ +26.9° (c=2.6, $CHCl_3$). IR ν_{max} (neat): 3352, 1717, 1684, 1530, 1369, 1244, 1163, 1076, 868 cm^{-1}. ^1H NMR δ 1.38 (s, 3H), 1.46 (s, 9H), 1.47 (s, 3H), 1.98 (m, 1H), 2.24 (m, 1H), 2.49 (br t, 2H, J=7.9 Hz), 3.93 (m, 1H), 4.4-4.5 (m, 2H), 4.80 (br, 1H). Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.70; H, 8.19; N, 4.85.

(1*R*,4*S*)-3-*t*-Butoxycarbonyl-3-aza-2-oxabicyclo[2.2.2]oct-5-ene (47). The oxazine (**36**) (4.96 g, 33.627 mmol) was protected as described for the protection of **42** to give **47** (6.91 g, 97%) as a colorless oil, bp 120°C /3 mmHg. $[\alpha]_D^{22}$ +21.3° (c=1.5, $CHCl_3$). IR ν_{max} (neat): 1703, 1617, 1456, 1368, 1260, 1074, 1053, 991, 959, 916, 880 cm^{-1}. ^1H NMR δ 1.1-1.6 (m, 2H), 1.46 (s, 9H), 2.17 (m, 2H), 4.73 (br, 2H), 6.54 (m, 2H). Anal. Calcd for $C_{11}H_{17}NO_3$: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.18; H, 8.12; N, 6.63.

(1*R*,4*S*,5*R*,6*R*)-5,6-Dihydroxy-3-*t*-butoxycarbonyl-3-aza-2-oxabicyclo[2.2.2]octane (48). To a solution of **47** (6.29 g, 29.81 mmol) in 90% aq. acetone (298 mL) were added OsO_4 (0.1 M solution in toluene, 29.8 mL, 2.98 mmol) and 50% aq. NMO (17.5 mL, 74.526 mmol). After being stirred at rt for 2 h, the mixture was added to 1M Na_2SO_3 and stirred at ambient temperature for 30 min. NaCl was added to the

mixture, and the mixture was extracted with EtOAc (x 3) and washed with brine. The extracts were dried over Na_2SO_4 then concentrated *in vacuo*. The residue was recrystallized from EtOAc-acetone-hexane to give **48** (2.982 g, 41%) as colorless needles. Then the mother liquid was purified by silica gel column chromatography (BW-200, 200 g, EtOAc:hexane=7:11) to give **48** (2.172 g, 30%) as a colorless solid, mp. 117-119°C. $[\alpha]_D^{20}$ -5.4° (c=0.5, $CHCl_3$). IR ν_{max} ($CHCl_3$) 3750-3100, 1705, 1458, 1370, 1256, 1167, 1121, 1088, 937, 839 cm^{-1}. ^1H NMR δ 1.50 (s, 9H), 1.87 (br, 2H), 2.05 (m, 2H), 3.55 (br, 2H), 4.10 (br, 1H), 4.16 (br, 1H), 4.22 (br, 2H). Anal. Calcd for $C_{11}H_{19}NO_5$: C, 53.87; H, 7.81; N, 5.71. Found: C, 53.73; H, 7.84; N, 5.79.

(1*R*,4*S*,5*R*,6*R*)-5,6-Dihydroxy-3-benzyloxycarbonyl-3-aza-2-oxabicyclo[2.2.2]octane (49). The olefin (**37**) (7.058 g, 28.808 mmol) was dihydroxylated as described for the oxidation of **47** to give crude **49**, which was purified by silica gel column chromatography (BW-200, 300 g, EtOAc:hexane=2:1) to give **49** (6.528 g, 81%) as a colorless viscous oil. $[\alpha]_D^{23}$ -3.6° (c=1.0, $CHCl_3$). IR ν_{max} (neat): 3700-3100, 1700, 1499, 1456, 1399, 1341, 1273, 1243, 1118, 1087, 1069 cm^{-1}. ^1H NMR δ 1.8-1.9 (m, 2H), 1.9-2.1 (m, 2H), 3.0-3.7 (br, 2H), 4.14 (m, 4H), 5.21 (s, 2H), 7.36 (s, 5H).

(1*R*,4*S*,5*R*,6*R*)-5,6-*O*-Isopropylidenedioxy-3-*t*-butoxycarbonyl-3-aza-2-oxabicyclo-[2.2.2]octane (50). To a solution of **49** (4.994 g, 20.38 mmol) in CH_2Cl_2 (160 mL) were added 2,2-dimethoxypropane (DMP) (20 mL, 160.30 mmol) and pyridinium *p*-toluenesulfonate (410 mg, 1.63 mmol) at ambient temperature. After being stirred at ambient temperature for 12 h, the mixture was added to H_2O. The whole mixture was extracted with CH_2Cl_2 (x 2), and washed with H_2O and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (BW-200, 250 g, EtOAc:hexane=1:5) to give **50** (5.723 g, 99%) as a white solid, mp 64-65°C. $[\alpha]_D^{20}$ -4.2° (c=1.3, $CHCl_3$). IR ν_{max} ($CHCl_3$): 1698, 1456, 1368, 1266, 1210, 1161, 1127, 1063, 934, 866 cm^{-1}. ^1H NMR δ 1.39 (s, 3H), 1.51 (s, 9H), 1.54 (s, 3H), 1.7-2.1 (m, 4H), 4.28 (br, 2H), 4.43 (m, 2H). Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.91; H, 8.18; N, 4.92.

(1*R*,4*S*,5*R*,6*R*)-5,6-*O*-Isopropylidenedioxy-3-benzyloxycarbonyl-3-aza-2-oxabicyclo-[2.2.2]octane (51). The diol (**49**) (6.414 g, 22.989 mmol) was protected as described for the protection of **48** to give crude **51**, which was purified by silica gel column chromatography (BW-200, 250 g, EtOAc:hexane=1:4) to give **51** (6.618 g, 90%) as a white solid, mp 66-69°C. $[\alpha]_D^{23}$ -4.1° (c=1.1, $CHCl_3$). IR ν_{max} ($CHCl_3$): 1700, 1499, 1385, 1347, 1267, 1209, 990, 870, 754, 698 cm^{-1}. ^1H NMR δ 1.37 (s, 3H), 1.53 (s, 3H), 1.7-2.1 (m, 4H), 4.3-4.5 (m, 4H), 5.23 (s, 2H), 7.36 (s, 5H). Anal. Calcd for $C_{17}H_{21}NO_5$: C, 63.94; H, 6.63; N, 4.39. Found: C, 63.87; H, 6.69; N, 4.40.

(1*R*,2*S*,3*R*,4*S*)-2,3-*O*-Isopropylidenedioxy-4-(*t*-butoxycarbonylamino)cyclohexanol (46b). The oxazine (**50**) (5.65 g, 19.825 mmol) was reduced as described for the reduction of **44** to give **46b** (6.22 g, quant.) as a hygroscopic viscous oil. $[\alpha]_D^{20}$ +7.5° (c=1.0, $CHCl_3$). Anal. Calcd for

$C_{14}H_{25}NO_5 \cdot {}^1/_2H_2O$: C, 56.74; H, 8.84; N, 4.73. Found: C, 56.65; H, 8.60; N, 4.88.

(1*R*,2*S*,3*R*,4*S*)-2,3-*O*-Isopropylidenedioxy-4-benzyloxycarbonylaminocyclohexanol (52). The oxazine (**51**) (6.57 g, 20.60 mmol) was reduced as described for the reduction of **44** to give crude residue, which was purified by silica gel column chromatography (BW-200, 200 g, EtOAc:hexane=3:2) to give **52** (6.377 g, 96%) as a white solid, mp 74-77°C. $[\alpha]_D^{20}$ +15.3° (c=1.0, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3432, 3341, 1701, 1541, 1456, 1374, 1296, 1244, 1057, 997, 876, 754 cm^{-1}. ^1H NMR δ 1.35 (s, 3H), 1.52 (s, 3H), 1.59 (m, 2H), 1.82 (m, 2H), 2.16 (br, 1H), 3.86 (m, 1H), 4.04 (m, 3H), 4.99 (d, 1H, J=8.3 Hz), 5.10 (ABq, 2H, J=12.2 Hz) 7.35 (s, 5H). Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.36. Found: C, 63.48; H, 7.27; N, 4.38.

(1*R*,2*R*,3*R*,4*S*)-1-(3,5-Dinitrobenzoyloxy)-2,3-*O*-isopropylidenedioxy-4-*t*-butoxy-carbonylaminocyclohexane (53b). To a solution of **46b** (29 mg, 0.102 mmol) in CH_2Cl_2 (1.0 mL) were added 3,5-dinitrobenzoyl chloride (28 mg, 0.123 mmol), Et_3N (21 μl, 0.153 mmol), and DMAP (1 mg, 0.01 mmol) at rt. After being stirred at ambient temperature for 2 h, H_2O was added to the mixture. The whole mixture was extracted with Et_2O, and washed with H_2O and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo* to give pale yellow oil. The residue was purified by silica gel column chromatography (BW-200, 15 g, Et_2O:hexane=2:3) to give 53b (44 mg, 89%) as a pale yellow amorphous solid. $[\alpha]_D^{20}$ -27.21° (c=0.9, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3443, 1732, 1703, 1630, 1549, 1507, 1456, 1347, 1275, 1215, 1165, 1065, 922 cm^{-1}. ^1H NMR δ 1.39 (s, 3H), 1.47 (s, 9H), 1.57 (s, 3H), 1.7-1.8 (m, 2H), 1.9-2.1 (m, 2H), 4.00 (br, 1H), 4.22 (br t, 1H, J=5.3mHz), 4.31 (brmt, 1H, J=5.6oHz), 4.71 (br, 1H), 5.39 (br, 1H), 9.15 (d, 2H, J=2.3 Hz), 9.24 (t, 1H, J=2.3 Hz).

(1*S*,2*S*,3*S*,4*R*)-1-(3,5-Dinitrobenzoyloxy)-2,3-*O*-Isopropylidenedioxa-4-*t*-butoxy-carbonylaminocyclohexane (53a). The alcohol **46a** (31 mg, 0.108 mmol) was condensed as described for **46b** to give **53a** (30 mg, 58%) as a pale yellow amorphous solid. $[\alpha]_D^{20}$ +28.1° (c=1.3, $CHCl_3$).
HPLC analysis for 53b and 53a: column, DAICEL CHIRALCEL OD-H; eluate, *i*-PrOH: hexane=1:3; detect, UV (254 nm); flow, 0.75ml/min; retention time, **53b**; 21.7min, **53a**; 25.9min. From **46b**; 98:2 (96%ee). From **46a**; 3:97 (94%ee)

(2*R*,3*R*,4*S*)-2,3-*O*-Isopropylidenedioxy-4-(benzyloxycarbonylamino)cyclohexanone (54). The alcohol **52** (6.34 g, 19.75 mmol) was oxidized as described for the oxidation of *trans*-4-*t*-butoxycarbonylaminocyclohexanol to give the crude **54**, which was purified by silica gel column chromatography (BW-200, 300 g, acetone:hexane=5:12) to give **54** (5.907 g, 94%) as a colorless viscous oil. $[\alpha]_D^{23}$ -18.9° (c=2.1, $CHCl_3$). IR ν_{max} (neat) 3445, 1728, 1700, 1538, 1534, 1456, 1388, 1379, 1310, 1242, 1225, 1163, 1040, 980, 895, 876 cm^{-1}. ^1H NMR δ 1.37 (s, 3H), 1.47 (s, 3H), 1.99 (m, 1H), 2.22 (br, 1H), 2.47 (d, 1H, J=7.9 Hz), 2.49 (d, 1H, J=5.9 Hz), 3.99 (m, 1H), 4.40 (d, 1H, J=6.6 Hz), 4.45 (m, 1H), 5.12 (ABq, 2H, J=12.5 Hz), 5.1-5.2 (m, 1H), 7.36 (s, 5H). Anal. Calcd for $C_{17}H_{21}NO_5 \cdot {}^1/_2H_2O$: C, 62.18; H, 6.75; N, 4.27. Found: C, 61.82; H, 6.51; N, 4.33.

Methyl 2-Methoxyimino-3-[(2*R*,3*S*,4*R*)-2,3-*O*-isopropylidenedioxy-4-*t*-butoxycarbonylaminocyclohexylidene]propionate (55a). To a solution of **32a** (10.42 g, 43.60 mmol) in DME (150 mL) was added *n*-BuLi (1.56 M in hexane, 28.0 mL, 43.60 mmol) at -78°C under argon. The mixture was stirred for 1 h, then a solution of **41** (4.97 g, 17.44 mmol) in DME (50 mL) was added. After being stirred at -78°C for 2 h, the mixture was warmed to rt during 2 h, and stirred at rt for 11 h. The mixture was quenched with sat. aq. NH_4Cl and extracted with EtOAc (x 2). The extracts were washed successively with 10% aq. citric acid, sat. aq. $NaHCO_3$, and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 300 g, EtOAc:hexane=2:5 and BW-300, 300 g, Et_2O:hexane=3:2) to give **55a** (5.95 g, 86%; *E*:*Z*=94:6) as a colorless waxy solid, mp. 35-39°C. $[\alpha]_D^{25}$ +60.3° (c=1.1, $CHCl_3$, pure *E* isomer). IR ν_{max} ($CHCl_3$): 3391, 1709, 1507, 1439, 1368, 1313, 1163, 927, 868 cm^{-1}. ^1H NMR δ 1.38 (s, 3H), 1.44 (s, 9H), 1.54 (s, 3H), 1.4-1.6 (m, 1H), 2.0-2.2 (m, 2H), 2.2-2.4 (m, 1H), 3.79 (m, 1H), 3.86 and 3.87 (s and s; 3H), 4.06 and 4.09 (s and s; 3H), 4.0-4.2 (m, 1H), 4.54 (d, 0.06H, J=4.3 Hz), 4.61 (d, 0.94H, J=8.3Hz), 4.93 (br d, 1H, J=8.3 Hz), 6.06 (s, 0.06H), 6.17 (s, 0.94H). Anal. Calcd for $C_{19}H_{30}N_2O_7$: C, 57.27; H, 7.59; N, 7.03. Found: C, 57.12; H, 7.70; N, 6.97.

Methyl 2-Methoxyimino-3-[(2*S*,3*R*,4*S*)-2,3-*O*-isopropylidenedioxy-4-benzyloxycarbonylaminocyclohexylidene]propionate (55b). The ketone (**54**) (75 mg, 0.235 mM) was condensed with **32a** as described for the preparation of **55a** to give an oily residue, which was purified by silica gel column chromatography (BW-200, 20 g, EtOAC:hexane=1:2) to give **55b** (59 mg, 58%) as a viscous oil. In addition, 13 mg of the starting material (**54**) (17%) was recovered.

Compound (55b): a colorless viscous oil. IR ν_{max} ($CHCl_3$): 3440, 1720, 1518, 1456, 1439, 1385, 1373, 1289, 1159, 1134, 1030, 930 cm^{-1}. ^1H NMR δ 1.2-1.6 (m, 2H), 1.30 and 1.38 (s and s, 3H), 1.47 and 1.55 (s and s, 3H), 2.0-2.2 (m, 2H), 2.2-2.4 (m, 1H), 3.76 and 3.7-4.0 (dd, J=12.2, 6.9 Hz and m; 1H), 3.85 (br s, 3H), 3.95 and 4.05 (s and s; 3H), 4.17 and 4.33 (m and dd, J=14.2, 7.3Hz; 1H), 4.52 (d, 0.14H, J=6.6 Hz), 4.62 (d, 0.86H, J=6.3 Hz), 5.12 (m, 2H), 5.0-5.2 and 5.35 (m and br; 1H), 6.05 (s, 0.14H), 6.16 (d, 0.86H, J=1.3 Hz), 7.35 (s, 5H). DIFNOE: 9.4% δ 6.16 (C3-H) to 4.62 (C5-H).

Methyl 2-Methoxyimino-3-[(2*R*,3*S*,4*R*)-2,3-dihydroxy-4-*t*-butoxycarbonylaminocyclohexylidene]propionate (56) and (7*R*,8*S*,8'*R*)-3-Methoxyimino-7-*t*-butoxycarbonylamino-8-hydroxy-3,5,6,7,8,8a-hexahydro-4-dehydrocoumarin (57). To a solution of **55a** (14.8 g, 37.19 mmol) in 90% aq. MeOH (372 mL) was treated with *p*-TsOH·H_2O (3.54 g, 18.59 mmol) at ambient temperature. After being stirred at rt for 3 days, the mixture was concentrated *in vacuo*. The whole residue was extracted with EtOAc (x 2) and washed with H_2O and brine. The extracts were combined and dried over Na_2SO_4, then concentrated *in vacuo* to give an oily residue. The residue was purified by silica gel column chromatography (BW-300, 300 g, EtOAc:hexane=2:1) to give **56** (12.49 g, 83%) and **57** (879 mg, 6%).

Compound (56): a hygroscopic viscous oil. $[\alpha]_D^{27}$ +72.56° (c=1.0, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3750-3100, 3400, 1732, 1715, 1510, 1439, 1367, 1165, 1051, 949, 926 cm^{-1}. ^1H NMR δ 1.1-1.3 (m, 1H), 1.46 (s, 9H), 1.9-2.1 (m, 2H), 2.3-2.5 (m, 1H), 2.80 (br, 1H), 3.49 (m, 1H), 3.86 (s, 3H), 3.7-4.0 (m,

1H), 4.06 (s, 3H), 4.31 (br, 2H), 4.53 (br, 1H), 6.03 (s, 1H). Anal. Calcd for $C_{16}H_{26}N_2O_7$: C, 53.62; H, 7.31; N, 7.82. Found: C, 53.48; H, 7.63; N, 7.47.

Compound (57): a white solid, mp 189°C (decomp). $[\alpha]_D^{27}$ -12.37° (c=1.0, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3750-3100, 3445, 1732, 1700, 1568, 1507, 1446, 1395, 1367, 1320, 1248, 1165, 1078, 939, 911, 877 cm^{-1}. ^1H NMR δ 1.46 (s, 9H), 1.77 (m, 1H), 2.0-2.2 (m, 1H), 2.2-2.6 (m, 2H), 2.70 (br, 1H), 3.99 (br, 1H), 4.12 (s, 3H), 4.43 (d, 1H, J=3.6 Hz), 4.66 (br, 1H), 5.16 (br, 1H), 6.82 (s, 1H). Anal. Calcd for $C_{15}H_{22}N_2O_6 \cdot 1/4H_2O$: C, 54.45; H, 6.85; N, 8.47. Found: C, 54.70; H, 6.87; N, 8.08.

Methyl 2-Methoxyimino-3-[(2*R*,3*S*,4*R*)-2,3-*O*-thiocarbonyldioxy-4-*t*-butoxycarbonyl-aminocyclohexylidene]propionate (59). To a solution of **56** (3.3 g, 9.23 mmol) in benzene (92 mL) was added 1,1'-thiocarbonyldiimidazole (TCDI **58**) (1.87 g, 10.16 mmol) at ambient temperature under argon. After being stirred at rt for 12 h, the mixture was quenched with 10% aq. citric acid. The whole was extracted with EtOAc (x 2), and washed with sat. aq. $NaHCO_3$ and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo*. The whole residue was purified by silica gel column chromatography (BW-200, 250 g, EtOAc:hexane=4:5) to give **59** (3.371 g, 91%) as a colorless amorphous solid, mp 45-52°C. $[\alpha]_D^{25}$ +122.7° (c=1.0, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3391, 1713, 1510, 1441, 1316, 1275, 1248, 1159, 1051, 910 cm^{-1}. ^1H NMR δ 1.45 (s, 9H), 1.83 (m, 1H), 1.9-2.2 (m, 1H), 2.2-2.5 (m, 2H), 3.90 (s, 3H), 3.8-4.0 (m, 1H), 4.13 (s, 3H), 5.08 (br, 1H), 5.35 (br d, 2H, J=7.9 Hz), 5.26 (d, 1H, J=1.3 Hz). Anal. Calcd for $C_{17}H_{24}N_2O_7S$: C, 50.99; H, 6.04; N, 7.00. Found: C, 51.09; H, 6.04; N, 6.90.

Methyl 2-Methoxyimino-3-(4*R*-*t*-butoxycarbonylamino-2-cyclohexenylidene)propionate (61). To a solution of **59** (635 mg, 1.588 mmol) in CH_2Cl_2 (7 mL) was added the phosphine (**60**) (923 mg, 4.763 mmol) at ambient temperature under argon. After being stirred at rt for 12 h, the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 80 g, Et_2O:hexane=4:5) to give **61** (479 mg, 93%) as a colorless viscous oil. $[\alpha]_D^{28}$ +34.8° (c=1.2, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3443, 1705, 1499, 1439, 1368, 1320, 1242, 1217, 1163, 1051, 1015, 926 cm^{-1}. ^1H NMR δ 1.45 (s, 9H), 1.54 (m, 1H), 1.9-2.1 (m, 1H), 2.25 (m, 2H), 3.87 (s, 3H), 4.07 (s, 3H), 4.32 (br, 1H), 4.52 (br, 1H), 5.89 (dd, 1H, J=9.9, 3.3 Hz), 5.98 (s, 1H), 6.22 (dd, 1H, J=9.9, 1.7 Hz). Anal. Calcd for $C_{16}H_{24}N_2O_5$: C, 59.24; H, 7.46; N, 8.64. Found: C, 58.95; H, 7.60; N, 8.35.

Methyl 2-Methoxyimino-3-(4*R*-benzyloxycarbonylamino-2-cyclohexenylidene)propionate (62). A solution of **61** (1.82 g, 5.617 mmol) in CH_2Cl_2 (14.0 mL) was treated with TFA (5.06 mL) at ambient temperature for 1.5 h. The mixture was concentrated *in vacuo*, and toluene was added to the residue, then the mixture was concentrated *in vacuo*. This work-up was repeated three times. The residue was dissolved in $CHCl_3$ (56.2 mL), and Et_3N (1.88 mL, 13.48 mmol) and ZCl (963 μL, 6.741 mmol) were added at 0°C. After being stirred at 0°C for 2 h, the mixture was extracted with EtOAc (x 2). The extracts were washed with 10% aq. citric acid, sat. aq. $NaHCO_3$, and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 200 g, Et_2O:hexane=3:2) to give **62** (1.823 g, 91%) as a colorless viscous oil. $[\alpha]_D^{27}$ +46.9° (c=1.1, $CHCl_3$). IR

ν_{max} ($CHCl_3$): 3440, 1721, 1717, 1504, 1439, 1320, 1302, 1157, 1024, 926 cm^{-1}. ^1H NMR δ 1.5-1.7 (m, 1H), 1.9-2.1 (m, 1H), 2.25 (br, 2H), 3.86 (s, 3H), 4.07 (s, 3H), 4.39 (br, 1H), 4.86 (br d, 2H, J=8.3 Hz), 5.10 (s, 2H), 5.88 (dd, 1H, J=9.9, 3.3 Hz), 5.98 (s, 1H), 6.22 (dd, 1H, J=9.9, 1.7 Hz), 7.35 (s, 5H). Anal. Calcd for $C_{19}H_{22}N_2O_5 \cdot 1/4CHCl_3$: C, 59.55; H, 5.78; N, 7.22. Found: C, 59.43; H, 5.94; N, 7.39. FABMS m/z=359 (MH^+).

Methyl 2-Methoxyimino-3-(4*R*-acetamido-2-cyclohexenylidene)propionate (63). A solution of **61** (2.01 g, 6.20 mmol) in CH_2Cl_2 (15.5 mL) was treated with TFA (5.6 mL) at ambient temperature for 1 h. The mixture was concentrated *in vacuo*, and toluene was added to the residue, then the mixture was concentrated *in vacuo*. This work-up was repeated three times. The residue was dissolved in CH_2Cl_2 (62 mL), and then Et_3N (2.15 mL, 15.51 mmol), Ac_2O (704 μL, 7.45 mmol), and DMAP (76 mg, 0.62 mmol) were added. After being stirred at rt for 7 h, H_2O was added to the mixture and the mixture was extracted with EtOAc (x 2). The extracts were washed with 10% aq. citric acid, sat. aq. $NaHCO_3$, and brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-200, 200 g, EtOAc:hexane=6:1) to give **63** (1.632 g, 99%) as a white solid, mp 105-108°C. $[\alpha]_D^{27}$ +22.6° (c=1.0, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3440, 1732, 1657, 1539, 1439, 1372, 1318, 1157, 1051, 962, 841 cm^{-1}. ^1H NMR δ 1.52 (m, 1H), 1.99 (s, 3H), 1.9-2.1 (m, 1H), 2.25 (m, 2H), 3.87 (s, 3H), 4.08 (s, 3H), 4.65 (m, 1H), 5.59 (br d, 1H, J=8.3 Hz), 5.86 (dd, 1H, J=9.6, 3.3 Hz), 5.99 (s, 1H), 6.26 (dd, 1H, J=9.6, 1.3 Hz). Anal. Calcd for $C_{13}H_{18}N_2O_4$: C, 58.63; H, 6.81; N, 10.52. Found: C, 58.44; H, 6.68; N, 10.53.

Methyl 2-Methoxyimino-3-(4*aS*-acetamidocyclohexylidene)propionate (64). To a solution of **63** (100 mg, 0.376 mmol) in MeOH (10 mL) was added 5% Pd-$CaCO_3$ poisoned with Pb (Lindlar catalyst: 50 mg), and the mixture was stirred at rt for 5 h under H_2 (1 atm). The mixture was filtered through the pad of celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (BW-300, 20g, acetone:hexane=3:2) to give **64** (63 mg, 63%) as a colorless viscous oil. $[\alpha]_D^{25}$ +8.0° (c=1.1, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3438, 1732, 1651, 1549, 1439, 1372, 1320, 1217, 1148, 1109, 972, 912, 735 cm^{-1}. ^1H NMR δ 1.2-1.5 (m, 2H), 1.96 (s, 3H), 1.9-2.2 (m, 4H), 2.2-2.5 (m, 2H), 3.85 (s, 3H), 3.98 (m, 1H), 4.05 (s, 3H), 5.64 (br, 1H), 5.79 (s, 1H). Anal. Calcd for $C_{13}H_{20}N_2O_4 \cdot 1/4CH_3CO_2C_2H_5$: C, 57.92; H, 7.64; N, 9.65. Found: C, 57.86; H, 7.68; N, 9.75. FABMS: m/z=269 (MH^+).

Methyl 2-*t*-Butoxycarbonylamino-3-(4*aS*-acetamidocyclohexylidene)propionate (65). The oxime (**64**) (250 mg, 0.933 mmol) was converted to the Boc amino acid in two step sequence, as described for the preparation of **34** to give crude **65**, which was purified by silica gel column chromatography (BW-300, 10 g, $CHCl_3$:MeOH=30:1) to give **65** (275 mg, 87%) as an amorphous powder, mp 51-57°C. IR ν_{max} ($CHCl_3$): 3440, 3305, 1743, 1700, 1659, 1539, 1447, 1368, 1254, 1169, 1049, 1026, 914, 864, 772 cm^{-1}. ^1H NMR δ 1.2-1.4 (m, 2H), 1.43 (s, 4.5H), 1.44 (s, 4.5H), 1.96 (s, 3H), 1.9-2.3 (m, 5H), 2.6-2.8 (m, 1H), 3.72 (s, 1.5H), 3.74 (s, 1.5H), 3.8-4.1 (m, 1H), 4.9-5.3 (m, 3H), 5.64

(br, 1H). Anal. Calcd for $C_{17}H_{28}N_2O_5 \cdot {}^1/_5CHCl_3 \cdot {}^1/_4H_2O$: C, 56.02; H, 7.84; N, 7.60. Found: C, 56.36; H, 7.94; N, 7.60. FABMS: m/z=341 (MH^+).

2-Methoxyimino-3-(4*R*-benzyloxycarbonylamino-2-cyclohexenylidene)propionyl-(2*S*,7*aS*)-Aacp(Ac)-OMe (66a) and 2-Methoxyimino-3-(4*R*-benzyloxycarbonylamino-2-cyclohexenylidene)propionyl-(2*R*,7*aS*)-Aacp(Ac)-OMe (66b). To a solution of **62** (101 mg, 0.283 mmol) in THF-H_2O (2.5 mL-0.8 mL) was added a solution of 0.5*N* aq. LiOH (847 μL, 0.424 mmol) at 0°C and the mixture was stirred for 1 h. After the mixture was washed with EtOAc, the aqueous layer was acidified with 1M $KHSO_4$ (pH=3), extracted with EtOAc (x 2), and washed with brine. The extracts were dried over Na_2SO_4, then concentrated *in vacuo* to give the crude acid (90 mg). The crude acid was used for the condensation without further purification.

A solution of **65** (80 mg, 0.235 mmol) in CH_2Cl_2 (588 μL, 2.5 mL/mmol) was treated with TFA (212 μL, 0.9 mL/mmol) at ambient temperature for 1 h. The mixture was concentrated *in vacuo* to give a pale yellow residue. Toluene was added to the residue and the mixture was concentrated *in vacuo*. This work-up was repeated three times to remove the excess of TFA completely. The crude amine TFA salt (116 mg) was used for condensation without further purification.

The above acid and the amine TFA salt were dissolved in DMF (700 μL) and cooled to 0°C. DEPC (43 μL, 0.283 mmol) and then *i*-Pr_2NEt (90 μL, 0.517 mmol) were added to the solution. After being stirred at 0°C for 4 h, then rt for 3 days, the mixture was quenched with 10% aq. citric acid. The whole mixture was extracted with $CHCl_3$ (x 3) and washed with sat. aq. $NaHCO_3$ and brine. The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a yellow residue. The residue was triturated with $CHCl_3$-Et_2O to give **66a** as a white amorphous powder. The mother liquid was purified by silica gel column chromatography (BW-300, 20 g, acetone:hexane=10:9) to give **66b** as a colorless amorphous solid. The yield of **66** was 113 mg, 85%, and the ratio was determined by HPLC analysis (DAICEL CHIRALCEL OJ, eluate: *i*-PrOH:hexane=1:3, flow rate: 0.5 mL/min, detect: UV 254 nm, **66a**:**66b**=55:45; 18.1 min:10.9 min). Each diastereomer was used for the next step after complete separation.

Compound (66a): a white amorphous powder, mp 215-219°C. $[\alpha]_D^{24}$ +25.5° (c=2.1, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3441, 3330, 1749, 1682, 1647, 1528, 1508, 1456, 1307, 1199, 1169, 1055, 1026 cm^{-1}. ^1H NMR δ 1.27 (m, 2H), 1.57 (m, 1H), 1.97 (s, 3H), 2.06 (m, 4H), 2.24 (m, 4H), 2.75 (br d, 1H), 3.75 (s, 3H), 3.97 (m, 1H), 4.02 (s, 3H), 4.38 (br, 1H), 4.77 (br d, 1H, J=8.6 Hz), 5.10 (s, 2H), 5.14 (d, 1H, J=9.2 Hz), 5.2-5.4 (m, 2H), 5.85 (dd, 1H, J=9.9, 3.3 Hz), 5.91 (s, 1H), 6.23 (dd, 1H, J=9.9, 1.3 Hz), 7.10 (d, 1H, J=6.9 Hz), 7.36 (s, 5H). Anal. Calcd for $C_{30}H_{38}N_4O_7 \cdot {}^1/_5CHCl_3 \cdot {}^1/_2CH_3OH$: C, 60.79; H, 6.68; N, 9.24. Found: C, 60.43; H, 6.54; N, 9.54. HRFABMS Calcd for $C_{30}H_{39}N_4O_7$ (MH^+): 567.2819. Found: 567.2779.

Compound (66b): a colorless amorphous solid. $[\alpha]_D^{24}$ +70.6° (c=1.1, $CHCl_3$). IR ν_{max} ($CHCl_3$) 3440, 1740, 1707, 1676, 1509, 1458, 1439, 1217, 1207, 1174, 1053 cm^{-1}. ^1H NMR δ 1.2-1.4 (m, 2H), 1.4-1.7 (m, 1H), 1.94 (s, 3H), 1.8-2.4 (m, 8H), 2.81 (brd, 1H), 3.75 (s, 3H), 4.00 (m, 1H), 4.03 (s, 3H), 4.36 (br, 1H), 4.80 (brd, 1H), 5.10 (m, 3H), 5.30 (dd, 1H, J=9.3, 1.7Hz), 5.48 (br, 1H), 5.86 (dd, 1H, J=9.9, 3.3Hz), 5.94 (s, 1H), 6.24 (dd, 1H, J=9.9, 1.7Hz), 7.18 (m, 1H), 7.35 (s, 5H). HRFABMS Calcd for

$C_{30}H_{39}N_4O_7$ (MH^+): 567.2819. Found: 567.2783.

(2*S*,7*R*)-Ac-Aayp(Z)-(2*S*,7*aS*)-Aacp(Ac)-OMe (67a) and (2*R*,7*R*)-Ac-Aayp(Z)-(2*S*,7*aS*)-Aacp(Ac)-OMe (67b).

i) From 66a: The oxime (**66a**) (178 mg, 0.315 mmol) was reduced as described for the preparation of **65** to give crude (2S,R,7R)-H-Aayp(Z)-(2S,7aS)-Aacp(Ac)-OMe·HCO_2H, which was treated with Ac_2O-pyridine (0.4 mL-2.0 mL) at ambient temperature for 10 h. The mixture was concentrated in vacuo to give a yellow residue. The crude residue was purified by silica gel column chromatography (BW-300, 20 g, $CHCl_3$:MeOH=35:1 to 15:1) to give 67a and 67b (173 mg, 95%) as a white powder, in a ratio of 55:45 diastereomixture. The diastereomer was separated by preparative HPLC (YMC Pack R&D D-SIL-5-06, 250 x 20 mm; eluate: $(CH_2Cl)_2$:EtOH=8:1, flow rate: 10 mL/min, detect: UV 254 nm 67a: 31 min; 67b: 33 min).

ii) From radiosumin (1): A solution of **1** (16.5 mg, 0.03 mmol) in H_2O-dioxane (0.5 mL-0.5 mL) was treated with $NaHCO_3$ (6.4 mg, 0.076 mmol) and ZCl (6.5 μL, 0.045 mmol) at 0°C. After being stirred vigorously at 0°C for 2 h, the mixture was washed with Et_2O (x 3). The H_2O layer was neutralized with AcOH and concentrated *in vacuo* to give pale yellow residue. The residue was dissolved in MeOH (2.0 mL) and $TMSCHN_2$ (1.59 M in hexane solution, 190 μL, 0.3 mmol) was added at ambient temperature. After being stirred at rt for 2 h, the mixture was concentrated *in vacuo*, to give a yellow residue. The crude residue was purified by silica gel column chromatography (BW-300, 10 g, $CHCl_3$:MeOH=35:1 to 15:1) to give **67a** (6.7 mg, 38%) as a white amorphous solid.

Compound (67a) from 66a: a white amorphous powder. $[\alpha]_D^{17}$ +118.5° (c=0.1, $CHCl_3$:MeOH=9:1). IR ν_{max} (nujol): 3304, 1742, 1687, 1684, 1651, 1636, 1539, 1310, 1252, 1061, 1026, 847 cm^{-1}. ^1H NMR δ 1.1-1.4 (m, 2H), 1.60 (m, 2H), 1.96 (s, 3H), 2.01 (s, 3H), 1.9-2.2 (m, 3H), 2.2-2.3 (m, 2H), 2.3-2.5 (m, 1H), 2.6-2.9 (m, 2H), 3.74 (s, 3H), 3.95 (m, 1H), 4.40 (m, 1H), 4.77 (br d, 1H, J=8.6 Hz), 5.03 (br d, 1H, J=9.9 Hz), 5.11 (s, 2H), 5.1-5.2 (m, 2H), 5.31 (br d, 2H, J=7.9 Hz), 5.76 (dd, 1H, J=9.9, 3.3 Hz), 6.10 (dd, 1H, J=9.9, 1.0 Hz), 6.26 (br d, 1H, J=7.3 Hz), 6.40 (br d, 1H, J=6.3 Hz), 7.36 (s, 5H). HRFABMS Calcd for $C_{31}H_{41}N_4O_7$ (MH^+): 581.2975. Found: 581.2950.

Compound (67a) from 1: a white amorphous powder. $[\alpha]_D^{21}$ +117.2 (c=0.07, $CHCl_3$:MeOH=9:1). ^1H NMR δ 1.1-1.4 (m, 2H), 1.60 (m, 2H), 1.96 (s, 3H), 2.01 (s, 3H), 1.9-2.2 (m, 3H), 2.2-2.3 (m, 2H), 2.3-2.5 (m, 1H), 2.6-2.9 (m, 2H), 3.74 (s, 3H), 3.95 (m, 1H), 4.40 (m, 1H), 4.77 (br d, 1H, J=8.6 Hz), 5.03 (br d, 1H, J=9.2 Hz), 5.11 (s, 2H), 5.1-5.2 (m, 2H), 5.3-5.4 (m, 2H changed with D_2O), 5.32 (br d, 2H, J=7.9 Hz), 5.76 (dd, 1H, J=9.9, 3.3 Hz), 6.10 (dd, 1H, J=9.9, 1.0 Hz), 6.32 (br d, 1H, J=6.6 Hz), 6.45 (br d, 1H, J=6.6 Hz), 7.36 (s, 5H).

Compound (67b): a white amorphous powder. $[\alpha]_D^{20}$ -68.6° (c=0.08, $CHCl_3$:MeOH= 9:1). IR ν_{max} (nujol): 3291, 1742, 1693, 1684, 1645, 1636, 1317, 1252, 1202, 1138, 1059, 1026, 868, 802, 722, 696, 670 cm^{-1}. ^1H NMR δ 1.1-1.4 (m, 2H), 1.4-1.7 (m, 2H), 1.95 (s, 3H), 2.00 (s, 3H), 2.05 (m, 3H), 2.20 (m, 2H), 2.55 (m, 2H), 2.70 (m, 1H), 3.73 (s, 3H), 3.95 (s, 3H), 4.37 (m, 1H), 4.79 (br d, 1H, J=7.6 Hz), 5.04 (br d, 1H, J=8.9 Hz), 5.11 (s, 2H), 5.1-5.2 (m, 2H), 5.30 (d, 1H, J=8.3 Hz), 5.39 (d, 1H, J=9.6 Hz), 5.78 (dd, 1H, J=9.9, 3.6 Hz), 6.12 (dd, 2H, J=9.9, 1.3 Hz), 6.47 (br d, 1H, J=6.9 Hz), 7.36 (s, 5H).

(2*S*,7*R*)-Ac-Aayp(Z)-(2*R*,7*aS*)-Aacp(Ac)-OMe (67c) and (2*R*,7*R*)-Ac-Aayp(Z)-(2*R*,7*aS*)-Aacp(Ac)-OMe (67d). The oxime (**66b**) (23 mg, 0.04 mmol) was converted as described for the conversion of **66a** to give crude **67c** and **67d.** The crude residue was purified by silica gel column chromatography (BW-300, 20 g, $CHCl_3$:MeOH=20:1 to 15:1) to give **67c** and **67d** (17.4 mg, 74 %) as a white powder, a 44:56 ratio diastereomixture.

67c and 67d mixture. HRFABMS Calcd for $C_{31}H_{41}N_4O_7$ (MH^+): 581.2975. Found: 581.2958.

HPLC analysis for 67a-d: column, DAICEL CHIRALPAK AD; eluate: *i*-PrOH:hexane=1:2; flow rate: 0.5 ml/min; detect: UV (254 nm). **67a**: 9.5 min; **67b**: 12.4 min; **67c**: 16.2 min; **67d**: 19.6 min.

(2*S*,7*R*)-Ac-Aayp-(2*S*,7*aS*)-Aacp(Ac)-OH·xTFA (1, radiosumin). To a suspension of **67a** (29 mg, 0.05 mmol) in benzene (30 mL) was added $(Bu_3Sn)_2O$ (150 μL, 0.3 mmol) and the mixture was refluxed for 3 days under argon. After cooling, the mixture was quenched with AcOH and concentrated *in vacuo*. The residue was washed with Et_2O (x 3), and $CHCl_3$-MeOH-EtOAc was added to the precipitates, then the whole was centrifuged (2000 rpm, 3 min). The supernatant was separated from the precipitates, and concentrated *in vacuo* to give crude **67a**. The precipitate was passed through the ODS column (ODS, 5 g, H_2O to H_2O:MeOH=4:1) to give 15 mg of the crude product. The crude product was separated by preparative HPLC (YMC-Pack R&D, D-ODS-5-A, 250 x 20mm, aq. 8% MeCN:0.05% TFA) to give **1** (7 mg, 26%), and radiosumin methyl ester (6 mg, 22%) after lyophilized, respectively. In addition to these, starting material (**67a**) (12 mg, 41%) was recovered after purification.

Synthetic (1): a white amorphous powder, $[\alpha]_D^{17}$ +74.4° (c=0.1, H_2O). [lit.,[1] $[\alpha]_D^{20}$ +96°(c=0.77, H_2O).] IR ν_{max} (nujol): 3700-2000, 3283, 1717, 1684, 1653, 1636, 1559, 1541, 1509, 1204, 1136, 1043, 841, 801, 723, 669 cm^{-1}. ^1H NMR (270 MHz, DMSO-d_6) δ 1.21 (m, 2H), 1.53 (m, 1H), 1.76 (s, 3H), 1.7-2.0 (m, 3H), 1.82 (s, 3H), 2.0-2.4 (m, 4H), 2.54 (m, 1H), 2.72 (m, 1H), 3.71 (br m, 1H), 3.86 (brm, 1H), 4.85 (dd, 1H, J=8.9, 7.3 Hz), 5.13 (d, 1H, J=9.2 Hz), 5.16 (dd, 1H, J=8.9, 5.6 Hz), 5.33 (d, 1H, J=9.2 Hz), 5.68 (br dd, 1H, J=9.2, 2.3 Hz), 6.25 (d, 1H, J=9.2 Hz), 7.75 (d, 1H, J=7.5 Hz), 7.91 (br d, 3H, J=3.6 Hz), 8.26 (d, 1H, J=7.9 Hz), 8.46 (d, 1H, J=6.9 Hz). HRFABMS Calcd for $C_{22}H_{33}N_4O_5$ (MH^+): 433.2451. Found: 433.2438.

Natural 1: a white amorphous powder. $[\alpha]_D^{14}$ +99.9° (c=0.06, H_2O) and $[\alpha]_D^{16}$ +79.2° (c=0.1, H_2O; after preparative HPLC). ^1H NMR (270 MHz, DMSO-d_6) δ 1.20 (m, 2H), 1.53 (m, 1H), 1.76 (s, 3H), 1.7-2.0 (m, 3H), 1.82 (s, 3H), 2.0-2.4 (m, 4H), 2.4-2.6 (m, 1H), 2.75 (m, 1H), 3.7 (br m, 1H), 3.86 (br m, 1H), 4.85 (dd, 1H, J=8.6, 7.9 Hz), 5.13 (d, 1H, J=8.9 Hz), 5.16 (dd, 1H, J=8.6, 4.6 Hz), 5.33 (d, 1H, J=9.2 Hz), 5.68 (br dd, 1H, J=9.2, 2.6 Hz), 6.25 (d, 1H, J=9.2 Hz), 7.76 (d, 1H, J=7.9 Hz), 7.95 (br, 3H), 8.26 (d, 1H, J=7.9 Hz), 8.46 (d, 1H, J=7.3 Hz). [lit.,[19] ^1H NMR (500 MHz, DMSO-d_6) δ 1.19 (m, 2H), 1.52 (m, 1H), 1.76 (s, 3H), 1.77 (m, 2H), 1.82 (s, 3H), 1.85 (m, 1H), 2.00 (m, 1H), 2.02 (m, 1H), 2.16 (m, 1H), 2.31 (m, 1H), 2.53 (m, 1H), 2.71 (m, 1H), 3.70 (m, 1H), 3.85 (br m, 1H), 4.86 (dd, 1H, J=9.1, 6.6 Hz), 5.13 (d, 1H, J=9.1 Hz), 5.15 (dd, 1H, J=9.3, 7.9 Hz), 5.33 (d, 1H, J=9.3 Hz), 5.70 (br dd, 1H, J=9.7, 2.8 Hz), 6.23 (d, 1H, J=9.7 Hz), 7.75 (d, 1H, J=7.6 Hz), 8.08 (br d, 3H, J=7.6 Hz), 8.24 (d, 1H, J=7.9 Hz), 8.40 (d, 1H, J=6.6 Hz)].

ACKNOWLEDGEMENTS

Special thanks are due to Professor M. Murakami at University of Tokyo for the gift of natural radiosumin and valuable suggestions. This work is supported in part by Grants-in Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan and Japan Society for the Promotion of Science.

† Present address: Pfizer Central Research Laboratories, Taketoyo, Chita, Aichi 470-2393, Japan.

‡ Present address: Graduate School of Environmental and Human Sciences, Meijo University, Shiogama-guchi, Tempaku-ku, Nagoya 468-8502, Japan

* Corresponding author. Tel. & Fax: +81-52-832-1555; e-mail: shioiri@ccmfs.meijo-u.ac.jp

REFERENCE AND NOTES

1. H. Matsuda, T. Okino, M. Murakami, and K. Yamaguchi, *J. Org. Chem.*, 1996, **61**, 8648.
2. For a recent review, see T. Shioiri and Y. Hamada, *Synlett*, 2001, 184.
3. A preliminary account of this work was already reported: H. Noguchi, T. Aoyama, and T. Shioiri, *Peptide Chemistry 1996*, 1997, **34**, 5; H. Noguchi, T. Aoyama, and T. Shioiri, *Tetrahedron Lett.*, 1997, **38**, 2883.
4. (a) R. F. W. Jackson, N. Wishart, A. Wood, K. James, and M. J. Wythes, *J. Org. Chem.*, 1992, **57**, 3397. (b) T. Itaya, A. Mizutani, and T. Iida, *Chem. Pharm. Bull.*, 1991, **39**, 1407.; T. Itaya and A. Mizutani, *Tetrahedron Lett.*, 1985, **26**, 347. (c) N. A. Sasaki, C. Hashimoto, and R. Pauly, *Tetrahedron Lett.*, 1989, **30**, 1943. (d) M. P. Sibi and P. A. Renhowe, *Tetrahedron Lett.*, 1990, **31**, 7407; M. P. Sibi, D. Rutherford, and R. Sharma, *J. Chem Soc., Perkin Trans. 1*, 1994, 1675. (e) E. C. R. Smith, L. A. McQuaid, J. W. Paschal, and J. DeHoniesto, *J. Org. Chem.*, 1990, **55**, 4472.
5. (a) P. Garner and J. M. Park, *J. Org. Chem.*, 1987, **52**, 2361 and *Org. Synth.*, 1991, **70**, 18. (b) T. Moriwake, S. Hamano, S. Saito, and S. Torii, *Chem. Lett.*, 1987, 2085. (c) L. D. Arnold, C. G. Drover, and J. C. Vederas, *J. Am. Chem. Soc.*, 1987, **109**, 4649.; S. V. Pansare, G. Huyer, L. D. Armond, and J. C. Vederas, *Org. Synth.*, 1991, **70**, 1 and 10.
6. (a) M. Clarembeau, A. Cravador, W. Dumont, L. Hevesi, A. Krief, J. Lucchetti, and D. Van Ende, *Tetrahedron*, 1985, **41**, 4793; A. Krief, *Tetrahedron*, 1980, **36**, 2531 and references cited therein. (b) N. Komatsu, S. Matsunaga, T. Sugita, and S. Uemura, *J. Am. Chem. Soc.*, 1993, **115**, 5847.
7. (a) S. E. Denmark and I. Rivera, *J. Org. Chem.*, 1994, **59**, 6887. (b) A. Abiko and S. Masamune, *Tetrahedron Lett.*, 1996, **37**, 1077.
8. *Cf.* S. Knapp, J. J. Hale, M. Bastos, A. Molina, and K. Y. Chen, *J. Org. Chem.*, 1992, **57**, 6239.
9. T. Aoyama, N. Sonoda, M. Yamauchi, K. Toriyama, A. Anzai, A. Ando, and T. Shioiri, *Synlett*, 1998, 35 and references cited therein.
10. (a) M. Hayashi, Y. Miyamoto, T. Inoue, and N. Oguni, *J. Org. Chem.*, 1993, **58**, 1515. (b) E. J. Corey and Z. Wang, *Tetrahedron Lett.*, 1993, **34**, 4001.
11. (a) O. R. Duthaler, *Tetrahedron* 1994, **50**, 1539. (b) S. Harusawa, Y. Hamada, and T. Shioiri, *Synthesis*, 1979, 716. (c) S. Harusawa, Y. Hamada, and T. Shioiri, *Tetrahedron Lett.*, 1979, **20**, 4663 and 1980, **21**, 2202.

12. (a) D. A. Evans, T. C. Britton, J. A. Ellman, and R. Dorow, *J. Am. Chem. Soc.*, 1990, **112**, 4011. (b) D. A. Evans, T. C. Britton, R. L. Dorow, and J. F. D. Dellaria, Jr., *Tetrahedron*, 1988, **44**, 5525.
13. A. J. Bicknell, G. Burton, and J. S. Elder, *Tetrahedron Lett.*, 1988, **29**, 3361.
14. M. S. VanNieuwenhze and K. B. Sharpless, *J. Am. Chem. Soc.*, 1993, **115**, 7864 and references cited therein.
15. (a) T. Mukaiyama, K. Yorozu, K. Kato, and T. Yamada, *Chem. Lett.*, 1992, 181. (b) S. Itsuno, Y. Sakurai, K. Shimizu, and K. Ito, *J. Chem. Soc., Perkin Trans. 1*, 1990, 1859 and references cited therein. (c) Y. Sakito, Y. Yoneyoshi, and G. Suzukamo, *Tetrahedron Lett.*, 1988, **29**, 223.
16. (a) M. Sabuni, G. Kresze, and H. Braun, *Tetrahedron Lett.*, 1984, **25**, 5377. (b) H. Felber, G. Kresze, H. Braun, and A. Vasella, *ibid.,* 1984, **25**, 5381. (c) H. Felber, G. Kresze, R. Prewo, and A. Vasella, *Helv. Chim Acta*, 1986, **69**, 1137. (d) H. Braun, R. Charles, G. Kresze, M. Sabuni, and J. Winkler, *Liebigs Ann. Chem.*, 1987, 1129. (e) O. Werbitzky, K. Klier, and H. Felber, *ibid.,* 1990, 267. (f) H. Braun, H. Felber, G. Kresze, F. P. Schmidtchen, R. Prewo, and A. Vasella, *Liebigs Ann. Chem.*, 1993, 261. (f) For a review, see H. Waldmann, 'Organic Synthesis Highlights II' ed. by H. Waldmann, VCH, 1995, pp. 35-47.
17. G. E. Keck, S. Fleming, D. Nickell, and P. Weider, *Synth. Commun.*, 1979, **9**, 281.
18. K. Schürrle, B. Beier, and W. Piepersberg, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2407.
19. (a) N. C. Yang, M. Chen, and P. Chen, *J. Am. Chem. Soc.*, 1984, **106**, 7310. (b) K. Ramesh, M. S. Wolfe, Y. Lee, D. V. Velde, and R. T. Borchardt, *J. Org. Chem.*, 1992, **57**, 5861. (c) For a review, see T. Hudlicky and J. W. Reed, 'Advances in Asymmetric Synthesis' ed. by A. Hassner, JAI Press Ltd, Greenwich, CT., 1995, Vol. 1, pp. 271-312 and references cited therein.
20. (a) E. J. Corey and P. B. Hopkins, *Tetrahedron Lett.*, 1982, **23**, 1979. (b) M. K. Das and J. J. Zuckerman, *Inorg. Chem.*, 1971, **10**, 1028. (c) For a review, see E. Block, *Organic Reactions* 1974, **30**, 457.
21. Catalytic hydrogenation of **63** over 5% Pd/C mainly gave the overreduction product and the desired cyclohexane derivative (**64**) was obtained in 32% yield. Only the overreduction product was formed in the analogous hydrogenation of **61**. The imide reduction did not proceed at all, and only the starting material was recovered.
22. S. Takuma, Y. Hamada, and T. Shioiri, *Chem. Pharm. Bull.*, 1982, **30**, 3147 and references cited therein.
23. N. Hashimoto, T. Aoyama, and T. Shioiri, *Chem. Pharm. Bull.*, 1981, **29**, 1475.
24. C. J. Salomon, E. G. Mata, and O. A. Mascaretti, *J. Chem. Soc., Perkin Trans. 1*, 1996, 995 and references cited therein.
25. During the preparation of this manuscript, radiosumin B, composed of Aayp and N-Me-Aayp, was isolated from the blue-green alga *Microcystis aeruginosa* Kützing: J. E. Coleman and J. L. C. Wright, *J. Nat. Prod.*, 2001, **64**, 668.