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Determination of the Absolute Configuration and Total Synthesis of Radiosumin, a Trypsin Inhibitor from a Freshwater Blue-green Alga

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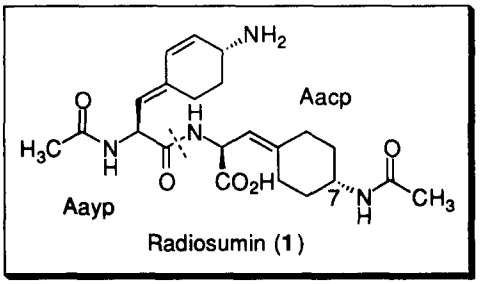
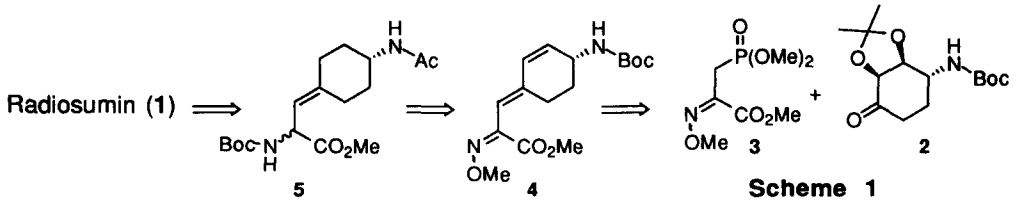
Abstract: Radiosumin (**1**), a novel potent trypsin inhibitory dipeptide isolated from a freshwater blue-green alga *Plectonema radiosum* (NIES-515), was synthesized for the first time, which unambiguously determined the absolute configuration. © 1997 Elsevier Science Ltd.

Radiosumin (**1**) was isolated by Murakami *et al.*[1] from a blue-green alga *Plectonema radiosum* (NIES-515) living in freshwater and was shown to be highly active against trypsin (IC_{50}=0.14 μg/ml) and moderately active against plasmin (IC_{50}=6.2 μg/ml) and thrombin (IC_{50}=88 μg/ml). This structurally intriguing dipeptide is composed of two unusual, novel α-amino acids: 2-amino-3-(4-amino-2-cyclohexen-1-ylidene)propionic acid (Aayp) and 2-amino-3-(4-amino-2-cyclohexylidene)propionic acid (Aacp). The absolute configuration at the C-7 position of Aacp, the axially chiral center, had remained unsolved. As a part of our program for the synthesis and study of aquatic natural products, we have investigated the structure elucidation and total synthesis of radiosumin.[2] We now report the determination of the unidentified axial chirality at the C-7 position of Aacp to be (*S*) and the first total synthesis of this structurally unique and biologically intriguing molecule **1**.



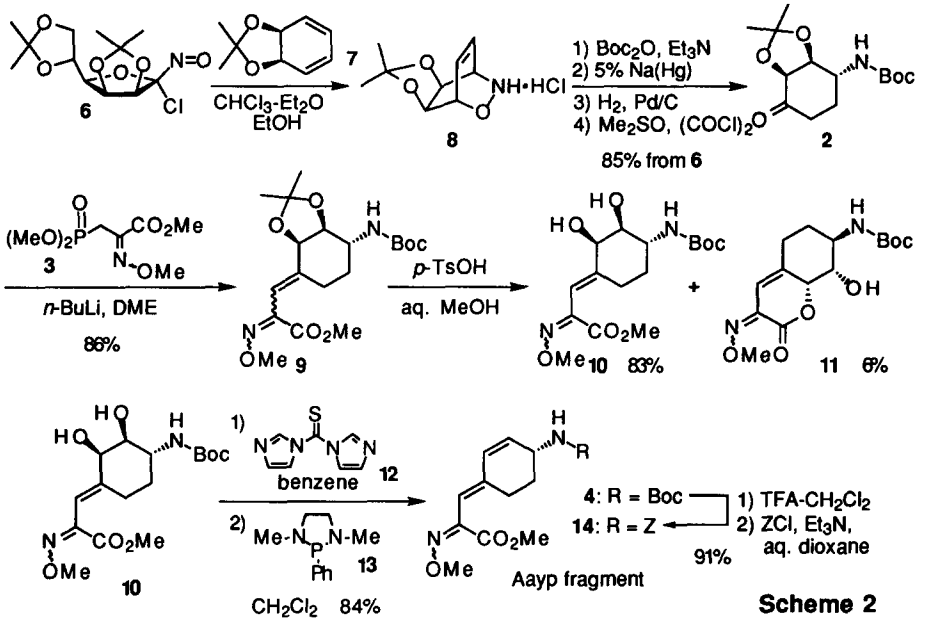
Radiosumin (**1**)

Consideration on the biosynthesis of radiosumin suggested that it would be formed in nature by the coupling of two amino acid units having the same absolute configuration, which led us to synthesize **1** containing (7*S*)-Aacp. Key features of our synthesis are (1) the stereoselective Horner-Emmons olefination of the aminocyclitol ketone **2** with Elder's phosphonate **3**[3] and (2) the regioselective hydrogenation of the Aayp derivative **4** to the Aacp one **5**, as shown in Scheme 1. The ketal function of the ketone **2** plays important roles in the protection of the double bond, the stereodirecting effect at the Horner-Emmons reaction, and easy separation of the diastereomers.



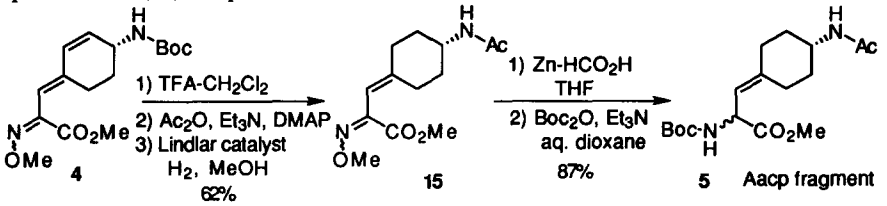
Scheme 1

Our synthesis started from the optically active oxazine **8** which was prepared by the asymmetric hetero Diels-Alder reaction of α-chloro nitroso compound **6** with the dihydrocatechol derivative **7** according to the literature,[4] as shown in Scheme 2. The oxazine **8** (94% ee) was efficiently converted to the amino cyclitol ketone **2** by the sequential amine protection with di-tert-butyl dicarbonate (Boc_2O), reductive fission of the N-O bond with sodium amalgam, catalytic reduction of the double bond over palladium carbon, and the Swern oxidation. The Horner-Emmons reaction of the ketone **2** with Elder's phosphonate **3** smoothly proceeded with efficient diastereoselectivity to give the ester **9** (*E*:*Z*=94:6).[5] Treatment of the E-Z mixture with p-toluenesulfonic acid in methanol afforded a mixture of the *vic*-diol **10** and the lactone **11**, which were easily separated on a silica gel column. After the *vic*-diol **10** was treated with 1,1-thiocarbonyldiimidazole (**12**), the Corey-Winter reaction by use of the Corey-Hopkins reagent, 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine (**13**),[6] gave the synthetic intermediate **4** common to Aayp and Aacp. The replacement of the Boc group of **4** with the benzyloxycarbonyl (Z) group was smoothly achieved to give **14**.



Scheme 2

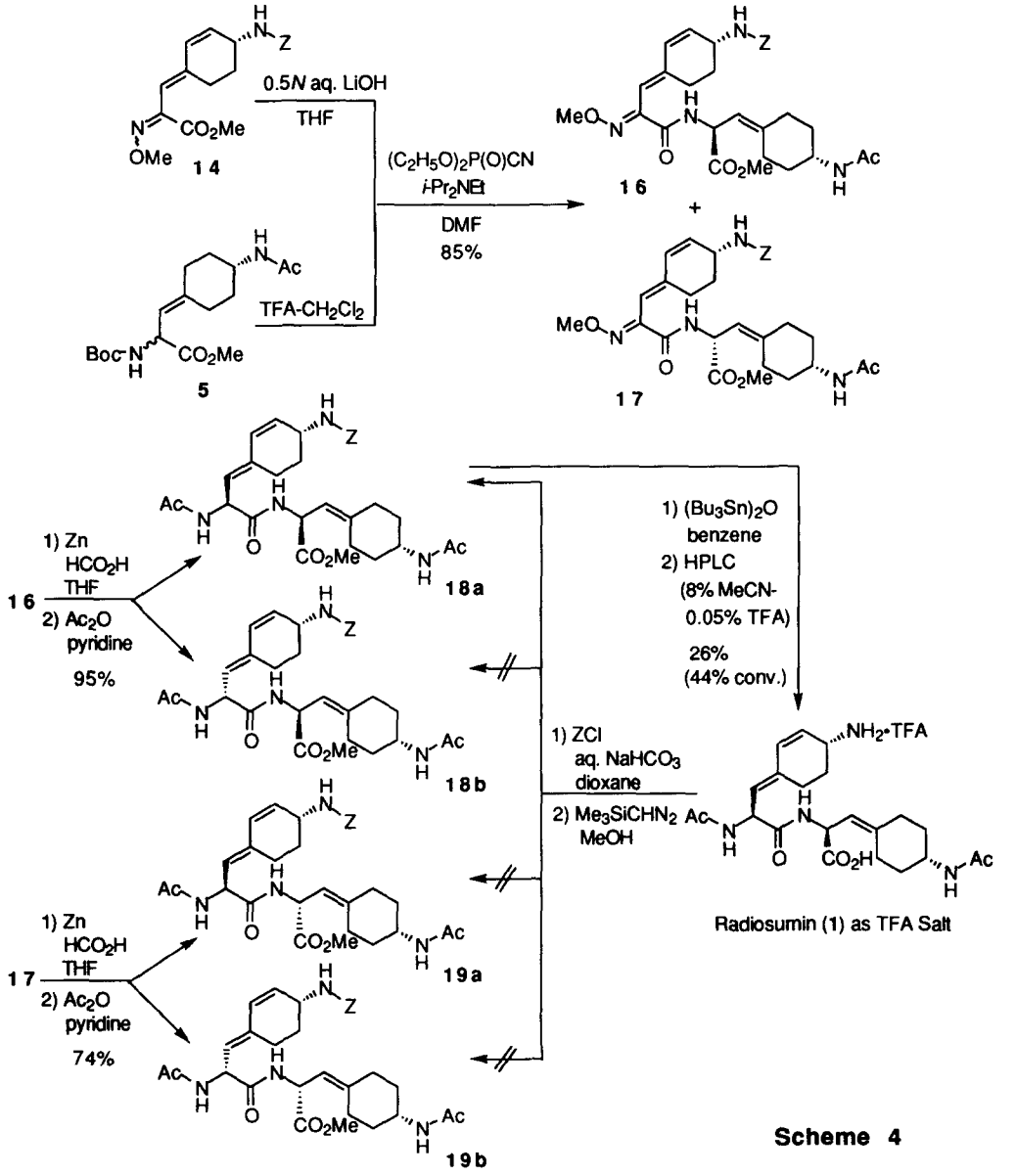
The (7*S*)-Aacp derivative **5** was prepared from the common intermediate **4** in 5 steps, as shown in Scheme 3. The replacement of the Boc group with the acetyl one, followed by the regioselective hydrogenation over the Lindlar catalyst afforded the acetyl derivative **15**. Reduction of the oxime ether and then the Boc protection produced the (7*S*)-Aacp derivative **5**.



Scheme 3

After deprotection of the Aayp derivative **14** with alkaline treatment and the Aacp one **5** with acidic treatment, the coupling of both deprotected fragments was smoothly achieved with diethyl phosphorocyanidate (DEPC, $(EtO)_2P(O)CN$)[7] to give a mixture of the dipeptide **16** and its Aacp C-2 diastereomer **17** in a ratio of

55:45,[5] which were completely separated from each other by trituration with chloroform-diethyl ether. The dipeptide **16**, a more polar major isomer, was treated with zinc-formic acid, followed by acetylation to give the N,O-protected radiosumin **18a** and its Aayp isomer **18b** in a ratio of 55:45.[5] Analogously, the dipeptide **17** was converted to a mixture of the Aacp C-2 isomers **19a** and **19b**. To determine the absolute configuration at the C-7 position of the Aacp moiety in radiosumin, natural radiosumin (**1**) was converted to the N,O-protected compound by treatment with benzyloxycarbonyl chloride (ZCl) followed by trimethylsilyldiazomethane in the presence of methanol.[8] The N,O-protected compound thus obtained was identical with **18a** among the four isomers.[9] Thus, the absolute configuration at the C-7 position of the Aacp moiety was unambiguously determined to be (*S*), hence the absolute stereostructure of radiosumin was determined to be **1**.



Scheme 4

The total synthesis of radiosumin was finally finished by treatment of **18a** with bis(tri-n-butyltin)oxide[10] under neutral conditions. The synthetic radiosumin obtained in this way was completely identical to the natural one by direct comparison as its trifluoroacetic acid (TFA) salt.[11,12]

Thus, we have accomplished the determination of the unidentified chiral center to be (*S*) and completed the first total synthesis of radiosumin (**1**). The method employed will be useful for the synthesis of other biologically interesting molecules.

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References and Notes

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2. Presented in part at the 34th Symposium on Peptide Chemistry held in Tsukuba on October 16-18; *Peptide Chemistry 1996* (ed. by C. Kitada), Protein Research Foundation, Osaka, **1997**, 6.
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4. (a) Braun, H.; Felber, H.; Kresze, G.; Schmidtchen, F. P.; Prewo, R.; Vasella, A. *Liebigs Ann. Chem.* **1993**, 261. (b) Schürrle, K.; Beier, B.; Piepersberg, W. *J. Chem. Soc. Perkin Trans. 1* **1991**, 2407.
5. The *E*, *Z* ratio of **9** was determined by 270 MHz ^{1}H NMR. The ratio of the other isomers was determined by HPLC analysis.
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9. Compound **18a**: $[\alpha]_D^{21}$+117.2° (c=0.07, $CHCl_3$/MeOH=9/1; from natural radiosumin). $[\alpha]_D^{17}$+118.5° (c=0.33, $CHCl_3$/MeOH=9/1; from **16**). ^{1}H NMR (270MHz, $Me_4Si/CDCl_3$) 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 (brd, 1H, J=8.6 Hz), 5.03 (brd, 1H, J=9.9 Hz), 5.11 (s, 2H), 5.1-5.2 (m, 2H), 5.31 (brd, 2H, J=7.9 Hz), 5.76 (dd, 1H, J=9.9, 3.0 Hz), 6.10 (dd, 1H, J=9.9, 1.0 Hz), 6.26 (brd, 1H, J=7.3 Hz), 6.40 (brd, 1H, J=6.3 Hz), 7.36 (s, 5H). HR FAB-MS. Calcd. for $C_{31}H_{41}N_4O_7$: 581.2975 (MH^+). Found: 581.2950 (MH^+). Compound **18b**: $[\alpha]_D^{20}$-68.6° (c=0.08, $CHCl_3$/MeOH=9:1).
   Retention time on HPLC of **18** and **19**: **18a** (9.5 min), **18b** (12.4 min), **19a** (16.2 min), and **19b** (19.6 min) on DAICEL CHIRAL PAK AD; *i*-PrOH:hexane =1:2, UV(254 nm). The synthetic **18a** was identical with the sample derived from natural radiosumin on HPLC analyses (DAICEL CHIRALCEL OD-H and YMC -Pack R&D R-SIL-5-06).
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11. Radiosumin thus obtained was actually its TFA salt because 8% aq. MeCN-0.05% TFA was used as an eluent for purification 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.[1b] 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.
12. The radiosumin methyl ester•TFA salt was also obtained in 22% yield together with 41% recovery of the starting material **18a**.

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