Analysis of Amide Bond Formation with an α-Hydroxy-β-amino Acid Derivative, 3-Amino-2-hydroxy-4-phenylbutanoic Acid, as an Acyl Component: Byproduction of Homobislactone

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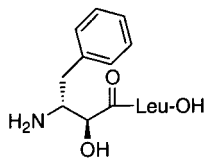
In the synthesis of peptidomimetics containing α-hydroxy-β-amino acid, the coupling of this N^{β}-protected β-amino acid with amine components was generally performed without the protection of its α-hydroxyl group. However, the formation of dipeptides in low yield was often observed when sterically hindered amine components were used. Boc-Apns-OH [Apns: (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoic acid, allophenylnorstatine] (**6**), which is one of such β-amino acid derivatives, is intensively employed as a core structure in the development of HIV-1 protease inhibitors. There have been no precise studies, to date, that have examined amide bond formation with α-hydroxy-β-amino acid derivatives as an acyl component. To determine the cause of this low-yield reaction, we studied the amide bond formation focusing on the activation step of N^{β}-protected α-hydroxy-β-amino acid by using a model coupling reaction between **6** and H-Dmt-OR [Dmt: (*R*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid] (**7**). A significant amount of homobislactone **9** was formed through the activation of the carboxyl group of **6** to the benzotriazole-type active esters such as OBt and OAt. In addition, this homobislactone formation was markedly increased in the presence of a catalytic amount of a base, which exhibited good correlation with the low yield of the amide bond formation, suggesting that homobislactone formation is one major reason for the low yield of the amide bond formation. Moreover, homobislactones were also formed in other derivatives of the N^{β}-protected α-hydroxy-β-amino acid, suggesting a common feature of this type of amino acids. The use of a strong activation method like EDC−HOAt without base addition enhanced amide bond formation, although a small amount of homobislactone may be formed during the coupling reaction.

Introduction

α-Hydroxy-β-amino acids are well-known as inhibitory machinery involved in the development of protease inhibitors. Representative derivatives include 3-amino-2-hydroxy-4-phenylbutanoic acid (AHPBA) and 3-amino-2-hydroxy-5-methylhexanoic acid (AHMHA), which can recognize the active site of proteases. Bestatin (**1**),[1] an aminopeptidase B and leucine-aminopeptidase inhibitor, and amastatin (**2**),[2] an aminopeptidase A inhibitor, are naturally occurring compounds containing AHPBA and AHMHA with one and three amino acid residues to the C-terminal of these residues, respectively. In a search for potent aspartyl protease inhibitors, compounds with (2*S*,3*S*)-AHPBA (allophenylnorstatine; Apns) as a P1 moiety were recently found to exhibit potent inhibitory efficacy against HIV-1 protease (**3**−**5**). Apns is known to interact with two aspartic acid residues of the active site by forming a stable transition-state intermediate, thus leading to potent inhibition of HIV-1 protease, which then inhibits replication of the causative agent of AIDS.[3−5]

Since the presence and the correct absolute configuration of the AHPBA residue in each of the compounds are crucial for the biological activities of these compounds, many stereoselective synthetic strategies have been reported.[6] These methods have effectively contributed to the synthesis of **1**, which contains (2*S*,3*R*)-AHPBA, referred to as Ubenimix,[7] a clinical chemotherapy agent for cancer, and KNI-764 (**4**, JE-2147), which contains Apns, a potent HIV-1 protease inhibitor in phase I clinical study for AIDS therapy.[3]



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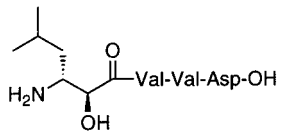

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(1) Umezawa, H.; Aoyagi, T.; Suda, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1976**, *29*, 97.

(2) Aoyagi, T.; Tobe, H.; Kojima, F.; Hamada, M.; Takeuchi, T.; Umezawa, H. T *J. Antibiot.* **1978**, *31*, 636.

(3) Mimoto, T.; Kato, R.; Takaku, H.; Nojima, S.; Terashima, K.; Misawa, S.; Fukazawa, T.; Ueno, T.; Sato, H.; Shintani, M.; Kiso, Y.; Hayashi. H. *J. Med. Chem.* **1999**, *42*, 1789.

(4) Mimoto, T.; Hattori, N.; Takaku, H.; Kisanuki, S.; Fukazawa, T.; Terashima, K.; Kato, R.; Nojima, S.; Misawa, S.; Ueno, T.; Imai, J.; Enomoto, H.; Tanaka, S.; Sakikawa, H.; Shintani, M.; Hayashi, H.; Kiso, Y. *Chem. Pharm. Bull.* **2000**, *48*, 1310.

(5) Komai, T.; Higashida, S.; Sakurai, M.; Nitta, T.; Kasuya, A.; Miyamoto, S.; Yagi, R.; Ozawa, Y.; Handa, H.; Mohri, H.; Yasuoka, A.; Oka, S.; Nishigaki, T.; Kimura, S.; Shimada, K.; Yabe. Y. *Bioorg. Med. Chem.* **1996**, *4*, 1365. Takashiro, E.; Hayakawa, I.; Nitta, T.; Kasuya, A.; Miyamoto, S.; Ozawa, Y.; Yagi, R.; Yamamoto, I.; Shibayama, T.; Nakagawa, A.; Yabe, Y. *Bioorg. Med. Chem.* **1999**, *7*, 2063 references cited therein.)

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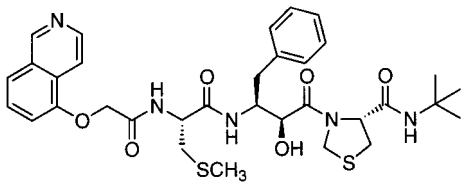

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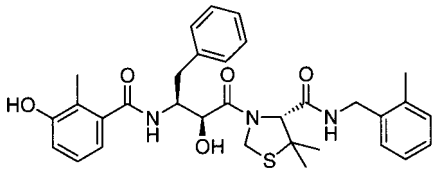

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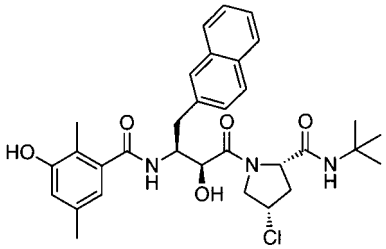

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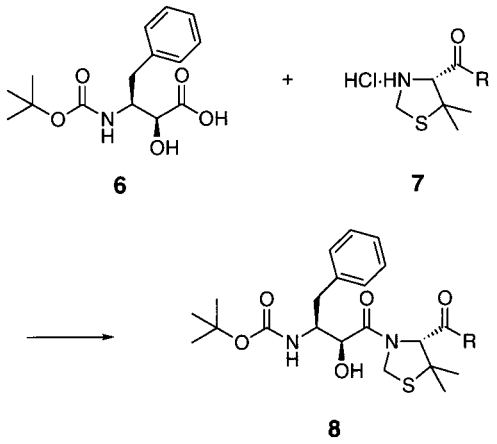
For the synthesis of these compounds, the coupling of N$^{\beta}$-protected AHPBA with amine components has been performed with the α-hydroxyl group of AHPBA unprotected by assuming the low reactivity of this secondary hydroxyl group. The yield of the amide bond formation is dependent on the nature of the amine component. In the case of sterically hindered amine components, the yield is relatively low compared to that of nonsterically hindered amine components probably because of steric hindrance.[3−5,8] Nevertheless, it has been reported that there are no problems in peptide bond formation with α-hydroxy-*β*-amino acid derivatives as an acyl component.

In our ongoing efforts for the synthesis of effective HIV-1 protease inhibitors (in accordance with compound

Scheme 1



6 **7** **8**

4), low yields were often observed during the condensation of Boc-Apns-OH (**6**) with C-terminal-modified Dmt [(R)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid] (**7**) residues yielding Boc-Apns-Dmt-R (**8**) (Scheme 1). Our preliminary observations were as follows: (1) the yield of **8** with H-Dmt-amide was better than with H-Dmt-ester, (2) during workup or in gram-scale reaction mixtures, an insoluble white precipitate is often formed, (3) the yield is influenced by the addition of base and the reaction seems to be better under acidic conditions rather than the normally used neutral or basic conditions, and (4) coupling using the BOP method[9] seems less effective than the EDC−HOBt method.[10]

With the increase in demand for the development of new aspartyl protease inhibitors such as *β*-secretase inhibitors,[11] malarial aspartyl protease inhibitors[12] and HTLV (human T cell leukemia virus)-1 protease inhibitors,[13] compounds with α-hydroxy-*β*-amino acid derivatives would be more significant from a medicinal chemistry point of view. Henceforth, a precise analysis of the condensation reaction of the AHPBA unit with sterically hindered P1′ units will be of great value for the synthesis and development of protease inhibitors. Because of the low yield in the condensation step, two aspects must be considered. These include (i) activation of the acyl component (α-hydroxy-*β*-amino acid derivatives) and (ii) reactivity of the amine component. In the present study, we have focused on the activation step of the acyl component and considered the side reaction, which occurs during the activation, using a model reaction between Boc-Apns-OH (**6**) and H-Dmt-R (**7**).[3]

Results and Discussion

Many methods for peptide bond formation with genetically coded amino acids have already been developed, and some of them are utilized in an automated-solid phase synthesizer to prepare peptides and proteins. On the other hand, there is increasing diversified requirement in peptide chemistry for the preparation of peptidomi-

(6) (a) Reetz, M. T.; Drewes, M. W.; Harms, K.; Reif, W. *Tetrahedron Lett.* **1988**, *29*, 3295. (b) Herranz, R.; Castro-Pichel, J.; Vinuesa, S.; Garcia-Lopez, M. T. *J. Chem. Soc., Chem. Commun.* **1989**, 938. Herranz, R.; Castro-Pichel, J.; Vinuesa, S.; Garcia-Lopez, M. T. *J. Org. Chem.* **1990**, *55*, 2232. (c) Pearson, W. H.; Hines, J. V. *Ibid.* **1989**, *54*, 4235. (d) Kobayashi, Y.; Takemoto, Y.; Kamijo, T.; Harada, H.; Ito, Y.; Terashima, S. *Tetrahedron* **1992**, *48*, 1853. (e) Norman, B. H.; Morris, M. L. *Tetrahedron Lett.* **1992**, *33*, 6803. (f) Patel, R. N.; Banerjee, A.; Howell, J. M.; McNamee, C. G.; Brozozowski, D.; Mirfakhrae, D.; Nanduri, V.; Thottathil, J. K.; Szarka, L. J. *Tetrahedron*: *Asymmetry* **1993**, *4*, 2069. (g) Kawabata, T.; Kiryu, Y.; Sugiura, Y.; Fuji, K. *Tetrahedron Lett.* **1993**, *34*, 5127. (h) Dondoni, A.; Perrone, D. *Synthesis* **1993**, 1162. Dondoni, A.; Perrone, D.; Semola, T. *Ibid.* **1995**, 181. (i) Bunnage, M. E.; Davies, S. G.; Goodwin, C. J. *Synlett* **1993**, 731. (j) Kearns, J.; Kayser, M. M. *Tetrahedron Lett.* **1994**, *35*, 2845. (k) Sasaki, H.; Kim, W.-S.; Suzuki, T.; Shibasaki, M. *Tetrahedron Lett.* **1994**, *35*, 6123. Hamashima, Y.; Sawada, D.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc.* **1999**, *121*, 2641. (l) Ojima, I.; Delaloge, F. *Chem. Soc. Rev.* **1997**, *26*, 377. (m) Keding, S. J.; Dales, N. A.; Lim, S.; Beaulieu, D.; Rich, D. H. *Synth. Commun.* **1998**, *28*, 4463. (n) Seki, M.; Matsumoto, K. *Synthesis* **1999**, 924.

(7) Ishizuka, M.; Masuda, T.; Kanbayashi, N.; Fukasawa, S.; Takeuchi, T.; Aoyagi, T.; Umezawa, H. *J. Antibiot.* **1980**, *33*, 642. Umezawa, H. *Small Molecular Immunomodifiers of Microbial Origin. Fundamental and Clinical Studies of Bestatin*; Japan Scientific Societies Press: Tokyo, and Pergamon Press: Oxford, 1981.

(8) Nishizawa, R.; Saino, T.; Takita, T.; Suda, H.; Aoyagi, T.; Umezawa, H. *J. Med. Chem.* **1977**, *20*, 510. Nishizawa, R.; Saino, T.; Suzuki, M.; Fujii, T.; Shirai, T. Aoyagi, T.; Umezawa, H. *J. Antibiot.* **1983**, *36*, 695. Saino, T.; Seya, K.; Nishizawa, R.; Takita, T.; Aoyagi, T.; Umezawa, H. *J. Antibiot.* **1987**, *40*, 1165.

(9) Nguyen, D. L.; Seyer, R.; Heitz, A.; Castro, B. *J. Chem. Soc., Perkin Trans. 1* **1985**, 1025.

(10) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788; **1970**, *103*, 2024;. **1973**, *106*, 3626.

(11) Ghosh, A. K.; Shin, D.; Downs, D.; Koelsch, G.; Lin, X.; Ermolieff, J.; Tang, J. *J. Am. Chem. Soc.* **2000**, *122*, 3522.

(12) Gluzman, I. Y.; Francis, S. E.; Oksman, A.; Smith, C. E.; Duffin, K. L.; Goldberg, D. E. *J. Clin. Invest.* **1994**, *93*, 1602.

(13) Louis, J. M.; Oroszlan, S.; Tözsér, J. *J. Biol. Chem.* **1999**, *274*, 6660.

Table 1. Synthetic Yield (%)[a] of Boc-Apns-Dmt-R (8)

	−R			
reagents	−NHBzl	−OBzl (**12**)	−NHMe	−OMe
EDC-HOBt[b]	69	40	78	31
BOP[c]	43	13	54	17

[a] Isolation yield. [b] Reaction conditions: **6** (1.1 equiv), **7**·HCl (1.0 equiv), HOBt (1.1 equiv), EDC·HCl (1.1 equiv), Et_3N (1.0 equiv), DMF, rt, 16 h. [c] Reaction conditions: **6** (1.1 equiv), **7**·HCl (1.0 equiv), BOP (1.1 equiv), Et_3N (2.5 equiv), DMF, rt, 16 h.

metics with nongenetically coded and/or unnatural amino acids. In the amide bond formation with these special amino acid residues, unexpected side reactions should be carefully taken into account. The case of α-hydroxy-*β*-amino acid derivatives is one of many such examples.

In our ongoing search for HIV-1 protease inhibitors, Apns-Dmt is considered as the core unit, based on the transition state analogue theory. Boc-Apns-Dmt-R (**8**) is a synthetic key intermediate compound, which is generally prepared from Boc-Apns-OH (**6**) and HCl·Dmt-R (HCl·**7**) by employing either the EDC−HOBt or the BOP method in coupling reactions. The Dmt residue is a highly constrained amino acid with a secondary amino group and a geminal dimethyl group on the thiazolidine ring; accordingly, we selected this core structure as a model for the analysis of the sterically hindered amide bond formation in N^{β}-protected α-hydroxy-*β*-amino acid derivatives.

Formation of Boc-Apns-Dmt-R (8) in a Conventional Manner. The effect of the two conventional coupling methods mentioned above on the yield of **8** was studied with **7**·HCl containing different R moieties (Scheme 1). Triethylamine was used for in situ neutralization of the HCl salt of **7**. As shown in Table 1, the isolation yields of **8** after column chromatography on silica gel were, in general, relatively low. The EDC method gave higher yields compared to the BOP method in all cases. Interestingly, the yields were also influenced by the C-terminal structure of the amine components. The benzyl- and methylamide structures produced higher yields compared to the corresponding esters in both coupling methods. These results indicate that the reactivity of the amine component is affected by the C-terminal structure, and the amide structure is preferable to attain higher yields. The reason for the low reactivity of H-Dmt-OR is unclear.

Formation of Homobislactone during Activation of Boc-Apns-OH. During the above coupling reaction or subsequent workup for the preparation of **8**, we frequently observed the production of an insoluble precipitate in both the aqueous and organic layers. To determine its chemical structure, we analyzed the precipitate using physicochemical methods and found it to be a homobislactone **9** with an energetically favorable symmetric six-membered ring structure derived from two **6** molecules. This result clearly indicated that a side reaction occurred during the amide bond formation, which was caused by the activation of the secondary α-hydroxyl group of **6**, suggesting that this α-hydroxyl group is reactive under the conditions required for amide bond formation. Two hydrophilic carbamate moieties and the rigid hydrophobic homobislactone structure of **9** may contribute to its insolubility in both organic and aqueous solvents.

Formation of Homobislactone under Different Coupling Conditions. To evaluate the mechanism by which **9** is formed and to find a suitable coupling method to circumvent this side reaction, we first carried out the HPLC analysis of the formation of **9** by the activation of **6** with different types of coupling agents and additives in the presence or absence of a base. As coupling agents, we used EDC·HCl, BOP, 2-chloro-1,3-dimethylimidazolidium hexafluorophosphate (CIP),[14] 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU),[15] and (benzotriazolyloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP),[16] and as additives, 1-hydroxybenzotriazole hydrate (HOBt·H_2O), 1-hydroxy-7-azabenzotriazole (HOAt),[17] 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HODhbt),[18] *N*-hydroxysuccinimide (HOSu),[19] and 4-(dimethylamino)pyridine (DMAP). In addition, Et_3N and *N*,*N*′-diisopropylethylamine (DIEA) were used as the base in varying proportions depending on the type of coupling agents.

Table 2. Homobislactone 9 Formation under Various Coupling Conditions

coupling agents	additives	Et_3N (equiv)	DIEA (equiv)	formation of **9** (%)[a]
EDC[b]				0
EDC		1.0		0
EDC	HOAt[c]			6.0 ± 0.4
EDC	HOAt	1.0		47.6 ± 1.2
EDC	HOAt		1.0	48.7 ± 1.1
EDC	HOBt[d]			6.0 ± 0.2
EDC	HOBt	1.0		42.4 ± 1.2
EDC	HOBt		1.0	41.2 ± 0.2
EDC	HODhbt[e]			<1.0
EDC	HODhbt	1.0		33.3 ± 2.1
EDC	HOSu[f]			0
EDC	HOSu	1.0		3.3 ± 0.4
EDC	DMAP[g]			3.1 ± 0.2
EDC	DMAP	1.0		<1.0
BOP[i]	HOBt	2.0		54.5 ± 3.6
PyBOP[j]	HOBt	2.0		58.9 ± 1.6
HBTU[k]		2.0		24.1 ± 2.8
CIP[h]				0
CIP		2.0		0
CIP		3.0		2.1 ± 0.4
CIP	HOAt	2.0		<1.0
CIP	HOAt	3.0		43.2 ± 1.6

[a] Yields were calculated by HPLC analysis using a working curve from the standard DMF solution of homobislactone, which was analyzed using the same HPLC conditions. Values are the mean value of three independent experiments with the standard error of the mean (SEM). [b] EDC: 1-ethyl-3-(3,3-dimethylaminopropyl)carbodiimide hydrochloride. [c] HOAt: 1-hydroxy-7-azabenzotriazole. [d] HOBt: 1-hydroxybenzotriazole. [e] HODhbt: 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine. [f] HOSu: *N*-hydroxysuccinimide. [g] DMAP: 4-(dimethylamino)pyridine. [h] CIP: 2-chloro-1,3-dimethyl-2-imidazolinium hexafluorophosphate. [i] BOP: benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate. [j] PyBOP: (benzotriazolyloxy)tripyrrolidinophosphonium hexafluorophosphate. [k] HBTU: 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. Reaction conditions: Boc-Apns-OH (1 equiv), coupling agent (1.0 or 1.2 equiv depending on the type of coupling agents), additive (1 equiv when used), Et_3N or DIEA (varying amounts depending on the type of coupling agents; see Table 1) in DMF solution (total volume of 2 mL), rt, 2 h.

As shown in Table 2, **9** was not formed when only EDC was employed. However, the addition of additives such as HOAt, HOBt, and DMAP resulted in a significant

(14) Akaji, K.; Kuriyama, N.; Kiso, Y. *Tetrahedron Lett.* **1994**, *35*, 3315.
(15) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.
(16) Caste, J.; Nguyen, D. L.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205.
(17) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
(18) (a) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2034. (b) Acerton, E.; Cameron, L.; Meldal, M.; Sheppard, R. J. *J. Chem. Soc., Chem. Commun.* **1986**, 1763.
(19) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. *J. Am. Chem. Soc.* **1964**, *86*, 1839.

formation of **9**, indicating that the formation of **9** is influenced by the addition of additives, with HOAt and HOBt being a strong promoter. On the other hand, the addition of HODhbt showed only a trace amount of **9** and the addition of HOSu did not result in **9** formation. The compound **9** was also not detected on TLC with ninhydrin staining in the latter case. These results indicated that the yield of **9** in the presence of additives decreased in the order HOBt = HOAt > DMAP > HODhbt ≥ HOSu.

Next, we examined the effect of base addition on the formation of **9**. Homobislactone formation was markedly increased by the addition of a base in conjunction with all of the above-mentioned additives except for DMAP, which reduced **9** formation (<1%), and HOSu, which exhibited only 3% formation of **9**. More than 40% of **6** was converted to **9** in the EDC−HOBt and −HOAt methods in the presence of 1 equiv of Et_3N or DIEA. In the case of HODhbt, 33% formation of **9** was observed in the presence of Et_3N, although only a trace amount of **9** was detected in the absence of the base. Thus, the extent of **9** formation promoted by additives in the presence of Et_3N decreases in the order HOAt ≥ HOBt > HODhbt ≫ HOSu > DMAP.

A high-yield generation of **9** was also observed in the BOP−HOBt, PyBOP−HOBt, and HBTU methods, which utilize the agents responsible for the conversion of a carboxylic acid into the corresponding HOBt esters directly in the presence of a tertiary amine. In the BOP and PyBOP methods, more than 50% of **6** was converted to homobislactone **9**.

We also examined CIP, an effective coupling agent developed in our laboratory for amide bond formation with sterically hindered amino acids. No homobislactone was formed with 0−2 equiv of Et_3N in the absence of additives, i.e., conditions that CIP could effectively promote the symmetrical anhydride of **6**, although 2% of **9** was formed in the presence of 3 equiv of Et_3N. On the other hand, in the presence of HOAt, under conditions that CIP promotes the formation of the HOAt ester of **6**, the use of 2 equiv of a base resulted in the formation of only a trace amount of **9**. However, the addition of 3 equiv of Et_3N markedly increased **9** formation comparable to that of EDC−HOBt or −HOAt with the addition of 1 equiv of Et_3N. Since 2 equiv of a base is consumed for the neutralization of hexafluorophosphate and the resulted chloride cations in the CIP case, 3 equiv of the base in the system may be equal to 1 equiv of the base in the EDC-additive systems.

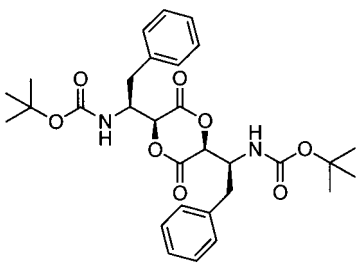
From these studies, activation with EDC or CIP resulted in almost no homobislactone formation independent of Et_3N (Table 1), suggesting that EDC or CIP-activated species such as the symmetric anhydride of Boc-Apns-OH are not involved in the formation of homobislactone. On the other hand, the addition of additives such as HOBt and HOAt significantly increased homobislactone formation, although HODhbt and HOSu hardly promoted the formation. The marked homobislactone formation upon the addition of 1 molar equiv of a base observed in the case of HOAt, HOBt, and HODhbt indicates that benzotriazole- and benzotriazine-type active esters specifically contribute to the formation of homobislactone **9**. This was also seen in the case of the BOP, PyBop, and HBTU methods and in the case of the CIP-HOAt methods with 3 equiv of the base.

One possible explanation for the homobislactone formation arising from benzotriazole- and benzotriazine-type active esters is thought to be the neighboring-group participation of the nitrogen atom in these esters. In the case of HODhbt, the formation of a small amount of homobislactone **9** was observed, which might be due to the low electron density prevailing over the nitrogen atom due to the existence of an electron-withdrawing carbonyl group. This observation is also supported by the extent of homobislactone formation among active esters, consistent with the known reactivity of each ester in the amide bond formation (HOAt > HOBt > HOSu).[10,17,18,20]

Effect of Base Concentration on Homobislactone Formation. Since the above results indicate that the addition of a base significantly increased homobislactone formation, to determine the role of the added base, we studied the effect of Et_3N concentration on the formation of **9** based on four coupling methods, i.e., the EDC−HOAt, EDC−HOBt, EDC−HODhbt, and CIP−HOAt methods. The amount of Et_3N varied from 0 to 4 equiv except for the CIP−HOAt method, where 0−5 equiv was used, and the formation of **9** in the reaction mixture after 2 h was analyzed by HPLC.

As shown in Figure 1A, the formation of homobislactone varied with increasing amount of Et_3N in the EDC−HOAt method. In particular, the addition of a small amount of Et_3N (0.1 equiv) still resulted in the formation of a substantial amount of **9** (33%), which reached a maximum (44%) at 1 equiv of Et_3N. Interestingly, further addition of Et_3N decreased the yield of **9** with increased formation of a more hydrophilic compound (as seen in the HPLC analysis). A similar profile was also observed in other coupling methods (Figure 1B,C), indicating that formation of **9** proceeds via a common mechanism irrespective of the coupling methods employed. Structural analysis of this unknown product revealed that this compound **10** was a half-ester dimer of **6**. The maximum production of **10** occurred with addition of around 2.5 equiv of Et_3N and gradually decreased with further addition of Et_3N. Most of **6** was converted to homobislactone or the half-ester dimer in the presence of 1.5−2.5 equiv of Et_3N in the EDC-additive methods. With the addition of a larger amount of Et_3N, HPLC chromatograms showed some minor peaks corresponding to the molecular weights of the tandemly linked trimer and tetramer of **6**, as detected by mass analysis (data not shown). The CIP−HOAt method also significantly increased homobislactone formation with more than 2 equiv of Et_3N similar to other coupling methods (Figure 1D).

These results indicate that a small amount of a base can accelerate the homobislactone **9** formation in all cases tested (Figure 1), suggesting that the base catalyzes homobislactone formation in conjunction with benzotriazole- and benzotriazine-type additives. However, the



9

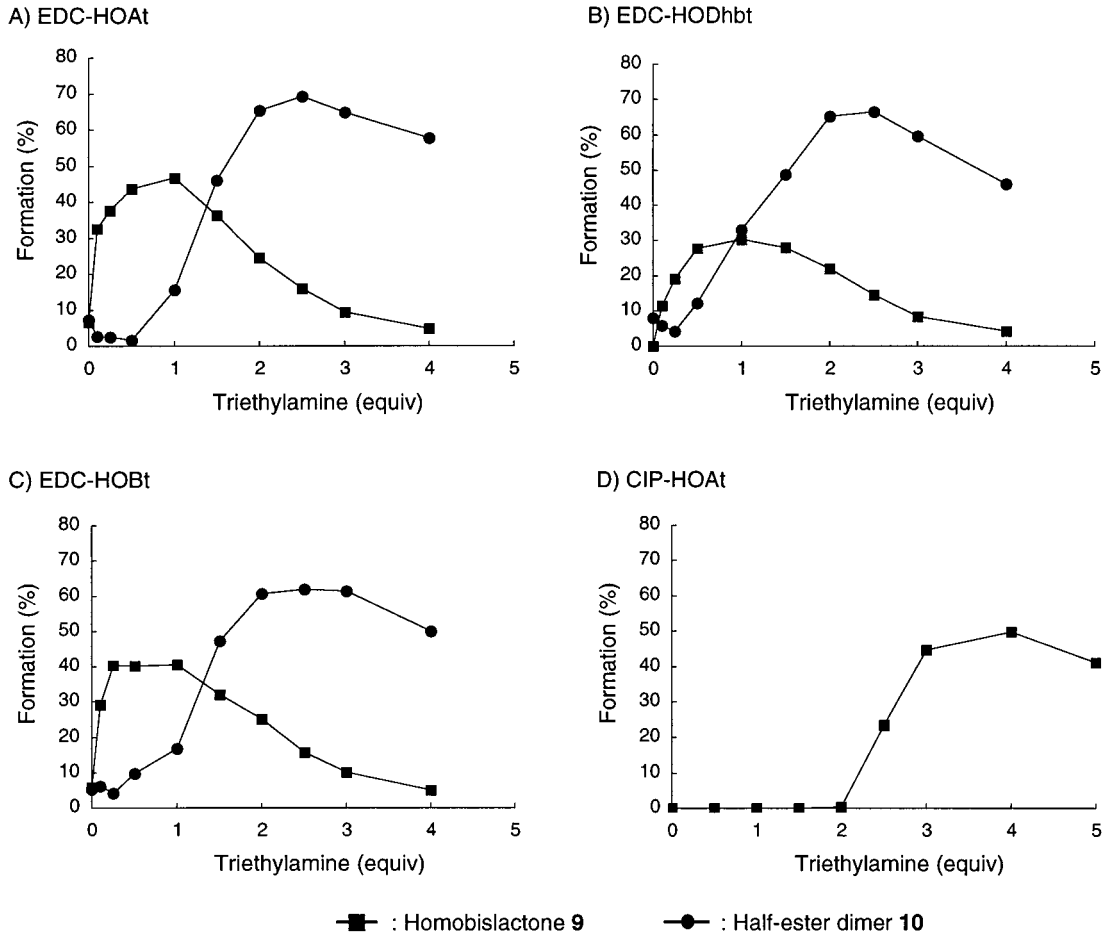
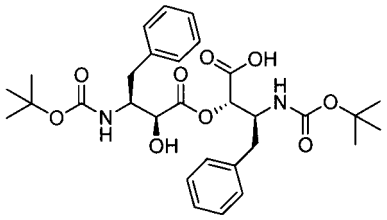


Figure 1. Effect of base concentration on the homobislactone **9** formation. This experiment was carried out using almost the same procedure mentioned in Table 2 but with different Et_3N concentrations (0−4 equiv in every coupling method except for the CIP-HOAt method; 0−5 equiv). Yields of **9** and **10** were calculated by HPLC analysis using a working curve from the standard DMF solution of the respective compound, which was analyzed under the same HPLC conditions.

formation of homobislactone reached the maximum at almost 1 equiv of base addition, and further addition of the base decreased the formation of homobislactone and caused the formation of the half-ester dimer of Boc-Apns-OH, which was the predominant product, the amount of which was inversely proportional to the homobislactone formation. This is probably due to the presence of a small amount of water molecules, which hydrolyze the active ester resulting in half-ester formation in the presence of a larger amount of the base.

On the basis of these findings, we postulate that homobislactone **9** formation could be explained by the following mechanism (Figure 2). Activation by EDC results in the formation of the Boc-Apns-OBt ester, wherein the α-hydroxyl group attacks the activated carbonyl group of another active ester molecule through the electron-donating influence of the active ester nitrogen (neighboring-group participation), resulting in the formation of a dimer (II). The base can abstract a proton from the α-hydroxyl group, which can further attack the carbonyl, resulting in the formation of an energetically favorable six-membered ring homobislactone with the elimination of an HOBt molecule (route A). On the other hand, higher base concentrations increased the water-mediated destruction of the active ester in the dimer (II), which results in the formation of the half-ester dimer **10** (route B).



10

Effect of Homobislactone Formation on the Yield of Boc-Apns-Dmt-OBzl (12). To determine the effect of homobislactone formation on the amide bond formation, a coupling reaction between Boc-Apns-OH and H-Dmt-OBzl (**11**) to form Boc-Apns-Dmt-OBzl (**12**) was carried out using the EDC method with three different additives, HOAt, HODhbt, and HOBt. H-Dmt-OBzl was used as an amine component instead of its hydrochloric acid salt in order to avoid in situ neutralization with the base. After 24 h reaction at room temperature, the formation of **12** was detected by HPLC. Increasing the amount of added Et_3N resulted in a dose-dependent inhibition of **12** production, and a complete inhibition of the production was observed in the presence of more than 1.5 equiv of Et_3N in all cases tested (Figure 3). These results correlate closely with those obtained for the accelerated formation of both homobislactone and the half-ester dimer with increased base concentration, sug-

(20) Hudson, D. *J. Org. Chem.* **1988**, *53*, 617.

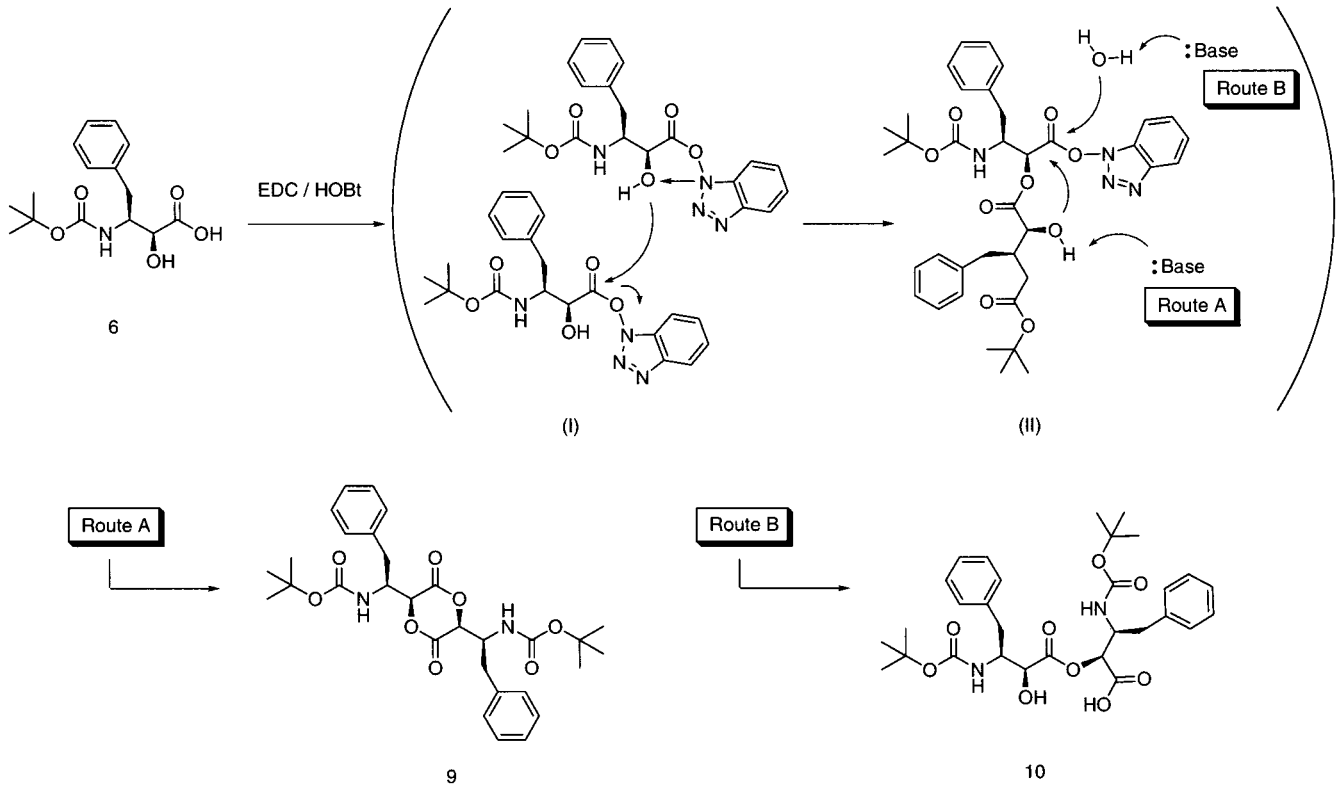


Figure 2. Proposed mechanism for the formation of homobislactone **9** and half-ester dimer **10**.

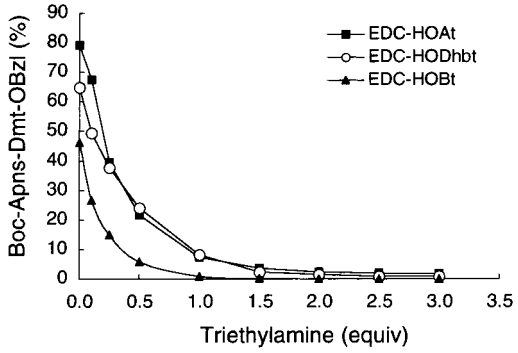


Figure 3. Effect of base concentration on the yield of Boc-Apns-Dmt-OBzl (**12**). Yields were calculated based on HPLC analysis using a working curve from the standard DMF solution of **12**, which was analyzed by the same HPLC system. Reaction conditions: To a solution of **6** (100 mg, 0.34 mmol) and **11** (127 mg, 0.51 mmol) in DMF were added Et_3N (0.1, 0.25, 0.5, 1, 1.5, 2, 2.5, or 3 equiv), additive (HOBt, HOAt, or HODhbt, 0.34 mmol), and EDC·HCl (65 mg, 0.34 mmol) to a total volume of 2 mL, and the mixture was stirred at room temperature for 24 h.

gesting that homobislactone formation is one major reason for the low yield in the amide bond formation with α-hydroxy-*β*-amino acid.

The effect of additives on the formation of **12** was slightly different from the homobislactone **9** formation mentioned above. The level of **12** formation decreases in the order HOAt > HODhbt > HOBt with these three additives studied.

Optimization of Boc-Apns-Dmt-OBzl Formation (12). To optimize the reaction conditions for **12** on the basis of the above findings, the reaction was performed under conditions that minimized homobislactone production.

Table 3. Yield (%)[a] of Boc-Apns-Dmt-OBzl (12) Using Various Coupling Procedures

coupling agents	additives	Et_3N (equiv)	yield[a] (%)
EDC[b,c]			4.4 ± 0.2 (15)
EDC	HOAt[b]		86.7 ± 5.1 (92)
EDC	HODhbt[b]		66.9 ± 1.4 (75)
EDC	HOBt[b]		57.3 ± 6.2 (64)
CIP[b,d]		2.0	2.0 ± 0.8
CIP	HOAt	2.0	72.8 ± 7.2
CIP	HODhbt	2.0	51.2 ± 1.1
CIP	HOBt	2.0	37.8 ± 2.5

[a] HPLC yields, which were calculated based on HPLC analysis using a working curve from the standard DMF solution of **12**, which was analyzed using the same HPLC conditions. Values are mean ± SEM of three experiments. Isolation yields (%) are shown in parentheses. [b] Abbreviations, see Table 2. [c] Reaction conditions: **6** (1.0 equiv), **11** (1.5 equiv), additive (1.0 equiv), EDC·HCl (1.0 equiv) in DMF solution (total volume of 2 mL), rt, 24 h. [d] Reaction conditions: **6** (1.0 equiv), **11** (1.5 equiv), additive (1.0 equiv), CIP (1.2 equiv), Et_3N (2.0 equiv) in DMF solution (total volume of 2 mL), rt, 24 h.

As shown in Table 3, higher yield was obtained using the EDC−HOAt, EDC−HODhbt and CIP−HOAt methods, and the efficacy was in the order HOAt > HODhbt > HOBt. Although the EDC and CIP methods rarely showed homobislactone **9** formation, the reaction with only the EDC or CIP reagent was ineffective for the amide bond formation probably due to the lower reactivity of the activated carboxyl group, suggesting that appropriate additives are required to increase the reactivity of the acyl component.

The addition of additives effectively improved the yield of the desired dipeptide in both methods. The EDC-additive method showed preferable yields for the CIP-additive method with all additives tested. Although the reason for the difference between the two reagents is not known, the EDC method seems to have some advantages,

namely, the reaction can progress without a base, while in the CIP method, the use of a base even in small excess increases homobislactone formation. The EDC−HOAt method produces about 6% homobislactone in the absence of Et_3N and the amine component, and the observed high-yield production of **12** may be due to its strong activation of the corresponding carboxylic acid for amide bond formation. This conclusion is also supported by the result showing that the addition of HOBt, which is known to be a weaker agent than HOAt, gave only a moderate yield. These results indicate that the EDC−HOAt method is one of the best procedures among the known coupling methods used here for the preparation of **12** and that the interference of a small amount of homobislactone production in the coupling reaction is negligible.

Homobislactone Formation in Other N^{β}-Protected α-Hydroxy-β-amino Acid Derivatives. Next, to determine the generality of homobislactone formation, we investigated the activation of two other N^{β}-protected α-hydroxy-β-amino acid derivatives, i.e., Boc-Pns-OH [Pns: phenylnorstatine, (2*R*,3*S*)-AHPBA] and Boc-Chns-OH [Chns: cyclohexylnorstatine, (2*R*,3*S*)-3-amino-2-hydroxy-4-cyclohexylbutanoic acid], using the reaction conditions of EDC, HOAt, and Et_3N (1 equiv each) in DMF at room temperature for 2 h. On the basis of HPLC analysis of the reaction mixture, the formation of homobislactones was observed in 48 and 44% yields for Boc-Pns-OH and Boc-Chns-OH, respectively. The extent of the homobislactone formation was similar to that of Boc-Apns-OH under the same reaction conditions, suggesting that homobislactone formation in N^{β}-protected α-hydroxy-β-amino acid seems to be a common feature and it could be formed easily when amine components with low reactivity are used.

Conclusion

As one of the core units of aspartyl protease inhibitors, α-hydroxy-β-amino acid derivatives are important compounds in medicinal chemistry. On the basis of the analysis of peptide bond formation with these units as acyl components in a model reaction, we found a significant amount of homobislactone formation during activation of the carboxyl group via the benzotriazole- and benzotriazine-type active ester formation of **6**. Such homobislactone formation was accelerated by a catalytic amount of a base. The lower yield in the amide bond formation is attributed mainly to the production of homobislactone. Moreover, such homobislactone formation was a common feature prevalent when these α-hydroxy-β-amino acids were used as acyl components. A strong activation method like EDC−HOAt without base addition is recommended to increase the yield in the amide bond formation for the synthesis of these α-hydroxy-β-amino acid derivatives for the development of clinically important aspartyl protease inhibitors.

Experimental Section

General Information. All solvents were reagent grade and dried prior to use. Column chromatography was performed using 70−230 mesh silica gel. Melting points (mp) were not corrected. ^1H NMR spectra were recorded at 270 and 300 MHz, and ^{13}C NMR spectra were recorded at 67.5 and 75 MHz, respectively. High-resolution mass spectra were performed using the electron impact (EI) or fast atom bombardment (FAB) method. HPLC was carried out using a Hitachi system (L-6200) equipped with a UV/vis detector and an integrator. The solvent system used for analytical HPLC was a binary system: water containing 0.1% TFA and acetonitrile containing the same amount of TFA as the organic modifier.

General Procedure for Preparation of Boc-Apns-Dmt-R (8) Using EDC−HOBt Method. To a solution of **6** (325 mg, 1.1 mmol) and HCl salt of H-Dmt-NH-Bzl (287 mg, 1.0 mmol) in DMF (5 mL) were added 1-hydroxybenzotriazole ($HOBt·H_2O$, 168 mg, 1.1 mmol), Et_3N (140 μL, 1.0 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 211 mg, 1.1 mmol) at 0 °C, and the mixture was stirred at room temperature for 18 h. After removal of the solvent in vacuo, the residue was dissolved in EtOAc (50 mL), washed with 5% citric acid, 5% sodium bicarbonate, and saturated NaCl, dried over Na_2SO_4, and concentrated in vacuo. The residual oil was applied to a silica gel (20 g) column and eluted with EtOAc−hexane (1:4) to yield 363 mg (69%) of Boc-Apns-Dmt-NHBzl as a white solid: mp 89−91 °C; ^1H NMR (270 MHz, DMSO-d_6) δ 8.41 (t, J = 6 Hz, 1H), 7.08−7.35 (m, 10H), 6.78 (d, J = 9 Hz, 1H), 5.39 (d, J = 7 Hz, 1H), 4.95 (dd, J = 9, 15 Hz, 2H), 4.40 (s, 1H), 4.16−4.48 (m, 3H), 3.38−3.96 (m, 1H), 2.54−2.85 (m, 2H), 1.49 (s, 3H), 1.32 (s, 3H), 1.27 (s, 9H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 170.1 167.8, 155.0, 139.1, 129.3, 128.0, 127.7, 127.1, 126.5, 125.6, 77.6, 71.8, 71.5, 54.2, 51.1, 47.7, 42.2, 34.0, 30.0, 28.2, 24.7; HRMS (FAB) calcd for $C_{28}H_{38}N_3O_5S$ $(M + H)^+$ 528.2532, found 528.2539. Anal. Calcd for $C_{28}H_{37}N_3O_5S·0.5H_2O$: C, 62.66; H, 7.14; N, 7.83. Found: C, 62.65; H, 7.08; N, 7.70.

A series of compounds **8** were prepared according to the general procedure described for the preparation of Boc-Apns-Dmt-NHBzl starting from **6** by coupling **7**·HCl with *N*-methylamide, benzyl ester, and methyl ester.

Boc-Apns-Dmt-OBzl: white solid; mp 112−114 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 7.09−7.40 (m, 10H), 4.84−5.01 (m, 3H), 4.63 (s, 1H), 4.59−4.72 (m, 1H), 4.01−4.17 (m, 1H), 3.63 (br, 1H), 2.50−2.83 (m, 2H), 1.60 (s, 3H), 1.38 (s, 3H), 1.34 (s, 9H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 170.4, 167.8, 155.6, 137.8, 134.9, 129.1, 128.6, 128.5, 128.4, 128.3, 126.2, 79.6, 71.7, 71.3, 67.3, 54.1, 51.5, 48.9, 28.6, 28.3, 25.3. HRMS (FAB) calcd for $C_{28}H_{37}N_2O_6S$ $(M + H)^+$ 529.2372, found 529.2365. Anal. Calcd for $C_{28}H_{36}N_2O_6S·0.25H_2O$: C, 63.08; H, 6.90; N, 5.25. Found: C, 63.15; H, 6.65; N, 5.57.

Boc-Apns-Dmt-NHMe: white solid; mp 97−99 °C; ^1H NMR (270 MHz, DMSO-d_6) δ 7.69 (q, J = 5 Hz, 1H), 7.09−7.32 (m, 5H), 6.76 (d, J = 9 Hz, 1H), 5.36 (d, J = 7 Hz, 1H), 4.92 (s, 1H), 4.26−4.36 (m, 1H), 4.28 (s, 1H), 3.79−3.95 (m, 1H), 2.59 (d, J = 5 Hz, 1H), 2.54−2.81 (m, 2H), 1.48 (s, 3H), 1.30 (s, 3H), 1.28 (s, 9H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 170.0, 168.1, 155.0, 139.0, 129.3, 127.7, 125.6, 77.6, 71.8, 71.3, 54.1, 51.0, 47.7, 34.2, 29.7, 28.2, 25.5, 24.7; HRMS (FAB) calcd for $C_{22}H_{34}N_3O_5S$ $(M + H)^+$ 452.2219, found 452.2228. Anal. Calcd for $C_{22}H_{33}N_3O_5S·0.5H_2O$: C, 57.37; H, 7.44; N, 9.12. Found: C, 57.40; H, 7.32; N, 8.87.

Boc-Apns-Dmt-OMe: white solid; mp 56−57 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 7.11−7.34 (m, 5H), 4.84−5.01 (m, 3H), 4.68 (d, J = 5 Hz, 1H), 4.61 (s, 1H), 4.05−4.17 (m, 1H), 3.75 (s, 3H), 3.64 (d, J = 7 Hz, 1H), 2.54−2.83 (m, 2H), 1.64 (s, 3H), 1.48 (s, 3H), 1.34 (s, 9H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 170.4, 168.4, 155.6, 137.8, 129.4, 129.0, 128.3, 128.2, 126.3, 79.6, 71.8, 71.5, 54.2, 52.4, 51.4, 48.9, 33.8, 28.6, 28.3, 25.4; HRMS (FAB) calcd for $C_{22}H_{33}N_2O_6S$ $(M + H)^+$ 453.2059, found 453.2054. Anal. Calcd for $C_{22}H_{32}N_2O_6S$: C, 58.39; H, 7.13; N, 6.19. Found: C, 58.53; H, 7.18; N, 6.03.

The protocol carried out for the BOP method was similar to that of the EDC−HOBt method except for the reaction conditions with BOP reagent (1.1 equiv) instead of EDC·HCl and 2.5 equiv of Et_3N.

Homobislactone 9: white solid; mp 226 °C dec; ^1H NMR (270 MHz, DMSO-d_6) δ 7.37 (d, J = 8 Hz, 2H), 7.44−7.13 (m, 10H), 5.49 (br, 2H), 4.33 (br, 2H), 2.90−2.65 (m, 4H), 1.30 (s, 18H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 165.8, 154.9, 138.3, 128.8, 128.0, 126.1, 78.2, 77.8, 52.4, 33.9, 28.2; HRMS (FAB) calcd for $C_{30}H_{39}N_2O_8$ $(M + H)^+$ 555.2706, found 555.2699. Anal. Calcd for $C_{30}H_{38}N_2O_8$: C, 64.97; H, 6.91; N, 5.05. Found: C, 64.96; H, 7.02; N, 5.25.

Half-ester dimer of 6: white solid; mp 128−130 °C; ^{1}H NMR (300 MHz, DMSO-*d6*) δ 7.10−7.36 (m, 10H), 6.67 (d, J = 8 Hz, 1H), 5.07 (d, J = 4 Hz, 1H), 4.37 (d, J = 3 Hz, 1H), 4.04−4.28 (m, 2H), 2.60−2.88 (m, 4H), 1.26 (s, 18H); HRMS (FAB) calcd for $C_{30}H_{40}N_2O_9Na$ $(M + Na)^+$ 595.2632, found 595.2635. Anal. Calcd for $C_{30}H_{40}N_2O_9 \cdot 2H_2O$: C, 59.20; H, 7.29; N, 4.60. Found: C, 58.87; H, 7.00; N, 4.46.

Homobislactone of Boc-Pns-OH: white solid; mp 180.5−182 °C; ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.15−7.33 (m, 10H), 6.60 (d, J = 8 Hz, 2H), 5.21 (d, J = 4 Hz, 2H), 4.20−4.36 (m, 2H), 2.84 (d, J = 7 Hz, 4H), 1.30 (s, 18H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 165.0, 155.0, 137.6, 129.5, 128.1, 126.2, 78.0, 75.7, 52.2, 36.4, 28.1; HRMS (FAB) calcd for $C_{30}H_{39}N_2O_8$ $(M + H)^+$ 555.2706, found 555.2700. Anal. Calcd for $C_{30}H_{38}N_2O_8 \cdot 0.5H_2O$: C, 63.93; H, 6.97; N, 4.97. Found: C, 63.71; H, 6.95; N, 4.94.

Homobislactone of Boc-Chns-OH: white solid; mp 106−107 °C; ^{1}H NMR (270 MHz, DMSO-d_6) δ 6.67 (d, J = 9 Hz, 2H), 5.18 (d, J = 4 Hz, 2H), 4.07−4.22 (m, 2H), 1.36, (s, 18H), 0.73−1.88 (m, 26H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 165.35, 155.11, 77.85, 76.20, 47.42, 37.83, 33.50, 33.13, 32.04, 28.16, 26.09, 25.90, 25.74; HRMS (FAB) calcd for $C_{30}H_{51}N_2O_8$ $(M + H)^+$ 567.3645, found 567.3649. Anal. Calcd for $C_{30}H_{50}N_2O_8$: C, 63.58; H, 8.89; N, 4.94. Found: C, 63.31; H, 8.91; N, 5.29.

HPLC Analysis of Homobislactone 9 Formation under Different Coupling Conditions. To a solution of **6** (100 mg, 0.34 mmol) in DMF were added a base (Et_3N or DIEA), an additive ($HOBt \cdot H_2O$, HOAt, HODhbt, HOSu, or DMAP, 0.34 mmol), and a coupling agent (EDC·HCl, CIP, BOP, PyBOP, or HBTU) to a total volume of 2 mL, and the mixture was stirred at room temperature for 2 h. Triethylamine was used in various proportions depending on the type of coupling agent (Table 2), and DIEA (0.34 mmol) was used in the EDC−HOAt and −HOBt methods. The amount of coupling agents was 1.2 equiv (0.41 mmol) except for EDC·HCl (1.0 equiv, 0.34 mmol). Aliquots of samples were diluted with DMF and filtered using a 0.2 μm PVDF membrane filter. A 10 μL portion of the resultant filtrate was injected for HPLC. The solvent system used for HPLC analysis consisted of a binary system: water containing 0.1% TFA and acetonitrile containing the same amount of TFA as the organic modifier. The dimensions of the column used were 4.6 × 150 mm (YMC-pack ODS-AM). The analytical conditions were a gradient of acetonitrile with 45% for 2 min and then 45−80% for 30 min with 0.1% TFA at a flow rate of 0.9 mL/min using 230 nm for detection. Homobislactone **9** was eluted with a retention time of 22 min under these conditions. The yield of homobislactone **9** was calculated using a working curve from the standard DMF solution of homobislactone **9**, which was analyzed using the same HPLC conditions. Data are expressed as the mean value of three experiments with the standard error of the mean (SEM). Homobislactone formation of Boc-Pns-OH and Boc-Chns-OH was carried out using the EDC−HOAt method and one equivalent of Et_3N under the same reaction conditions mentioned above. The same HPLC gradient conditions were employed for the analysis of homobislactone from Boc-Pns-OH. HPLC analysis of homobislactone from Boc-Chns-OH was carried out using the same system, i.e., a gradient of acetonitrile of 55% for 2 min and then 55−80% for 30 min with 0.1% TFA. Homobislactone was eluted at retention time of 28 min under these conditions.

Effect of Base Concentration on Homobislactone 9 Formation. This experiment was carried out using almost the same procedure mentioned above with 0−4 equiv of triethylamine in every coupling method studied except for the CIP−HOAt method (0−5 equiv). EDC−HOAt, −HODhbt, −HOBt, and CIP−HOAt methods were employed. The formation of homobislactone was analyzed by HPLC using the conditions described above. The formation of half-ester dimer (**10**) arising from Boc-Apns-OH was analyzed by HPLC with the same gradient conditions as those for the homobislactone formation. A peak eluted at 18 min, which corresponds to **10**, was measured, and the yield of **10** was calculated by using a working curve from the standard DMF solution of **10**, which was analyzed using the same HPLC conditions.

HPLC Analysis of Boc-Apns-Dmt-OBzl (12) Formation under Various Coupling Conditions. To a solution of **6** (100 mg, 0.34 mmol) and **11** (127 mg, 0.51 mmol) in DMF were added Et_3N (0.1, 0.25, 0.5, 1, 1.5, 2, 2.5, or 3 equiv), additive ($HOBt \cdot H_2O$, HOAt, or HODhbt, 0.34 mmol), and EDC·HCl (65 mg, 0.34 mmol) to a total volume of 2 mL, and the mixture was stirred at room temperature for 24 h. Aliquots of samples were diluted with DMF and filtered using a 0.2 μm PVDF membrane filter. A 10 μL portion of the resulting filtrate was injected to HPLC. The formation of **12** was analyzed by HPLC using the same system mentioned above with a gradient of acetonitrile of 15% for 2 min, then 15−35% for 4 min, 35−40% for 4 min, 40−50% for 6 min, 50−65% for 4 min, and then 60−85% for 16 min with 0.1% TFA at a flow rate of 0.9 mL/min with detection of absorbance at 230 nm. Compound **12** was eluted at 26 min under these conditions. The yield of **12** was calculated using a working curve from the standard DMF solution of **12** that was analyzed using the same HPLC conditions.

Acknowledgment. This work was supported by grants from the Ministry of Education, Science and Culture of Japan and Frontier Research Program of the Ministry of Education, Science and Culture of Japan.

Supporting Information Available: Copies of both ^{1}H and ^{13}C NMR spectra of compounds **8** (Boc-Apns-Dmt-NHBzl and Boc-Apns-Dmt-OBzl) and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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