Stereoselective Synthesis of CF_2-Substituted Phosphothreonine Mimetics and Their Incorporation into Peptides Using Newly Developed Deprotection Procedures[†,||]

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Stereoselective syntheses of all four stereoisomers of CF_2-substituted nonhydrolyzable phosphothreonine derivatives (**33**, **39**, and their enantiomers) and their incorporation into peptides are described herein. Key to the synthesis of these amino acids was construction of secondary phosphate-mimicking difluoromethylphosphonate units along with generation of two stereocenters. The former was achieved using a Cu(I)-mediated cross-coupling reaction of $BrZnCF_2P(O)(OEt)_2$ (**8**) and β-iodo-α,β-unsaturated ester **12**, with stereochemistry of both α- and β-stereocenters being established using bornane-10,2-sultam as a chiral auxiliary. Diastereoselective hydrogenation of a chiral α,β-unsaturated acylsultam (for the β-center) (e.g., **16a**) and subsequent stereoselective bromination (for the α-center of the threo derivative) or amination (for the α-center of erythro (allo) derivative) were utilized. Transesterification of the bromide to the benzyl ester followed by azide displacement of the halogen, then reduction of the resulting azide, followed by Boc-protection and finally removal of the benzyl group, afforded protected both L- and D-phosphothreonine mimetics (**39** and its enantiomer). On the other hand, protected both L- and D-*allo*-phosphothreonine mimetics (**33** and its enantiomer) were synthesized via transesterification of the above-mentioned amination product, followed by hydrogenolytic removal of the benzyl group. Key to utilization of these amino acid analogues in peptide synthesis was removal of ethyl protection from the difluoromethylphosphonate moiety. A two-step deprotection methodology, consisting of a combination of a first-step reagent [0.3 M BSTFA−TBAI in CH_2Cl_2, BF_3·Et_2O] followed by a second-step reagent [1 M TMSOTf−thioanisole in TFA, *m*-cresol, EDT] was developed for use in solid-phase protocols. A 12-residue Cdc (cell division cycle) 2-peptide **41**, possessing two nonhydrolyzable phosphoamino acid mimetics (F_2Pmab **6** and F_2Pmp **4**), was subjected to this deprotection procedure and was obtained in 25% yield based on the protected resin. The present synthetic method affords nonhydrolyzable phosphoamino acid mimetics-containing peptides in high yield without accompanying side reactions.

Introduction

Phosphorylation and dephosphorylation of proteins serve as posttranslational modifications that are critical for intracellular signal transduction.[1] Phosphopeptides[2] have provided useful biochemical tools for evaluating the roles of phosphorylation events in cellular signaling; however, the phosphate moiety is easily hydrolyzed by the action of protein phosphatases. For this reason, nonhydrolyzable phosphoamino acid mimetic-containing peptides[3] have gained much attention as useful agents for exploring signaling events. Among various nonhydrolyzable phosphoamino acid analogues, those employing the difluoromethylene unit (CF_2)[4,5] as a replacement for the phosphoryl ester oxygen have shown particular utility. For example, 4-phosphonodifluoromethyl phenylalanine (F_2Pmp **4**)[6] as CF_2-substituted pTyr (phosphotyrosine) **1** mimetic has served as a nonhydrolyzable pTyr analogue where the acidity of F_2Pmp is comparable to that of pTyr, whereas the corresponding CH_2-analogue (phosphonomethyl phenylalanine, Pmp) is less acidic. Similarly, CF_2-substitution in pSer (phosphoserine) **2** or pThr (phosphothreonine) **3** would seem to provide potential nonhydrolyzable phosphoamino acid mimetic,

[†] This paper is dedicated to the memory of Prof. Toshiro Ibuka, who unexpectedly passed away on January 20, 2000.

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[||] Abbreviations. TMSOTf = trimethylsilyl trifluoromethanesulfonate, TMSI = trimethylsilyl iodide, TFMSA = trifluoromethanesulfonic acid, TFA = trifluoroacetic acid, DMS = dimethyl sulfide, EDT = 1,2-ethanedithiol, NaHMDS = sodium bis(trimethylsilyl)amide, IS-MS = Ion-spray mass spectrometry, ClZ = 2-chlorobenzyloxycarbonyl, Cl_2-Bzl = 2,6-dichlorobenzyl, PAM = 4-(oxymethyl)phenylacetamidomethyl, MBHA = 4-methylbenzhydrylamine, DIPCDI = *N*,*N*-diisopropylcarbodiimide, HOBt = 1-hydroxybenzotriazole, DIPEA = *N*,*N*-diisopropylethylamine, WSCDI = water soluble carbodiimide (1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide), Cdc = cell division cycle.

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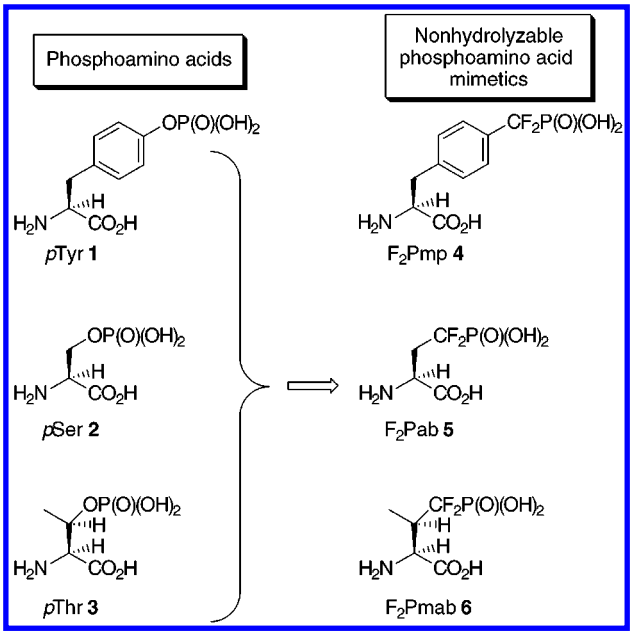

Figure 1.

which led us to engage the synthesis of the pSer and pThr mimetics (Figure 1).

Synthetic approaches toward CF_2-substituted phosphoamino acid mimetics by us and others have provided F_2Pmp **4**[6] and 2-amino-4,4-difluoro-4-phosphonobutanoic acid (F_2Pab **5**)[7] as nonhydrolyzable pTyr and pSer mimetics, respectively.[8] Alternatively, synthesis of 2-amino-4,4-difluoro-3-methyl-4-phosphonobutanoic acid (F_2Pmab **6**) as a CF_2-substituted pThr mimetic initially was lacking due to a paucity of efficient methodologies for construction of the secondary CF_2 phosphonate unit. However, Berkowitz and co-workers[9] did described a stereoselective synthesis of this pThr mimetic through the reaction of CH_3MgBr onto a keto difluoromethylphosphonate. We have also accomplished the preparation of racemic protected F_2Pmab[10] by the $CeCl_3$-mediated conjugate addition of diethyl difluoromethylphosphonate anion onto a nitroalkene.[11] We then successfully incorporated these phosphoamino acid mimetics into peptides using a combination of solid-phase peptide synthesis and a two-step deprotection protocol, which consisted of high acidic and low acidic treatments.[12] Recently, copper(I)-mediated coupling reactions of (diethylphosphonodifluoromethyl)zinc bromide (**8**) with alkenyl halides have been reported,[13] which prompted us to utilize similar methodology in combination with Oppolzer's sultam chemistry[14] for the stereoselective synthesis of F_2Pmab. We chose to employ side chain ethyl protection of F_2Pmab similar to that used for F_2Pmp and F_2Pab,[15] since the requisite difluoromethylphosphonate reagent bearing ethyl groups, $BrCF_2P(O)(OEt)_2$ (**7**),[16] is easily obtainable and cleanly converted to the corresponding Zn reagent.[13,17] Furthermore, ethyl protection is stable toward synthetic transformations employed in our study. However, incorporation of such ethyl-protected derivatives into peptides should be done with extreme caution, since this type of phosphonate protection may behave differently relative to other protecting groups normally employed in peptide chemistry.[18] Increasing the acidity of deprotection reagents typically leads to facile removal of commonly used protecting groups; however, this is not the case with phosphonate- or phosphate-alkyl protecting groups, where low acidic S_N2-type deprotecting methodologies have been shown to be critical for removal of side chain protecting groups on phosphoamino acids. Thus, we developed a two-step deprotecting methodology[19] consisting of high acidic (1 M TMSOTf−thioanisole in TFA[20]), followed by low acidic (1 M TMSOTf−thioanisole in TFA + TMSOTf−DMS), treatment, which is suitable for dimethyl-protected pTyr-, pSer-, and/or pThr-containing peptide resins. Application of this methodology to the deprotection of ethyl-protected F_2Pmp, or F_2Pab-containing peptide resins affords fully deprotected peptides with some success.[12] However, incomplete removal of ethyl groups, especially on F_2Pmab, has been encountered. Moreover, use of alternative low acidic treatment (TMSOTf−DMS−*m*-cresol−EDT), which provides effective removal of ethyl groups, sometimes hampers the isolation of fully deprotected peptide from reaction mixtures.[10]

We report herein the stereoselective synthesis of all four isomers of protected F_2Pmab as well as an examination of deprotecting reagent systems suitable for CF_2-substituted phosphoamino acid mimetics-containing peptide resins.

Results and Discussion

Recently, Yokomatsu et al. have reported the CuBr-mediated coupling of $BrZnCF_2P(O)(OEt)_2$ **8** with alkenyl halides to yield (α,α-difluoroallyl)phosphonates.[13] We undertook the stereoselective synthesis of all four F_2-

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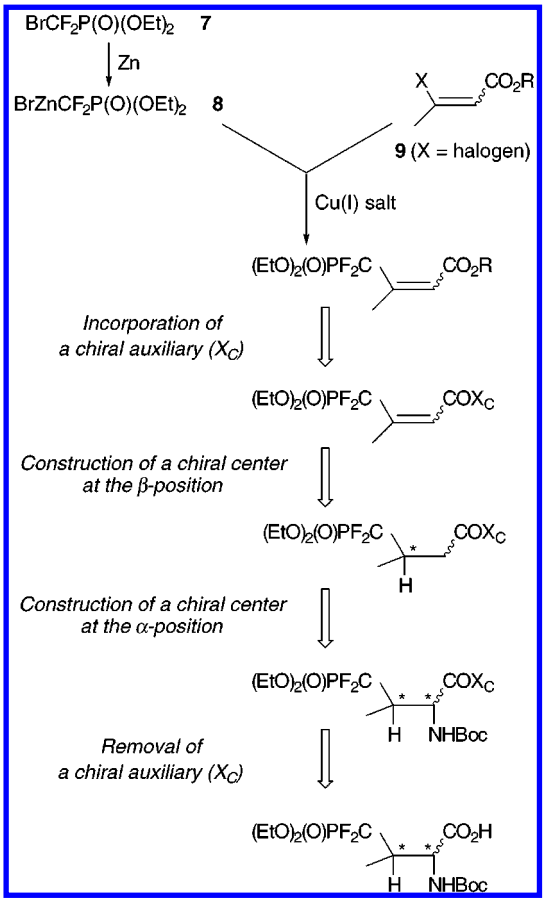
Scheme 1. Synthetic Plan for F_2Pmab Derivatives

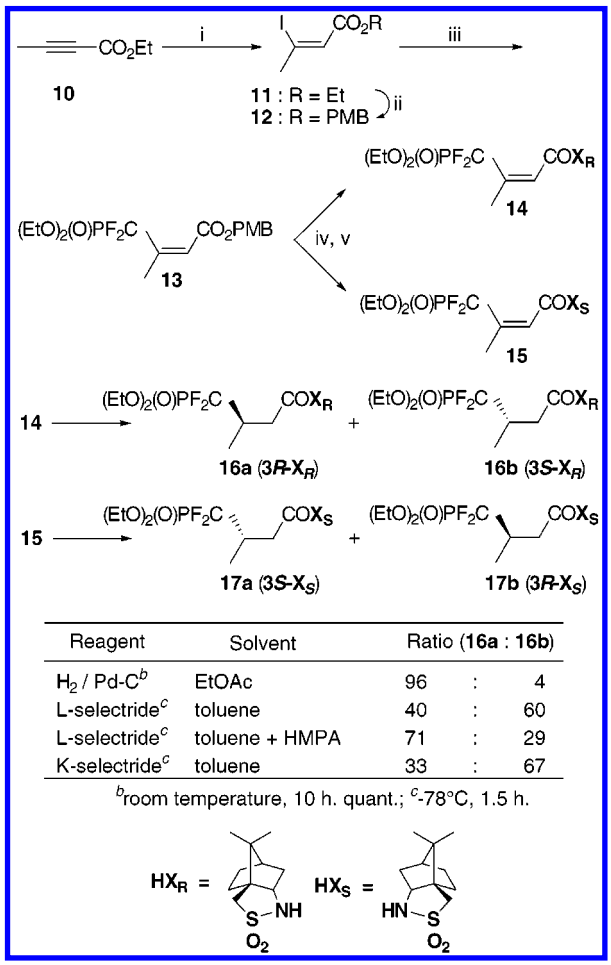


Pmab isomers by applying this coupling reaction to β-halo-α,β-unsaturated carboxylic acid derivatives **9**, followed by diastereoselective hydrogenation and amination with the aid of a chiral auxiliary, as conceptually outlined in Scheme 1.

Ethyl (*Z*)-3-iodo-2-butenoate (**11**) was employed as a requisite β-halo alkenoic acid derivative, which could be easily obtained in a highly regio- and stereoselective manner by the reaction of ethyl 2-butynoate (**10**) with NaI in AcOH (Scheme 2).[21] Prior to introduction of the difluoromethylphosphonate unit, **11** was converted to the corresponding *p*-methoxybenzyl ester derivative **12** through transesterification using Ti(O*i*Pr)$_4$ in PMB−OH (PMB = *p*-methoxybenzyl).[22] This was done to allow subsequent selective removal of PMB groups without affecting phosphonate ethyl esters. The CuBr-mediated coupling of **8** with **12** in DMF at room temperature, afforded adduct **13** in nearly quantitative yield, with retention of the starting olefinic geometry.[23] Alternative attempts at coupling **8** with (*Z*)-3-iodo-2-butenoic acid did not give a desired coupling product in satisfactory yield. Treatment of **13** with 95% aqueous TFA, followed by coupling with (2*R*)- or (2*S*)-bornane-10,2-sultam according to published procedures,[14] yielded sultam-imide conjugated alkenes **14** or **15**, respectively (Scheme 2).

Oppolzer et al. reported high diastereoface discrimination in the hydrogenation of a trisubstituted sultam-imide

Scheme 2[a]



Reagent	Solvent	Ratio (**16a** : **16b**)
H_2 / Pd-C[b]	EtOAc	96 : 4
L-selectride[c]	toluene	40 : 60
L-selectride[c]	toluene + HMPA	71 : 29
K-selectride[c]	toluene	33 : 67

[b]room temperature, 10 h. quant.; [c]-78°C, 1.5 h.

[a] Key: (i) NaI (1.0 equiv), AcOH; (ii) Ti(O*i*Pr)$_4$ (2.0 equiv), PMB-OH (12 equiv); (iii) $BrZnCF_2P(O)(OEt)_2$ (2.0 equiv), CuBr (2.0 equiv), DMF; (iv) 95% TFA aq; (v) LiX_R (or LiX_S) (1.5 equiv), PivaCl (1.2 equiv), Et_3N (1.2 equiv), THF.

olefinic bond in the presence of Pd−C.[24] Furthermore, 1,4-hydride addition to olefinic sultam-imides using lithium tri-*s*-butylborohydride (L-Selectride) has been reported to proceed with high opposite π-face discrimination, as compared with H_2/Pd−C.[25] These reports prompted us to utilize similar methodologies for construction of the stereogenic centers at the β-position. Hydrogenation of the olefinic sultam-imide derivative **14** with H_2/Pd−C in EtOAc resulted in high stereoface discrimination, affording a mixture of major (**16a**) and minor (**16b**) isomers (96:4) in quantitative yield (Scheme 2). Following flash chromatographic purification, **16a** was crystallized from EtOAc, with the stereochemistries of **16a** and **16b** being determined as follows: X-ray analysis of **16a** showed that it possessed (3*R*-X_R)-configuration. When **16a** and **16b** were converted to their corresponding benzyl ester derivatives (**16a′** and **16b′**) using Ti(O*i*Pr)$_4$ in benzyl alcohol,[22,26] physical and chemical data for both compounds were identical, except that the signs of optical rotation were reversed. Based on this, we concluded that **16b** possessed the (3*S*-X_R)-configuration. Analogously,

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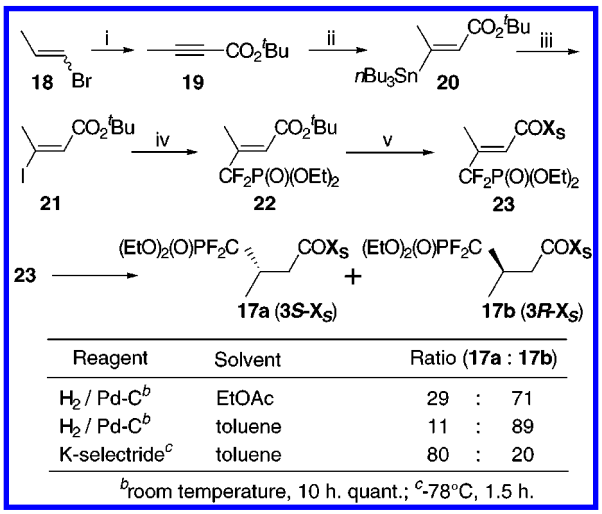
Scheme 3[a]



Reagent	Solvent	Ratio (**17a** : **17b**)
H_2 / Pd-C[b]	EtOAc	29 : 71
H_2 / Pd-C[b]	toluene	11 : 89
K-selectride[c]	toluene	80 : 20

[b]room temperature, 10 h. quant.; [c]-78°C, 1.5 h.

[a] Key: (i) *n*BuLi (2.2 equiv), $(Boc)_2O$ (1.55 equiv), THF; (ii) $nBu_3Sn(nBu)Cu(CN)Li_2$ (1.3 equiv), EtOH, THF, (iii) I_2(1.0 equiv), CH_2Cl_2; (iv) $BrZnCF_2P(O)(OEt)_2$ (2.0 equiv), CuBr (2.0 equiv), DMF, (v) 95% TFA aq.; (vi) LiX_S (1.5 equiv), Piva-Cl (1.2 equiv), Et_3N (1.2 equiv), THF.

compounds **17a** (major) and **17b** (minor) obtained hydrogenolytically from **15**, the antipode of **14**, possessed (3*S*-X_S)- and (3*R*-X_S)-configurations, respectively. To obtain a diastereomer **16b** (3*S*-X_R) in a highly stereoselective manner, 1,4-hydride addition to **14** using L-Selectride or K-Selectride was attempted, however high diastereofacial discrimination was not observed (Scheme 2). This necessitated alternative synthetic approaches to **16b** and **17b** (Scheme 3).

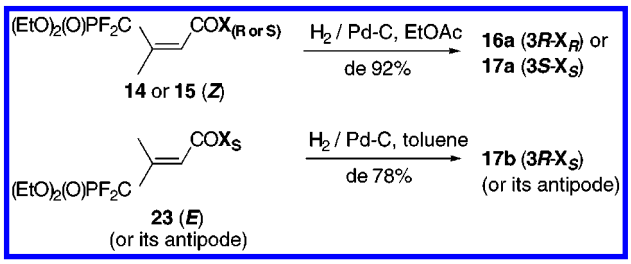
Treatment of 1-bromo-1-propene (**18**) with *n*BuLi in THF at −78 °C afforded propynyllithium, which was subsequently carried forward in a one-pot reaction using $(Boc)_2O$ to yield *tert*-butyl 2-butynoate (**19**).[27] Kinetically controlled stannyl cupration of **19** with $nBu_3Sn(nBu)Cu(CN)Li_2$ at −78 °C in the presence of EtOH, gave *tert*-butyl (*E*)-3-tributylstannyl-2-butenoate (**20**),[28] which upon treatment with iodine in CH_2Cl_2 yielded *tert*-butyl (*E*)-3-iodo-2-butenoate (**21**). Transformation of **21** to sultamimide conjugated alkene **23** was achieved in a fashion similar to that employed in the conversion of **12** to **14**. The resulting conjugated alkene **23** was hydrogenated over Pd−C in toluene with moderate π-face discrimination to afford a mixture of major **17b** (3*R*-X_S) and minor **17a** (3*S*-X_S) (89:11) isomers in quantitative yield. These results indicated that hydrogenation of (*Z*)- or (*E*)-3-diethylphosphonodifluoromethyl-2-butenimide derivatives over Pd−C gave diastereoselectively (3*R*-X_R)-**16a** and its antipode **17a** or (3*S*-X_R)-**16b** and its antipode **17b**, respectively (Scheme 4).

Having routes to the four stereochemically pure isomers of 3-diethylphosphonodifluoromethylbutanimide in hand, we next examined the diastereoselective introduction of amino functionality using two protocols (Scheme 5).

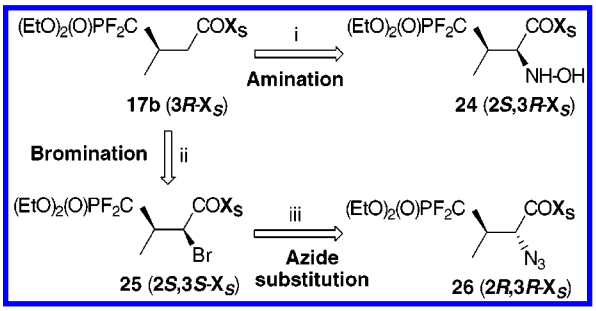
One approach toward diastereoselective amino introduction (Scheme 5, i) employs a π-face selective electrophilic enolate amination using 1-chloro-1-nitrosocyclohexane as an NH_2^+ equivalent,[29] while the other (Scheme 5, ii + iii) utilizes a diastereoselective enolate bromination followed by an S_N2 substitution using azide anion.[26,30] The former reaction is reported to proceed via a transition state which features a chelated (*Z*)-enolate that is attacked by the nitroso electrophile opposite to the lone pair on the sultam nitrogen. The latter bromination reaction proceeds from the same face. This implies that both electrophilic amination and bromination of enolates derived from X_S-sultams should furnish (2*S*)-products.[14] Thus, we speculated that the reaction of the enolate derived from **17b** (3*R*-X_S) with 1-chloro-1-nitrosocyclohexane, followed by hydrolysis of the resulting unisolated nitrone, should afford **24** (2*S*,3*R*-X_S), which is a precursor to the L-F_2Pmab derivative. However, attempted reactions based on the above considerations resulted in recovery of starting material. Alternatively, electrophilic amination of Na-enolates derived from **17a** (3*S*-X_S) by treatment with NaHMDS in THF at −78 °C, with 1-chloro-1-nitrosocyclohexane, followed by addition of aqueous 1 N HCl, proceeded with high stereofacial discrimination to afford hydroxylamine **27** (2*S*,3*S*-X_S). Without isolating, this was subjected to a sequence of reactions to furnish Boc-protected L-*allo*-F_2Pmab derivative **ent-33**. The difference in reactivity of Na-enolates resulting from **17a** and **17b** potentially may be attributed to differences in reactive conformations (TS (transition state) **28** and TS **29**) (Figure 2).

In TS **29**, both positioning of the difluoromethylphosphonate group on the *Si*-face due to 1,3-allylic strain[31,32] and shielding of *Re*-face by the chiral auxiliary may prevent attack of the nitroso electrophile from both sides, thereby blocking product formation. On the other hand,

Scheme 4



Scheme 5. Strategies for Diastereoselective Introduction of an Amino Functionality[a]



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(32) Bromination (NaHMDS then NBS) of PMB ester, resulting from treatment of **16a** (3*R*-X_R) with $Ti(OiPr)_4$ in PMB−OH, proceeded with moderate diastereoselectivity (de 79%) to mainly afford the (2*R*)-product, which can be attributed to the *Si*-face shielding by diethylphosphonodifluoromethyl group.

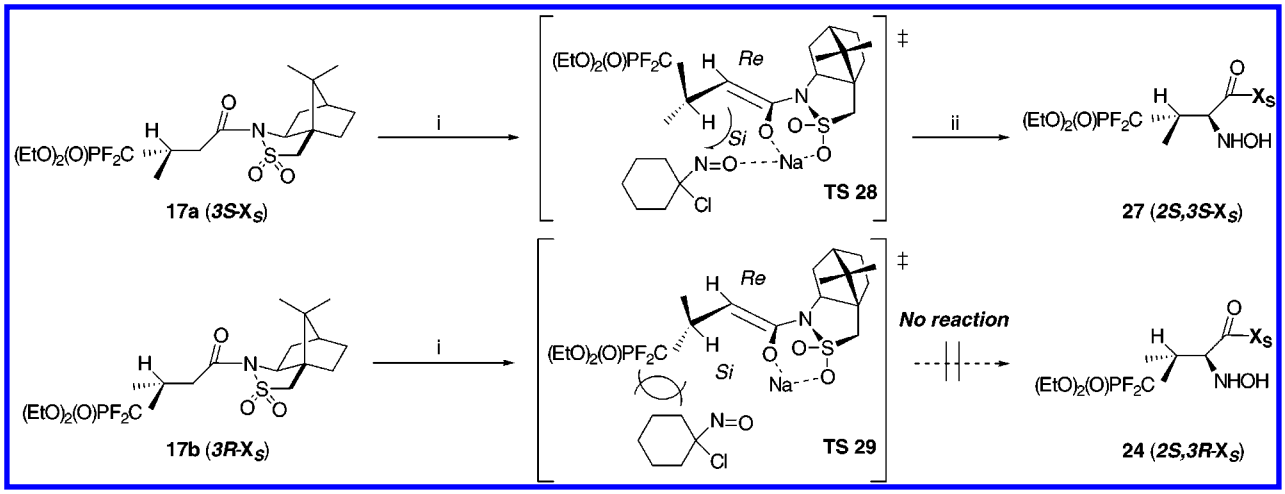
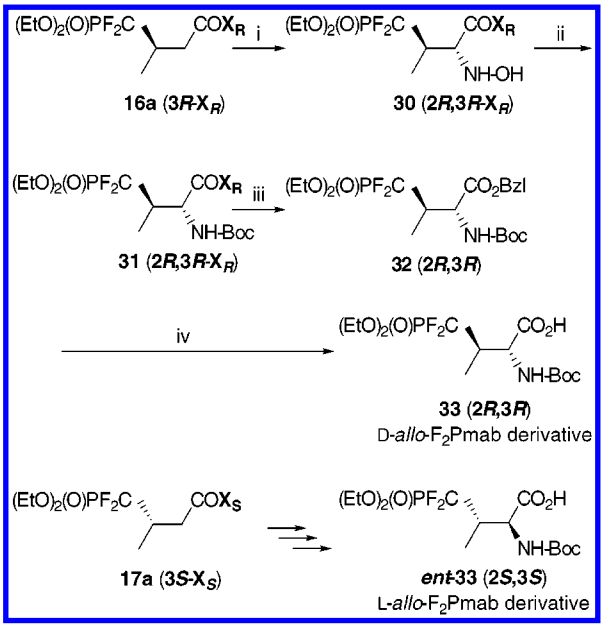


Figure 2. Reagents are as follows: (i) NaHMDS, THF, 1-chloro-1-nitrosocyclohexane, −78 °C; (ii) 1 N HCl (aq).

Scheme 6. Synthesis of Nonhydrolyzable *allo*-pThr Mimetics[a]



[a] Key: (i) NaHMDS (1.1 equiv), 1-chloro-1-nitrosocyclohexane (1.1 equiv), THF then 1 N HCl aq.; (ii) Zn (40 equiv), AcOH (50 equiv) then $(Boc)_2O$ (2.0 equiv), CH_3CN; (iii) $Ti(OiPr)_4$ (2.0 equiv), Bzl-OH (44 equiv), toluene; (iv) H_2/Pd-C, EtOAc.

shielding of the *Re*-face in TS **28** by both the difluoromethylphosphonate group and the sultam moiety should permit facile attack by 1-chloro-1-nitrosocyclohexane from the *Si*-face with high π-face discrimination. These results show that while erythro (allo)-amino acids ((2*R*,3*R*) and its antipode) could be obtained by direct amination of **16a** (3*R*-X_R) and **17a** (3*S*-X_S) utilizing the nitroso electrophile, similar procedures could not be applied to the transformation of **17b (**3*R*-X_S) and its antipode **16b** (3*S*-X_R) to yield the corresponding threo derivatives **24** (2*S*,3*R*-X_S) and its antipode. Therefore, for the preparation of threo amino acid derivatives, we resorted to successive transformations of **16a** or **17a** utilizing diastereoselective bromination and azide substitution with inversion of configuration.

In Scheme 6 is summarized the synthesis of erythro (allo) derivatives, in which the preparation of optically pure protected D-*allo*-F_2Pmab derivative **33** (2*R*,3*R*; erythro) was achieved from **16a** (3*R*-X_R). Deprotonation of **16a** with NaHMDS in THF at −78 °C, followed by slow addition of 1-chloro-1-nitrosocyclohexane (blue), instantaneously afforded a colorless solution of nitrone. Treatment of this solution with aqueous 1 N HCl, followed by an extractive workup, gave crude hydroxylamine **30**, which was taken to the next step without further purification. Reduction of **30** with Zn−AcOH in THF, followed by introduction of Boc protection onto the resulting NH_2 group using $(Boc)_2O$, gave Boc-protected **31** (2*R*,3*R*-X_R), with transformation of **31** to the corresponding benzyl ester **32** being accomplished utilizing $Ti(OiPr)_4$−benzyl alcohol in toluene at 120 °C.[26,33] Use of toluene was critical due to low solubility of **31**. Hydrogenolytic debenzylation (H_2/10% Pd−C in EtOAc) gave the Boc-protected D-allo-amino acid **33**. Application of the same reaction sequence to **17a** (3*S*-X_S) afforded the corresponding protected L-allo-amino acid **ent-33** (2*S*,3*S*) (Scheme 6). Data obtained from NMR measurements of **33** and **ent-33** were identical to those published[9] and of racemic *allo*-F_2Pmab derivative synthesized by us.[10]

In Scheme 7 is shown an alternative synthetic route for the threo derivatives utilizing bromination and azide displacement. Diastereoselective bromination with NBS in THF of sodium enolates resulting from **16a**, proceeded with high diastereoselectivity (de 98.8%), to give bromide **34** (2*R*,3*S*-X_R) in 96% yield. Conversion of **34** to the corresponding benzyl ester **35** was achieved using $Ti(OiPr)_4$−benzyl alcohol in toluene at 120 °C for 5 h. Transformation at this stage is critical for accomplishing the synthesis of threo derivatives.[34,35] Treatment of **35** with tetramethylguanidinium azide (TMGA)[36] in CH_3CN at room temperature gave azide derivative **36** (2*S*,3*R*) in 50% yield accompanied by an E2-elimination product **37** (**36**:**37** = 3:1). It would be expected that repulsive $-CF_2P(O)(OEt)_2$/$-CO_2Bzl$ interactions in **35** could make C(α)-Br and C(β)-H groups antiperiplanar, which could

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(34) Azide displacement of sultam-imide **34** gave much more amount of an E2-elimination product than that resulting from benzyl ester **35**.

(35) Transesterification of Boc-protected sultam-imide, resulting from **34** via the following sequence (azide displacement, reduction, and Boc protection), with $Ti(OiPr)_4$−benzyl alcohol in toluene at 120 °C recovered the starting material. Furthermore, the reaction at elevated temperature gave decomposed products.

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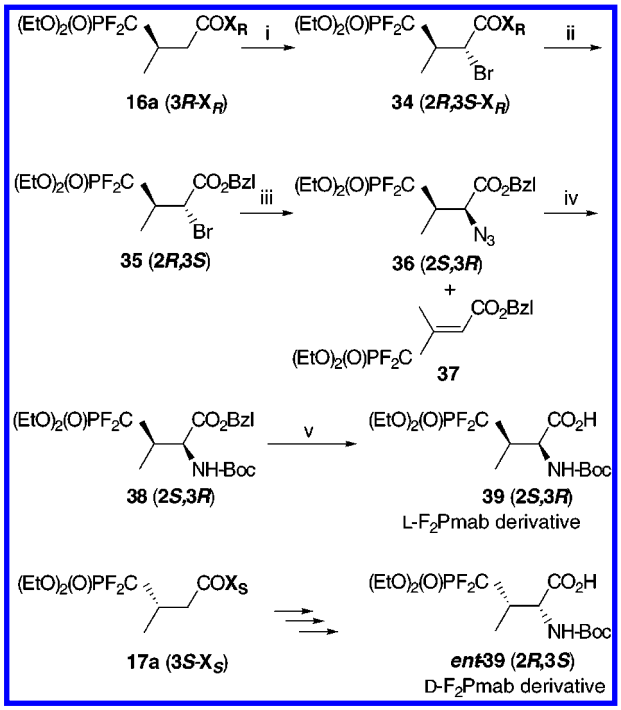
Scheme 7. Synthesis of Nonhydrolyzable pThr Mimetics[a]



[a] Key: (i) NaHMDS (1.2 equiv), NBS (3.0 equiv), THF; (ii) $Ti(OiPr)_4$ (2.0 equiv), Bzl-OH (44 equiv), toluene; (iii) TMGA (3.0 equiv), CH_3CN; (iv) Zn (40 equiv), AcOH (50 equiv) then Et_3N, $(Boc)_2O$ (2.0 equiv), CH_3CN; (v) H_2/Pd-C, EtOAc.

account for the formation of a significant amount of the elimination product **37**. Conversion of the azide to amine (Zn−AcOH in THF), followed by introduction of Boc protection using $(Boc)_2O/Et_3N$, gave Boc-protected threo derivative **38** (2*S*,3*R*) in 82% yield. Catalytic hydrogenation of **38** (H_2/Pd−C) yielded the desired L-F_2Pmab derivative **39** in quantitative yield, suitable for solid-phase peptide synthesis. The D-F_2Pmab derivative **ent-39** (2*R*,3*S*) was also obtained from **17a** using the same reaction sequence. Data obtained from NMR measurements of **39** and **ent-39** were identical to those published[9] and of racemic F_2Pmab derivative synthesized by us.[10]

Next, we examined the synthesis of F_2Pmab-containing peptides. As mentioned above, ethyl phosphonate esters are difficult to deprotect using reagent systems commonly employed for peptide synthesis. We were therefore prompted to examine reagent systems applicable to ethyl-protected F_2Pmp, F_2Pab, and/or F_2Pmab-containing peptide resins, which lead to the development of two deprotecting protocols. The first method consisted of a one-pot, two-step methodology which employed high acidic (1 M TMSOTf−thioanisole in TFA) and low acidic (1 M TMSOTf−thioanisole in TFA + TMSOTf−DMS) treatments.[19] The second method involved successive treatment with 1 M TMSOTf−thioanisole in TFA followed by ethyl deprotection of the ether-precipitated crude peptide resulting from the first reaction mixture, using TMSOTf−DMS−*m*-cresol−EDT.[12] The use of the former procedure sometimes resulted in incompletely deprotected peptide, while the latter protocol has ethyl-removing ability superior to that of the former system. It should be noted however that partially deprotected peptide must be precipitated and washed with ether prior to the second step. Furthermore, difficulty in isolating fully deprotected peptide from the second reaction mixture by precipitation with ether can be encountered. In developing the former methodology, we have previously reported that operation under S_N2 conditions[19,37] is crucial for removal of phosphate or phosphonate protecting groups. Additionally, we have found that against dimethyl-protected phosphate-containing peptides replacement of TMSOTf by TFMSA[38] results in substantial formation of incompletely demethylated product. During the deprotecting reaction, conversion of monoalkyl phosphate species to fully deprotected forms is rate determing. Therefore, differences between TMSOTf- and TFMSA-based reagents may be attributed to structural variations in intermediary monoalkyl-protected forms. That is, silylated intermediates resulting from TMSOTf-based treatment may more easily undergo nucleophilic attack by sulfides to form fully deprotected products as compared to nonsilylated intermediates.[39] We speculated that a deprotecting system capable of completely silylating monoalkyl phosphonates as well as inducing nucleophilic attack, could potentially represent a highly efficient deprotective methodology for ethyl protected CF_2-phosphonate derivatives. Gordeev et al. reported that *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA)−TMSI in CH_2Cl_2 effectively removes ethyl groups from protected Fmoc-F_2Pmp amino acid, and the ethyl-deprotected Fmoc amino acid was successfully applied to practical F_2Pmp peptide synthesis.[40] Removal of ethyl groups from protected Pmp (phosphonomethyl phenylalanine) peptide was also achieved TMSI in MeCN.[41] However, direct application of BSTFA−TMSI in CH_2Cl_2 or TMSI in MeCN to protected peptide resins has some disadvantage, which might afford peptide mixtures involving incompletely deprotected peptide and resin-bound peptide since TMSI has ability to remove partly several protecting groups including peptide−resin linkers. We therefore attempted to develop a practical deprotecting system, followed by easy workup, which could be directly used for protected peptide resins. Since TMSI potentially serves as both an activator of BSTFA and a nucleophile in Gordeev's method, we examined Lewis-acid−nucleophile combinations as substituents for TMSI. For this purpose, an F_2-Pmab$(OEt)_2$-containing model peptide resin **40**, corresponding to the partial sequence of the phosphrylated domain of Cdc2,[42] was prepared using standard Boc-based solid-phase techniques (Figure 3).

Starting from unsubstituted MBHA resin,[43] the protected peptide resin was synthesized using manual Boc methodology with a combination of TFA-mediated Boc deprotection, followed by neutralization with DIPEA and

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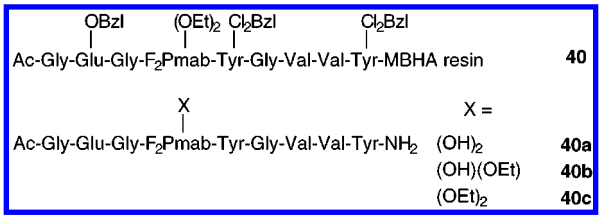

Figure 3. Protected peptide resin of a model peptide and resulting deprotected peptides.

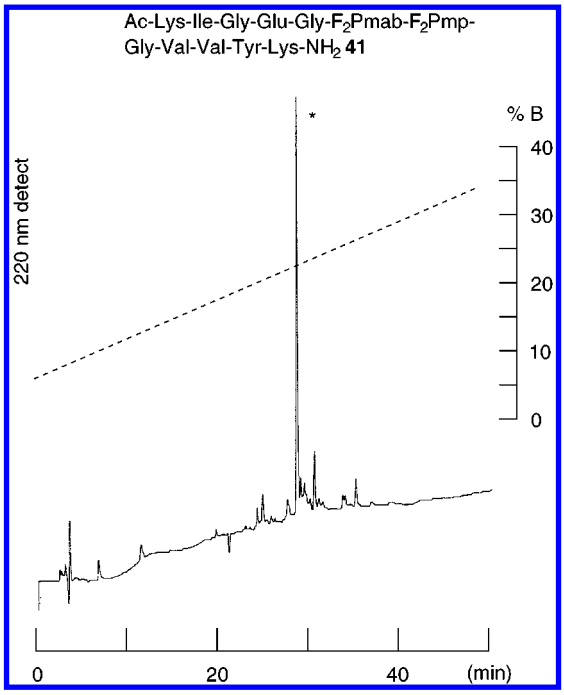

Figure 4. HPLC profile of crude **41**. An asterisk denotes the desired peptide: column, Cosmosil $5C_{18}$-AR (4.6 × 250 mm); buffer A, 0.1% aqueous TFA; B, CH_3CN (0.1% TFA); linear gradient, 5−34% B over 50 min; flow rate, 1.0 mL/min, detection at 220 nm.

DIPCDI/HOBt-mediated coupling of Boc-amino acids. The following side chain protecting groups were utilized: Bzl for Glu, Cl_2Bzl for Tyr,[44] and Et for the F_2Pmab. To obtain incompletely deprotected standard peptides (mono-Et form **40b** and di-Et form **40c**, Figure 3), the protected resin was treated with 1 M TMSOTf in TFA, *m*-cresol, EDT (Table 1, run 1) or 1 M TMSOTf−thioanisole in TFA, *m*-cresol, EDT (Table 1, run 2). The former reagent system without sulfide as a nucleophile, operates under S_N1 conditions to yield a mixture of **40b** and **40c** in a ratio of 14:86. Deprotection of **40** with the latter thioanisole-mediated system may proceed by a mixed mode consisting of S_N1 and S_N2 to afford crude peptides in a ratio of fully deprotected peptide **40a**:**40b** (39:61).

Using standard peptides generated in this fashion for HPLC analyses of crude deprotected mixtures, several deprotection conditions were examined. Deprotection with previously published one-pot two-step methodology gave fully deprotected peptide as a main product; however, a significant amount of monoethyl peptide **40b** was obtained (**40a**:**40b** = 55:45 Table 1, run 3). The use of other low-acidic treatment, for example, successive treatment of **40** with 1 M TMSOTf−thioanisole in TFA, followed by TMSOTf−DMS−*m*-cresol−EDT (4:6:0.5:0.5, v/v), predominantly gave **40a** (Table 1, run 4). However, difficulties in product isolation due to lack of ether-induced peptide precipitate formation were encountered. We next evaluated deprotection conditions based on the silylation−deprotection concept. Here we initially planned to use BSTFA as a silylating agent, BF_3·Et_2O as an activator of BSTFA, and $(n\text{Bu})_4$NI (TBAI) as a soft nucleophile. Dichloromethane (CH_2Cl_2) was used as a reaction solvent to prevent hydrolysis of the TMS group on monoethtyl intermediates. After preliminary experiments, it was found that treatment of protected peptide resins with 0.3 M BSTFA−TBAI (molar ratio 1:1) in CH_2Cl_2 (200 equiv), BF_3·Et_2O (20 equiv), followed by filtration of the resin and successive washing with solvent (CH_2Cl_2, DMF, and Et_2O), provided peptide resin possessing fully deprotected F_2Pmab residues. Cleavage of resin with 1 M TMSOTf−thioanisole in TFA gave fully deprotected F_2Pmab peptides. Application of this sequence to **40** gave fully deprotected peptide **40a** without accompanying **40b** and **40c** (Table 1, run 5). In this BSTFA−TBAI−BF_3 methodology, the combination of the three reagents is critical for efficient deprotection. As shown in Table 1, omission of a single reagent resulted in the formation of incompletely deprotected peptide (Table 1, runs 6−8). To verify the general applicability of the newly developed two-step deprotection protocol, we attempted the synthesis of Ac-Lys-Ile-Gly-Glu-Gly-F_2Pmab-F_2Pmp-Gly-Val-Val-Tyr-Lys-NH_2 **41**, which is a partial sequence of Cdc2 and a potential candidate as an inhibitor of Cdc25[45] phosphatase. Starting from unsubstituted MBHA resin, the protected peptide resin was elongated using standard Boc methodology. For side chain protection, the following protecting groups were utilized: Bzl for Glu, Cl_2Bzl for Tyr, ClZ for Lys,[46] and Et for F_2Pmab and F_2Pmp. This peptide sequence contained two nonhydrolyzable CF_2-substituted phosphoamino acid mimetics (F_2Pmab and F_2Pmp). Treatment of the completed peptidyl resin with

Table 1. Examination of Deprotecting Conditions

run	reagent	ratio (%) of components **40a**	**40b**	**40c**
1	1 M TMSOTf in TFA, *m*-cresol, EDT[a]	0	14	86
2	1 M TMSOTf-thioanisole in TFA[a]	39	61	0
3	First step (run 2[a]) + Second step (addition of DMS−TMSOTf (30:20)[b])	55	45	0
4	First step (run 2[a]) + Second step (TMSOTf−DMS−*m*-cresol−EDT (4:6:0.5:0.5)[b])	93	7	0
5	First step (0.3 M BSTFA−TBAI in CH_2Cl_2, BF_3·Et_2O[c]) + Second step (run 2[a])	100	0	0
6	First step (0.3 M BSTFA−TBAI in CH_2Cl_2[c]) + Second step (run 2[a])	49	51	0
7	First step (0.3 M BSTFA in CH_2Cl_2, BF_3·ET_2O[c]) + Second step (run 2[a])	71	29	0
8	First step (0.3 M TBAI in CH_2Cl_2, BF_3·Et2O[c]) + Second step (run 2[a])	58	41	0

[a] At 4 °C for 1.5 h then at room temperature for 0.5 h. [b] At 4 °C for 2 h. [c] At room temperature for 1.5 h.

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0.3 M BSTFA−TBAI in CH_2Cl_2, $BF_3{\cdot}Et_2O$ at room temperature for 1.5 h, followed by washing with solvent, and exposure to 1 M TMSOTf−thioanisole in TFA, *m*-cresol, EDT at 4 °C for 1.5 h with additional stirring for 0.5 h at room temperature, resulted in the release of completely deprotected peptide. Analyses of resulting crude peptide by HPLC and IS-MS showed that no significant side reactions had occurred.[47] After HPLC purification, the purified peptide was obtained in 25% yield based on the protected peptide resin. These results indicate that the newly developed two-step protocol, utilizing a BSTFA−TBAI−$BF_3{\cdot}Et_2O$ in CH_2Cl_2 system, has wide applicability for the synthesis of CF_2-substituted nonhydrolyzable phosphoamino acid mimetics-containing peptides.

That no loss of peptide from the resin occurred during the first deprotection treatment was supported by model experiments using Boc-Leu-PAM-Gly resin (Gly, internal standard for amino acids analyses), where cleavage under the deprotection condition was not observed. PAM linker[48] is known to be sensitive to acidic treatment as compared to MBHA linker used in this work, indicating that resin cleavage would be highly unlikely using MBHA resin.

In conclusion, we have achieved the stereoselective synthesis of all four isomers of protected F_2Pmab. Furthermore, deprotection methodology operating under S_N2 conditions based on a silylation−deprotection concepts, which is suitable for removal of Et groups on F_2Pmab residues, was developed. A combination of this new system and 1 M TMSOTf−thioanisole in TFA provides a general and efficient procedure for the practical synthesis of F_2Pmab-, F_2Pmp-, and/or F_2Pab-containing peptides. Biological evaluation of synthetic Cdc2 peptide is now in progress.

Experimental Section

General Methods. All reactions were carried out under a positive pressure of argon except for deprotective reactions for protected peptide resins. All glassware and syringes were dried in an electric oven at 100 °C prior to use. All melting points are uncorrected. All NMR spectra were recorded in $CDCl_3$. Chemical shifts are reported in parts per million downfield from internal Me_4Si (s = singlet, d = doublet, dd = double doublet, t = triplet, q = quadruplet, m = multiplet). For flash chromatographies, silica gel 60H (silica gel for thin-layer chromatography, Merck) or silica gel 60 (finer than 230 mesh, Merck) was employed. All HPLC separations were carried out using either Cosmosil 5C_{18}-AR analytical (4.6 × 250 mm) or Cosmosil 5C_{18}-AR preparative column (20 × 250 mm). Eluting products were detected by UV at 220 nm. Solvent A was 0.1% TFA (v/v) in water, and solvent B was 0.1% TFA in CH_3CN unless otherwise specified. A flow rate of 1 mL/min was used for all analytical separations and a 7 mL/min flow rate for preparative runs. Both isomers of bornane-10,2-sultam were prepared according to the literature.[49] Protected amino acids, MBHA resin and other peptide synthesis chemicals were purchased from Nova Biochmicals or Watanabe Chemical Inc. All other chemicals were purchased from either Nacalai Tesque, Wako Pure Chemical Industries, Kanto Chemicals, Aldrich, or Merck.

4-Methoxybenzyl (*Z*)-3-Iodobut-2-enoate (12) from 11. A solution of 18.4 mL (62.5 mmol) of $Ti(OiPr)_4$ in 4-methoxybenzyl alcohol (50 mL, 362 mmol) was stirred under vacuum for 1 h. To the solution at room temperature under argon was added 7.5 g (31.2 mmol) of **11**, and the mixture was allowed to warm 70 °C and to stir at this temperature for 2 h. The reaction was quenched at room temperature by addition of aqueous 1 N HCl. The mixture was extracted with Et_2O, and the extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to yield an oil. Flash chromatographic purification of the oil over silica gel with *n*-hexanes−EtOAc (3:1) gave 8.7 g (83.6% yield) of the title compound **12** as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.72 (d, J = 1.4 Hz, 3H), 3.81 (s, 3H), 5.13 (s, 2H), 6.31 (q, J = 1.4 Hz, 1H), 6.86−6.92 (m, 2H), 7.28−7.36 (m, 2H); HRMS (FAB) *m*/*z* calcd for $C_{12}H_{13}O_3I$ (MH^+) 331.9911, found 331.9917.

4-Methoxybenzyl (*Z*)-3-(Diethylphosphonodifluoromethyl)but-2-enoate (13) from 12. To a stirred suspension of 7.5 g (115 mmol) of Zn dust, activated according to published procedure,[50] in DMF (150 mL) at room temperature under argon was slowly added 18.3 mL (104 mmol) of diethyl (bromodifluoromethyl)phosphonate (**7**). An exothermic reaction occurring with the addition of **7** was maintained around 55 °C by controlling the addition rate. After addition was completed, additional stirring for 3 h at room temperature gave a solution of (diethylphosphonodifluoromethyl)zinc bromide (**8**). To the solution at room temperature was added a solid of 14.9 g (104 mmol) of CuBr in one portion. After an additional 30 min of stirring, a solution of 17.3 g (52.1 mmol) of **12** in DMF (50 mL) was added to the above solution. The reaction was stirred for 3 h at room temperature and then quenched at 4 °C by addition of aqueous 1 N HCl. The mixture was passed through Celite and extracted with EtOAc. The extract was successively washed with 1 N HCl, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo. Flash chromatographic purification of the crude materials over silica gel with *n*-hexanes−EtOAc (2:1) gave 19.6 g (95.9% yield) of the title compound **13** as a pale yellow oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.34 (t, J = 7.0 Hz, 3H), 2.02 (t, J = 1.8 Hz, 3H), 3.80 (s, 3H), 4.18−4.32 (m, 4H), 5.10 (s, 2H), 6.05 (t, J = 1.8 Hz, 1H), 6.84−6.91 (m, 2H), 7.28−7.34 (m, 2H); HRMS (FAB) *m*/*z* calcd for $C_{17}H_{24}O_6F_2P$ (MH^+) 393.1267, found 393.1278.

(2*R*)-*N*-[(*Z*)-3′-(Diethylphosphonodifluoromethyl)but-2-enoyl]bornane-10,2-sultam (14) from 13. Treatment of 10 g (25.5 mmol) of **13** with TFA−H_2O (95:5, 50 mL) at 4 °C for 1 h, followed by concentration in vacuo, afforded a crude carboxylic acid derivative as a gelatinous residue. The residue was dissolved with EtOAc. The organic phase was washed with brine (5 times), dried over $MgSO_4$, and concentrated to leave a gelatinous residue, which was redissolved with THF (100 mL). To the solution were successively added 4.3 mL (30.6 mmol) of Et_3N and 3.8 mL (30.6 mmol) of pivaloyl chloride at −78 °C under argon. After 15 min of stirring at −78 °C, the solution was allowed to warm to 4 °C and stirred for 1 h to yield a mixed anhydride. In separate flask lithiated sultam (LiX_R) was prepared by treatment of 8.2 g (38.3 mmol) of sultam (HX_R) in THF (100 mL) with 29.8 mL (45.9 mmol) of 1.54 M *n*-BuLi in hexane at −78 °C under argon followed by an additional 15 min of stirring. To recooled solution of the mixed anhydride to −78 °C was added the solution of lithiated sultam, and stirring was continued for 1 h at −78 °C. The reaction was quenched with saturated NH_4Cl solution and extracted with EtOAc. The extract was successively washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo to yield an yellow oil. The oily material was purified by flash chromatography over silica gel with *n*-hexanes−EtOAc (1:1) to give 9.65 g (81.1% yield) of the title compound **14** as an yellow oil: $[\alpha]^{23}_D$ −59.3° (*c* 1.87, $CHCl_3$); ^{1}H NMR (270M Hz, $CDCl_3$) δ 0.97 (s, 3H), 1.16 (s, 3H), 1.37 (t, J = 7.1 Hz, 6H), 1.34−1.45 (m, 2H), 1.85−1.93 (m, 3H), 2.09 (t, J = 1.7 Hz, 3H), 2.14−2.26 (m, 2H), 3.40 (d, J = 7.0 Hz, 1H), 3.48 (d, J = 7.0 Hz, 1H), 3.87 (dd, J = 8.0, 5.0 Hz, 1H), 4.25−4.38 (m, 4H), 6.33 (t, J = 1.2 Hz, 1H); HRMS (FAB) *m*/*z* calcd for $C_{19}H_{31}O_6NF_2PS$ (MH^+) 470.1578, found 470.1570. The

(47) The crude peptide was ascertained not to accompany Et-remaining peptides by co-injection HPLC analysis.

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enantiomer **15** ($[\alpha]^{23}_D$ +58.0° (*c* 1.89, $CHCl_3$)) of the title compound **14** described above was prepared in an identical fashion.

(2*R*,3′*R*)-*N*-[3′-(Diethylphosphonodifluoromethyl)butanoyl]bornane-10,2-sultam (16a, 3*R*-X$_R$) and (2*R*,3′*S*)-*N*-[3′-(diethylphosphonodifluoromethyl)butanoyl]bornane-10,2-sultam (16b, 3*S*-X$_R$) from 14. A suspension of 10% Pd−C (200 mg) and **14** (1.0 g, 2.13 mmol) in EtOAc was subjected to catalytic hydrogenation at room temperature for 10 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give a mixture of major (**16a**) and minor (**16b**) isomers. Diastereomer analysis [HPLC; H_2O-CH_3CN (52:48)] of the unpurified product gave a **16a** (t_R = 31.8 min):**16b** (t_R = 29.2 min) ratio of 96:4. Flash chromatographic purification of the mixture over silica gel with *n*-hexanes−EtOAc gave **16a** (891 mg, 88.8% yield) and **16b** (36.8 mg, 3.7% yield), respectively. Recrystallization of **16a** from Et_2O gave colorless crystals. An X-ray structural analysis of the crystals (obtained from EtOAc) indicated that **16a** possesses (3′*R*)-configuration. For **16a**: mp 130−131 °C; $[\alpha]^{27}_D$ −49.0° (*c* 1.14, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.98 (s, 3H), 1.16 (s, 3H), 1.17 (d, *J* = 7.5 Hz, 3H), 1.38 (t, *J* = 7.0 Hz, 6H), 1.34−1.45 (m, 2H), 1.85−1.93 (m, 3H), 2.08−2.13 (m, 2H), 2.79 (dd, *J* = 16.0, 9.5 Hz, 1H), 2.83−3.07 (m, 1H), 3.16 (dd, *J* = 16.0, 2.5 Hz, 1H), 3.43 (d, *J* = 13.5 Hz, 1H), 3.51 (d, *J* = 13.5 Hz, 1H), 3.88 (dd, *J* = 7.0, 5.9 Hz, 1H), 4.26 (q, *J* = 7.0 Hz, 2H), 4.29 (q, *J* = 7.0 Hz, 2H). Anal. Calcd for $C_{19}H_{32}NO_6SPF_2$: C, 48.40; H, 6.84; N, 2.97. Found: C, 48.29; H, 6.77; N, 2.90. Crystal data for **16a**: orthorhombic, *a* = 13.0428(8) Å, *b* = 22.948(1) Å, *c* = 7.6546(8) Å, *V* = 2291.3(2) Å^3, *Z* = 4, space group $P2_12_12_1$, D_{calc} = 1.367 mg m^{-3}. Data collection: A total of 2243 reflections were measured by Rigaku AFC7R diffractometer with graphite monochromated Cu Kα radiation (λ = 1.54178 Å) at room temperature. Structure analysis and refinement: The crystal structure was solved by the direct methods with the program SnB[51] and refined by full-matrix least squares. Non-hydrogen atoms were refined with anisotropic temperature factors, and hydrogen atoms were included at calculated positions and refined atoms with isotropic temperature factors. The final *R* value for 2119 reflections with $I > 2\sigma(I)$ was 0.056 and 0.069. The maximum and minimum peaks in final difference Fourier map were 0.46 and −0.48 eÅ^{-3}, respectively. Keeping of the purified **16b** in refrigerator for several days afforded colorless crystals: mp 58−60 °C; $[\alpha]^{21}_D$ −68.2° (*c* 2.05, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.97 (s, 3H), 1.15 (s, 3H), 1.20 (d, *J* = 7.0 Hz, 3H), 1.38 (t, *J* = 7.0 Hz, 6H), 1.34−1.47 (m, 2H), 1.84−1.94 (m, 3H), 2.01−2.18 (m, 2H), 2.73 (dd, *J* = 17.3, 8.4 Hz, 1H), 2.83−3.10 (m, 1H), 3.20 (dd, *J* = 17.3, 4.1 Hz, 1H), 3.43 (d, *J* = 13.8 Hz, 1H), 3.51 (d, *J* = 13.8 Hz, 1H), 3.88 (dd, *J* = 7.3, 5.1 Hz, 1H), 4.26 (q, *J* = 7.0 Hz, 2H), 4.29 (q, *J* = 7.0 Hz, 2H). Anal. Calcd for $C_{19}H_{32}NO_6SPF_2$: C, 48.40; H, 6.84; N, 2.97. Found: C, 48.59; H, 7.04; N, 2.96; HRMS (FAB) *m*/*z* calcd for $C_{19}H_{33}O_6NF_2PS$ (MH^+) 472.1734; found 472.1726. To reconfirm the 3′-configuration of **16b**, **16a** and **16b** were respectively converted to the corresponding benzyl ester derivatives using a procedure similar to that described for the conversion of **11** to **12**. Benzyl ester **16a′** from **16a**: a colorless oil; $[\alpha]^{21}_D$ +7.23° (*c* 1.23, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.18 (d, *J* = 6.8 Hz, 3H), 1.37 (t, *J* = 7.0 Hz, 6H), 2.33 (dd, *J* = 15.9, 9.7 Hz, 1H), 2.70−2.90 (m, 1H), 2.95 (dd, *J* = 15.9, 3.8 Hz, 1H), 4.25 (q, *J* = 7.0 Hz, 2H), 4.28 (q, *J* = 7.0 Hz, 2H), 5.14 (s, 2H), 7.33−7.38 (m, 5H); HRMS (FAB) *m*/*z* calcd for $C_{16}H_{24}O_5F_2P$ (MH^+) 365.1329; found 365.1338. Benzyl ester **16b′** from **16b**: a colorless oil; $[\alpha]^{21}_D$ −6.31° (*c* 1.39, $CHCl_3$). Both NMR and FABMS analyses of **16b** gave the same results as those of **16a**. From these results, we concluded that the minor isomer (**16b**) possesses (3′*S*)-configuration.

(2*S*,3′*S*)-*N*-[3′-(Diethylphosphonodifluoromethyl)butanoyl]bornane-10,2-sultam (17a, 3*S*-X$_S$) and (2*S*,3′*R*)-*N*-[3′-(Diethylphosphonodifluoromethyl)butanoyl]bornane-10,2-sultam (17b, 3*S*-X$_S$) from 15. By a procedure identical with that described above, the title compound **17a** (major) and **17b** (minor) were prepared. For **17a** (**3*S*-X$_S$**): colorless crystals; $[\alpha]^{26}_D$ +49.0° (*c* 1.14, $CHCl_3$). For **17b** (**3*R*-X$_S$**): colorless crystals; $[\alpha]^{21}_D$ +74.0° (*c* 2.15, $CHCl_3$). Other physical and chemical data for **17a** and **17b**, except for optical rotation, were identical to those of **16a** and **16b**, respectively.

***tert*-Butyl But-2-ynoate (19) from 18.** To a solution of 38.0 g (314 mmol) of **18** in THF (250 mL) at −78 °C under argon was added 285 mL (440 mL) of 1.54 M *n*-BuLi in hexane. After the mixture was stirred at −78 °C for 2 h, a solution of 44 g (220 mmol) of $(Boc)_2O$ in THF (100 mL) was added. The reaction was allowed to warm to 4 °C, stirred for 10 h, and quenched with saturated NH_4Cl solution. The mixture was extracted with Et_2O, and the extract was successively washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure to leave residues. Distillation (20 mmHg, 77 °C) of the crude materials gave 22.2 g (72.1% yield) of the title compound **19** as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.49 (s, 9H), 1.96 (s, 3H); HRMS (CI) *m*/*z* calcd for $C_8H_{13}O_2$ (MH^+) 141.0915, found 141.0914.

***tert*-Butyl (*E*)-3-Iodobut-2-enoate (21) from 19.** To a stirred suspension of CuCN (8.6 g, 96.3 mmol) in THF (300 mL) at −78 °C was added by syringe 131 mL (202 mmol) of 1.54 M *n*-BuLi in hexane, and the mixture was allowed to warm to 4 °C so that a homogeneous solution was obtained. The mixture was recooled to −78 °C, where neat *n*-Bu_3SnH (49.9 mL, 185 mmol) was added, and the mixture was stirred for 15 min. To the solution of resulting stannylcuprate was added 20 mL (354 mmol) of EtOH. After 5 min, a solution of **19** (10.0 g, 71.3 mmol) in THF (50 mL) was added by syringe at −78 °C, and the mixture was stirred at the same temperature for 2 h. The reaction was quenched with saturated NH_4Cl−5% NH_4OH (1:1) solution. The mixture was extracted with EtOAc, and the extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give an oily product. Without further purification, solid iodine (27 g, 106 mmol) was added piecemeal at room temperature over 5 h to a solution of the above crude material (**20**) in CH_2Cl_2 (100 mL). After 24 h, the reaction mixture was concentrated to yield a reddish brown oil. Distillation (8 mmHg, 86 °C) of the crude materials gave 11.5 g (59.9% yield) of the title compound **21** as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.47 (s, 9H), 2.95 (d, *J* = 1.4 Hz, 3H), 6.55 (q, *J* = 1.4 Hz, 1H); HRMS (CI) *m*/*z* calcd for $C_8H_{14}O_2I$ (MH^+) 269.0040, found 269.0049.

***tert*-Butyl (*E*)-3-(Diethylphosphonodifluoromethyl)-but-2-enoate (22) from 21.** By a procedure identical with that described for the synthesis of **13** from **12**, 11.5 g (42.9 mmol) of **21** was converted to 14.0 g (99.4% yield) of the title compound **22** as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.3 Hz, 6H), 1.50 (s, 9H), 2.24−2.27 (m, 3H), 4.27 (q, *J* = 7.3 Hz, 2H), 4.30 (q, *J* = 7.3 Hz, 2H), 6.13−6.18 (m, 1H); HRMS (FAB) *m*/*z* calcd for $C_{26}H_{47}O_{10}F_4P_2$ (2M + H^+) 657.2580, found 657.2565.

(2*S*)-*N*-[(*E*)-3′-(Diethylphosphonodifluoromethyl)but-2-enoyl]bornane-10,2-sultam (23) from 22. By a procedure identical with that described for the synthesis of **14** from **13**, 10.0 g (30.5 mmol) of **22** was converted to 10.9 g (75.5% yield) of the title compound **23**. For **23**: colorless crystals from EtOAc−*n*-hexane; mp 113−115 °C; $[\alpha]^{23}_D$ +58.0 (*c* 1.89, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.98 (s, 3H), 1.17 (s, 3H), 1.34−1.41 (m, 6H), 1.35−1.45 (m, 2H), 1.87−1.97 (m, 3H), 2.10−2.16 (m, 2H), 2.27 (t, *J* = 1.6 Hz, 3H), 3.43 (d, *J* = 13.8 Hz, 1H), 3.51 (d, *J* = 13.8 Hz, 1H), 3.93 (dd, *J* = 6.8, 5.7 Hz, 1H), 4.22−4.37 (m, 4H), 6.80−6.86 (m, 1H). Anal. Calcd for $C_{19}H_{30}NO_6SPF_2$: C, 48.61; H, 6.44; N, 2.98. Found: C, 48.52; H, 6.30; N, 2.92.

Catalytic Hydrogenation of 23. A suspension of 10% Pd−C (500 mg) and **23** (8.0 g, 17.0 mmol) in toluene (80 mL) was subjected to catalytic hydrogenation at room temperature for 5 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give a mixture of minor (**17a**) and major (**17b**) isomers. Diastereomer analysis [HPLC; H_2O-CH_3CN (52:48)] of the unpurified product gave a **17a** (t_R = 33.0 min):**17b** (t_R = 29.8 min) ratio of 11:89. Flash chromatographic purification of the mixture over silica gel with

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n-hexanes−EtOAc gave **17a** (0.23 g, 2.9% yield) and **17b** (7.0 g, 87.1% yield), respectively. Each compound obtained was identical to that derived from **15** by catalytic hydrogenation over 10% Pd−C in EtOAc.

(2*R*,2′*R*,3′*R*)-*N*-[2′-(*tert*-Butoxycarbonyl)amino-3′-(diethylphosphonodifluoromethyl)butanoyl]bornane-10,2-sultam (31, 2*R*,3*R*-X_R) from 16a. To a solution of 500 mg (1.2 mmol) of **16a** in THF (8 mL) at −78 °C under argon was added by syringe 1.4 mL (1.4 mmol) of 1 M NaHMDS in THF. After the mixture was stirred at this temperature for 1 h, where a blue solution of 205 mg (1.4 mmol) of 1-chloro-1-nitrosocyclohexane in THF (1.4 mL) was added with additional stirring at −78 °C for 30 min. To the mixture at −78 °C was added aqueous 1 N HCl (6.6 mL). The mixture was allowed to warm to room temperature, and the stirring was continued for 30 min. The mixture then was concentrated in vacuo to leave residues, to which *n*-hexane (40 mL) and aqueous 1 N HCl (40 mL) were added. The organic phase was washed with aqueous 1 N HCl (40 mL). The combined aqueous phase was alkalinized with solid $NaHCO_3$ and extracted with EtOAc. The extract was dried over $MgSO_4$ and concentrated to give an oil of a hydroxylamine derivative **30**. Without further purification, the residue was dissolved with THF (5 mL). To the solution at room temperature were successively added AcOH (3.3 mL), aqueous 1 N HCl (6.6 mL), and Zn dust (3.03 g, 46.4 mmol). After the suspension was stirred for 2 h, the reaction was alkalinized with solid $NaHCO_3$. The reaction mixture was passed through Celite, and then the filtrate was extracted with EtOAc. The extract was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to yield an oil of a crude amine derivative. To a solution of the amine in CH_3CN (5 mL) was added at room temperature a solution of 506 mg (2.3 mmol) of $(Boc)_2O$ in CH_3CN (5 mL). After the reaction was stirred at room temperature for 12 h, the mixture was directly purified by flash chromatography over silica gel with *n*-hexanes−EtOAc (2:3) to yield 372 mg (56% yield) of the title compound **31** as a colorless oil. Diastereomer analysis [HPLC; H_2O−CH_3CN (50:50)] of the unpurified product gave a **31** (t_R = 40.6 min):(**2′*S***) isomer (t_R = 48.7 min) ratio of 97.7:2.3. For **31**: $[\alpha]^{25}_D$ −27.2° (*c* 0.74, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.96 (s, 3H), 1.18 (s, 3H), 1.25 (d, J = 7.3 Hz, 3H), 1.34−1.41 (m, 6H), 1.41 (s, 9H), 1.38−1.45 (m, 3H), 1.85−1.95 (m, 3H), 2.02−2.30 (m, 2H), 3.08−3.32 (broad, 1H), 3.41 (d, J = 13.5 Hz, 1H), 3.49 (d, J = 13.5 Hz, 1H), 3.92 (dd, J = 7.0, 5.8 Hz, 1H), 4.26 (q, J = 7.3 Hz, 2H), 4.29 (q, J = 7.3 Hz, 2H), 4.91−5.00 (m, 1H), 5.26 (d, J = 7.3 Hz, 1H); HRMS (FAB) *m*/*z* calcd for $C_{24}H_{40}O_8N_2F_2PS$ ($M-H^-$) 585.2211, found 585.2215. The (**2′*S***) isomer for diastereomer analysis was prepared from bromide **34** via the following sequence: (i) azide displacement; (ii) reduction; (iii) Boc protection.

Benzyl (2*R*,3*R*)-2-(*tert*-Butoxycarbonyl)amino-3-(diethylphosphonodifluoromethyl)butanoate (32) from 31. A solution of 0.34 mL (1.14 mmol) of Ti(O*i*Pr)$_4$ in benzyl alcohol (2.4 mL, 22.8 mmol) was stirred under vacuum for 1 h. To the solution at room temperature under argon was added a solution of 334 mg (0.57 mmol) of **31** in toluene (3 mL), and the mixture was allowed to warm to 120 °C and to stir at this temperature for 5 h. The reaction was quenched at room temperature by addition of aqueous 1 N HCl. The mixture was extracted with EtOAc, and the extract was washed with 1 N HCl and brine, dried over $MgSO_4$, and concentrated in vacuo to leave an oil containing **32**, benzyl alcohol, and the sultam. The oil was treated with 4.3 mL (45.6 mmol) of Ac_2O and 0.92 mL (11.4 mmol) of pyridine at room temperature for 20 min. The reaction was quenched at 4 °C by slow addition of saturated $NaHCO_3$, followed by extraction with EtOAc. The extract was successively washed with saturated $NaHCO_3$, 1 N HCl, and brine, dried over $MgSO_4$, and concentrated to give crude materials. Flash chromatographic purification of the crude materials over silica gel with *n*-hexanes−EtOAc (1:1) gave 204 mg (74.5% yield) of the title compound **32** as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.14 (d, J = 7.3 Hz, 3H), 1.36 (t, J = 7.3 Hz, 6H), 1.43 (s, 9H), 2.75−3.00 (m, 1H), 4.24 (q, J = 7.3 Hz, 2H), 4.27 (q, J = 7.3 Hz, 2H), 4.85 (dd, J = 8.9, 2.7 Hz, 1H), 5.10−5.25 (m, 3H), 7.33−7.37 (m, 5H); HRMS (FAB) *m*/*z* calcd for $C_{16}H_{33}O_9N_3F_2P$ (MH^+) 480.1922, found 480.1953.

(2*R*,3*R*)-2-(*tert*-Butoxycarbonyl)amino-3-(diethylphosphonodifluoromethyl)butanoic Acid (33, D-allo-Form) from 32. A solution of 150 mg (0.31 mmol) of **32** in EtOAc (3 mL) in the presence of 10% Pd−C was subjected to debenzylation under H_2 at room temperature for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give 120.5 mg (98.9% yield) of the title compound **33** as a colorless oil: $[\alpha]^{19}_D$ −13.7° (*c* 1.41, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (d, J = 7.3 Hz, 3H), 1.38 (t, J = 7.0 Hz, 3H), 1.39 (t, J = 7.0 Hz, 3H), 1.44 (s, 9H), 2.79−3.05 (broad, 1H), 4.26−4.36 (m, 4H), 4.86 (dd, J = 9.0, 1.5 Hz, 1H), 5.17 (d, J = 9.0 Hz, 1H); HRMS (FAB) *m*/*z* calcd for $C_{14}H_{27}O_7NF_2P$ (MH^+) 390.1493, found 390.1481.

The antipode (L-allo-form, **ent-33**) of **33** was also prepared from **17a** according to the sequence of reactions employed for the conversion of **16a** to **33**. L-allo-form: $[\alpha]^{19}_D$ +14.4° (*c* 1.32, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.20 (d, J = 7.3 Hz, 3H), 1.38 (t, J = 7.0 Hz, 3H), 1.39 (t, J = 7.0 Hz, 3H), 1.45 (s, 9H), 2.80−3.05 (broad, 1H), 4.26−4.37 (m, 4H), 4.87 (dd, J = 8.9, 1.5 Hz, 1H), 5.18 (d, J = 8.9 Hz, 1H), 7.90−8.60 (broad, 1H); HRMS (FAB) *m*/*z* calcd for $C_{14}H_{27}O_7NF_2P$ (MH^+) 390.1493, found 390.1485. Data obtained from NMR measurements of **33** and **ent-33** were identical to those published in the literature[9] and of racemic *allo*-F_2Pmab derivative synthesized by us.[10] Enantio- and diastereomeric purities of **33** were ascertained by comparison of NMR data between D-allo-form (**33**) and other isomers derivatized as Mosher amides, obtained via the following sequence: (i) $TMSCH_2N_2$, MeOH, benzene;[52] (ii) TFA then Et_3N, THF; (iii) (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid ((*S*)-MTPA),[53] WSCDI, HOBt, THF. From this experiment, no other isomers were detected in synthetic D-allo-form **33**.

(2*R*,2′*R*,3′*S*)-*N*-[2′-Bromo-3′-(diethylphosphonodifluoromethyl) butanoyl]bornane-10,2-sultam (34, 2*R*,3*S*-X_R) from 16a. To a solution of 3.0 g (6.40 mmol) of **16a** in THF (30 mL) at −78 °C under argon was added by syringe 7.7 mL (7.70 mmol) of 1 M NaHMDS in THF. After the mixture was stirred at this temperature for 1 h, a precooled (−78 °C) suspension of 3.42 g (19.2 mmol) of NBS in THF (30 mL) was added with additional stirring at −78 °C for 90 min. The reaction was quenched at −78 °C by addition of aqueous 0.5 M $NaHSO_3$. After warming to room temperature, the mixture was extracted with EtOAc. The extract was washed with 0.5 M $NaHSO_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure to give an oil. Flash chromatography of the crude material over silica gel with *n*-hexanes−EtOAc (1:1) yielded 3.37 g (95.7% yield) of the title compound **34** as a pale yellow oil: $[\alpha]^{23}_D$ −42.7° (*c* 1.28, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.98 (s, 3H), 1.20 (s, 3H), 1.38 (t, J = 7.0 Hz, 6H), 1.37−1.45 (m, 2H), 1.49 (d, J = 7.0 Hz, 3H), 1.87−1.97 (m, 3H), 2.07−2.20 (m, 2H), 3.19 (m, 1H), 3.45 (d, J = 14.0 Hz, 1H), 3.53 (d, J = 14.0 Hz, 1H), 3.94 (dd, J = 7.5, 5.0 Hz, 1H), 4.27 (q, J = 7.0 Hz, 2H), 4.30 (q, J = 7.0 Hz, 2H), 5.17 (d, J = 7.0 Hz, 1H); HRMS (FAB) *m*/*z* calcd for $C_{19}H_{32}O_6NBrF_2PS$ (MH^+) 550.0840, found 550.0831. Diastereomer analysis was carried out after conversion of the crude products to the corresponding *p*-methoxybenzyl esters using Ti(OPMB)$_4$ in toluene. An HPLC analysis of the PMB (H_2O−CH_3CN (55:45)) afforded 99.4:0.6 ratio of (2*R*) and (2*S*) diastereomers (t_R 61.8 and 53.5 min, respectively). Authentic samples were prepared as follows: (i) conversion of **16a** to the corresponding PMB ester, (ii) bromination, and (iii) separation of diastereomers on HPLC.

Benzyl (2*S*,3*R*)-[2-Azide-3-(diethylphosphonodifluoromethyl)]butanoate (36, 2*S*,3*R*) from 34. Conversion of **34** to the corresponding benzyl ester derivative **35** was achieved according to a procedure identical with that described for the transesterification of **31** to **32**. Treatment of 8.0 g (14.5

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(53) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543−2549.

mmol) of **34** in toluene (60 mL) with Ti(O*i*Pr)$_4$ (4 equiv) in benzyl alcohol (32 equiv) at 120 °C for 5 h, followed by the usual workup gave a mixture of **35**, benzyl alcohol, and the sultam (HX_R) as an oil. The oil was treated with 110 mL (1.16 mol) of Ac_2O and 23.3 mL (0.29 mol) of pyridine at room temperature for 20 min. The reaction was quenched by slow addition of saturated $NaHCO_3$, followed by extraction with EtOAc. The extract was successively washed with saturated $NaHCO_3$, 1 N HCl, and brine, dried over $MgSO_4$, and concentrated to give an oil. Flash chromatographic purification of the oil over silica gel with *n*-hexanes−EtOAc (3:2) gave a ca. 1:1 mixture of **35** and the sultam (HX_R). For **35**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.87 (d, J = 7.0 Hz, 3H), 1.26 (s, 9H), 1.39 (t, J = 7.3 Hz, 6H), 2.98−3.15 (m, 1H), 4.24 (q, J = 7.3 Hz, 2H), 4.27 (q, J = 7.3 Hz, 2H), 4.78 (d, J = 6.2 Hz, 1H), 5.20 (s, 2H), 7.35−7.40 (m, 5H); HRMS (FAB) *m*/*z* calcd for $C_{16}H_{23}O_5BrF_2P$ (MH^+) 443.0435, found 443.0428. Without further purification, the mixture was subjected to next step. To a solution of the mixture (containing ca. 9.8 mmol of **35**) in CH_3CN (40 mL) was added at room temperature a solution of 4.6 g (29.3 mmol) of tetramethylguanidinium azide in CH_3CN (30 mL). After the reaction was stirred for 12 h, quenched by addition of aqueous saturated $NaHCO_3$. The mixture was extracted with EtOAc. The extract was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to give a mixture of azide displaced product **36**, an elimination product **37**, and HX_R. Flash chromatography of the mixture over silica gel with *n*-hexanes−EtOAc (3:2) yielded, in order of elution, 1.65 g of an oil consisting of **37** and HX_R, and 1.98 g (35% yield from **34**) of **36** as an oil. For **36**: $[\alpha]^{23}_D$ −2.91 (*c* 2.54, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.24 (d, J = 7.3 Hz, 3H), 1.35 (t, J = 7.3 Hz, 3H), 1.37 (t, J = 7.3 Hz, 3H), 2.80−3.05 (m, 1H), 4.14 (d, J = 6.5 Hz, 1H), 4.22 (q, J = 7.3 Hz, 2H), 4.28 (q, J = 7.3 Hz, 2H), 5.23 (s, 2H), 7.33−7.42 (m, 5H); HRMS (FAB) *m*/*z* calcd for $C_{16}H_{23}O_5N_3F_2P$ (MH^+) 406.1343, found 406.1349.

Benzyl (2*S*,3*R*)-[2-(*tert*-Butoxycarbonyl)amino-3-(diethylphosphonodifluoromethyl)]butanoate (38, 2*S*,3*R*) from 36. To a solution of 1.97 g (4.9 mmol) of **36** in THF (20 mL) were successively added Zn dust (6.35 g, 97.2 mmol) and AcOH (2.8 mL, 49 mmol) at room temperature. After 2 h of stirring, the suspension was neutralized by addition of Et_3N. To the mixture was added a solution of 2.1 g (9.7 mmol) of $(Boc)_2O$ in THF (5 mL). After the stirring was continued for 12 h, the solid precipitate was removed by filtration. The filtrate was extracted with EtOAc, followed by the usual workup. The crude material was subjected to flash chromatographic purification over silica gel with *n*-hexanes−EtOAc (1:1) to yield 1.9 g (81.9% yield) of the title compound **38** as a colorless oil: $[\alpha]^{23}_D$ +15.9° (*c* 3.53, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.29 (d, J = 7.6 Hz, 3H), 1.33 (t, J = 7.0 Hz, 3H), 1.35 (t, J = 7.0 Hz, 3H), 1.44 (s, 9H), 3.03−3.25 (m, 1H), 4.22 (m, 4H), 4.56 (dd, J = 10.0, 2.2 Hz, 1H), 5.16 (s, 2H), 5.47 (d, J = 10.0 Hz, 1H), 7.32−7.38 (m, 5H); HRMS (FAB) *m*/*z* calcd for $C_{21}H_{33}O_7NF_2P$ (MH^+) 480.1963, found 480.1953.

(2*S*,3*R*)-2-(*tert*-Butoxycarbonyl)amino-3-(diethylphosphonodifluoromethyl)butanoic Acid (39, L-F$_2$Pmab Derivative) from 38. A suspension of 10% Pd−C (50 mg) and **38** (200 mg, 0.42 mmol) in EtOAc (2 mL) was subjected to catalytic hydrogenation at room temperature for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give 161 mg (98.9% yield) of the title compound **39** as a colorless oil: $[\alpha]^{22}_D$ +22.7° (*c* 1.00, MeOH) [lit.[9], $[\alpha]^D$ +21.0° (c 1.10, MeOH)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.27 (d, J = 7.3 Hz, 3H), 1.35−1.45 (m, 6H), 1.45 (s, 9H), 2.97−3.23 (broad, 1H), 4.24−4.40 (m, 4H), 4.61 (dd, J = 8.9, 3.0 Hz, 1H), 5.47 (d, J = 8.9 Hz, 1H); HRMS (FAB) *m*/*z* calcd for $C_{14}H_{26}O_7NF_2PNa$ (MNa^+) 412.1313, found 412.1308.

The antipode (D-F$_2$Pmab derivative **ent-39**) of **39** was also prepared from **17a** according to the sequence of reactions employed for the conversion of **16a** to **39**. For the D-form: $[\alpha]^{22}_D$ −23.7° (*c* 0.99, MeOH); HRMS (FAB) *m*/*z* calcd for $C_{14}H_{27}O_7NF_2P$ (MH^+) 390.1493, found 390.1497. Data obtained from NMR measurements of **39** and **ent-39** were identical to those published on literature[9] and of racemic F$_2$Pmab derivative synthesized by us.[10] Optical purity of **39** was judged to be >99% ee from an HPLC analysis of its Mosher amide, obtained via the following sequence: (i) $TMSCH_2N_2$, MeOH, benzene; (ii) TFA then Et_3N, THF; (iii) (*S*)-MTPA, WSCDI, HOBt, THF.

Synthesis of Protected Peptide Resin 40. Protected peptide resin was manually constructed using the Boc-based solid-phase method on MBHA resin (0.10 mmol, NH_2 0.46 mmol/g). Boc deprotection was achieved using 50% TFA−2% anisole in toluene (1 × 1 min, 1 × 15 min); 5% DIPEA in toluene (2 × 1 min) was used for neutralization of the TFA salts. Boc-protected amino acids including Boc-F$_2$Pmab-(OEt)$_2$-OH **39** (2.5-fold molar excess, 0.25 mmol) were sequentially condensed using DIPCDI (0.25 mmol)−HOBt (0.25 mmol) in DMF. Standard Boc protocols were applicable to the incorporation of F$_2$Pmab derivative into protected peptide resins.

Treatment of Protected Peptide Resin 40 with Acidic Deprotecting Reagents. Run 1: 1 M TMSOTf in TFA, *m*-Cresol, EDT System. To 10 mg (3.12 μmol) of the protected resin **40** were added *m*-cresol (32 μL) and EDT (32 μL). After stirring for 5 min at room temperature, the reaction mixture was cooled to 4 °C with an ice-chilled bath, and then precooled TFA (0.5 mL) and TMSOTf (121 μL, 0.624 mmol) were successively added, and the stirring was continued at 4 °C (1.5 h) and then at room temperature (0.5 h). After removal of the resin by filtration, ether was added to the filtrate to precipitate the crude product. The precipitate was collected by centrifugation and washed with ether (3 times) to remove scavengers. The crude peptides were dissolved in 0.1% TFA in H_2O−CH_3CN (3:1, 2.0 mL). The peptide solution was subjected to an HPLC analysis using analytical HPLC (B; 15−30% over 40 min). Components, corresponding to **40b** (t_R = 23.5 min) and **40c** (t_R = 41.0 min) obtained by analytical HPLC purification, were identified using IS-MS analyses. The ratio of components was determined by comparison of peak areas, where no significant side reactions were observed which could prevent the comparison of the peak areas. IS-MS (reconstructed): *m*/*z* calcd for $C_{48}H_{69}O_{17}N_{10}PF_2$ (**40b**) 1127.11, found 1127.99; *m*/*z* calcd for $C_{50}H_{73}O_{17}N_{10}PF_2$ (**40c**) 1155.17, found 1154.49.

Run 2: 1 M TMSOTf−Thioanisole in TFA, *m*-Cresol, EDT System. To 10 mg (3.12 μmol) of **40** were added *m*-cresol (32 μL), thioanisole (73 μL, 0.624 mmol), and EDT (32 μL). After 5 min of stirring at room temperature, the reaction mixture was cooled to 4 °C with an ice-chilled bath, and then precooled TFA (0.43 mL) and TMSOTf (121 μL, 0.624 mmol) were successively added. The reaction was stirred at 4 °C for 1.5 h with additional stirring at room temperature for 0.5 h. A procedure identical to that described above was utilized to obtain the crude peptides and to determine the ratio of products. IS-MS (reconstructed): *m*/*z* calcd for $C_{46}H_{65}O_{17}N_{10}PF_2$ (**40a**) 1099.06, found 1099.99.

Run 3: 1 M TMSOTf−Thioanisole in TFA, *m*-Cresol, EDT System (first step), Followed by Addition of TMSOTf−DMS (second step). Protected peptide resin **40** (10 mg, 3.12 μmol) was treated with 1 M TMSOTf−thioanisole in TFA (0.624 mL) in the presence of *m*-cresol (32 μL) and EDT (32 μL) at 4 °C for 1.5 h with additional stirring at room temperature for 0.5 h. The reaction mixture was recooled to 4 °C, where DMS (187 μL) and TMSOTf (125 μL) were successively added. The reaction was stirred for at 4 °C for 2 h. A procedure identical to that described above was utilized to obtain the crude peptides and to determine the ratio of products.

Run 4: 1 M TMSOTf−Thioanisole in TFA, *m*-Cresol, EDT System (first step), Followed by TMSOTf−DMS−*m*-Cresol−EDT Treatment (second step). Protected peptide resin **40** (10 mg, 3.12 μmol) was treated with 1 M TMSOTf-thioanisole in TFA (0.624 mL) in the presence of *m*-cresol (32 μL) and EDT (32 μL) at 4 °C for 1.5 h with additional stirring at room temperature for 0.5 h. Crude deprotected peptide were obtained by a procedure identical to that mentioned above. To the crude deprotected peptide was successively added at 4 °C *m*-cresol (15 μL), EDT (15 μL), DMS (182 μL), and TMSOTf (121 μL, 0.624 mmol). After 2 h of stirring at 4 °C, the reaction was quenched by successive addition of Et_2O (5 mL) and H_2O

(2 mL). The aqueous phase was washed with Et_2O (3 times) and filtered. The filtrate was subjected to an HPLC analysis to determine the ratio of products.

Run 5: 0.3 M BSTFA−TBAI in CH_2Cl_2, BF_3·Et_2O System (first step), Followed by 1 M TMSOTf−Thioanisole in TFA, *m*-Cresol, EDT System (second step). To a suspension of **40** (10 mg, 3.12 μmol) in CH_2Cl_2 (1.9 mL) were successively added TBAI (230 mg, 0.624 mmol), BSTFA (166 μL, 0.624 mmol), and BF_3·Et_2O (7.7 μL, 62.4 μmol) at room temperature. The mixture was shaken at room temperature for 1.5 h, and then passed through filter. The remaining resin on the filter was successively washed with CH_2Cl_2, DMF, MeOH, and Et_2O, and dried in vacuo. The peptide attached resin was treated with 1 M TMSOTf−thioanisole in TFA (0.624 mL) in the presence of *m*-cresol (32 μL) and EDT (32 μL) at 4 °C for 1.5 h and then at room temperature for 0.5 h, followed by a usual workup and HPLC analysis. As other runs (runs 6−8), protected resin **40** was treated under conditions similar to the title condition (run 5), where only one of the above three agents (TBAI, BSTFA, or BF_3·Et_2O) was omitted.

Synthesis of Ac-Lys-Ile-Gly-Glu-Gly-F_2Pmab-F_2Pmp-Gly-Val-Val-Tyr-Lys-NH_2 (41). A protected peptide resin corresponding to the Cdc2 peptide **41** was prepared by standard Boc-based solid-phase techniques on MBHA resin (0.23 mmol, NH_2 0.46 mmol/g) as previously described, where 2.5-fold excess of Boc-F_2Pmab$(OEt)_2$-OH **39** and Boc-F_2Pmp-$(OEt)_2$-OH were used for incorporation of F_2Pmab and F_2Pmp residues, respectively. The completed resin (56 mg, 14.7 μmol) was treated with 0.3 M BSTFA−TBAI in CH_2Cl_2 (8.6 mL, 200 equiv) in the presence of BF_3·Et_2O (72.8 μL, 40 equiv) at room temperature. After 1.5 h of shaking, the resin was washed with solvents described above and subjected to the second step with 1 M TMSOTf−thioanisole in TFA (2.95 mL), *m*-cresol (141 μL), and EDT (141 μL) with stirring for 1.5 h at 4 °C and then for 0.5 h at room temperature. A workup similar to that already mentioned above was utilized to obtain the crude peptide. HPLC purifications (linear gradient of B, 15−40% over 30 min) of the crude product gave the pure title peptide **41** as a white powder: 3.7 mg (25.2% yield, based on the protected peptide resin); IS-MS (reconstructed) *m*/*z* calcd for $C_{65}H_{101}O_{22}N_{15}P_2F_4$ **41** 1582.56, found 1582.49.

Examination of Stability of Peptide−Resin Bond under Treatment with 0.3 M BSTFA−TBAI in CH_2Cl_2, BF_3·Et_2O. To each suspension of Boc-Leu-PAM-Gly resin (10 mg each, 7.8 μmol) in CH_2Cl_2 (2.4 mL) was successively added TBAI (288 mg, 0.78 mmol), BSTFA (207 μL, 0.78 mmol), and BF_3·Et_2O (9.6 μL, 78 μmol) at room temperature. Each reaction mixture was shaken at room temperature for 30 min, 1 or 2 h, and passed through a filter. Each remaining resin on the filter was washed as described above, and a part of the resin was hydrolyzed with 6 N HCl at 110 °C for 18 h. The resulting hydrolysates were subjected to an amino acid analysis. Remained Leu on the resin was quantified compared with amino acid analysis of hydrolysate of the intact resin. Leu remained on the PAM resin; 95.3% (30 min), 91.1% (1 h), and 94.1% (2 h).

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Supporting Information Available: Tables of atomic coordinates, anisotropic displacement parameters, bond length and angles, and ORTEP diagram for **16a**. HPLC charts of the diastereomer analysis of products obtained from following reactions; (i) hydrogenation of **14** and **23**, (ii) amination of **16a** (including supporting NMR charts of resulting isomers), and (iii) bromination of **16a** (including supporting NMR charts of resulting isomers). NMR charts of the enantiomer and diastereomer analysis of **33** based on MTPA method. HPLC charts of the enantiomer and diastereomer analysis of **39** based on MTPA method. Copies of ^{1}H NMR spectra of compounds **13**, its (*E*)-isomer, **16a**, **16b**, **15**, **23**, **33**, and **39**. HPLC charts of deprotection reactions shown in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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