# A Concise β-Lactam Route to Short Peptide Segments Containing β,β-Disubstituted β-Amino Acids

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An efficient epimerization-free route toward β,β-disubstituted β-amino acid-containing peptides is described. The methodology involves the use of 4,4-disubstituted-*N*-Boc β-lactams as acylating agents, which upon coupling with amino acid esters, promoted by potassium cyanide, give rise to dipeptides with no appreciable racemization. By this procedure short peptide segments containing a four-, five-, or six-membered ring at the β-position of the β-amino acid residue have been prepared. The method has also proven to be valuable for the preparation of tripeptides. In addition the sterically hindered amino terminus of the β-amino acid can undergo peptide couplings under standard conditions.

The concept of structural modification in peptide fragments to confer them specific properties is of current interest in the study and design of new bioactive targets.[1] A well-known example is the incorporation of α-aminoisobutyric acid (Aib) into peptides and/or proteins which leads to important conformational restrictions, often providing an increase in the helix content[2] or the formation of specific β-turns.[3] The reduced flexibility of the peptide backbone usually facilitates the search for the active conformation recognized by a physiological receptor.[4] While the majority of the investigations in this area have dealt with the synthesis and use of α-branched α-amino acids, relatively little work has been conducted with β,β-disubstituted β-amino acids.[5] In general, the limited metabolism of β-amino acids suggests that β-amino acid analogues of α-amino acids will be metabolically stable in vivo. The incorporation of β-amino acids into peptides of pharmacological interest has often been found advantageous in terms of biological activity and/or metabolic stability.[6] In addition, recent studies on β-peptide oligomers have revealed new opportunities for the development of specific helical conformations or β-sheet-type structures.[7] Within this context we have been interested in the study of amino acids incorporating a cycloalkane structure as a model for conformationally restricted β,β-branched β-amino acids.

We investigated the incorporation of 1-aminocyclobutylacetic acid and the higher cycloalkyl homologues into small peptide segments.[8] Although there exist many methods for the synthesis of β-amino acids, only few of them appear to be appropriate for the generation of a quaternary center at the β-position.[9] Herein we describe the preparation of the desired β,β-disubstituted β-amino acid concomitant with its incorporation into a peptide.[10] Independent studies from this laboratory[11] and one other[12] suggest that this goal could be achieved by using β-lactams as acylating agents. However, earlier work in this area[13] indicated that $C_4$-disubstituted *N*-sulfonyl β-lactams, readily obtained by the sulfonyl isocyanate−alkene cycloaddition approach,[14] proved to be of very little value in β-amino acid peptide synthesis. Recently, Bhupathy and co-workers[15] have proposed a solution to this

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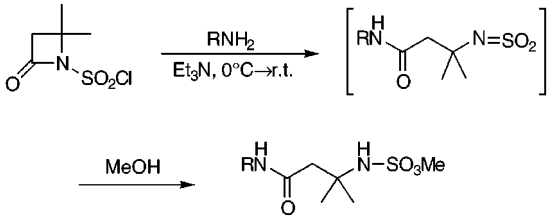
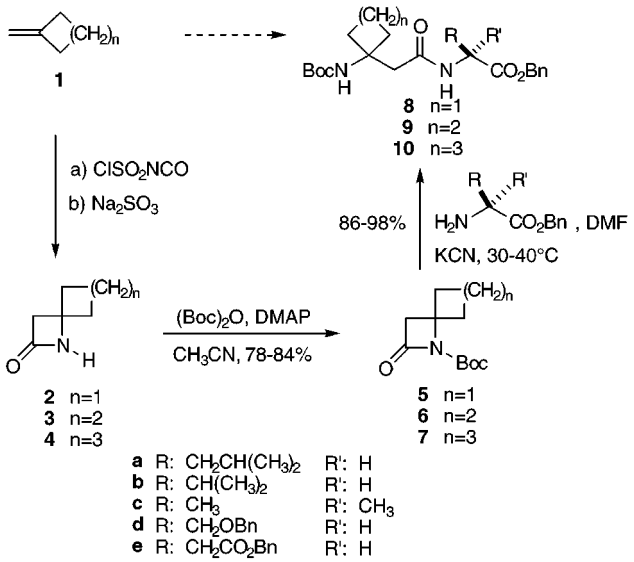
**Scheme 1**



**Scheme 2**[a]



problem based on the reaction of a *N*-chlorosulfonyl $\beta$-lactam with an amino compound to give a highly reactive intermediate (Scheme 1) which reacts rapidly with methanol to give the corresponding $\beta$-methoxysulfonyl protected amino derivative. We wish to present here a complementary route toward short peptide segments containing $\beta,\beta$-disubstituted $\beta$-amino acids that involves the coupling of *N*-Boc $\beta$-lactams with $\alpha$-amino acid esters. The key to this approach is the use of an *N*-activated $\beta$-lactam and potassium cyanide as the promoter of the acylation reaction.[16] In our approach (Scheme 2) the chlorosulfonyl moiety of the corresponding $\beta$-lactam is replaced by the more readily removable *tert*-butoxycarbonyl (Boc) group often employed as a protecting group in amino acid chemistry.

The starting $\beta$-lactams were prepared by the addition of chlorosulfonyl isocyanate (CSI) to the corresponding alkenes **1** according to established methods.[17] Subsequent treatment of each $\beta$-lactam product **2**, **3**,[18] and **4** with di-*tert*-butyl dicarbonate and DMAP[19] in acetonitrile overnight provided the *N*-Boc $\beta$-lactams **5**, **6**, and **7** in yields of 80%, 78%, and 84%, respectively. With these $\beta$-lactams in hand, our first aim was to establish the optimum reaction conditions to achieve their coupling with some representative $\alpha$-amino acid esters. With regard to this, we had previously observed that the ring opening of both 3-oxy and 3-amino $\beta$-lactams is strongly influenced by the substitution pattern at the $C_4$ position.[11] Thus, while 3-oxy and 3-amino 4-monoalkylated $\beta$-lactams were efficiently coupled in DMF with $\alpha$-amino acid esters without any additives or using sodium azide as the promoter, the corresponding 4-dialkylated counterparts were unreactive under these conditions, and potassium cyanide was required to catalyze the coupling reaction.[11] Taking these observations into account, it was not surprising to observe that $C_4$-disubstituted $\beta$-lactams **5**, **6**, and **7**, upon treatment with (L)-LeuOBn either in the presence or the absence of sodium azide, were completely resistant to $\beta$-lactam ring opening. However, contrary to our expectations, the coupling also did not proceed in the presence of potassium cyanide, and only unreacted $\beta$-lactam was recovered after 16 h of stirring at room temperature. We were gratified to observe that, by simply performing the reaction at 40 °C in the presence of a stoichiometric amount of potassium cyanide, the corresponding dipeptide products were obtained in excellent yields and without loss of optical purity,[20] vide infra. For example, under these conditions the coupling of **5** with both (L)-LeuOBn and (L)-ValOBn gave **8a** and **8b** after 16 h of reaction in 90% and 93% yields, respectively. Even the bulky AibOBn could efficiently be coupled with **5** to afford the dipeptide **8c** in 70% yield. Similarly, the assistance of potassium cyanide proved to be crucial for the coupling of both O-benzyl (L)-SerOBn and (L)-AspOBn with the $\beta$-lactam **5** to give rise to products **8d** (76%) and **8e** (75%), respectively. Likewise, treatment of both **6** and **7** with (L)-LeuOBn afforded, after column chromatography, the respective **9a** and **10a** in 98% and 90% isolated yields although in the latter case a longer reaction time was needed. The lower reactivity of $\beta$-lactam **7** could be overcome by performing the reaction at higher temperatures. Thus coupling of **7** with (L)-LeuOBn was complete at 70 °C in 16 h affording the corresponding peptide with little reduction in chemical

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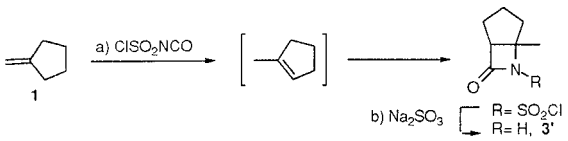
(18) During preparation of compound **3** an impurity (10−20%) was detected in the reaction. This impurity could be separated from **3** only after forming the Boc-derivative. From the spectroscopic data we assigned structure **3′**, which would likely have arised from an acid-catalyzed isomerization of the terminal olefin to the endocyclic olefin, to this impurity.



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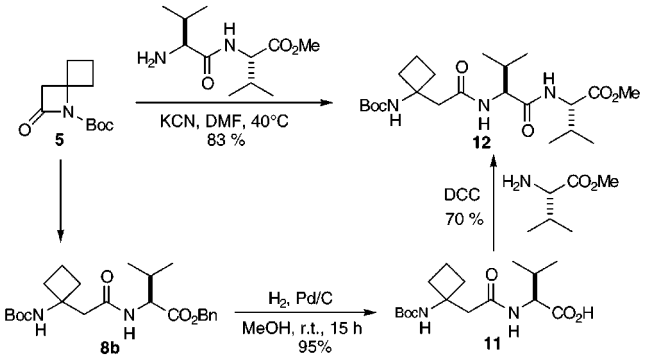
(20) Potassium cyanide has also been employed as a catalyst in transesterification processes to remove peptides from the Merrifield resin without racemization of the C-terminal amino acid. For more detailed information, see: Moore, G.; McMaster, D. *Int. J. Pept. Protein Res.* **1978**, *11*, 140.

**Scheme 3**

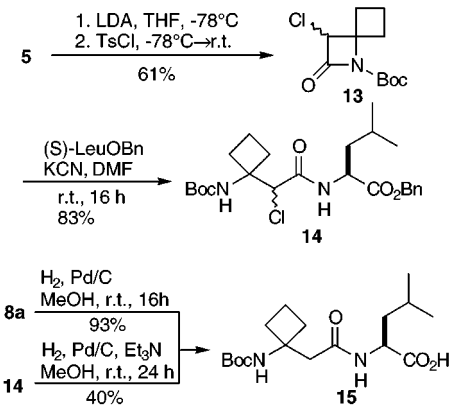



yield and, again, without appreciable racemization. Alternatively, coupling reactions could also be performed at room temperature in DMF−$H_2O$ (10:1) albeit chemical yields of isolated peptides fell to 50−60%. Decreasing the amount of potassium cyanide from 1 to 0.1 equiv resulted in the incomplete reaction (25% conversion after 16 h).

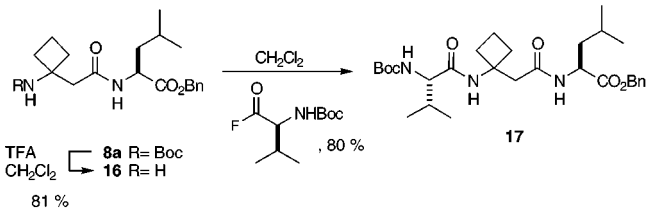
As Scheme 3 illustrates, exposure of the dipeptide product **8b** to $H_2$ and 10% Pd/C provided the *N*-Boc dipeptide **11** in 95% yield and ready for a subsequent coupling step with α-amino acid esters. For example, the DCC-assisted coupling of **11** with (L)-ValOMe furnished the tripeptide **12** in 70% isolated yield and without formation of epimerization products. In this context, it should be mentioned that coupling of (L)-ValOMe with **11** via its acid fluoride, prepared according to Carpino's procedure,[21] gave the corresponding coupling product as an equimolar mixture of diastereomers. The next question we addressed was whether β-lactam **5** could be coupled with dipeptides. If so, tripeptide units incorporating β,β-disubstituted β-amino acids at the *N*-terminus would be produced efficiently and, therefore, in better chemical yields. Under the same conditions employed for the preparation of dipeptides, β-lactam **5** efficiently coupled with (L)-Val-ValOMe to produce, in 83% isolated yield, the tripeptide **12** without detectable epimerization.

As mentioned above, the potential of β-lactams to act as acylating agents of amino acid esters seems to be influenced by the presence of electron-withdrawing substituents at the $C_3$ position of the azetidinone nucleus. To confirm this, the 3-chloro β-lactam **13** was prepared by treatment of the lithium enolate of **5** with an excess of *p*-toluensulfonyl chloride according to the method of Kühlein,[22] and the product **13** was then submitted to treatment with (S)-LeuOBn in DMF as solvent (Scheme 4). Indeed, unlike the result with 3-unsubstituted β-lactams, attempted amino acid coupling at room temperature for 16 h afforded detectable dipeptide formation (15% conversion judged by $^1$H NMR) in the absence of any promoter. Further, when the same coupling was carried out under identical conditions but with the assistance of 1 equiv of sodium azide, the yield rose to 85%. Finally, substituting potassium cyanide for sodium azide afforded complete reaction at room temperature and gave rise to dipeptide **14** in 83% isolated yield. Subsequent exposure of the crude product **14** to $H_2$ and 10% Pd/C led to the

**Scheme 4**




**Scheme 5**




compound **15** which showed identical chiroptic and spectroscopic data to those of the product previously obtained from **8a**. Therefore, these results along with those previously reported[11] clearly indicate, from a qualitative standpoint, that the presence of an electron-withdrawing group at the $C_3$ position of the β-lactam ring facilitates the $N_1-C_2$ carbon bond cleavage by nitrogen nucleophiles.[23]

The preceding results also demonstrate that both dipeptide and tripeptide units incorporating β,β-disubstituted β-amino acids are directly accessible from β-lactams and α-amino acid derivatives. We then examined the formation of peptide bonds at the hindered *N*-terminus of β,β-branched dipeptides.[24] To this end we again evaluated the acid fluoride methodology developed by Carpino and found that the dipeptide **16** coupled efficiently with Boc-ValF in the presence of *N*-methylmorpholine to give **17** in 80% yield (Scheme 5).

A remaining question related to this coupling methodology concerns the optical integrity of the compounds under the reaction conditions used. To assess this latter aspect the crude products **8a**, **8b**, **9a**, and **10a**, selected as representative examples, were subjected to HPLC analysis using a chiral column. In all cases, judged by the unique absortion signal exhibited on the respective chromatogram, the above-described procedure appeared to be free of racemization. To ensure the validity of this optical purity assay, we have also prepared the corresponding peptides **8a**, **8b**, **8c**, **9a**, and **10a** in their racemic forms by potassium cyanide mediated coupling of each *N*-Boc β-lactam **5**, **6**, and **7** with the corresponding

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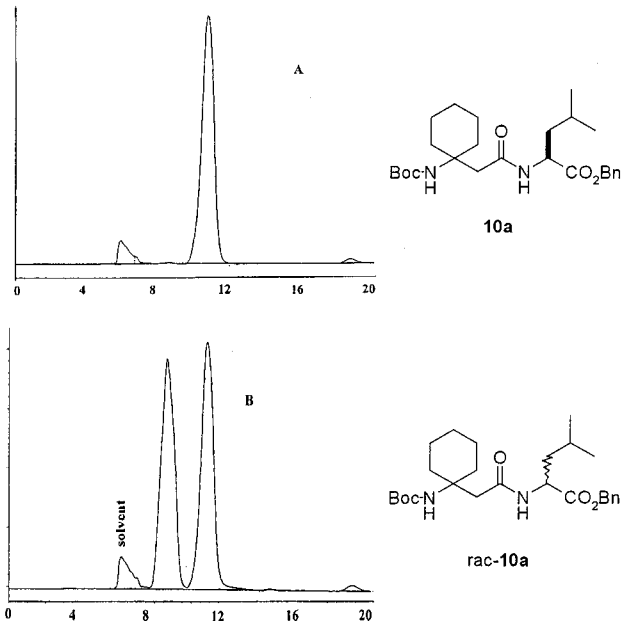


**Figure 1.** Determination of enantiomeric purity of coupling products by chiral HPLC analysis. Examples of chromatograms corresponding to (A) nonracemic **10a** and (B) racemic **10a**.

racemic α-amino acid esters. The resulting crude products were then submitted to HPLC analysis under the same conditions as above. Comparison of each pair of chromatograms corresponding to the nonracemic and racemic samples, as in the example shown in Figure 1, confirmed that no appreciable epimerization had occurred during coupling reactions.[25] It, thus, appears that potassium cyanide facilitates the ring opening of *β*-lactams by generating an acyl cyanide intermediate which then reacts with the corresponding α-amino acid ester under racemization free conditions. Although this intermediate has not been isolated yet, the results presented herein indicate that the procedure should be readily extended to further applications in *β*-lactam-derived *β*-amino acid peptide synthesis.[26] An additional attractive feature of the method is that the overall process involves a very concise way to build-up peptide fragments from alkenes.

## Experimental Section

Melting points were determined with a Büchi SMP-20 capillary apparatus and are uncorrected. Proton nuclear magnetic resonance spectra (200 and 300 MHz) and $^{13}C$ spectra (75 MHz) were recorded at room temperature in $CDCl_3$, unless otherwise stated. All chemical shifts are reported as $\delta$ values (ppm) relative to residual $CHCl_3$ $\delta_H$ (7.26 ppm) and $CDCl_3$ $\delta_C$ (77.0 ppm) as internal standards. Mass spectra (EIMS) were obtained on a Finnigan GCQ mass spectrometer (70 eV) using GC-MS coupling (column: fused silica gel, 15 m, 0.25 mm, 0.25 mm phase SPB-5). Optical rotations were measured at 25 ± 0.2 °C in methylene chloride unless otherwise stated. HPLC analyses were performed on analytical columns (25 cm, phase Lichrosorb-Si60) and (25 cm, phase Chiralcel OD) with flow rates of 1 mL/min and 0.5 mL/min, respectively, using a DAD detector. Flash chromatography was executed with Merck Kiesegel 60 (230−400 Mesh) using mixtures of ethyl acetate and hexane as eluants. Ether was distilled over sodium and benzophenone. Methylene chloride was shaken with concentrated sulfuric acid, dried over potasium carbonate, and distilled. DMF was distilled over BaO. $CH_3CN$ was dried by refluxing over $CaH_2$ and distilled. MeOH was dried over magnessium metal and iodine. Commercially available compounds were used without further purification. Caution! During the workup of the *N*-Boc *β*-lactam opening reaction some quantities of HCN might be produced. A good ventilated hood was used, and the remaining aqueous phases were combined and treated as indicated in *The Sigma-Aldrich Library of Chemical Safety Data*, 2nd ed.; Vol. 2, p 2898B, before wasting.

**General Procedure for the Preparation of *β*-Lactams 2 and 4.** Chlorosulfonyl isocyanate (1.40 g, 9.9 mmol) was added dropwise at 0 °C to a solution of the corresponding alkene (10 mmol) in dry $Et_2O$ (4 mL). The reaction mixture became semisolid with fine needles. The oily residue resulting from the evaporation of the volatiles was dissolved in $Et_2O$ (23 mL) and added dropwise to a mixture of two parts 25% aqueous $Na_2SO_3$ (13 mL) and one part of $Et_2O$. The aqueous phase was kept slightly basic by addition of 10% KOH solution as the reduction proceeded. After 1 h of stirring, the $Et_2O$ phase was separated and dried and the solvent evaporated to give the corresponding *β*-lactam as a colorless oil.

**1-Azaspiro[3.3]heptan-2-one (2)**: yield, 0.80 g (72%). Oil. IR (film) 1767 $cm^{-1}$, 1734 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 6.9−6.5 (bs, 1H), 2.92 (d, 2H, $J$ = 1.46 Hz), 2.5−2.1 (m, 4H), 1.8−1.5 (m, 2H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 167.6, 55.5, 49.8, 33.9, 12.9. EIMS $m/z$ 112 $(M + 1)^+$.

**1-Azaspiro[3.4]octan-2-one (3)**: yield, 0.81 g (65%). Oil. IR (film) 1743 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 6.7−6.5 (bs, 1H), 2.82 (d, 2H, $J$ = 1.7 Hz), 2.0−1.5 (m, 8H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 167.8, 59.5, 48.1, 36.0, 22.9. EIMS $m/z$ 126 $(M + 1)^+$.

**1-Azaspiro[3.5]nonan-2-one (4)**: Yield, 1.15 g (83%). Oil. IR (film) 1748 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 6.9−6.7 (bs, 1H), 2.57 (d, 2H, $J$ = 1.66 Hz), 1.8−1.3 (m, 10H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 168.1, 54.3, 47.5, 36.2, 24.3, 23.5. EIMS $m/z$ 140 $(M + 1)^+$.

**General Procedure for the Preparation of *N*-Boc *β*-Lactams 5, 6, and 7.** $Boc_2O$ (4.32 g, 20 mmol) and DMAP (0.12 g, 1 mmol) were added to a solution of the corresponding azetidin-2-one (10 mmol) in acetonitrile (24 mL) at 0 °C, and the mixture was stirred at room temperature overnight. Then, methylene chloride (25 mL) was added, and the mixture was washed with 1 M $NaHSO_3$ (2 × 25 mL), a saturated solution of $NaHCO_3$ (25 mL), and a saturated solution of NaCl (25 mL). The organic layer was dried over $MgSO_4$, and the solvent was removed in vacuo. Products were purified by column chromatography (silica gel, hexane/EtOAc 5:1) and crystallized from hexane.

***N*-Boc-1-Azaspiro[3.3]heptan-2-one (5).** The general procedure was followed starting from **2** (0.80 g, 7.2 mmol). Yield, 1.22 g (80%). Mp: 61−62 °C. IR (KBr) 1787 (CO), 1710 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 2.99 (s, 2H), 2.87 (ddd, 2H, $J$ = 19.8, 9.9, 2.7 Hz), 2.2−2.0 and 1.9−1.6 (m, 2H), 1.52 (s, 9H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 164.4, 147.7, 82.9, 59.1, 50.0, 31.2, 28.0, 12.9. Anal. Calcd for $C_{11}H_{17}NO_3$ (211.26): C, 62.54; H, 8.11; N, 6.63. Found: C, 62.30; H, 8.09; N, 6.68.

***N*-Boc-1-Azaspiro[3.4]octan-2-one (6).** The general procedure was followed starting from **3** (0.81 g, 6.5 mmol). Yield, 1.02 g (70%). Mp: 64−65 °C. IR (KBr) 1804 (CO), 1713 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 2.87 (s, 2H), 2.4−2.2 (m, 2H), 1.9−1.6 (m, 6H), 1.52 (s, 9H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 164.7, 147.1, 82.4, 65.4, 51.4, 34.4, 27.7, 24.0. Anal. Calcd for $C_{12}H_{19}NO_3$ (225.29): C, 63.98; H, 8.50; N, 6.22. Found: C, 63.70; H, 8.68; N, 6.35.

***N*-Boc-1-Azaspiro[3.5]nonan-2-one (7).** The general procedure was followed starting from **4** (1.15 g, 8.3 mmol). Yield, 1.67 g (84%). Mp: 87−88 °C. IR (KBr) 1814 (CO), 1708 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 2.69 (s, 2H), 2.3−2.0 and 1.9−1.8 (m, 2H), 1.7−1.6 (m, 3H), 1.52 (s, 9H), 1.3−1.1 (m, 3H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 165.1, 147.7, 82.7, 60.9, 47.9, 33.9, 28.0, 24.7,

(25) Chiral HPLC analyses were performed using a 25 cm phase Chiralcel OD analytical column, with a 0.5 mL/min flow of a mixture of ethyl acetate and hexane. In all cases the *R* enantiomer showed a shorter retention time.

(26) For a recent review on *β*-amino acids and their derivatives from *β*-lactams, see: Palomo, C.; Aizpurua, J. M.; Ganboa, I., in ref 9d, p 279.

23.9. Anal. Calcd for $C_{13}H_{21}NO_3$ (239.31): C, 65.25; H, 8.84; N, 5.85. Found: C, 65.62; H, 8.90; N, 6.07.

**General Procedure for the Coupling of *N*-Boc-β-Lactams with α-Amino Acid Esters.** To a solution of the *N*-Boc-β-lactam (1 mmol) in dry DMF were added the corresponding α-amino acid benzyl ester (1.2 mmol) and KCN (1 mmol), and the resulting mixture was stirred under a $N_2$ atmosphere at 40 °C. When no more starting material was observable by TLC, $Et_2O$ (20 mL) was added and the mixture washed with brine (2 × 20 mL), 1 N HCl (20 mL), and $NaHCO_3$ (saturated solution, 20 mL). The organic layer was dried over $MgSO_4$ and filtered, and the solvent was evaporated at reduce pressure to give the corresponding peptide, which was purified by column chromatography.

**8a.** Yield, 90%. Mp: 79−80 °C. $[\alpha]^{25}_D$ −9.23 ($c$ = 1.0, $CH_2Cl_2$). IR (KBr) 1748 (CO), 1679 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 7.35 (s, 5H), 5.94 (d, 1H, $J$ = 5.4 Hz), 5.15 (m, 2H), 5.07 (s, 1H), 4.63 (ddd, 1H, $J$ = 12.3, 5.8, 3.2 Hz), 2.75 (s, 2H), 2.3−2.1 (m, 4H), 2.0−1.7 (m, 2H), 1.7−1.5 (m, 3H), 1.42 (s, 9H), 0.91 (d, 6H, $J$ = 4.2 Hz). $^{13}C$ NMR ($CDCl_3$, $\delta$) 172.7, 170.7, 154.7, 135.4, 128.6, 128.4, 128.2, 79.2, 67.0, 54.9, 50.6, 43.1, 41.4, 33.4, 33.2, 28.4, 24.8, 22.8, 21.8, 14.6. Anal. Calcd for $C_{24}H_{36}N_2O_5$ (432.56): C, 66.64; H, 8.39; N, 6.48. Found: C, 66.84; H, 8.63; N, 6.43.

**8b.** Yield, 83%. Mp: 93−94 °C. $[\alpha]^{25}_D$= −2.4 ($c$ = 1.0, $CH_2Cl_2$). IR (KBr) 1723 (CO), 1691 (CO), 1666 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 7.35 (s, 5H), 6.06 (d, 1H, $J$ = 8.8 Hz), 5.16 (m, 2H), 5.11 (s, 1H), 4.58 (dd, 1H, $J$ = 8.8, 4.8 Hz), 2.78 (s, 2H), 2.3−2.1 (m, 5H), 2.0−1.7 (m, 2H), 1.42 (s, 9H), 0.91 (d, 3H, $J$ = 6.8 Hz), 0.85 (d, 3H, $J$ = 6.8 Hz). $^{13}C$ NMR ($CDCl_3$, $\delta$) 171.6, 170.7, 154.5, 135.1, 128.4, 128.3, 128.2, 79.1, 66.8, 56.7, 54.8, 43.0, 33.1, 33.0, 30.9, 28.2, 18.9, 17.5, 14.5. Anal. Calcd for $C_{23}H_{34}N_2O_5$ (418.53): C, 66.00; H, 8.19; N, 6.69. Found C, 65.68; H, 8.30; N, 6.43.

**8c.** Yield, 66%. Mp: 117−119 °C. IR (KBr) 1743 (CO), 1680 (CO), 1659 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 7.33 (s, 5H), 6.17 (s, 1H), 5.14 (s, 2H), 4.96 (s, 1H), 2.66 (s, 2H), 2.3−2.0 (m, 4H), 1.9−1.7 (m, 2H), 1.51 (s, 6H), 1.41 (s, 9H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 174.0, 170.2, 154.7, 135.7, 128.4, 128.1, 128.1, 79.1, 67.0, 56.1, 55.0, 43.1, 33.3, 28.4, 24.8, 14.6. Anal. Calcd for $C_{22}H_{32}N_2O_5$ (404.50): C, 65.32; H, 7.97; N, 6.92. Found C, 65.09; H, 8.10; N, 7.20.

**8d.** Yield, 76%. Mp: 76−78 °C. $[\alpha]^{25}_D$ = +2.89 ($c$ = 1.0, $CH_2Cl_2$). IR (KBr) 1738 (CO), 1703 (CO), 1674 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 7.4−7.1 (m, 10H), 6.40 (d, 1H, $J$ = 8.2 Hz), 5.3−5.0 (m, 3H), 4.78 (dt, 1H, $J$ = 8.2, 3.0 Hz), 4.45 (m, 2H), 3.91 (dd, 1H, $J$ = 9.4, 3.0 Hz), 3.65 (dd, 1H, $J$ = 9.4, 3.0 Hz), 2.75 (m, 2H), 2.3−2.1 (m, 4H), 2.0−1.7 (m, 2H), 1.40 (s, 9H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 170.7, 169.9, 154.6, 137.2, 135.2, 128.5, 128.4, 128.2, 127.8, 127.6, 79.1, 73.2, 69.6, 67.2, 54.8, 52.4, 43.1, 33.2, 32.8, 28.3, 14.6. Anal. Calcd for $C_{28}H_{36}N_2O_6$ (496.60): C, 67.72; H, 7.31; N, 5.64. Found C, 68.18; H, 7.31; N, 5.75.

**8e.** Yield, 75%. Mp: 86−88 °C. $[\alpha]^{25}_D$ = +3.72 ($c$ = 1.0, $CH_2Cl_2$). IR (KBr) 1753 (CO), 1737 (CO), 1731 (CO), 1674 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 7.4−7.2 (m, 10H), 6.65 (d, 1H, $J$ = 8.2 Hz), 5.16 (bs, 1H), 5.11 (s, 2H), 5.05 (s, 2H), 4.91 (dt, 1H, $J$ = 8.2, 4.7 Hz), 3.05 (dd, 1H, $J$ = 17.0, 4.7 Hz), 2.85 (dd, 1H, $J$ = 17.0, 4.7 Hz), 2.71 (s, 2H), 2.3−2.0 (m, 4H), 2.0−1.7 (m, 2H), 1.43 (s, 9H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 170.5, 170.5, 170.3, 154.5, 135.1, 134.9, 128.5, 128.3, 128.3, 128.2, 128.2, 79.1, 67.4, 66.7, 54.7, 48.3, 43.0, 36.2, 33.0, 32.8, 28.3, 14.5. Anal. Calcd for $C_{29}H_{36}N_2O_7$ (524.61): C, 66.39; H, 6.92; N, 5.34. Found C, 66.86; H, 6.57; N, 5.61.

**9a.** Yield, 80%. Syrup. $[\alpha]^{25}_D$ = −15.22 ($c$ = 1.0, $CH_2Cl_2$). IR ($CH_2Cl_2$) 1736 (CO), 1706 (CO), 1671 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 7.35 (s, 5H), 6.04 (d, 1H, $J$ = 5.4 Hz), 5.15 (m, 2H), 4.69 (s, 1H), 4.61 (ddd, 1H, $J$ = 11.4, 6.0, 3.4 Hz), 2.71 (m, 2H), 2.0−1.5 (m, 11H), 1.43 (s, 9H), 0.91 (d, 6H, $J$ = 5.2 Hz). $^{13}C$ NMR ($CDCl_3$, $\delta$) 172.6, 170.9, 155.2, 135.4, 128.5, 128.3, 128.2, 79.2, 66.9, 62.1, 50.7, 43.6, 41.3, 38.4, 38.1, 28.4, 24.7, 23.1, 23.1, 22.7, 21.8. EIMS $m/z$ $M^+$ 446.

**10a.** Yield, 86%. Syrup. $[\alpha]^{25}_D$ = −11.13 ($c$ = 1.0, $CH_2Cl_2$). IR (NaCl, film) 1736 $cm^{-1}$ (CO), 1706 $cm^{-1}$ (CO), 1669 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 7.30 (s, 5H), 6.13 (d, 1H, $J$ = 7.8 Hz), 5.10 (m, 2H), 4.6−4.4 (m, 1H), 4.5 (s, 1H), 2.62 (m, 2H), 2.01−1.70 (m, 2H), 1.7−1.0 (m, 11H), 1.39 (s, 9H), 0.86 (d, 6H, $J$ = 4.0 Hz). $^{13}C$ NMR ($CDCl_3$, $\delta$) 172.5, 170.3, 154.9, 135.4, 128.4, 128.2, 128.1, 79.1, 66.7, 53.7, 50.6, 44.6, 41.1, 35.2, 35.1, 28.3, 25.3, 24.6, 22.7, 21.7, 21.4. EIMS $m/z$ $M^+$ 460.

**Preparation of 12. Method A.** The β-Lactam **5** (0.5 mmol) was treated with (L)-Val-(L)-ValOMe (0.75 mmol) in DMF under the same conditions described above for coupling reactions. Yield, 83%. Mp: 138−139 °C. $[\alpha]^{25}_D$ = −15.6 ($c$ = 1.0, $CH_2Cl_2$). IR (KBr) 1744 $cm^{-1}$ (CO), 1709 $cm^{-1}$ (CO), 1686 $cm^{-1}$ (CO), 1645 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 6.87 (d, 1H, $J$ = 8.6 Hz), 6.40 (d, 1H, $J$ = 8.2 Hz), 5.56 (s, 1H), 4.47 (dd, 1H, $J$ = 8.6 Hz, 5.2 Hz), 4.29 (dd, 1H, $J$ = 8.2 Hz, 6.8 Hz), 3.69 (s, 3H), 2.74 (m, 2H), 2.4−2.0 (m, 6H), 2.0−1.7 (m, 2H), 1.38 (s, 9H), 1.0−0.8 (m, 12H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 172.3, 171.2, 171.1, 154.7, 79.3, 58.7, 57.13, 55.0, 52.1, 43.2, 33.5, 32.9, 31.1, 30.5, 28.3, 19.2, 18.9, 18.10; 17.9; 14.4. Anal. Calcd for $C_{22}H_{39}N_3O_6$ (441.57): C, 59.84; H, 8.90; N, 9.52. Found C, 60.15; H, 9.11; N, 9.59.

**Method B.** To a solution of peptide **8b** (0.417 g, 1 mmol) in MeOH (10 mL) was added 10% palladium on charcoal (42 mg), and the mixture was kept under hydrogen (1 atm) at room temperature for 18 h. The suspension was then filtered through a pad of Celite, and evaporation of the solvent gave product **11**. Yield, 95%. $^1H$ NMR ($CD_3OD$, $\delta$) 7.0−6.8 (bs, 1H), 6.2−6.0 (bs, 1H), 6.0−5.7 (bs, 1H), 4.3−4.1 (m, 1H), 2.8−2.6 (m, 2H), 2.4−2.0 (m, 5H), 1.9−1.6 (m, 2H), 1.39 (s, 9H), 1.0−1.8 (m, 6H). This crude product was coupled with (L)-ValOMe (0.125 g, 0.95 mmol) by using DCC (0.206 g, 1 mmol) and HOBt (0.128 g, 0.95 mmol) in THF (3 mL) at 0 °C for 1 h and additional 1 h at rt. The reaction mixture was filtered, the solvent evaporated, and the residue redissolved in EtOAc (40 mL) and washed with a saturated solution of $NaHCO_3$ (20 mL), 1 N citric acid (20 mL), saturated $NaHCO_3$ (20 mL), and water (20 mL). The solution was dried over $MgSO_4$ and filtered, and the solvent was evaporated at reduced pressure to give **12**, which was purified by column chromatography. Yield, 0.296 g (70%). All physical and spectroscopic data were identical to those of the material obtained following method A.

**Preparation of 13.** To a solution of $i$Pr$_2$NH (0.08 mL, 0.6 mmol) in dry THF (1 mL) at −78 °C under a nitrogen atmosphere was added 1.6 M BuLi in hexane (0.37 mL, 0.6 mmol), and the solution was stirred for 30 min. β-Lactam **5** (0.106 g, 0.5 mmol) in THF (0.5 mL) was dropped in, and the mixture was stirred at −78 °C for 30 min. A solution of TsCl (0.154 g, 0.8 mmol) in THF (0.5 mL) was added dropwise, and the cold bath was removed to allow the mixture to rise to rt over 1 h. The resulting mixture was partitioned between a saturated solution of $NH_4Cl$ (10 mL) and $Et_2O$ (10 mL), the organic phase was dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue obtained was purified by flash column chromatography (eluant $CH_2Cl_2$/EtOAc 200:1. Yield, 0.754 g (61%). Mp: 67−68 °C. IR (KBr) 1809 (CO), 1720 $cm^{-1}$ (CO). $^1H$ NMR ($CDCl_3$, $\delta$) 4.67 (s, 1H), 3.1−2.8 (m, 1H), 2.8−2.5 (m, 2H), 2.3−2.1 (m, 1H), 2.0−1.6 (m, 2H), 1.55 (s, 9H). $^{13}C$ NMR ($CDCl_3$, $\delta$) 160.9, 147.4, 84.1, 65.2, 64.7, 31.0, 28.0, 26.8, 13.2. Anal. Calcd for $C_{11}H_{16}ClNO_3$ (245.70): C, 53.77; H, 6.56; N, 5.70. Found C, 53.53; H, 6.59; N, 5.75.

**Preparation of 15. Method A:** A solution of **8a** (0.432 g, 1 mmol) in MeOH (10 mL) was kept under $H_2$ in the presence of 0.043 g of Pd on charcoal (10% w/w) at rt until disappearance of the starting material (16 h). The catalyst was then filtered through a pad of Celite and the solvent removed under reduced pressure to give **15** which was crystallized from $Et_2O$ to afford a white foamy solid. Yield, 0.325 g (93%). Mp: 164−165 °C. $[\alpha]^{25}_D$ = −14.5 ($c$ = 1.0, MeOH). IR (KBr) 1729 (CO), 1682 (CO), 1635 $cm^{-1}$ (CO). $^1H$ NMR ($CD_3OD$, $\delta$) 4.5−4.4 (m, 1H), 2.72 (m, 2H), 2.3−2.1 (m, 4H), 1.9−1.6 (m, 5H), 1.44 (s, 9H), 0.96 (d, 3H, $J$ = 6.3 Hz), 0.93 (d, 3H, $J$ = 6.3 HZ). $^{13}C$ NMR ($CD_3OD$, $\delta$) 176.2, 173.2, 156.8, 80.2, 56.3, 52.2, 44.0, 42.0, 34.0, 33.7, 28.9, 26.0, 23.3, 22.0, 15.3. Anal. Calcd for $C_{17}H_{30}N_2O_5$ (342.43): C, 59.63; H, 8.83; N, 8.18. Found C, 60.01; H, 8.93; N, 8.10.

**Method B.** The coupling of **13** (0.246 g, 1 mmol) with (*S*)-LeuOBn was carried out first by the same procedure than that

used for coupling of 3-unsubstituted $\beta$-lactams, except the reaction was conducted at room temperature. Yield of the 1:1 epimeric mixture of **14** after column chromatography was 0.388 g (83%). To a solution of chloride **14** (0.388 g, 0.83 mmol) in MeOH (10 mL) were added $Et_3N$ (0.210 g, 2.1 mmol) and 10% Pd/C, and the resulting mixture was kept under $H_2$ at rt until the starting material was consumed (24 h). Filtration of the catalyst through a pad of Celite and evaporation of the solvent gave an oil which was partitioned between $CH_2Cl_2$ and 0.1 N HCl. The organic layer was separated and dried over $MgSO_4$, and the solvent was evaporated to afford **15** which exhibited identical physical and spectroscopic data to those of the product obtained by the method A. Yield, 0.145 g (51%).

**Preparation of 17.** To a solution of peptide **8a** (0.143 g, 0.33 mmol) in $CH_2Cl_2$ (2 mL) was added $CF_3CO_2H$ (1.0 mL, 13 mmol), and the resulting solution was stirred at rt for 1.5 h. After evaporation of all the volatiles, the resulting residue was dissolved in $CH_2Cl_2$ (5 mL), the solution was washed with $NaHCO_3$ (saturated, 4 mL), the organic layer was dried over $MgSO_4$, and the solvent was evaporated under reduce pressure to afford **16**. Yield, 0.075 g (70%). To crude **16** in dry $CH_2Cl_2$ (1 mL) were added a solution of *N*-Boc-Val-F (0.053 g, 0.24 mmol) in $CH_2Cl_2$ (0.5 mL) and *N*-methylmorpholine (0.04 mL, 0.33 mmol). After 2 h of stirring at rt, additional $CH_2Cl_2$ (1 mL) was added and the resulting solution washed with $H_2O$ (1 mL), 1 N HCl (1 mL), and $NaHCO_3$ (saturated, 1 mL). The organic layer was dried over $MgSO_4$ and the solvent evaporated under reduced pressure. Yield, 0.079 g (70%). Syrup. $[\alpha]^{25}_D = +27.6$ ($c = 1.0$, $CH_2Cl_2$). IR (KBr) 1742 (CO), 1721 (CO), 1651 (CO). $^1$H NMR ($CDCl_3$, $\delta$) 7.34 (s, 5H), 7.00 (d, 1H, $J = 8.6$ Hz), 6.85 (s, 1H), 5.15 (m, 2H), 5.06 (d, 1H, $J = 8.0$ Hz), 4.8−4.6 (m, 1H), 3.6−3.5 (m, 1H), 2.84 (d, 1H, $J = 12.8$ Hz), 2.50 (d, 1H, $J = 12.8$ Hz), 2.5−2.3 (m, 2H), 2.3−2.1 (m, 1H), 2.1−1.8 (m, 4H), 1.8−1.5 (m, 3H), 1.41 (s, 9H), 0.97 (m, 6H), 0.89 (d, 6H, $J = 5.7$ Hz). $^{13}$C NMR ($CDCl_3$, $\delta$) 174.4, 171.3, 171.1, 156.6, 135.4, 128.5, 128.3, 128.0, 79.9, 67.1, 61.0, 56.2, 50.9, 43.5, 39.4, 33.9, 31.2, 30.1, 28.2, 24.8, 22.9, 21.1, 19.5, 18.7, 15.4. HRMS (FAB) *m*/*z* calcd for $C_{29}H_{46}N_3O_6$ (M + H) 532.3387, found 532.3388.

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