Solution-Phase Synthesis of a Hindered *N*-Methylated Tetrapeptide Using Bts-Protected Amino Acid Chlorides: Efficient Coupling and Methylation Steps Allow Purification by Extraction

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N-Benzothiazole-2-sulfonyl (Bts)-protected amino acid chlorides were used to prepare the hindered cyclosporin 8−11 tetrapeptide subunit **1**. The synthesis was performed via **3a** and the deprotected amines **5a**, **13**, and **19**, including three repeated cycles involving *N*-methylation using iodomethane/potassium carbonate, deprotection of the Bts group, and *N*-acylation with a *N*-Bts-amino acid chloride such as **9b** or **9c**. Among three Bts cleavage methods compared (H_3PO_2/THF; $NaBH_4$/EtOH; PhSH/K_2CO_3), the third gave somewhat higher overall yields. *N*-Acylation of **5a** with the Bts-protected *N*-methylamino acid chloride **10b** followed by deprotection was also highly efficient and could be used as an alternative route to **11**. Each of the deprotected amines was isolated without chromatography using simple extraction methods to remove neutral byproducts. The tetrapeptide **1** was obtained in analytically pure form as the monohydrate.

Optimized methods for peptide synthesis are now available that allow the coupling of hindered *N*-methylamino acids with minimal racemization. For example, Akaji et al. reported that treatment of Cbz-MeLeu-OH with 2-chloro-1,3-dimethyl-2-imidazolinium hexafluorophosphate (CIP), 1-hydroxy-7-azabenzotriazole (HOAt), and MeVal-OMe affords the hindered dipeptide Cbz-MeLeu-MeVal-OMe with <1.5% of the epimeric dipeptide derived from racemization.[1] In earlier work, similar results were reported by Wenger et al..[2] or Rich et al.[3a] using a mixed pivaloyl anhydride or the BOP-Cl coupling agent, respectively, for the synthesis of analogous dipeptides. These workers also described further conversion to cyclosporin via a sequence of difficult coupling reactions involving several additional *N*-methylamino acid subunits. Chromatographic purification was typically used after the coupling reactions. Alternatively, synthesis on a solid support could be employed to control product purity.[3b]

We have been interested in the use of highly reactive aminoacyl chlorides protected at nitrogen by the Bts (benzothiazole-2-sulfonyl) group as reagents for the synthesis of hindered peptides.[4] Because these reagents require no organic additives to effect peptide coupling, product purification should be relatively easy. Furthermore, the Bts-protected peptides should be subject to *N*-methylation under conditions similar to those used in "site specific methylation".[5] The latter process utilizes an *N*-arylsulfonyl amino acid for peptide synthesis and exploits that greater acidity of the resulting $ArSO_2NH$ subunit to effect selective *N*-methylation in the presence of amide NH. In this way, a difficult peptide coupling step can be performed using the $ArSO_2NH$-containing amino acid, and the *N*-methyl group can be introduced later. Site-selective *N*-methylation has been demonstrated in solid-phase peptide synthesis applications, including the synthesis of cyclosporin derivatives.[3b] Analogous solution-phase *N*-alkylations of *N*-arylsulfonylamino acid derivatives have also been reported,[6] and the corresponding reactions of *o*-nitrobenzenesulfonamides have been used for the synthesis of *N*,*N*-dialkylamines.[7,8]

The purpose of the work described below is to determine whether Bts-protected amino acid chlorides would allow practical synthesis of hindered peptides containing *N*-methylamino acid subunits using solution-phase methodology. In particular, it was hoped that highly efficient peptide coupling, *N*-alkylation, and deprotection steps would allow purification of the product peptides using simple extraction methods instead of chromatography or solid-phase techniques. The studies outlined below demonstrate the feasibility of this approach in a synthesis of the hindered cyclosporin 8−11 tetrapeptide subunit **1** (D-Ala-MeLeu-MeLeu-MeVal-*O*-*tert*-Bu). The corresponding FMOC-protected tetrapeptide has been used as an intermediate in the synthesis of cyclosporin A by Rich et al.[3]

Results and Discussion

The starting Bts-protected amino acids **2** were prepared from Bts-Cl and the corresponding amino acid using two-phase conditions with aqueous NaOH to

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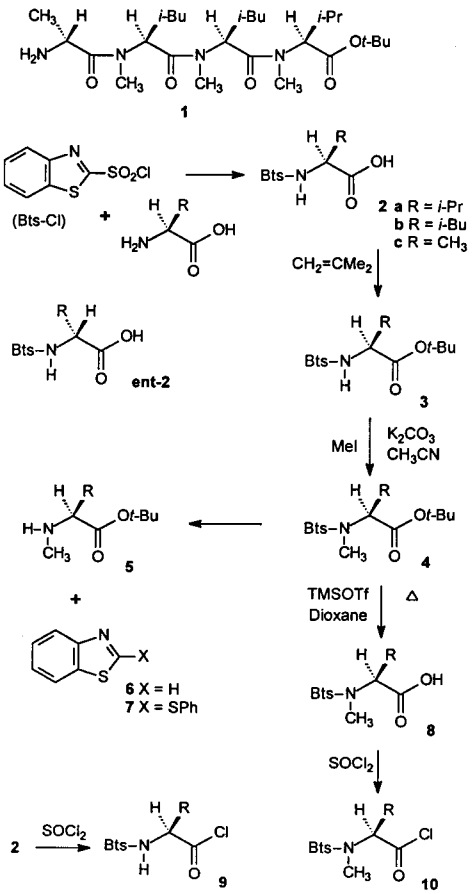
Scheme 1



maintain pH 10−10.5.[4] This procedure consistently gave 87−91% of crystallized **2a**−**c** (Scheme 1). The enantiomeric protected amino acids *ent*-**2a**−**c** were made in the same way. Treatment of **2a** or **2b** with isobutylene in the presence of acid catalyst gave **3a** or **3b** in high yield, and subsequent reaction with iodomethane/K_2CO_3 afforded the *N*-methylated products **4a** or **4b**. The *N*-methylation step occurred in excellent yields in polar aprotic solvents including DMF, propionitrile, or acetonitrile. Acetonitrile was most convenient in terms of yield and product quality and was adopted in the standard procedure for this and subsequent *N*-methylations. Nonpolar solvents such as dichloromethane or toluene were relatively ineffective, and the rate of *N*-methylation was considerably slower. Compared to other sulfonamide *N*-methylations, the K_2CO_3/MeI/CH_3CN procedure reported here for the Bts-protected amino acids has advantages in the simplicity of reagents and the high (>95%) yield. Indeed, the *N*-methylation consistently gave complete conversion within the limits of careful NMR and TLC assay despite the heterogeneous reaction conditions (suspension of K_2CO_3 in acetonitrile).

In an earlier report, cleavage of the *N*-Bts protecting group was achieved using reducing agents such as Al−Hg, Zn, or H_3PO_2.[4] The latter procedure appeared better suited for the acid/base extraction scheme that was planned for product purification, so it received the initial focus. However, the *N*-methylated Bts derivative **4a** proved less reactive compared to the Bts-NH analogues studied earlier,[4] and the deprotection step was more sensitive to concentration effects. The conversion of **4a** to **5a** required slow addition of a large excess (>20 equiv) of 50% aqueous H_3PO_2 to a dilute solution of **4a** in THF. The best result (95% conversion to **5a** and **6**) was achieved at an initial concentration of 0.02 M for the substrate **4a**, while higher concentrations resulted in progressively lower conversions (0.05 M, 90%; 0.3 M, 77%; 0.8 M, 43%).

Subsequent experiments with *N*-methylated dipeptides also encountered concentration effects and somewhat lower conversions with H_3PO_2, so alternative methods for *N*-Bts cleavage were evaluated at the stage of **4a**. By far the simplest procedure was the reductive cleavage using $NaBH_4$/ethanol. This reaction was complete within 3h at room temperature and gave the same yield of **5a** as in the best H_3PO_2 experiment. The $NaBH_4$ method was also effective in several other applications involving the cleavage of *N*-methylated *N*-Bts analogues. However, the unmethylated Bts-dipeptide **12** gave only 35% of the deprotected dipeptide using this technique, apparently due to competing deprotonation of the acidic Bts-N−H subunit.

Deprotection of **4a** was also examined under Fukuyama's conditions for cleavage of *N*-(*o*-nitrophenylsulfonyl) protecting groups.[7] Thus, **4a** was stirred with benzenethiol/K_2CO_3 in DMF to afford **5a** (92%) and **7**. All three deprotection methods were comparable in terms of yield with **4a** as the substrate. On the other hand, the PhSH/K_2CO_3 technique proved more reliable with other substrates and cleaved methylated as well as unmethylated *N*-Bts peptides (see below). Wuts has reported a similar procedure for the deprotection of a variety of *N*-Bts amines using PhSH/DMF and diisopropylamine or K*O*-*tert*-butoxide as the base.[9]

With several alternatives established for nitrogen deprotection, the issue of peptide coupling could be addressed. Brief heating of the *N*-Bts amino acids **2** or **8** with thionyl chloride in CH_2Cl_2 gave the acid chlorides **9** and **10**, respectively. Excellent results in peptide coupling were obtained using crude **9** or **10** after evaporation of the thionyl chloride.

The option of coupling the *N*-methylamino ester **5a** with **10b** was investigated initially (Scheme 2). Excellent (>98%) yields of **11** resulted using the Schotten−Bauman procedure at 0° in water−dichloromethane (30 min; Na_2CO_3−$NaHCO_3$). Clearly, the high reactivity of the acid chloride **10b** is more than sufficient to overcome steric hindrance by the *N*-methyl substituent in **5a** in the coupling step. On the other hand, significant work was required to prepare **10b** because of the need to protect the carboxylic acid prior to *N*-methylation,[10] four steps overall from **2b**. We therefore explored the alternative where **9b** is used in the peptide-coupling step, and *N*-methylation is performed subsequently.

As expected, treatment of **5a** with the acid chloride **9b** proceeded smoothly to afford **12**, and subsequent methylation using the MeI/K_2CO_3−CH_3CN conditions afforded **11** in nearly quantitative yield. For convenience, a similar strategy was used in subsequent steps as a way to avoid the oxygen protection; deprotection required for preparation of **10**. However, the direct coupling of **5a** with **10b** is high yielding and either approach affords **11** with excellent purity.

Deprotection of the dipeptide **11** was investigated using the most promising conditions that had been established

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(10) Reaction of **2a** with $TMSCHN_2$ gave faster *O*-methylation compared to *N*-methylation.

Scheme 2

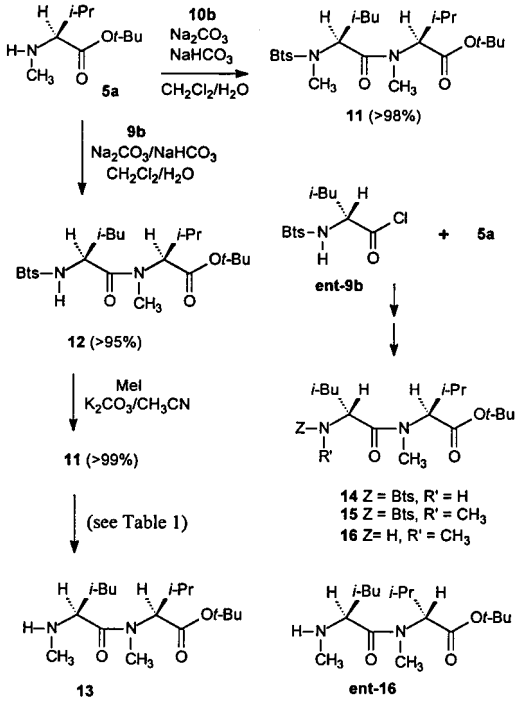


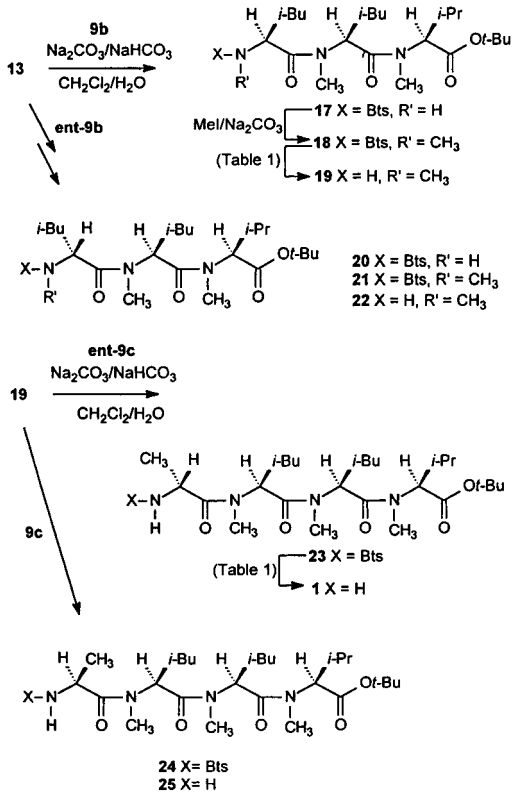
Table 1. Deprotection of *N*-Bts Peptides (% yield based on *N*-Bts precursor)

Bts-peptide	product[a]	H_3PO_2[b]	$NaBH_4$[c]	$PhSH/K_2CO_3$[d]
4a	**5a**	91	93	92
11	**13**	91	97	95
18	**19**	83	89	99
23	**1**	82	*e*	95

[a] Peptide isolated by acid extraction, neutralization, and extraction with HPLC grade dichloromethane. [b] Slow addition of excess aqueous 50% H_3PO_2 to a 0.025 M solution of the peptide in refluxing THF, 3 h. [c] 5 equiv of $NaBH_4$ in ethanol, 0 °C to room temperature, 1−3 h. [d] 2−3 equiv of PhSH, 3 equiv K_2CO_3, DMF, 3−5 h at room temperature. [e] Not attempted due to the presence of acidic Bts-NH.

with the simpler substrate **4a**. As summarized in Table 1, the H_3PO_2 method gave **13** in 91% yield. However, this result required dilute conditions (0.02 M in **11**), while similar experiments using 0.08 or 0.12 M **11** afforded **13** in 80% and 70% yield, respectively, together with recovered starting material **11**. By comparison, the $NaBH_4$/EtOH procedure was much better in terms of conversion and convenience and produced **13** in 97% yield. The $PhSH/K_2CO_3$ method also worked well (95% of **13** isolated), and all three methods gave product of excellent quality after acid/base extraction using HPLC grade solvents during workup. No extraneous signals were detected in NMR spectra of **13**, but the presence of *E*/*Z* amide isomers in this and related *N*-methyl peptides resulted in a relatively complex spectrum. Because the purity of **13** after acid−base extraction is critical for the intended application, additional assays were therefore performed, and the dipeptide diastereomer **16** was prepared for comparison. The latter was made by a similar sequence from **5a** and *ent*-**9b** to give **14** followed by *N*-methylation and deprotection with $PhSH/K_2CO_3$. No cross-contamination between diastereomers **13** and **16** or **12** and **14** could be detected by NMR or HPLC methods (>99.5% ds). Thus, the acid chloride **9b** couples without measurable racemization, as in the analogous N−H series using **9a**.[4] To test for epimerization via reversible

Scheme 3



enolization in the *N*-methylvaline subunit, an authentic sample of *ent*-**16** was also prepared starting from *ent*-**2a** via *ent*-**14** and *ent*-**15**. Both **16** and *ent*-**16** were obtained with >99.5% ee and ds according to HPLC assay on a chiral solid phase. Therefore, each amino acid subunit survives coupling, *N*-methylation, and deprotection without change of α-carbon configuration at the dipeptide stage.

The same sequence of peptide coupling; *N*-methylation was repeated to introduce a second *N*-methylleucine subunit (Scheme 3). Deprotected dipeptide **13** was used with no purification beyond the acid/base extraction, and the coupling and *N*-methylation steps were carried out according to the standard procedures. The resulting Bts-protected tripeptide **18** was subjected to the three techniques for deprotection as described earlier. In this case, the $PhSH/K_2CO_3$ procedure was significantly better than the $NaBH_4$/EtOH or H_3PO_2 alternatives for the synthesis of **19** (Table 1). Comparisons with independently prepared epimer **22** (from **13** and *ent*-**9b** via **20** and **21**) confirmed retention of leucine configuration in **19** (>99.5% ds).

To complete the synthesis of the cyclosporin 8−11 tetrapeptide, the last coupling reaction requires the incorporation of a D-alanine subunit (Scheme 3). The corresponding Bts-protected acid chloride *ent*-**9c** was prepared in the usual way and was coupled with **19** to give **23**. Deprotection to give the desired **1**[11] proceeded smoothly with the $PhSH/K_2CO_3$ reagent and could also be performed with H_3PO_2 under dilute conditions (Table 1). However, the $NaBH_4$/EtOH was not tried in this case due to the presence of the acidic Bts-NH subunit as discussed earlier.

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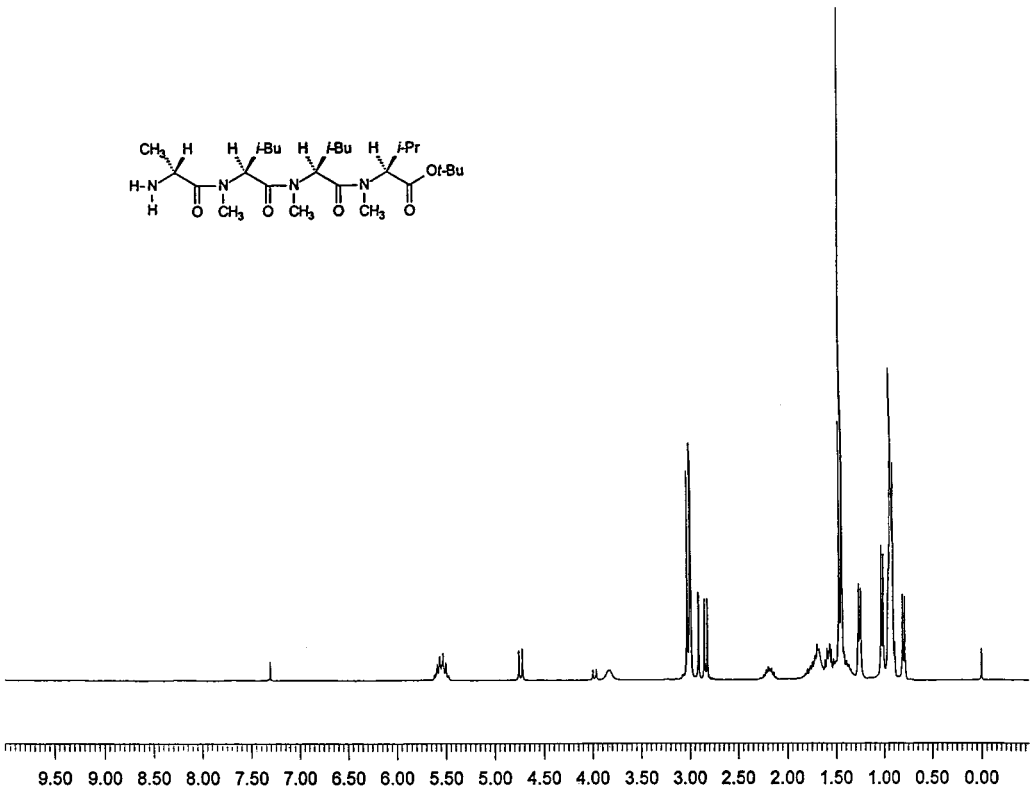


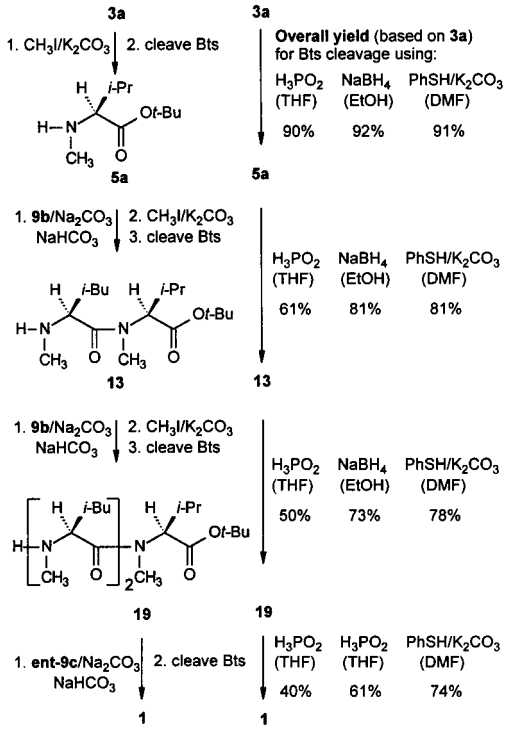
Figure 1. ^{1}H NMR spectrum of **1** after acid/base extraction.

The final sample of **1** was obtained using the same acid/base extraction method as in the case of intermediates **13** and **19**. An authentic sample of the epimer **25** was also prepared from **19** via **24**. As in all the previous examples, there was no cross-contamination of epimers (>99.5% ds according to HPLC assay), and the spectra of the final products indicated excellent purity. In particular, only two doublets for the characteristic valine α-proton were found between 4−5 ppm, suggesting major and minor rotamers (3:1 ratio) at the amide linkage (Figure 1). A similar pair of doublets was seen at the dipeptide stage (**13**; 2:1 ratio of *E*/*Z* isomers).

Despite 10 linear steps from **2a** to **1** without chromatography or crystallization, the NMR spectrum of the final product **1** contained no discernible extraneous signals. However, the integral of the complex region between 1.2−2.0 ppm indicated the presence of ca. 2 additional protons unless the sample was dried (molecular sieves). Elemental analysis was therefore performed on a deprotected sample after drying at 40 °C, but the analysis matched a monohydrate of **1** within 0.3% for C, H, and N.

For further comparison of the three deprotection methods, the synthetic sequence from **2a** to **1** was performed as a linear stepwise process using the same deprotection method at each stage where possible. The results are summarized in Scheme 4 and the yield numbers are given at each of the four deprotected peptide stages. These yields are known with better precision than the yields for each deprotection step because the deprotected peptides were purified by acid/base extraction to remove neutral impurities, while the precursor *N*-Bts peptides were used as the crude products from the *N*-methylation step. Overall yields are given at each stage based on **3a**. It is apparent that the PhSH/K_2CO_3 method is the best overall. The $NaBH_4$/EtOH technique is also

Scheme 4



effective in cases where the acidic Bts-NH is absent. The overall yield of 61% in the $NaBH_4$ column was obtained using the H_3PO_2 reagent for cleavage of **19** to **1** at the tetrapeptide stage.

One final optimization was performed in an attempt to avoid the solvent change from acetonitrile in the *N*-methylation to DMF in the BTS deprotection step. Acetonitrile proved to be less effective than DMF for the BTS cleavage using PhSH/K_2CO_3, resulting in a consid-

erably slower reaction. On the other hand, DMF could be substituted for acetonitrile in the *N*-methylation. The *N*-methylation and deprotection steps could be carried out in one pot, provided that the excess iodomethane was removed prior to addition of PhSH. This variation of Scheme 4 (combine steps 2 and 3 using DMF; MeI/K_2CO_3 followed by PhSH/K_2CO_3 deprotection) was more convenient and gave deprotected peptides **13**, **19**, and **1** with indistinguishable NMR spectra compared to the original stepwise sequence. The overall yield of **1** was 80% compared with 76% in the stepwise procedure shown in Scheme 4.

Summary

Peptide coupling using the highly reactive Bts-protected acid chlorides is demonstrated with *N*-methylamino acid-containing substrates. Hindered *N*-methyl-*N*-Bts amino acid chlorides such as **10b** react well with *tert*-butyl *N*-methylvalinate **4a**, but it is easier to use the unmethylated acid chloride **9b**, followed by *N*-methylation at the stage of the dipeptide **12**. By repetition of the coupling, *N*-methylation, and Bts cleavage steps, it is possible to assemble the cyclosporin 8−11 tetrapeptide **1** in 74−80% overall yield. Simple solution-phase coupling and *N*-methylation procedures afford the final product with excellent purity after 10 linear steps without chromatography or crystallization. If desired, the *N*-methylation and deprotection steps can be combined into one operation by carrying out both reactions in DMF.

Depending on the deprotection method, benzothiazole **6** or the corresponding sulfide **7** is formed as the byproduct of Bts cleavage, but the neutral heterocycle was easily removed by acid/base extraction. Products derived from incomplete peptide coupling or incomplete *N*-methylation were not detected by HPLC or NMR at the stage of the deprotected peptides. Furthermore, epimeric peptides were not formed above the HPLC detection limits (0.25%) according to comparisons with authentic material prepared at each coupling stage.

The reactivity advantages of conventionally protected amino acid chlorides for peptide synthesis are well-known from the work of Carpino et al.,[12] but the Bts-protected analogues **9** or **10** have additional benefits. The acid chlorides are formed cleanly using the standard $SOCl_2$ procedure from the carboxylic acid, and no organic additives are needed to prepare or to couple **9** or **10** to the peptide *N*-terminus. Indeed, the entire 10-step synthesis of **1**[11] uses no organic reagents other than the solvents, the amino acid-derived reactants, and the iodomethane that is required to introduce essential *N*-methyl groups. Purity of the peptide therefore depends on the efficiency of coupling and *N*-methylation steps and on the extraction process. After deprotection, any unmethylated H_2N-peptide would be extracted along with the *N*-methylated product because of the similar acid solubility, but this problem was not encountered. The overall effectiveness of the technique is underscored by the NMR spectrum of the final product **1** after extraction (Figure 1), and by spectra of similar quality at each deprotection stage.

The combination of highly efficient bond-forming steps and solubility-based purification techniques has considerable potential for repetitive reaction cycles involving amines. A similar concept has been described for solution-phase combinatorial synthesis of amide libraries by Boger et al. using conventional *N*-protection strategy and standard coupling reagents, and products of good quality were obtained over a route of six linear steps.[13] In the present work, a related approach is used in the context of peptide synthesis to prepare the cyclosporin tetrapeptide **1** in a sequence of 10 steps, including three repeated cycles of *N*-methylation, deprotection, and peptide coupling. Further studies designed to probe the limits of the Bts-mediated approach to peptide synthesis in solution phase will be reported in due course.

Experimental Section

General. Dichloromethane and acetonitrile were distilled from P_2O_5. Dioxane was distilled from Na/benzophenone ketyl. Toluene, diisopropylethylamine, triethylamine, and dimethylformamide were distilled from CaH_2 and were stored over 4 Å molecular sieves.

Benzothiazole-2-sulfonyl Chloride (Bts-Cl). To a magnetically stirred solution of 33% aqueous acetic acid (60 mL) cooled to 0−5 °C (ice−salt bath; internal thermometer) was introduced Cl_2 gas. After a yellow precipitate formed, a suspension of powdered 2-mercaptobenzothiazole (10.0 g, 60 mmol) in 33% aqueous acetic acid (60 mL) was added over 2 h while the internal temperature was maintained between 0 and 5 °C. A large excess of Cl_2 gas is crucial for minimizing product contamination with partially converted intermediates. The yellow suspension was stirred at 0 °C for an additional 15 min, and the reaction mixture was then suction-filtered through an ice-cooled funnel. The precipitate was washed with ice−water (100 mL) and dissolved in cold CH_2Cl_2 (100 mL). The resulting CH_2Cl_2 solution was washed successively with cold saturated aqueous $NaHCO_3$ (100 mL) and cold brine (100 mL). The dichloromethane layer was then dried over Na_2SO_4 at −10 °C for 1 h, filtered through Celite, and concentrated under diminished pressure until a thick slurry was formed. Dry ether (20 mL) was added, and the suspension was cooled in a dry ice−acetone bath and then filtered by suction. The fluffy solid was washed with cold ether (10 mL) and dried under vacuum for 10 min before storage in the freezer. The benzothiazole-2-sulfonyl chloride (Bts-Cl) was obtained as an off-pink fluffy solid (8.0 g, 57%): mp 105−108 °C dec (lit. mp 108−110 °C;[14a] 101−105 °C[14b]); 300 MHz NMR ($CDCl_3$, ppm) δ 8.35−8.26 (1H, m) 8.10−8.01 (1H, m) 7.76−7.66 (2H, m). A highly crystalline sample of Bts-Cl can be stored in the freezer for a few months. It slowly decomposes by the extrusion of sulfur dioxide. The decomposition of crystalline Bts-Cl occurs within 1−2 days at room temperature. Solutions in $CHCl_3$ are increasingly unstable as the concentration increases. Thus, a 0.1 M solution in $CDCl_3$ survived for weeks at room temperature with <5% change in NMR signal ratios, but a 1 M solution was >95% decomposed after 3 days. Stability of the 1 M solution in $CHCl_3$ was improved upon addition of 1% BHT (2,6-di-*tert*-butyl-4-methylphenol), 6% decomposition after 8 days.

General Procedure for the Preparation of *N*-Benzothiazole-2-sulfonyl-Protected Amino Acids. An amino acid hydrochloride (3.20 mmol) was dissolved in 0.25 M aqueous NaOH (11 mL, 2.8 mmol) at 10 °C. Solid Bts-Cl (1.10 g, 4.72 mmol) was added in one portion, and the suspension was stirred for 10 h at 10 °C. The reaction was monitored using a pH meter and was maintained at pH = 10−10.5 by addition of 1.3 M aqueous NaOH (total 5 mL, 6.5 mmol) as needed. The cloudy solution was extracted with ether (10 mL) to remove organic soluble materials. The aqueous phase was then

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acidified to pH 1 with concentrated HCl and was extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were dried under Na_2SO_4, evaporated, and crystallized from $CHCl_3$/hexane. This general procedure was used to prepare benzothiazole-2-sulfonyl derivatives of amino acids as listed below.

***N*-Bts-(*S*)-Valine (2a).** (*S*)-Valine (0.375 g, 3.20 mmol) gave *N*-Bts-(*S*)-valine (**2a**) (0.919 g, 91%): analytical TLC on silica gel, 10:20:1 hexane/EtOAc/HOAc, R_f = 0.65. Pure material was obtained by crystallization from chloroform/hexane: mp 100−101 °C; $[\alpha]^{25}_D$ +29.2 (*c* = 2.3, EtOH); IR (KBr, cm^{-1}) 3200, N−H; 1714, C=O; 300 MHz NMR (CD_3CN, ppm) δ 8.13−8.08 (2H, m) 7.67−7.57 (2H, m) 6.58 (1H, br s) 4.09 (1H, br d, *J* = 4.5 Hz) 2.21−2.08 (1H, m) 0.96 (3H, d, *J* = 7.2 Hz) 0.84 (3H, d, *J* = 6.9 Hz). *N*-Bts-(*R*)-Valine (*ent*-**2a**) was prepared in the same way: mp 100.5−101.5 °C; $[\alpha]^{25}_D$ −28.3 (*c* = 2.3, EtOH). No parent ion for $C_{12}H_{14}N_2O_4S_2$; M + 1, 315.0500, error = 8 ppm; base peak = 135 amu.

***N*-Bts-(*S*)-Leucine (2b).** (*S*)-Leucine (0.419 g, 3.20 mmol) gave *N*-Bts-(*S*)-leucine (**2b**) (0.917 g, 87%): analytical TLC on silica gel, 10:20:1 hexane/EtOAc/HOAc, R_f = 0.54. Pure material was obtained by crystallization from chloroform/hexane: mp 146−147 °C; $[\alpha]^{25}_D$ +11.7 (*c* = 2.3, EtOH). No parent ion for $C_{13}H_{16}N_2O_4S_2$; M + 1, 329.0636, error = 2 ppm; base peak = 135 amu; IR (KBr, cm^{-1}) 3245, N−H; 1735, C=O; 300 MHz NMR (CD_3CN, ppm) δ 8.15−8.08 (2H, m) 7.69−7.57 (2H, m) 6.66 (1H, br s) 4.18 (1H, dd, *J* = 7.2, 7.2 Hz) 1.79−1.63 (1H, m) 1.55 (2H, dd, *J* = 7.2, 6.9 Hz) 0.89 (3H, d, *J* = 6.6 Hz) 0.84 (3H, d, *J* = 6.3 Hz). *N*-Bts-(*R*)-Leucine (*ent*-**2b**) was prepared in the same way: mp 147−148 °C; $[\alpha]^{25}_D$ −11.3 (*c* = 2.3, EtOH).

***N*-Bts-(*S*)-Alanine (2c).** (*S*)-Alanine (0.248 g, 3.20 mmol) was employed to provided *N*-Bts-(*S*)-alanine (**2c**) (0.815 g, 89%): analytical TLC on silica gel, 10:20:1 hexane/EtOAc/HOAc, R_f = 0.27. Pure material was obtained by crystallization from ethyl acetate/hexane: mp 142−143 °C; $[\alpha]^{25}_D$ −3.1 (*c* = 3.5, EtOH); IR (KBr, cm^{-1}) 3213, N−H; 1725, C=O; 300 MHz NMR (CD_3CN, ppm) δ 8.17−8.08 (2H, m) 7.69−7.57 (2H, m) 6.55 (1H, br s) 4.28 (1H, q, *J* = 7.2 Hz) 1.39 (3H, d, *J* = 7.5 Hz). *N*-Bts-(*R*)-Alanine (*ent*-**2c**) was prepared in the same way: mp 143−144 °C; $[\alpha]^{25}_D$ +3.1 (*c* = 3.5, EtOH).

General Procedure for the Preparation of *tert*-Butyl *N*-Benzothiazole-2-sulfonyl-Protected Amino Acid Esters. Into a stoppered thick-walled round-bottom flask, charged with *N*-Bts-protected amino acid (1.0 mmol), dry CH_2Cl_2 (3.0 mL) were introduced 5 drops of concentrated H_2SO_4 and isobutylene gas at −78 °C to make the increase in volume approximately 5 mL. The container was stoppered with a septum secured by wire and stirred vigorously for 4 days at ambient temperature. After the flask was cooled in an ice-bath, the internal pressure was released by puncturing the septum with a needle. The reaction mixture was then poured into a solution of saturated $NaHCO_3$ (10 mL) and extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with brine (10 mL), dried over Na_2SO_4, and concentrated (aspirator). Pure materials were obtained by crystallization from ethyl acetate/hexane.

***N*-Bts-(*S*)-Valine-*O*-*tert*-Bu (3a).** *N*-Bts-(*S*)-Valine (**2a**) (0.320 g, 1.0 mmol) was employed to provide *N*-Bts-(*S*)-valine-*O*-*tert*-Bu (**3a**) (0.329 g, 89%): analytical TLC on silica gel, 7:3 hexane/EtOAc, R_f = 0.39. Pure material was obtained by crystallization from ethyl acetate/hexane: mp 119−120 °C; $[\alpha]^{25}_D$ +62.6 (*c* = 2.3, $CHCl_3$). HRMS, M + 1, 371.1081, error = 5 ppm; IR (KBr, cm^{-1}) 3258, N−H; 1731, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.16−8.11 (1H, m) 7.98−7.94 (1H, m) 7.62−7.51 (2H, m) 5.61 (1H, d, *J* = 9.6 Hz) 4.18 (1H, dd, *J* = 9.0, 4.5 Hz) 2.26−2.14 (1H, m) 1.20 (9H, s) 1.07 (3H, d, *J* = 6.6 Hz) 0.88 (3H, d, *J* = 7.2 Hz). *N*-Bts-(*R*)-Valine-*O*-*tert*-Bu (*ent*-**3a**) was prepared in the same way: mp 119.5−120 °C; $[\alpha]^{25}_D$ −61.9 (*c* = 2.3, $CHCl_3$).

***N*-Bts-(*S*)-Leucine-O-*tert*-Bu (3b).** *N*-Bts-(*S*)-Leucine (**2b**) (0.328 g, 1.0 mmol) was employed to provide *N*-Bts-(*S*)-leucine-O-*tert*-Bu (**3b**) (0.372 g, 97%): analytical TLC on silica gel, 4:1 hexane/EtOAc, R_f = 0.25. Pure material was obtained by crystallization from CH_2Cl_2/hexane, mp 97−98 °C; $[\alpha]^{25}_D$ +50.6 (*c* = 1.04, $CHCl_3$). HRMS, M + 1, 385.1244, error = 3 ppm; base peak = 283 amu; IR (KBr, cm^{-1}) 3284, N−H; 1719, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.17−8.12 (1H, m) 7.99−7.94 (1H, m) 7.62−7.51 (2H, m) 5.50 (1H, d, *J* = 9.0 Hz) 4.28 (1H, ddd, *J* = 9.0, 9.0, 5.7 Hz) 1.98−1.83 (1H, m) 1.64−1.47 (2H, m) 1.21 (9H, s) 0.99 (3H, d, *J* = 6.0 Hz) 0.96 (3H, d, *J* = 6.6 Hz).

General Procedure A for *N*-Methylation of *tert*-Butyl *N*-Bts-Protected Amino Acid Esters and Peptides. To a suspension of *N*-Bts-protected amino acid or peptide and K_2CO_3 (4 equiv) in dry CH_3CN (ca. 0.17 M) under N_2 was added excess iodomethane (10 equiv) in one portion at room temperature. The reaction mixture was brought to 35 °C for ca. 3 h and was monitored by TLC. After conversion was complete, the mixture was diluted with HPLC grade CH_2Cl_2 (10 mL) and water (10 mL). The aqueous layer was separated and extracted with HPLC grade CH_2Cl_2 (3 × 5 mL), and the combined CH_2Cl_2 was washed with brine (10 mL), dried (Na_2SO_4), and concentrated to give a crude product with >99% conversion by ^1H NMR assay.

General Procedure B for Deprotection of *tert*-Butyl *N*-Bts-Amino Acid Esters or Peptides by PhSH. To a suspension of *tert*-butyl *N*-Bts-protected amino acid ester or peptide and K_2CO_3 (3 equiv) in dry DMF (ca. 0.2 M in substrate) under N_2 was added thiophenol (3 equiv for the tetrapeptide; 2 equiv in the other examples) in one portion at room temperature. The suspension was vigorously stirred for 3−5 h and diluted with ether (10 mL) and water (10 mL), the aqueous phase was extracted with ether (2 × 10 mL), and the combined ether extracts were washed with water (3 × 10 mL). The organic phase was extracted with 1% aqueous hydrochloric acid (3 × 5 mL), washed with brine (10 mL), dried over Na_2SO_4, and evaporated to give 2-phenylthiobenzothiazole (**7**) and diphenyl disulfide as byproducts. The combined aqueous hydrochloric acid extracts were washed with ether (10 mL) and neutralized with saturated $NaHCO_3$ (10 mL). The resulting aqueous phase was extracted with distilled HPLC grade CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 layer was washed with brine (10 mL), dried over Na_2SO_4, and evaporated (aspirator) to provide the *N*-deprotected amino acid ester or peptide.

2-Phenylthiobenzothiazole (7). Purified by preparative TLC on silica gel (20 × 10 × 0.25 cm), 9:1 hexane/EtOAc eluent: analytical TLC on silica gel, 4:1 hexane/EtOAc, R_f = 0.45. Molecular ion calcd for $C_{13}H_9NS_2$: 243.01770; found *m/e* = 243.0131; base peak = 242 amu; 300 MHz NMR ($CDCl_3$, ppm) δ 7.88 (1H, d, *J* = 8.7 Hz) 7.77−7.70 (2H, m) 7.64 (1H, d, *J* = 8.4 Hz) 7.55−7.35 (4H, m) 7.30−7.22 (1H, m).

Deprotection of *tert*-Butyl *N*-Bts-*N*-Methylamino Acid Esters or Peptides by Sodium Borohydride (Procedure C). To a stirred suspension of $NaBH_4$ (5 equiv) in EtOH (2 mL) at 0 °C was added a solution of *tert*-butyl *N*-Bts-amino acid ester or peptide in EtOH (3 mL) via cannula. The solution was brought to rt for 1−3 h and monitored by TLC before quenching with 1% aqueous hydrochloric acid (ca. 20 mL) and evaporation (aspirator) to remove EtOH. The residue was diluted with ether (10 mL) and extracted with H_2O (3 × 5 mL). The combined aqueous phase was washed with ether (2 × 5 mL) and neutralized by aqueous $NaHCO_3$ (10 mL). The resulting aqueous phase was extracted with HPLC grade CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 extracts were washed with brine (10 mL), dried over Na_2SO_4, and evaporated to give the *N*-deprotected amino acid ester or peptide.

Deprotection of *tert*-Butyl *N*-Bts-Amino Acid Esters or Peptides by Hypophosphorous Acid (H_3PO_2) (Procedure D). To a refluxing solution of *tert*-butyl *N*-Bts-amino acid ester or peptide in THF (ca. 0.025 M) under N_2 was added dropwise 50% H_3PO_2 (22 equiv) over 3 h. The solution was cooled and diluted with ether (10 mL). The reaction was extracted with H_2O (3 × 5 mL), and the combined aqueous extracts were washed with ether (2 × 5 mL). The organic phase was combined, washed with brine (10 mL), dried over Na_2SO_4, and concentrated. Assay by ^1H NMR indicated incomplete conversion and formation of benzothiazole. The aqueous phase was neutralized with saturated $NaHCO_3$ (10 mL) and extracted with HPLC grade CH_2Cl_2 (3 × 5 mL), and the combined CH_2Cl_2 extracts were washed with brine (10 mL), dried (Na_2-

SO_4), and evaporated to give the *N*-deprotected amino acid ester or peptide.

General Procedure E for Peptide Bond Formation. To a stirred suspension of *N*-Bts-protected amino acid (1.1 equiv) in dry CH_2Cl_2 (ca. 0.2 M) under N_2 was added $SOCl_2$ (3 equiv) at room temperature, and the reaction mixture was brought to reflux for 2 h. After cooling, the dichloromethane was removed (aspirator), the resulting residue was dissolved in dry toluene (2 mL), and the toluene and residual $SOCl_2$ were evaporated to give the corresponding acyl chloride which was used without further purification in the next step.

To a vigorously stirred mixture of *tert*-butyl amino acid ester or peptide, $NaHCO_3$ (3.2 equiv), and Na_2CO_3 (2 equiv) in 1:1 CH_2Cl_2 and H_2O (ca. 0.1 M) at 0−5 °C was added the acid chloride in dry CH_2Cl_2 (3 mL) via cannula over 1 min. The reaction mixture was maintained at 0−5 °C for an additional 30 min, and the aqueous phase was then extracted with CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 extracts were washed successively with 0.5% aqueous hydrochloric acid (10 mL) and brine (10 mL) and were then dried over Na_2SO_4 followed by evaporation (aspirator).

Bts-(*S*)-MeVal-O-*tert*-Bu (4a). According to the general procedure A, Bts-(*S*)-Val-*O*-*tert*-Bu (**3a**) (0.074 g, 0.2 mmol) was employed to produce Bts-(*S*)-MeVal-*O*-*tert*-Bu (**4a**) (observed 0.085 g, theoretical yield 0.077 g, >99% conversion): analytical TLC on silica gel, 4:1 hexane/EtOAc, R_f = 0.37; analytical HPLC, CHIRALCEL AD (95 hex/IPA, 1 mL/min, P =203.1 psi) T_R = 6.59 min; >99.75:0.25 er, >99.5% ee. Pure material was obtained by crystallization from hexane: mp 68.5−69 °C; $[\alpha]^{25}_D$ −23.1 (c = 1, $CHCl_3$). HRMS, M + 1, 385.1269, error = 3 ppm; IR (KBr, cm^{-1}) 1733, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.18−8.13 (1H, m) 7.98−7.94 (1H, m) 7.62−7.51 (2H, m) 4.13 (1H, d, J = 10.5 Hz) 3.19 (3H, s) 2.24−2.10 (1H, m) 1.12 (9H, s) 1.00 (3H, d, J = 6.6 Hz) 0.99 (3H, d, J = 6.6 Hz).

Bts-(*R*)-MeVal-*O*-*tert*-Bu (*ent*-4a). Bts-(*R*)-MeVal-O-*tert*-Bu (*ent*-**4a**) (observed 0.080 g, theoretical yield 0.077 g, >99% conversion) was prepared in the same way from Bts-(*R*)-Val-O-*tert*-Bu (*ent*-**3a**) (0.074 g, 0.2 mmol): analytical HPLC, CHIRALCEL AD (95 hex/IPA, 1 mL/min, P = 203.1 psi) T_R = 7.67 min; >99.75:0.25 er, >99.5% ee; mp 68.5−69.5 °C; $[\alpha]^{25}_D$ +24.5 (c = 1.1, $CHCl_3$).

Bts-(*S*)-MeLeu-*O*-*tert*-Bu (4b). According to the general procedure A, Bts-(*S*)-Leu-*O*-*tert*-Bu (**3b**) (0.311 g, 0.80 mmol) was employed to produce Bts-(*S*)-MeLeu-*O*-*tert*-Bu **(4b)** (observed 0.320 g, theoretical yield 0.319 g, >99% conversion): analytical TLC on silica gel, 4:1 hexane/EtOAc, R_f = 0.34. Pure material was obtained by crystallization from hexane: mp 58.5−59.5 °C. $[\alpha]^{25}_D$ +24.5 (c = 1.1, $CHCl_3$). HRMS, M + 1, 399.1453, error = 10 ppm; IR (KBr, cm^{-1}) 1736, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.18−8.13 (1H, m) 7.98−7.93 (1H, m) 7.62−7.50 (2H, m) 4.71 (1H, dd, J = 7.6, 7.6 Hz) 3.11 (3H, s) 1.76−1.62 (3H, m) 1.18 (9H, s) 1.01 (3H, d, J = 6.3 Hz) 0.98 (3H, d, J = 6.3 Hz).

(*S*)-MeVal-*O*-*tert*-Bu (5a). According to the general procedure B, Bts-(*S*)-MeVal-*O*-*tert*-Bu (**4a**) (0.076 g, 0.198 mmol) and PhSH (41 μL, 0.4 mmol) were employed, and the reaction was performed for 3 h to produce (*S*)-MeVal-*O*-*tert*-Bu (**5a**) (0.034 g, 91% based on **3a**): analytical TLC on silica gel, 4.5:1:0.5 *n*-BuOH/H_2O/HOAc, R_f = 0.51; >99.75:0.25 er, >99.5% ee after *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ +4.1 (c = 1.0, $CHCl_3$) [lit. $[\alpha]^{25}_D$ +4.5 (c = 1.0, $CHCl_3$)[2b], $[\alpha]^{25}_D$ +4.4 (c = 1.0, $CHCl_3$)[3a]]; HRMS, M + 1, 188.1640, error = 6 ppm; IR (neat, cm^{-1}) 3342, N−H; 1726, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 2.76 (1H, d, J = 6.0 Hz) 2.37 (3H, s) 1.95−1.78 (1H, m) 1.48 (9H, s) 1.37 (1H, br s) 0.95 (3H, d, J = 6.9 Hz) 0.94 (3H, d, J = 6.9 Hz); ^{13}C NMR: ($CDCl_3$, ppm) δ 174.4, 80.8, 69.9, 35.2, 28.1, 19.2, 18.8.

(*R*)-MeVal-*O*-*tert*-Bu (*ent*-5a). (*R*)-MeVal-*O*-*tert*-Bu (*ent*-**5a**) (0.035 g, 93% based on *ent*-**3a**), the enantiomer of **5a**, was prepared in the same way from Bts-(*R*)-MeVal-*O*-*tert*-Bu (*ent*-**4a**) (0.076 g, 0.02 mmol) and PhSH (41 μL, 0.4 mmol): >99.75:0.25 er, >99.5% ee after *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ −4.6 (c = 1.0, $CHCl_3$).

Preparation of Bts-(*S*)-MeLeu-OH (8b). To a stirred solution of Bts-(*S*)-MeLeu-*O*-*tert*-Bu (**4b**) (0.198 g, 0.49 mmol) and Et_3N (0.16 mL, 1.18 mmol) in dry dioxane (2 mL) was added TMSOTf (0.9 mL, 1.03 mmol) dropwise at room temperature. The reaction was then brought to reflux for 0.5 h, solvent was evaporated (aspirator), and the residue was diluted with water (10 mL) and extracted with EtOAc (3 × 5 mL). The combined EtOAc was washed with brine (10 mL), dried over Na_2SO_4, and evaporated to give Bts-(*S*)-MeLeu-OH (**8b**) (0.154 g, 92%). For characterization, the product was purified by preparative TLC on silica gel (20 × 10 × 0.25 cm), 10:20:0.25 hexane/EtOAc/HOAc eluent: analytical TLC on silica gel, 10:20:1 hexane/EtOAc/HOAc, R_f = 0.50. Pure material was obtained by crystallization from ether/hexane: mp 114−115 °C; $[\alpha]^{25}_D$ −71.2 (c = 1.22, $CHCl_3$). HRMS, M + 1, 343.0804, error = 5 ppm; base peak = 100 amu; IR (KBr, cm^{-1}) 1724, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 9.70−8.80 (1H, broad) 8.21−8.14 (1H, m) 8.02−7.96 (1H, m) 7.64−7.54 (2H, m) 4.90−4.83 (1H, m) 2.97 (3H, s) 1.79−1.64 (3H, m) 1.02 (3H, d, J = 5.7 Hz) 0.99 (3H, d, J = 6.3 Hz).

Bts-(*S*)-Leu-(*S*)-MeVal-O-*tert*-Bu (12). Following procedure E, Bts-(*S*)-leucine (**2b**) (0.065 g, 0.198 mmol) was coupled with (*S*)-MeVal-*O*-*tert*-Bu (**5a**) (0.034 g, 0.18 mmol) to produce Bts-(*S*)-Leu-(*S*)-MeVal-*O*-*tert*-Bu (**12**) (observed 0.091 g, theoretical yield 0.090 g; >95% conversion): analytical TLC on silica gel, 7:3 hexane/EtOAc, R_f = 0.32; >99.75:0.25 dr after *N*-methylation and HPLC assay; $[\alpha]^{25}_D$ +25.2 (c = 1.1, $CHCl_3$). HRMS, M-73, 424.1344, error = 5 ppm; IR (neat, cm^{-1}) 3248, N−H; 1732, C=O; 1646, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.21−8.14 (0.3H, m) 8.14−8.06 (0.7H, m) 7.97−7.89 (1H, m) 7.60−7.46 (2H, m) 6.22 (0.7H, d, J = 7.8 Hz) 5.88 (0.3H, d, J = 9.6 Hz) 4.87 (0.3H, br t, J = 9.9 Hz) 4.69 (0.7H, br t, J = 8.1 Hz) 4.51 (0.7H, d, J = 9.9 Hz) 3.67 (0.3H, d, J = 10.5 Hz) 2.95 (2.1H, s) 2.85 (0.9H, s) 2.41−2.27 (0.3H, m) 2.20−1.82 (1.7H, m) 1.62−1.23 (2H, m) 1.48 (2.7H, s) 1.40 (6.3H, s) 1.20−0.90 (7.8H, m) 0.77 (2.1H, d, J = 6.6 Hz) 0.19 (2.1H, d, J = 6.9 Hz).

Bts-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (11). According to the general procedure A, Bts-(*S*)-Leu-(*S*)-MeVal-*O*-*tert*-Bu (**12**) (0.085 g, 0.171 mmol) was employed to produce Bts-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**11**) (observed 0.103 g, theoretical yield 0.087 g, >99% conversion): analytical TLC on silica gel, 4:1 hexane/EtOAc, R_f = 0.26; analytical HPLC, CHIRALCEL AD (98 hex/IPA, 1 mL/min, P = 203.1 psi) T_R = 19.93 min; >99.75:0.25 dr, $[\alpha]^{25}_D$ −82.7 (c = 1.13, $CHCl_3$). HRMS, M − 73, 438.1498, error = 6 ppm; IR (neat, cm^{-1}) 1731, C=O; 1659, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.20−8.13 (1H, m) 7.99−7.93 (1H, m) 7.64−7.50 (2H, m) 5.12 (1H, t, J = 7.2 Hz) 4.70 (0.8H, d, J = 10.2 Hz) 4.15 (0.2H, d, J = 10.2 Hz) 3.15 (2.4H, s) 3.14 (2.4H, s) 3.12 (0.6H, s) 2.82 (0.6H, s) 2.36−2.26 (0.2H, m) 2.23−2.09 (0.8H, m) 1.81−1.30 (3H, m) 1.52 (1.8H, s) 1.43 (7.2H, s) 1.10 (0.6H, d, J = 6.6 Hz) 1.00−0.87 (9H, m) 0.77 (2.4H, d, J = 6.6 Hz).

Preparation of Bts-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (11) from the Coupling Reaction of Bts-(*S*)-MeLeu-OH (8b) and (*S*)-MeVal-*O*-*tert*-Bu (5a). Following procedure E, Bts-(*S*)-MeLeu-OH (**8b**) (0.065 g, 0.19 mmol) was coupled with (*S*)-MeVal-*O*-*tert*-Bu (**5a**) (0.035 g, 0.19 mmol) to produce Bts-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**11**) (observed 0.10 g, theoretical yield 0.097 g; >99% conversion). Assay by 300 MHz ^{1}H NMR in $CDCl_3$ detected no epimer formation.

(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (13). According to procedure B, Bts-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**11**) (0.087 g, 0.17 mmol) and PhSH (37 μL, 0.36 mmol) were employed, and the reaction was performed for 3 h to produce (*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**13**) (0.051 g, 89% based on **5a**): analytical TLC on silica gel, 4.5:1:0.5 *n*-BuOH/H_2O/HOAc, R_f = 0.53; >99.75:0.25 dr after *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ −128.3 (c = 1.03, $CHCl_3$) [lit. $[\alpha]^{25}_D$ −118 (c = 1.0, $CHCl_3$),[2b] $[\alpha]^{25}_D$ −115.4 (c = 1.0, $CHCl_3$)[3a]]. Molecular ion calcd for $C_{17}H_{34}N_2O_3$: 314.2570; found m/e = 314.2574, error = 1 ppm; base peak = 100 amu. Anal. Calcd: C, 64.91; H, 10.92; N, 8.91, found: C, 64.89; H, 10.72; N, 8.91; IR (neat, cm^{-1}) 3321, N−H; 1731, C=O; 1651, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 4.89 (0.7H, d, J = 10.5 Hz) 3.78 (0.3H, d, J = 11.1 Hz) 3.55 (0.7H, dd, J = 10.5, 2.7 Hz) 3.43 (0.3H, dd, J = 8.7, 4.8 Hz) 2.98 (2.1H, s) 2.97 (0.9H, s) 2.35−2.14 (1H, m) 2.28 (2.1H, s) 2.26 (0.9H, s) 1.99−1.67 (2H, m) 1.47 (2.7H, s) 1.45 (6.3H, s) 1.48−1.14

(2H, m) 1.06−0.85 (12H, m); partial ^{13}C NMR: ($CDCl_3$, ppm; minor rotamer signals marked *) δ 176.7* 176.1, 170.1, 169.4*, 82.1*, 81.2, 65.8*, 62.5, 58.6, 58.4*, 43.3*, 42.8.

Bts-(*R*)-Leu-(*S*)-MeVal-*O*-*tert*-Bu (14). Bts-(*R*)-Leu-(*S*)-MeVal-*O*-*tert*-Bu (**14**) (observed 0.092 g, theoretical yield 0.085 g; >95% conversion) was prepared following procedure E from Bts-(*R*)-leucine (*ent*-**3b**) (0.062 g, 0.187 mmol) and (*S*)-MeVal-*O*-*tert*-Bu (**5a**) (0.0324 g, 0.17 mmol); >99.75:0.25 dr after *N*-methylation and HPLC assay; $[\alpha]^{25}_D$ −106 (c = 1.05, $CHCl_3$). HRMS, M − 73, 424.1365, error = 7 ppm; IR (neat, cm^{-1}) 3251, N−H; 1731, C=O; 1643, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.19−8.14 (0.6H, m) 8.08−8.04 (0.4H, m) 7.96−7.89 (1H, m) 7.60−7.47 (2H, m) 6.30 (0.4H, br d, J = 9.0 Hz) 6.20 (0.6H, br d, J = 9.0 Hz) 4.83 (0.6H, ddd, J = 11.0, 8.0, 2.7 Hz) 4.58 (0.4H, ddd, J = 11.0, 8.0, 2.7 Hz) 4.41 (0.6H, d, J = 10.2 Hz) 3.64 (0.4H, d, J = 10.2 Hz) 3.16 (1.8H, s) 2.81 (1.2H, s) 2.22−1.97 (2H, m) 1.78−1.22 (2H, m) 1.48 (3.6H, s) 1.24 (5.4H, s) 1.08−0.72 (12H, m).

Bts-(*S*)-Leu-(*R*)-MeVal-*O*-*tert*-Bu (*ent*-14). Following procedure E, Bts-(*S*)-leucine (**2b**) (0.065 g, 0.198 mmol) was coupled with (*R*)-MeVal-*O*-*tert*-Bu (*ent*-**5a**) (0.035 g, 0.18 mmol) to produce Bts-(*S*)-Leu-(*R*)-MeVal-*O*-*tert*-Bu (*ent*-**14**) (0.077 g, 89%): analytical TLC on silica gel, 7:3 hexane/EtOAc, R_f = 0.28; >99.75:0.25 er, >99.5% ee after *N*-methylation and HPLC assay; $[\alpha]^{25}_D$ +105.7 (c = 1.25, $CHCl_3$).

Bts-(*R*)-MeLeu-(*S*)-MeVal-O-*tert*-Bu (15). Bts-(*R*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**15**) (observed 0.097 g, theoretical yield 0.083 g, >99% conversion) was prepared using procedure A from Bts-(*R*)-Leu-(*S*)-MeVal-*O*-*tert*-Bu (**14**) (0.081 g, 0.162 mmol): analytical HPLC, CHIRALCEL AD (98 hex/IPA, 1 mL/min, P = 203.1 psi) T_R = 14.95 min; >99.75:0.25 dr, $[\alpha]^{25}_D$ −33.4 (c = 1.1, $CHCl_3$). HRMS, M + 1, 512.2267, error = 3 ppm; IR (neat, cm^{-1}) 1731, C=O; 1653, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.21−8.14 (1H, m) 8.00−7.94 (1H, m) 7.64−7.51 (2H, m) 5.17−5.06 (1H, m) 4.67 (0.6H, d, J = 10.5 Hz) 4.33 (0.4H, d, J = 10.5 Hz) 3.17 (1.8H, s) 3.12 (1.8H, s) 3.08 (1.2H, s) 2.88 (1.2H, s) 2.38−2.14 (1H, m) 2.02−1.90 (0.4H, m) 1.88−1.77 (0.6H, m) 1.52−1.35 (1H, m) 1.49 (3.6H, s) 1.44 (5.4H, s) 1.31−0.99 (5H, m) 0.91−0.79 (8H, m).

Bts-(*S*)-MeLeu-(*R*)-MeVal-*O*-*tert*-Bu (*ent*-15). According to procedure A, Bts-(*S*)-Leu-(*R*)-MeVal-*O*-*tert*-Bu (*ent*-**14**) (0.077 g, 0.155 mmol) was employed to produce Bts-(*S*)-MeLeu-(*R*)-MeVal-*O*-*tert*-Bu (*ent*-**15**) (observed 0.080 g, theoretical yield 0.079 g, >99% conversion): analytical TLC on silica gel, 4:1 hexane/EtOAc, R_f = 0.28; analytical HPLC, CHIRALCEL AD (98 hex/IPA, 1 mL/min, P = 203.1 psi) T_R = 16.71 min; >99.75:0.25 dr, $[\alpha]^{25}_D$ +34.6 (c = 1.12, $CHCl_3$) ($CDCl_3$, ppm; minor rotamer signals marked *)

(*R*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (16). (*R*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**16**) (0.050 g, 93% based on **5a**), the enantiomer of *ent*-**16**, was prepared using procedure B from Bts-(*R*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**15**) (0.083 g, 0.162 mmol) and PhSH (35 μL, 0.34 mmol); >99.75:0.25 dr after *N*-benzoylation and HPLC assay: $[\alpha]^{25}_D$ −101 (c = 1.09, $CHCl_3$). Molecular ion calcd for $C_{17}H_{34}N_2O_3$: 314.2570; found m/e = 314.2560, error = 3 ppm; base peak = 100 amu; IR (neat, cm^{-1}) 3322, N−H; 1733, C=O; 1652, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 4.84 (0.6H, d, J = 10.5 Hz) 3.85 (0.4H, d, J = 10.5 Hz) 3.48−3.38 (1H, m) 2.99 (1.8H, s) 2.91 (1.2H, s) 2.36−2.13 (1H, m) 2.30 (1.8H, s) 2.26 (1.2H, s) 2.04−1.80 (1H, m) 1.73 (1H, br s) 1.52−1.20 (2H, m) 1.48 (3.6H,s) 1.46 (5.4H, s) 1.05−0.80 (12H, m); partial ^{13}C NMR: ($CDCl_3$, ppm; minor rotamer signals marked *) δ 176.8, 176.2*, 170.2, 169.0*, 82.3*, 81.2, 65.7*, 62.6, 58.7*, 58.5, 43.0, 42.2*.

(*S*)-MeLeu-(*R*)-MeVal-*O*-*tert*-Bu (*ent*-16). According to procedure B, Bts-(*S*)-MeLeu-(*R*)-MeVal-*O*-*tert*-Bu (*ent*-**15**) (0.078 g, 0.153 mmol) and PhSH (32 μL, 0.32 mmol) were employed, and the reaction was performed for 3 h to produce (*S*)-MeLeu-(*R*)-MeVal-*O*-*tert*-Bu (*ent*-**16**) (0.045 g, 77% based on *ent*-**5a**): analytical TLC on silica gel, 4.5:1:0.5 *n*-BuOH/H_2O/HOAc, R_f = 0.53; >99.75:0.25 dr after *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ +98.5 (c = 1.13, $CHCl_3$).

Bts-(*S*)-Leu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (17). Following procedure E, Bts-(*S*)-leucine (**2b**) (0.059 g, 0.179 mmol) was coupled with (*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**13**) (0.051 g, 0.162 mmol) to produce Bts-(*S*)-Leu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**17**) (observed 0.118 g, theoretical yield 0.101 g; >98% conversion): analytical TLC on silica gel, 7:3 hexane/EtOAc, R_f = 0.32; >99.75:0.25 dr after *N*-methylation and HPLC assay. Pure material was obtained by crystallization from ether/hexane: mp 99−100 °C; $[\alpha]^{25}_D$ −58.8 (c = 1.69, $CHCl_3$). HRMS, M − 73, 551.2465; base peak = 100 amu; IR (KBr, cm^{-1}) 3178, N−H; 1732, C=O; 1635, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.17−8.08 (1H, m) 8.01−7.94 (1H, m) 7.66−7.52 (2H, m) 6.22 (0.9H, d, J = 9.2 Hz) 6.19 (0.1H, d, J = 9.2 Hz) 5.25−5.15 (1H, m) 4.77−4.61 (1H, m) 4.66 (0.9H, d, J = 10.2 Hz) 3.80 (0.1H, d, J = 9.6 Hz) 3.03 (2.7H, s) 3.00 (0.3H, s) 2.86 (2.7H, s) 2.75 (0.3H, s) 2.24−1.99 (2H, m) 1.62−1.37 (2H, m) 1.45 (0.9H, s) 1.40 (8.1H, s) 1.28−1.17 (1H, m) 1.11−0.91 (10H, m) 0.84−0.70 (4H, m) 0.59−0.41 (6H, m).

Bts-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (18). According to procedure A, noncrystallized Bts-(*S*)-Leu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**17**) (0.098 g, 0.16 mmol) was employed to produce Bts-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**18**) (observed 0.117 g, theoretical yield 0.101 g, >99% conversion): analytical TLC on silica gel, 7:3 hexane/EtOAc, R_f = 0.37; analytical HPLC, CHIRALCEL AD (96 hex/IPA, 1 mL/min, P = 203.1 psi) T_R = 28.08 min; >99.75:0.25 dr, $[\alpha]^{25}_D$ −111.1 (c = 1.01, $CHCl_3$). HRMS, M − 73, 565.2557, error = 7 ppm; base peak = 297 amu; IR (neat, cm^{-1}) 1733, C=O; 1652, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.21−8.14 (1H, m) 8.00−7.94 (1H, m) 7.65−7.52 (2H, m) 5.38 (1H, br t, J = 7.3 Hz) 5.11 (1H, br t, J = 7.1 Hz) 4.71 (0.8H, d, J = 10.2 Hz) 3.86 (0.2H, d, J = 10.2 Hz) 3.18 (2.4H, s) 3.15 (2.4H, s) 3.11 (0.6H, s) 3.09 (0.6H, s) 2.91 (2.4H, s) 2.80 (0.6H, s) 2.23−2.08 (1H, m) 1.78−1.18 (6H, m) 1.46 (1.8H, s) 1.42 (7.2H, s) 1.04−0.68 (18H, m).

(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (19). According to procedure B, Bts-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**18**) (0.100 g, 0.156 mmol) and PhSH (33 μL, 0.326 mmol) were employed, and the reaction was performed for 4 h to produce (*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**19**) (0.069 g, 96% based on **13**): analytical TLC on silica gel, 4.5:1:0.5 *n*-BuOH/H_2O/HOAc, R_f = 0.49; >99.75:0.25 dr after *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ −181.1 (c = 1.0, $CHCl_3$) [lit. $[\alpha]^{25}_D$ −183 (c = 1.0, $CHCl_3$)].[3a] HRMS, M + 1, 442.3666, error = 5 ppm; base peak = 100 amu; IR (neat, cm^{-1}) 3322, N−H; 1732, C=O 1645; 300 MHz NMR ($CDCl_3$, ppm) δ 5.70−5.60 (1H, m) 4.74 (0.9H, d, J = 10.2 Hz) 4.28 (0.1H, d, J = 9.6 Hz) 3.45−3.33 (1H, m) 3.02 (2.7H, s) 3.00 (2.7H, s) 2.97 (0.3H, s) 2.78 (0.3H, s) 2.30−2.11 (1H, m) 2.28 (2.7H, s) 2.27 (0.3H, s) 1.94−1.60 (4H, m) 1.58−1.15 (3H, m) 1.46 (0.9H, s) 1.45 (8.1H, s) 1.10−0.75 (18H, m); partial ^{13}C NMR: ($CDCl_3$, ppm; minor rotamer signals marked *) δ 175.8, 175.1*, 171.7, 171.4*, 170.0, 169.0*, 82.0*, 81.3, 65.6*, 62.5, 58.8, 58.7*, 51.2*, 50.8, 42.9, 41.8*, 38.8*, 37.9, 35.1, 34.9*.

Bts-(*R*)-Leu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (20). Following procedure E, Bts-(*R*)-leucine (*ent*-**2b**) (0.062 g, 0.185 mmol) was coupled with (*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**13**) (0.053 g, 0.168 mmol) to produce Bts-(*R*)-Leu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**20**) (observed 0.110 g, theoretical yield 0.105 g; >95% conversion): analytical TLC on silica gel, 7:3 hexane/EtOAc, R_f = 0.32; >99.75:0.25 dr after *N*-methylation and HPLC assay. Pure material was obtained by crystallization from ether/hexane, mp 143−144 °C; $[\alpha]^{25}_D$ −122 (c = 1.17, $CHCl_3$). HRMS, M − 73, 551.2363, error = 0 ppm; base peak = 100 amu; IR (KBr, cm^{-1}) 3165, N−H; 1726, C=O; 1639, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.25−8.15 (1H, m) 7.97−7.90 (1H, m) 7.65−7.48 (2H, m) 6.34 (0.9H, d, J = 8.1 Hz) 6.26 (0.1H, d, J = 7.8 Hz) 5.47 (0.1H, dd, J = 10.2, 7.6 Hz) 5.29 (0.9H, t, J = 7.2 Hz) 4.75 (1H, ddd, J = 11.0, 8.1, 2.7 Hz) 4.49 (0.9H, d, J = 10.2 Hz) 3.91 (0.1H, d, J = 10.2 Hz) 3.13 (0.3H, s) 3.09 (2.7H, s) 2.68 (0.3H, s) 2.58 (2.7H, s) 2.08−1.95 (1H, m) 1.89−1.75 (1H, m) 1.74−1.24 (5H, m) 1.41 (8.1H, s) 1.26 (0.9H, s) 1.04−0.81 (15.3H, m) 0.32 (2.7H, d, J = 6.9 Hz); partial ^{13}C NMR: ($CDCl_3$, ppm; minor rotamer signals marked *) δ 175.9, 174.7, 171.7, 170.9*, 169.9, 168.8*, 82.0*, 81.3, 65.5*, 62.4, 58.7, 51.9*, 50.8, 42.8, 42.5*, 38.7*, 38.0, 35.1*, 35.1.

Bts-(*R*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (21). According to procedure A, noncrystallized Bts-(*R*)-Leu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**20**) (0.10 g, 0.16 mmol) was employed to produce Bts-(*R*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**21**) (observed 0.118 g, theoretical yield 0.102 g, >99% conversion): analytical TLC on silica gel, 7:3 hexane/EtOAc, R_f = 0.37; analytical HPLC, CHIRALCEL AD (96 hex/IPA, 1 mL/min, P = 203.1 psi) T_R = 12.43 min; >99.75:0.25 dr, $[\alpha]^{25}_D$ −62.1 (c = 1.37, $CHCl_3$). Pure material was obtained by crystallization from ether/hexane, mp 74.5−75 °C. HRMS, M − 64, 574.3549, error = 1 ppm; base peak = 297 amu; IR (KBr, cm^{-1}) 1730, C=O; 1645, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.21−8.16 (1H, m) 8.00−7.95 (1H, m) 7.65−7.54 (2H, m) 5.49 (1H, br t, J = 7.2 Hz) 5.09 (1H, br t, J = 7.2 Hz) 4.74 (0.9H, d, J = 10.2 Hz) 4.08 (0.1H, d, J = 9.9 Hz) 3.22 (0.3H, s) 3.16 (2.7H, s) 3.13 (2.7H, s) 3.11 (0.3H, s) 2.94 (2.7H, s) 2.79 (0.3H, s) 2.22−2.08 (1H, m) 1.84−1.60 (2H, m) 1.55−1.30 (4H, m) 1.45 (8.1H, s) 1.40 (0.9H, s) 1.08−0.75 (18H, m).

(*R*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-O-*tert*-Bu (22). According to procedure B, noncrystallized Bts-(*R*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**21**) (0.101 g, 0.158 mmol) and PhSH (35 μL, 0.34 mmol) were employed, and the reaction was performed for 4 h to produce (*R*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**22**) (0.069 g, 96% based on **13**): analytical TLC on silica gel, 4.5:1:0.5 *n*-BuOH/H_2O/HOAc, R_f = 0.61; >99.75:0.25 dr after *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ −154 (c = 1.04, $CHCl_3$). HRMS, M + 1, 442.3642, error = 1 ppm; base peak = 100 amu; IR (neat, cm^{-1}) 3324, N−H; 1733, C=O; 1645, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 5.62 (0.8H, d, J = 7.3 Hz) 5.54 (0.2H, d, J = 7.3 Hz) 4.76 (0.8H, d, J = 10.2 Hz) 4.06 (0.2H, d, J = 10.2 Hz) 3.46−3.35 (1H, m) 3.05 (2.4H, s) 3.02 (2.4H, s) 2.90 (0.6H, s) 2.79 (0.6H, s) 2.30−2.14 (1H, m) 2.27 (0.6H, s) 2.25 (2.4H, s) 2.05−1.65 (4H, m) 1.62−1.12 (3H, m) 1.45 (9H, s) 1.18−0.70 (18H, m).

Bts-(*R*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (23). Following procedure E, Bts-(*R*)-alanine (*ent*-**2c**) (0.051 g, 0.176 mmol) was coupled with (*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**19**) (0.069 g, 0.156 mmol) to produce Bts-(*R*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**23**) (observed 0.124 g, theoretical yield 0.111 g; >99% conversion); analytical TLC on silica gel, 1:1 hexane/EtOAc, R_f = 0.47; analytical HPLC, CHIRALCEL AD (94 hex/IPA, 1 mL/min, P = 232.1 psi) T_R = 44.31 min; >99.75:0.25 dr, $[\alpha]^{25}_D$ −166.4 (c = 1.04, $CHCl_3$). HRMS, M − 73, 636.2886, error = 1 ppm; base peak = 396 amu; IR (KBr, cm^{-1}) 3188, N−H; 1732, C=O; 1641, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.19−8.13 (1H, m) 7.99−7.94 (1H, m) 7.69−7.52 (2H, m) 6.39 (1H, d, J = 6.9 Hz) 5.49−5.47 (2H, m) 4.83 (1H, quintet, J = 7.2 Hz) 4.72 (0.7H, d, J = 10.5 Hz) 3.89 (0.3H, d, J = 10.5 Hz) 3.06 (2.1H, s) 2.97 (2.1H, s) 2.92 (0.9H, s) 2.80 (0.9H, s) 2.78 (2.1H, s) 2.49 (0.9H, s) 2.23−2.09 (1H, m) 1.77−1.10 (18H, m) 1.02−0.75 (18H, m).

Bts-(*S*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-O-*tert*-Bu (24). Following procedure E, Bts-(*S*)-alanine (**2c**) (0.048 g, 0.165 mmol) was coupled with (*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**19**) (0.068 g, 0.15 mmol) to produce Bts-(*S*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**24**) (observed 0.118 g, theoretical yield 0.106 g; >99% conversion): analytical TLC on silica gel, 1:1 hexane/EtOAc, R_f = 0.41; analytical HPLC, CHIRALCEL AD (94 hex/IPA, 1 mL/min, P = 232.1 psi) T_R = 49.90 min; >99.75:0.25 dr, $[\alpha]^{25}_D$ −130.7 (c = 1.05, $CHCl_3$). HRMS, M + 1, 710.3613, error = 1 ppm; base peak = 396 amu; IR (KBr, cm^{-1}) 3182, N−H; 1732, C=O; 1639, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 8.14−8.04 (1H, m) 8.00−7.95 (1H, m) 7.64−7.53 (2H, m) 6.31 (0.3H, d, J = 8.1 Hz) 6.27 (0.7H, d, J = 8.4 Hz) 5.53−5.41 (1H, m) 5.35−5.22 (1H, m) 4.83 (1H, quintet, J = 6.6 Hz) 4.69 (0.7H, d, J = 10.2 Hz) 3.90 (0.3H, d, J = 10.2 Hz) 3.02 (2.1H, s) 2.96 (2.1H, s) 2.95 (0.9H, s) 2.89 (2.1H, s) 2.79 (0.9H, s) 2.74 (0.9H, s) 2.24−2.08 (1H, m) 1.73−1.24 (8H, m) 1.43 (6.3H, s) 1.41 (2.7H, s) 1.08−0.53 (19H, m).

(*S*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (25). According to the general procedure B, Bts-(*S*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**24**) (0.105 g, 0.148 mmol) and PhSH (46 μL, 0.45 mmol) were employed and the reaction was performed for 5 h to produce (*S*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**25**) (0.075 g, 97% based on **19**): analytical TLC on silica gel, 4.5:1:0.5 *n*-BuOH/H_2O/HOAc, R_f = 0.60; >99.75:0.25 dr after *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ −265.2 (c = 1.22, $CHCl_3$). Molecular ion calcd for $C_{27}H_{52}N_4O_5$: 512.39380; found m/e = 512.3928, error = 2 ppm; base peak = 100 amu; IR (neat, cm^{-1}) 3373, N−H; 1734, C=O; 1653, C=O; 300 MHz NMR ($CDCl_3$, ppm) δ 5.64−5.46 (2H, m) 4.72 (0.7H, d, J = 10.5 Hz) 4.00 (0.3H, d, J = 10.2 Hz) 3.80 (1H, q, J = 6.9 Hz) 2.99 (4.2H, s) 2.97 (2.1H, s) 2.90 (0.9H, s) 2.82 (1.8H, s) 2.25−2.11 (1H, m) 1.80−1.21 (8H, m) 1.47 (2.7H, s) 1.44 (6.3H, s) 1.23 (3H, d, J = 7.2 Hz) 1.05−0.76 (18H, m); partial ^{13}C NMR: ($CDCl_3$, ppm; minor rotamer signals marked *) δ 176.4, 176.2*, 171.5, 171.2, 170.8*, 170.1*, 170.0, 168.6*, 81.9*, 81.3, 65.5, 65.4*, 51.6*, 51.4*, 51.2, 50.9, 47.3, 38.5*, 37.7, 37.69, 37.2*.

(*R*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (1). According to procedure B, Bts-(*R*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**23**) (0.111 g, 0.155 mmol) and PhSH (49 μL, 0.48 mmol) were employed, and the reaction was performed for 5 h to produce (*R*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O-tert*-Bu (**1**) (0.076 g, 94% based on **19**): analytical TLC on silica gel, 4.5:1:0.5 *n*-BuOH/H_2O/HOAc, R_f = 0.53; >99.75:0.25 dr after *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ −202 (c = 1.06, $CHCl_3$) or $[\alpha]^{25}_D$ −213 (c = 1.04, $CHCl_3$) and −218 (c = 1.05, MeOH) after drying the sample 12 h under vacuum; lit.[11] −126 (c = 1.2, MeOH). Molecular ion calcd for $C_{27}H_{52}N_4O_5$: 512.39380; found m/e = 512.3928, error = 2 ppm; base peak = 100 amu; Anal. calcd for $C_{27}H_{52}N_4O_5(H_2O)$: C, 61.09; H, 10.27; N, 10.56; found (after 2−3 days drying under vacuum at 40 °C): C, 61.36; H, 10.26; N, 10.64; IR (neat, cm^{-1}) 3374, N−H; 1732, C=O; 1646, C=O; 300 MHz NMR ($CDCl_3$; spectrum recorded after 30 min drying over molecular sieves; ppm) δ 5.62−5.46 (2H, m) 4.74 (0.7H, d, J = 10.2 Hz) 3.98 (0.3H, d, J = 10.5 Hz) 3.82 (1H, br s) 3.03 (2.1H, s) 3.00 (2.1H, s) 2.99 (2.1H, s) 2.91 (0.9H, s) 2.85 (0.9H, s) 2.82 (0.9H, s) 2.25−2.11 (1H, m) 1.82−1.24 (8H, m) 1.47 (2.7 H, s) 1.44 (6.3H, s) 1.27 (1H, d, J = 6.6 Hz) 1.26 (2H, d, J = 6.6 Hz) 1.03−0.77 (18H, m); partial ^{13}C NMR: ($CDCl_3$, ppm; minor rotamer signals marked *) δ 176.5, 176.1*, 171.4, 171.3, 170.7*, 169.92*, 169.88, 168.6*, 81.6*, 81.3, 65.4*, 62.5, 51.6*, 51.5*, 51.3, 50.8, 47.2, 38.6*, 37.9, 37.8, 37.6*.

General Procedure F for One-Pot *N*-Methylation-Deprotection of *tert*-Butyl *N*-Bts-Protected Amino Acid Esters and Peptides. To a suspension of *N*-Bts protected amino acid or peptide and K_2CO_3 (5 equiv) in anhydrous DMF (ca. 0.17 M) under N_2 was added excess iodomethane (10 equiv) in one portion at room temperature. The reaction mixture was brought to 35 °C for ca. 3 h and was monitored by TLC. After the conversion was complete, the excess iodomethane was removed under a N_2 stream, followed by stirring at room temperature for 30 min under vacuum (ca. 0.2 mmHg). The concentration (ca. 0.17 M) of the reaction mixture was then readjusted by adding dry DMF. Thiophenol (3 equiv) was added in one portion at room temperature. The suspension was vigorously stirred for 3 h and was monitored by TLC. The mixture was diluted with ether (10 mL) and water (10 mL). The aqueous phase was extracted with ether (2 × 10 mL), and the combined ether extracts were washed with water (3 × 10 mL). The organic phase was extracted with 1% aqueous hydrochloric acid (3 × 5 mL), washed with brine (10 mL), dried over Na_2SO_4, and evaporated to give 2-phenylthiobenzothiazole (**7**) and diphenyl disulfide as neutral byproducts. The combined aqueous hydrochloric acid extracts were washed with ether (10 mL) and neutralized with saturated $NaHCO_3$ (10 mL). The resulting aqueous phase was extracted with HPLC grade CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 layer was washed with brine (10 mL), dried over Na_2SO_4, and evaporated (aspirator) to provide the *N*-deprotected amino acid ester or peptide.

Sequence from (*S*)-MeVal-*O-tert*-Bu (5a) to 1 via One-Pot Methylation/Deprotection. According to procedure F, Bts-(*S*)-Val-*O-tert*-Bu (**3a**) (0.0746 g, 0.20 mmol) was employed to produce (*S*)-MeVal-O*t*-Bu (**5a**) (0.036 g, 96% based on **3a**). Following procedure E, Bts-(*S*)-leucine (**2b**) (0.073 g, 0.21 mmol) was coupled with **5a** (0.036 g, 0.19 mmol) to produce Bts-(*S*)-Leu-(*S*)-MeVal-*O-tert*-Bu (**12**) (observed 0.099 g, theoretical yield 0.096 g; >95% conversion). Following procedure

F, **12** (0.183 mmol) was employed to produce (*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**13**) (0.0527 g, 0.167 mmol, 86% based on **5a**). According to procedure E, Bts-(*S*)-leucine (**2b**) (0.0634 g, 0.184 mmol) was coupled with **13** (0.0527 g, 0.167 mmol) to produce Bts-(*S*)-Leu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**17**) (observed 0.112 g, theoretical yield 0.104 g; >98% conversion). According to procedure F, **17** (0.164 mmol) was employed to produce (*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**19**) (0.0714 g, 0.162 mmol, 97% based on **13**). Following procedure E, Bts-(*R*)-alanine (*ent*-**2c**) (0.052 g, 0.178 mmol) was coupled with **19** (0.0714 g, 0.162 mmol) to produce Bts-(*R*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**23**) (observed 0.125 g, theoretical yield 0.115 g, >98% conversion). According to procedure B, **23** (0.158 mmol) was employed to produce (*R*)-Ala-(*S*)-MeLeu-(*S*)-MeLeu-(*S*)-MeVal-*O*-*tert*-Bu (**1**) (0.0824 g, 0.16 mmol, 98% based on **19**), identical to **1** prepared above by NMR; $[\alpha]^{25}_{D}$ −223 (*c* = 1.03, $CHCl_3$) and −214 (*c* = 1.22, MeOH) after drying the sample 12 h under vacuum The overall yield calculated from **5a** was 80%.

General Procedure for *N*-Benzoylation of Amino Acid Esters and Peptides for HPLC Assay. To a solution of free amine in dry CH_2Cl_2 (ca. 0.1 M) were added *i*-Pr_2NEt (1.3 equiv) and BzCl (1.2 equiv). After stirring under N_2 at room temperature for 2 h, the reaction mixture was diluted with $CHCl_3$ (5 mL) and dilute citric acid (10 mL). The aqueous phase was extracted with $CHCl_3$ (3 × 5 mL). The combined $CHCl_3$ extracts were washed successively with H_2O (10 mL), saturated $NaHCO_3$ (10 mL), and brine (10 mL). The resulting solution was then dried over Na_2SO_4 and concentrated to give the corresponding benzoyl-protected product (see Supporting Information for assay details and characterization).

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Supporting Information Available: Assay and characterization data for *N*-benzoyl derivatives of deprotected amines; NMR spectra of synthetic intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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