An Alkanesulfonamide "Safety-Catch" Linker for Solid-Phase Synthesis

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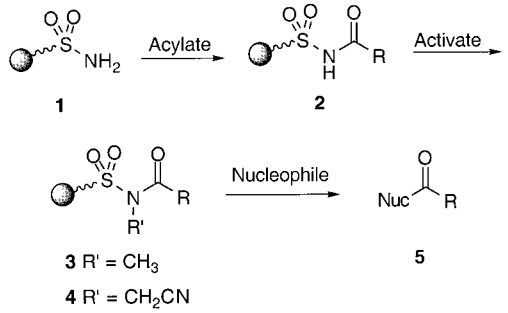
An alkanesulfonamide "safety-catch" linker has been developed for tethering carboxylic acids to support. Acylation of the sulfonamide support provides a support-bound *N*-acylsulfonamide that is stable to both basic and strongly nucleophilic reaction conditions. At the end of a solid-phase synthesis sequence, treatment with iodoacetonitrile provides *N*-cyanomethyl derivatives that can be cleaved by nucleophiles under mild reaction conditions to release the target compounds. Coupling conditions have been developed to load Boc- and Fmoc-amino acids to the alkanesulfonamide resin with high loading efficiencies and minimal racemization. A number of support-bound amino acids incorporating diverse side-chain functionality have been subjected to a short synthesis sequence, followed by iodoacetonitrile activation and nucleophilic displacement to provide dipeptide products. All 20 of the proteinogenic amino acids, when suitably protected, are compatible with the loading and activation steps. Finally, displacement with various nucleophiles including amines, α-amino acid methyl esters, and α-amino acid coumarin derivatives is demonstrated.

Introduction

The utility of small molecule libraries in drug research programs has caused a resurgence in the field of solid-phase organic synthesis.[1,2] The emergence of novel linkers and linkage strategies has been a central component of these developments.[3] "Safety-catch" linker strategies in which a stable linkage is activated for cleavage by a discrete chemical modification have proven to be particularly useful.[4,5] These linkers are often compatible with a broad range of reactions, yet target compounds can be released from support under mild conditions after the activation step. In addition, the activation step can potentially provide a reactive linkage functionality for cleavage with diverse nucleophiles. Therefore, an additional element of diversity can be added to the structure of the target compounds in the cleavage step. Activation can also facilitate the synthesis of cyclic compounds by intramolecular attack of a nucleophile upon the linkage functionality liberating the target compounds from support.

Kenner's sulfonamide "safety-catch" linker[6] **1** for tethering carboxylic acids to support is unique in that it shows stability to both strongly acidic and strongly basic reaction conditions (Scheme 1). Kenner employed an arenesulfonamide linker for solid-phase peptide chemistry in which activation by diazomethane treatment provided an *N*,*N*-methylacylsulfonamide **3** that can be cleaved with forcing conditions by aminolysis, hydrazinolysis, and saponification to provide primary amides, hydrazones, and carboxylic acids, respectively. Limitations encountered in Kenner's work include poor loading efficiencies, racemization in the loading step, and poor reactivity of the *N*,*N*-methylacylsulfonamide **3** that is obtained upon diazomethane activation. We have utilized a modified version of this linker for small-molecule organic synthesis efforts to prepare substituted aryl acetic acid derivatives that represent an important class of antiinflammatory drugs.[7] Basic reaction conditions including enolate alkylation and Suzuki cross-coupling reaction conditions were demonstrated. We have also developed an activation method in which *N*-alkylation of the *N*-acylsulfonamide with a haloacetonitrile provides an extremely reactive *N*,*N*-cyanomethylacylsulfonamide **4** for mild nucleophilic displacement.[8,9] The linkage is reactive enough to allow for the use of limiting quantities of nucleophilic amines in the cleavage step to provide pure amide products in solution. Thus, target compounds can be isolated without purification, greatly simplifying compound isolation.[10]

Scheme 1



We were interested in extending the use of sulfonamide linkers to include the loading, activation, and cleavage

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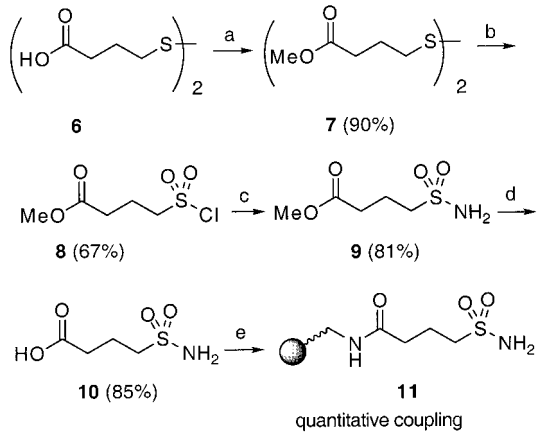
of amino acid derivatives. The use of an amino acid as the initial, linker-bound building block has been the most widely employed strategy for the solid-phase synthesis of small organic molecules.[1,2,11] Amino acids are utilized extensively in organic synthesis,[12] and the commercial availability of numerous, diverse derivatives makes them ideal for use in combinatorial synthesis efforts.[13] Extension of the safety-catch linker to include amino acids would also clearly be applicable to solid-phase peptide synthesis for segment condensation strategies[14] and for the synthesis of cyclic peptides[15] and peptide amides.[16]

In a previous paper,[8] we demonstrated that the haloacetonitrile activation method can be extended to include carboxylic acids that possess α-electron-withdrawing groups, such as *N*-protected amino acids, by employing an alkanesulfonamide linker.[17] After activation of support-bound amino acids by iodoacetonitrile treatment, cleavage was demonstrated with amines and amino acid methyl esters. Here, a detailed experimental procedure for the synthesis of an alkanesulfonamide handle and optimized procedures for the attachment of the initial amino acid that provide minimal racemization and high loading efficiencies are reported. As well, the generality of the iodoacetonitrile activation procedure is demonstrated by the preparation of a number of dipeptide amides that incorporate diverse functionality. Finally, displacement of the support-bound *N*,*N*-cyanomethylacylsulfonamide active ester is accomplished with a variety of nucleophiles including amines, α-amino methyl esters, and α-amino acid coumarin derivatives.

Results and Discussion

Synthesis of an Alkanesulfonamide Handle. Two criteria were considered for the synthesis of an alkanesulfonamide handle: (1) The synthesis sequence must be short and simple and allow for the preparation of significant quantities of material without the need for a column chromatography step. (2) The handle must be loaded to support in an efficient manner to provide an alkanesulfonamide resin uncontaminated with byproducts. To meet these criteria, 3-carboxypropanesulfonamide[18] was prepared as a handle for loading to aminomethyl resins (Scheme 2). Starting from commercially available 4,4′-dithiobutyric acid (**6**), esterification with thionyl chloride in MeOH followed by distillation provides diester **7** in high yield. Oxidation with Cl_2 gas in water followed by extractive isolation then gives 2 equiv of sulfonyl chloride **8**. Aminolysis of **8** provides sulfonamide **9** with product isolation accomplished by extraction. Saponification and isolation by precipitation of the KCl salts provides the 3-carboxypropanesulfonamide handle **10** in good yield. The handle can be quantitatively loaded to aminomethyl resin using standard DICI (*N*,*N*′-diisopropylcarbodiimide) and HOBt (*N*-hydroxybenzotriazole) coupling conditions to provide alkanesulfonamide resin **11** as monitored by a bromophenol blue test.[19,20] Oligomerization does not occur in the coupling step since the alkanesulfonamide does not react with HOBt esters (vide infra).

Scheme 2[a]



[a] Key: (a) $SOCl_2$, MeOH; (b) Cl_2, H_2O; (c) NH_3, Et_2O; (d) KOH, H_2O; (e) aminomethyl polystyrene resin, DICI, HOBt, DMF.

Optimization of First Amino Acid Loading. Coupling conditions were sought to load the first amino acid to alkanesulfonamide linker **11** with high loading efficiencies and low levels of racemization. In initial studies, **11** proved quite unreactive, failing to react using DICI and HOBt activation. Acylation with HOBt esters proceeds only with the addition of catalytic DMAP, indicating that the reactivity of the alkanesulfonamide is comparable to that of an alcohol. Linker **11** was subjected to several coupling conditions (Table 1), followed by activation with iodoacetonitrile and nucleophilic displacement with MeO-Leu-NH_2 to provide dipeptide **14** (Scheme 3). Reversed-phase HPLC analysis was used to determine the extent of racemization (% D,L-epimer observed). Acylation of **11** using symmetrical anhydrides

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(13) Gordon, E. M.; Gallop, M. A.; Patel, D. V. *Acc. Chem. Res.* **1996**, *29*, 144.

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(15) For the use of Kaiser's oxime linker see: Osapay, G.; Taylor, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 6046.

(16) For the use of Kaiser's oxime linker, see: (a) Voyer, N.; Lavoie, A.; Pinette, M.; Bernier, J. *Tetrahedron Lett.* **1994**, *35*, 355. For "safety-catch" strategies to prepare peptide amides and cyclic peptides, see: (b) Marshall, D. L.; Liener, I. E. *J. Org. Chem.* **1970**, *35*, 867. (c) Flanigan, E.; Marshall, G. R. *Tetrahedron Lett.* **1970**, *21*, 2403. (d) Lewalter, J.; Birr, C. *Liebigs Ann. Chem.* **1970**, *740*, 48. (e) Weieland, T.; Lewalter, J.; Birr, C. *Liebigs Ann. Chem.* **1970**, *740*, 31.

(17) In a previous report (ref 8), we demonstrated that in the iodoacetonitrile step an alkanesulfonamide linker, in contrast to arenesulfonamide linkers, provides for the complete *N*-alkylation of *N*-acylsulfonamides derived from carboxylic acids that possess α-electron-withdrawing groups. This is due to the greater basicity and, therefore, greater nucleophilicity of the alkanesulfonamide (methanesulfonamide DMSO pK_a 17.5) vs the arenesulfonamide (benzenesulfonamide DMSO pK_a 16.0). Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456.

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(19) Bunin, B. A. *The Combinatorial Index*; Academic Press: San Diego, 1998; pp 213−236.

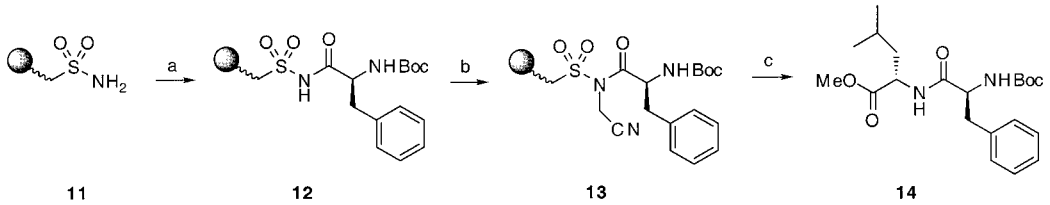
(20) This resin is now commercially available; Novabiochem catalog no. 01-64-0152.

Table 1. Optimization of First Amino Acid Loading of Alkanesulfonamide Resin 11 (Scheme 3)[a]

entry	coupling method[b]	base	solvent	yield **14**,[c] %	DL,[d] %
1	(Boc-Phe-O)$_2$ (5 equiv), DMAP (cat.)	*i*-Pr$_2$EtN (10 equiv)	DMF	90	7−10
2	(Boc-Phe-O)$_2$ (5 equiv), DMAP (cat.)	*i*-Pr$_2$EtN (10 equiv)	CH_2Cl_2	nd	5.5
3	Boc-Phe-OH (5 equiv), HATU (5 equiv)	*i*-Pr$_2$EtN (10 equiv)	DMF	nd	2.5
4	Boc-Phe-OH (5 equiv), PyBrop (5 equiv)	*i*-Pr$_2$EtN (10 equiv)	DMF	nd	3.1
5	Boc-Phe-OPfp (5 equiv), DMAP (cat.)	*i*-Pr$_2$EtN (10 equiv)	DMF	<5	<0.5
6	2 × Boc-Phe-OH (5 equiv), PyBop (5 equiv), HOBt (5 equiv)	*i*-Pr$_2$EtN (10 equiv)	DMF	<10	2.5
7	2 × Boc-Phe-OH (5 equiv), PyBop (5 equiv), HOAt (5 equiv)	*i*-Pr$_2$EtN (10 equiv)	DMF	<10	2.0
8	2 × Boc-Phe-OH (5 equiv), PyBop (5 equiv)	*i*-Pr$_2$EtN (10 equiv)	DMF	80	1.4
9	2 × Boc-Phe-OH (5 equiv), PyBop (5 equiv)	*i*-Pr$_2$EtN (10 equiv)	DMF	<30	1.5
10	2 × Boc-Phe-OH (5 equiv), PyBop (5 equiv)	collidine (10 equiv)	DMF	<10	<0.5
11	2 × Boc-Phe-OH (5 equiv), PyBop (5 equiv)	Proton Sponge (10 equiv)	DMF	<20	0.85
12	2 × Boc-Phe-OH (5 equiv), PyBop (5 equiv)	NMM (10 equiv)	DMF	nd	5.2
13	2 × Boc-Phe-OH (5 equiv), PyBop (5 equiv)	*i*-Pr$_2$EtN (10 equiv)	THF	<50	0.7
14	2 × Boc-Phe-OH (5 equiv), PyBop (5 equiv)	*i*-Pr$_2$EtN (10 equiv)	CH_2Cl_2, −20 °C	90	<0.5

[a] Abbreviations used: nd = not determined; pfp = pentafluorophenyl; Proton Sponge = 1,8-bis(*N*,*N*-dimethylamino)naphthalene; NMM = *N*-methylmorpholine. [b] Couplings endured 2−4 h. [c] Yields are based upon the initial aminomethyl resin substitution level and represent a mass balance of purified material. [d] % D,L-epimer is determined by reversed-phase HPLC analysis.

Scheme 3[a]



[a] Key: (a) coupling conditions (Table 1); (b) ICH$_2$CN, *i*-Pr$_2$EtN, NMP; (c) Leu-OMe, CH_2Cl_2.

with DMAP in DMF or CH_2Cl_2 (Table 1, entries 1 and 2) provides excellent loading efficiency, but significant epimerization. The use of coupling agents HATU (*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) and PyBrop (bromotris(pyrrolidino)phosphonium hexafluorophosphate) also results in significant levels of racemization (Table 1, entries 3 and 4). Acylation with the pentafluorophenyl active ester of Boc-Phe-OH and catalytic DMAP leads to significantly less racemization but poor loading efficiency (Table 1, entry 5). Moderate success is realized using PyBOP (benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate) with *i*-Pr$_2$EtN in DMF (Table 1, entry 8), in which a double coupling provides a 80% yield of **14** with only 1.4% of the D,L-epimer being observed. Attempts to lower the level of racemization obtained with PyBOP using different bases (Table 1, entries 10−12) or solvents (Table 1, entry 13) did not reduce racemization and resulted in lower yields. In general, the PyBOP coupling yields fall with the addition of HOBt and HOAt (7-aza-1-benzotriazole) (Table 1, entries 6 and 7), and the coupling yields are most dependable when PyBOP is added directly to the reaction mixture without a premix protocol. This indicated that the *O*-acyl phosphonium adduct is the active intermediate. Indeed, Patel and co-workers have utilized PyBOP and *i*-Pr$_2$EtN in CH_2Cl_2 at −20 °C for the acylation of alcohols in high yields.[21] Under these conditions, the *O*-acyl phosphonium adduct is proposed to be the reactive intermediate. When these conditions are employed (Table 1, entry 14), a good yield of dipeptide **14** is observed and racemization is not detected. Further optimization of loading efficiencies has been performed using Fmoc-amino acids since quantitation of the loading level by spectrophotometric methods[19] is both accurate and straightforward.

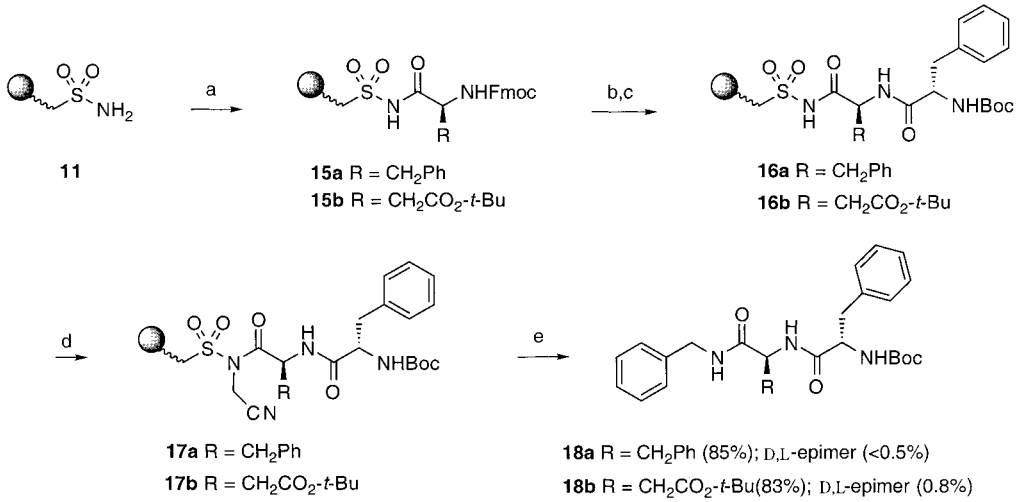
Loading Efficiency for Fmoc-Amino Acids. When our most effective loading conditions in CH_2Cl_2 are employed (Table 1, entry 14), a double coupling procedure utilizing 5 equiv of amino acid in each step is required to obtain high loading efficiencies. As well, many Fmoc-amino acid derivatives are insoluble in CH_2Cl_2 at −20 °C. Dramatic improvements in both coupling yields and solubility properties are realized when $CHCl_3$ rather than CH_2Cl_2 is employed as solvent in the coupling step. Treatment of resin **11** with Fmoc-Phe-OH (3 equiv), PyBOP (3 equiv), and *i*-Pr$_2$EtN (5 equiv) with $CHCl_3$ as solvent at −20 °C for 8 h provides excellent loading efficiencies (89%).[22] This is noteworthy since Fmoc-Phe-OH is one of the most insoluble Fmoc-amino acids.[23] Coupling with Fmoc-Asp(O-*t*-Bu)-OH employing the identical coupling procedure results in a loading efficiency of 92%. To determine the extent of racemization for each of these couplings, dipeptide amides **18a**,**b** have been prepared (Scheme 4). The α-amino group of Fmoc-amino acids **15a**,**b** are deprotected with 20% piperidine in DMF for 30 min. After several DMF washes, Boc-L-Phe-OH is coupled using DICI, HOBt activation in DMF with a short premixing period of 2 min. Activation with 1 M iodoacetonitrile and *i*-Pr$_2$EtN with NMP (1-methyl-2-pyrrolidinone) as solvent provides the support-bound *N*,*N*-cyanomethylacylsulfonamide active esters **17a**,**b**. The support-bound dipeptides are cleaved with 5 equiv of benzylamine in THF to provide dipeptide amides **18a** in 85% yield (<0.5% D,L-epimer) and **18b** in 83% yield

(21) Kim, M. H.; Patel, D. V. *Tetrahedron Lett.* **1994**, *35*, 5603.

(22) The loading yields are determined by piperidine treatment of small aliquots of resin followed by spectrophotometric analysis of the piperidine-bifulvulene adduct in solution (ref 19).

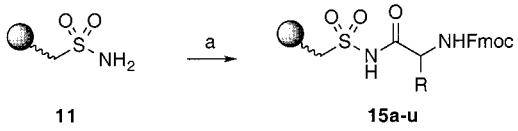
(23) Fields G. B.; Noble R. L. *Int. J. Peptide Protein Res.* **1990**, *35*, 161.

Scheme 4[a]



[a] Key: (a) Fmoc-Phe-OH (3 equiv) or Fmoc-Asp(O-*t*-Bu)-OH (3 equiv), PyBOP (3 equiv), *i*-Pr_2EtN (5 equiv), $CHCl_3$ −20 °C, 8 h; (b) 20% piperidine in DMF; (c) Boc-Phe-OH, DICI, HOBt, DMF (2 min premix); (d) ICH_2CN, *i*-Pr_2EtN, NMP; (e) $BnNH_2$ (5 equiv), THF.

Scheme 5[a]



[a] Key: (a) Fmoc-amino acid (3 equiv), PyBOP (3 equiv), *i*-Pr_2EtN (5 equiv), $CHCl_3$ −20 °C, 8 h.

(0.8% D,L-epimer). *It is important that the coupling reaction is filtered immediately after the 8 h coupling period.* Couplings allowed to proceed for longer reaction times, or allowed to come to room temperature, while providing a modest increase in loading efficiencies, result in increased levels of racemization (**18a**, 1.7% D,L-epimer).

To demonstrate the generality of our coupling conditions, a number of Fmoc-protected amino acids have been coupled to the alkanesulfonamide resin **11** (Scheme 5, Table 2). In most cases, good loading efficiencies are achieved in a single coupling step. For sterically hindered Fmoc-amino acids where couplings are less efficient, such as Fmoc-Ile-OH (65%), a double coupling provides useful levels of substitution (80%). For Fmoc-Gly-OH it is necessary to employ DMF as solvent to increase solubility.

Synthesis of Dipeptide Amides. Fmoc-amino acid loaded resins **15a**−**u** have been subjected to the short peptide amide synthesis sequence described previously (Scheme 4) to demonstrate the compatibility of the iodoacetonitrile activation step with various side chain functionality. Fmoc removal, Boc-Phe-OH coupling, iodoacetonitrile activation, and benzylamine cleavage are performed as described in the previous section with the exception that the activated support-bound dipeptides **17a**−**u** are cleaved with a limiting amount of benzylamine (ca. 0.5 equiv) in THF to provide dipeptides amides **18a**−**u** in solution (Table 3). The THF solutions are then filtered through a small plug of silica to remove resin particulates followed by elution with excess THF. Reversed-phase HPLC analysis at 254 nm is utilized to gauge the purity of the peptide products. The dipeptide amide THF solutions are concentrated and the yields are determined on the basis of the limiting reagent, benzylamine, for the mass balance of recovered material.

Table 2. Coupling of Fmoc-Amino Acids to Alkylsulfonamide Resin 11 (Scheme 5)[a]

resin product	Fmoc-amino acid	yield,[b,c] %
15c	Fmoc-Ala-OH	93
15d	Fmoc-Arg(Pbf)-OH	85
15e	Fmoc-Asn(Trt)-OH	81
15b	Fmoc-Asp(O-*t*-Bu)-OH	92
15f	Fmoc-Cys(Trt)-OH	>95
15g	Fmoc-Glu(O-*t*-Bu)-OH	81
15h	Fmoc-Gln(Trt)-OH	77
15i	Fmoc-Gly-OH	89[e]
15j	Fmoc-His(Trt)-OH	>95
15k	Fmoc-His(Boc)-OH	89
15l	Fmoc-Ile-OH	65 (80)[d]
15m	Fmoc-Leu-OH	80
15n	Fmoc-Lys(Boc)-OH	80
15o	Fmoc-Met-OH	>95
15a	Fmoc-Phe-OH	89
15p	Fmoc-Pro-OH	44 (65)[d]
15q	Fmoc-Ser(O-*t*-Bu)-OH	77
15r	Fmoc-Thr(O-*t*-Bu)-OH	55 (70)[d]
15s	Fmoc-Trp(Boc)-OH	80
15t	Fmoc-Tyr(O-*t*-Bu)-OH	76
15u	Fmoc-Val-OH	69 (84)[d]

[a] Abbreviation used: Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl. [b] Yields determined by Fmoc analysis. [c] Couplings were terminated after 8 h. [d] Yield for a double coupling. [e] DMF is employed as solvent with Fmoc-Gly-OH (4 equiv) and *i*-Pr_2EtN (6 equiv).

Concentrated samples were directly submitted for elemental analysis without further purification. High levels of purity are observed for most products. It should be noted that for cases in which the amino acid-substituted resin employed may contain free, unreacted alkanesulfonamide (Fmoc-Ile **15l**), no benzylamide product derived from Boc-Phe-OH coupling to the free alkylsulfonamide is observed. Yields are uniformly high for the dipeptide products **18a**−**u**, and satisfactory elemental analyses were obtained for a significant number of derivatives (18 out of 20). For those dipeptide products where elemental analyses were not satisfactory, ^1H NMR showed a high level of purity. It is notable that significant *S*-alkylation of the thioether-containing side chains of Met and Cys(Trt) did not occur in the iodoacetonitrile activation step, and dipeptide products **18o** and **18f** were obtained in good yields. While loading proceeds in high efficiency for Fmoc-His(Trt)-OH (Table 2, >95%), no

Table 3. Coupling, Activation, and Nucleophilic Displacement with Support-Bound Fmoc-Amino Acids (Scheme 4)[a]

resin starting material	support-bound Fmoc-amino acid	dipeptide product	yield,[b] %	HPLC purity,[c] %
15c	Fmoc-Ala	**18c**	90	94
15d	Fmoc-Arg(Pbf)	**18d**	83	93
15e	Fmoc-Asn(Trt)	**18e**	69[d]	89
15b	Fmoc-Asp(O-*t*-Bu)	**18b**	78	90
15f	Fmoc-Cys(Trt)	**18f**	54[d]	87
15g	Fmoc-Glu(O-*t*-Bu)	**18g**	90	92
15h	Fmoc-Gln(Trt)	**18h**	93	94
15i	Fmoc-Gly	**18i**	89	96
15j	Fmoc-His(Trt)	**18j**	–	–
15k	Fmoc-His(Boc)	**18k**	91	83
15l	Fmoc-Ile	**18l**	89	90
15m	Fmoc-Leu	**18m**	91	95
15n	Fmoc-Lys(Boc)	**18n**	89	94
15o	Fmoc-Met	**18o**	78	75
15a	Fmoc-Phe	**18a**	89	91
15p	Fmoc-Pro	**18p**	95	93
15q	Fmoc-Ser(*t*-Bu)	**18q**	96	95
15r	Fmoc-Thr(*t*-Bu)	**18r**	96	94
15s	Fmoc-Trp(Boc)	**18s**	83	97
15t	Fmoc-Tyr(*t*-Bu)	**18t**	89	94
15u	Fmoc-Val	**18u**	88	92

[a] Abbreviations used: Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl. [b] Yields of analytically pure material based upon $BnNH_2$ as the limiting agent. [c] Reversed-phase HPLC analysis with detection at 254 nm. [d] Unsatisfactory elemental analysis.

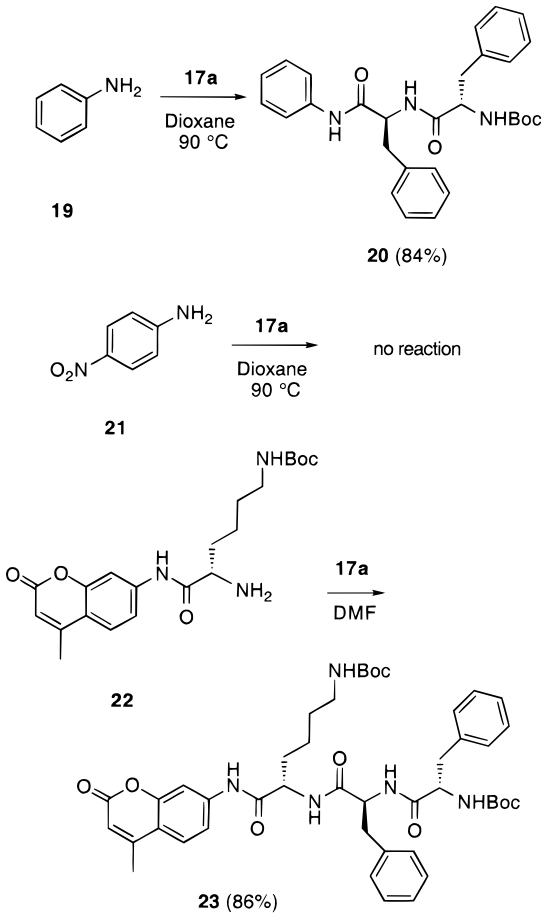
dipeptide product was obtained upon activation and cleavage. It is likely that, in this case, alkylation of the imidazole functionality occurs in the iodoacetonitrile activation step and decomposition follows. However, high loading efficiencies and high yields of dipeptide product are observed when commercially available Fmoc-His(Boc)-OH is employed.

Displacement with Various Nucleophiles. The condensation of amino acid methyl esters with activated support-bound Boc-amino acids to provide dipeptide esters (Table 1) and the cleavage of support-bound dipeptides **17a**–**u** with benzylamine (Scheme 4, Table 3) occur at ambient temperature and have been demonstrated to be racemization-free and high yielding. Poor nucleophiles such as aniline also displace **17a** to provide peptide anilide **20** in 84% yield, although forcing conditions are required (Scheme 6). Attempts to displace **17a** with 4-nitroaniline failed to give the desired product. Fluorogenic peptide substrates derived from 4-nitroaniline and 7-amino-4-methylcoumarin have many uses in enzymology. Because displacement of **17a** with 7-amino-4-methylcoumarin also failed to provide the anilide product, we sought an alternative route to provide these fluorogenic peptide substrates. Anilide **22**, prepared by coupling 7-amino-4-methylcoumarin and lysine, can be condensed with **17a** at ambient temperatures to provide a high yield of tripeptide **23**.

Conclusion

An alkanesulfonamide linker has been developed for solid-phase synthesis efforts. Numerous Fmoc-amino acid derivatives can be loaded efficiently without significant levels of racemization being observed. To load sterically hindered amino acids that typically provide less efficient couplings, a double coupling protocol provides useful levels of substitution. A number of the support-bound

Scheme 6



amino acids have been subjected to a short synthesis sequence to provide support-bound dipeptides. Treatment with iodoacetonitrile then provides an activated *N*-cyanomethyl derivative that can be cleaved with a variety of nucleophiles to provide dipeptide *C*-terminal derivatives. Cleavage with amines, amino acid esters, and aniline proceeds in high yields to provide amide, peptide, and anilide products, respectively. All 20 of the proteinogenic amino acids, when suitably protected, are compatible with the loading and activation steps.

These methods should be applicable to the solid-phase synthesis of several classes of small organic molecules in which amino acids are employed as the initial linker-bound building block. As well, these methods are clearly applicable to solid-phase peptide chemistry for use in segment condensation strategies and for the synthesis of cyclic peptides and peptide amides. In future work, we will apply these general strategies to synthesize fluorogenic peptide substrates for the preparation of positional-scanning combinatorial libraries to determine protease specificities.[24]

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Aminomethyl Merrifield resin was purchased from Novabiochem, and the aminomethyl substitution level of the resin was determined (0.84 mequiv/gram) by exhaustive

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acylation with Fmoc-Ala-OH followed by a spectrophotometric Fmoc-quantitation assay.[19] Fmoc-amino acids were purchased from Novabiochem. Anhydrous NMP and DMF were purchased from Aldrich. Tetrahydrofuran (THF) was distilled under N_2 from sodium/benzophenone prior to use, and CH_2Cl_2 was distilled under N_2 from CaH_2. Iodoacetonitrile and chloroform were filtered through a small plug of basic alumina prior to use. Chromatography was carried out using Merck 60 230−240 mesh silica gel according to the procedure of Still.[25] Thin-layer chromatography was carried out on Merck 60 F_{254} 250-μm silica gel plates. IR spectra were recorded neat (for oils) and as films from CH_2Cl_2 or $CHCl_3$ (for crystalline compounds), and only partial data are reported. ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$, unless otherwise stated. NMR chemical shifts are reported in ppm downfield from an internal solvent peak, or TMS, and J values are in hertz. Elemental analyses were performed by M−H−W Labs, Phoenix, AZ. All solid-phase yields reported are based upon the initial aminomethyl substitution level of the resin and constitute a mass balance of analytically pure material from support, unless otherwise stated. All HPLC assays were performed utilizing a Rainin Dynamax Microsorb C18 reversed-phase analytical column.

General Methods for Solid-Phase Synthesis. Overhead mechanical stirring was utilized for reactions in which >2 g resin was employed. *When employing overhead stirring, it is important that the stirring paddle does not contact the bottom of the flask to prevent degradation of the resin.* Reactions employing <2 g of resin were performed in 24/40 50 mL round-bottom flasks or fritted polypropylene syringe cartridges with agitation provided by a magnetic stir bar (Fisher Scientific, 13 mm × 3.2 mm octagonal stir bar) or a stream of N_2, respectively. Solvents were not distilled for resin washes and were removed from the resin by a filtration cannula (Pharmacia, Uppsala, Sweden) for reactions performed in round-bottom flasks or by suction provided by an aspirator for reactions performed in syringe cartridges. In cases where >2 g of resin was employed, washes were applied to the resin in a fritted glass funnel. Individual resin washes endured 4−5 min, unless otherwise stated.

Dimethyl 4,4′-Dithiobutyrate (7). To a 500 mL round-bottom flask were added 4,4′-dithiobutyric acid **6** (21 g, 0.089 mol), $SOCl_2$ (15 mL, 0.20 mol), and MeOH (200 mL). The flask was fitted with a reflux condenser, and the solution was heated at reflux for 4 h, after which the flask was cooled with an ice bath. Water (100 mL) was added very slowly, and the solution was extracted with CH_2Cl_2 (2 × 200 mL). The organic layer was washed with 1 M $NaHCO_3$ (3 × 200 mL), dried with Na_2SO_4, and concentrated to give 23.5 g of unpurified material. The crude material was distilled (bp 136−138 °C, 0.65 mm) to afford 21.4 g (90%) of **7** as a colorless oil: IR 1738 cm^{-1}; ^{1}H NMR (400 MHz) δ 2.00 (quintet, 4, J = 7.2), 2.42 (t, 4, J = 7.2), 2.69 (t, 4, J = 7.2), 3.65 (s, 6); ^{13}C NMR (101 MHz) δ 24.2, 32.3, 37.8, 51.6, 173.4. Anal. Calcd for $C_{10}H_{18}S_2O_4$: C, 45.09; H, 6.81. Found: C, 44.88; H, 6.64.

Methyl 4-(Chlorosulfonyl)butyrate (8). To a 300 mL three-neck round-bottom flask were added **7** (14.6 g, 55.0 mmol) and H_2O (100 mL). Cl_2 gas was bubbled in slowly with vigorous stirring for 5 h. It is important that the chlorine gas is added at a slow rate. After N_2 gas was bubbled through the solution for 1 h to remove excess Cl_2, the solution was extracted with CH_2Cl_2 (2 × 100 mL), dried with Na_2SO_4, and concentrated to give 14.6 g (67%) of unpurified material. The product was sufficiently pure by NMR analysis and was taken on to the next step. A small amount was distilled (bp 98−99 °C, 0.65 mm) to provide a sample for elemental analysis: IR 1733 cm^{-1}; ^{1}H NMR (400 MHz) δ 2.33 (quintet, 2, J = 6.9), 2.42 (t, 2, J = 6.9), 3.68 (s, 3), 3.78 (t, 2, J = 6.9); ^{13}C NMR (101 MHz) δ 19.8, 31.0, 52.0, 64.1, 172.1. Anal. Calcd for $C_5H_9SO_4$: C, 29.93; H, 4.52. Found: C, 29.74; H, 4.61.

Methyl 4-Sulfamoylbutyrate (9). To a 100 mL round-bottom flask were added **8** (4.65 g, 23.2 mmol) and Et_2O (50 mL). The flask was cooled with an ice bath, and ammonia gas was bubbled in slowly until the formation of precipitate ceased. The solution was concentrated, and H_2O (10 mL) was added. The aqueous layer was extracted with hot EtOAc (3 × 50 mL), and the organic layers were pooled, dried with Na_2SO_4, and concentrated to give 3.4 g (81%) of a light yellow oil that solidified upon standing. The product was sufficiently pure by NMR analysis and was taken on to the next step. To provide a sample of analytical purity, 2 g of material was purified by column chromatography (5 cm × 20 cm eluted with 70:30 EtOAc/hexanes) to yield **9** as a colorless solid: mp 49−50 °C; IR 3323, 1734 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.89 (quintet, 2, J = 7.5), 2.45 (t, 2, J = 7.5), 2.96 (t, 2, J = 7.5), 3.56 (s, 3), 6.78 (s, 2); ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.7, 31.9, 51.8, 53.9, 173.2. Anal. Calcd for $C_5H_{11}SO_4N$: C, 33.14; H, 6.19; N, 7.73. Found: C, 32.95; H, 6.04; N, 7.50.

3-Carboxypropanesulfonamide (10). To a 100 mL round-bottom flask were added **9** (3.85 g, 21.3 mmol) and an aqueous KOH solution (1.8 M, 25 mL). The flask was fitted with a condenser, and the mixture was heated at reflux for 4 h followed by cooling with an ice bath. An aqueous HCl solution was added dropwise (1.8 M, 25 mL), and the solution was concentrated under aspirator pressure to a volume of 2−3 mL. An equal volume of acetone was then added, and the KCl salts were filtered. The solution was concentrated and dried in a desiccator under vacuum with P_2O_5 to give 3.0 g (85%) of **10** as a colorless solid. A sample was prepared for elemental analysis by filtration through a plug of silica (20:80 MeOH/CH_2Cl_2): mp 93−95 °C; IR 1738 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.84 (quintet, 2, J = 7.4), 2.34 (t, 2, J = 7.4), 2.95 (t, 2, J = 7.4), 6.75 (s, 2); ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.7, 32.7, 54.1, 78.7, 174.3. Anal. Calcd for $C_4H_9SO_4N$: C, 28.73; H, 5.42; N, 8.38. Found: C, 28.66; H, 5.52; N, 8.16.

Alkanesulfonamide Resin 11. To a 250 mL three-neck round-bottom flask fitted with an overhead stirrer were added aminomethyl polystyrene resin (15 g, 5.9 mmol), **10** (3.1 g, 16 mmol), DICI (2.4 mL, 16 mmol), HOBt (2.1 g, 16 mmol), and DMF (130 mL). After the slurry was stirred for 4 h, the reaction was complete as determined by a bromophenol blue test.[19] The resin was washed with DMF (3 × 100 mL), CH_2Cl_2 (3 × 100 mL), and MeOH (3 × 100 mL) and dried in a desiccator under vacuum with P_2O_5.

Iodoacetonitrile Activation. A general procedure for iodoacetonitrile activation to prepare a support-bound *N*,*N*-cyanomethylacylsulfonamide is described. The resin-bound *N*-acylsulfonamide (500 mg, 0.25 mmol) was washed with several portions of NMP. To the swollen resin were added NMP (4 mL) and *i*-Pr_2EtN (240 μL, 1.25 mmol). After filtration through an alumina basic plug proir to use, iodoacetonitrile (367 μL, 5 mmol) was added to the reaction mixture, and the reaction flask was shielded from light. The resin was agitated for 24 h, filtered, and washed with NMP (5 × 5 mL, >10 min wash times) and CH_2Cl_2 (3 × 5 mL) or THF (3 × 5 mL).

Optimization of Resin 11 Loading Procedure (Table 1). Coupling experiments were performed using a symmetrical anhydride, a pentafluorophenol ester, or Boc-Phe-OH (0.3−0.5 M) and the solvent, base, and additives listed in Table 1 with resin **11** (200 mg, 0.14 mmol). Typically, a 2−4 h reaction time was employed, after which the resin was washed with NMP (3 × 4 mL) and activated for cleavage with iodoacetonitrile. Leu-OMe (124 mg, 0.85 mmol) in CH_2Cl_2 (1.5 mL) that had been free-based by extraction was added to the reaction flask, and the mixture was stirred for 12 h. The resin was filtered and washed with CH_2Cl_2 (3 × 5 mL), and the organic layers were combined, washed with 0.1 M $NaHSO_4$ (2 × 20 mL), and dried with Na_2SO_4. The unpurified material was subjected to HPLC analysis to determine the percent racemization in the initial coupling step. Optically pure L,L- and D,L-epimers of Boc-Phe-Leu-OMe were prepared as standards in solution using HATU and collidine in DMF[26] and subjected to HPLC analysis (MeOH/H_2O−0.1% TFA, 10%−100% for 60 min, 1 mL/min, 260 nm). The L,L-epimer eluted at 48.6 min,

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(26) Carpino, L. A.; Elfaham, A. *J. Org. Chem.* **1994**, *59*, 695.

and the D,L-epimer eluted at 49.3 min. For cases (Table 1) in which yields are given, purification on silica gel (1.5 × 10 cm eluted with 20:80 EtOAc/hexanes) provided **Boc-L-Phe-L-Leu-OMe (11)**: mp 101−104 °C; IR 3294, 2959, 1751, 1684, 1652 cm^{-1}; ^1H NMR (300 MHz) δ 0.90 (d, 6, J = 6.6), 1.40−1.44 (m, 10), 1.50−1.53 (m,1), 1.52−1.55 (m, 1), 3.07 (t, 2, J = 7.0), 3.69 (s, 3), 4.33−4.35 (m, 1), 4.50−4.52 (m, 1) 4.97 (bs, 1), 6.25 (d, 1, J = 8.1), 7.18−7.31 (m, 5); ^{13}C NMR (101 MHz) δ 21.9, 22.7, 24.6, 28.2, 38.1, 41.7, 50.7, 55.7, 52.2, 80.2, 126.9, 128.6, 129.4, 136.6, 155.6, 170.9, 172.8.

Optimized Resin 11 Loading Procedure for Fmoc-Amino Acids. To a 50 mL round-bottom flask were added resin **11** (200 mg, 0.15 mmol), $CHCl_3$ (1.7 mL) that had been filtered through an alumina basic plug, *i*-Pr_2EtN (143 μL, 0.750 mmol), and an Fmoc-amino acid (0.45 mmol). The reaction mixture was stirred for 10 min followed by cooling to −20 °C. After 20 min, PyBop (234 mg, 0.450 mmol) was added to the reaction mixture as a solid, and the reaction mixture was stirred for 8 h, filtered, and washed with $CHCl_3$ (3 × 5 mL). *It is important to terminate the reaction after 8 h in order to minimize racemization.* For coupling with Fmoc-Gly-OH, 4 equiv of amino acid and 6 equiv of *i*-Pr_2EtN were employed with DMF as solvent, and the reaction mixture was allowed to come to room temperature overnight after the 8 h coupling period.

General Procedure for the Preparation of Dipeptide Products 18a and 18b. To a 12 mL syringe reactor cartridge was added a support-bound Fmoc-amino acid resin **15a** or **15b** (150 mg, ca. 0.075 mmol), followed by solvation with DMF. A 20% piperidine in DMF solution (2 mL) was added to the reactor, and the mixture was agitated for 40 min. The resin was then filtered and washed with DMF (3 × 2 mL). To a separate 10 mL flask were added Boc-L-Phe-OH (160 mg, 0.60 mmol), HOBt (81 mg, 0.60 mmol), DICI (94 μL, 0.60 mmol), and DMF (2 mL). After a 2−3 min premix period, the solution was added to the resin-containing syringe reactor followed by agitation for 2−3 h. A bromophenol blue test was employed to determine reaction completion.[19] The resin was filtered and washed with DMF (3 × 3 mL) and NMP (3 × 3 mL). After activation with iodoacetonitrile, the resin was washed with NMP (4 × 3 mL, 10 min/wash), CH_2Cl_2 (3 × 3 mL), and THF (3 × 3 mL) and transferred to a 50 mL round-bottom flask. To the resin-containing flask were added THF (2 mL) and benzylamine (40 μL, 0.38 mmol), and the reaction mixture was stirred for 4 h. The mixture was taken up in CH_2Cl_2 (20 mL), extracted with 1 M $NaHSO_4$ (3 × 20 mL), dried with Na_2SO_4, and concentrated. The unpurified material was analyzed for epimerization by HPLC assay in which authentic samples of L,L-epimers and D,L-epimers of the dipeptide amides served as standards. Silica gel column chromatography followed to provide the respective products in analytically pure form.

Boc-L-Phe-L-Phe-NHBn (18a). Cleavage of resin **17a** (150 mg, 0.088 mmol) provided 39 mg (85%) of **18a** as a colorless solid after purification on silica gel (1.5 × 10 cm eluted with 30:70 EtOAc/hexanes): mp 129−131 °C; IR 1687, 1645, 1530 cm^{-1}; ^1H NMR (300 MHz) δ 1.23 (s, 9), 2.87 (dd, 1, J = 7.0, J = 13.5), 2.98−3.01 (m, 2), 3.25 (dd, 1, J = 4.0, J = 13.5), 4.22−4.29 (m, 2), 4.33 (dd, 1, J = 5.8, J = 13.8), 4.66−4.4.67 (m, 1), 4.77 (bs, 1), 6.3 (bs, 1), 6.44 (bs, 1), 7.00−7.03 (m, 2), 7.09 (d, 2, J = 7.8), 7.14 (d, 2, J = 6.0), 7.16−7.20 (m, 9); ^{13}C NMR (101 MHz) δ 27.2, 37.2, 37.4, 42.7, 54.6, 57.9, 79.7, 126.4, 126.4, 126.7, 127.0, 127.1, 128.0, 128.1, 128.2, 128.9, 129.0, 136.9, 138.1, 165.1, 171.7, 172.8. Anal. Calcd for $C_{30}H_{35}N_3O_4$: C, 71.80; H, 7.03; N, 8.38. Found: C, 71.60; H, 6.83; N, 8.39.

Boc-L-Phe-L-Asp(O-*t*-Bu)-NHBn (18b). Cleavage of resin **17b** (150 mg, 0.085 mmol) provided 37 mg (83%) of **18b** as a colorless solid after purification on silica gel (1.5 × 10 cm eluted with 30:70 EtOAc/hexanes): mp 153−155 °C; IR 1724, 1691, 1648, 1535 cm^{-1}; ^1H NMR (300 MHz) δ 1.32 (s, 9), 1.39 (s, 9), 2.43 (dd, 1, J = 5.7, 17.0), 2.97−2.99 (m, 2), 3.10 (dd, 1, J = 5.4, 13.5), 4.26−4.28 (m, 2), 4.40 (dd, 1, J = 6.1, 15.0), 4.74−4.76 (m, 1), 4.86 (bs, 1), 6.99 (bs, 1), 7.17−7.30 (m, 11); ^{13}C NMR (101 MHz) δ 26.9, 27.2, 36.5, 37.3, 42.8, 49.8, 56.4, 79.6, 81.2, 126.4, 126.8, 127.0, 128.0, 128.1, 129.0, 137.1, 138.2, 156.6, 170.2, 171.1, 172.8. Anal. Calcd for $C_{29}H_{39}N_3O_6$: C, 66.20; H, 7.48; N, 7.99. Found: C, 66.08; H, 7.60; N, 7.66.

Preparation of Dipeptide Amides 18a−u. The general procedures for Fmoc deprotection, Boc-Phe-OH coupling, iodoacetonitrile activation, and $BnNH_2$ displacement were followed with the exception that a limiting amount of $BnNH_2$ was employed in the cleavage step. A stock solution of freshly distilled $BnNH_2$ in THF (100 mg in a 25 mL volumetric flask, 0.037 M) was prepared, and 1.00 mL (0.037 mmol) of this solution was added to resins **17a−u**. The resin-containing flask was flushed with argon gas, and the reaction mixture was stirred for 24 h. The solution was then filtered through a small silica plug, and several volumes of THF (total volume 25 mL) were applied. The solution was subjected to reversed-phase HPLC analysis (MeOH/H_2O−0.1% TFA, 10−100% for 30 min, 1 mL/min, 254 nm detection for 40 min) to determine the purity. The samples were then concentrated to obtain a yield based upon $BnNH_2$. The sample was submitted for ^1H NMR and elemental analysis without further purification.

Boc-L-Phe-L-Ala-NHBn (18c). To *N*-cyanomethylated resin **17c** was added benzylamine in THF (0.028 M solution, 1.67 mL, 0.047 mmol) to provide 18 mg (90%) of compound **18c** as a colorless solid: reversed-phase HPLC analysis (22.4 min, 94%); TLC (40:60 EtOAc/hexanes, R_f = 0.44); ^1H NMR (300 MHz) δ 1.34 (d, 3, J = 7.4), 1.35 (s, 9), 3.01−3.04 (m, 2), 4.29−4.31 (m, 1) 4.38 (d, 2, J = 5.8), 4.44−4.51 (m, 1), 4.89 (bs, 1), 6.36 (d, 1, J = 7.2), 6.59 (bs, 1), 7.15 (d, 2, J = 6.1), 7.22−7.34 (m, 8). Anal. Calcd for $C_{24}H_{31}N_3O_4$: C, 67.70; H, 7.34; N, 9.87. Found: C, 67.90; H, 7.17; N, 9.68.

Boc-L-Phe-L-Arg(Pbf)-NHBn (18d). To *N*-cyanomethylated resin **17d** was added benzylamine in THF (0.037 M solution, 1.0 mL, 0.037 mmol) to provide 25 mg (86%) of compound **18d** as a colorless solid: reversed-phase HPLC analysis (26.1 min, 93%); TLC (70:30 EtOAc/hexanes, R_f = 0.20); ^1H NMR (300 MHz) δ 1.30 (s, 9), 146−1.53 (m, 4), 165−1.69 (m, 1), 1.81 (m, 6), 1.83−1.89 (m, 1), 2.08 (m, 3), 2.47 (s, 3), 2.55 (s, 3), 2.83−2.93 (m,3), 3.07 (dd, 1, J = 5.3, J = 13.7), 3.22−3.25 (m, 2), 4.29−4.36 (m, 3), 4.47−4.50 (m, 1), 5.18 (bs, 1), 6.19−6.26 (m, 3), 7.10−7.12 (m, 2), 7.17−7.31 (m, 8), 7.33−7.42 (m, 2). Anal. Calcd for $C_{41}H_{56}N_6SO_7$: C, 63.30; H, 7.26; N, 10.80. Found: C, 63.53; H, 7.31; N, 10.91.

Boc-L-Phe-L-Asn(Trt)-NHBn (18e). To *N*-cyanomethylated resin **17e** was added benzylamine in THF (0.037 M solution, 1.0 mL, 0.037 mmol) to provide 16 mg (69%) of compound **18e** as a colorless solid: reversed-phase HPLC analysis (31.5 min, 89%); TLC (70:30 EtOAc/hexanes, R_f = 0.50); ^1H NMR (300 MHz) δ 1.26 (s, 9), 2.50 (dd, 1, J = 5.5, J = 15.3), 2,87 (dd, 1, J = 8.0, J = 13.7), 3.04−3.15 (m, 2), 4.22−4.29 (m, 2), 4.40−4.49 (m, 1), 4.73−4.76 (m, 1), 4.80 (d, 1, J = 4.1), 6.89 (s, 1), 7.05 (bs, 1), 7.11−7.17 (m, 11), 7.22−7.33 (m, 14), 7.58−7.60 (bs, 1).

Boc-L-Phe-L-Asp(O-*t*-Bu)-NHBn (18b). To *N*-cyanomethylated resin **17b** was added benzylamine in THF (0.037 M solution, 1.0 mL, 0.037 mmol) to provide 15 mg (79%) of compound **18b** as a colorless solid: reversed-phase HPLC analysis (26.4 min, 91%); TLC (40:60 EtOAc/hexanes, R_f = 0.36); ^1H NMR data were identical to those described previously. Anal. Calcd for $C_{29}H_{39}N_3O_6$: C, 66.20; H, 7.48; N, 7.99. Found: C, 66.02; H, 7.49; N, 7.76.

Boc-L-Phe-L-Cys(Trt)-NHBn (18f). To *N*-cyanomethylated resin **17f** was added benzylamine in THF (0.037 M solution, 1.0 mL, 0.037 mmol) to provide 14 mg (54%) of compound **18f** as a colorless solid: reversed-phase HPLC analysis (22.4 min, 87%); TLC (40:60 EtOAc/hexanes, R_f = 0.42); ^1H NMR (300 MHz) δ 1.23 (s, 9), 2.41 (dd, 1, J = 5.0, 12.4), 2.84 (dd, 1, J = 7.8, 14.0), 2.90−2.93 (m, 1) 3.03 (dd, 1, J = 5.9, 14.0), 4.20−4.33 (m, 3), 4.45 (dd, 1, J = 5.7, 16.6), 4.76 (m, 1), 6.29 (d, 1, J = 7.0), 6.73 (bs, 1), 7.13 (d, 2, J = 7.6), 7.18−7.33 (m, 15) 7.36−7.39 (m, 8).

Boc-L-Phe-L-Gln(Trt)-NHBn (18g). To *N*-cyanomethylated resin **17g** was added benzylamine in THF (0.037 M solution, 1.00 mL, 0.037 mmol) to provide 25 mg (93%) of compound **18g** as a colorless solid: reversed-phase HPLC analysis (29.1 min, 94%); TLC (70:30 EtOAc/hexanes, R_f = 0.46); ^1H NMR (300 MHz) δ 1.30 (s, 9), 1.82−1.99 (m, 2), 2.25−

2.34 (m, 1), 2.45−2.53 (m, 1), 2.86 (dd, 1, J = 8.0, 13.9), 3.06 (dd, 1, J = 5.4, 13.9), 4.23−4.29 (m, 2), 4.31−4.35 (m, 2), 4.85 (d, 1 J = 6.9), 6.92 (bs, 1), 7.04 (s, 1), 7.10−7.13 (m, 2), 7.17−7.31 (m, 23), 7.37 (bs, 1). Anal. Calcd for $C_{45}H_{48}N_4O_5$: C, 74.50; H, 6.67; N, 7.73. Found: C, 74.48; H, 6.57; N, 7.54.

Boc-L-Phe-L-Glu(O-*t*-Bu)-NHBn (18h). To *N*-cyanomethylated resin **17h** was added benzylamine in THF (0.037 M solution, 1.00 mL, 0.037 mmol) to provide 18 mg (90%) of compound **18h** as a colorless solid: reversed-phase HPLC analysis (24.5 min, 92%); TLC (40:60 EtOAc/hexanes, R_f = 0.32); ^{1}H NMR (300 MHz) δ 1.35 (s, 9), 1.42 (s, 9), 1.90−1.95 (m, 1), 2.05−2.09 (m, 1), 2.21−2.23 (m, 1), 2.30−2.33 (m, 1), 3.04 (d, 2, J = 6.6), 4.30−4.33 (m, 1), 4.37−4.46 (m, 3), 4.93 (d, 1, J = 4.8), 6.86 (bs, 1), 7.08 (bs, 1), 7.14−7.17 (m, 2), 7.19−7.27 (m, 8). Anal. Calcd for $C_{30}H_{41}N_3O_6$: C, 66.70; H, 7.66; N, 7.79. Found: C, 67.00; H, 7.78; N, 7.86.

Boc-L-Phe-L-Gly-NHBn (18i). To *N*-cyanomethylated resin **17i** was added benzylamine in THF (0.028 M solution, 1.67 mL, 0.047 mmol) to provide 17 mg (89%) of compound **18i** as a colorless solid: reversed-phase HPLC analysis (23.4 min, 96%); TLC (70:30 EtOAc/hexanes, R_f = 0.32); ^{1}H NMR (300 MHz) δ 1.33 (s, 9), 2.97 (dd, 1, J = 5.5, 15.5), 3.09 (dd, 1, J = 6.6, 15.5), 3.82 (dd, 1, J = 5.7, 16.7), 3.96 (dd, 1, J = 6.1, 16.7), 4.22−4.29 (m, 1), 4.35 (dd, 1, J = 5.3, 15.0), 4.44 (dd, 1, J = 6.2, 15.0), 6.52 (bs, 1), 4.97 (d, 1, J = 6.3), 6.81 (bs, 1), 7.15−7.18 (m, 2), 7.22−7.33 (m, 8). Anal. Calcd for $C_{23}H_{29}N_3O_4$: C, 67.10; H, 7.10; N, 10.20. Found: C, 67.20; H, 6.90; N, 10.02.

Boc-L-Phe-L-His(Boc)-NHBn (18k). To *N*-cyanomethylated resin **17k** was added benzylamine in THF (0.028 M solution, 1.67 mL, 0.047 mmol) to provide 20 mg (91%) of compound **18k** as a colorless solid: reversed-phase HPLC analysis (26.4 min, 83%); TLC (70:30 EtOAc/hexanes, R_f = 0.28); ^{1}H NMR (300 MHz) δ 1.22 (s, 9), 1.66 (s, 9), 2.77 (dd, 1, J = 4.9, 14.7), 2.99 (dd, 1, J = 8.6, 14.1), 3.19 (dd, 1, J = 4.9, 14.1), 3.29 (dd, 1, J = 4.9, 14.7), 4.28 (dd, 1, J = 4.7, 15.3), 4.33−4.36 (m, 1), 4.52 (dd, 1, J = 7.1, 15.3), 4.70−4.79 (m, 1), 5.01 (d, 1, J = 4.7), 6.92 (d, 2, J = 7.5), 7.14−7.36 (m, 10), 7.88 (s, 1), 8.31 (d, 1, J = 7.2). Anal. Calcd for $C_{32}H_{41}N_5O_6$: C, 64.96; H, 6.98; N, 11.84. Found: C, 64.96; H, 7.18; N, 11.84.

Boc-L-Phe-L-Ile-NHBn (18l). To *N*-cyanomethylated resin **17l** was added benzylamine in THF (0.037 M solution, 1.00 mL, 0.037 mmol) to provide 18 mg (89%) of compound **18l** as a colorless solid: reversed-phase HPLC analysis (27.3 min, 90%); TLC (40:60 EtOAc/hexanes, R_f = 0.38); ^{1}H NMR (300 MHz) δ 0.83−0.87 (m, 6), 0.92−0.96 (m, 1), 1.35 (s, 9), 1.36−1.39 (m, 1), 2.02−2.04 (m, 1), 3.03 (dd, 1, J = 4.5, 17.8), 3.06 (dd, 1, J = 3.9, 17.8), 4.26−4.28 (m, 2), 4.38 (d, 2, J = 5.7), 4.9 (bs, 1), 6.43 (d, 1, J = 8.3), 6.53 (bs, 1), 7.75−7.17 (m, 2), 7.19−7.33 (m, 8). Anal. Calcd for $C_{27}H_{37}N_3O_4$: C, 69.30; H, 7.98; N, 8.94. Found: C, 69.45; H, 8.03; N, 8.87.

Boc-L-Phe-L-Leu-NHBn (18l). To *N*-cyanomethylated resin **17m** was added benzylamine in THF (0.028 M solution, 1.67 mL, 0.047 mmol) to provide 21 mg (91%) of compound **18m** as a colorless solid: reversed-phase HPLC analysis (26.2 min, 91%); TLC (40:60 EtOAc/hexanes, R_f = 0.40); ^{1}H NMR δ 0.89 (d, 6, J = 6.4), 1.36 (s, 9), 1.41−1.50 (m, 2), 1.67−1.73 (m, 1), 3.03 (dd, 1, J = 2.8, 13.0), 3.06 (dd, 1, J = 2.3, 13.0), 4.28−4.31 (m, 1), 4.44 (d, 2, J = 4.7), 4.48−4.50 (m, 1), 4.90 (bs, 1), 6.21 (d, 1, J = 8.3), 6.55 (bs, 1), 7.17−7.19 (m, 2), 7.21−7.34 (m, 8). Anal. Calcd for $C_{27}H_{37}N_3O_4$: C, 69.30; H, 7.98; N, 8.99. Found: C, 69.32; H, 7.77; N, 8.93.

Boc-L-Phe-L-Lys(Boc)-NHBn (18n). To *N*-cyanomethylated resin **17n** was added benzylamine in THF (0.028 M solution, 1.67 mL, 0.047 mmol) to provide 24 mg (89%) of compound **18n** as a colorless solid: reversed-phase HPLC analysis (25.3 min, 94%); TLC (70:30 EtOAc/hexanes, R_f = 0.50); ^{1}H NMR (300 MHz) δ 1.23 (pentet, 2, J = 7.5), 1.36 (s, 9) 1.40−1.42 (m, 10), 1.53−1.63, (m, 1), 1.86−1.98 (m, 2), 2.95−3.05 (m, 5), 4.31−4.43 (m, 4), 4.64−4.66 (m, 1), 5.02 (bs, 1), 6.54 (d, 1, J = 8.0), 6.70 (bs, 1), 7.14 (d, 2, J = 5.8), 7.21−7.34 (m, 8). Anal. Calcd for $C_{31}H_{46}N_4O_6$: C, 65.20; H, 8.12; N, 9.82. Found: C, 65.38; H, 7.96; N, 9.67.

Boc-L-Phe-L-Met-NHBn (15o). To *N*-cyanomethylated resin **17o** was added benzylamine in THF (0.037 M solution, 1.00 mL, 0.037 mmol) to provide 14 mg (78%) of compound **18o** as a colorless solid: reversed-phase HPLC analysis (25.1 min, 75%); TLC (40:60 EtOAc/hexanes, R_f = 0.24); ^{1}H NMR (300 MHz) δ 1.34 (s, 9), 2.00−2.08 (m, 5), 2.42−2.48 (m, 2), 3.06 (d, 2, J = 6.5), 4.26−4.32 (m, 1), 4.38 (d, 2, J = 5.6), 4.59−4.63 (m, 1), 4.88 (bs, 1), 6.81−6.83 (m, 2), 7.15−7.18 (m, 2), 7.22−7.33 (m, 8). Anal. Calcd for $C_{26}H_{35}N_3O_4S$: C, 64.30; H, 7.26; N, 8.65. Found: C, 64.15; H, 7.33; N, 8.38.

Boc-L-Phe-L-Phe-NHBn (18p). To *N*-cyanomethylated resin **17p** was added benzylamine in THF (0.028 M solution, 1.67 mL, 0.047 mmol) to provide 21 mg (88%) of compound **18p** as a colorless solid: reversed-phase HPLC analysis (24.9 min, 91%); TLC (40:60 EtOAc/hexanes, R_f = 0.32); ^{1}H NMR data were identical to those provided previously. Anal. Calcd for $C_{30}H_{35}N_3O_4$: C, 71.80; H, 7.03; N, 8.38. Found: C, 71.69; H, 6.87; N, 8.34.

Boc-L-Phe-L-Pro-NHBn (18p). To *N*-cyanomethylated resin **17p** was added benzylamine in THF (0.028 M solution, 1.67 mL, 0.047 mmol) to provide 20 mg (95%) of compound **18p** as a colorless solid: reversed-phase HPLC analysis (24.3 min, 93%); TLC (70:30 EtOAc/hexanes, R_f = 0.44); ^{1}H NMR (300 MHz) δ 1.40 (s, 9), 1.82−1.86 (m, 2), 2.32−2.35 (m, 1), 2.92 (d, 2, J = 7.3), 2.66−2.98 (m, 1), 3.35−3.43 (m, 1), 3.50−3.60 (m, 1), 4.3 (dd, 1, J = 5.5, 14.9), 4.47 (dd, 1, J = 6.1, 14.9), 4.57−4.69 (m, 2), 5.26 (d, 1, J = 10.5), 7.07 (bs, 1), 7.10−7.35 (m, 10). Anal. Calcd for $C_{26}H_{33}N_3O_4$: C, 69.10; H, 7.37; N, 9.31. Found: C, 69.01; H, 7.10; N, 9.33.

Boc-L-Phe-L-Ser(*t*-Bu)-NHBn (18q). To *N*-cyanomethylated resin **17q** was added benzylamine in THF (0.028 M solution, 1.67 mL, 0.047 mmol) to provide 22 mg (96%) of compound **18q** as a colorless solid: reversed-phase HPLC analysis (27.1 min, 95%); TLC (40:60 EtOAc/hexanes, R_f = 0.30); ^{1}H NMR (300 MHz) δ 1.09 (s, 9), 1.28 (s, 9), 3.05 (dd, 1, J = 7.6, 14.0), 3.12 (dd, 1, J = 6.1, 14.0), 3.26−3.30 (m, 1), 3.93−3.95 (m, 1), 4.30−4.34 (m, 2), 4.44−4.46 (m, 1), 4.57 (dd, 1, J = 6.3, 15.2), 4.92 (bs, 1), 6.75 (d, 1, J = 7.4), 6.98 (bs, 1), 7.17−7.20 (m, 2), 7.22−7.40 (m, 8). Anal. Calcd for $C_{28}H_{39}N_3O_4$: C, 67.50; H, 7.90; N, 8.44. Found: C, 67.34; H, 7.75; N, 8.29.

Boc-L-Phe-L-Thr(*t*-Bu)-NHBn (18r). To *N*-cyanomethylated resin **17r** was added benzylamine in THF (0.028 M solution, 1.67 mL, 0.047 mmol) to provide 23 mg (96%) of compound **18r** as a colorless solid: reversed-phase HPLC analysis (28.9 min, 94%); TLC (40:60 EtOAc/hexanes, R_f = 0.42); ^{1}H NMR (300 MHz) δ 0.96 (d, 2, J = 6.2), 1.16 (s, 9), 1.36 (s, 9), 3.05−3.10 (m, 2), 4.25−4.27 (m, 2), 4.35−4.37 (m, 1), 4.41 (d, 2, J = 5.7), 4.9 (bs, 1), 6.96 (d, 1, J = 5.7), 7.17−7.19 (d, 2, J = 8.0), 7.22−7.34 (m, 9). Anal. Calcd for $C_{29}H_{40}N_3O_5$: C, 68.20; H, 7.90; N, 8.23. Found: C, 67.96; H, 7.96; N, 8.24.

Boc-L-Phe-L-Trp(Boc)-NHBn (18s). To *N*-cyanomethylated resin **17s** was added benzylamine in THF (0.037 M solution, 1.00 mL, 0.037 mmol) to provide 20 mg (83%) of compound **18s** as a colorless solid: reversed-phase HPLC analysis (29.2 min, 97%); TLC (40:60 EtOAc/hexanes, R_f = 0.40); ^{1}H NMR (300 MHz) δ 1.06 (s, 9), 1.64 (s, 9), 2.96−3.01 (m, 3), 3.36−3.38 (m, 1), 4.19−4.22 (m, 2), 4.33−4.35 (m, 1), 4.68 (d, 1, J = 5.9), 4.75−4.79 (m, 1), 6.40−6.45 (m, 2), 7.0 (d, 2, J = 6.1), 7.14−7.37 (m, 12), 8.10 (d, 1, J = 8.1). Anal. Calcd for $C_{38}H_{44}N_4O_6$: C, 69.90; H, 6.79; N, 8.58. Found: C, 69.56; H, 6.84; N, 8.81.

Boc-L-Phe-L-Tyr(*t*-Bu)-NHBn (18t). To *N*-cyanomethylated resin **17t** was added benzylamine in THF (0.028 M solution, 1.67 mL, 0.047 mmol) to provide 24 mg (89%) of compound **18t** as a colorless solid: reversed-phase HPLC analysis (28.6 min, 94%); TLC (40:60 EtOAc/hexanes, R_f = 0.38); ^{1}H NMR (300 MHz) δ 1.27 (s, 9), 1.31 (s, 9), 2.86 (dd, 1, J = 7.3, J = 13.6), 2.96−3.05 (m, 2), 3.12 (dd, 1, J = 8.8, J = 14.0), 4.22−4.38 (m, 3), 4.61−4.63 (m, 1), 4.83 (d, 1, J = 6.0), 6.34 (bs, 1), 6.82 (d, 2, J = 8.5), 6.92 (d, 2, J = 8.3), 7.12−7.20 (m, 4), 7.30−7.35 (m, 7). Anal. Calcd for $C_{34}H_{43}N_3O_5$: C, 71.10; H, 7.55; N, 7.32. Found: C, 71.10; H, 7.55; N, 7.32.

Boc-L-Phe-L-Val-NHBn (18u). To *N*-cyanomethylated resin **17u** was added benzylamine in THF (0.037 M solution, 1.00 mL, 0.037 mmol) to provide 15 mg (88%) of compound **18u** as a colorless solid: reversed-phase HPLC analysis (23.7 min,

92%); TLC (40:60 EtOAc/hexanes, $R_f = 0.36$); ^{1}H NMR (300 MHz) δ 0.82 (d, 3, $J = 6.9$), 0.89 (d, 3, $J = 6.9$), 1.34 (s, 9), 2.27−2.29 (m, 1), 3.02 (dd, 1, $J = 5.3, 19.4$), 3.05 (dd, 1, $J = 6.2, 19.4$), 4.25−4.40 (m, 4), 4.49, (bs, 1), 6.43 (d, 1, $J = 8.2$), 6.53 (bs, 1), 7.17−7.20 (m, 2), 7.22−7.33 (m, 8). Anal. Calcd for $C_{26}H_{35}N_3O_4$: C, 68.80; H, 7.78; N, 9.26. Found: C, 68.65; H, 7.67; N, 9.10.

Boc-L-Phe-L-Phe-NHPh (20). To a 50 mL round-bottom flask were added *N*-cyanomethylated resin **17a** (150 mg, 0.086 mmol), dioxane (1.2 mL), and aniline **20** (128 mg, 1.2 mmol). The mixture was heated with stirring for 15 h at reflux, diluted with CH_2Cl_2 (20 mL), extracted with 1 M $NaHSO_4$ (3 × 20 mL), dried with Na_2SO_4, and concentrated. Purification on silica gel (1.5 × 10 cm eluted with 30:70 to 50:50 EtOAc/hexanes) provided 33 mg (84%) of **23** as a colorless solid: mp 183−185 °C; IR 1690, 1648, 1600, 1534 cm^{-1}; ^{1}H NMR (300 MHz) δ 1.25 (s, 9), 2.96−3.04 (m, 3), 3.33−3.36 (m, 1), 4.28−4.30 (m, 1), 4.81−4.85 (m, 2), 6.39 (bs, 1), 7.02−7.18 (m, 3), 6.39 (bs, 1), 7.22−7.32 (m, 10), 7.31 (d, 2, $J = 6.9$), 8.09 (s, 1); ^{13}C NMR (101 MHz) δ 28.1, 37.5, 37.7, 54.3, 56.2, 80.9, 120.2, 124.4, 127.1, 127.3, 128.7, 128.8, 128.9, 129.3, 129.4, 136.0, 136.1, 137.6, 155.8, 168.8, 171.3. Anal. Calcd for $C_{29}H_{33}N_3O_4$: C, 73.80; H, 7.05; N, 8.91. Found: C, 73.78; H, 7.17; N, 8.81.

Coumarin Tripeptide (23). To a 50 mL round-bottom flask were added *N*-cyanomethylated resin **17a** (150 mg, 0.086 mmol), DMF (1.5 mL), and coumarin derivative **22** (128 mg, 0.340 mmol). After being stirred for 15 h, the reaction mixture was diluted with EtOAc (20 mL), extracted with 1 M $NaHSO_4$ (3 × 20 mL), dried (Na_2SO_4), and concentrated. Purification on silica gel (1.5 × 10 cm eluted with 50:50 to 90:10 EtOAc/hexanes) provided 59 mg (86%) of **23** as a colorless solid: mp 143−145 °C; IR 1719, 1707, 1686, 1648, 1581, 1530 cm^{-1}; ^{1}H NMR (300 MHz) δ 1.22−1.29 (m, 11), 1.41−1.56 (m, 10), 1.56−1.62 (m, 1), 2.01−2.05 (m, 2), 2.38 (d, 2, $J = 1.0$), 2.93−3.08 (m, 2), 3.10−3.17 (m, 2), 3.24−3.27 (m, 1), 4.16−4.17 (m, 1), 4.50−4.59 (m, 2), 4.67 (bs, 1), 4.98 (bs, 1), 6.16 (d, 2, $J = 1.0$), 6.54 (bs, 1), 6.98−7.00 (m, 2), 7.11−7.19 (m, 3), 7.22−7.26 (m, 3), 7.29−7.34 (m, 2), 7.50 (d, 2, $J = 9.1$), 7.83 (s, 1), 7.98 (s, 2), 8.99 (s, 1); ^{13}C NMR (101 MHz) δ 18.6, 23.5, 28.1, 28.4, 29.4, 30.9, 36.4, 37.5, 40.2, 54.4, 54.7, 57.0, 79.1, 81.3, 107.6, 113.2, 115.9, 116.2, 124.9, 127.5, 128.7, 129.0, 129.1, 129.2, 129.3, 131.2, 135.4, 141.9, 152.5, 154.1, 156.0, 156.5, 161.4, 170.6, 171.0, 172.8. Anal. Calcd for $C_{44}H_{55}N_5O_9$: C, 66.20; H, 6.95; N, 8.78. Found: C, 66.01; H, 6.59; N, 8.80.

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