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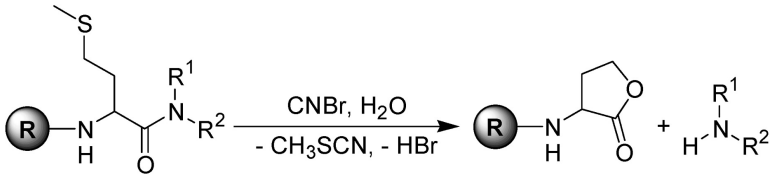

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A Convenient Orthogonally Cleavable Methionine Handle for Anchoring Amines to Polymeric Supports

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Methionine has been used to anchor amines to polymeric supports for solid-phase synthesis. Either a "preformed handle" or a stepwise elongation strategy was followed. Cyanogen bromide (CNBr) treatment then released amines into solution. CNBr reaction variables were evaluated in order to converge to optimal cleavage conditions, and the strategy was shown to be effective for a range of primary and secondary amines, but not for aromatic amines.

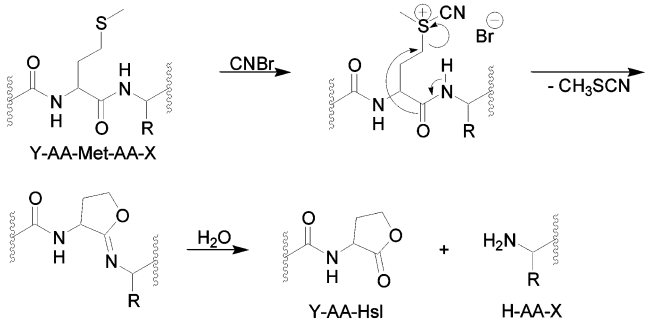
Introduction

In solid-phase synthesis, handles (linkers) provide a means for anchoring a substrate onto a polymeric support and for later releasing the target molecule into solution upon treatment with a carefully optimized cleavage cocktail.[1] In the final step, the target compound acquires the appropriate terminal functionality, the nature of which is dependent on the substrate−handle bond and the chemical mode of cleavage. Peptides, the traditional end product of solid-phase synthesis, are invariably prepared as C-terminal acids or amides, and many handles exist to accommodate these functionalities. New anchoring chemistry that provides access to further types of functionality is needed to expand the scope of solid-phase chemistry.

A staple procedure in analytical protein biochemistry is the selective cleavage of polypeptide chains at the C-terminal side of methionine (Met) residues upon treatment with cyanogen bromide (CNBr) under acidic conditions.[2] This highly selective transformation begins with S-alkylation of Met by CNBr, followed by an intramolecular cyclization and ultimate hydrolysis to provide a C-terminal homoserine lactone (Hsl) residue in place of the original Met plus the corresponding N-terminal amino moiety from the cleavage site (Scheme 1).

Handles are often designed by adapting known solution chemistry, including protecting group manipulations, that gives rise to a desired functional group with high yield and specificity. According to this general concept, it was envisaged that a Met-containing handle, in conjunction with the CNBr cleavage reaction,[3] could be applied effectively for reversible anchoring of amines to polymeric supports. The simple design and the mild conditions for both anchoring and cleavage would then provide an alternative method for solid-phase preparation of amines. Previously reported linker strategies for this purpose include (i) adaptation of acid-labile linkers and resins originally designed for amides, but applying harsher conditions (e.g., Rink,[4,5] Knorr resin,[6] 2-chlorotrityl,[7] PhFl,[8] PAL,[4] and BAL[9] supports); (ii) use of carbamate-type handles, cleavable by acid,[10] Pd(0),[11] or light;[12] (iii) direct use of Merrifield resin, which is cleaved after quaternization with α-chloroethyl chloroformate;[13] (iv) hydrazinolyzable formamidine-based anchoring[14] or 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde)-based handles;[15] (v) modification of the acidolyzable *O*-(tetrahydropyranyloxymethyl)benzamide protecting group;[16] (vi) reductively cleavable sulfonamide anchoring;[17] (vii) 2,3-dichlor-5,6-dicyano-1,4-benzoquinone (DDQ)-labile *para*-methoxybenzyl (PMB)-derived anchoring;[18] (viii) hydrazine[19] and triazine-based handles;[20] (ix) a safety-catch linker based on internal diketopiperazine release;[21] and (x) a trimellitic anhydride linker that is cleaved under a variety of selective conditions.[22]

Scheme 1. CNBr Cleavage of Peptides and Proteins



Results and Discussion

Initial experiments reported herein examined Met-containing "preformed handles", which were prepared according to

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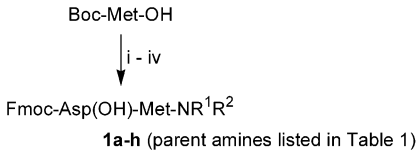
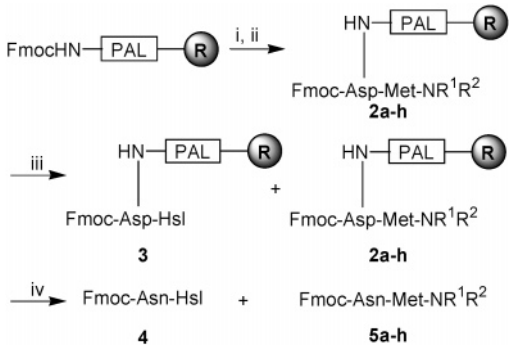
Scheme 2 Synthesis of Met-Containing "Preformed Handles" with Various Amines[a]



[a] (i) HNR^1R^2 (2.5 equiv), HBTU (1.1 equiv), HOBt·H_2O (1.1 equiv), DIEA (2.6 equiv), DMF, 25 °C, 2 h; (ii) TFA−CH_2Cl_2 (1:1), 25 °C, 0.5 h; (iii) Fmoc-Asp(O*t*Bu)-OH (1 equiv), HBTU (1.1 equiv), HOBt·H_2O (1.1 equiv), DIEA (2.6 equiv), DMF, 25 °C, 2 h; (iv) TFA−CH_2Cl_2 (1:1), 25 °C, 0.5 h.

Scheme 3. CNBr Cleavage Model Study[a]



[a] (i) Piperidine−DMF (1:4, 3 × 1 min, 3 × 5 min), 25 °C; (ii) **1a**−**h** (5 equiv), HATU (5 equiv), DIEA (10 equiv), DMF, 25 °C, 2 h; (iii) CNBr (60 equiv), CH_3CN−HOAc−H_2O (5:4:1), 25 °C, 12 h; (iv) TFA−H_2O (19:1), 25 °C, 1 h. The parent amines are listed in Table 1.

Scheme 2. Boc-Met-OH was coupled in solution to various amines, as mediated by HBTU and HOBt plus *N*,*N*-diisopropylethylamine (DIEA) in DMF. After Boc removal with trifluoroacetic acid (TFA)−CH_2Cl_2 (1:1), the Met derivatives were coupled to Fmoc-Asp(O*t*Bu)-OH by the same HBTU/HOBt/DIEA protocol. Acid removal of the *t*Bu ester provided the required handles **1a**−**h**, which were then loaded via their free β-carboxyl onto tris(alkoxy)benzylamine (PAL) resin, as mediated by HATU (5 equiv) plus DIEA (10 equiv) in DMF (Scheme 3). The resultant resin-bound amines **2a**−**h** were treated with CNBr (60 equiv) in CH_3CN−HOAc−H_2O (5:4:1) to release the amines from the support while concurrently generating resin-bound homoserine lactone **3**. In principle, depending on the completeness of the reaction, a mixture of starting resin (**2a**−**h**) plus **3** was possible. Subsequent treatment of the CNBr-cleaved peptide-resin with TFA−H_2O (19:1) cleaved both the corresponding homoserine lactone dipeptide **4** and the Met-containing dipeptides (**5a**−**h**), and these were observed in a ratio that reflected the initial on-resin values. Thus, analysis of the cleavage filtrates by high performance liquid chromatography (HPLC) determined the ratios of **4** to **5a**−**h**, and from these, the CNBr cleavage yields could be calculated. Using this assay (Scheme 3, Table 1), it was found that primary aliphatic amines (Table 1, entries a−c and f) were cleaved in high yields (83−99%). Secondary amines (Table 1, entries d and e) were also cleaved in good yields (70−95%). However, poor yields were obtained with aromatic amines (Table 1, entries g and h).

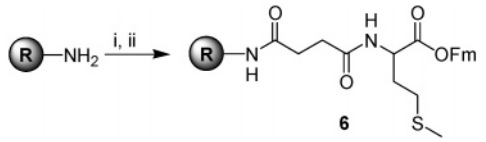
Encouraged by these results, a simpler approach to prepare a Met handle-containing resin was developed (Scheme 4). Amino-functionalized PEG-PS resin was treated with succinic anhydride in the presence of DIEA, providing pendant carboxyl sites, following which HCl·H-Met-OFm was introduced by HATU/DIEA-mediated coupling in CH_2Cl_2−DMF (1:1) to provide the protected Met resin **6**.

Table 1. CNBr Cleavage of Amines Anchored via Met-Containing "Preformed" Handles

entry	amine	percent cleavage[a]
a	methylamine	87
b	ethylamine	99
c	tetrahydrofurfurylamine	88
d	diethylamine	95
e	*N*,*O*-dimethylhydroxylamine	70
f	benzylamine	83
g	2,6-dimethylaniline	15
h	aniline	4

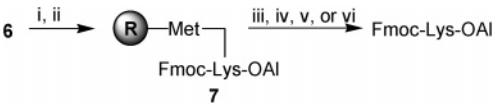
[a] Overall experimental design is in Scheme 3. Cleavage yields calculated on the basis of HPLC comparison of the relative peak areas (220 nm) of **4** and **5a**−**h**.

Scheme 4. Preparation of Met-Handle Resin[a]



[a] (i) Succinic anhydride (10 equiv), DIEA (20 equiv), CH_2Cl_2, 25 °C, 0.5 h; (ii) HCl·H-Met-OFm (5 equiv), HATU (5 equiv), DIEA (10 equiv), CH_2Cl_2−DMF (1:1), 25 °C, 0.5 h.

Scheme 5. Analysis of CNBr Cleavage Variables[a]



[a] (i) Piperidine−DMF (1:4, 3 × 1 min, 3 × 5 min), 25 °C; (ii) Fmoc-Lys-OAl·HCl (5 equiv), HATU (5 equiv), DIEA (10 equiv), CH_2Cl_2−DMF (1:1), 25 °C, 0.5 h; (iii) CNBr (60 equiv), X−HOAc−H_2O (5:4:1) (X = CH_3CN, CH_2Cl_2, $CHCl_3$, DMF, THF, dioxane), 25 °C, 12 h; (iv) CNBr (60 equiv), CH_3CN−HOAc−H_2O (5:X:1) (X = 0, 1, 2, 3, 4, 5), 25 °C, 12 h; (v) CNBr (X equiv) (X = 1, 5, 10, 20, 45, 60, 180), CH_3CN−HOAc−H_2O (5:4:1), 25 °C, 12 h; (vi) CNBr (60 equiv), CH_3CN−HOAc−H_2O (5:4:1), 25 °C, *X* h (*X* = 1, 5, 12, 24, 48).

Conditions for use of CNBr to cleave amines from the support were then tested and compared (Scheme 5). First, resin **6** was treated with piperidine−DMF (1:4) to remove the 9-fluorenylmethyl (Fm) group, and HATU-mediated coupling of Fmoc-Lys-OAl·HCl (5 equiv) generated the resin-bound Lys derivative **7**. Cleavage yields were determined by quantifying the Fmoc content (301 nm) of resin **7** and comparing this to the Fmoc content of the resin following CNBr treatment. The variables analyzed were (1) nature of the organic solvent used in the cleavage solution, (2) amount of HOAc in the cleavage solution, (3) amount of CNBr, and (4) time needed for maximum cleavage.

For the first series of experiments, six organic solvents, CH_3CN, CH_2Cl_2, $CHCl_3$, DMF, THF, and dioxane, were used in cleavage mixture solutions consisting of organic solvent−HOAc−H_2O (5:4:1). Resin **7** was treated separately with each of the six solvent mixtures, plus CNBr (60 equiv), at 25 °C for 12 h. DMF gave the best result, with 68% cleavage, and dioxane was the worst at 5%. The other solvents (CH_3CN, CH_2Cl_2, $CHCl_3$, and THF) were effective, with cleavage yields ranging from 42 to 53%.

In the second series of experiments, the amount of HOAc in the $CH_3CN-HOAc-H_2O$ (5:*X*:1) mixtures was varied so that $X = 5$, 4, 3, 2, 1, or 0. Resin **7** was treated separately with the solvent mixtures plus CNBr (60 equiv) at 25 °C for 12 h. The cleavage yields decreased as the amount of HOAc increased so that the best cleavage was with no HOAc. Yields were 73 ($X = 0$), 66 ($X = 1$), 60 ($X = 2$), 56 ($X = 3$), 53 ($X = 4$), and 37% ($X = 5$).

The amount of CNBr was varied in the third series of experiments. Six reactions were performed in $CH_3CN-HOAc-H_2O$ (5:4:1) with CNBr for 12 h at 25 °C, where the equivalents of CNBr were 1, 5, 10, 20, 45, 60, and 180, respectively. As the amount of CNBr increased, so, too, did the cleavage yield, up to a point. The maximum yield was with 45 equiv, and a slight decrease was noted at higher concentrations. The yields were 17 (1 equiv), 25 (5 equiv), 37 (10 equiv), 49 (20 equiv), 50 (45 equiv), 44 (60 equiv), and 42% (180 equiv).

For the last series of experiments, the time of reaction was varied so that resin **7** was treated with CNBr (60 equiv) in $CH_3CN-HOAc-H_2O$ (5:4:1) at 25 °C for 1, 5, 12, 24, and 48 h. The cleavage yields increased until reaching a maximum at 24 h. The yields for each reaction were 13 (1 h); 33 (5 h); 55 (12 h); 64 (24 h), and 64% (48 h).

On the basis of all of these results, it was concluded that the optimal conditions for cleaving amines from the solid support involve $DMF-H_2O$ (5:1) as solvent plus CNBr (20−45 equiv), for 24 h. Treatment of resin **7** using these conditions with 20 equiv of CNBr gave a cleavage yield of 70%, and with 45 equiv, gave a cleavage yield of 73%.

Summary and Conclusions

The amino acid methionine (Met) has been applied as a key component in a novel strategy for solid-phase anchoring of a variety of amines, predicated on the use of CNBr as the cleavage reagent. We expect this approach to be of value to synthetic peptide and combinatorial chemists who require additional versatility in management of the important amine functionality.

Experimental Section

General Procedures. Materials, solvents, instrumentation, and general methods were essentially as described in previous publications from our laboratory.[23−25] Organic transformations and washes were at 25 °C unless indicated otherwise. Flash chromatography was performed using ICN silica gel 32−63, 60 Å. Thin-layer chromatography was performed on Merck Silica gel 60 F_{254} plates, and compounds were observed by fluorescence quenching, by spraying with a dilute ethanolic ninhydrin solution, or both. Room temperature polymer-supported reactions were carried out using plastic syringes (3, 5, and 10 mL) fitted with polypropylene frits. PEG-PS·HCl and PAL-PEG-PS resins were obtained from PE Biosystems (Framingham, MA). Boc-Met-OH, Fmoc-Asp(O*t*Bu)-OH, HBTU, HATU, and HOBt·H_2O, were obtained from Advanced ChemTech (Louisville, KY). All other reagents were obtained from Aldrich (Milwaukee, WI). CH_2Cl_2 was freshly distilled from anhydrous calcium hydride. Methylamine and ethylamine *N*-hydroxysuccinimide salts were prepared by mixing the amine (1 equiv; 2 M in MeOH) with *N*-hydroxysuccinimide (1 equiv) in MeOH, followed by concentration to one-half volume, filtration, and washing with cold MeOH. ^{1}H NMR spectra were obtained at ambient temperature on Varian VI 500 or Varian VI 300 spectrophotometers. Chemical ionization mass spectroscopy (CIMS) was performed on a Perkin-Elmer Sciex API III triple quadrupole mass spectrometer equipped with ionspray interface, and fast atom bombardment mass spectroscopy (FABMS) was performed on a VG7070E-HF mass spectrometer. Analytical HPLC was performed using a Vydac C_{18} reversed-phase column (0.46 × 25 cm) on a Beckman instrument configured with two 112 pumps and a 165 variable wavelength detector set at 220 and 280 nm. Linear gradients of 0.1% aqueous TFA and 0.1% TFA in CH_3CN were run at 1.0 mL/min flow rate from 9:1 to 0:1 over 30 min, then 0:1 for 5 min.

Synthesis of Preformed Methionine Linkers (1a−1h). Boc-Met-OH (1 equiv) was dissolved in DMF (3 mL/mmol of Boc-Met-OH). HBTU (1.1 equiv), HOBt·H_2O (1.1 equiv), DIEA (2.6 equiv), and the amine (2.5 equiv) were then added sequentially to the solution. After 2 h at 25 °C, the solution was diluted with EtOAc (10 mL × vol of DMF) and was washed with 5% aqueous $NaHCO_3$ (×3), 10% aqueous citric acid (×3), and brine (×3); dried over $MgSO_4$; and concentrated in vacuo. Emulsions formed during the aqueous washings were broken up by adding brine. The Boc-protected intermediate was then dissolved in CH_2Cl_2−TFA (1:1, 4 mL/mmol), and the reaction was stirred for 30 min at 25 °C. The reaction was concentrated under a stream of N_2, dissolved in CH_2Cl_2, and reconcentrated. The deprotected methionine intermediate was then coupled with Fmoc-Asp(O*t*Bu)-OH using HBTU as described above. Deprotection of the *t*Bu ester was accomplished with CH_2Cl_2−TFA (1:1, 4 mL/mmol) at 25 °C for 30 min.

Fmoc-Asp-Met-NH(CH_3) (1a). Prepared as described above using methylamine *N*-hydroxysuccinimide salt (0.18 g, 1.23 mmol) to provide the title compound as a white powder (0.10 g, 41% overall). HPLC t_R 16.1 min; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.35 (d, $J = 7.5$ Hz, 1H), 7.79 (d, $J = 7.5$ Hz, 2H), 7.65 (d, $J = 7.2$ Hz, 2H), 7.28−7.41 (m, 4H), 4.43−4.48 (m, 2H), 4.37 (d, $J = 7.2$ Hz, 2H), 4.23 (t, $J = 6.8$ Hz, 1H), 2.93 (d, $J = 7.5$ Hz, 1H), 2.87 (d, $J = 7.5$ Hz, 1H), 2.72 (s, 3H), 2.41−2.61 (m, 2H), 2.13−2.20 (m, 1H), 2.01 (s, 3H), 1.85−1.94 (m, 1H). FABMS calcd for $C_{25}H_{29}N_3O_6S$ 499.2, found 500.2 $[M + H]^+$.

Fmoc-Asp-Met-NH(CH_2CH_3) (1b). Prepared as described above using ethylamine *N*-hydroxysuccinimide salt (0.05 g, 0.33 mmol) to provide the title compound as a white powder (0.028 g, 42% overall). HPLC t_R 16.9 min; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.35 (d, $J = 7.8$ Hz, 1H), 7.80 (d, $J = 7.8$ Hz, 2H), 7.65 (d, $J = 7.2$ Hz, 2H), 7.28−7.41 (m, 4H), 4.45 (t, $J = 6.6$ Hz, 2H), 4.37 (d, $J = 6.9$ Hz, 2H), 4.24 (d, $J = 5.4$ Hz, 1H), 3.21 (t, $J = 7.2$ Hz, 2H), 2.86−2.94 (m, 1H), 2.69−2.75 (m, 1H), 2.45−2.57 (m, 2H), 2.08−2.22 (m, 1H), 2.01 (s, 3H), 1.82−1.96 (m, 1H), 1.11 (t, $J = 7.2$ Hz, 3H). FABMS calcd for $C_{26}H_{31}N_3O_6S$ 513.2, found 514.2 $[M + H]^+$.

Fmoc-Asp-Met-NH−CH_2(−$CHOCH_2CH_2CH_2$−) (1c). Prepared as described above using tetrahydrofurfurylamine (0.37 g, 3.7 mmol) to provide the title compound as a white solid (0.16 g, 19% overall). HPLC t_R 17.2 min; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.75 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 6.6 Hz, 2H), 7.39 (t, J = 7.2 Hz, 2H), 7.29 (t, J = 6.5 Hz, 2H), 6.50−6.84 (m, 2H), 6.06 (t, J = 7.2 Hz, 1H), 4.66−4.75 (m, 2H), 4.38 (d, J = 7.2 Hz, 2H), 4.19 (t, J = 6.9 Hz, 1H), 3.98−4.10 (m, 1H), 3.75−3.84 (m, 2H), 3.49−3.62 (m, 1H), 2.76−3.18 (m, 4H), 2.5 (t, J = 6.8 Hz, 2H), 1.90−2.11 (m, 8H), 1.51−1.57 (m, 1H); FABMS calcd for $C_{29}H_{35}N_3O_7S$ 569.2, found 570.2 $[M + H]^+$.

Fmoc-Asp-Met-N(CH_2CH_3)$_2$ (1d). Prepared as described above using diethylamine (0.40 g, 5.45 mmol) to provide the title compound as a yellow oil (0.73 g, 62% overall). HPLC t_R 18.6 min; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74 (d, J = 6.6 Hz, 2H), 7.57 (d, J = 7.2 Hz, 2H), 7.27−7.43 (m, 4H), 5.08 (d, J = 5.1 Hz, 1H), 4.64 (d, J = 6.6 Hz, 1H), 4.36 (d, J = 7.5 Hz, 2H), 4.11−4.25 (m, 3H), 2.48−3.47 (m, 8H), 2.07 (d, J = 10.8 Hz, 3H), 1.93 (d, J = 6.9 Hz, 2H), 1.26 (t, J = 7.1 Hz, 6H). FABMS calcd for $C_{28}H_{35}N_3O_6S$ 541.2, found 542.1 $[M + H]^+$.

Fmoc-Asp-Met-N(CH_3)(OCH_3) (1e). Prepared as described above using *N*,*O*-dimethylhydroxylamine hydrochloride (0.12 g, 1.25 mmol) to provide the title compound as a colorless oil (0.12 g, 45% overall). HPLC t_R 16.4 min; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.74 (d, J = 7.6 Hz, 2H), 7.57 (d, J = 6.6 Hz, 2H), 7.25−7.42 (m, 4H), 6.24 (d, J = 8.6 Hz, 1H), 5.12 (d, J = 4.4 Hz, 1H), 4.70 (d, J = 6.6 Hz, 1H), 4.36−4.60 (m, 1H), 4.39 (d, J = 7 Hz, 2H), 4.19 (t, J = 6.9 Hz, 1H), 3.77 (s, 3H), 3.22 (s, 3H), 2.39−3.04 (m, 4H), 2.02 (s, 3H), 1.82−2.08 (m, 2H). FABMS calcd for $C_{26}H_{31}N_3O_7S$ 529.2, found 552.2 $[M + Na]^+$.

Fmoc-Asp-Met-NH(CH_2Ph) (1f). Prepared as described above using benzylamine (0.81 g, 7.55 mmol) to provide the title compound as white powder (1.47 g, 85% overall). HPLC t_R 19.2 min; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.06−7.75 (m, 13H), 6.05 (s, 1H), 3.95−4.77 (m, 9H), 2.31−3.01 (m, 6H), 1.98 (s, 3H). FABMS calcd for $C_{31}H_{33}N_3O_6S$ 575.2, found 576.2 $[M + H]^+$.

Fmoc-Asp-Met-NH[(2,6-dimethyl)Ph] (1g). Prepared as described above using 2,6-dimethylaniline (0.11 g, 0.93 mmol) to provide the title compound as a white solid (0.12 g, 55% overall). HPLC t_R 19.7 min; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.59 (s, 1H), 7.80 (s, 2H), 7.64 (s, 2H), 7.32−7.38 (m, 4H), 7.05 (s, 3H), 4.46−4.56 (m, 2H), 4.30−4.40 (m, 2H), 4.16−4.26 (m, 1H), 2.57−2.90 (m, 6H), 2.16−2.27 (m, 1H), 2.16 (s, 3H), 2.05 (s, 1H), 1.18 (s, 6H). FABMS calcd for $C_{32}H_{35}N_3O_6S$ 589.2, found 590.0 $[M + H]^+$.

Fmoc-Asp-Met-NH(Ph) (1h). Prepared as described above using aniline (0.17 g, 1.83 mmol) to provide the title compound as a white powder (0.14 g, 34% overall). HPLC t_R 19.7 min; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.80 (d, J = 7.5 Hz, 2H), 7.65 (t, J = 6 Hz, 2H), 7.62 (d, J = 7.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.28−7.33 (m, 4H), 7.10 (t, J = 7.3 Hz, 1H), 4.63 (dd, J_1 = 4.0 Hz, J_2 = 9.5 Hz, 1H), 4.50 (t, J = 6.8 Hz, 1H), 4.31−4.42 (m, 2H), 4.24 (t, J = 6.8 Hz, 1H), 2.92 (dd, J_1 = 7.5 Hz, J_2 = 17.0 Hz, 1H), 2.75 (dd, J_1 = 6.5 Hz, J_2 = 16.5 Hz, 1H), 2.60−2.65 (m, 1H), 2.50−2.56 (m, 1H), 2.25−2.27 (m, 1H), 2.04 (s, 3H), 1.99−2.01 (m, 1H). FABMS calcd for $C_{30}H_{31}N_3O_6S$ 561.2, found 584.2 $[M + Na]^+$.

CNBr Cleavage Model Study. Fmoc-PAL-PEG-PS (100 mg, 0.15 mmol/g) was treated with piperidine−DMF (1:4, 3 × 1 min, 3 × 5 min), then washed with DMF (10 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min). Next, a solution of the preformed methionine linker (**1a**−**h**) (5 equiv) in DMF (0.4 mL) and DIEA (27 μL, 10 equiv) was added to the resin. The coupling was then initiated by the addition of HATU (30 mg, 5 equiv) in solid form. After 2 h at 25 °C, the resin was washed with DMF (8 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min). A small aliquot of the resin (**2a**−**h**) (10 mg) was cleaved with TFA−H_2O (19:1) (0.5 mL) for 1 h to give Fmoc-Asn-Met-NR^1R^2 (**5a**−**h**), which was collected and concentrated under N_2, then characterized by analytical HPLC. Separately, the resin (**2a**−**h**) (20 mg) was treated with CNBr (18 mg, 60 equiv) in CH_3CN−HOAc−H_2O (5:4:1, 0.5 mL). After mixing for 12 h, the resulting resin mixture (**3** + **2a**−**h**) was filtered and washed with CH_3CN (5 × 0.5 min), DMF (5 × 0.5 min), and CH_2Cl_2 (5 × 0.5 min) and cleaved with TFA−H_2O (19:1) (1 mL) for 1 h. The filtrate [Fmoc-Asn-Hsl (**4**) plus Fmoc-Asn-Met-NR^1R^2 (**5a**−**h**)] was collected and concentrated under N_2, then characterized by analytical HPLC.

Fmoc-Asn-Hse (4). From resin **2a**, HPLC t_R 14.2 min (68%) [+ **5a**, t_R 15.3 min (10%)]; from resin **2b**, HPLC t_R 14.2 min (35%); from resin **2c**, HPLC t_R 14.2 min (70%) [+ **5c**, t_R 16.3 min (10%)]; from resin **2d**, HPLC t_R 14.2 min (69%) [+ **5d**, t_R 17.8 min (4%)]; from resin **2e**, HPLC t_R 14.2 min (35%) [+ **5e**, t_R (16.7 min (15%)]; from resin **2f**, HPLC t_R 14.2 min (50%) [+ **5f**, t_R 18.5 min (10%)]; from resin **2g**, HPLC t_R 14.2 min (7%) [+ **5g**, t_R 19.2 min (41%)]; from resin **2h**, HPLC t_R 14.2 min (3%) [+ **5h**, t_R 15.9 min (66%)].

Fmoc-Asn-Met-NH(CH_3) (5a). Prepared as described above from resin **2a**; HPLC t_R 15.3 min; FABMS calcd for $C_{25}H_{30}N_4O_5S$ 498.6, found 499.3 $[M + H]^+$.

Fmoc-Asn-Met-NH(CH_2CH_3) (5b). Prepared as described above from resin **2b**; HPLC t_R 20.0 min; FABMS calcd for $C_{26}H_{32}N_4O_5S$ 512.6, found 512.2 $[M + H]^+$.

Fmoc-Asn-Met-NH−CH_2(−$CHOCH_2CH_2CH_2$−) (5c). Prepared as described above from resin **2c**; HPLC t_R 16.3 min; FABMS calcd for $C_{29}H_{36}N_4O_6S$ 568.7, found 569.3 $[M + H]^+$.

Fmoc-Asn-Met-N(CH_2CH_3)$_2$ (5d). Prepared as described above from resin **2d**; HPLC t_R 17.8 min; FABMS calcd for $C_{28}H_{36}N_4O_5S$ 540.7, found 541.3 $[M + H]^+$.

Fmoc-Asn-Met-N(CH_3)(OCH_3) (5e). Prepared as described above from resin **2e**; HPLC t_R 16.7 min; FABMS calcd for $C_{26}H_{32}N_4O_6S$ 528.6, found 529.3 $[M + H]^+$.

Fmoc-Asn-Met-NH(CH_2Ph) (5f). Prepared as described above from resin **2f**; HPLC t_R 18.5 min; FABMS calcd for $C_{31}H_{34}N_4O_5S$ 574.7, found 575.3 $[M + H]^+$.

Fmoc-Asn-Met-NH[(2,6-dimethyl)Ph] (5g). Prepared as described above from resin **2g**; HPLC t_R 19.0 min; FABMS calcd for $C_{32}H_{36}N_4O_5S$ 588.7, found 589.2 $[M + H]^+$.

Fmoc-Asn-Met-NH(Ph) (5h). Prepared as described above from resin **2h**; HPLC t_R 15.9 min; FABMS calcd for $C_{30}H_{32}N_4O_5S$ 560.7, found 561.3 $[M + H]^+$.

L-Methionine α-Fluorenylmethyl Ester, Hydrochloride Salt (H-Met-OFm·HCl). Boc-L-methionine (2.00 g, 8.02 mmol) was dissolved in CH_2Cl_2 (130 mL), and *N*,*N*′-dicyclohexylcarbodiimide (1.99 g, 9.63 mmol), 9-fluorenylmethanol (1.89 g, 9.63 mmol), and 4-(dimethylamino)-pyridine (0.20 g, 1.60 mmol) were added in turn. After stirring for 48 h at 25 °C, the reaction mixture was filtered and concentrated under reduced pressure. The resultant residue was purified by silica gel chromatography [neat hexane to hexanes−ethyl acetate (7.5:2.5)], giving 1.88 g (55%) of N^{α}-Boc-L-methionine α-fluorenylmethyl ester as a white solid; R_f 0.61 [hexanes−ethyl acetate, (8:2)]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.79 (d, J = 7.5 Hz, 2H), 7.59−7.63 (m, 2H), 7.31−7.45 (m, 4H), 5.09 (d, J = 8.1 Hz, 1H), 4.46−4.61 (m, 3H), 4.24 (t, J = 6.6 Hz, 1H), 2.43 (t, J = 7.2 Hz, 2H), 2.06 (s, 3H), 1.97−2.10 (m, 1H), 1.77−1.86 (m, 1H), 1.46 (s, 9H). A portion of this material (0.50 g, 1.17 mmol) was dissolved in 4 N HCl−dioxane (5 mL), stirred at 25 °C for 1 h, and then concentrated under reduced pressure and chased with Et_2O [6 × 10 mL, followed by reconcentration]. The title product was obtained as a white solid (0.39 g, 92%). R_f 0.68 [ethyl acetate]; HPLC t_R 15.1 min; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.81 (d, J = 7.5 Hz, 2H), 7.63 (t, J = 8.6 Hz, 2H), 7.29−7.43 (m, 4H), 5.07 (dd, J_1 = 11.1 Hz, J_2 = 4.6 Hz, 1H), 4.71 (dd, J_1 = 11.1, Hz, J_2 = 4.6 Hz, 1H), 4.30 (t, J = 4.4 Hz, 1H), 3.96 (t, J = 6.5 Hz, 1H), 2.1−2.3 (m, 2H), 1.87 (s, 3H), 1.65 (q, J = 7.1 Hz, 2H); FABMS calcd for $C_{19}H_{21}NO_2S$ 327.1, found 328.1 $[M + H]^+$.

Met Handle Resin (6). Fmoc-Ile-PEG-PS resin (1.0 g, 0.2 mmol/g) was treated with piperidine−DMF (1:4, 3 × 1 min, 3 × 5 min), and then washed with DMF (10 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min). The resin was then treated with succinic anhydride (0.20 g, 10 equiv) and DIEA (0.7 mL, 20 equiv) in CH_2Cl_2 (3 mL), for 30 min at 25 °C, followed by washing with CH_2Cl_2 (5 × 0.5 min) and DMF (5 × 0.5 min). Next, a solution of H-Met-OFm·HCl (0.36 g, 5 equiv) in CH_2Cl_2−DMF (1:1, 2.5 mL), and DIEA (0.4 mL, 10 equiv), was added to the resin. The coupling was then initiated by the addition of HATU (0.38 g, 5 equiv) in solid form. After 30 min at 25 °C, the resin was washed with DMF (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min).

***N*$^{\alpha}$-9-Fluorenylmethyloxycarbonyl-lysine Allyl Ester, Hydrochloride Salt (Fmoc-Lys-OAl·HCl).** *N*,*N*-Diisopropylethylamine (DIEA) (3.5 mL, 20 mmol) was added to a solution of Fmoc-Lys(Boc)-OH (4.69 g, 10 mmol) in CH_3CN−allyl bromide (1:1, 20 mL), and the mixture was heated at 60 °C for 90 min. After cooling, the reaction mixture was diluted with EtOAc (200 mL); washed with 10% aqueous citric acid (3 × 50 mL), 5% aqueous $NaHCO_3$ (3 × 50 mL), and brine (3 × 50 mL); dried ($MgSO_4$); and concentrated in vacuo. The residue was purified by silica gel chromatography [hexane (neat) to hexanes−ethyl acetate (7:3)], giving 4.40 g (86%) of Fmoc-Lys(Boc)-OAl as a white solid. HPLC (t_R 23.8 min, 99% purity); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.76 (d, J = 6.8 Hz, 2H), 7.60 (d, J = 7.0 Hz, 2H), 7.28−7.44 (m, 4H), 5.8−6.0 (m, 1H), 5.42 (s, 1H), 5.33 (dd, J_1 = 17.4 Hz, J_2 = 1.2 Hz, 1H), 5.26 (dd, J_1 = 10.6 Hz, J_2 = 1.2 Hz, 1H), 4.64 (d, J = 5.8 Hz, 2H), 4.32−4.58 (m, 2H), 4.22 (t, J = 6.8 Hz, 1H), 3.09 (t, J = 6.2 Hz, 2H), 1.66−1.90 (m, 2H), 1.3−1.6 (m, 4H), 1.43 (s, 9H). The Boc-protected intermediate (1.0 g, 1.97 mmol) was dissolved in 4 N HCl−dioxane (5 mL) and stirred at 25 °C for 1 h. Next, the mixture was concentrated and chased with Et_2O (5 × 10 mL) to provide the title product as a white powder (0.90 g, quantitative). HPLC (t_R 16.2 min, >99% purity); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.80 (d, J = 7.5 Hz, 2H), 7.67 (t, J = 8.0 Hz, 2H), 7.40 (t, J = 7.3 Hz, 2H), 7.32 (t, J = 7.3 Hz, 2H), 5.90−5.96 (m, 1H), 5.33 (d, J = 17 Hz, 1H), 5.22 (d, J = 10 Hz, 1H), 4.63 (d, J = 4.5 Hz, 2H), 4.41−4.45 (m, 1H), 4.34 (t, J = 8.8 Hz, 1H), 4.22 (q, J = 6.7 Hz, 2H), 2.91 (s, 2H), 1.86−1.90 (m, 2H), 1.66−1.74 (m, 2H), 1.46−1.50 (m, 2H); FABMS calcd for $C_{24}H_{28}N_2O_4$ 408.2, found 409.2 $[M + H]^+$.

Fmoc-Lys(Met-Resin)OA1 (7). Met-handle resin **6** (250 mg, 0.17 mmol/g) was deprotected with piperidine−DMF (1:4, 3 × 1 min, 3 × 5 min), followed by washing with DMF (10 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min). A solution of Fmoc-Lys-OAl·HCl (111 mg, 5 equiv) in CH_2Cl_2−DMF (1:1, 1.2 mL) was then added to the resin, followed in turn by DIEA (75 mL, 10 equiv) and solid HATU (95 mg, 5 equiv). After 30 min at 25 °C, the resin was washed with DMF (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min) and dried in vacuo.

Analysis of CNBr Cleavage Variables: Organic Solvent (Scheme 5, Step iii). Resin **7** (30 mg, 0.17 mmol/g) was treated with CNBr (32 mg, 60 equiv) in X−HOAc−H_2O (5:4:1) (0.5 mL), where X = CH_3CN, CH_2Cl_2, $CHCl_3$, DMF, THF, or dioxane, at 25 °C for 12 h. The filtrate was collected and characterized by analytical HPLC. The resin was washed with the reaction solvent (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min) and dried in vacuo, and the cleavage yield was determined by Fmoc quantitation [(Fmoc loading of the CNBr-treated resin/Fmoc loading of starting resin) × 100%]. Results of HPLC analysis and Fmoc quantitation/percent cleavage for each of the solvents are as follows: CH_3CN, HPLC t_R 16.4 min (96%), resin substitution 0.111 mmol/g (42% cleavage); CH_2Cl_2, HPLC t_R 16.4 min (95%), resin substitution 0.098 mmol/g (47% cleavage); $CHCl_3$, HPLC t_R 16.3 min (96%), resin substitution 0.092 mmol/g (53% cleavage); DMF, HPLC t_R 16.4 min (95%), resin substitution 0.059 mmol/g (68%); THF, HPLC t_R 16.3 min (95%), resin substitution 0.092 mmol/g (53% cleavage); dioxane, HPLC t_R 16.4 min (24%), resin substitution 0.175 mmol/g (5% cleavage). Substitution of starting resin **7** was 0.187 mmol/g via Fmoc quantification.

Analysis of CNBr Cleavage Variables: HOAc (Scheme 5, Step iv). Resin **7** (30 mg, 0.17 mmol/g) was treated with CNBr (32 mg, 60 equiv) in CH_3CN−HOAc−H_2O (5:*X*:1) (0.5 mL), where *X* = 5, 4, 3, 2, 1, or 0, at 25 °C for 12 h. The filtrate was collected and characterized by analytical HPLC. The resin was washed with CH_3CN (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min) and dried in vacuo, and the cleavage yield was determined by Fmoc quantitation [(Fmoc

loading of the CNBr-treated resin/Fmoc loading of starting resin) × 100%]. Results of HPLC analysis and Fmoc quantitation/percent cleavage are as follows: $CH_3CN-HOAc-H_2O$ (5:5:1), HPLC t_R 16.1 min (98%), resin substitution 0.092 mmol/g (37% cleavage); $CH_3CN-HOAc-H_2O$ (5:4:1), HPLC t_R 16.1 min (97%), resin substitution 0.065 mmol/g (56% cleavage); $CH_3CN-HOAc-H_2O$ (5:3:1), HPLC t_R 16.1 min (94%), resin substitution 0.068 mmol/g (53% cleavage); $CH_3CN-HOAc-H_2O$ (5:2:1), HPLC t_R 16.1 min (98%), resin substitution 0.058 mmol/g (60% cleavage); $CH_3CN-HOAc-H_2O$ (5:1:1), HPLC t_R 16.1 min (98%), resin substitution 0.051 mmol/g 66% cleavage); $CH_3CN-HOAc-H_2O$ (5:0:1), HPLC t_R 16.1 min (98%), resin substitution 0.039 mmol/g (73% cleavage). Substitution of starting resin **7** was 0.145 mmol/g via Fmoc quantification.

Analysis of CNBr Cleavage Variables: CNBr Equivalents (Scheme 5, Step v). Resin **7** (30 mg, 0.17 mmol/g) was treated with CNBr [(0.5 mg, 1 equiv), (2.7 mg, 5 equiv), (5.3 mg, 10 equiv), (10.6 mg, 20 equiv), (31.8 mg, 60 equiv), or (95.4 mg, 180 equiv)] in $CHCl_3-HOAc-H_2O$ (5:4:1) (0.5 mL) at 25 °C for 12 h. The filtrate was collected and characterized by analytical HPLC. The resin was washed with CH_3CN (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min) and dried in vacuo, and the cleavage yield was determined by Fmoc quantitation [(Fmoc loading of the CNBr-treated resin/Fmoc loading of starting resin) × 100%]. Results of HPLC analysis and Fmoc quantitation/percent cleavage are as follows: CNBr (1 equiv), HPLC t_R 16.3 min (74%), resin substitution 0.155 mmol/g (17% cleavage); CNBr (5 equiv), HPLC t_R 16.3 min (96%), resin substitution 0.139 mmol/g (25% cleavage); CNBr (10 equiv), HPLC t_R 16.3 min (95%), resin substitution 0.118 mmol/g (37% cleavage); CNBr (20 equiv), HPLC t_R 16.1 min (95%), resin substitution 0.095 mmol/g (49% cleavage); CNBr (45 equiv), HPLC t_R 16.3 min (95%), resin substitution 0.094 mmol/g (50% cleavage); CNBr (60 equiv), HPLC t_R 16.3 min (98%), resin substitution 0.105 mmol/g (44% cleavage); CNBr (180 equiv), HPLC t_R 16.3 min (96%), resin substitution 0.109 mmol/g (42% cleavage). Substitution of starting resin **7** was 0.187 mmol/g via Fmoc quantification.

Analysis of CNBr Cleavage Variables: Reaction Time (Scheme 5, Step vi). Resin **7** (30 mg, 0.17 mmol/g) was treated with CNBr (32 mg, 60 equiv) in $CH_3CN-HOAc-H_2O$ (5:4:1) (0.5 mL) at 25 °C for 1, 5, 12, 24, or 48 h. The filtrate was collected and characterized by analytical HPLC. The resin was washed with $CHCl_3$ (5 × 0.5 min) and CH_2Cl_2 (5 × 0.5 min) and dried in vacuo, and the cleavage was yield determined by Fmoc quantitation [(Fmoc loading of the CNBr-treated resin/Fmoc loading of starting resin) × 100%]. Results of HPLC analysis and Fmoc quantitation/percent cleavage are as follows: **1h**, HPLC t_R 16.4 min (91%), resin substitution 0.136 mmol/g (13% cleavage); **5h**, HPLC t_R 16.4 min (97%), resin substitution 0.104 mmol/g (33% cleavage); **12h**, HPLC t_R 16.3 min (95%), resin substitution 0.070 mmol/g (55% cleavage); **24h**, HPLC t_R 16.3 min (96%), resin substitution 0.056 mmol/g (64% cleavage); **48h**, HPLC t_R 16.3 min (95%), resin substitution 0.056 mmol/g (64% cleavage). Substitution of starting resin **7** was 0.155 mmol/g via Fmoc quantification.

Abbreviations

The following abbreviations are used: AA, generic amino acid; BAL, backbone amide linker; Dde, 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; Fm, 9-fluorenylmethyl; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU, *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAc, acetic acid; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; Hsl, homoserine lactone; PAL, peptide amide linker; PEG-PS, poly(ethylene glycol)-*graft*-polystyrene polymeric support; PhFl, 9-phenylfluoren-9-yl; PMB, *para*-methoxybenzyl; *t*Bu, *tert*-butyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran. Amino acid symbols denote the L-configuration unless indicated otherwise. Abbreviations used for amino acids and the designations of peptides follow the IUPAC−IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* **1972**, *247*, 977−983.

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