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Convenient Synthesis of *N*-Methylamino Acids Compatible with Fmoc Solid-Phase Peptide Synthesis

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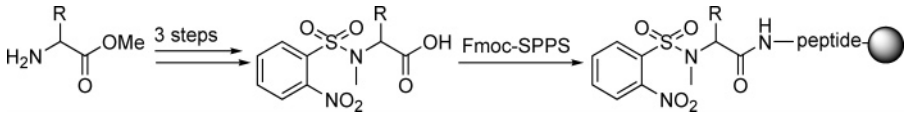

N^{α}-Methylamino acid containing peptides exhibit interesting therapeutic profiles and are increasingly recognized as potentially useful therapeutics. Unfortunately, their synthesis is hampered by the high price and unavaibility of many N^{α}-methylamino acids. An efficient and practical preparation of N^{α}-methyl-N^{α}-(*o*-nitrobenzenesulfonyl)-α-amino acids without extensive purification is described. The procedure is based on the well-known *N*-alkylation of N^{α}-arylsulfonylamino esters which was improved by using dimethyl sulfate and DBU as base. Ester cleavage is efficiently achieved by using an S_N2-type saponification with lithium iodide, avoiding racemization observed with lithium hydroxide hydrolysis. Compatibility of the synthesized N^{α}-methylamino acids with Fmoc solid-phase peptide synthesis is demonstrated by using normal coupling conditions to efficiently prepare *N*-methyl dipeptides. The described procedure allows the preparation of N^{α}-methylamino acids in a very short period of time and a rapid synthesis of *N*-methyl peptides using Fmoc solid-phase peptide synthesis.

Introduction

N^{α}-Methylamino acids are found in a wide range of natural peptides and depsipeptides exhibiting a great range of biological effects, including antibiotic,[1] anticancer,[2] antiviral,[3] and immunosuppressive activity.[4] Incorporation of N^{α}-methylamino acids into biologically active peptides changes the chemical and physical properties and thus may provide important information about backbone conformation.[5] It may also result in enhanced potency,[6] new receptor subtype selectivity,[7] and conversion of an agonist into an antagonist.[8] N^{α}-Methylamino acid containing peptides are also potentially useful therapeutics since *N*-methylation has been shown to improve important pharmacological parameters such as lipophilicity,[9,10] bioavailability,[10b] proteolytic stability,[9,11] conformational rigidity,[5,9] and duration of action.[8]

Commercially available N^{α}-methylamino acids are still very expensive even if various methods have been developed for the synthesis of optically active N^{α}-methylamino acids.[12] The most widely used methods are direct

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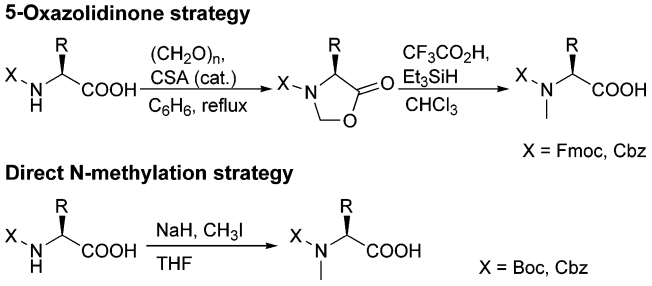

FIGURE 1. Amino acid *N*-methylation by two different procedures.[13,14]

N-methylation of N^{α}-protected α-amino acids or α-amino esters[13] and 5-oxazolidinone formation and reduction (Figure 1).[14] Methods involving reductive amination,[15] Mitsunobu reaction,[16] Diels−Alder/retro-Diels−Alder sequences,[17] and alternative procedures have also been developed.[18] Unfortunately, most of these methods are limited to aliphatic amino acids or are characterized by harsh reaction conditions or long synthetic sequences, and some cause partial racemization of the substrate.[13f,15b,19] In general, the methods above show limited or no application to N^{α}-methylamino acids with functionalized side-chains and are incompatible with Fmoc solid-phase peptide synthesis (Fmoc-SPPS) side-chain protecting groups (Boc, Trt, *t*-Bu). Therefore, we were interested in developing a fast, easy, and highly efficient procedure to prepare side-chain protected N^{α}-methylamino acids in large quantities with the possibility of using them directly in Fmoc-SPPS.

Methods for selective *N*-methylation of peptides on solid support have been reported.[20,21] The most efficient procedure is a three-step sequence involving amine activation by an *o*-nitrobenzenesulfonyl group (*o*-NBS), followed by alkylation[20a] or Mitsonobu reaction[20b,c] on the activated nitrogen and then removal of the sulfonamide group. Three steps are added to the normal coupling/deprotection sequence with this method and can be time-consuming and confusing when *N*-methylated peptide libraries are synthesized. Another problem is that only very small quantities can be prepared on solid support. It has been shown that *o*-NBS-amino acids are compatible with side-chain protections used in Fmoc-SPPS.[22] In this paper, we report the successful synthesis of *o*-NBS-protected N^{α}-methylamino acids and their direct utilization in Fmoc-SPPS.

Results and Discussion

The *o*-nitrobenzenesulfonyl group (*o*-NBS), first described by Fukuyama et al.,[23] has been commonly used for selective *N*-alkylation.[13l,20,22−24] Our strategy was to use the *o*-NBS group to protect and activate the amino function of the amino acid derivatives allowing the use of weaker base and softer conditions for the *N*-methylation step. By doing every step in solution, N^{α}-methyl-N^{α}-*o*-NBS-α-amino acids could be prepared and directly used in Fmoc-SPPS. Therefore, three supplementary steps on solid support could be eliminated. Our initial attempts involved the direct *N*-methylation of N^{α}-*o*-NBS-α-amino acids (Scheme 1). Phenylalanine was protected with *o*-NBS-Cl using the Schotten−Baumann procedure.[25] *N*-Methylation of N^{α}-*o*-NBS-phenylalanine (**1**) was achieved by the Vigneaud and Behrens procedure[13b] using iodomethane in NaOH 0.5 N at 65 °C. Unfortunately, the reaction gave a mixture of N^{α}-*o*-NBS-phenylalanine methyl ester (**2a**), N^{α}-methyl-N^{α}-*o*-NBS-phenylalanine

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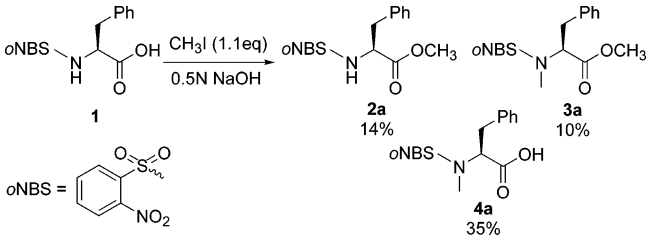
SCHEME 1. Direct Methylation of *o*-NBS-Phe-OH (1)



methyl ester (**3a**), and N^{α}-methyl-N^{α}-*o*-NBS-phenylalanine (**4a**). The desired N^{α}-methyl-N^{α}-*o*-NBS-phenylalanine (**3a**) was only obtained in 35% yield after purification. These low yields become expensive when using side-chain protected amino acids and led us to explore the possibility of using α-amino acid methyl esters.

The N^{α}-*o*-NBS amino acid methyl esters **2a**−**r** were prepared in 92−99% yields of isolated products (Table 1) by treatment of the corresponding methyl esters with *o*-nitrobenzenesulfonyl chloride in the presence of triethylamine in dry methylene chloride (Scheme 2).[20b,24] Protection of the amino acid methyl esters did not involve racemization as shown by ^{1}H NMR and HPLC of the crude product of N^{α}-*o*-NBS-isoleucine methyl ester (**2b**) and N^{α}-*o*-NBS-threonine-*O*-*tert*-butyl ether methyl ester (**2h**).

N-Alkylation of N^{α}-*o*-NBS amino acid methyl esters has already been reported and can be achieved with diazomethane in 24 h,[13l] with iodomethane and K_2CO_3 or Cs_2CO_3 in acetonitrile or dimethylformamide in 3 h,[20b,24] or under Mitsonobu conditions.[20b,c] Miller and Scanlan reported a site-selective *N*-methylation of *o*-NBS peptides on solid support using methyl *p*-nitrobenzenesulfonate in combination with the hindered, nonionic guanidium base MTBD (7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene) in DMF and completed in 30 min.[20a,22] This procedure was chosen and transferred into solution. A first investigation of the reaction with N^{α}-*o*-NBS-phenylalanine methyl ester (**2a**) revealed that a minimum of 2 equiv of MTBD is needed for full conversion to N^{α}-methyl-N^{α}-*o*-NBS-phenylalanine methyl ester (**3a**) and is achieved in 5 min in dimethylformamide. *N*-Methylation in tetrahydrofuran or dichloromethane was not achieved after 2 h. Unfortunately MTBD is too expensive to be used for the synthesis of N^{α}-methyl-α-amino acids at the gram scale. Therefore, TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene) and DBU (1,8-diazabicyclo[5.4.0]undec-7-ene, two structurally similar hindered bases, were tested.

Investigation of the reaction revealed that both bases achieve complete conversion of **2a** under same conditions as MTBD. The less expensive and readily available DBU was chosen and tested on the other N^{α}-*o*-NBS-amino acid methyl esters **2b**−**r** (Scheme 3). *N*-Methylation was then performed using DBU and dimethyl sulfate in DMF at 0 °C for 15 min and gave the corresponding N^{α}-methyl-N^{α}-*o*-NBS amino acid methyl esters **3b**−**o** in 92−99% yields of isolated product without any purification (Table 1). Lower yields were obtained for N^{α}-methyl-N^{α}-*o*-NBS-methionine methyl ester (**3p**) (79%), N^{α}-methyl-N^{α}-*o*-NBS-*S*-tritylcysteine methyl ester (**3q**) (65%), and N^{α}-methyl-N^{α}-*o*-NBS-N^{imid}-tritylhistidine methyl ester (**3r**) (73%). These lower yields could be possibly explained by the presence of side-chain methylation. For example, two products were observed after *N*-methylation of N^{α}-*o*-NBS-methionine methyl ester (**2p**). Both products were isolated, and NMR studies revealed that one is the expected N^{α}-methyl-N^{α}-*o*-NBS-methionine methyl ester (**3p**) and that the other one has been also methylated on the side-chain to give N^{α}-methyl-N^{α}-*o*-NBS-*S*-methyl-methionine methyl ester (**3p′**). This side-chain methylation can be observed in ^{1}H NMR by the presence of a new singlet at 3.01 ppm integrating for six protons due to two methyl groups on the δ-thio function plus the normal new singlet at 2.93 ppm corresponding to *N*-methylation and by the absence of the methyl thioether signal at 2.00 ppm and of the N−H resonance at 5.60. This overmethylated product is water soluble and explains the lower yields obtained for *N*-methylation of **2p**−**r**. HPLC analysis of N^{α}-methyl-N^{α}-*o*-NBS-isoleucine methyl ester (**3b**) and N^{α}-methyl-N^{α}-*o*-NBS-threonine-*O*-*tert*-butyl ether methyl ester (**3h**) showed the presence of only one diastereoisomer, and ^{1}H NMR showed the presence of signals corresponding to only one diastereoisomer.

It is known that N^{α}-protected-N^{α}-methylamino acid methyl esters racemize more readily during saponification than the corresponding amino acid esters.[15c,19] Racemization was also observed in our case during saponification of **3b** and **3h** using 2 M LiOH in THF or methanol. Racemization could be observed by ^{1}H NMR with the appearance of a new doublet next to the normal αH doublet. 8% racemization has been observed for N^{α}-methyl-N^{α}-*o*-NBS-isoleucine (**4b**) and 10% racemization for N^{α}-methyl-N^{α}-*o*-NBS-threonine *O*-*tert*-butyl ether (**4h**). Racemization could also be observed by HPLC with the appearance of a new peak next to the main one. Higher racemization of N^{α}-methyl-N^{α}-*o*-NBS α-amino acid methyl esters during saponification could be explained by the fact that the N−H will generally be more acidic than the α-C−H. Thus, in basic conditions, this group will be deprotonated first and so protect the neighboring C−H bond from deprotonation. With N^{α}-substituted N^{α}-methyl-α-amino acid esters, no such effect is obtained, and the α-C−H will ionize more readily. Similarly, ionization of the free carboxyl group of N^{α}-substituted N^{α}-methyl-α-amino acids will prevent further ionization at the α-carbon atom.

To overcome this problem, methyl ester cleavage by S_N2 dealkylation was tested on the N^{α}-methyl-N^{α}-*o*-NBS-α-amino acid esters **3a**−**r** (Scheme 4). This mild and neutral method occurs with displacement of the carboxylate by S_N2 dealkylation and is usually used for selective methyl ester cleavage.[26] Ester cleavage works best when a powerful nucleophile such as iodide or cyanide is used in a dipolar aprotic solvent. S_N2 methyl ester cleavage using lithium iodide in pyridine has been used lately to minimize racemization during synthesis.[27] A first investigation of the reaction was achieved by treating **3a** with LiI in refluxing pyridine for 30 min.[27] Unfortunately, three products were observed after 30 min. It appears that the methyl on the nitrogen is also electrophile due to the neighboring *o*-NBS group and can also undergo substitution. To overcome this nonselective substitution,

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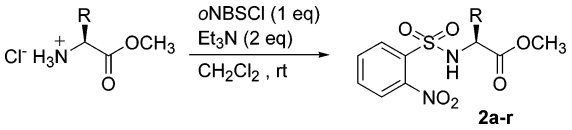
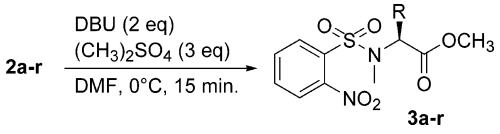
TABLE 1. Three-Step Synthesis of N^{α}-Methyl-N^{α}-*o*-NBS Amino Acids 4a−r

		o-NBS protection	*N*-methylation	saponification
R^1	amino acid	entry (yield (%))	entry (yield (%))	entry (yield (%))
CH_2Ph	Phe	**2a** (97)	**3a** (98)	**4a** (96)
$CH(CH_3)CH_2CH_3$	Ile	**2b** (96)	**3b** (96)	**4b** (85)
CH_3	Ala	**2c** (90)	**3c** (99)	**4c** (90)
Ph	PHg	**2d** (95)	**3d** (98)	**4d** (94)
$(CH_2)_4NHZ$	Lys(Z)	**2e** (93)	**3e** (98)	**4e** (97)
$(CH_2)_4NHBoc$	Lys(Boc)	**2f** (96)	**3f** (99)	**4f** (93)
CH_2OtBu	Ser(tBu)	**2g** (98)	**3g** (92)	**4g** (83)
$CH(CH_3)OtBu$	Thr(tBu)	**2h** (95)	**3h** (97)	**4h** (88)
$CH_2COOtBu$	Asp(tBu)	**2i** (86)	**3i** (96)	**4i** (88)
$CH_2CH_2COOtBu$	Glu(tBu)	**2j** (95)	**3j** (93)	**4j** (91)
$(CH_2)_3NHC(NH)NHPbf$	Arg(Pbf)	**2k** (98)	**3k** (98)	**4k** (95)
$CH_2CONHTrt$	Asn(Trt)	**2l** (97)	**3l** (99)	**4l** (93)
$CH_2CH_2CONHTrt$	Gln(Trt)	**2m** (97)	**3m** (98)	**4m** (89)
CH_2indol-3-yl	Trp	**2n** (97)	**3n** (96)	**4n** (99)
$CH_2PhOtBu$	Tyr(tBu)	**2o** (98)	**3o** (95)	**4o** (88)
$CH_2CH_2SCH_3$	Met	**2p** (90)	**3p** (79)	**4p** (85)
CH_2STrt	Cys(Trt)	**2q** (95)	**3q** (65)	**4q** (35)
CH_2-N^{im}-Trt-imidazol-4-yl	His(Trt)	**2r** (99)	**3r** (73)	**4r** (33)

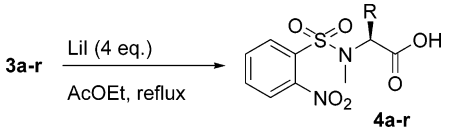
SCHEME 2. Synthesis of 2a−r



SCHEME 3 *N*-Methylation of 2a−r



SCHEME 4. Methyl Ester Cleavage of 3a−r



pyridine was replaced by ethyl acetate which has a lower boiling point and is neutral.[28] N^{α}-Methyl-N^{α}-*o*-NBS-phenylalanine (**4a**) was isolated in 96% yield after treatment of **3a** with LiI in refluxing ethyl acetate for 16 h. Methyl ester cleavage with lithium iodide was tested on the other N^{α}-methyl-N^{α}-*o*-NBS amino acid methyl esters **3b**−**r**, and the corresponding N^{α}-methyl-N^{α}-*o*-NBS amino acids **4b**−**p** were obtained in 83−99% yield without purification (Table 1). Both compounds **3p** and **3p′** gave the desired N^{α}-methyl-N^{α}-*o*-NBS-methionine (**4p**) in 85% yield after methyl ester cleavage. Lower yields were obtained again for **4q** (35%) and **4r** (33%). These lower yields could be possibly explained by the sensibility of the trityl protecting group. Methyl ester cleavage did not involve racemization as shown by ^{1}H NMR and HPLC of the crude product of N^{α}-methyl-N^{α}-*o*-NBS-isoleucine (**4b**) and N^{α}-methyl-N^{α}-*o*-NBS-threonine *O*-*tert*-butyl ether (**4h**).

Compatibility with Fmoc-SPPS was investigated by coupling the synthesized N^{α}-methyl-N^{α}-*o*-NBS amino acids **4a**−**r** to resin-bound leucine using normal Fmoc-SPPS coupling conditions.[29] Racemization of N^{α}-protected N^{α}-methylamino acids during peptide bond formation with mixed anhydride coupling has been reported.[30] Racemization was expected to be less important when using the benzotriazole-based coupling reagent TBTU in combination with HOBt in the presence of a very weak base. The N^{α}-methyl-N^{α}-*o*-NBS-amino acids **4a**−**r** were coupled to the amino free resin-bound leucine using 3 equiv of amino acid, TBTU, HOBt, and collidine in NMP for 30 min. Resulting dipeptides **5a**−**r** were cleaved from the resin, and only one diastereoisomer was observed by HPLC analysis (96−99% purity). In an additional experiment, N^{α}-methyl-N^{α}-*o*-NBS-D-phenylalanine (**4a′**) and N^{α}-methyl-N^{α}-*o*-NBS-D-tryptophan (**4n′**) were also coupled to resin-bound leucine. HPLC analysis of **5a′** and **5n′** showed a different retention time compared to their corresponding diastereoisomeric dipeptides **5a** and **5n** excluding any detectable racemization throughout the entire synthetic procedure. To continue the peptide synthesis, the *o*-NBS protecting group can be easily and selectively removed with mercaptoethanol and DBU in 30 min.[20a,22] Couplings on resin-bound *N*-methyl peptide can be achieved with HATU/HOAt or with triphosgene methods.[20a,31]

By comparing the herein described procedure with other known procedures to prepare *N*-methylamino acids, it can be easily observed that no procedure is the best one to prepare every amino acid with respect to yield, number of steps, and economy (Table 2). Therefore, it is crucial to choose the right procedure to prepare the desired amino acid. For example, the 5-oxazolidinone procedure is without a doubt the best method to prepare Fmoc-N^{α}-methyl aliphatic amino acids (Table 2). Only two steps and 2 days are needed to prepare the N^{α}-methylamino acids. On the other hand, the *o*-NBS

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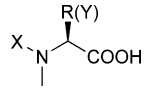
(31) (a) Falb, E., Yechezkel, J., Salitra, Y., Gilon, C. *J. Peptide Res.* **1999**, *53*, 507−517. (b) Thern, B.; Rudolf, J.; Jung, G. *Tetrahedron Lett*. **2002**, *43*, 5013−5016. (c) Thern, B.; Rudolf, J.; Jung, G. *Angew. Chem., Int. Ed*. **2002**, *41*, 2307−2309. (d) Sewald, N. *Angew. Chem., Int. Ed.* **2002**, *41*, 4661−4663.

TABLE 2. Preparation of N^{α}-Methylamino Acids by Different Procedures[a]



	*o*NBS procedure		5-oxazolidinone			direct *N*-methylation		
amino acid	Y	yield (%)	X	Y	% (no. of steps)	X	Y	% (no. of steps)
aliphatic		78−91	**Fmoc**		**51−96 (2)[14]**	Boc		83−90 (1)[13]
Asn	**Trt**	**89**	Cbz		62 (2)[14f]			
Arg	**Pbf**	**91**	Cbz	Boc_2	39 (7)[14h]	Boc	Cbz_2	62 (4)[13i]
Asp	**tBu**	**73**	Cbz	Bzl	38 (2)[14f]	**Cbz**	**tBu**	**86 (1)[13f]**
Cys	Trt	22	Cbz	Bzl	62 (2)[14i]			
Gln	**Trt**	**85**	Cbz		42 (2)[14i]			
Glu	**tBu**	**80**	Cbz		54 (2)[14f]	**Cbz**	**tBu**	**70 (1)[13f]**
His	Trt	24	**Cbz**	**DNP**	**30 (5)[14h]**			
Lys	**Boc**	**88**	Cbz	Bzl	21 (6)[14i]			
	Cbz	86	Fmoc	Pht	51 (2)[14c]			
Met		**75**	Cbz		64 (4)[14i]	**Cbz**		**71 (1)[13f]**
Ser	tBu	75	Fmoc	Bzl	94 (2)[14c]	Boc	Bzl	80 (1)[13d]
Thr	tBu	81	Cbz		64 (5)[14i]	Boc	Bzl	75 (1)[13d]
Trp		**92**						
Tyr	**tBu**	**82**	**Fmoc**	**Bzl**	**60 (2)[14i]**	Boc	Bzl	70 (1)[13d]

[a] Suggested procedures to prepare the N^{α}-methylamino acid are shown in boldface type.

procedure is the method of choice to prepare directly Fmoc-SPPS compatible tryptophan, lysine, asparagine, glutamine, and arginine. It is important to note that some N^{α}-methylamino acids reported in Table 2 still need some transformation to be compatible with Fmoc-SPPS. One advantage of the *o*-NBS procedure is that *o*-NBS-Cl is actually less expensive than Fmoc-Cl. Another advantage is that the starting material (side-chain protected amino acid methyl esters) is also quite inexpensive or can be easily prepared from the less expensive amino acid methyl esters or side-chain protected amino acids. Unfortunately, cysteine and histidine are still problematic and are obtained in low yields or with Fmoc-SPPS incompatible protecting groups in every reported procedure.

Conclusion

N^{α}-Methyl-N^{α}-*o*-NBS-amino acids are prepared from amino acid methyl esters in three steps and excellent overall yields without any purification. Side-chain protected amino acid methyl esters are commercially available or can be easily obtained from inexpensive amino acid methyl esters or side-chain protected amino acids. The *o*-NBS procedure is also an inexpensive way to prepare N^{α}-methyl-D- and unnatural amino acids. After protection and activation of the amino function of the amino acid methyl esters with *o*-NBS-Cl, the *N*-methylation reaction is performed by treatment of *o*-NBS derivatives with DBU and dimethyl sulfate. Methyl ester hydrolysis of the N^{α}-methyl-N^{α}-*o*-NBS-amino acid methyl esters with lithium hydroxide causes partial racemization. To overcome this problem, a clean and racemization-free S_N2 methyl ester cleavage of N^{α}-methyl-N^{α}-*o*-NBS-amino acid methyl esters is achieved with lithium iodide in boiling ethyl acetate as confirmed by HPLC and ^{1}H NMR spectroscopy. It has been shown, by comparing literature data, that no single procedure is best for the synthesis of all derivatives, suggesting that the right procedure should be chosen to prepare the desired *N*-methyl amino acid. N^{α}-Methyl-N^{α}-*o*-NBS-amino acids can be directly used in Fmoc-SPPS with normal coupling conditions to synthesize *N*-methyl peptide analogues. The entire synthetic sequence is compatible with Fmoc-SPPS protecting groups and can be done in 1 day, allowing a rapid and efficient synthesis of *N*-methyl peptide analogues.

Experimental Section

N^{α}-Methyl-N^{α}-(*o*-nitrobenzenesulfonyl)-L-phenylalanine Methyl Ester (3a). To an ice-cold solution of N^{α}-(*o*-nitrobenzenesulfonyl)phenylalanine methyl ester **2a** (1 mmol) in dimethylformamide (30 mL) were slowly added first dimethyl sulfate (3 mmol) and then 1,8-diazabicyclo[5,4,0]undec-7-ene (2 mmol), and the mixture was stirred at 0 °C for 15 min. After the addition of acetic acid (1.1 mmol), the solvent was evaporated under reduced pressure and the residue taken up in ethyl acetate and washed with a 10% aqueous solution of $NaHCO_3$ (3 × 25 mL) and water, dried (Na_2SO_4), and evaporated to dryness. Diethyl ether was added and evaporated two times, and the residue was precipitated with $CHCl_3$ and *n*-hexanes to afford a pale yellow solid (98%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.81−7.75 (m, 1H), 7.69−7.51 (m, 3H), 7.31−7.19 (m, 5H), 5.01−4.92 (m, 1H), 3.65 (s, 3H), 3.42−3.31 (dd, J = 6.2 Hz, 14.2 Hz, 1H), 3.08−2.91 (m, 4H); MS (ESI) *m/z* 379.1 $(M + H)^+$, 401.1 $(M + Na)^+$; RP-HPLC t_R = 23.9 (10−100%).

Synthesis of *N*-Methyl-*N*-(*o*-nitrobenzenesulfonyl)-amino Acids 4a−r. General Procedure. The *N*-methyl-*N*-(*o*-nitrobenzenesulfonyl)-α-amino acid methyl ester **3a**−**r** (1 mmol) and LiI (5 mmol) were dissolved in ethyl acetate (5 mL). The reaction mixture was protected from light and heated at reflux for 16 h or until full conversion was observed by TLC. Water was added (5 mL), and the solution was acidified with 0.1 M HCl and quickly extracted with ethyl acetate. The organic phase was washed with water and a saturated aqueous solution of NaCl and extracted with a solution 0.1 M NaOH (3 × 15 mL). The aqueous phase was washed with DCM (3 × 15 mL), acidified with 1 M HCl, and quickly extracted with ethyl acetate (3 × 15 mL). The organic phase was dried and evaporated to dryness to afford a pale yellow solid or a pale yellow oil. To obtain the amino acids as powder, they were precipitated with $CHCl_3$ and *n*-hexanes (Phe, Phg, Thr, Arg, Asn, Gln, Trp, Cys, His) or lyophilized with a mixture of dioxane/water (Ala, Lys, Ser, Asp, Glu, Tyr, Met). They can also be recrystallized with ethyl acetate−benzene or ethyl

acetate−*n*-hexanes (Phe, Phg, Thr, Arg, Asn, Gln, Trp, Cys, His) and EtOH−water (Ala, Lys, Asp, Glu, Tyr). Isoleucine, serine, and methionine derivatives could not be recrystallized.

***N*-Methyl-*N*-(*o*-nitrobenzenesulfonyl)-L-phenylalanine (4a):** pale yellow solid (96%); mp 56−59 °C; $[\alpha]^{25}_{D}$ +64 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.55 (br s, 1H), 7.78−7.52 (m, 4H), 7.31−7.19 (m, 5H), 5.08−4.97 (m, 1H), 3.46−3.35 (dd, J = 5.4 Hz, 14.5 Hz, 1H), 3.12−2.95 (m, 4H); ^{13}C δ 175.79, 147.81, 135.73, 133.57, 132.35, 131.71, 130.62, 128.96, 128.66, 127.19, 124.35, 60.72, 35.37, 30.86; MS (ESI) *m/z* 365.1 (M + H)$^+$, 387.1 (M + Na)$^+$; RP-HPLC t_R = 20.8 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-L-isoleucine (4b):** pale yellow oil (85%); ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.55 (br s, 1H), 8.05−7.98 (m, 1H), 7.74−7.60 (m, 3H), 4.24 (d, J = 9.3 Hz, 1H), 3.04 (s, 3H), 2.04−1.83 (m, 1H), 1.59−1.44 (m, 1H), 1.28−1.04 (m, 1H), 1.02−0.82 (m, 6H); ^{13}C δ 176.0, 147.9, 133.9, 132.2, 131.7, 130.9, 124.2, 63.7, 34.5, 31.1, 25.6, 15.4, 10.9; MS (ESI) *m/z* 331.1 (M + H)$^+$, 353.1 (M + Na)$^+$; RP-HPLC t_R = 20.9 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-L-alanine (4c):** pale yellow solid (90%); mp 48−50 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.12−8.06 (m, 1H), 7.76−7.64 (m, 3H), 7.00 (br s, 1H), 4.78−4.69 (m, 1H), 2.91 (s, 3H), 1.45 (d, J = 7.5 Hz, 3H). ^{13}C δ 177.0, 147.9, 133.7, 132.5, 132.0, 131.1, 124.4, 55.5, 30.2, 15.2; MS (ESI) *m/z* 311.0 (M + Na)$^+$, 599.1 (2M + Na)$^+$; RP-HPLC t_R = 16.9 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-L-phenylglycine (4d):** pale yellow solid (94%); mp 55−59 °C; $[\alpha]^{25}_{D}$ +12.5 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.23 (br s, 1H), 8.08−8.02 (m, 1H), 7.75−7.67 (m, 3H), 7.45−7.31 (m, 5H), 6.03 (s, 1H), 2.82 (s, 3H); ^{13}C δ 175.2, 147.7, 133.9, 132.8, 132.2, 131.8, 130.7, 129.1, 129.0, 129.0, 124.5, 62.9, 31.3; MS (ESI) *m/z* 351.1 (M + H)$^+$, 373.1 (M + Na)$^+$, 701.2 (2M + H)$^+$, 723.2 (2M + Na)$^+$; RP-HPLC t_R = 20.6 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*N*$^{\epsilon}$-benzyloxycarbonyl-L-lysine (4e):** pale yellow solid (97%); mp 85−87 °C; $[\alpha]^{25}_{D}$ +46.5 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.37 (br s, 1H), 8.09−8.01 (m, 1H), 7.73−7.60 (m, 3H), 7.43−7.32 (m, 5H), 6.11 (m, 1H), 5.10 (s, 2H), 4.71−4.61 (m, 1H), 3.25−3.09 (m, 2H), 2.95 (s, 3H), 2.07−1.88 (m, 1H), 1.84−1.32 (m, 5H); ^{13}C δ 174.7, 147.9, 133.6, 132.4, 131.6, 130.8, 128.5, 128.1, 124.3, 66.7, 59.1, 40.6, 30.3, 29.0, 28.5, 23.0; MS (ESI) *m/z* 480.1 (M + H)$^+$, 502.1 (M + Na)$^+$, 959.2 (2M + H)$^+$, 981.2 (2M + Na)$^+$; RP-HPLC t_R = 21.6 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*N*$^{\epsilon}$-*tert*-butyloxycarbonyl-L-lysine (4f):** pale yellow solid (93%); mp 54−55 °C; $[\alpha]^{25}_{D}$ +67.5 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.85 (br s, 1H), 8.09−8.01 (m, 1H), 7.76−7.64 (m, 3H), 6.03 (br s, 1H), 4.68−4.62 (m, 1H), 3.17−3.01 (m, 2H), 2.96 (s, 3H), 2.08−1.93 (m, 1H), 1.84−1.65 (m, 1H), 1.58−1.31 (m, 13H); ^{13}C δ 174.8, 156.0, 147.9, 133.6, 132.4, 131.6, 130.8, 124.3, 79.0, 59.2, 40.1, 30.3, 29.0, 28.4, 23.1; MS (ESI) *m/z* 446.1 (M + H)$^+$, 468.1 (M + Na)$^+$, 991.2 (2M + H)$^+$, 1013.2 (2M + Na)$^+$; RP-HPLC t_R = 21.1 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*O*-*tert*-butyl-L-serine (4g):** pale yellow gummy solid (83%); $[\alpha]^{25}_{D}$ +47.1 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.08 (br s, 1H), 8.17−8.08 (m, 1H), 7.76−7.63 (m, 3H), 4.93−4.86 (m, 1H), 3.92−3.76 (m, 2H), 3.09 (s, 3H), 1.18 (s, 9H); ^{13}C δ 173.2, 148.0, 133.5, 132.5, 131.6, 130.8, 127.3, 74.6, 60.9, 59.6, 32.1, 27.1; MS (ESI) *m/z* 361.3 (M + H)$^+$, 383.3 (M + Na)$^+$, 721.6 (2M + H)$^+$, 743.6 (2M + Na)$^+$; RP-HPLC t_R = 21.3 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*O*-*tert*-butyl-L-threonine (4h):** pale yellow solid (88%); mp 142−145 °C; $[\alpha]^{25}_{D}$ +156.3 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.44 (br s, 1H), 8.11−8.00 (m, 1H), 7.79−7.61 (m, 3H), 4.70 (d, J = 3.6 Hz, 1H), 4.47−4.35 (m, 1H), 3.20 (s, 3H), 1.31 (d, J = 6.3, 3H), 1.16 (s, 9H); ^{13}C δ 175.1, 147.7, 133.5, 131.6, 131.5, 130.9, 124.5, 74.8, 69.2, 64.8, 33.8, 28.4, 20.3; MS (ESI) *m/z* 375.1 (M + H)$^+$, 396.1 (M + Na)$^+$, 749.2 (2M + H)$^+$, 771.2 (2M + Na)$^+$; RP-HPLC t_R = 24.1 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-L-aspartic acid β-*tert*-butyl ester (4i):** pale yellow solid (88%); mp 75−77 °C; $[\alpha]^{25}_{D}$ +22.9 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.01 (br s, 1H), 8.10−8.03 (m, 1H), 7.78−7.64 (m, 3H), 5.13 (t, J = 7.1 Hz, 1H), 3.02−2.87 (m, 4H), 2.71 (dd, J = 7.6 Hz, 16 Hz, 1H), 1.45 (s, 9H); ^{13}C δ 174.6, 168.8, 147.9, 133.8, 132.2, 131.7, 131.0, 124.4, 82.4, 56.4, 36.4, 31.3, 27.9; MS (ESI) *m/z* 389.3 (M + H)$^+$, 411.3 (M + Na)$^+$, 777.2 (2M + H)$^+$, 799.2 (2M + Na)$^+$; RP-HPLC t_R = 20.9 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-L-glutamic acid γ-*tert*-butyl ester (4j):** pale yellow solid (91%); mp 58−62 °C; $[\alpha]^{25}_{D}$ +66.9 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.87 (br s, 1H), 8.06−8.00 (m, 1H), 7.77−7.64 (m, 3H), 4.80−4.72 (m, 1H), 2.96 (s, 3H), 2.54−2.26 (m, 3H), 2.04−1.91 (m, 1H), 1.47 (s, 9H); ^{13}C δ 175.4, 171.8, 147.8, 133.8, 131.9, 131.6, 130.7, 124.3, 81.2, 58.7, 31.6, 30.4, 28.0, 24.0; MS (ESI) *m/z* 403.1 (M + H)$^+$, 425.1 (M + Na)$^+$; RP-HPLC t_R = 21.8 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*N*^{G}-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginine (4k):** white solid (95%); mp 93−101 °C dec; $[\alpha]^{25}_{D}$ +49.3 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.12−8.04 (m, 1H), 7.75−7.56 (m, 3H), 6.23 (s, 2H), 6.05 (s, 1H), 4.68−4.59 (m, 1H), 3.37−3.23 (m, 2H), 2.99 (s, 2H), 2.92 (s, 3H), 2.52−2.47 (d, 6H), 1.89−1.54 (m, 4H), 1.48 (s, 6H); ^{13}C δ 174.1, 159.6, 156.5, 148.2, 139.0, 134.2, 132.5, 131.2, 125.5, 124.6, 118.2, 87.1, 59.5, 43.5, 41.0, 29.0, 26.4, 19.7, 18.2, 12.9; MS (ESI) *m/z* 626.5 (M + H)$^+$, 648.5 (M + Na)$^+$; RP-HPLC t_R = 21.9 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*N*$^{\beta}$-trityl-L-asparagine (4l):** pale yellow solid (93%); mp 105−112 °C dec; $[\alpha]^{25}_{D}$ +11.5 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.25 (br s, 1H), 8.16−8.05 (m, 1H), 7.64−7.58 (m, 3H), 7.31−7.17 (m, 15H), 6.83 (s, 1H), 4.99−4.95 (m, 1H), 3.07−2.81 (m, 5H); ^{13}C δ 170.8, 170.4, 147.9, 143.7, 133.7, 132.2, 132.0, 130.9, 128.7, 128.0, 127.2, 124.3, 71.3, 56.3, 38.1, 31.8; MS (ESI) *m/z* 574.5 (M + H)$^+$, 596.7 (M + Na)$^+$; RP-HPLC t_R = 25.1 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*N*$^{\gamma}$-trityl-L-glutamine (4m):** pale yellow solid (89%); mp 105−112 °C dec; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.33 (br s, 1H), 8.00−7.94 (m, 1H), 7.68−7.57 (m, 3H), 7.35−7.20 (m, 15H), 6.90 (s, 1H), 4.68−4.61 (m, 1H), 2.93 (s, 3H), 2.56−2.41 (m, 2H), 2.39−2.21 (m, 1H), 2.02−1.83 (m, 1H); ^{13}C δ 171.9, 171.7, 148.0, 145.0, 133.5, 132.4, 132.0, 130.2, 128.5, 127.4, 126.2, 123.8, 69.1, 61.7, 33.9, 30.2, 26.0; MS (ESI) *m/z* 588.4 (M + H)$^+$, 610.4 (M + Na)$^+$; RP-HPLC t_R = 25.3 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-L-tryptophan (4n):** pale yellow solid (99%); mp 83−85 °C; $[\alpha]^{25}_{D}$ +40.1 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, DMSO-d_6) δ 13.10 (br s, 1H), 10.84 (s, 1H), 7.82−7.65 (m, 2H), 7.52 (d, J = 7.7 Hz, 1H), 7.46−7.41 (m, 2H), 7.33 (d, J = 7.7 Hz, 1H), 7.21−7.16 (m, 1H), 7.12−6.95 (m, 2H), 4.85−4.76 (m, 1H), 3.42−3.31 (m, 1H), 3.17 (dd, J = 10.1 Hz, 15.2 Hz, 1H), 3.00 (s, 3H); ^{13}C δ 171.5, 147.2, 136.1, 133.9, 131.6, 131.2, 129.4, 126.7, 123.9, 123.7, 121.0, 118.5, 117.8, 111.5, 108.9, 59.7, 30.1, 24.9; MS (ESI) *m/z* 404.1 (M + H)$^+$, 426.1 (M + Na)$^+$, 807.2 (2M + H)$^+$, 829.2 (2M + Na)$^+$; RP-HPLC t_R = 20.1 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*O*-*tert*-butyl-L-tyrosine (4o):** pale yellow solid (88%); mp 75−80 °C; $[\alpha]^{25}_{D}$ +50.9 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.97 (br s, 1H), 7.91−7.53 (m, 4H), 7.12 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 8.5 Hz, 2H), 5.01−4.92 (m, 1H), 3.33 (dd, J = 5.4 Hz, 14.5 Hz, 1H), 3.07−2.90 (m, 4H), 1.34 (s, 9H); ^{13}C δ 175.4, 154.3, 147.7, 133.5, 132.3, 131.6, 130.6, 130.5, 129.4, 124.3, 124.2, 78.6, 60.7, 34.7, 30.8, 28.8; MS (ESI) *m/z* 437.2 (M + H)$^+$, 459.2 (M + Na)$^+$, 873.4 (2M + H)$^+$, 895.4 (2M + Na)$^+$; RP-HPLC t_R = 23.5 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*S*-methyl-L-methionine (4p):** pale yellow solid (85%); mp 109−111 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.66 (br s, 1H), 8.10−8.05 (m, 1H), 7.77−7.66 (m, 3H), 4.88−4.82 (m, 1H), 2.99 (s, 3H), 2.62−2.46

(m, 2H), 2.33−2.18 (m, 1H), 2.14−1.95 (m, 4H); ^{13}C δ 175.8, 147.8, 133.8, 132.3, 131.8, 131.1, 124.5, 58.3, 30.6, 30.3, 28.8, 15.4; MS (ESI) *m/z* 349.0 $(M + H)^+$, 371.0 $(M + Na)^+$, 697.0 $(M + H)^+$, 719.0 $(M + Na)^+$; RP-HPLC t_R = 19.6 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*S*-trityl-L-cysteine (4q):** pale yellow solid (35%); mp 62−66 °C dec; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.09−7.96 (m, 1H), 7.67−7.50 (m, 3H), 7.44−7.13 (m, 15H), 4.57−4.44 (m, 1H), 2.85−2.68 (m, 4H), 2,55−2.42 (m, 1H); ^{13}C δ 170.7, 147.9, 143.8, 134.0, 133.2, 132.2, 130.8, 128.6, 128.0, 126.9, 123.9, 69.3, 62.3, 31.2, 24.8; MS (ESI) *m/z* 563.1 $(M + H)^+$, 586.1 $(M + Na)^+$, 1125.2 $(2M + H)^+$, 1147.2 $(2M + Na)^+$; RP-HPLC t_R = 26.2 (10−100%).

***N*$^{\alpha}$-Methyl-*N*$^{\alpha}$-(*o*-nitrobenzenesulfonyl)-*N*^{imid}-trityl-L-histidine (4r):** white solid (33%); mp 81−85 °C dec; $[\alpha]^{25}_D$ +37.5 (*c* 0.1, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.15−8.06 (m, 1H), 7.72−7.55 (m, 3H), 7.48−7.38 (m, 9H), 7.20−7.10 (m, 6H), 7.00 (s, 1H), 5.21−5.10 (m, 1H), 3.66−3.50 (m, 1H), 3.34−3.18 (m, 1H), 2.94 (s, 3H). ^{13}C δ 170.0, 148.1, 139.4, 134.7, 133.7, 132.0, 131.7, 130.2, 129.2, 127.9, 124.0, 121.9, 79.0, 59.2, 30.9, 24.9, 15.4; MS (ESI) *m/z* 597.3 $(M + H)^+$, 619.3 $(M + Na)^+$, 1193.6 $(2M + H)^+$, 1215.6 $(2M + Na)^+$; RP-HPLC t_R = 21.5 (10−100%).

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Supporting Information Available: General procedure for the synthesis of N^{α}-*o*-NBS amino acid methyl esters **2a**−**r**, synthesis of N^{α}-methyl-N^{α}-*o*-NBS amino acid methyl esters **3b**−**r**, compound characterization data of **2a**−**r**, ^{1}H NMR spectra of **2**−**4b** and **2**−**4h**, general procedure for the synthesis of dipeptides **5a**−**r**, and HPLC analyses of **5a**−**a′**,**b**,**f**,**n**−**n′**. This material is available free of charge via the Internet at http://pubs.acs.org.

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