Synthesis of 1,1-Dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl (Bsmoc) Protected *N*-Methyl Amino Acids by Reduction of Bsmoc-5-Oxazolidinones and Their Use in Peptide Synthesis

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Abstract: The synthesis of Bsmoc-*N*-methyl amino acids is presented. The first step involves *p*-toluenesulfonic acid (TsOH) catalysed condensation of a Bsmoc-amino acid with p*ara*formaldehyde to furnish *N*-Bsmoc-5-oxazolidinone under MW irradiation. This intermediate is reduced to the corresponding *N*-methyl amino acid using triethylsilane (Et_3SiH) and trifluoroacetic acid (TFA) at r.t. The *N*-methyl amino acids are converted into corresponding acid fluorides using diethylaminosulfur trifluoride (DAST) and employed as coupling agents in the synthesis of dipeptides. The peptide coupling was mediated by KOAt in CH_2Cl_2.

Keywords: Bsmoc *N* methyl amino acids, 5-Oxazolidinone, Amino acid fluorides, KOAt.

INTRODUCTION

The *N*-methyl amino acids are wide spread in nature as part of larger peptidic natural products [1-3], alamethicin peptides and cyclosporine family [4]. They find broad application in the design and development of biologically potent molecules in medicinal chemistry [5, 6]. In addition, they are inserted into biologically active peptides to enhance potency and duration of action or to convert an agonist to an antagonist or to obtain information about backbone conformation or to increase the aqueous solubility of a peptide [7]. For the purpose of peptide synthesis, it is essential to have *N*-urethane protected *N*-methyl amino acids as building blocks. Several procedures are available for the synthesis of *N*-carbamate protected *N*-methyl amino acids [8]. Benoiton procedure [9] involving NaH and MeI for abstraction of proton followed by methylation is the method of choice being utilized to prepare base stable Boc and Z protected *N*-methyl amino acids. An improvement has been made to the above method by using KH/18-crown ether and alkyl halide. Ben-Ishai [10] demonstrated the first synthesis and utility of 5-oxazolidinones as a tool for simultaneous protection of the amino and carboxyl groups. Recently, Sureshbabu *et al.* [11] demonstrated the utility of MW irradiation for the rapid, high yielding synthesis of 5-oxazolidinones. The entire protocol is complete in 2 min. Govender and Arvidsson [12] further fine tuned by selecting acetonitrile in place of toluene as a solvent due to its poor dielectric heating capacity. Weinreb *et al.* [13] had developed a procedure for the reduction of methylol derivatives of simple amides using triethylsilane – trifluoroacetic acid. This involves reduction of N-acyliminium ion followed by ionic hydrogenation. Freidinger *et al.* [14] is the first one to synthesize Fmoc-*N*-methylated amino acids *via* 5-oxazolidinones and their reduction employing Et_3SiH–TFA. This procedure was extended for the synthesis of *N*-methyl Trp, Asn, His and Arg and then to all other amino acids by Aurelio *et al.* [15,16]. Zhang *et al.* [17] accomplished the reductive ring opening of the 5-oxazolidinone's using Lewis acid catalysis ($AlCl_3$ or $ZnBr_2$). This paper deals with the hitherto unreported Bsmoc-*N*-methyl amino acids synthesis employing Freidinger procedure.

Bsmoc group [18] , introduced by Louis Carpino, for amino protection has significant advantages over the Fmoc group. The key difference of this invention is the mode of cleavage mechanism through Michael-like attack of base on α,β unsaturated sulfones [19] followed by elimination of CO_2 and amino component (Scheme **1**). As Bsmoc group can be effectively cleaved with very low concentration of a weak base, its usage can circumvent several base catalyzed side reactions. Upon cleavage of the Fmoc group, the acidic phosphate buffer wash is necessary for the removal of DBF adduct formed. On the other hand, the use of either water or brine wash is sufficient for the complete removal of all by products formed in Bsmoc chemistry. This leads to a clear phase separation and minimal loss of carefully built peptide chain into aqueous phase. Ultimately, increase in yields of the target peptides can be achieved. The utility of Bsmoc group in rapid, continuous solution-phase peptide synthesis was demonstrated by the synthesis of a human parathyroid hormone fragment (hPTH, 21-29) [20]. The synthesis of peptides employing acid fluorides is well known in peptide chemistry. In contrast to the corresponding acid chlorides, the acid fluorides have greater stability and are suitable for carrying out reactions bearing acid labile groups such as ^{t}Bu,

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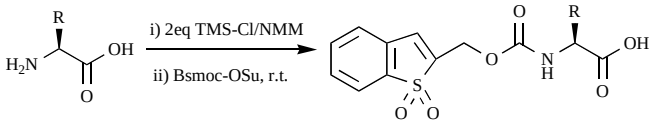
Boc or trityl groups in the side chain [21]. Acid fluorides have been shown to accomplish difficult esterifications such as between a weakly nucleophilic secondary alcohol and a cyclohexyl amino acid, where other methods of activation such as DCC/DMAP, mixed anhydride, BOP-Cl or BOP have failed [22]. Successful synthesis of acyl carrier protein 65-74 fragment (ACP), magainin-II-amide, human corticotropin-releasing factor (*h*-CRF) and peptide related to Alzheimers disease Aβ (1-42) which are known as difficult sequences to synthesize, have been accomplished efficiently employing acid fluorides [23]. Very difficult sequence containing sterically hindered $C^{\alpha\alpha}$-dialkylamino acids such as Aib [24], 1-aminocyclohexane-1-carboxylic acid and 4-amino-piperidine-4-carboxylic acid [25] have also been achieved with greater efficiency by the use of corresponding acid fluorides. A comparative study of activation methods for the synthesis of the model tetrapeptide H-Aib-Aib-Aib-Aib-OH by symmetric anhydride, PyBroP, acid fluoride and UNCA methods revealed that the acid fluoride coupling perform exceptionally well [26]. The acid fluorides are synthesized using cyanuric fluoride in presence of pyridine according to the general technique of Olah [27]. An alternative procedure for the synthesis of Fmoc-amino acid fluorides involves DAST [28] which is advantageous owing to the formation of water soluble byproducts, thus facilitating the isolation of the acid fluorides. The synthesis of Bsmoc N-methyl aminoacid fluorides using DAST and their coupling in the presence of KOAt is described.

RESULTS AND DISCUSSION

Towards the first step, benzothiophene-2-methanol, prepared using literature procedure, was oxidized using *m*-chloroperbenzoic acid (*m*-CPBA) at r.t. A simple work up of the reaction resulted the sulfone in 88% yield. Earlier description of the oxidation involved in the use of excess of acetic acid and sodium perborate. During the preliminary rounds of preparation of sulfone we followed the reported protocol. In such crops, during the removal of acetic acid considerable amount of the product was decomposed resulting in low yield of sulfone. Treatment of 1,1-dioxo-benzothiophene-2-methanol with triphosgene resulted in 77% of Bsmoc-Cl (Scheme **2**) which was then converted into its succinimide. Bsmoc- amino acids were prepared by the reported procedure .

For the synthesis of Bsmoc-amino acids, Bolin's technique of *N,O-bis*-trimethyl amino acids was employed. Bsmoc-OSu was added to a solution of freshly prepared *N,O-bis*-trimethyl amino acid in DCM and the reaction mixture was stirred at r.t. for 2 hr. Workup of the reaction mixture resulted in Bsmoc amino acids in good yield (Scheme **3**).

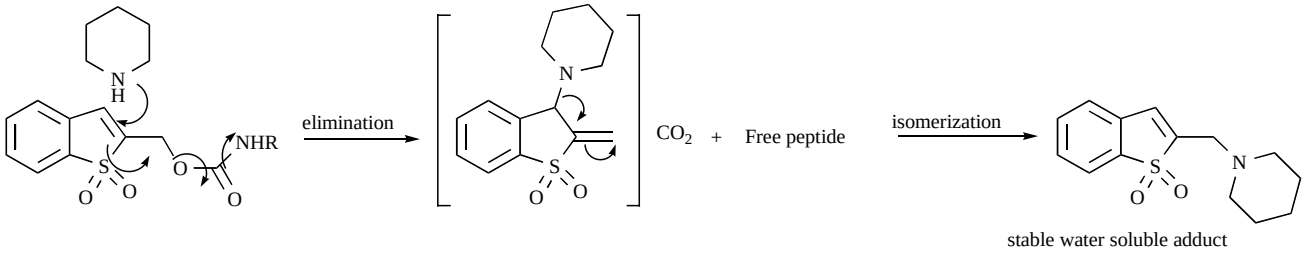


R = H, CH_3, $CH(CH_3)_2$, $CH_2C_6H_5$, $CH_2CH(CH_3)_2$, $CH(C_2H_5)CH_3$

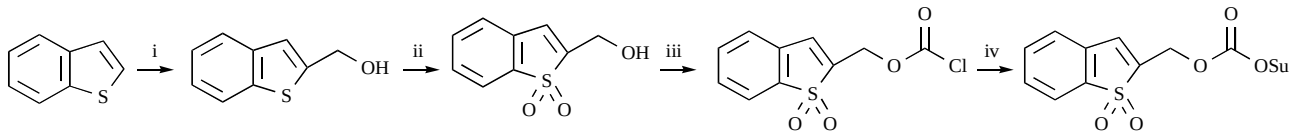
Scheme 3. Synthesis of Bsmoc-amino acid.

The synthesis of Bsmoc-5-oxazolidinones was carried out starting from Bsmoc-amino acids. Bsmoc-amino acid was condensed with paraformaldehyde in presence of catalytic amount of TsOH under microwave irradiation. The reaction was complete within 3 min and workup of the reaction mixture resulted in 1a-f (Scheme **4**, Table **1**) in 89 to 95% yield. These oxazolidinones were stable at room temperature and had a strong IR stretching frequency at 1800 cm^{-1}. Further, the ^1H NMR specta of the same also showed a signal in the vicinity of gamma amino alcohol δ 5.1 ppm which is the characteristic peak of 5-oxazolidinone.

The 5-oxazolidinones were reduced into their corresponding *N*-methyl amino acids by treatment with 2.5 eq of Et_3SiH in presence of TFA-$CHCl_3$ (1:1). This has resulted in simultaneous ring opening along with reduction to obtain Bsmoc-*N*Me-amino acids 2a-f in 80 to 93% yield (Scheme **5**, Table **2**). The reaction was complete after stirring for 22 hr at r.t. The IR specta of Bsmoc-*N*-methyl amino acids showed the absence of IR stretching band at 1800 cm^{-1} corresponding to 5-oxazolidinones. The ^1H NMR of Bsmoc-*N*-methyl

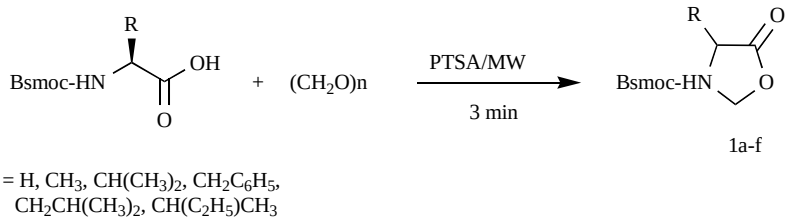


Scheme 1. Cleavage mechanism of Bsmoc group.



i. n-BuLi, $(CH_2O)n$, -78°C; ii. m-CPBA/DCM;
iii. Triphosgene, 0°C; iv. N-hydroxysuccnimide, DCM

Scheme 2. Synthesis of Bsmoc-Cl.

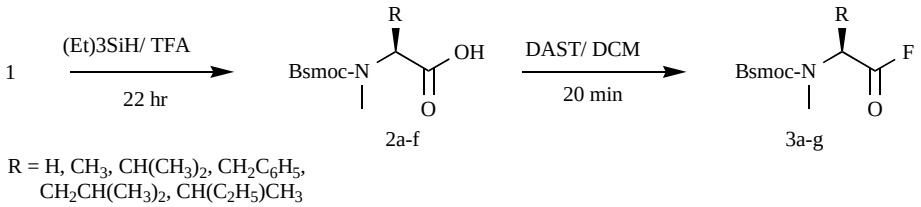


Scheme 4. Synthesis of Bsmoc -5-oxazolidinones.

Table 1. List of Bsmoc-5-Oxazolidinones

Entry	Bsmoc-5-oxazolidinones R	Yield* (%)	IR (cm^{-1})	M.P. (^oC)	$[\alpha]^{25}_D$ (C1, $CHCl_3$)
1a	H	91	1801,1718	100-03	-
1b	CH_3	93	1801,1720	Gum	+79.8
1c	$CH_2C_6H_5$	92	1799,1719	95-97	+91.9
1d	$CH(CH_3)CH_2CH_3$	92	1799,1717	123-25	+74.8
1e	CH_2 CH $(CH_3)_2$	95	1800,1720	40-42	+81.4
1f	CH $(CH_3)_2$	95	803,1720	120-22	+77.3

*The reaction time was 3 minutes for all the entries



Scheme 5. Reduction of N-Bsmoc-5-oxazolidinones to *N*-methyl amino acids and convertion into corresponding acid fluorides.

Table 2. List of Bsmoc-*N*-Methyl Amino Acids

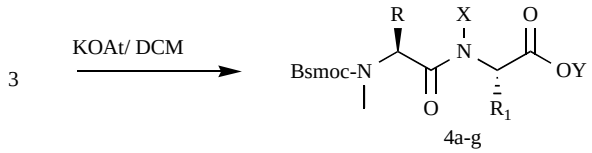
Entry	Bsmoc-*N*Me-amino acids	Yield (%)	M.P. (^oC)	$[\alpha]^{25}_D$ (c 1, $CHCl_3$)
2a	Bsmoc-Sar-OH	80	gum	-
2b	Bsmoc-MeAla-OH	91	140-42	-27.9
2c	Bsmoc-MePhe-OH	89	95-97	-20.0
2d	Bsmoc-MeIle-OH	88	123-25	-31.5
2e	Bsmoc-MeLeu-OH	93	40-42	-26.8
2f	Bsmoc-MeVal-OH	92	105-08	-38.9

amino acids showed the presence of singlet peak at around δ 3.0 ppm pertaining to N-methyl protons.

On treatment of Bsmoc-*N*-methyl amino acids with DAST in DCM resulted in Bsmoc-*N*-methyl amino acid fluorides 3a-g in good yield (Scheme **5**, Table **3**). The IR analysis of acid fluorides showed a strong stretching absorption band at 1780 cm^{-1}.

Potassium Salt of 7-Aza-1-hydroxybenzotriazole (KOAt), introduced by Sureshbabu and Gopi, was used to mediate the coupling of Bsmoc –*N*-methyl amino acids to prepare dipeptides (Scheme **6**, Table **4**). The coupling was carried out in CH_2Cl_2 at r.t. and was found be free from racemisation. All the isolated peptides were fully charactaerized. In order to test susceptibility of the method towards racemization, the synthesis of two diastereomeric dipeptides Bsmoc-*L*-Phg-Phe-OMe and Bsmoc-*D*-Phg-Phe-OMe was carried out. Bsmoc-*L* or *D*-Phg-F was coupled with H_2N-Phe-OMe in presence of KOAt. The ^1H NMR analysis of the two diastereomers revealed that the C-methyl doublets of the

dipeptides differed from each other and also the methyl ester singlet was separated (δ 3,59; 3,66) by 0.07 ppm . Further, the HPLC studies of these dipeptides Bsmoc-*L*-Phg-Phe-OMe (R_t value 9.7) and Bsmoc-*D*-Phg-Phe-OMe (R_t value 10.7), synthesized by the present method, revealed that they are optically pure and the coupling is completely free from racemization.



Scheme 6. Synthesis of peptides.

CONCLUSION

In summary, this paper describes the preparation of Bmoc-*N*-methyl amino acids in two step procedure. Key *N*-Bsmoc-5-oxazolidinones have been isolated in good yields. The reduction of *N*-Bsmoc-5-oxazolidinones was carried using Et_3SiH and TFA in CH_2Cl_2 at r.t. Bsmoc-*N*-methyl amino acid fluorides are prepared using DAST which are isolated in almost quantitative yield. The coupling of Bsmoc-*N*-methyl amino acids was accomplished by KOAt successfully.

EXPERIMENTAL PROCEDURES

All the reactions were monitored by TLC with precoated silica gel plates purchased from Merk. Column chromatography was performed with Merk silica gel (100-240) at normal atmospheric pressure. The optical rotations were determined with an automatic digital AA-10 polarimeter (Optical activity, U.K). Analytical HPLC was performed on a Shimadzu Class VP- V6.1 with PDA detector; Column: Agilent Zorbax C-18, 250 x 4.0mm column; Mobile phase: CH_3CN (65%) :: H_2O (35%); Absorbance : 230 nm; flow rate : 1 mL/min. ^1H NMR was recorded on a Bruker AMX 400 MHz instrument with Me_4Si as an internal standed. Mass spectra were recorded on Kratos PCKompact SEQ V1.2.2 spectrometer.

General Procedure for the Synthesis of Bsmoc-Amino Acids

To a suspension of amino acid (5 mmol) in DCM (10 mL), NMM (0.55 mL, 5 mmol) and TMS-Cl (0.9 mL, 12 mmol) were added in one portion. The reaction mixture was refluxed for 2 hr and cooled in an ice bath. Bsmoc-OSu (1.29 g, 5 mmol) was added at that temperature over a period of 10 min. The reaction mixture was stirred for 1-1.5 hr at r.t. The solvent was removed *in vacuo* and the resulting oil was distributed between diethyl ether and 10% $NaHCO_3$ solution. The combined aqueous layers were acidified to pH 2 with

Table 3. List of the Acid Fluorides

Entry	Bsmoc-amino acids	Yield (%)	M.P. (^oC)	$[\alpha]^{25}_D$ (c 1, $CHCl_3$)
3a	Bsmoc-Nva-F	89	70-72	-6.3
3b	Bsmoc-Sar-F	79	Gum	-
3c	Bsmoc-MeAla-F	-	140-42	-6.8
3d	Bsmoc-Me-Leu-F	91	Gum	-11.5
3e	Bsmoc-Me-Ile-F	82	Gum	-14.1
3f	Bsmoc-Me-Val-F	90	100-02	12.7
3g	Bsmoc-Me-Phe-F	89	110-02	-17.8

Table 4. List of the Bsmoc Dipeptides

Entry	R	R_1	X	Y	Yield (%)	Mass[1] $[M+Na]^+$
4a	$CH_2CH(CH_3)_2$	CH_3	H	$CH_2C_6H_5$	89	551.3
4b	CH3	$CH_2CH(CH_3)_2$	H	CH_3	92	475.2
4c	$(CH_2)_2CH_3$	H	CH_3	CH_3	91	461.2
4d	$CH_2CH(CH_3)_2$	$CH_2CH(CH_3)_2$	CH_3	CH_3	89	531.3
4e	$CH(CH_3)_2$	$(CH_2)_2CH_3$	CH_3	CH_3	87	503.1
4f	$CH_2CH(CH_3)_2$	$CH(CH_3)_2$	CH_3	CH_3	85	517.1
4g	$CH_2C_6H_5$	$CH(CH_3)CH_2CH_3$	CH_3	CH_3	89	565.2

[1] MS [MALDI-TOF]

conc HCl and the resulting Bsmoc-amino acids was extracted with ethyl acetate (10 mL X 3 times). The combined organic layer was washed with water (20 mL X 3 times) and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* and recrystallization of the resulting residue from DCM: *n*-hexane (1:1) gave Bsmoc-amino acids in good yield.

General Procedure for the Synthesis of Bsmoc-5-Oxazolidinones 1a-f:

A slurry of Bsmoc-amino acid (10 mmol), paraformaldehyde (2 g) and *p*-TsOH (100 mg) in toluene (10 mL) was subjected to microwave irradiation in an unmodified domestic microwave oven operating at 2450 MHz frequency at its full power. After completion of the reaction, the residue was diluted with $CHCl_3$ (25 mL), washed with 10% aqueous $NaHCO_3$ (10 mL X 3 times) and water (10 mL X 3 times). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated *in vacuo* to obtain the 5-oxazolidinones in almost quantitative yield.

Bsmoc-Ala-5-oxazolidinone 1b. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 1.29 (d, J= 6.7, 3H), 3.92 (m, 1H), 4.54 (s, 2H), 5.17 (s, 2H), 7.20-7.80 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 18.2, 55.3, 65.9, 71.2, 120.8, 122.2, 123.1, 127.5, 130.1, 132.2, 137.5, 139.3, 159.2, 169.1; ES-MS Cald for $C_{14}H_{13}NO_6S$ m/z: 324.3 $[M+1]^+$, found: 324.3 $[M+1]^+$.

Bsmoc-Phe-5-oxazolidinone 1c. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 2.85-3.05 (m, 2H), 4.50 (s, 2H), 4.65 (m, 1H), 5.21 (s, 2H), 7.15-7.95 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) 31.2, 57.5, 69.0, 72.5, 120.5, 122.4, 123.1, 123.9, 124.6, 125.3, 126.1, 127.1, 127.5, 130.1, 132.2, 137.2, 137.5, 139.3, 162.2, 170.1; ES-MS Cald for $C_{20}H_{17}NO_6S$ m/z: 400.4 $[M+1]^+$, found: 400.3 $[M+1]^+$.

Bsmoc-Ile-5-oxazolidinone 1d. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 0.86-1.05 (m, 6H), 1.59-1.63 (m, 3H), 4.56 (s, 2H), 4.70 (m, 1H), 5.20 (s, 2H), 7.20-7.85 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 14.2, 17.5, 27.0, 35.1, 66.5, 69.8, 75.5, 120.7, 122.8, 123.9, 124.6, 126.1, 130.1, 137.2, 162.2, 170.1; ES-MS Cald for $C_{20}H_{17}NO_6S$ m/z: 400.4 $[M+1]^+$, found: 400.3 $[M+1]^+$.

Bsmoc-Leu-5-oxazolidinone 1e. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 0.95 (m, 6H), 1.29-1.53 (m, 3H), 4.60 (s, 2H), 4.75 (m, 1H), 5.25 (s, 2H), 7.18-7.80 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 14.2, 17.5, 27.0, 35.1, 66.5, 69.8, 75.5, 120.7, 122.8, 123.9, 124.6, 126.1, 130.1, 137.2, 162.2, 170.1; ES-MS Cald for $C_{17}H_{19}NO_6S$ m/z: 366.4 $[M+1]^+$, found: 366.3 $[M+1]^+$.

Bsmoc-Val-5-oxazolidinone 1f. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 1.15 (d, J= 6.7, 6H), 1.71-1.80 (m, 1H), 4.10 (m, 1H), 4.55 (s, 2H), 5.15 (s, 2H), 7.21-7.80 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 21.1, 21.3, 27.6, 65.5, 70.1, 79.2, 120.4, 122.9, 124.0, 126.1, 127.2, 129.1, 130.5, 138.2, 162.5, 171.1; ES-MS Cald for $C_{16}H_{17}NO_6S$ m/z: 352.4 $[M+1]^+$, found: 352.4 $[M+1]^+$.

General Procedure for the Synthesis of Bsmoc-*N*-Methyl Amino Acids 2a-f:

Bsmoc-5-oxazolidinone (10 mmol) was dissolved in $CHCl_3$ (15 mL). To this solution was added TFA (15 mL) and Et_3SiH (3 mL) and stirred at r.t. for 24 hr. After completion of the reaction mixture, it was concentrated in vacuo and the residue was portioned between ether (20 mL) and 10% aqueous $NaHCO_3$ (20 mL). The combined aqueous layer was acidified with concentrated HCl and extracted with EtOAc (10 mL X 3 times). The combined organic layer was washed with water (30 mL) and dried over anhydrous $NaSO_4$. Evaporation of the solvent resulted in Bsmoc-*N*-methyl amino acids in good yield.

Bsmoc-Sar-OH 2a. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 3.02 (s, 3H), 3.60 (s, 2H), 5.25 (s, 2H), 7.25-7.82 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 32.5, 55.2, 67.5, 119.1, 120.7, 121.5, 126.8, 130.1, 132.2, 137.6, 139.8, 152.2, 172.2; ES-MS Cald for $C_{13}H_{13}NO_6S$ m/z: 311.1 $[M+1]^+$, found: 312.2 $[M+1]^+$.

Bsmoc-MeAla-OH 2b. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 1.29 (d, J= 6.7, 3H), 2.98 (s, 3H), 4.35 (m, 1H), 5.21 (s, 2H), 7.15-7.70 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 15.1, 35.1, 55.9, 69.2, 119.5, 123.5, 126.1, 126.9, 129.5, 132.5, 138.1, 138.9, 160.5, 172.1; ES-MS Cald for $C_{14}H_{15}NO_6S$ m/z: 325.1 $[M+1]^+$, found: 326.3 $[M+1]^+$.

Bsmoc-MePhe-OH 2c. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 3.00 (s, 3H), 3.21 (m, 2H), 4.25 (m, 1H), 5.30 (s, 2H), 7.10-7.95 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) 32.1, 33.8, 59.7, 69.0, 119.5, 120.9, 121.5, 121.4, 122.7, 122.9, 123.4, 125.9, 126.1, 129.0, 131.9, 132.0, 132.6, 159.2, 174.1; ES-MS Cald for $C_{20}H_{19}NO_6S$ m/z: 401.1 $[M+1]^+$, found: 402.2 $[M+1]^+$.

Bsmoc-MeIle-OH 2d. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 0.85-1.15 (m, 8H), 2.25 (m, 1H), 2.95 (s, 3H), 4.45 (m, 1H), 5.10 (s, 2H), 7.20-7.85 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 13.2, 16.6, 26.9, 33.0, 62.5, 67.8, 121.4, 121.9, 125.4, 127.2, 128.0, 129.5, 131.1, 138.1, 159.0, 173.1; ES-MS Cald for $C_{17}H_{21}NO_6S$ m/z: 367.1 $[M+1]^+$, found: 368.0 $[M+1]^+$.

Bsmoc-MeLeu-OH 2e. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 0.92 (d, J=6.7, 6H), 1.29 (m, 2H), 2.10 (m, 1H), 3.00 (s, 3H), 4.20 (m, 1H), 5.17 (s, 2H), 7.18-7.80 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 18.2, 22.0, 27.1, 33.7, 65.5, 67.8, 121.0, 122.7, 123.1, 123.9, 127.7, 133.2, 135.6, 137.2, 165.2, 172.4; ES-MS Cald for $C_{17}H_{21}NO_6S$ m/z: 367.1 $[M+1]^+$, found: 368.4 $[M+1]^+$.

Bsmoc-MeVal-OH 2f. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 0.95 (d, J= 6.7, 6H), 2.22 (m, 1H), 2.98 (s, 3H), 4.35 (m, 1H), 5.15 (s, 2H), 7.21-7.75 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 15.3, 29.2, 33.3, 67.3, 72.1, 121.1, 122.0, 123.4, 125.9, 129.0, 132.1, 132.9, 133.4, 165.5, 173.7; ES-MS Cald for $C_{16}H_{19}NO_6S$ m/z: 353.1 $[M+1]^+$, found: 354.3 $[M+1]^+$.

Bsmoc-*N*-Methyl Amino Acid Fluorides 3a-g:

To a suspension of Bsmoc-*N*-methyl amino acid (1 mmol) in DCM (5 mL) was added DAST (0.2 mL, 1.5 mmole) under nitrogen and stirred for an hr at r.t. After the completion of the reaction, it was further diluted with DCM (10 mL) and washed with cold water (10 mL X 2 times). The solvent was dried over anhydrous Na_2SO_4 and was evaporated under reduced pressure to result in a residue which was

recrystallized using DCM: n-hexane to yield Bsmoc-amino acid fluorides as solids.

General Procedure for the Coupling of Bsmoc-*N*-Methyl Amino Acid Fluorides Mediated by KOAt 4a-g

To a stirred solution of Bsmoc-*N*-methyl amino acid fluoride (2 mmole) and KOAt (1.1 mmole) in dry DCM (5 mL) was added a solution of amino free amino acid ester in dry DCM (5 mL) and the reaction mixture was stirred at r.t. The progress of the reaction was monitored by TLC. After the completion of the reaction, it was concentrated and the resulting residue was diluted with CH_2Cl_2 (10 mL), washed thrice with 5 mL portions of 5% HCl, 5% $NaHCO_3$ and water and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* and recrystallization of the residue from CH_2Cl_2: petroleum ether mixture (1:3) yielded the peptide.

Bsmoc-*N*methyl-Leu-Ala-OBzl 4a. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.82-1.08 (m, 6H), 1.31 (d, J=5.9 Hz, 3H), 1.51(m, 2H), 1.72 (m, 1H), 2.98 (s, 3H), 4.49 (m, 1H), 4.68 (m, 1H), 4.97-5.28 (m, 3H), 5.35 (s, 2H), 7.32-7.77 (m, 10H); MS (MALDI-TOF) calcd. for $C_{27}H_{32}N_2O_7S$ m/z: 551.2 $[M + Na]^+$, 567.7 $[M + K]^+$, found: 551.3 $[M + Na]^+$, 567.5 $[M + K]^+$; Anal. Calcd for $C_{27}H_{32}N_2O_7S$ (%): C, 61.34; H, 6.10; N, 5.29; found: C, 61.21; H, 6.01; N, 5.14.

Bsmoc-NMeVal-Leu-OMe 4b. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.89- 1.05 (m, 6H), 1.35 (d, J=5.7, 3H), 1.55-1.75 (m, 3H), 3.00 (s, 3H), 3.60 (s, 3H), 4.54 (m, 1H) 5.13-5.29 (m, 3H), 6.32 (br, 1H), 7.35-7.80 (m, 4H) MS (MALDI-TOF) calcd. for $C_{21}H_{28}N_2O_7S$ m/z: 475.2 $[M + Na]^+$, 491.1 $[M + K]^+$, found: 475.2 $[M + Na]^+$, 491.1 $[M + K]^+$.

Bsmoc-Nva-Sar-OMe 4c. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.85 (t, J= 6.7, 3H), 1.32-167 (m, 4H), 2.97 (s, 3H), 3.01 (s, 3H), 3.65 (s, 3H) 4.24 (m, 1H), 5.17 (s, 2H), 5.65 (m, 1H), 7.30-7.79 (m, 4H); MS (MALDI-TOF) calcd. for $C_{20}H_{26}N_2O_7S$ m/z: 461.1 $[M + Na]^+$, 477.1 $[M + K]^+$, found: 461.2 $[M + Na]^+$, 477.1 $[M + K]^+$.

Bsmoc-*N*MeLeu-*N*MeLeu-OMe 4d. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.85- 1.01 (m, 12H), 1.27-1.39 (m, 3H), 1.52-1.76 (m, 3H), 2.95 (s, 3H), 3.01 (s, 3H), 3.63 (s, 3H), 4.92 (m, 1H) 5.12 (m, 1H), 5.20 (s, 2H), 7.35-7.77 (m, 4H); MS (MALDI-TOF) calcd. for $C_{25}H_{36}N_2O_7S$ m/z: 531.2 $[M + Na]^+$, 547.2 $[M + K]^+$, found: 531.3 $[M + Na]^+$, 547.0 $[M + K]^+$.

Bsmoc-*N*MeVal-Nva-OMe 4e. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.89 (t, J=5.9, 3H), 1.15-1.45 (m, 10H), 1.97 (m, 1H), 2.97 (s, 3H), 3.05 (s, 3H), 3.67 (s, 3H), 4.74 (m, 1H) 5.35 (m, 1H), 5.18 (s, 2H), 7.30-7.75 (m, 4H); MS (MALDI-TOF) calcd. for $C_{23}H_{32}N_2O_7S$ m/z: 503.2 $[M + Na]^+$, 519.2 $[M + K]^+$, found: 503.1 $[M + Na]^+$, 519.1 $[M + K]^+$.

Bsmoc-*N*MeLeu-*N*MeVal-OMe 4f. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.85- 1.02 (m, 6H), 1.32 (m, 2H), 1.47-1.68 (m, 7H), 1.85 (m, 1H), 2.95 (s, 3H), 3.02 (s, 3H), 3.60 (s, 3H), 4.52 (m, 1H) 5.65 (m, 1H), 5.17 (s, 2H), 7.29-7.77 (m, 4H); MS (MALDI-TOF) calcd. for $C_{24}H_{34}N_2O_7S$ m/z: 517.2 $[M + Na]^+$, 533.2 $[M + K]^+$, found: 517.1 $[M + Na]^+$, 533.3 $[M + K]^+$.

Bsmoc-*N*MePhe-*N*MeIle-OMe 4g. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.01-1.27 (m, 8H), 1.56 (m, 1H), 2.72 (m, 2H), 2.97 (s, 3H), 3.00 (s, 3H), 3.67 (s, 3H), 4.67 (m, 1H) 5.32 (m, 1H), 5.20 (s, 2H), 7.19-7.89 (m, 10H); MS (MALDI-TOF) calcd. for $C_{28}H_{34}N_2O_7S$ m/z: 565.2 $[M + Na]^+$, 581.2 $[M + K]^+$, found: 565.2 $[M + Na]^+$, 581.1 $[M + K]^+$.

Racemization Studies were Carried Out by Preparing Following Compounds

Bsmoc-L-*Phg-Phe-OMe*

To a stirred solution of Bsmoc-Phg-F (0.75 g, 2 mmoles) and KOAt (0.38 g, 2.2 mmoles) in dry DCM (5 mL) was added a solution of H_2N-Phe-OMe (0.36 g, 2 mmoles) dissolved in dry DCM (5 mL). The reaction mixture was stirred for 30 min at r.t. and its workup, following the general procedure yielded the dipeptide as a white crystalline solid (0.93 g, 88%); m. p. 125-27°C; TLC R_f (EtOAc : *n*-hexane :: 3:7), 0.43; $[\alpha]^{25}_D$ -14.0° (c 0.5, DMF); ^{1}H NMR ($CDCl_3$): δ 2.9 (2H, d), 3.64 (3H,s), 4.5 (1H, m), 5.10 (2H, d), 5.25 (1H, d), 6.25 (2H, 2d), 7.1-7.8 (15 ArH , m). Anal. Calcd. for $C_{28}H_{26}N_2O_7S_1$ (534.58): C, 62.91; H, 4.90; N, 5.24; found: C, 62.82; H, 4.81; N, 5.14. MS (MALDI-TOF): m/z calcd. 557.5 $[M + Na]^+$, 573.6 $[M + K]^+$, found: 557.7 $[M + Na]^+$, 573.1 $[M + K]^+$.

Bsmoc-D-*Phg-Phe-OMe*

To a stirred solution of Bsmoc-*D*-Phg-F (0.75 g, 2 mmoles) and KOAt (0.38 g, 2.2 mmoles) in dry DCM (5 mL) was added a solution of H_2N-Phe-OMe (0.36 g, 2 mmoles) dissolved in dry DCM (5 mL). The reaction mixture was stirred for 30 min at r.t. and its workup yielded the dipeptide as a white crystalline solid (0.95 g, 90%), m. p. 127-30°C; TLC R_f (EtOAc : *n*-hexane :: 3:7), 0.45; $[\alpha]^{25}_D$ +14.4° (c 0.5, DMF); ^{1}H NMR ($CDCl_3$): δ 3.0 (2H, d), 3.73 (3H, s), 4.5 (1H, m), 5.10 (2H, d), 5.25 (1H, d), 6.25 (2H, two d), 7.1-7.8 (15 ArH , m). Anal. Calcd. for $C_{28}H_{26}N_2O_7S_1$ (534.58): C, 62.91; H, 4.90; N, 5.24. found: C, 62.85; H, 4.88; N, 5.16. MS (MALDI-TOF): m/z calcd. 557.5 $[M + Na]^+$, 573.6 $[M + K]^+$, found: 557.9 $[M + Na]^+$, 573.5 $[M + K]^+$.

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