Facile Synthesis of 2-(4-Nitrophenylsulfonyl)ethoxycarbonyl-*O*-*t*-butyl-L-serine, Threonine, and Tyrosine

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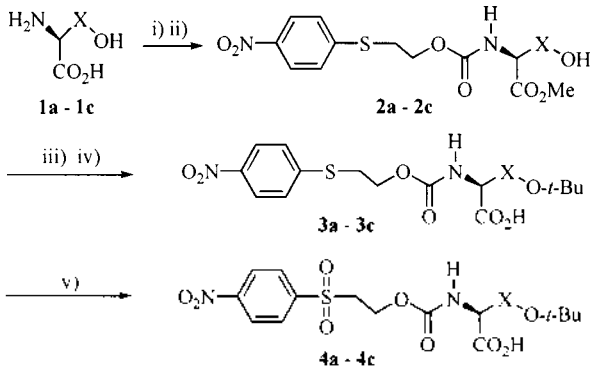
N$^{\alpha}$-Protected amino acids, especially N$^{\alpha}$-9-fluorenylmethoxycarbonyl (Fmoc)-amino acids have been the most widely investigated for the synthesis of biologically active peptides by solid phase method.[1] As an alternative to the Fmoc, new base-labile 2-(4-nitrophenylsulfonyl)ethoxycarbonyl (Nsc) group was developed, which was demonstrated to be useful and advantageous to solid phase peptide synthesis.[2]

Among the N$^{\alpha}$-Nsc-amino acids, serine (Ser), threonine (Thr), and tyrosine (Tyr) which have a reactive hydroxyl function were prepared in combination with acid-labile *t*-butyl (*t*-Bu) group. We synthesized the Nsc-*O*-*t*-butyl amino acids by Bolin method[3] starting from commercially available intermediates; H-Ser(*t*-Bu)-OH, H-Thr(*t*-Bu)-OH, and H-Tyr(*t*-Bu)-OH. This route, however, was not cost-effective to obtain large quantities of the products. So we intended to develope the new synthetic route for large-scale preparation of the products from amino acids.

A convenient procedure for the preparation of Fmoc-*O*-*t*-butyl amino acids by temporary α-carboxyl protection using methyl esters had been developed.[1] This procedure, however, had the disadvantage that the hydrolysis of methyl esters could not be completed under the weakly basic condition to protect unfavorable Fmoc detachment. We had tried to synthesize Nsc-*O*-*t*-butyl-amino acids *via* the same 4-step route as above. As a result, this route was found to be not so good in scale-up process because of longer time of *t*-butylation (3 day) notwithstanding shorter route.



a: X = CH_2, **b**: X = $CH(CH_3)CH_2$, **c**: X = CH_2Ph

Scheme 1. i) $SOCl_2$, MeOH. ii) **2a-b**: Ntc-Cl[5], TEA, dioxane, 90-95%, **2c**: Ntc-Cl, TMS-Cl, TEA, $CHCl_3$, 85%. iii) isobutylene, BF_3·Et_2O (**3b-c**; additional *p*-TsOH·H_2O), $CHCl_3$, rt, 5-24 h. iv) 1 N NaOH, MeOH, rt, 2 h, 85-90%. v) 0.3 M Na_2MoO_4, H_2O_2, acetone or EtOH, rt, 5 h, 85-95%.

From our continuing study of N$^{\alpha}$-Nsc-amino acids, we have found that the 2-(4-nitrophenylthio)ethoxycarbonyl (Ntc) group is more stable than Nsc under basic condition. Considering this fact, we designed the synthetic route of Nsc-*O*-*t*-butyl amino acids introducing more base resistant Ntc group before *t*-butylation and hydrolysis (Scheme 1). As expected, the *t*-butylation and the hydrolysis of methyl esters underwent smoothly to give the acids in high yields. Based on optimized reaction conditions, we were able to prepare large quantities of the products in good yields without any serious loss.

Methyl esters of amino acids were quantitatively obtained by esterification with thionyl chloride in MeOH. The Ntc group was introduced to α-position of HCl·H-Ser-OMe and HCl·H-Thr-OMe by modified Schotten-Baumann method. For HCl·H-Tyr-OMe, Bolin method was applied to protect undesired di-substitution (15-20%) of Ntc group. The *t*-butylation of hydroxyl side functions was carried out using isobutylene in the presence of catalytic amount of BF_3·Et_2O for a short period of time in high yield. In order to avoid *t*-butylation on *ortho*-position of Tyr, reaction temperature did not exceed 25 °C. Notably, *t*-butylation of Thr and Tyr was enhanced by the addition of *p*-TsOH·H_2O. Ntc-*O*-*t*-butyl amino acid methyl esters thus obtained without purification were hydrolyzed by 1 N NaOH at room temperature for 2 h to afford **3a-3c** in high yields, of which **3b** and **3c** were crystallized in the form of cyclohexylamine (CHA) salt. These salts were directly oxidized by H_2O_2 in acetone or EtOH into **4a** and **4c** after desalting. Compound **4a-4c** had good crystallinity and solubility to be easily isolated from appropriate solvents in 75%, 60%, and 45% overall yields, respectively.

In summary, we have developed new efficient synthetic route for the preparation of Nsc-*O*-*t*-butyl amino acids by the introduction of base-resistant Ntc-group starting from methyl esters of amino acids. It is noteworthy that this route can be applicable to the other N$^{\alpha}$-Nsc-protected amino acids with reactive side function.

Experimental Section

Melting points were determined on Buchi 535 without correction. Optical rotations were obtained on a JASCO DIP 1000. Elemental anaylses were obtained on elementar analysensystem GmbH vario EL. FAB mass spectrometry were carried out on a VG70-VSEQ, which were ionized by 35eV Cs+ ion beam with 3-nitrobenzyl alcohol as matrix. ^{1}H-NMR spectra were recorded on Bruker (400 MHz or 500

MHz). IR spectra were recorded on JASCO SSP-300 spectrophotometer.

2-(4-Nitrophenylthio)ethoxycarbonyl-L-serine methyl ester (2a). HCl·H-Ser-OMe (77.8 g, 0.5 mol) prepared by the known procedure[6] using Ser (52.5 g, 0.5 mol), thionyl chloride (43.8 mL, 0.6 mol), and MeOH (1.5 L) was dissolved in EtOH/water (9/1, 500 mL). To the solution was added 146 mL of TEA, and cooled in ice-bath for 30 min. A solution of Ntc-Cl (130.2 g, 0.5 mol) in 300 mL of dioxane was added dropwise with vigrous stirring and kept for 1 h. The reaction mixture was quenched by 5 mL of AcOH, and concentrated. The residual oil was diluted with water (300 mL), and extracted with EtOAc (500 mL×2). Combined organic layer was washed with water (400 mL×3) and brine (400 mL×2), dried over anhydrous Na_2SO_4, and concentrated. The residue was crystallized from Et_2O and hexane to afford 154 g (90%) of **2a** as light yellow powder: mp 98-99 °C; $[\alpha]^{25}_D$ = -9.27 (C 1.05, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.31 (t, *J*=7.0 Hz, 2H, -SCH_2), 3.83 (s, 3H, -OCH_3), 3.94 (dd, J_1=9.0 Hz, J_2=4.5 Hz, 1H, -CH_β) 4.03 (dd, J_1=9.0 Hz, J_2=2.9 Hz, 1H, -$CH_{\beta'}$), 4.32-4.39 (m, 2H, -OCH_2), 4.45 (m, 1H, -CH_α), 5.70 (d, *J*=6.8 Hz, 1H, -NH), 7.45 (d, *J*=8.8 Hz, 2H, Ar_{Ntc}), 8.19 (d, *J*=8.8 Hz, 2H, Ar_{Ntc}).

2-(4-Nitrophenylthio)ethoxycarbonyl-L-threonine methyl ester (2b). HCl·H-Thr-OMe (84.8 g, 0.5 mol) was processed as described above (synthesis of **2a**) to give **2b** (170.5 g, 95%) as yellow powder: mp 119-120 °C; $[\alpha]^{25}_D$ = -18.4 (C 0.96, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28 (d, *J* 6.4 Hz, 3H, -CH_3), 3.32 (t, *J*=7.0 Hz, 2H, -SCH_2), 3.81 (s, 3H, -OCH_3), 4.29-4.40 (m, 4H, -OCH_2, -CH_α, and -CH_β), 5.51 (d, *J*=8.5 Hz, 1H, -NH), 7.52 (d, *J*=8.9 Hz, 2H, Ar_{Ntc}), 8.19 (d, *J*=8.8 Hz, 2H, Ar_{Ntc}).

2-(4-Nitrophenylthio)ethoxycarbonyl-L-tyrosine methyl ester (2c). To a suspension of HCl H-Tyr-OMe (115.8 g, 0.5 mol) in 600 mL of $CHCl_3$ were added 208.7 mL of TEA and 125.5 mL of TMS-Cl at room temperature. The reaction mixture was stirred at room temperature for 3 h, and cooled to 0-5 °C. A solution of Ntc-Cl (130.5 g, 0.5 mol) in 500 mL of $CHCl_3$ was added dropwise and allowed to warm to room temperature for 2 h. The reaction mixture was concentrated, partitioned between EtOAc (600 mL) and water (300 mL), and acidified to pH 2 with 0.5 M HCl. The organic layer was separated, washed with 0.1 M HCl (500 mL×2), water (400 mL×3), and brine (300 mL×2), dried over anhydrous $MgSO_4$, and concentrated. The residue was crystallized from Et_2O and hexane to give **2c** (175.3 g, 85%) as white powder: mp 90-91 °C; $[\alpha]^{25}_D$ = +3.1 (C 0.99, MeOH); ^{1}H NMR ($CDCl_3$, 400 Hz) δ 2.9-3.1 (m, 2H, -$CH_{2\beta\beta'}$), 3.26 (t, *J*=6.9 Hz, 2H, -SCH_2), 3.76 (s, 3H, -OCH_3), 4.28-4.29 (m, 2H, -OCH_2), 4.60 (m, 1H, -CH_α), 5.16 (d, *J*=7.9 Hz, 1H, -NH), 6.77 (d, *J*=8.2 Hz, 2H, Ar_{Tyr}), 6.99 (d, *J*=8.3 Hz, 2H, Ar_{Tyr}), 7.46 (d, *J*=8.8 Hz, 2H, Ar_{Ntc}), 8.15 (d, *J*=8.7 Hz, 2H, Ar_{Ntc}).

2-(4-Nitrophenylthio)ethoxycarbonyl-*O*-*t*-butyl-L-serine (3a). A mixture of **2a** (172.2 g, 0.5 mol), BF_3·Et_2O (20 mL), and $CHCl_3$ (600 mL) was stirred under isobutylene gas (2.5 psi) at room temperature for 5 h, quenched by 1 N NaOH (25 mL), and evaporated to oil. The residue was dissolved in 500 mL of MeOH, followed by portionwise addition of 1 N NaOH (550 mL, 1.1 eq.). After standing at room temperature for 2 h, the mixture was concentrated to dryness. To the residue were added Et_2O (400 mL) and water (400 mL). The aqueous layer was washed with Et_2O (300 mL×2), acidified to pH 2 with 1 N HCl, and extracted with EtOAc (500 mL×2). The organic layer was washed with water (400 mL×3) and brine (400 mL×2), dried over anhydrous $MgSO_4$, and concentrated to oil. The residue was crystallized from Et_2O and petroleum ether to give 172.6 g (90%) of **3a** as white powder: mp 108-109 °C; $[\alpha]^{25}_D$ = +11.98 (C 1.08, MeOH); ^{1}H NMR ($CDCl_3$, 400 Hz) δ 1.20 (s, 9H, -$C(CH_3)_3$), 3.30 (t, *J*=7.0 Hz, 2H, -SCH_2), 3.58 (dd, J_1=8.8 Hz, J_2=4.6 Hz, 1H, -CH_β), 3.91 (dd, J_1=8.9 Hz, J_2=3.0 Hz, 1H, -$CH_{\beta'}$), 4.33 (m, 2H, -OCH_2), 4.44-4.46 (m, 1H, CH), 5.59 (d, *J*=8.2 Hz, 1H, -NH), 7.43 (d, *J*=8.8 Hz, 2H, Ar_{Ntc}), 8.15 (d, *J*=8.8 Hz, 2H, Ar_{Ntc}).

2-(4-Nitrophenylthio)ethoxycarbonyl-*O*-*t*-butyl-L-threonine (3b). **2b** (207.2 g, 0.5 mol) was *t*-butylated by the similar procedure as described above (synthesis of **3a**) in the presence of BF_3·Et_2O (10 mL) and *p*-TsOH·H_2O (47.5 g) to give **3b** as oil. The oil was isolated in the formation of salt by addition of CHA in $CHCl_3$ and Et_2O: 85% yield; mp 170-171 °C; $[\alpha]^{25}_D$ = +0.63 (C 0.96, MeOH); ^{1}H NMR ($CDCl_3$, 400 Hz) δ 1.19 (m, 12H, -$C(CH_3)_3$ and -CH_3), 3.29 (m, 2H, -SCH_2), 3.88 (dd, J_1=8.2 Hz, J_2=2.3 Hz, 1H, -CH_α), 4.22-4.32 (m, 3H, -CH_β, and -OCH_2), 7.45 (d, *J*=8.8 Hz, 2H, Ar_{Ntc}), 8.16 (d, *J*=8.8 Hz, 2H, Ar_{Ntc}).

2-(4-Nitrophenylthio)ethoxycarbonyl-*O*-*t*-butyl-L-tyrosine (3c). **2c** (238.3 g, 0.5 mol) was *t*-butylated by the similar procedure as described above (synthesis of **3a**) in the presence of BF_3·Et_2O (10 mL) and *p*-TsOH·H_2O (95 g) to give **3c** as oil. The residual oil was isolated in the formation of salt by addition of CHA in MeOH and Et_2O: 85% yield; mp 163-164 °C; $[\alpha]^{25}_D$ = +28.6 (C 0.92, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.19 (s, 9H, -$C(CH_3)_3$), 3.28 (t, *J*=6.9 Hz, 2H, -SCH_2), 4.29 (m, 2H, -OCH_2), 4.59-4.60 (m, 1H, -CH_α), 5.16 (d, *J*=7.9 Hz, 1H, -NH), 6.77 (d, *J*=8.2 Hz, 2H, Ar_{Tyr}), 7.0 (d, *J*=8.3 Hz, 2H, Ar_{Tyr}), 7.46 (d, *J*=8.8 Hz, 2H, Ar_{Ntc}), 8.16 (d, *J*=8.7 Hz, 2H, Ar_{Ntc}).

2-(4-Nitrophenysulfonyl)ethoxycarbonyl-*O*-*t*-butyl-L-serine (4a). To a solution of **3a** (193.2 g, 0.5 mol) in 500 mL of acetone were added 0.3M Na_2MoO_4 (200 mL) and H_2O_2 (35%, 200 mL), and kept at room temperature for 5 h. The reaction mixture was concentrated, and distributed between EtOAc (600 mL) and water (300 mL). The organic layer was washed with 1% $NaHCO_3$ (300 mL×2), water (400 mL×3), and brine (200 mL), dried over anhydrous $MgSO_4$, and concentrated to dryness. The residue was crystallized from Et_2O and hexane to give 197.6 g (95%) of **4a** as white powder: mp 109-111 °C; $[\alpha]^{25}_D$ = +3.3 (C 0.99, DMF); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.15 (s, 9H, -$C(CH_3)_3$), 3.48-3.62 (m, 3H, -SCH_2 and -CH_β), 3.82 (dd, J_1=9.1 Hz, J_2=2.9 Hz, 1H, -$CH_{\beta'}$), 4.31 (m, 1H, -CH_α), 4.42-4.55 (m, 2H, -OCH_2), 5.25 (d, *J*=8.4 Hz, 1H, -NH), 8.41 (d, *J*=8.7 Hz, 2H, Ar_{Nsc}), 8.16 (d, *J*=8.9 Hz, 2H, Ar_{Nsc}); FAB/MS m/z = 419 $[M+H]^+$; Anal.

Calcd. for $C_{16}H_{22}N_2O_9S$: C 45.93, H 5.30, N 6.70, S 7.66 Found C 45.93, H 5.34, N 6.46, S 7.93; IR (KBr, cm^{-1}) 3312, 1748, 1724.

2-(4-Nitrophenysulfonyl)ethoxycarbonyl-*O-t*-butyl-L-threonine (4b). The CHA salt (249.8 g, 0.5 mol) of **3b** was processed as described above (synthesis of **4a**) in EtOH (500 mL) to give **4b** (184.7 g, 86%) as white powder: mp 68-70 °C; $[\alpha]^{25}_D$ = -7.9 (C 0.97, DMF); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.1 (d, *J*=6.4 Hz, 3H, $-CH_{3\gamma}$), 1.23 (s, 9H, $-C(CH_3)_3$), 3.54-3.57 (m, 2H, $-SCH_2$), 4.1-4.12 (m, 1H, $-CH_\alpha$), 4.21-4.22 (m, 1H, $-CH_\beta$), 4.43-4.54 (m, 2H, $-OCH_2$), 5.29 (d, *J*=7.0 Hz, 1H, -NH), 8.42 (d, *J*=8.9 Hz, 2H, Ar_{Nsc}), 8.15 (d, *J*=8.7 Hz, 2H, Ar_{Nsc}); FAB/MS m/z = 433 $[M+H]^+$; Anal. Cald. for C_1-$H_{21}N_2O_9S$ C 47.21, H 5.59, N 6.48, S 7.42 Found C 46.88, H 5.83, N 6.23, S 7.55; IR (KBr, cm^{-1}) 3329, 1748, 1724.

2-(4-Nitrophenysulfonyl)ethoxycarbonyl-*O-t*-butyl-L-tyrosine (4c). The CHA salt (280.1 g, 0.5 mol) of **3c** was processed as described above (synthesis of **4a**) in EtOH (500 mL) to give **4c** (206.2 g, 84%) as white powder: mp 82-84 °C; $[\alpha]^{25}_D$ = -4.9 (C 0.96, DMF); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.35 (s, 9H, $-C(CH_3)_3$), 2.98 (dd, J_1=14.2 Hz, J_2=6.6 Hz, 1H, $-CH_\beta$), 3.11 (dd, J_1=14.2 Hz, J_2=5.3 Hz, 1H, $-CH_{\beta'}$), 3.45-3.58 (m, 2H, $-SCH_2$), 4.36-4.41 (m, 1H, $-CH_\alpha$), 4.46-4.49 (m, 2H, $-OCH_2$), 4.89 (d, *J*=7.9 Hz, 1H, -NH), 6.93 (d, *J*=8.4 Hz, 2H, Ar_{Tyr}), 7.02 (d, *J*=8.3 Hz, 2H, Ar_{Tyr}), 8.09 (d, *J*=8.7 Hz, 2H, Ar_{Nsc}), 8.34 (d, *J*=8.6 Hz, 2H, Ar_{Nsc}); FAB/MS m/z = 495 $[M+H]^+$; Anal. Cald. for $C_{22}H_{26}N_2O_9S$ C 53.43, H 5.30, N 5.66, S 6.48 Found C 52.92, H 5.59, N 5.18, S 6.86; IR (KBr, cm^{-1}) 3321, 1748, 1725.

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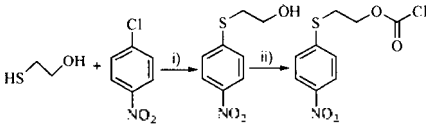
4. Adamson, J. G.; Blaskovich, M. A.; Groenevelt, H.; Lajoie, G. A. *J. Org. Chem.* **1991**, *56*, 3447.
5. Ntc-Cl was prepared by the following procedure.



i) 4M KOH, 70 °C, quantitative. ii) $COCl_2$, THF, -20 °C, 95%.

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