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The 2-(4-trifluoromethylphenylsulfonyl)ethoxycarbonyl (Tsc) amino-protecting group: use in the solid-phase synthesis of pyrrole-imidazole polyamides

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Abstract—The development of the 2-(4-trifluoromethylphenylsulfonyl)ethoxycarbonyl (Tsc) function, a novel base-sensitive amino-protecting group, and its application to the preparation of DNA-binding polyamides are described. Pyrrole–imidazole polyamides were synthesized by an efficient solid-phase method under conditions compatible with Fmoc chemistry using two Tsc-protected amino acids, Tsc-Py-OH **1a** and Tsc-Im-OH **1b**.

Recently, we reported the use of the 2-(4-nitrophenylsulfonyl)ethoxycarbonyl (Nsc) base-labile amino-protecting group for the preparation of DNA-binding polyamides containing pyrrole (Py) amino acids.[1] The Nsc group differs from the 9-fluorenylmethoxycarbonyl (Fmoc) group regarding its greater chemical and thermal stability when installed on the exocyclic amino group of the heteroaromatic pyrrole amino acid.[1,2] However, the general utility of the Nsc group in the solid-phase synthesis of polyamides was hampered by the poor solubility of the Nsc-protected imidazole (Im) amino acid, Nsc-Im-OH, in organic solvents.[1] To overcome this problem, we set out to develop a different protecting group superior in solubility to Nsc without compromising its stability. With this new protection scheme, polyamides containing both pyrrole and imidazole amino acids could be produced.

As a first attempt, we have chosen the 2-(4-trifluoromethylphenylsulfonyl)ethoxycarbonyl (Tsc) group as a more soluble analog of the Nsc group. Substitution of the trifluoromethyl group for the nitro group would make the protecting group more soluble. Both groups have similar electron-withdrawing abilities, so the substitution should not effect the base-promoted β-elimination reaction. Here, we report the development of Tsc, a novel amino-protecting group, and its use in the efficient solid-phase synthesis of pyrrole–imidazole polyamides. The results reveal that Tsc is superior in solubility to Nsc and in stability to Fmoc in the protection of exocyclic (hetero)aromatic amines. Tsc is expected to envision its extensions to numerous amine protections.

In order to implement the Tsc methodology for the synthesis of pyrrole–imidazole polyamides, two Tsc-protected monomers, Tsc-Py-OH **1a** and Tsc-Im-OH **1b**, were prepared by a modified route used for the synthesis of Nsc-protected aliphatic and aromatic amino acids (Scheme 1).[1,3] In contrast to the Fmoc group, we could readily introduce the Tsc protecting group into Py and Im amino acids starting from methyl and ethyl esters **5a** and **5b** via base-resistant 2-(4-trifluoromethylphenylthio)ethoxycarbonyl (Ttc) protection. The Ttc group could be oxidatively converted to the corresponding base-labile Tsc group. For the synthesis of **1a** and **1b**, the starting nitropyrrole **5a** and nitroimidazole **5b** were reduced by catalytic hydrogenation to provide aminopyrrole **6a** and aminoimidazole **6b**, respectively.[4] These amine derivatives were not stable as the free base and were used immediately without further purification. The aromatic amino group of **6a** and **6b** was readily protected by reaction with 4-nitrophenyl-2-(4-trifluoromethylphenylthio)ethyl carbonate (**4**)[5] to give the corresponding carbamates **7a** and **7b**.

Keywords: Tsc group; amino protecting group; amino acid; distamycin; DNA.

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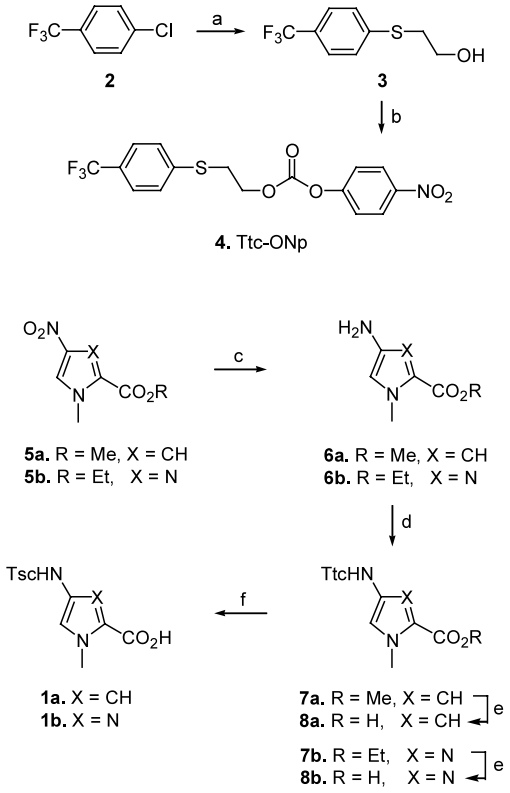


Scheme 1. *Reagents and conditions*: (a) mercaptoethanol, K_2CO_3, DMF, reflux, 68%; (b) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2, rt, 86%; (c) 10% Pd/C, 40 psi H_2, EtOAc (**5a**) or MeOH (**5b**), rt; (d) **4**, DIEA, DMAP, HOBt, CH_2Cl_2, rt, (**7a**, 86% from **5a**; **7b**, 77% from **5b**); (e) LiOH, THF:H_2O (2:1), rt, (**8a**, 66%; **8b**, 81%); (f) Na_2MoO_4, H_2O_2, acetone, rt, (**1a**, 65%; **1b**, 68%).

N-Ttc amino acid esters thus obtained were hydrolyzed under alkaline conditions to afford the acids **8a** and **8b** in high yields. Subsequent oxidation of the Ttc moiety with H_2O_2/Na_2MoO_4 in acetone gave the desired products, Tsc-Py-OH **1a** and Tsc-Im-OH **1b**.[6,7]

As expected, Tsc-Im-OH exhibited much greater solubility than Nsc-Im-OH in DMF, NMP and 1:1 DMF/NMP (>0.15 M versus <0.01 M).[1,8] Tsc-Py-OH was also more soluble than its Nsc analog (>0.15 M versus >0.05/<0.15 M). The solubility of Tsc-Im-OH and Tsc-Py-OH was sufficient to allow their use for the solid-phase synthesis of polyamides containing both Im and Py amino acids.

Premature deprotection of Tsc protected amino acids was investigated under various coupling reaction conditions.[9,10] Careful HPLC monitoring of the decomposition of Tsc-Py-OH and Tsc-Im-OH at room temperature revealed that >98% of the Tsc group remained intact even after 4 h under coupling reaction conditions, whereas about 25–30% of the Fmoc groups were eliminated from Fmoc-Py-OH and Fmoc-Im-OH.[1,9] Tsc-Py-OH and Tsc-Im-OH were also less prone to decomposition than their Fmoc analogs at higher temperature under the same condition (>95% versus

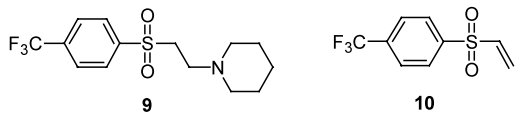


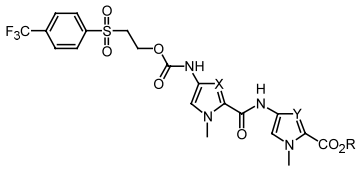
Figure 1. The piperidine adduct **9** that forms presumably via **10** upon deprotection of **1a** and **1b** with a 20% (v/v) solution of piperidine in DMF.

<50% intact after 2 h at 55°C). These results indicate that Tsc is superior to Fmoc and similar to Nsc in its chemical and thermal stability when used to protect the exocyclic amino group of heteroaromatic amino acids.[1,9] Based on this result, the general application of Tsc chemistry to the protection of exocyclic (hetero)aromatic amines was deemed feasible.

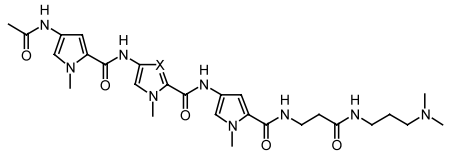
The deprotection of Tsc with piperidine and related nitrogen bases was performed by essentially the same procedure to that of Fmoc and Nsc. Periodic TLC monitoring of the deprotection of **1a** and **1b** revealed that the cleavage of the Tsc group by treatment with 20% (v/v) piperidine in DMF proceeded to completion within 5 min at comparable rates.[11] The primary byproduct that liberated upon treatment of **1a** and **1b** with excess piperidine was a stable compound whose ^1H NMR, ^{13}C NMR and mass spectra were consistent with the structure **9** (Fig. 1).[12] According to HPLC and ^1H NMR analyses of the deprotection reaction, no β-elimination byproduct **10** was observed at any point in the reaction. Presumably the initially formed vinyl sulfone **10** underwent rapid conversion to the adduct **9** by Michael-like attack by piperidine. This piperidine adduct was easily removed from a DMF solution of the deprotected amines by wash several times with DMF, CH_2Cl_2 and MeOH. Thus, Tsc is similar to Nsc in that the deprotection and adduct-formation occur simultaneously, alleviating the byproduct removal problem encountered in the Fmoc deprotecting reaction.[1] Analogous to Fmoc and Nsc chemistry, the presence of the chromophore in the piperidine adduct **9** allows facile UV detection and quantitation. This provides a simple method for estimation of stepwise yields in coupling of Py and Im aromatic amino acids whose amino group does not react in the quantitative ninhydrin test.

Peptide coupling of the Tsc protected Py and Im amino acids was investigated under various standard reaction conditions in solution using coupling reagents such as HOBt, HOAt, PyBOP, TFFH, TFFH/HOAt, PyBroP and EDCI/DMAP.[13] We found that EDCI/DMAP is superior to other reagents by quantitatively affording dipeptides **11a–d** in the solution-phase coupling reaction between **1** and **6** (Fig. 2). The similar results were observed for Nsc-Py-OH.[1] Based on these results, EDCI/DMAP was used for the solid-phase synthesis of polyamides.

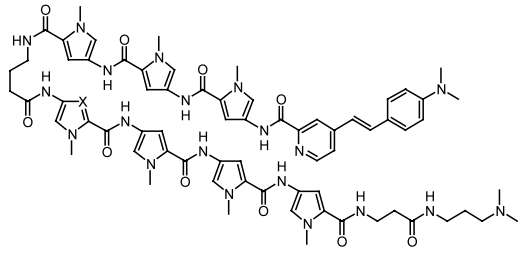
With Tsc-Py-OH and Tsc-Im-OH in hand, we set out to demonstrate their use in polyamide synthesis. Our approach involves standard Fmoc-based solid-phase synthesis of polyamides using Fmoc-β-alanine-Wang



11a. Tsc-PyPy-OMe (R = Me, X = CH, Y = CH)
11b. Tsc-PyIm-OEt (R = Et, X = CH, Y = N)
11c. Tsc-ImPy-OMe (R = Me, X = N, Y = CH)
11d. Tsc-ImIm-OEt (R = Et, X = N, Y = N)



12a. Ac-PyPyPy–β-Dp (X = CH)
12b. Ac-PyImPy–β-Dp (X = N)



13a. PrPyPyPy-γ-PyPyPyPy-β-Dp (X = CH)
13b. PrPyPyPy-γ-ImPyPyPy-β-Dp (X = N)

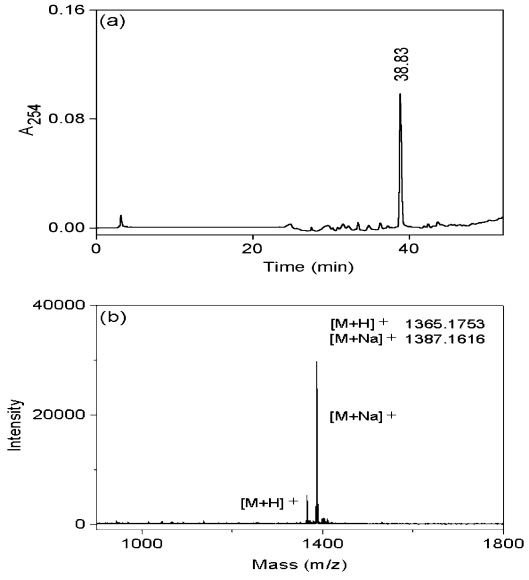


Figure 2. Structures of polyamides **11**–**13**. HPLC[14] (a) and MALDI-TOF mass spectral (b) analyses of the polyamide **13b**.

resin.[1,2,4] Each coupling cycle consists of a solvent wash, removal of the Tsc group with 20% piperidine in DMF for 30 min, a solvent wash, addition of monomer (2.5 equiv.) preactivated with EDCI and DMAP, coupling for 4 h, a solvent wash, capping with acetic anhydride/DIEA, and a final solvent wash. Washing was performed with DMF, MeOH, DCM and then DMF. An additional DCM wash was employed right before and after capping reactions. The resin was cleaved by aminolysis with 3-(dimethylamino)propylamine at 55°C for 24 h. Polyamides **12a** and **12b** were synthesized in nine steps using the manual solid-phase protocol (Fig. 2).[14] The stepwise yield for both polyamides was established as >99% by HPLC analysis. A single HPLC purification of these three-ring polyamides afforded an overall recovery of 61% and 55% for **12a** and **12b**, respectively, and a final purity greater than 98% as determined by analytical HPLC and mass spectrometry. We also successfully synthesized the eight-ring polyamides **13a** and **13b** containing one fluorescent probe Pr[1] in >98% yield of each coupling step and an overall recovery of 48% and 36%, respectively.[14] We could not obtain comparable high coupling and overall yields for **13b** using Fmoc-Py-OH and Fmoc-Im-OH under reaction conditions as described (we obtained an overall recovery of 5% for **13b** with Fmoc chemistry. Dervan's group reported an overall recovery of 9% for ImPyPyPy-γ-ImPyPyPy-β-Dp).[2] These results validate our Tsc strategy for the incorporation of imidazole amino acids into polyamides in an efficient manner.

In conclusion, we have demonstrated the suitability of the Tsc methodology for the preparation of polyamides containing heteroaromatic pyrrole and imidazole amino acids. The Tsc group alleviates a solubility problem associated with the Nsc protecting group. In addition, the Tsc method shows advantages over Fmoc chemistry in that the Tsc group is less sensitive to premature deprotection when installed on the exocyclic (hetero)aromatic amines and its deprotection byproduct is easy to remove. The milder Tsc method will allow the preparation of polyamides with a wide range of modifications that are incompatible with Boc chemistry. We anticipate that Tsc will be widely used as an alternative to Fmoc and Nsc amino-protecting groups.

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5. Ttc-ONp **4**. TLC (EtOAc:hexane = 1:3) $R_f = 0.37$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, $J = 9.3$ Hz, 2H), 7.56 (d, $J = 8.7$, 2H), 7.47 (d, $J = 8.1$, 2H), 7.36 (d, $J = 8.7$, 2H), 4.47 (t, $J = 6.9$, 2H), 3.35 (t, $J = 7.1$, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.05, 152.08, 145.24, 139.89, 129.29, 128.75, 128.32, 128.17, 127.89, 127.45, 125.87, 125.82, 125.77, 125.72, 125.69, 125.15, 122.09, 121.55, 118.48, 66.78, 30.95.
6. Tsc-Py-OH **1a**. TLC (EtOAc:hexane = 4:1) $R_f = 0.38$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.15 (brs, 1H), 9.21 (brs, 1H), 8.14 (d, $J = 8.1$, 2H), 8.00 (d, $J = 8.1$, 2H), 6.95 (s, 1H), 6.56 (s, 1H), 4.34 (t, $J = 5.6$, 2H), 3.84 (t, $J = 5.4$, 2H), 3.77 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.98, 152.61, 143.37, 134.28, 133.85, 133.42, 132.99, 128.87, 126.67, 126.63, 126.23, 122.14, 121.61, 120.04, 118.98, 117.99, 107.84, 57.61, 54.43, 36.15; HRMS (FAB$^+$) for $C_{16}H_{15}F_3N_2O_6S$ (M^+), calcd 420.0603, found 420.0609.
Tsc-Im-OH **1b**. TLC (EtOAc:MeOH:H_2O = 24:5:4) R_f = 0.17; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.82 (brs, 1H), 8.14 (d, $J = 7.8$, 2H), 8.00 (d, $J = 7.8$, 2H), 7.94 (brs, 1H), 7.06 (s, 1H), 4.36 (brs, 2H), 3.87 (brs, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.60, 152.41, 143.24, 136.34, 134.64, 134.15, 133.72, 133.29, 132.86, 128.90, 126.62, 125.16, 121.55, 117.93, 111.83, 57.77, 54.22, 35.28; HRMS (FAB$^+$) for $C_{15}H_{14}F_3N_3O_6SNa$ (MNa$^+$), calcd 444.0453, found 444.0464.
7. **1a** and **1b** could also be synthesized by treatment of *tert*-butyl esters, H-Py-OtBu and H-Im-OtBu, with Tsc-Cl using literature protocols.[2] However, *tert*-butyl esters are more expensive and require harsher conditions for reduction (500 versus >40 psi) and ester deprotection (0.2 M $TiCl_4$ versus 0.1N LiOH) than methyl and ethyl esters.
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10. The amount of intact **1a** and **1b** (0.1 M) after treatment with NH_2-Gly-OEt·HCl (0.2 M) and DIEA (0.3 M) in DMF was determined by analytical HPLC on a C_{18} column using UV absorption at 254/268 nm. tBoc-Py-OH[4] (0.1 M) was added as an internal standard. Fmoc-Py-OH and Fmoc-Im-OH[2] were used for comparison experiments and its cleavage was monitored by UV absorption at 254/290 nm.
11. The deprotection rate was slightly faster by addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (1% v/v) to a 20% piperidine–DMF solution.
12. Piperidine adduct **9**. TLC (EtOAc:hexane = 1:1) $R_f = 0.38$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, $J = 8.4$, 2H), 7.83 (d, $J = 8.4$, 2H), 3.33 (t, $J = 7.2$, 2H), 2.74 (t, $J = 7.2$, 2H), 2.27 (s, 4H), 1.35 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.37, 135.87, 135.44, 135.00, 134.57, 128.65, 126.12, 126.07, 126.02, 125.97, 124.93, 121.32, 117.72, 54.08, 53.62, 51.95, 25.66, 23.97; HRMS (FAB$^+$) for $C_{14}H_{19}F_3NO_2S$ (MH$^+$), calcd 322.1089, found 322.1082; UV (DMF) $\lambda_{max} = 268$ nm ($\varepsilon = 1.6\times10^3$ M^{-1} cm^{-1}).
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14. The polyamides **12** and **13** were synthesized on Fmoc-β-Ala-Wang resin (30 μmol) in a stepwise fashion by a manual solid-phase method as described.[1,15] Purity of the polyamide was determined to be >98% by reverse-phase HPLC on a C_{18} analytical column (4 μm, 0.39×15 cm, Nova-Pak, Waters, MA) under gradient conditions: **12**, 3.33% CH_3CN/min, 1 mL min^{-1} flow rate, Rv = 9.06 mL (**12a**) and 9.14 mL (**12b**); **13**, 0–10 min 100% H_2O, 10–60 min 2% CH_3CN/min, 1 mL min^{-1} flow rate, Rv = 37.50 mL (**13a**) and 38.83 mL (**13b**). The observed molecular mass agreed to within 0.1% of the calculated polyamide mass. MALDI-TOF: **12a**, $C_{28}H_{40}N_9O_5$ (MH$^+$), calcd 582.3152, found 582.4471; **12b**, $C_{27}H_{39}N_{10}O_5$ (MH$^+$), calcd 583.3105, found 583.7133; **13a**, $C_{70}H_{83}N_{20}O_{10}$/ $C_{70}H_{82}N_{20}O_{10}$Na (MH$^+$/MNa$^+$), calcd 1363.6601/ 1385.6420, found 1364.4369/1386.4111; **13b**, $C_{69}H_{82}N_{21}O_{10}$/ $C_{69}H_{81}N_{21}O_{10}$Na (MH$^+$/MNa$^+$), calcd 1364.6554/1386.6373, found 1365.1753/1387.1616.
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