Pergamon

Tetrahedron Letters 43 (2002) 4295–4299

TETRAHEDRON LETTERS

Nsc-mediated solid-phase synthesis of polyamides containing pyrrole amino acid

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Accepted 24 April 2002

Abstract—The synthesis of the Nsc-protected amino acid, Nsc-Py-OH **1a**, and its oligomerization are described.

Polyamides for the specific recognition of DNA sequences can be designed by the linear alignment of *N*-methylpyrrole (Py), *N*-methylimidazole (Im), and *N*-methyl-3-hydroxypyrrole (Hp) amino acids.[1] The sequence specificity of the polyamides depends on side-by-side ring pairings of these aromatic amino acids in the minor groove of DNA. The four amino acid pairs complete the minor-groove recognition code for all four Watson–Crick base pairs. The ability of polyamides to bind DNA with specificities and affinities comparable to those of naturally occurring DNA-binding proteins has generated considerable interests owing to their potential roles in regulating gene expressions.[1,2]

Previous reports on polyamide synthesis utilized *tert*-butoxycarbonyl (Boc)-based protocols.[1e] This involves acidic procedures such as repeated exposure of the polyamide to TFA in the removal of the temporary Boc amino protecting group. Such conditions preclude the incorporation of various acid-sensitive modifications into the standard polyamide structures. An alternative strategy for polyamide synthesis may employ the 9-fluorenylmethoxycarbonyl (Fmoc) protecting group.[3] The Fmoc protection scheme would complement the Boc strategy for polyamide synthesis because of its stability to acid and lability to base. Interestingly, relatively little information on Fmoc protections of the exocyclic (hetero)aromatic amine is available in the literature.

Recently, several groups tried to apply the Fmoc protection method in the preparation of polyamides.[4] However, we have found that the synthesis of two Fmoc-protected monomer building blocks, Fmoc-Py-OH and Fmoc-Im-OH amino acids, required longer synthetic steps and showed a substantially lower overall chemical yield than that of Boc-protected Py and Im amino acids. This might be attributed to facile decarboxylation of Py/Im heteroaromatic (amino) acids and insufficient stability of the Fmoc group installed on the exocyclic (hetero)aromatic amine.[2b,5] We therefore explored the alternative established for amine protection but still retaining compatibility with Fmoc chemistry. The use of the 2-(4-nitrophenylsulfonyl)ethoxycarbonyl (Nsc) group may be more suitable for polyamide synthesis because of its increased stability when compared to the Fmoc group.[6,7] Here, we describe the efficient synthesis of Py and Im monomers bearing Nsc protection, followed by studies to optimize conditions for coupling/deprotection cycles.

To test the applicability of the Nsc group for polyamide synthesis, two monomers, Nsc-Py-OH **1a** and Nsc-Im-OH **1b** were prepared by a modified route used for synthesis of Nsc-protected aliphatic amino acids (Scheme 1).[6] In contrast to the Fmoc group, we could readily introduce the Nsc protecting group into Py and Im amino acids starting from methyl and ethyl esters **5a** and **5b** via base-resistant 2-(4-nitrophenylthio)ethoxycarbonyl (Ntc) protection. The Ntc group could be oxidatively converted to the corresponding base-labile Nsc group. For the synthesis of **1a** and **1b**, the starting nitropyrrole **5a** and nitroimidazole **5b** were reduced by catalytic hydrogenation to provide aminopyrrole **6a**

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

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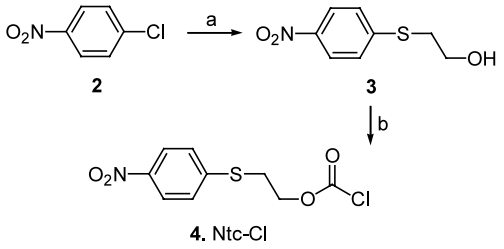
PII: S0040-4039(02)00809-2



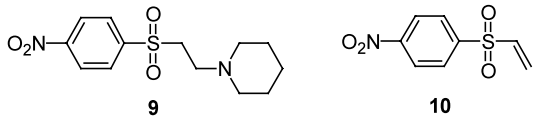
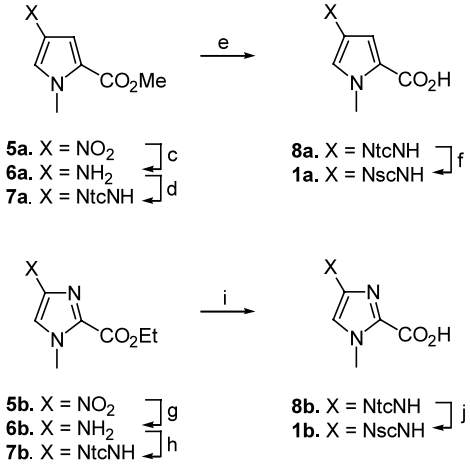


Figure 1. The piperidine adduct **9** that forms upon deprotection of **1a** and **1b** with a 20% (v/v) solution of piperidine in DMF.



Scheme 1. *Reagents and conditions*: (a) mercaptoethanol, KOH, 70°C, quantitative; (b) $COCl_2$, THF, −20°C, 95%; (c) 10% Pd/C, 40 psi H_2, EtOAc, rt; (d) **4**, TEA, EtOAc, rt, 78% from **5a**; (e) LiOH, THF:H_2O (2:1), rt, 79%; (f) Na_2MoO_4, H_2O_2, 1,4-dioxane, rt, 64%; (g) 10% Pd/C, 40 psi H_2, EtOAc/EtOH (1:2), rt; (h) **4**, TEA, EtOAc, rt, 50% from **5b**; (i) NaOH, 1,4-dioxane, rt, 98%; (j) Na_2MoO_4, H_2O_2, 1,4-dioxane/AcOH (4:1), rt, 31%.

and aminoimidazole **6b**, respectively.[1e] These amine derivatives were not stable as the free base and thus used as, formed immediately without further purification. The aromatic amino group of **6a** and **6b** was readily protected by reaction with chloro-2-(4-nitrophenylthio)ethyl carbonate (**4**) to give the corresponding carbamates **7a** and **7b**, respectively. *N*-Ntc amino acid esters thus obtained were hydrolyzed under alkaline conditions to afford the acids **8a** and **8b**. Subsequent oxidation of the Ntc moiety with H_2O_2/Na_2MoO_4 gave the desired products, Nsc-Py-OH **1a** and Nsc-Im-OH **1b**.[8]

Nsc-Py-OH exhibited greater solubility than Nsc-Im-OH in DMF, NMP, and DMF/NMP (1:1). In fact, the poor solubility (<0.01 M) of Nsc-Im-OH in these and other common organic solvents such as $CHCl_3$, MeOH and THF proved to be the major obstacle to its utility in the solid-phase synthesis of polyamides.[9] Therefore, only Nsc-Py-OH was used for solid-phase polyamide synthesis. Premature deprotection of Nsc protected amino acids was investigated under various coupling reaction conditions.[10,11] Careful HPLC monitoring of the decomposition of Nsc-Py-OH at room temperature revealed that >98% of the Nsc 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.[11] Nsc-Py-OH was also less prone to decomposition than Fmoc-Py-OH at higher temperature under the same solution conditions (>95% versus <50% intact after 2 h at 55°C). These results indicate that the Nsc group differs from the Fmoc group regarding its increased chemical and thermal stability when used to protect the exocyclic amino group of heteroaromatic amino acids.[10] Based on this result, general application of Nsc chemistry to exocyclic (hetero)aromatic amine protection was deemed feasible.

The deprotection of Nsc with piperidine and related nitrogen bases was just like that of Fmoc. Periodic TLC monitoring of the deprotection of **1a** and **1b** revealed that the cleavage of the Nsc group by treatment with 20% (v/v) piperidine in DMF proceeded to completion within 5 min at comparable rates.[12] The primary byproduct that liberated upon treatment of **1a** and **1b** with excess piperidine was a stable compound whose ^1H, ^{13}C NMR and mass spectra were consistent with the structure **9** (Fig. 1).[13] According to HPLC and ^1H NMR analyses of the deprotection reaction, no β-elimination byproduct **10** was observed at any point of 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 extraction or wash several times with DMF, CH_2Cl_2 and MeOH. Thus, Nsc alleviates the byproduct removal problem associated with the Fmoc protecting group.[7] Analogous to Fmoc 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 aromatic amino acid whose amino group does not react in the quantitative ninhydrin test.

Peptide coupling of the Nsc protected Py amino acid was investigated under various standard reaction conditions in solution using coupling reagents such as HOBt, HOAt, PyBOP, TFFH, TFFH/HOAt and PyBroP.[14] The coupling reagents, TFFH/HOAt and PyBroP showed comparable moderate efficiency (ca. 60% isolated yield) in the synthesis of dipeptide **11** from **1a** and **6a** (Fig. 2).[15] On the other hand, other reagents gave a lower yield than that obtained with TFFH/HOAt and PyBroP (ca. 30–40% isolated yield). Since all these coupling efficiencies were somewhat unsatisfactory for

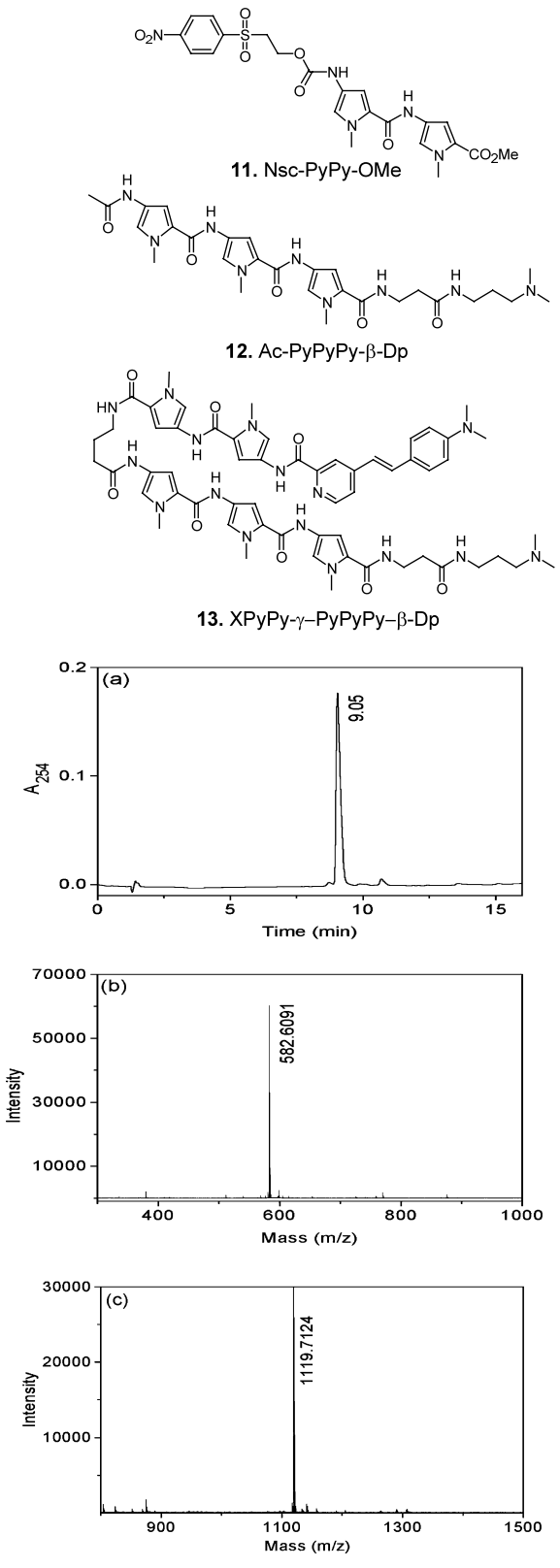


Figure 2. Structures of Nsc-PyPy-OMe **11**, Ac-PyPyPy-β-Dp **12**, and XPyPy-γ-PyPyPy-β-Dp **13**. HPLC analysis of **12** (a) and MALDI-TOF mass spectral analyses of purified polyamides **12** (b) and **13** (c).

their use in solid-phase polyamide synthesis, we explored more effective coupling reagents. We found that EDCI in combination with DMAP demonstrated the superiority over TFFH/HOAt and PyBroP by affording the quantitative yield in the solution phase coupling reaction between **1a** and **6a**.[16] Based on these results, the solid-phase coupling reaction was performed using a combination of EDCI and DMAP.

The polyamides **12** and **13** were prepared by solid-phase synthesis using Fmoc-β-alanine-Wang resin. Each coupling cycle consists of a solvent wash, removal of the Nsc 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 and DIEA and a final solvent wash. Washing were 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. Ac-PyPyPy-β-Dp **12** was synthesized in nine steps using the protocols as described (Fig. 2).[17] The stepwise yield was established as >99% by HPLC analysis. A single HPLC purification of the three-ring polyamide **12** afforded a 57% overall recovery and a final purity greater than 98% as determined by analytical HPLC and mass spectroscopy. We also successfully synthesized the polyamide **13** containing five pyrroles and one fluorescent label X **14**[18] in >98% yield of each coupling step and an overall 48% recovery. We could not obtain comparable high coupling and overall yields using Fmoc-Py-OH under reaction conditions as described[4a] (<80–90% coupling yield and <5–10% recovery for Fmoc-Py-OH).

In conclusion, we have demonstrated the suitability of the Nsc methodology for preparation of polyamides containing heteroaromatic amino acids. The Nsc group alleviates a stability problem associated with the Fmoc protecting group due to the less sensitivity of the Nsc group toward premature deprotection when installed on the exocyclic (hetero)aromatic amine. In addition, the Nsc method shows advantages over the Fmoc chemistry in that it eliminates difficulties in removing the deprotection byproduct. The milder Nsc method will allow for preparation of polyamides with a wide range of modifications which are incompatible with Boc chemistry.

Acknowledgements

This work was financially supported by the Korea Research Foundation Grant (KRF-2000-015-DP0254). Fellowship support from the BK21 Program (J.S.C.

and Y.L.) is gratefully acknowledged. We are grateful for the instrumental support from the equipment facility of CRM-KOSEF, Korea University.

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8. Nsc-Py-OH **1a**. TLC (EtOAc:hexane = 3:1) $R_f = 0.30$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.15 (brs, 1H), 9.03 (brs, 1H), 8.40 (d, $J = 8.7$ Hz, 2H), 8.17 (d, $J = 8.7$, 2H), 6.88 (s, 1H), 6.50 (s, 1H), 4.34 (t, $J = 5.4$, 2H), 3.89 (t, $J = 5.6$, 2H), 3.76 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.59, 152.13, 150.16, 144.59, 129.27, 124.48, 121.79, 119.78, 118.69, 107.46, 57.59, 54.28, 36.12; HRMS (FAB$^+$) for $C_{15}H_{15}N_3O_8S$ (M^+), calcd 397.0580, found 397.0582. Nsc-Im-OH **1b**. TLC (EtOAc:MeOH:H_2O = 25:5:4) R_f = 0.40; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.77 (brs, 1H), 8.37 (d, $J = 7.8$, 2H), 8.16 (d, $J = 8.4$, 2H), 7.15 (s, 1H), 4.37 (brs, 2H), 3.91 (brs, 2H), 3.86 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.64, 152.16, 150.09, 144.55, 136.86, 131.74, 129.27, 124.45, 113.30, 57.91, 54.20, 35.48; LRMS (FAB$^+$) for $C_{14}H_{15}N_4O_8S$ (MH^+), calcd 399.1, found 399.0.
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11. The amount of intact Nsc-Py-OH **1a** (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 at 254 and 268 nm. tBoc-Py-OH (0.1 M) was added as an internal standard. Fmoc-Py-OH was selected as a substrate for comparision experiments.[4a] Cleavage of the Fmoc group was performed as described above, with the exception of monitoring the absorbance at 254 and 290 nm.
12. 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.
13. Piperidine adduct **9**. TLC (EtOAc:hexane = 3:1) $R_f = 0.39$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (d, $J = 8.7$, 2H), 8.14 (d, $J = 9.0$, 2H), 3.36 (t, $J = 6.9$, 2H), 2.75 (t, $J = 6.8$, 2H), 2.26 (brs, 4H), 1.33 (brs, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.60, 145.73, 129.62, 124.12, 54.01, 53.56, 51.95, 25.52, 23.79; HRMS (FAB$^+$) for $C_{13}H_{19}N_2O_4S$ (MH^+), calcd 299.1066, found 299.1076; UV (DMF) $\lambda_{max} = 267$ nm ($\varepsilon = 4.7 \times 10^3$).
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15. Nsc-PyPy-OMe **11**. TLC (EtOAc:hexane = 3:1) $R_f = 0.22$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (brs, 1H), 9.20 (brs, 1H), 8.43 (d, $J = 7.5$, 2H), 8.19 (d, $J = 7.5$, 2H), 7.46 (s, 1H), 6.89 (s, 1H), 6.81 (s, 1H), 6.77 (s, 1H), 4.37 (brs, 2H), 3.90 (brs, 2H), 3.83 (s, 3H), 3.80 (s, 3H), 3.74 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.81, 158.28, 152.52, 150.43, 144.74, 129.47, 124.67, 122.90, 122.76, 121.56, 120.76, 118.48, 117.36, 108.32, 103.85, 57.49, 54.29, 50.98, 36.19, 36.12.
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17. Ac-PyPyPy-β-Dp **12** and XPyPy-γ-PyPyPy-β-Dp **13**. The polyamides **12** and **13** were synthesized on Fmoc-β-Ala-Wang resin (60 μmol) in a stepwise fashion by a manual solid-phase method as described.[19] Nsc-Py-OH (150

μmol) was activated with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI, 300 μmol) and 4-dimethylaminopyridine (DMAP, 300 μmol) and coupled for 4 h. Deprotection of the Nsc groups was performed using 20% (v/v) piperidine in DMF for 30 min. Polyamide **12** was acetylated at the N-terminus. Polyamide resin cleavage was performed using 3-(dimethylamino)-propylamine (3 mL) in a 4 mL glass vial at 55°C for 24 h. After evaporation of the solvent, the crude polyamide was dissolved in DMF (4 mL). Purification was achieved by reverse-phase HPLC with a Vydac C_{18} column and a linear water/acetonitrile gradient containing 0.1% (v/v) trifluoroacetic acid. Polyamides were detected by monitoring the absorbance at 254 nm. Polyamides were recovered upon lyophilization of the appropriate fractions as a solid (**12**, 20 mg, 57% recovery; **13**, 32 mg, 48% recovery). 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 mL; **13**, 0–10 min 100% H_2O, 10–60 min 3.33% CH_3CN/min, 1 mL min^{-1} flow rate, Rv = 39 mL). The molecular mass of each polyamide was measured using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) on a PerSeptive Biosystems Voyager-DE™RP mass spectrometer. The observed molecular mass agreed to within 0.1% of the calculated polyamide mass. MALDI-TOF: **12**, $C_{28}H_{40}N_9O_5$ (MH^+), calcd 582.3152, found 582.6091; **13**, $C_{58}H_{71}N_{16}O_8$ (MH^+), calcd 1119.5641, found 1119.7124.

18. Compound X-OH **14**. ^1H NMR (300 MHz, DMSO-d_6) δ 8.56 (d, J = 4.8, 1H), 8.13 (s, 1H), 7.68 (d, J = 4.8, 1H), 7.53 (d, J = 14.7, 1H), 7.52 (d, J = 8.7, 2H), 7.05 (d, J = 16.2, 1H), 6.74 (d, J = 8.1, 2H), 2.96 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.25, 150.76, 149.17, 146.91, 134.64, 128.61, 123.57, 122.71, 120.94, 119.83, 112.00; LRMS (FAB^+) for $C_{16}H_{17}N_2O_2$ (MH^+), calcd 269.1, found 269.0.
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