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Synthesis of photolabile 2-(2-nitrophenyl)propyloxycarbonyl protected amino acids

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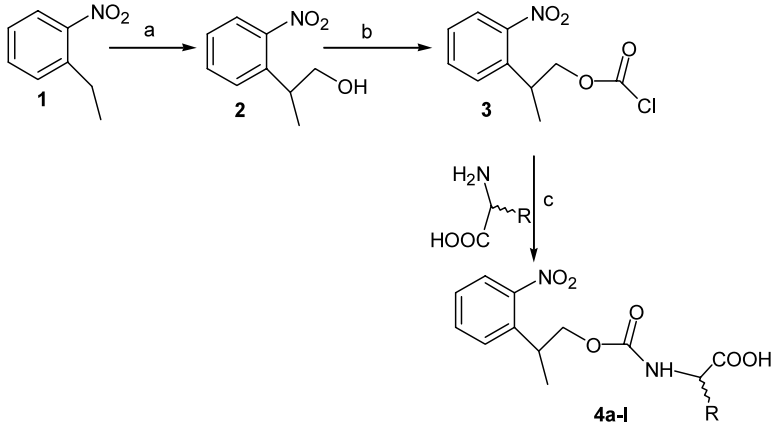
Abstract—The 2-(2-nitrophenyl)propyloxycarbonyl (NPPOC) group has been introduced as a photolabile amino protecting group for amino acids to be used as building blocks in photolithographic solid-phase peptide synthesis. NPPOC-protected amino acids were found to be cleaved in the presence of UV light about twice as fast as the corresponding *o*-nitroveratryloxycarbonyl (NVOC)-protected amino acids.

In recent years there has been growing interest in the synthesis of microarrays of oligonucleotides and peptides on glass or other surfaces utilizing photolithographic processes, for use in genomics and proteomics research, respectively.[1] In 1991, Fodor et al.[2] demonstrated for the first time that addressable arrays (e.g. peptides) could be synthesized on glass surfaces using building blocks with photolabile protecting groups. However, soon after this report synthetic efforts tended to shift to oligonucleotide arrays,[3] because of interest in genomics analysis, the relative ease of oligonucleotide synthesis and the fact that oligonucleotide synthesis requires only four building blocks, whereas peptide synthesis requires twenty. Now, however, with the burgeoning growth of proteomics,[4] attention is returning to peptide arrays.

In the earlier studies by Fodor and co-workers[2,5] amino acids were protected with the photolabile *o*-nitroveratryloxycarbonyl (NVOC) group, which was originally introduced by Patchornik et al. in 1970.[6] Unfortunately, the photolytic removal of NVOC is not very



Scheme 1. *Reagents and conditions*: (a) $(HCHO)_n$, Triton B (40% in MeOH), reflux, 6 h; (b) $COCl_2$, THF, 0°C, 3 h; (c) Na_2CO_3, 1,4-dioxane/water (1:1), rt, ~20 h (Table 1).

Keywords: peptide synthesis; photolabile protecting group; photolithography; microarray.
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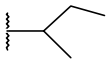
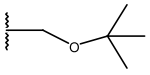
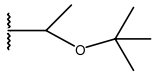
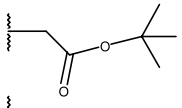
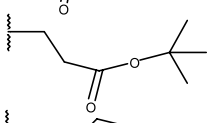
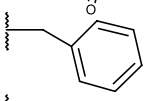
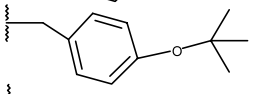
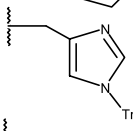
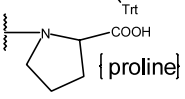
Table 1. Reaction times, yields and masses of products **4**

Entry	R	time (h)	yield[a] (%)	found[b] [M+H$^+$]/ calculated [M]
4a	H	18	83	283.0 / 282.0
4b		20	85	297.1 / 296.1
4c		19	84	325.1 / 324.1
4d		22	86	339.2 / 338.2
4e		20	84	369.3 / 368.3
4f		20	85	383.3 / 382.3
4g		23	81	397.3 / 396.3
4h		23	80	411.5 / 410.4
4i		18	87	373.2 / 372.2
4j		23	81	445.3 / 444.5
4k		24	79	605.2 / 604.2
4l	 {proline}	20	78	323.2 / 322.3

[a]isolated yield; [b]MS (CI$^+$)

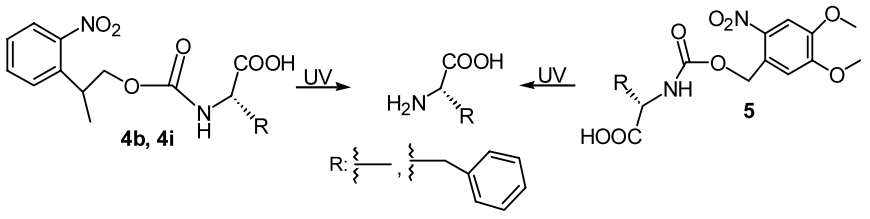
However, some improvement in the yield of photodeprotection has been reported by Holmes et al.,[7] through use of the α-methyl-*o*-nitropiperonyloxycarbonyl (MeNPOC) group. More recently Beier and Hoheisel[8] have demonstrated that the efficiency of photolytic cleavage of 2-(2-nitrophenyl)propyloxycarbonyl (NPPOC) protected nucleotides is significantly better than that for MeNPOC protected nucleotides. One difference between the NPPOC group and the NVOC and MeNPOC groups is that the former is a derivative of 2-(2-nitrophenyl)ethyl alcohol, whereas the latter two derive from 2-nitrobenzyl alcohol. The additional methylene group in the NPPOC group leads to a different photocleavage mechanism.[9] In addition, the photodegradation products of the NVOC and MeNPOC groups include carbonyl compounds[10] which can react with amino groups and reduce stepwise synthetic yields.

Since the NPPOC group seems to be beneficial in oligonucleotide synthesis, we decided to investigate its utility in peptide synthesis, with the aim of using NPPOC protected amino acids for the synthesis of peptide microarrays. This communication describes the synthesis of several NPPOC-amino acids.

To obtain NPPOC-protected amino acids **4**, we first devised an improved synthesis[11] of 2-(2-nitrophenyl)propanol **2** (Scheme 1), based on the method of Tsuji et al.[12] for preparation of 2-nitrophenethyl alcohol. The alcohol **2** was then treated with phosgene to give NPPOC chloride **3**.[13] Reaction of **3** with various amino acids in the presence of sodium carbonate (pH 9.5–10; reaction in sodium bicarbonate gave some dipeptide material) generated **4**.[14] The products **4** and their purity were assessed by ^{1}H NMR, CI-MS and LC-ESI-MS.[15]

Rates of photolysis of NPPOC-amino acids **4b**,**i** were compared with those of the corresponding NVOC-amino acids **5** in solution under identical conditions (irradiation at 365 nm, 2000 μW/cm^2, 5 mM solvent) (Scheme 2). Of the several solvent conditions tested for the photodeprotection in these preliminary studies, acidic methanol (2.5 mM semicarbazide hydrochloride in methanol) gave the best results. LC-MS analysis indicated that the NPPOC derivatives were cleaved about twice as fast as the corresponding NVOC derivatives. More detailed studies of the rates and overall yields of photolysis in solution and on glass surfaces are planned.

In conclusion, we have developed an efficient method for the synthesis of photolabile 2-(2-nitrophenyl)propyloxycarbonyl (NPPOC) protected amino acids for use as building blocks for photolithographic solid-phase peptide synthesis. These derivatives undergo light-promoted deprotection at a rate at least twice that of the earlier described[5] NVOC amino acids, due, presumably, to different cleavage mechanisms. Efforts to synthesize peptide arrays on glass surfaces using these photolabile amino acid derivatives are in progress.



Scheme 2. *Solvents for photodeprotection*: (a) 1,4-dioxane; (b) acetonitrile; (c) methanol; (d) 2.5 mM diisopropylethylamine in methanol; (f) 2.5 mM semicarbazide hydrochloride in methanol.

Acknowledgements

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11. *Procedure for preparation of 2-(2-nitrophenyl)propanol* **2**: Triton B (40% in MeOH, 8 mmol) was added to 2-ethylnitrobenzene (8 mmol) and paraformaldehyde (8.1 mmol), and the mixture was heated at reflux for 6 h. After concentration under vacuum, the reaction mixture was neutralized using 5% aqueous HCl. The mixture was extracted with ethyl acetate (3×10 mL), dried over Na_2SO_4 and concentrated at reduced pressure. The residue was purified by flash chromatography using hexane–ethyl acetate (4:1) to give compound **2** (96%, red oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm 7.73 (d, J=8.0 Hz, 1H, Ar-H), 7.56 (t, J=7.4 Hz, 1H, Ar-H), 7.48 (d, J=7.6 Hz, 1H, Ar-H), 7.35 (t, J=7.6 Hz, 1H, Ar-H), 3.77 (d, J=6.4 Hz, 2H, CH_2), 3.51 (m, 1H, CH), 1.79 (br s, 1H, OH), 1.32 (d, J=6.8 Hz, 3H, CH_3); MS (CI^+) m/z: 182.1 ($M+H^+$).
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13. *Procedure for preparation of NPPOC chloride* **3**: To a solution of **2** (6 mmol) in anhydrous THF (5 mL) at 0°C, was added a solution of phosgene (20% in toluene, 9 mmol) over a period of 15 min with stirring under a nitrogen atmosphere. After 45 min, the ice bath was removed and stirring was continued at room temperature for 2 h. A stream of N_2 was then bubbled through the solution for 1 h to remove the excess phosgene, after which the mixture was evaporated to dryness under vacuum to give compound **3** (99%, brown oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm 7.81 (d, J=8.0 Hz, 1H, Ar-H), 7.60 (t, J=7.4 Hz, 1H, Ar-H), 7.43 (d, J=7.6 Hz, 1H, Ar-H), 7.38 (t, J=7.6 Hz, 1H, Ar-H), 4.47 (d, J=6.4 Hz, 2H, CH_2), 3.77 (m, 1H, CH), 1.39 (d, J=6.8 Hz, 3H, CH_3); MS (CI^+) m/z: 243.6 ($M+H^+$).
14. *Typical procedure for the preparation of NPPOC protected amino acids* **4**: Na_2CO_3 (2.2 mmol) was added to the solution of L-amino acid (1 mmol) in 10 mL water/1,4-dioxane (1:1) at 0°C, followed by the dropwise addition of **3** (1 mmol, in 1 mL THF). After 20 min the ice bath was removed and stirring was continued for 18–24 h. The reaction mixture was evaporated to dryness, 3 mL of water was added and the mixture was extracted with ethyl acetate (2×5 mL) to remove **3** or its hydrolysis product. The aqueous layer was acidified by addition of 5% HCl at 0°C and extracted with ethyl acetate (3×10 mL); the extracts were dried over Na_2SO_4 and concentrated at reduced pressure to give a glassy substance that, in most cases was essentially pure (free of by-products), based on spectroscopic measurements.
15. Spectroscopic data for selected products: **4b**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm 7.71 (d, J=8.0 Hz, 1H, Ar-H), 7.54 (t, J=7.4 Hz, 1H, Ar-H), 7.43 (d, J=7.6 Hz, 1H, Ar-H), 7.34 (t, J=7.6 Hz, 1H, Ar-H), 5.28 (br d, 1H, NH), 4.28 (d, J=6.4 Hz, 2H, CH_2), 4.11 (m, 1H, CH), 3.67 (m, 1H, CH), 1.40 (d, J=7.6 Hz, 3H, CH_3), 1.31 (d, J=6.8 Hz, 3H, CH_3); LC-MS (ESI^+) m/z: 297.1 ($M+H^+$), 319.1 ($M+Na^+$).
4e: ^{1}H NMR ($CDCl_3$, 400 MHz): δ /ppm 7.70 (d, J=8.0 Hz, 1H, Ar-H), 7.52 (t, J=7.4 Hz, 1H, Ar-H), 7.42 (d, J=7.6 Hz, 1H, Ar-H), 7.32 (t, J=7.6 Hz, 1H, Ar-H), 5.42 (br d, 1H, NH), 4.25 (d, J=6.4 Hz, 2H, CH_2), 4.09 (m, 1H, CH), 3.98 (m, 1H, CH_2), 3.82 (m, 1H, CH_2), 3.53 (m, 1H, CH), 1.28 (d, J=6.8 Hz, 3H, CH_3), 1.10 (s, 9H, 3×CH_3); LC-MS (ESI^+) m/z: 369.1 ($M+H^+$), 391.1 ($M+Na^+$).
4h: ^{1}H NMR ($CDCl_3$, 400 MHz): δ /ppm 7.67 (d, J=8.0 Hz, 1H, Ar-H), 7.50 (t, J=7.4 Hz, 1H, Ar-H), 7.41 (d, J=7.6 Hz, 1H, Ar-H), 7.30 (t, J=7.6 Hz, 1H, Ar-H), 5.48 (br d, 1H, NH), 4.23 (d, J=6.4 Hz, 2H, CH_2), 4.16 (m, 1H, CH), 3.62 (m, 1H, CH), 2.28 (m, 2H, CH_2), 2.09 (m, 1H, CH_2), 1.90 (m, 1H, CH_2), 1.37 (s, 9H, 3×CH_3), 1.31 (d, J=6.8 Hz, 3H, CH_3); LC-MS (ESI^+) m/z: 411.1 ($M+H^+$), 433.1 ($M+Na^+$).
4j: ^{1}H NMR ($CDCl_3$, 400 MHz): δ /ppm 7.70 (d, J=8.0 Hz, 1H, Ar-H), 7.51 (t, J=7.4 Hz, 1H, Ar-H), 7.40 (d, J=7.6 Hz, 1H, Ar-H), 7.29 (t, J=7.6 Hz, 1H, Ar-H), 6.98 (d, J=8.2 Hz, 2H, Ar-H), 6.87 (d, J=8.2 Hz, 2H, Ar-H) 5.10 (br d, 1H, NH), 4.24 (d, J=6.4 Hz, 2H, CH_2), 4.16 (m, 1H, CH), 3.65 (m, 1H, CH_2), 3.06 (m, 1H, CH_2),

2.98 (m, 1H, CH_2), 1.30 (s, 9H, 3×CH_3), 1.26 (d, $J=6.8$ Hz, 3H, CH_3); LC-MS (ESI$^+$) m/z: 445.2 (M+H$^+$), 467.2 (M+Na$^+$).

4l: ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm 7.71 (d, $J=8.0$ Hz, 1H, Ar-H), 7.52 (t, $J=7.4$ Hz, 1H, Ar-H), 7.41 (d, $J=7.6$ Hz, 1H, Ar-H), 7.32 (t, $J=7.6$ Hz, 1H, Ar-H), 4.29 (d, $J=6.4$ Hz, 2H, CH_2), 4.15 (m, 1H, CH), 3.65 (m, 2H, CH_2), 3.46 (m, 1H, CH), 1.80–2.20 (m, 4H, 2×CH_2), 1.29 (d, $J=6.8$ Hz, 3H, CH_3); LC-MS (ESI$^+$) m/z: 323.1 (M+H$^+$), 345.1 (M+Na$^+$).