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Solid-phase synthesis and utilization of side-chain reactive unnatural amino acids

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Abstract—Alkylation of the benzophenone imine of glycine Wang resin with α,ω-dihaloalkanes yielded valuable reactive intermediates. These racemic ω-chloro or ω-bromo intermediates were converted to α-amino acids containing diverse side-chain functionalities (e.g. ω-chlorides, nitriles, and thioethers), proline and its ring homologs, and 1-aminocycloalkanecarboxylic acid derivatives.

Over the years, non-proteinogenic, unnatural α-amino acids have been widely used as components of peptides to enhance biological activity, proteolytic stability, and bioavailability.[1] Unique conformational constraints can also be induced into peptides or peptidomimetics by introducing an appropriate amino acid precursor. Unnatural amino acids are generally prepared by synthetic routes involving solution-phase techniques, and subsequently incorporated into a peptide sequence by solid-phase methods. We have recently published methods for the preparation of resin-bound α,α-disubstituted unnatural amino acids with diverse side-chain substitutions,[2] and α-substituted prolines and homologs.[3] This paper describes the solid-phase preparation of racemic α-monosubstituted amino acids with remote electrophilic centers. This is accomplished by alkylation of the Schiff base of a resin-bound glycinate with α,ω-dihaloalkanes.[4] The resulting intermediates can then be converted on-resin to unnatural α-amino acids of three different types: (i) amino acids with a variety of side-chain functionalities (**4** and **6**), (ii) proline and homologs[5] (**8**), and (iii) 1-aminocycloalkanecarboxylic acid derivatives[5] (**10**) (Scheme 1).

Alkylation of the activated benzophenone imine of glycine Wang resin **1** with α,ω-dihaloalkanes of different chain lengths ($n=2$–5) provided key resin-bound racemic intermediates **2**. The alkylation of the imine was carried out in NMP under mild reaction conditions using the nonionic Schwesinger base, BTPP[6] (**11**, 10 equiv.). In most cases, α-bromo-ω-chloro electrophiles (10 equiv.) were used for the alkylation in order to reduce side reactions such as alkene formation by elimination, premature cyclization on the nitrogen, or cross-linking. Acid-catalyzed hydrolysis of the imines **2** (THF/1 N aqueous HCl, 2:1), was followed by transformation to the resin-bound Fmoc derivatives (Fmoc-Cl, 10 equiv.; DIEA, 20 equiv.; NMP) using an in situ neutralization protocol to minimize competing intramolecular cyclization. Final cleavage (TFA/Et_3SiH, 95:5) gave the N^{α}-Fmoc protected ω-chloro-α-amino acids **4a**–**d** (Scheme 1 and Table 1) with HPLC purities of the crude products from 75% to 100% and purified, isolated yields from 63 to 74%. For compound **4b** ($n=3$), HPLC purity and isolated yield was lower because of the expected partial competing cyclization to form proline (25% by HPLC).

Following characterization of key intermediates **2** as described above, on-resin displacement of the ω-halide by a nucleophile to provide side-chain functionalized products **6** was investigated (Scheme 1 and Table 1). An homologous series of ω-nitrile derivatives (length of side chain $n=2$–5) was prepared by displacement of the halide intermediates **2** (Bu_4NCN and Bu_4NI, 10 equiv. each; NMP; 24 h; 25°C). For cases in which $n=3$ to 5 the intermediate **2** was the ω-chloride, while for $n=2$ the ω-bromide was used, since in this case the ω-chloride derivative was not of sufficient reactivity. Additionally, displacement of the chloride intermediate **2c** ($n=4$) with a less reactive nucleophile, benzyl mercap-

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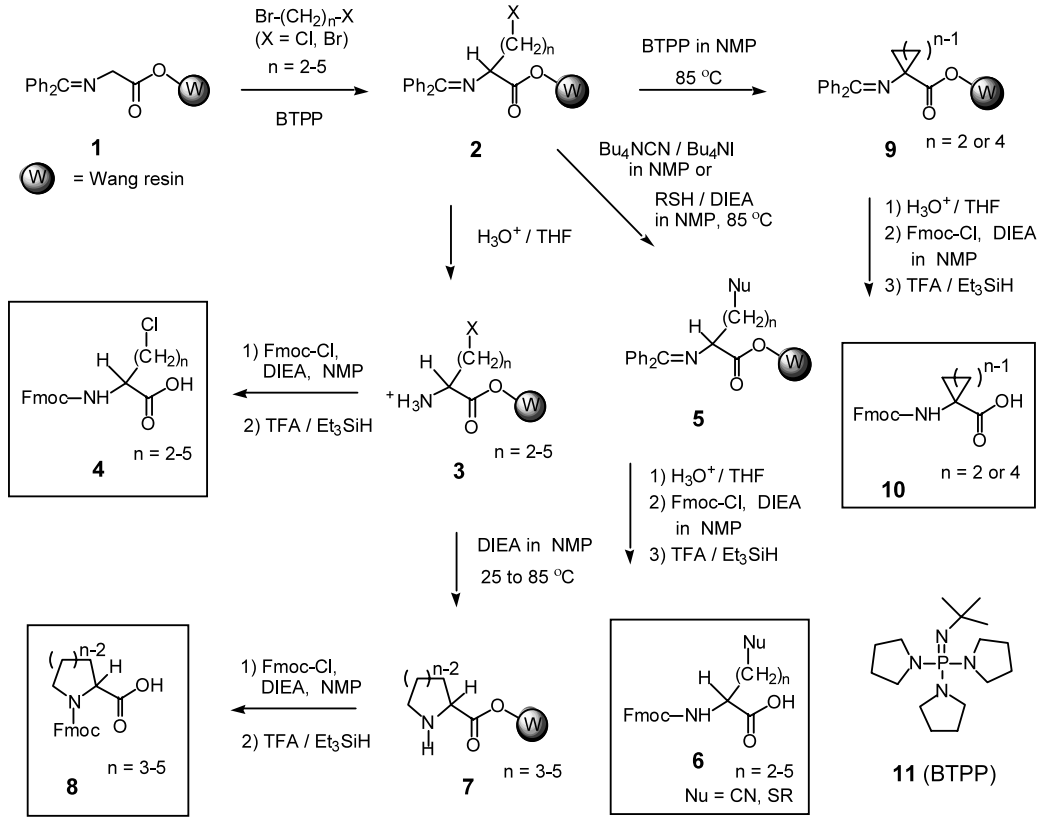
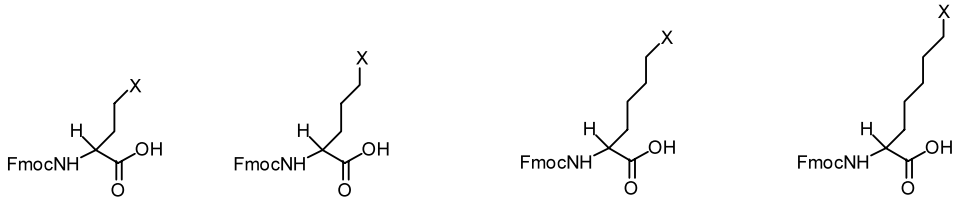


Scheme 1. Synthesis of side-chain reactive unnatural amino acids, proline and homologs, and spirocyclic amino acids, from key resin-bound intermediates **2**.

Table 1. N^{α}-Fmoc α-amino acids containing ω-chloride, nitrile or thioether functionalities, prepared from key resin-bound intermediates **2**



	n = 2		n = 3		n = 4			n = 5	
	4a	**6a**	**4b**	**6b**	**4c**	**6c**	**6c'**	**4d**	**6d**
	X = Cl	X = CN[a]	X = Cl	X = CN	X = Cl	X = CN	X = SCH_2Ph	X = Cl	X = CN
Crude HPLC Purity	89%	50%	75%	92%	93%	97%	63%	100%	100%
Purified Yield	67%	23%	63%	63%	71%	76%	35%	74%	71%

[a]The bromide was used as the halide precursor.

tide (20 equiv.; DIEA, 20 equiv.; NMP; 85°C; 36 h) provided the thioether derivative. Hydrolysis of **5**, acylation of the free α-amine with Fmoc-Cl, and TFA cleavage yielded products **6a–d** and **6c′** with crude HPLC purities ranging from 50 to 100%, and purified yields from 23 to 76% (Table 1).

Our next goal was to obtain cyclic imino acids (**8**) of different ring sizes.[7] Resin-bound five- to seven-membered proline ring homologs were prepared starting from intermediate **3**, by neutralization and intramolecular halide displacement with the α-amino group (Scheme 1). Cyclization conditions varied depending on the ring size. For $n=3$ (five-membered ring) and $n=4$ (six-membered ring), cyclization involved using the ω-chloro derivatives (10% DIEA in NMP, 25°C, 24 h). In addition, for $n=5$ (seven-membered ring), cyclization required using the ω-bromo derivative and higher temperature (85°C); for $n=2$ (four-membered ring), all experiments led to formation of the *N*-alkyl cross-link-

ing product by intermolecular halide displacement by the α-amino group. Acylation of the cyclic secondary amine with Fmoc-Cl, followed by TFA cleavage gave products **8a** and **8c**–**8d** with excellent crude HPLC purities (88–98%) and good purified yields (55–73%) (Table 2). Following identical procedures, N^{α}-Fmoc-4-methylproline **8b** (mixture of four stereoisomers) and N^{α}-Fmoc-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid **8e** were prepared in good purities and yields (Table 2) by using, respectively, (±)-1-bromo-3-chloro-2-methylpropane and α,α′-dichloro-*o*-xylene as the alkylating agents, and 10% DIEA in NMP for 24 h at 25°C for the cyclization.

1-Aminocycloalkanecarboxylic acid spiro derivatives **10** can also be synthesized starting from intermediate **2**, by an intramolecular C^{α}-alkylation (Scheme 1).[8] These conformationally constrained α-amino acid derivatives were prepared from the ω-chloro derivatives (BTPP, 10 equiv., NMP, 85°C, 24 h). Hydrolysis of **9**, followed by acylation with Fmoc-Cl, and TFA cleavage provided N^{α}-Fmoc protected 1-aminocyclopropane-1-carboxylic acid ($n=2$, **10a**), and homologs ($n=4$, **10b**, and 2-aminoindan-2-carboxylic acid, **10c**[9]) with crude HPLC purities ranging from 72 to 100%, and purified yields from 29 to 65% (Table 2).

In summary, alkylation of the benzophenone imine of glycine Wang resin with α,ω-dihaloalkanes gives access to key ω-halo intermediates. These intermediates were transformed on-resin to α-monosubstituted amino acids containing diverse side-chain functionalities, proline and its ring homologs, and 1-aminocycloalkanecarboxylic acid derivatives. In addition, the incorporation of the ω-haloalkanes **3** and **4** into peptides could provide potential alkylating agents for applications such as affinity labeling[10] and intramolecular cyclization.[11]

Experimental

Preparation of the benzophenone imine of Gly-Wang-resin (1). In a 5 mL syringe,[12] Fmoc-Gly-Wang-resin (0.2 g, 1.0 mmol/g) was washed with CH_2Cl_2 and DMF (2×1 min each), and then treated with piperidine–DMF (1:4, 3×5 min), followed by washings with DMF (6×0.5 min). Benzophenone imine (336 μL, 10 equiv.) in NMP (2 mL) was added to the resin, followed by the addition of glacial acetic acid (100 μL, 8.7 equiv.), and the reaction was allowed to proceed for 24 h with rotation. The resultant resin was washed with NMP and THF (4×0.5 min each).

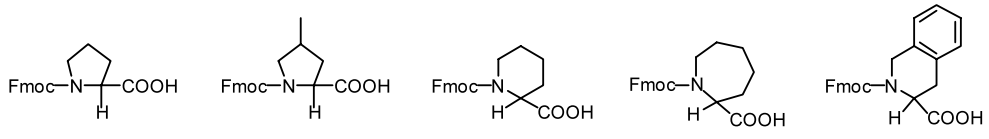
Alkylation of resin 1 with an α,ω-dihaloalkane. Resin-bound Schiff base **1** (200 μmol) was washed with NMP (2×0.5 min). The α,ω-dihaloalkane (10 equiv.) in NMP (2 mL) and BTPP (610 μL, 10 equiv.) were added, and the suspension was rotated for 24 h at 25°C. The resin was washed with NMP and CH_2Cl_2 (4×0.5 min each).

Nucleophilic displacement of the halide. The resin bound imine **2** (200 μmol) was swollen with NMP (2×0.5 min). Bu_4NI (925 mg, 10 equiv.) and Bu_4NCN (670 mg, 10 equiv.) were individually dissolved in NMP (1.5 mL each), combined, and added to the resin. The reaction mixture was rotated for 24 h at 25°C. The resulting resin **5** was washed with NMP and CH_2Cl_2 (6×0.5 min each).

Hydrolysis of the imine. Resin-bound imine **2** or **5** (200 μmol) was washed with THF (6×0.5 min). THF–1N HCl aqueous (2:1, 4 mL) was added, and the suspension was rotated for 4 h at 25°C. The resin was washed with THF and CH_2Cl_2 (4×0.5 min each).

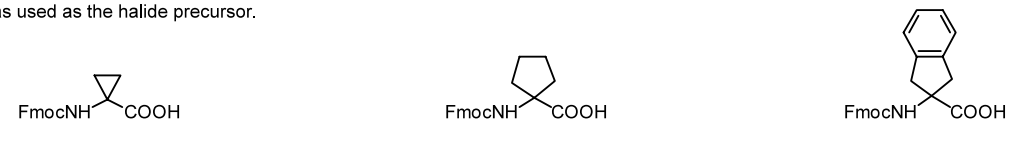
Acylation of resin-bound product with Fmoc-Cl. Resin-bound amine **3** (200 μmol) was washed with NMP

Table 2. Products from conversion of intermediates **2** to N^{α}-protected Fmoc proline, its ring homologs, and 1-aminocycloalkanecarboxylic acid derivatives



	8a	**8b**	**8c**	**8d**[a]	**8e**
Crude HPLC Purity	98%	98%	94%	88%	95%
Purified Yield	68%	77%	73%	55%	72%

[a]The bromide was used as the halide precursor.



	10a	**10b**	**10c**
Crude HPLC Purity	100%	73%	72%
Purified Yield	65%	58%	29%

(4×0.5 min). Fmoc-Cl (516 mg, 10 equiv.) was dissolved in NMP (2 mL), added to the resin, and acylation was started by addition of DIEA (680 μL, 20 equiv.). Reaction mixture was rotated for 24 h at 25°C. The resin was washed with NMP, THF, and CH_2Cl_2 (4×0.5 min each).

Cleavage and purification. The resin was cleaved with TFA–Et_3SiH (95:5, 1×2 h, 1×30 min). Filtrates were collected, combined with TFA–CH_2Cl_2 washes (1:3, 2×2 min) of the resin, and evaporated. Crude products were purified over silica gel with $CHCl_3$–THF–HOAc (92:8:1).

6-Chloro-2-[[(9*H*-fluoren-9-ylmethoxy)carbonyl] amino]-hexanoic acid (4c). Using 1-bromo-4-chlorobutane (230 μL, 10 equiv.) in the alkylation step. ^{1}H NMR (CD_3OD) δ 1.48–1.66 (m, 2H), 1.66–2.00 (m, 4H), 3.61 (t, $J=6.6$ Hz, 2H), 4.14–4.24 (m, 1H), 4.27 (t, $J=6.6$ Hz, 1H), 4.40 (d, $J=6.6$ Hz, 2H), 7.30–7.48 (m, 4H), 7.64–7.76 (m, 2H), 7.84 (d, $J=7.2$ Hz, 2H); ^{13}C NMR (CD_3OD) δ 24.3, 32.0, 33.2, 45.4, 48.6, 55.2, 67.9, 120.9, 126.2, 128.1, 128.7, 142.6, 145.2, 145.4, 158.7, 175.9.

6-Cyano-2-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]-hexanoic acid (6c). Using 1-bromo-4-chlorobutane (230 μL, 10 equiv.) in the alkylation step. ^{1}H NMR (CD_3OD) δ 1.50–1.65 (m, 2H), 1.65–1.85 (m, 3H), 1.85–2.00 (m, 1H), 2.49 (t, $J=6.9$ Hz, 2H), 4.15–4.25 (m, 1H), 4.27 (t, $J=7.5$ Hz, 1H), 4.41 (d, $J=7.5$ Hz, 2H), 7.30–7.52 (m, 4H), 7.62–7.78 (m, 2H), 7.84 (d, $J=7.5$ Hz, 2H); ^{13}C NMR (CD_3OD) δ 17.2, 26.0, 26.1, 31.9, 48.8, 55.1, 67.9, 120.9, 121.0, 126.3, 128.2, 128.8, 142.6, 145.2, 145.3, 158.7, 175.7.

Alternative synthetic procedure for proline derivatives

Intramolecular cyclization of the resin-bound alkylated products. The resin-bound amine **3** (200 μmol) was washed with NMP (4×0.5 min). 10% DIEA in NMP (4 mL) was added to the resin and the reaction mixture was rotated for 24 h at 25°C. The resin was washed with NMP and CH_2Cl_2 (4×0.5 min each).

1-(9*H*-Fluoren-9-ylmethyl) hydrogen 1,2-piperidine dicarboxylate (8c). Using 1-bromo-4-chlorobutane (230 μL, 10 equiv.) in the alkylation step. ^{1}H NMR (CD_3OD, 3:2 mixture of two rotamers) δ 1.24–1.54 (m, 2H), 1.54–1.82 (m, 3H), 2.16–2.36 (m, 1H), 2.94–3.20 (m, 1H), 3.88–4.10 (m, 1H), 4.22–4.36 (m, 1H), 4.36–4.52 (m, 2H), 4.68 (d, $J=5.1$ Hz, 0.4 H), 4.83 (d, $J=5.1$ Hz, 0.6 H), 7.28–7.50 (m, 4H), 7.56–7.72 (m, 2H), 7.84 (m, 2H); ^{13}C NMR (CD_3OD, mixture of two rotamers) δ 21.7, 25.7, 25.8, 27.8, 42.8. 43.0, 48.6, 55.6, 55.8, 68.8, 120.9, 126.1, 128.2, 128.8, 142.6, 145.2, 145.2, 145.3, 145.4, 157.8, 158.1, 174.6.

Alternative synthetic procedure for spiro derivatives

Intramolecular cyclization by C^{α}-alkylation of the resin-bound alkylated products. Resin **2** (200 μmol) was washed with NMP (2×0.5 min). Spiro formation was carried out in a glass vessel by adding BTPP (612 μL, 10 equiv.) in NMP (2 mL) to the resin. Reaction mixture was heated at 85°C for 24 h with occasional stirring. Resin was washed with NMP and CH_2Cl_2 (4×0.5 min each).

1-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]cyclo propanecarboxylic acid (10a). Using 1-bromo-2-chloroethane (166 μL, 10 equiv.) in the alkylation step. ^{1}H NMR (CD_3OD+$CDCl_3$, 3:1 mixture of two rotamers) δ 0.94–1.04 (m, 0.5H), 1.08–1.22 (m, 1.5H), 1.34–1.44 (m, 0.5H), 1.44–1.58 (m, 1.5H), 4.26 (t, $J=6.9$ Hz, 1H), 4.37 (d, $J=6.6$ Hz, 1.5H), 4.46 (d, $J=6.0$ Hz, 0.5H), 7.26–7.48 (m, 4H), 7.69 (d, $J=7.5$ Hz, 2H), 7.81 (d, $J=7.5$ Hz, 2H); ^{13}C NMR (CD_3OD+$CDCl_3$, mixture of two rotamers) δ 17.9, 34.8, 48.3, 67.8, 120.8, 126.1, 128.0, 128.6, 142.4, 145.1, 159.1, 176.7.

Acknowledgements

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