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Solid-phase synthesis of amino amides and peptide amides with unnatural side chains

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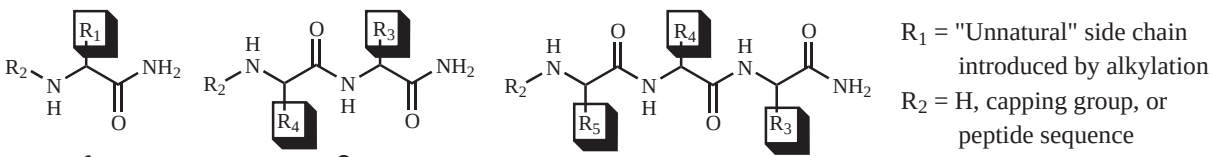
Abstract—Unnatural amino amides and peptide amides can be synthesized, using solid-phase chemistry, from glycine attached directly (or through an intervening peptide sequence) to a Rink resin. The glycine is converted to an activated benzophenone imine derivative, followed by *C*-alkylation and hydrolysis. This sequence provides a mild, high yielding route to Rink resin-bound racemic, unnatural, amino amides and di- and tripeptide amides. Cleavage with trifluoroacetic acid provides the final amide products in good yield and purity.

1. Introduction

We recently reported solid-phase routes to unnatural amino acids and peptides through on-resin alkylation, dialkylation, Michael and cation based chemistry.[1] We call this methodology 'UPS' for 'unnatural peptide synthesis'. Since classic peptide chemistry also produces primary amide derivatives of amino acids and peptides[2,3] by solid-phase syntheses (using Rink or equivalent resins), we decided to see if we could transfer our alkylation methodology to Rink based resins and obtain the corresponding unnatural amino and peptide amides (e.g. **1**–**3**). This paper describes the successful adaptation of our UPS methodology to the preparation of α-monosubstituted amino amides **1**, and peptide amides **2** and **3**, by simple solid-phase alkylation chemistry.[4]

Scheme 1 shows our route to monosubstituted amino amides and peptide amides. The chemistry outlined includes our basic UPS sequence of imine activation, alkylation and imine hydrolysis. This is followed by *N*-acylation and cleavage from the resin. We first prepared the model starting resin-bound imines **5a** (Gly: $n=0$), **5b** (Gly-Phe: $n=1$, R = benzyl), **5c** (Gly-Ala: $n=1$, R = methyl), **5d** (Gly-Phe-Ala: $n=2$, R = benzyl, methyl) and **5e** (Gly-Ala-Phe: $n=2$, R = methyl, benzyl). From Rink resin the peptide derivatives **4** were prepared in standard fashion, and activated to imines **5a**–**e**. Alkylations were probed with two alkylating agents of different reactivity, *p*-methyl benzyl bromide and the less reactive ethyl iodide. A neutral, non-ionic Schwesinger base, BEMP,[5] was again found to be effective in the alkylation reactions. After alkylation the imine was hydrolyzed under mild acid conditions to minimize cleavage from the resin. The resulting amine was acylated with the UV active Fmoc group to permit easy quantitation of starting material, product, and amine containing by-products. To produce internal unnatural residues, conventional peptide chemistry was



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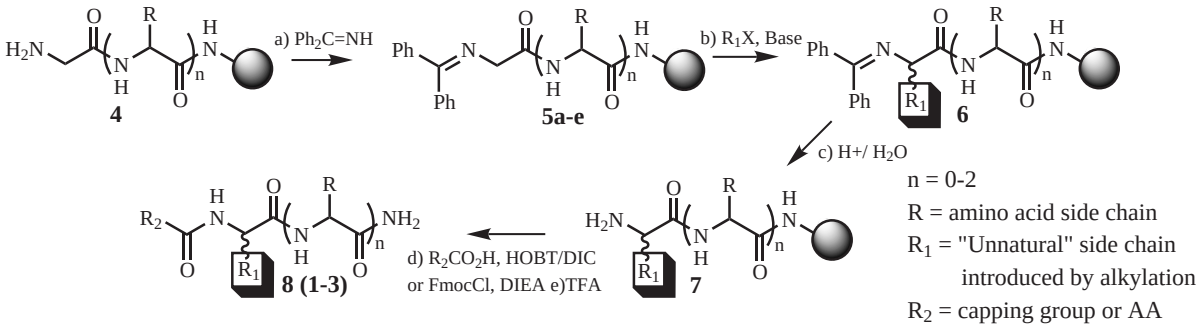


Scheme 1. Synthesis of mono-substituted amino amides and peptide amides (**8**).

performed on the free amine prior to capping. In exploratory work to **1** it was found that acylation with 2-naphthoic acid or quinaldic acid, followed by TFA cleavage from the resin, led to variable amounts (5–15%) of carboxylic acid by-product. In contrast, when an Fmoc protecting group was introduced the final product amides showed no detectable levels of acid. Therefore, for all subsequent experiments, the Fmoc group was used as the capping and UV reporter group R_2. Twenty four simultaneous reactions were carried out in a Billboard[6] apparatus. The structures of the products obtained are shown in Table 1.

This run produced two amino amides (**1a** and **1b**), eight dipeptide amides (**2a–d**, **2f–i**) and twelve tripeptide amides (**3a–l**). In this set there are examples of unnatural side chains at either the N-terminal, internal, or C-terminal position. Two control peptides (**2e** and **2j**) were also prepared. Data on crude yield and HPLC purity, xx (yy), are given under each compound. There was variable asymmetric induction in alkylations of derivatives with one or more pre-existing stereocenters, and purities are reported based on the combined diastereomeric products.[7] The alkylations and acylations to **1a** ($R_1 = p$-CH_3PhCH_2-, R_2 = Fmoc) and **1b** (R_1 = Et-, R_2 = Fmoc) proceeded in excellent overall yield and purity, 97% (98% purity by HPLC) and 93% (99% purity by HPLC), respectively. For all the other compounds shown in the table, crude yields were from 81 to 100% (average yield 95%). The HPLC purity of these products ranged from 56 to 99% (average purity 85%).

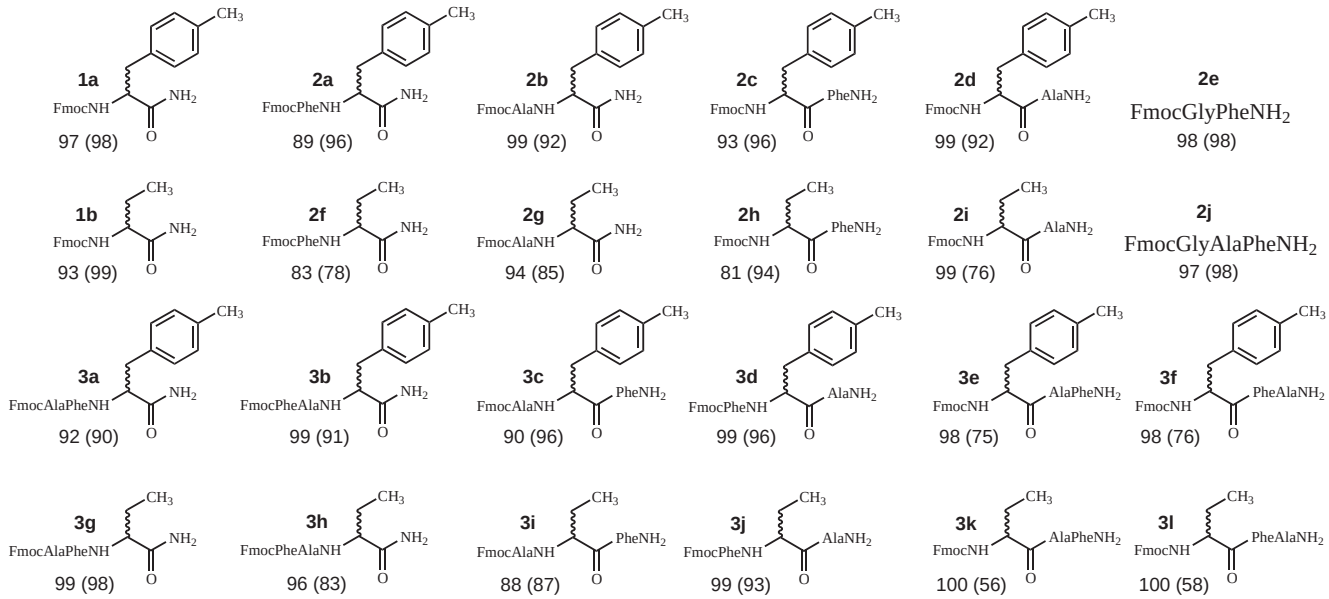
Using the mild procedures described in this manuscript (experimental given below) it is now possible to conduct the solid phase synthesis of α-monosubstituted unnatural amino amides (**1**) and peptide amides (**2** and **3**). The unnatural amino acid derivatives from this alkylation procedure can reside at the N-terminal amino acid residue, internal sites, or the C-terminal amide position.

2. Experimental

2.1. Preparation of the benzophenone imine of Gly-Axx-NH-Rink-resin

Fmoc-Gly-Axx-NH-MBHA-resin (prepared following standard Fmoc protocols from 1.0 g, 0.62 mmol, of commercially available Fmoc-Rink amide-MBHA-

Table 1. Mono-, di- and tripeptides from a single run with Rink-amide UPS chemistry



resin) was wetted with CH_2Cl_2 (10 mL) and drained. Piperidine (30% in NMP, 10 mL) was added, mixed briefly, and then drained. An additional 10 mL of piperidine (30% in NMP) was added and the resulting slurry was mixed by rotation for 30 min. The resin was filtered and washed with NMP, CH_2Cl_2, then NMP (3×10 mL each). Benzophenone imine (1.12 g, 6.2 mmol, 10 equiv.) in NMP (10 mL) was added to the resin, followed by glacial acetic acid (306 μL, 323 mg, 5.39 mmol, 8.7 equiv.) and the suspension was mixed by rotation for 18 h at room temperature. The resin was filtered and washed with NMP, THF, THF:H_2O (3:1), THF and then CH_2Cl_2 (3×10 mL each) and dried in vacuo (rt, overnight).

2.2. Monoalkylation of the benzophenone imine of Gly-Axx-NH-Rink-resin

The benzophenone imine of Gly-Axx-MBHA-Rink-resin (50 μmol) was washed with CH_2Cl_2 and then NMP (3×1.5–2 mL each). NMP (0.5 mL) was added to the resin, followed by a 1 M solution of RX (500 μL, 10 equiv.) and a 1 M solution of BEMP (500 μL, 10 equiv.) and the slurry was mixed by rotation for 24 h at room temperature. The resin was filtered and washed with NMP and then CH_2Cl_2 (3×1.5–2 mL each).

2.3. Hydrolysis of the imine

The resin-bound imine (50 μmol) was washed with THF and then THF/H_2O (3:1) (3×1.5–2 mL each). 1N $NH_2OH{\cdot}HCl$/THF (1:2) (1.5–2 mL) was added to the resin and the suspension was mixed by rotation for 5 h at room temperature.[8] The reaction mixture was filtered and washed with NMP (5×1.5–2 mL) and then neutralized with 10% DIEA/NMP (3×1.5–2 mL). This was followed by washing with NMP (10×1.5–2 mL).

2.4. Acylation of monosubstituted amino amides on MBHA Rink resin

To the resin-bound amine (50 μmol), was added NMP (1.25 mL) followed by a 1 M solution of FmocCl (250 μL, 5 equiv.) and DIEA (44 μL, 5 equiv.). The suspension was mixed by rotation for 18 h at room temperature. The reaction mixture was filtered and washed with NMP, THF, and then CH_2Cl_2 (3×1.5–2 mL each).

2.5. Product cleavage from MBHA Rink resin

To the resin (50 μmol) was added 95% TFA/H_2O (1.5–2 mL) and the reaction was mixed by rocking for 2 h at room temperature. After filtering cleaved product into a tared vial, the resin was rinsed with TFA/H_2O and then CH_2Cl_2 (3×1.5–2 mL each). The solvents were evaporated under a stream of argon and then the residue was dried in a vacuum oven at room temperature for several hours.

Representative ^1H NMR spectra (300 MHz, $CDCl_3$+ DMSO-d_6, δ): **1b**: 0.95 (t, 3H, $J=7.4$ Hz), 1.61–1.74 (m, 1H), 1.89 (ap. sext, 1H, $J=7.4$ Hz), 4.13–4.16 (m, 1H), 4.20 (t, 1H, $J=6.6$ Hz), 4.44–4.47 (m, 2H), 5.40 (d, 1H, $J=7.4$ Hz), 6.27 (s, 2H), 7.31 (ap. t, 2H, $J=7.4$ Hz), 7.41 (ap. t, 2H, $J=7.4$ Hz), 7.58 (d, 2H, $J=7.4$ Hz), 7.78 (d, 2H, $J=7.4$ Hz). **2c**: 2.26 (s, 3H), 2.77–3.12 (m, 4H), 4.10–4.21 (m, 2H), 4.29–4.39 (m, 2H), 4.62 (q, 1H, $J=7.3$ Hz), 6.25 (s, 2H), 6.63 (d, 1H, $J=8.1$ Hz), 6.79 (s, 1H), 7.00–7.06 (m, 4H), 7.12–7.23 (m, 5H), 7.28 (ap. t, 2H, $J=7.4$ Hz), 7.38 (ap. t, 2H, $J=7.4$ Hz), 7.52–7.63 (m, 2H), 7.75 (d, 2H, $J=7.4$ Hz).

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7. The diastereoselectivity of the alkylation of substrates containing a pre-existing stereocenter was not the focus of this study. However, in some cases varying levels of stereoselectivity were observed, primarily in the NMR spectra. For example, compounds **3j** and **3e** showed a doubling of the methyl triplets between δ 0.5 and 1.0, implying a diastereoselectivity of 2:1 and 8:1, respectively.
8. It is preferable to hydrolyze the imine with hydroxylamine hydrochloride rather than aqueous HCl. Premature hydrolysis from the Rink resin can occur if more acidic conditions are used. However, for several of the tripeptide amides (**3e**, **3f**, **3k**, and **3l**), hydrolysis was not complete with the hydroxylamine reagent. As a result, the product, after final cleavage from the resin, was contaminated with benzophenone and uncapped terminal amine. A repeat of the reaction to give product **3k**, using 1N aqueous HCl in THF (1:2) for 5 h at ambient temperature, resulted in complete removal of the benzophenone imine. The product was obtained in slightly lower yield (87%), but with excellent HPLC (95%) purity.