Solid-Phase Unnatural Peptide Synthesis (UPS)

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Solid-phase peptide synthesis (SPPS) has evolved into a powerful synthetic method since it was first reported in 1963.[1,2] It is considered the method of choice for the automated preparation of peptides in numerous laboratories around the world. SPPS is now being used to rapidly synthesize peptides in a combinatorial fashion.[3] Peptide libraries constructed from naturally occurring amino acids have been widely used to screen for biological activity. However, increased activity, bioavailability, and degradative resistance often require incorporation of unnatural residues in the peptide framework.[4] Currently, most synthetic routes to modified peptides incorporate the individually prepared unnatural amino acid residue[5] by normal peptide synthesis.

A more direct and flexible method for the preparation of peptides containing modified amino acid residues would be to build the new side chains onto the growing peptide chain during the peptide synthesis.[6] One major obstacle must be overcome to practically realize this goal: a mild methodology of carbon−carbon bond formation on solid phase is needed to *selectively* introduce the side chain at the α-carbon of a particular residue in the growing peptide chain. To accomplish this we have adapted the solution phase chemistry employed in the selective $C_\alpha-C_\beta$ bond formation reaction of Schiff base activated amino acids[7] and peptides[8] to a new methodology: "solid-phase unnatural peptide synthesis" or solid-phase UPS (Figure 1).

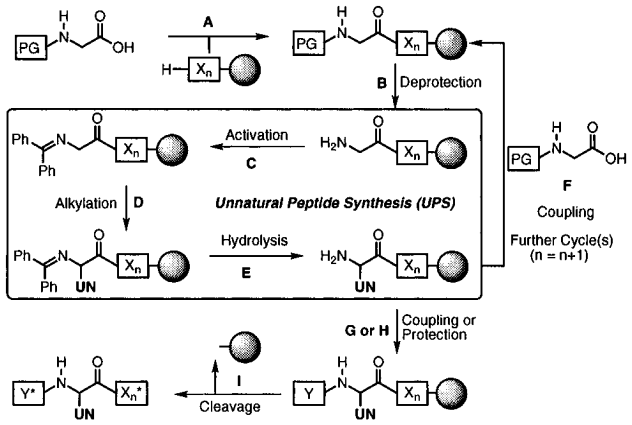

Figure 1. General scheme for solid-phase unnatural peptide synthesis (UPS). Abbreviations: shaded circle = resin (Merrifield, Wang,...); X_n = O, NH, peptide, etc.; PG = protecting group; UN = unnatural side chain (an electrophile, such as an alkyl halide UN−X, is used in the alkylation step); Y = natural amino acid or peptide or N-protected terminus; Y* = resin-free N-terminal end; X_n* = resin-free C-terminal end. For experimental conditions, see ref 9.

In this procedure,[9] three new steps (Figure 1, reactions in box), activation (C), alkylation (D), and imine hydrolysis of the resin-bound Schiff base (E), have been added to the normal SPPS sequence involving deprotection (B) and coupling (F or G) steps. Following step E in the loop a number of options are possible: repeat the steps in the UPS to add another unnatural residue to the growing peptide chain (step F), couple a natural amino acid or other residue via normal solid-phase methodology (step G), or protect the free amino group subsequent to other reactions on the resin-bound product (step H). Finally, the product is cleaved from the resin (step I).

To simplify analysis in the initial resin-bound alkylations, a known dipeptide [(D,L)-Phe−Leu] was constructed by backbone alkylation of a Gly−Leu derivative (Figure 2). The benzophenone imine of Gly−Leu−Merrifield resin (**1**) was conveniently prepared from TFA·H_2N−Gly−Leu−resin by reaction with benzophenone imine.[10,11] After unsuccessful attempts to effect benzylation of **1** with melted KOH/K_2CO_3 (incomplete conversion)[8] or strong ionic bases (racemization of peptides),[12] simultaneous deprotonation and alkylation of **1** with benzyl bromide was accomplished using the organic-soluble, nonionic

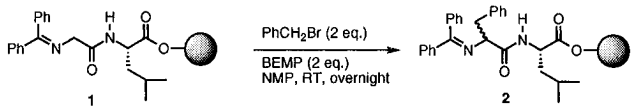

Figure 2. Alkylation step in solid-phase UPS.

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(9) Reaction conditions: **C**, (a) TFA·H_2N−Gly−Merrifield resin, Ph_2C=NH (1.5 equiv)/1-*N*-methyl-2-pyrrolidinone (NMP), overnight; (b) H_2N−Gly−Wang resin, Ph_2C=NH (1.5 equiv)/AcOH (1.3 equiv)/NMP, overnight. **D**, UN−X (2.0 equiv)/2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) (2.0 equiv)/NMP, overnight. **E**, (a) Merrifield resin, 1 N aqueous HCl/THF (3/7), overnight, then DIEA (10%)/NMP; (b) Wang resin, 1 N aqueous NH_2OH·HCl/THF (3/7), 5 h, then DIEA (10%)/NMP. **H**, (a) Merrifield resin, Boc_2O (3.0 equiv)/NMP or CbzOSu (3.0 equiv)/NMP; (b) Merrifield or Wang resin, FmocCl (3.0 equiv)/DIEA (6.0 equiv)/NMP. **I**, (a) Merrifield or Wang resin, Ti(OEt)$_4$ (5.0 equiv)/allyl alcohol, 120 °C, pressure tube, 2 h; (b) only for Wang resin, TFA (95%)/H_2O, 5 h.

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(12) Solution-phase model alkylation (solution UPS) of the dipeptide Schiff base ester Ph_2C=NCH_2CO−Phe−OMe with 4-bromobenzyl bromide showed that 15−68% racemization of the pre-existing chiral center was observed with potassium *tert*-butoxide, 9-lithium fluorene, or lithium 2,6-di-*tert*-butyl-4-methylphenoxide.

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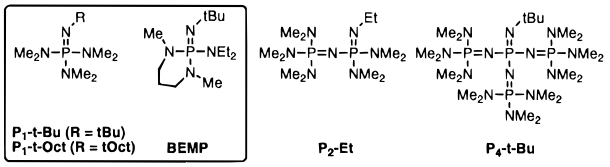

Figure 3. Organic-soluble, nonionic iminophosphorane bases.

bases P_1-*t*-Bu, P_1-*t*-Oct, ("Schwesinger bases"),[13] or BEMP (Figure 3) to yield the benzophenone imine of (D,L)-Phe−Leu−Merrifield resin (**2**).

Compound **2** was hydrolyzed (aqueous HCl, THF) and then neutralized to afford the free amino group, which was N-acylated with Fmoc-Cl. Titanate-mediated transesterification[14] conveniently cleaved the product from the resin to yield the O-allyl ester,[15] Fmoc-(D,L)-Phe−Leu−Oallyl (**3**), in 55% overall crude yield (54% purified yield).

The preparation of unnatural amino acids was demonstrated by the synthesis of **4a**−**c** (Figure 4). Thus, TFA·H_2N−Gly−Merrifield resin was activated as its benzophenone imine and alkylated to give the α-alkylated product. Hydrolysis and N-acylation (Boc_2O, Cbz-OSu or Fmoc-Cl), followed by titanate-mediated transesterification gave the O-allyl esters of the N-Boc, N-Cbz-, or N-Fmoc-protected unnatural amino acids (**4a**−**c**) in 42−54% overall yield.[16]

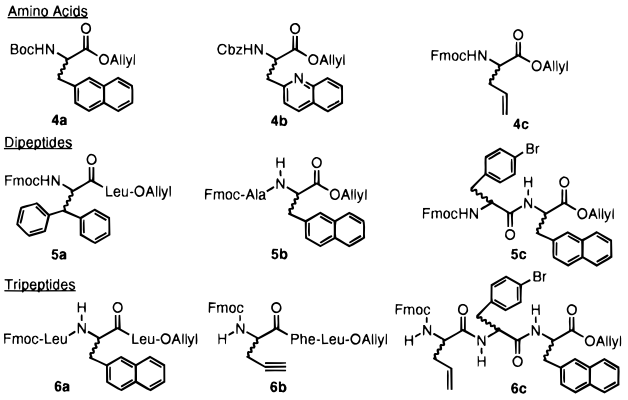

Figure 4. Preparation of amino acid derivatives (**4**) by solid-phase amino acid synthesis and di- and tripeptides (**5** and **6**, respectively) by solid-phase UPS.

Using this methodology (in conjunction with final Fmoc derivatization), dipeptides **5a** and **5b**, in which the unnatural residue was created as either the C- or N-terminal residue, were prepared. Similarly, tripeptides containing unnatural residues added during the second or third "loop" of the synthesis are represented by compounds **6a** and **6b**. It is also possible to prepare completely unnatural polypeptides (**5c** and **6c**) (Figure 4). The overall isolated yields for these products ranged from 37−55% based on the starting Boc-Gly−resin or Boc-Leu−resin.[16]

Solid-phase UPS can also be used with Wang resins and Fmoc-based SPPS methodology. Because of the acid sensitivity of the Wang resin linkage, it was necessary to develop methods for introduction and removal of the Schiff base group under essentially neutral conditions. The activation step is accomplished by standard piperidine cleavage of the N-terminal Fmoc group followed by reaction of the free amine with benzophenone imine (1.5 equiv) in the presence of acetic acid (1.3 equiv). Following the normal alkylation reaction, the imine is hydrolyzed (aqueous NH_2OH·HCl, THF, pH ≅ 6) and then neutralized (iPr_2NEt) to the free amino group,[9] which can be subjected to the various available options. For example, Fmoc-(D,L)-β-naphthylalanine allyl ester (**4d**) was prepared in 80% overall purified yield (83% crude yield) starting from Fmoc-Gly−Wang resin. Additionally, the free model dipeptide (D,L)-Phe−Leu (**7**) was obtained by UPS from Gly−Leu−Wang resin in 60% overall yield[17] using TFA to cleave the product from the resin.

The use of mild reagents and room temperature reactions in solid-phase UPS chemistry allows for the easy automation of this technique. In this regard, preliminary results are very encouraging. Thus, the dipeptide (D,L)-Phe−Leu (**7**) and a series of Phe-substituted Phe−Leu derivatives have been prepared from Fmoc-Leu−Wang resin (*via* the Gly−Leu−Wang resin intermediate). The reactions were performed on an automatic peptide synthesizer[18] using a variety of benzylic halides.

These preliminary studies demonstrate that UPS can be utilized to convert resin-bound amino acids or peptides to unnatural derivatives. The mild reagents and conditions used are compatible with both Boc- and Fmoc-based SPPS strategies, and the technique has been automated. A variety of structurally diverse combinatorial libraries[19] derived from unnatural amino acids or unnatural peptides are now accessible using this technique. We are currently exploring side chain compatibility, incorporation of α,α-disubstituted residues, and stereoselective UPS.

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Supporting Information Available: Experimental procedures for solid and solution phase UPS and ^{1}H-NMR spectra and mass spectra data for all new compounds (36 pages). Ordering information is given on any current masthead page.

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(13) No racemization was observed with P_1-*t*-Bu, P_1-*t*-Oct, or BEMP. In contrast, P_2-Et and P_4-*t*-Bu (Figure 3) resulted in overalkylation at the amide bond and extensive racemization. For other uses of "Schwesinger bases", see: Schwesinger, R.; Willaredt, J.; Schlemper, H.; Keller, M.; Schmidt, D.; Fritz, H. *Chem. Ber.* **1994**, *127*, 2435−2454.

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(16) Overall yields of amino acids from resin-bound reactions (crude yield, isolated yield): **4a**, 55%, 54%; **4b**, 50%, 48%; **4c**, 45%, 42%. Overall yields of di- and tripeptides from resin-bound reactions (crude yield, isolated yield): **5a**, 47%, 42%; **5b**, 55%, 53%; **5c**, 40%, 37%; **6a**, 67%, 56%; **6b**, 46%, 40%; **6c**, 50%, 43%. All products gave satisfactory TLC, ^{1}H-NMR, and HRMS.

(17) LC-MS analysis of crude product (D,L)-Phe−Leu **7** showed a 1:1 mixture of (D)-Phe−Leu and (L)-Phe−Leu [>80%, molecular ion: 279.2 (M+1)] containing (D,L)-Phe−Leu−Leu [molecular ion: 392.3 (M + 1), also found in an independently prepared sample of Phe−Leu from Leu−Wang resin by normal SPPS]. Elemental analysis of crude product **7**. Calcd for CF_3CO_2H·(D,L)-Phe−Leu: C, 52.04; H, 5.91; N, 7.14. Found: C, 49.90; H, 5.81; N, 7.04. Amino acid analysis: Phe/Gly = 97/3.

(18) The dipeptides **7a**−**f** were prepared (from benzyl bromide or the *o*-, *m*-, or *p*-methyl-, *p*-trifluoromethyl-, or *p*-nitrobenzyl bromides) on an Advanced ChemTech 357 automatic peptide synthesizer using conditions similar to those developed earlier. The products were identified by LC-MS and ^{1}H-NMR.

(19) Hydantoins **8** were prepared in 32−50% yields by solid-phase UPS followed by the Parke-Davis cyclization methodology, see: Dewitt, S. H.; Kiely, J. S.; Stankovic, C. J.; Schroeder, M. C.; Reynolds Cody, D. M.; Pavia, M. R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6909−6913.