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The Solid Phase Synthesis of α,α-Disubstituted Unnatural Amino Acids and Peptides (di-UPS)

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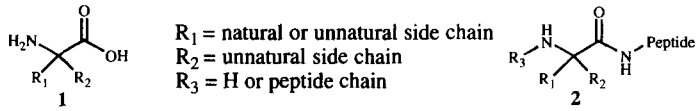
Abstract: This paper reports a new, mild procedure ("di-UPS") for the solid phase synthesis of racemic α,α-disubstituted amino acids (**1**) and epimeric α,α-disubstituted terminal amino acid residues (**2**, R_3=H) in a resin bound peptide. The synthetic route is compatible with most protected amino acid side chains and can be used in a continuing solid phase synthesis. Di-UPS should find wide applicability in the design and solid phase synthesis of hybrid amino acids and peptides, and the construction of basis units for combinatorial chemistry.

α,α-Disubstituted amino acid residues (**1**) play a critical role in determining the conformation, metabolic stability and biological activity of peptides. The disubstituted monomers are normally prepared by a variety of solution phase techniques before incorporation into a peptide.[1,2] This paper describes the preparation of racemic α,α–disubstituted amino acids (**1**), and epimeric peptides (**2**) by simple solid phase alkylation chemistry, termed "di-UPS" (for di-substituted unnatural peptide synthesis).



1 **2**

Together with our recently reported manual and automated solid phase synthesis of α-alkylated unnatural amino acids and peptides (UPS), solid phase alkylation routes are now available to a wide range of unnaturally substituted amino acids and peptides.[3-5] The synthesis of resin bound **1** (**6**) and its conversion to unnatural α,α–disubstituted amino acid derivatives **7** is outlined in Figure 1.

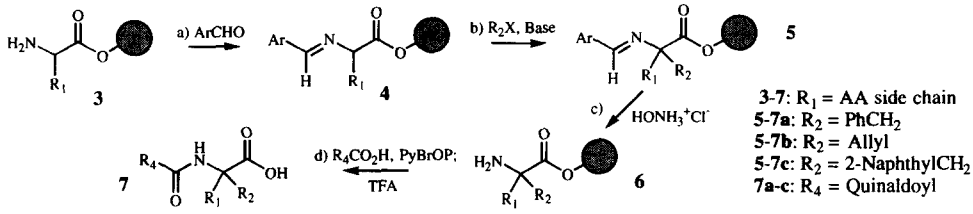


Figure 1. Synthesis of α,α–disubstituted amino acid derivatives by di-UPS. For product summary, see rows 1-3 in Table 1. For reaction conditions, see footnote 6.

Initial experiments explored the alkylation of phenylalanine with benzyl bromide to form the symmetrically disubstituted **7** (R_1, R_2 = -CH_2Ph). Several aromatic aldehydes were screened as candidates for the aldimine activating group in **4**:[7,8] benzaldehyde, 2- and 4-chlorobenzaldehyde; 3,4-, 3,5- and 2,5-dichlorobenzaldehyde; 2-naphthaldehyde, 4-nitrobenzaldehyde, mesitaldehyde, and 4-methoxybenzaldehyde.

These resin-bound Schiff base esters were then α-alkylated by di-UPS to give **5**, using BEMP[6] as base with benzyl bromide at room temperature. Transimination of **5** with hydroxylamine afforded the free amine **6**, which, for quantitation purposes, was converted to a UV active compound before being cleaved from the resin to give the α,α-disubstituted amino acid derivative **7** (R_1, R_2 = $-CH_2Ph$).[6] The best yield and purity of **7** was obtained with imine intermediate **4** formed from 3,4-dichlorobenzaldehyde, and this aldehyde was used in all the subsequent experiments.

We then examined the compatibility of twelve natural amino acid side chains (R_1 in **4**) with the alkylation conditions, using three different alkylating agents (benzyl bromide for **7a**, allyl bromide for **7b**, and 2-naphthylmethyl bromide for **7c**).[9] The qualitative results with these 36 combinations of R_1 and alkylating agents are summarized in the first three rows of Table 1.

4 or **8**: R_1* =	*Ala*	*Asp*	*Phe*	*Glu*	*Lys*	*Val*	*His*	*Trp*	*Tyr*	*Arg*	*Ser*	*Leu*
7a: R_2 = $-CH_2Ph$	A	A	A	B	A	D	D	B	A	D	B	A
7b: R_2 = -Allyl	A	A	B	A	B	D	D	C	A	D	B	A
7c: R_2 = $-CH_2$Naphth	A	A	A	B	A	D	D	C	A	D	B	A
9: R_2 = $-CH_2$Naphth	D	A[10]	A	D	B	D	B	A	A	D	B	A

Table 1. Qualitative results for the preparation of disubstituted amino acid derivatives **7** and tetrapeptides **9**. Key: A = Yield >60%, product purity >80%; B = Yield >60%, product purity 70-80%; C = Yield 40-60%, purity 70-80%; D = Several products and/or starting material (see text). *Three letter amino acid abbreviation used to identify side chain R_1 in **4** or **8** (e.g. "Ala" means R_1 = Me).

In all cases except valine, complete alkylation was obtained. This was confirmed by comparison of HPLC and mass spectra of quinaldolylated products (**7**, R_2 = alkyl group) with those of separately prepared quinaldolylated starting materials (**7**, R_2 = H). The side-chain protecting groups Asp(OtBu), Glu(OtBu), Lys(Boc), Trp(Boc), Tyr(OtBu), and Ser(OtBu) were compatible with alkylation, hydrolysis and coupling conditions. However, His(Trt) and Arg(Tos) gave mixtures of the desired product and overalkylated compounds.[12,13]

Next the α–alkylation was performed on terminal amino acid residues already incorporated in a resin bound peptide. The model tetrapeptide analogues **9** were prepared from **8**, using the di-UPS procedure and 2-naphthylmethyl bromide as the alkylating agent (Figure 2).[14] Qualitative results are summarized in row four of Table 1. The alkylation conditions were compatible with the pre-existing peptide components. Typically,

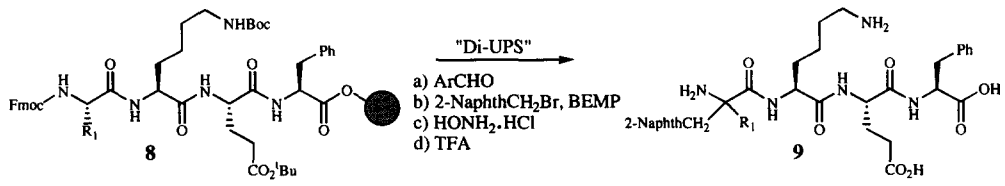


Figure 2. Synthesis of N-terminal α,α–disubstituted tetrapeptides by di-UPS. For product summary, see row four of Table 1. For reaction conditions, see footnote 14.

alkylations were complete and paralleled those obtained in the preparation of the disubstituted amino acid derivatives. As expected, N-terminal His(Trt) and Arg(Tos) gave mixtures of the corresponding mono-, di- and/or trialkylated tetrapeptides. Surprisingly, a mixture was obtained starting from N-terminal alanine.[15] In conclusion, we have developed a new, mild procedure for the solid phase synthesis of α,α-disubstituted amino acids and the α-alkylation of terminal amino acid residues in a peptide. The technique is compatible with most protected amino acids and can be incorporated in a continuing solid phase synthesis.

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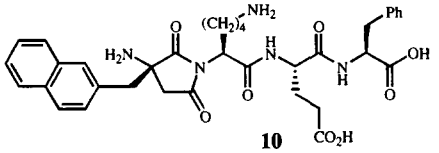
6. Fmoc-AA-Wang resins (100-200 mesh, 0.4-0.9 mmol/g) were used as starting materials. Reaction conditions (all at ambient temperature) for **3** to **7** were as follows: a) 3,4-dichlorobenzaldehyde (15 equiv), trimethyl

orthoformate (TMOF)/1-methyl-2-pyrrolidinone (NMP) (2:1), 12-15h; b) 2-[(1,1-dimethylethyl)imino]-N,N-diethyl-2,2,3,4,5,6-hexahydro-1,3-dimethyl-1,3,2-diazaphosphorin-2(1H)-amine (BEMP) (2.0 equiv)/R_2X (3.0 eq.)/NMP, 12-15 h; c) 1N aqueous $HONH_2$•HCl/THF (1:2), then diisopropylethyl amine (DIEA)/NMP; d) Quinaldic acid (10 equiv), bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBrOP) (10 equiv), DIEA (20 eq.)/NMP, 24 h; TFA (95%)/H_2O, 3 h. Yields are for the unpurified products. Product purity was determined by HPLC using a Vydac Protein and Peptide C18 column (150 x 4.6 mm) with mobile phases consisting of 0.1% (v/v) TFA in water (Solvent A) and 0.085% (v/v) TFA in acetonitrile (Solvent B), a flow rate of 1 mL/min., and a linear gradient from 0-80% solvent B over 20 min. Peaks were detected at 214 nm. Identity was confirmed by LC/MS, and selected products further characterized by HRMS.

7. Early solution-phase studies involving the preparation of α,α-dialkylamino acids by phase-transfer catalyzed (PTC) alkylation of aldimine esters involved use of the 4-chlorobenzaldehyde Schiff bases rather than benzaldehyde imines because the former were often crystalline solids (reference a) and were approximately an order of magnitude more acidic than the latter (reference b). More recently, these aldimine esters have also been used in the catalytic, enantioselective PTC alkylation reactions (reference c). (a) O'Donnell, M. J.; LeClef, B.; Rusterholz, D. B.; Ghosez, L.; Antoine, J. P.; Navarro, M. *Tetrahedron Lett* **1982**, *23*, 4259-4262. (b) O'Donnell, M. J.; Bennett, W. D.; Bruder, W. A.; Jacobsen, W. N.; Knuth, K.; LeClef, B.; Polt, R. L.; Bordwell, F. G.; Mrozack, S. R.; Cripe, T. A. *J. Am. Chem. Soc.* **1988**, *110*, 8520-5. (c) O'Donnell, M. J.; Wu, S. *Tetrahedron: Asymmetry* **1992**, *3*, 591-594.
8. (a) For preparation of resin-bound aldimines, see: Gordon, E. M.; Gallop, M. A.; Patel, D. V. *Acc. Chem. Res.* **1996**, *29*, 144-154. (b) Asymmetric monoalkylation of a Schiff base ester attached to a resin with pendant chirality has been reported. The substrate is bound to the resin by the imine bond and the reaction is conducted under anhydrous conditions (LDA, THF, -78 °C.). See: Calmes, M; Daunis, J.; Ismaili, H.; Jacquier, R.; Koudou, J.; Nkusi, G.; Zouanate, A. *Tetrahedron*, **1990**, *46*, 6021-6032.
9. A simple apparatus is used for manually conducting 24 parallel solid-phase reaction sequences. Reactions are done in fritted glass vials, on a 50-100 umolar scale, typically using 50-100 mg. of resin. The apparatus keeps the 24 separate reactions conveniently arranged for all the steps. It also allows easy cleavage and collection of products in 24 tared vials.
10. Alkylation of **8** (R_1 = CH_2CO_2tBu) provides an interesting example of unique structures available by di-UPS. After alkylation and cleavage, the aspartate side chain spontaneously closed to form the succinimide derivatives **10** and **11** as two separable diastereomers. Products **10** and **11** were separated by preparative HPLC.



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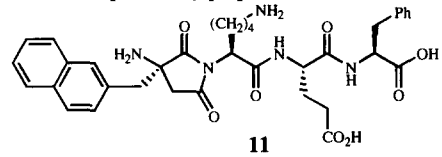

11

The overall purified yield from starting Fmoc-Phe-Wang resin to the 60:40 mixture of diastereomers was 43% (crude yield = 65%). To verify minimal epimerization during the di-UPS sequence the optical purity of the more readily epimerized Phe residue was determined. Starting material, crude product, and the individual diastereomers were subjected to amino acid hydrolysis conditions and analyzed for optical purity. All three samples gave identical ee's within experimental error (Footnote 11).

11. Determined by chiral HPLC, see: Imperiali, B.; Prins, T. J.; Fisher, S. L. *J. Org. Chem.* **1993**, *58*, 1613-1616. Phenylalanine cleaved directly from commercial Fmoc-Phe-Wang resin with 95% TFA/H_2O at ambient temperature gave 96% ee (L/D = 98/2). Phenylalanine from hydrolysis of crude tetrapeptides (**13** and **14**): 94% ee (L/D = 97/3) and from the purified diastereomeric mixture: 95% ee (L/D = 97.5/2.5).
12. A separate investigation showed that the desired monoalkylated product from Arg(Tos) was obtained in high purity by using shorter reaction times (< 8 h) and less base (BEMP $\leq$ 1.2 equiv).
13. In contrast to Ser(OtBu), no desired product was obtained starting from Cys(Trt), possibly due to elimination of tritylthiol anion from the corresponding Schiff base enolate.
14. Reaction conditions for **8** to **9**: after deprotection (20% piperidine/NMP/30 min) the reaction conditions and analyses were identical to those described in footnote 6.
15. In additional experiments, in which (4-NO_2)Phe, Met(O) or phenylglycine were introduced as the N-terminal residues of **8**, di-UPS again gave the desired α,α-dialkylated products **9** in good yield and purity.

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