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Improved Solid-Phase Peptide Synthesis Method Utilizing α-Azide-Protected Amino Acids

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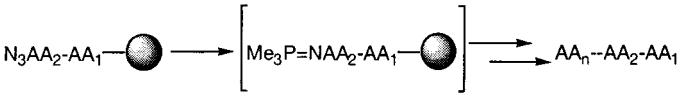
ABSTRACT



Pure α-azido acids were prepared using an efficient diazo transfer method followed by buffered workup. These building blocks were used to prepare small peptides on Wang resin by two approaches. Peptides prone to diketopiperazine formation were prepared in good yields by coupling acids to resin bound iminophosphoranes during Fmoc-Wang synthesis. The iminophosphoranes can also be hydrolyzed under neutral conditions to provide unprotected amines ready for further coupling.

Small peptides constitute a useful class of bioactive molecules despite efforts to find nonpeptide substrates for biological targets. In fact, analysis of the *Physicians' Desk Reference* reveals that there are currently several small peptides used for therapeutic indications in which no nonpeptide alternative treatments are available. While research quantities of larger peptides are generally obtained from natural sources or recombinant techniques, organic synthesis is the method of choice for smaller peptides and analogues containing nonnatural elements.[1]

Merrifield introduced solid-phase peptide synthesis (SPPS) in 1963 utilizing acid-labile protecting groups as well as a resin-substrate linker that was cleaved under strongly acidic conditions.[2] Similar work based on the 9-(fluorenylmethoxy)-carbonyl (Fmoc) protecting group in conjunction with the Wang linker has expanded the operation of peptide synthesis into the typical organic chemistry lab due to the mild conditions employed.[3] Although useful, lack of orthogonal protecting groups for resin-bound side chain manipulation and diketopiperazine (DKP) formation still present significant challenges for Fmoc-Wang-based SPPS.[4] A strategy to prevent DKP formation is known but not readily implemented due to the need for hindered linker preparation and *N*-protected amino acid chlorides.[5]

The use of azides as amine protecting groups in SPPS has been described.[6] The method is particularly useful for hindered couplings; however, the need for chromatographic purification of azido acids and deprotections involving mild heat, thiols, and tertiary amines present drawbacks.

Solution-phase amide bond formation from carboxylic acids and iminophosphoranes (prepared from the corresponding azides and phosphines) is well-known.[7] Although the condensation typically takes place at elevated temperatures, similar transformations have been shown to occur at room temperature with activated acids.[8] Modifications of this coupling procedure have recently been applied to solid-phase organic synthesis (SPOS)[9] as well as amide-forming ligation reactions.[10] Our goal was to combine and successfully modify these protocols for application toward key steps in Fmoc-Wang SPPS as well as improving methods utilizing *a*-azido acids as building blocks.

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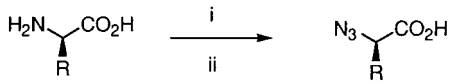
Table 1. α-Azido Acids Prepared in Scheme 1

entry	azido acid	$[\alpha]^{25}_D$[a]	yield	entry	azido acid	$[\alpha]^{25}_D$[a]	yield
1	N_3-Gly	-	68[f]	15	N_3-Gln	−73.3, MeOH	49
2	N_3-Phe	−74.2[b]	62	16	N_3-Asp(*t*-Bu)	−65.9	82
3	N_3-D-Phe	+64[c]	68	17	N_3-Glu(Bzl)	−46.8	42
4	N_3-Val	−47.8	75	18	N_3-Ser(*t*-Bu)	+29.3	84
5	N_3-D-Val	+50.8	80	19	N_3-D-Ser(*t*-Bu)	−27.4	84
6	N_3-Ala	+22.1	66	20	N_3-Thr(*t*-Bu)	+31.6	80
7	N_3-Leu	−13.0	68	21	N_3-Met	−98.5	86
8	N_3-Ile	−33.0	89	22	N_3-IBA	-	75
9	N_3-D-Ile	+38.3	73	23	N_3-Trp(*t*-Boc)	+31.0	86
10	N_3-D-*allo*-Ile	+108.6	76	24	N_3-Cys(4-MeO-Bzl)	−53.0	41
11	N_3-*tert*-Leu	−69.6	80	25	N_3-Tyr(*t*-Bu)	−40.7	70
12	N_3-Phg	+145.9[d]	85	26	N_3-Arg(Mtr)	−19.5, MeOH	46
13	N_3-D-Phg	−163.6[e]	87	27	N_3-Lys(*t*-Boc)	−19.0	66
14	N_3-Asn	−98.0, MeOH	48	28	N_3-His	−102.1, MeOH	86

[a] c = 1.0, $CHCl_3$ or MeOH if specified. [b] Lit.[16] $[\alpha]^{25}_D$ = −67.9 (c = 107, $CHCl_3$). [c] Lit.[16] $[\alpha]_D$ = +68.6 (c = 1.4, $CHCl_3$). [d] Lit.[16] $[\alpha]^{25}_D$ = +175 (c = 1.06, $CHCl_3$). [e] Lit.[16] $[\alpha]_D$ = −169 (c = 1.4, $CHCl_3$). [f] Synthesized trough nucleophilic substitution of bromoacetic acid by sodium azide.[17] Phenylglycine = Phg; Isobutyric Acid = IBA; 4-methoxybenzyl = 4-MeO-Bzl; 4-methoxy-2,3,6-trimethylbenzenesulfonyl = Mtr.

Successful implementation required readily available *a*-azido acids in chiral form (Table 1, **1**−**28**). We chose the copper(II)-catalyzed diazo transfer method of Wong[11] (Scheme 1) to prepare **1**−**28**,[6b,12] since other methods were less general

Scheme 1 α-Azido Acid Synthesis[a]



[a] Reagents and conditions: (i) Tf_2O, NaN_3; (ii) $CuSO_4$, K_2CO_3, H_2O, MeOH, CH_2Cl_2.

or involved cumbersome workups.[6,7c,13] Buffered extraction of the trifluoromethanesulfonamide byproduct during workup provided analytically pure azido acids in most cases without chromatography. Protection of the α-carboxylate was not required. Epimerization was negligible, and the method worked equally well on hindered substrates or amino acids possessing various side chain protecting groups (**16**−**20** and **23**−**27**). Additionally, substrates with unprotected nitrogen containing side chains were readily converted to α-azido acids (**14**, **15**, and **28**).

To prove useful to the field of SPPS, an ideal coupling method required good yields of products with little or no racemization. Preparation of peptides with the Glycine-Proline (GlyPro) *C*-terminus was reported to be unsuccessful due to DKP formation.[4a] Hence, Wang resin-bound FmocPro was deprotected, and the product was coupled to α-azido glycine (**1**) under standard conditions. This resin-bound azide was then treated with trimethylphosphine in dry dioxane and commercially available Fmoc-protected glycine *O*-succinimide ester. Cleavage from the support and purification by reversed-phase HPLC provided the desired FmocGlyGlyPro (**29**) in 70% yield (Scheme 2, path A). When the preparation of this compound was attempted with standard Fmoc-Wang chemistry, no product was detected (see Table 2, entry **29**). Other DKP-susceptible peptides[4,5] were also prepared using both methods. Substantial yield improvements were noted

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(12) Representative Azido Acid Synthesis by Diazo Transfer. Synthesis of Azido-Ile (**8**). The diazo transfer reactions utilize the method of Wong[11] for carbohydrates with an customized workup to accommodate the free acid products. Triflyl azide preparation: A solution of sodium azide (1.78 g, 27.45 mmol) was dissolved in distilled H_2O (4.5 mL) with CH_2Cl_2 (7.5 mL) and cooled on an ice bath. Triflyl anhydride (0.93 mL, 5.55 mmol) was added slowly over 5 min while stirring continued for 2 h. The mixture was placed in a separatory funnel and the CH_2Cl_2 phase removed. The aqueous portion was extracted with CH_2Cl_2 (2 × 3.75 mL). The organic fractions, containing the triflyl azide, were pooled and washed once with saturated Na_2CO_3 and used without further purification. L-Ile (366 mg, 2.79 mmol) was combined with K_2CO_3 (577.5 mg, 4.19 mmol), $Cu^{II}SO_4$ pentahydrate (6.98 mg, 27.9 μmol), distilled H_2O (9 mL), and CH_3OH (18 mL). The triflyl azide in CH_2Cl_2 (15 mL) was added, and the mixture was stirred at ambient temperature overnight. Subsequently, the organic solvents were removed under reduced pressure, and the aqueous slurry was diluted with H_2O (50 mL). This was acidified to pH 6 with concentrated HCl and diluted with 0.25 M, pH 6.2 phosphate buffer (50 mL) and extracted with EtOAc (4×) to remove sulfonamide byproduct. The aqueous phase was then acidified to pH 2 with concentrated HCl. The product was obtained from EtOAc extractions (3×). The organic extracts were combined, dried ($MgSO_4$), and evaporated to dryness giving 390 mg of the pale oil (**8**) in 89% yield with no need for further purification. $[\alpha]^{25}_D$ = −33.0 (c = 1.0 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.84 (d, J = 5.8 Hz, 1H), 2.15−1.95 (m, 2H), 1.70−1.55 (m, 1H), 1.45−1.25 (m, 1H), 1.04 (d, J = 6.8 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 66.9, 37.2, 24.9, 15.9, 11.5. Anal. Calcd for $C_6H_{11}N_3O_2$: C, 45.85; H, 7.05; N, 26.74. Found: C, 46.19; H, 7.29; N, 26.72.

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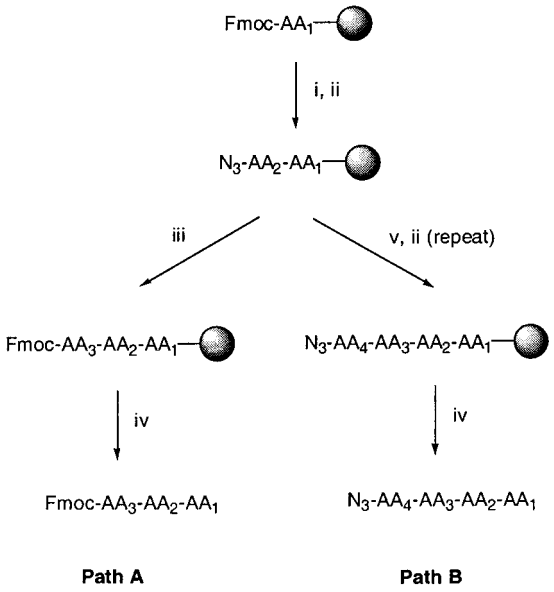
Scheme 2. Peptide Preparation from α-Azido Acids[a]



[a] Reagents and conditions: (i) 20% piperidine, DMF, 2 min., then 8 min.; (ii) azido acid, DCC, HOBT, (4.0 equiv each), DMF; (iii) trimethylphosphine (1.5 equiv), FmocAAOSu (4.0 equiv), dioxane; (iv) TFA−DCM(1:1); (v) trimethylphosphine (6.0 equiv), aq dioxane.

with the azide coupling procedure (Table 2, entries **30**−**32**). We attribute the increase in yields from the azido acid coupling method to suppresion of DKP formation since a closely related protocol was shown to inhibit the formation of δ-lactams.[9a] Although the reaction proceeds via an iminophosphorane intermediate, progress can be followed using the well-adopted Kaiser test.[14] Epimerization does not appear to be a significant problem since ^{1}H NMR inspection of crude tripeptides constructed with hindered isoleucine and D-*allo*-isoleucine in both the second and third positions indicated homogeneous products (Table 2, entries **33**−**36**). The alpha protons of the isoleucyl and D-*allo*-isoleucyl subunits in each case have unique signals coupled to the chiral β-proton. Hence, any racemization of the D-*allo*-isoleucyl subunit during the preparation of **33** would lead to its contamination with tripeptide **34**. The opposite would be true for the preparation of **34**. No cross contamination could be observed by 300 MHz ^{1}H NMR in either case. Similar rationale was applied to tripeptides **35** and **36** with the same results as above. The method confirmed that the stereochemical integrity of the amino acid components surrounding the newly formed amide bond was not compromised while coupling with the iminophosphorane.

Table 2. Peptides Prepared from α-Azidoacids

entry	sequence	yield[a,b]	epimerization
29	FmocGlyGlyPro	70 (0)	-
30	FmocGlyThrPro	81 (24)	-
31	FmocGly-D-ValPro	46 (0)	-
32	FmocValTyrPro	71 (0)	-
33	Gly-D-*allo*-IlePhe	-	<1%
34	GlyIlePhe	-	<1%
35	D-*allo*-IleGlyGly	-	<1%
36	IleGlyGly	-	<1%
37	N_3AspTyrLeuVal	76	-
38	N_3-D-Val-*t*-LeuTyrAla	20	-
39	N_3IleAibThrPhe	15	-
40	N_3-*t*-LeuVal-D-PheLeu	43	-
41	N_3LeuGlyPheVal	53	-
42	N_3MetAlaValPhe	21	-
43	N_3Ala-D-IleAlaPhe	46	-
44	N_3-*t*-LeuGlyIlePhe	66	-

[a] Yields are for purified products. [b] Yields in parentheses are for products prepared using the Fmoc-Wang SPPS method.

To expand the general utility of this methodology, N-terminal azidotetrapeptides were synthesized using azido acids as building blocks (Scheme 2, path B). Azides are readily converted to amines under mildly reductive conditions, employing phosphines in aqueous solvent systems.[15] Furthermore, the transformation proceeds under neutrality and avoids acid/base-catalyzed side reactions. A number of N-terminal azidotetrapeptides were prepared using standard coupling methods followed by the mild azide reduction technique described above (Scheme 2, path B and Table 2, entries **37**−**44**). The protocol suggests that a neutral, mild deprotection of resin-bound azides could add additional orthogonality to SPPS. Good yields were obtained in most cases except when hindered amino acid couplings were involved. The success of this method indicates that azido acids prepared by a more elaborate, asymmetric synthesis[16] could be incorporated directly into the peptides. This alleviates the need for reduction and then reprotection of the α-amino group of these valuable residues prior to coupling.

The methods described in this work show the robust and versatile nature of chiral α-azido acids as peptide building blocks. Increased yields for DKP susceptible peptides, flexibility in coupling methods, neutral reaction conditions, and the ability to complement standard Fmoc-Wang chemistry make the azido acid coupling strategy detailed here a useful addition to organic synthesis methodology.

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Supporting Information Available: Experimental procedures and analytical data for all azido acids (**1**−**28**) and peptides (**29**−**44**). This material is available free of charge via the Internet at http://pubs.acs.org.

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