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The azido acid approach to β-peptides: parallel synthesis of a tri-β-peptide library by fluorous tagging

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Abstract—A small tri-β-peptide library was prepared starting from three enantio- and diastereopure azido acids. Fluorous tagging followed by two cycles of azide reduction, fluorous solid phase extraction (f-SPE), peptide coupling with the original azido acids, and f-SPE provided 27 protected azido peptides. Reduction and HPLC purification provided 25 of the 27 targeted tri-β-peptides in acceptable yields and excellent purities.

1. Introduction

Oligomers and polymers of β-amino acids, called β-peptides, are of increasing interest because of their structures, folding patterns, and potential biological activities.[1] Most syntheses of β-peptides are patterned after the traditional approach to make α-peptides.[2] The key building blocks are *N*-protected β-amino acids, which are joined by an iterative sequence of N-deprotection and amide coupling (Fig. 1).

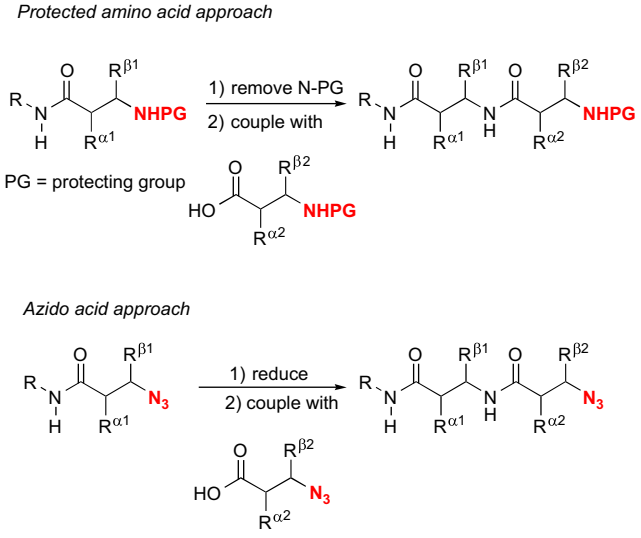

Figure 1. The 'amino acid' and 'azido acid' approaches to β-peptides.

Nelson and co-workers recently introduced an approach to β-peptides that starts from β-azido acids rather than β-amino acids and involves an iterative sequence of reduction of the azide to an amine followed by union of the amine with the next β-azido acid by amide bond formation.[3] α-Peptides have also been made from α-azido acids.[4] But the azide approach is not used very often because α-amino acids are much more readily available than α-azido acids and because α-azido acid derivatives activated for peptide coupling reactions are prone to racemization. This is unfortunate because atom economy and small size make the azide group attractive compared to protected amines.

For the β-peptide series, enantiopure β-azido acids are readily available by several routes,[5] and activated β-azido acids for peptide coupling cannot epimerize at the azide-bearing carbon. Thus, the azide approach merits serious consideration as a general approach to β-peptides.

Only a handful of di-β-peptides and a lone tri-β-peptide have been made by the azide approach to date.[3] We set out to extend the scope of the method by making a 27-member library of tri-β-peptides in a 3×3×3 matrix starting from three enantiopure β-azido acids. To expedite the library preparation, we used a light fluorous tag[6,7] on the C-terminus of the first β-azido acid so that intermediates could be quickly purified by fluorous SPE (f-SPE).[8] We describe herein the results of this study.

2. Results and discussion

The syntheses and fluorous tagging of the three β-azido acid building blocks are summarized in Scheme 1. β-Lactones **1a–c** are readily made in one step, on multi-gram scale, in

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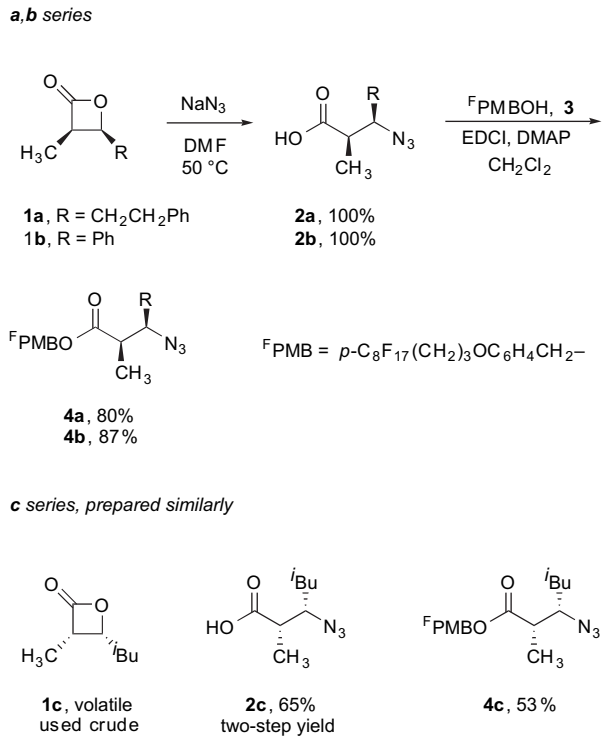
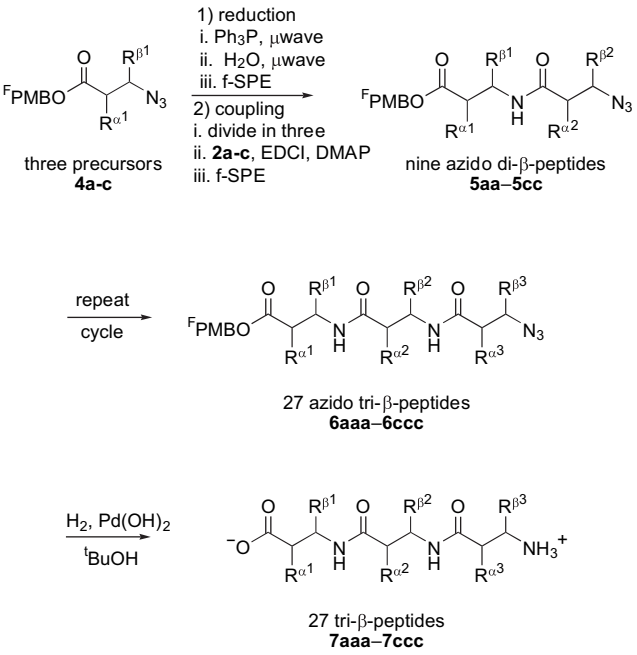


Scheme 1. Synthesis of azido acid precursors.

>90% ee by the cinchona-alkaloid catalyzed cyclocondensation reactions between acyl halides and aldehydes (AAC reaction).[3] Reactions of purified β-lactones **1a**,**b** with NaN_3 in DMF at 50 °C provided β-azido acids **2a**,**b** in quantitative yield. Due to its volatility, β-lactone **1c** was not purified; instead, the crude product of the AAC reaction was directly subjected to azide opening, and then the azide was purified. This provided **2c** in 65% yield over the two steps. To complete the precursor synthesis, the three β-azido acids (110–140 mg) were esterified with 1.2 equiv of the fluorous PMB alcohol **3** (^{F}PMB–OH)[9] in presence of 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide (EDCI) and *N*,*N*-dimethylaminopyridine (DMAP) to obtain the three azido-esters **4a–c**. These key precursors were purified by standard flash chromatography.

The synthesis of the tri-β-peptide library is summarized in Scheme 2 and involves two cycles of azide reduction and amide coupling (**4**→**5** and **5**→**6**), followed by a final hydrogenation/hydrogenolysis (**6**→**7**) to reduce the terminal azide and remove the fluorous PMB group.

The azides were reduced by a Staudinger procedure.[10,11] The three azido-esters **4a–c** were treated with 1.2 equiv of triphenylphosphine in THF under microwave irradiation at 120 °C, 250 W. After 5 min, ^{31}P NMR spectroscopic analysis showed complete conversion to the aza-ylide (see Supplementary data). Water (20 equiv) was added and the mixtures were again irradiated in the microwave instrument at 120 °C, 250 W. After 10 min, ^{31}P NMR analysis showed disappearance of the aza-ylide with formation of triphenylphosphine oxide. The samples were concentrated, taken up in a small amount of acetonitrile, and loaded into a fluorous SPE column.[9] First pass elution with 70/30 acetonitrile/water provided an organic fraction containing triphenylphosphine



Scheme 2. Synthesis of the tri-β-peptide library.

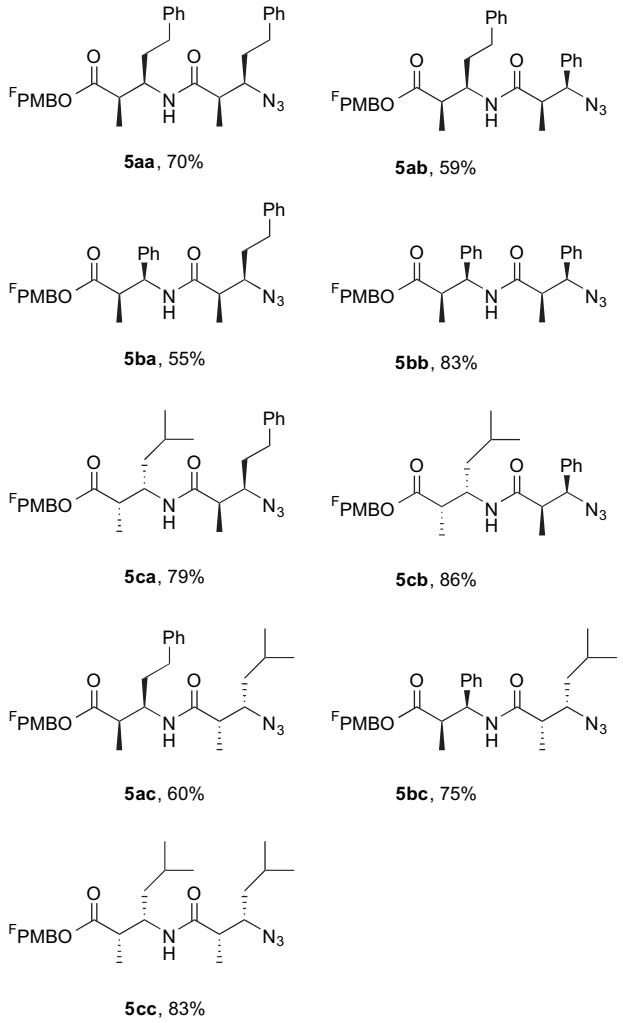
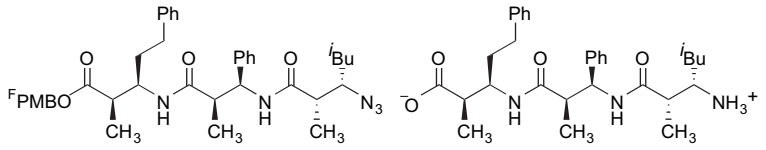


Figure 2. Structures of the nine dipeptide azides **5**.

Table 1. Yields and MS data for protected azides **6** and final tri-β-peptides **7**

Representative Structures



6abc **7abc**

Entry	**6** (%)	MS of **6** (calcd/obs)[a]	**7**[b] (f-SPE) (%)	**7**[c] (HPLC) (%)	MS of **7** (calcd/obs)[a]
aaa	33	1177.4/1178.2	92	—[d]	585.4/586.3
aab	37	1149.4/1150.0	89	—[d]	557.3/558.3
aac	55	1129.4/1130.2	80	76	537.4/538.3
aba	100	1149.4/1150.2	84	94	557.3/558.3
abb	99	1121.3/1122.0	71	—[d]	529.3/530.2
abc	94	1101.4/1102.2	64	45	509.3/510.3
aca	58	1129.4/1130.2	61	80	537.4/538.3
acb	74	1101.4/1102.2	72	67	509.3/510.2
acc	41	1081.4/1082.2	68	—[d]	489.4/490.4
baa	95	1149.4/1150.0	80	84	557.3/558.3
bab	84	1121.3/1122.2	70	94	529.3/530.2
bac	97	1101.4/1102.2	97	93	509.3/510.2
bba	84	1121.3/1122.2	51	99	529.3/530.2
bbb	87	1093.3/1094.2	60	—[d]	501.3/502.2
bbc	75	1073.3/1074.2	88	86	481.3/482.2
bca	30	1101.4/1102.2	82	64	509.3/537.3[e]
bcb	46	1073.3/1074.2	76	53	481.3/482.3
bcc	41	1053.4/1054.2	79	87	461.3/462.3
caa	42	1129.4/1130.2	87	91	537.4/538.3
cab	53	1101.4/1102.2	71	75	509.3/510.4
cac	69	1081.4/1082.2	55	78	489.4/490.3
cba	97	1101.4/1102.2	69	71	509.3/510.2
cbb	92	1073.3/1074.2	88	90	481.3/482.2
cbc	88	1053.4/1054.2	88	57	461.3/462.3
cca	37	1081.4/1082.2	82	40	489.4/490.4
ccb	38	1053.4/1054.2	97	83	461.3/469.2[e]
ccc	33	1033.4/1034.2	78	88	441.4/442.4

[a] *m/z* for M+1 ion.
[b] Yield of **7** after fluorous solid phase extraction.
[c] Recovery of **7** after HPLC purification.
[d] The product after f-SPE was pure by HPLC analysis and was not further purified.
[e] The mass spectrum is not consistent with the target product (see text).

oxide and unreacted triphenylphosphine, which was discarded. Second pass elution with THF provided the derived amines.

Without further characterization, each of the three intermediate amines was divided into three equal fractions, which were coupled in parallel with each of the three azido acids **2a–c** (1.2 equiv). Couplings were effected in dichloromethane with EDCI (1.2 equiv) and DMAP (0.5 equiv). EDCI was chosen because both EDCI and its derived urea are relatively polar and eluted very quickly under the first pass conditions of the fluorous solid phase extraction. The progress of the nine reactions was followed by TLC, and after about 30 min, the solvent was removed and the crude products were subjected to f-SPE as above. Again the first pass fraction was discarded and the second pass fraction (THF) was concentrated to provide the nine coupled azido β-peptides **5aa–5cc**, whose complete structures are shown in Figure 2. Yields of these crude products ranged from 55 to 86% over the two steps, and they all exhibited satisfactory ^{1}H and ^{13}C NMR spectra. Analyses by LC–MS also showed the expected molecular weights (see Supplementary data).

The nine products **5** were then subjected to a second cycle without further purification. First, reduction with triphenylphosphine and f-SPE provided nine amino di-β-peptides (80–100%). These were each divided into three equal portions followed by coupling with the three azido acids **2a–c** as above. Evaporation of the THF fraction following f-SPE provided the 27 azido tri-β-peptides **6aaa–6ccc** in the yields recorded in Table 1. Also shown in Table 1 is one representative structure (**6abc**); the full complement of structures is in Supplementary data. LC–MS analysis of all 27 samples showed the expected molecular weights for the products **6**, whose purities were typically 90% or better. Five of the samples selected by molecular weight (including highest, median, and lowest) were also analyzed by ^{1}H and ^{13}C NMR to provide additional support for structure and purity.

Finally, the terminal azides **6** were reduced and the ^{F}PMB groups were removed simultaneously by reduction with hydrogen in *tert*-butanol catalyzed by palladium hydroxide. Three initial reactions were conducted in vials and the crude products processed by f-SPE in cartridges as above to remove both the catalyst and the residual fluorous tag. This

time, the first pass fraction was concentrated and the second pass fraction was discarded. The remaining 24 samples were then hydrogenated together in a 24-well parallel synthesizer, followed by plate-to-plate solid phase extraction[12] and concentration.

After a preliminary analysis by LC–MS (reverse phase conditions), most of the crude tri-β-peptides **7** were purified by serial reverse phase HPLC under a standard set of conditions to provide purified products in the yields indicated in Table 1 (see Supplementary data for all structures). These zwitterionic products are generally white powders that were sparingly soluble in most organic solvents tested. They were then reanalyzed by LC–MS to confirm identity and purity. Twenty-five of the products exhibited essentially a single peak with the expected mass. Several of these products were again checked by ^{1}H NMR analysis (D_2O/CD_3CN) to confirm structure and purity (see Supplementary data).

Two of the products do not appear to have the expected structures based on MS analysis. The product **7bca** exhibited peaks for (M−H_2O+Na) and (M−H_2O+2Na), suggesting that it might be a dehydrated cyclic peptide. The MS for **7ccb** suggests that this product results from reductive deamination (hydrogenolysis) of the terminal benzylic amino group. These side reactions seem to be sequence specific, since they were not observed for any other members of the library.

3. Summary and conclusions

In summary, we have parlayed three readily available azido acids **2a–c** into a small library of tri-β-peptides **7** in amino acid form by fluorous tagging, two cycles of azide reduction and peptide coupling, and detagging with concomitant azide reduction. To expedite the synthesis, intermediates were purified only by fluorous solid phase extraction; however, the final products were purified by preparative HPLC to ensure good quality. This approach provided 25 of the 27 library members in acceptable yields and excellent purities.

The results suggest that the azido acid approach to make β-peptide oligomers deserves serious consideration as an alternative to the more traditional amino acid approach. The usefulness of the new approach will grow as a function of the availability of isomerically pure azido acids. The results further show the generality of fluorous solid phase extraction, whether in cartridges or in plate-to-plate format, as a method for rapid yet effective purification of molecules bearing light fluorous tags.

4. Experimental

4.1. General procedure 1: S_N2 addition of NaN_3 to β-lactones[3,5a]

The β-lactone (6.0 mmol) was added via syringe to a solution of NaN_3 (12.0 mmol) in anhydrous DMF (35 mL, 0.3 M in lactone) at 50 °C. The resulting homogeneous solution was stirred for 4–5 h. The reaction mixture was cooled to ambient temperature (25 °C), and saturated aqueous $NaHCO_3$ (30 mL) was added. The resulting heterogeneous mixture was diluted with water until all the precipitated salts dissolved. The resulting mixture was washed with ethyl acetate (2×50 mL) and the aqueous layer was acidified with 1 M aqueous HCl to pH≈1. The acidic aqueous layer was extracted with ethyl acetate (3×50 mL) and the combined organic portions were washed with water (2×50 mL) and brine (2×50 mL). The organic portion was dried (Na_2SO_4) and evaporated in vacuo to afford the β-azido acid.

4.2. General procedure 2: ^{F}PMB tagging

To a solution of β-azido acid (0.2 mmol) in CH_2Cl_2 (10 mL) was added *N*,*N*-dimethylaminopyridine (DMAP) (12 mg, 0.10 mmol). With stirring, ^{F}PMB–OH (140 mg, 0.240 mmol) was added to the solution followed by 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide (EDCI) (46 mg, 0.24 mmol). After 30 min, the mixture was partitioned between Et_2O (30 mL) and 1 M aqueous HCl (15 mL). The organic layer was separated and washed with H_2O (10 mL) and brine (10 mL), then dried over Na_2SO_4 and evaporated in vacuo to afford the crude ^{F}PMB ester. This was purified by flash chromatography (4:1 Hex/EtOAc).

4.3. General procedure 3: Staudinger reaction[10]

In a microwave tube, the azido ester or the azido β-peptide (0.2 mmol) was dissolved in dry THF (0.5 mL). A solution of triphenylphosphine (63 mg, 0.24 mmol) in dry THF (1 mL) was added into the microwave tube by syringe. The mixture was heated in the microwave reactor under stirring at 120 °C, 250 W, for 5 min. H_2O (72 mg, 4.0 mmol) was added into the microwave tube via syringe. The resulting mixture was microwaved for 10 min, at 120 °C, 250 W. The reaction progress can be monitored by ^{31}P NMR if desired. (^{31}P NMR spectra were recorded at 121.5 MHz. THF-d_8 was used as solvent. For calibration, the chemical shift of triphenylphosphine was set at −4.80 ppm. The chemical shifts of triphenylphosphine oxide and phosphorus ylide are at around 24 and 1 ppm, respectively.)[13] The solvent was removed in vacuo. A new f-SPE cartridge (5 g) was washed with THF (20 mL) under vacuum on the SPE manifold and preconditioned by passing through 70:30 MeCN/H_2O (30 mL). The crude product was dissolved in MeCN (1 mL) and loaded onto the cartridge using vacuum to ensure that the sample was completely adsorbed onto the silica. The cartridge was washed with 70:30 MeCN/H_2O (50 mL) to obtain the fraction containing the organic compounds, and washed with THF (30 mL) to obtain the fraction containing the fluorous compounds. The fluorous fraction was dried over Na_2SO_4 and the solvent was evaporated in vacuo to afford the amine.

4.4. General procedure 4: amide coupling

DMAP (12 mg, 0.10 mmol) was added to a solution of amine (0.2 mmol) in CH_2Cl_2 (2 mL). With stirring, β-azido acid (0.24 mmol) was added to the solution, followed by the addition of EDCI (46 mg, 0.24 mmol) and CH_2Cl_2 (1 mL). The mixture was stirred for 30 min, and then the solvent was removed in vacuo. A new f-SPE cartridge (5 g) was washed with THF (20 mL) under vacuum on the SPE manifold and preconditioned by passing through 70:30 MeCN/H_2O

(30 mL). The crude product was dissolved in MeCN (1 mL) and loaded onto the cartridge using vacuum to ensure that the sample was completely adsorbed onto the silica. The cartridge was washed with 70:30 MeCN/H_2O (50 mL) to obtain the fraction containing the organic compounds, and washed with THF (30 mL) to obtain the fraction containing the fluorous compounds. The fluorous fraction was dried over Na_2SO_4 and the solvent was evaporated in vacuo to afford the azido β-peptide.

4.5. General procedure 5: hydrogenation

In a GreenHouse Classic Parallel Synthesizer, a solution of each azido-tripeptide (8 mg) in *tert*-butanol (2 mL) was loaded to each test tube. $Pd(OH)_2/C$ (2 mg, 25 wt %) was added to each solution. The apparatus was evacuated and then filled with hydrogen gas by a balloon. With stirring, all reactions were done in 24 h according to TLC. The solvent was removed in vacuo using a vacuum centrifuge. The 24 reaction mixtures were directly loaded onto a preconditioned SPE cartridge plate, with each cartridge packed with Fluoro*Flash*™ silica gel (3 g).[14] The cartridges were first eluted with 70:30 MeCN/H_2O (2×5 mL). The solvent was removed in vacuo by vacuum centrifuge to afford the β-tripeptides. The cartridges were washed with THF (3×5 mL) to remove the ^{F}PMB residue and they were then ready for reuse.

4.6. Compound characterization

Complete characterization data, including copies of NMR spectra and HPLC traces, are provided in Supplementary data.

Acknowledgements

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Supplementary data

Contains full details of experiments and characterization (147 pages). This material is available free of charge via the Internet at http://www.sciencedirect.com. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.03.034.

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