Protocol

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Synthesis and alkylation of aza-glycinyl dipeptide building blocks

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Aza-glycinyl dipeptides are useful building blocks for the synthesis of a diverse array of azapeptides. The construction of the aza-glycine residue is however challenging, because of the potential for side reactions, such as those leading to formation of oxadiazalone, hydantoin and symmetric urea by-products. Employing *N,N′*-disuccinimidyl carbonate to activate benzophenone hydrazone, we have developed a more efficient approach for the synthesis of aza-glycinyl dipeptides. Alkylation of the semicarbazone of the resulting protected aza-glycinyl dipeptides using tetraethylammonium hydroxide and propargyl bromide provided an efficient entry into the aza-propargylglycinyl peptide building blocks, which have served previously in various reactions including Sonogashira cross-couplings, dipolar cycloadditions and intramolecular *exo-dig* cycloadditions to furnish a variety of azapeptide building blocks. Copyright © 2013 European Peptide Society and John Wiley & Sons, Ltd.

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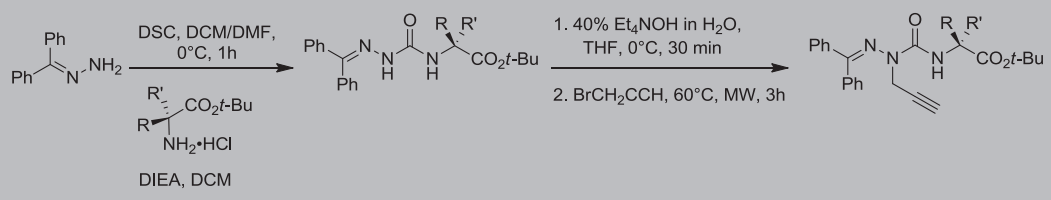
Keywords: aza-glycinyl dipeptide; benzophenone hydrazone; alkylation; *N,N′*-disuccinimidyl carbonate (DSC); azapeptide

REACTION SCHEME



GENERAL OPTIMIZED PROCEDURE

Benzophenone hydrazone was activated with *N,N′*-disuccinimidyl carbonate (DSC) and reacted with an amino *tert*-butyl ester to obtain the aza-glycinyl-dipeptide, which was alkylated with propargyl bromide and tetraethylammonium hydroxide without racemisation.

Scope and Comments

Azapeptides possess one or more semicarbazide residues from replacement of a backbone α-carbon by nitrogen in the peptide sequence (Figure 1) [1–11]. The conformation of the azapeptide is thus rigidified, because of the planarity of the urea and the lone-pair repulsion between adjacent nitrogen in the semicarbazide moiety, such that the φ and ψ dihedral angles of the aza-residue backbone have a tendency to adopt turn geometry, as shown by computational, spectroscopic and X-ray crystallography [1–7,11]. Moreover, the semicarbazide residue can bestow the azapeptide with improved pharmacokinetic properties, such as longer duration of action and resistance to proteases relative to natural peptides. [1,8–11]

Methods for the synthesis of azadipeptides have been reviewed with emphasis on the issues of side product formation, such as hydantoin from intramolecular cyclization of activated carbamato and isocyanato amides, and oxadiazalone on

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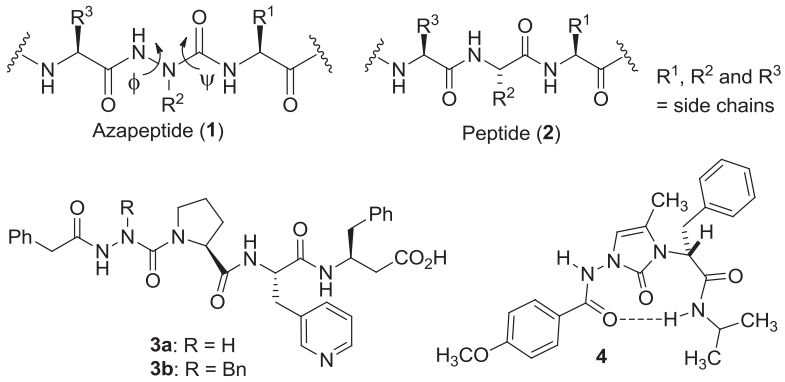

Figure 1. Azapeptide examples and structural comparison with peptides.

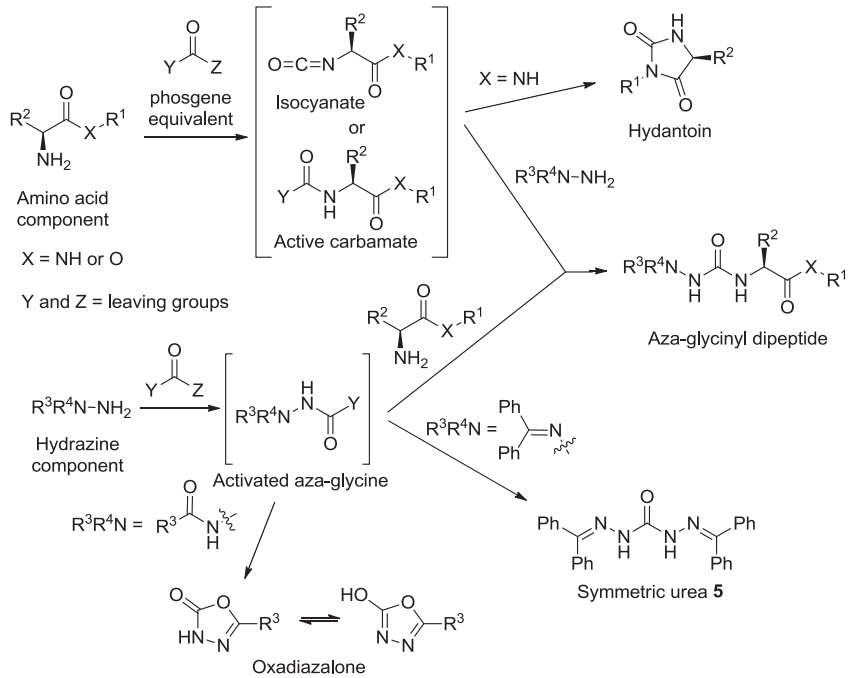

Figure 2. Strategies for the synthesis of azadipeptides.

activation of carbazates with phosgene equivalents (Figure 2) [1,11]. The issue of oxadiazalone formation has been surmounted in the synthesis of the aza-glycinyl dipeptide in solution by employing benzophenone hydrazone as the aza-Gly precursor [12]. The formation of symmetric urea **5** has however plagued this method, particularly, from using reactive activating agents, such as phosgene/triphosgene and carbonyldiimidazole. Symmetric urea **5** was minimized by using *p*-nitrophenyl chloroformate, as a safer agent than phosgene, to activate benzophenone hydrazone at 0 °C, because the *p*-nitrophenyl alkylidene carbazate intermediate was relatively stable and reacted effectively with amino esters and amino amides to provide aza-glycinyl dipeptides [12,13]. Removal of the *p*-nitrophenol by-product has however complicated this process.

Aza-glycinyl dipeptides have been employed in sub-monomer syntheses of a variety of structurally diverse and biologically active azapeptides by approaches featuring chemoselective alkylation and arylation of the semicarbazone to install various side chains at the aza-residue [13–17]. For example, benzhydrylidene aza-glycinyl-proline *tert*-butyl ester has served as a precursor in the synthesis of azapeptides **3** (Figure 1), which block myometrial contractions by modulating the activity of the prostaglandin F2α receptor through a mechanism featuring biased signalling and which exhibit potential as prototypes to develop inhibitors of preterm labour [18]. In the alkylation of benzhydrylidene aza-glycinyl phenylalanine *tert*-butyl ester **6a** with propargyl bromide (Scheme 1), racemisation has been avoided by employing tetraethylammonium hydroxide as a milder base, instead of potassium *tert*-butoxide or *tert*-butylaminotri(pyrrolidino)phosphorane [13,19]. The resulting aza-propargylglycinyl dipeptide **7a** and related analogues have served as versatile building blocks for broadening further the diversity of azapeptides, because the propargyl moiety has reacted effectively in Sonogashira cross-coupling chemistry with various aryl and heteroaryl halides [14,21], in copper-catalyzed azide-alkyne cycloadditions [20] and in 5-*exo*-*dig* cyclizations (Scheme 2) [14,21]. The latter reaction has provided a series of *N*-amino imidazolin-2-one dipeptide building blocks, which were shown by X-ray

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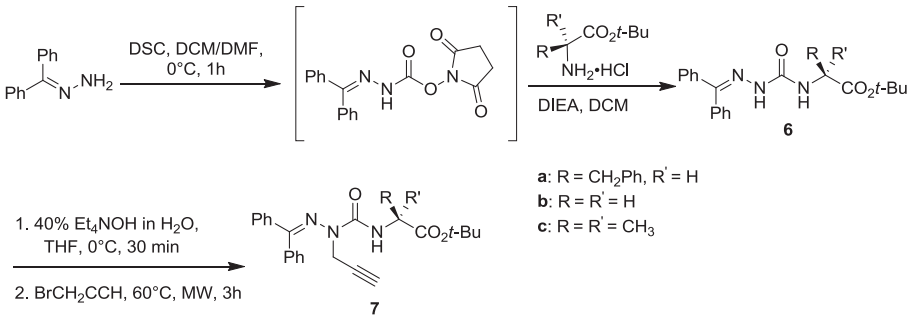
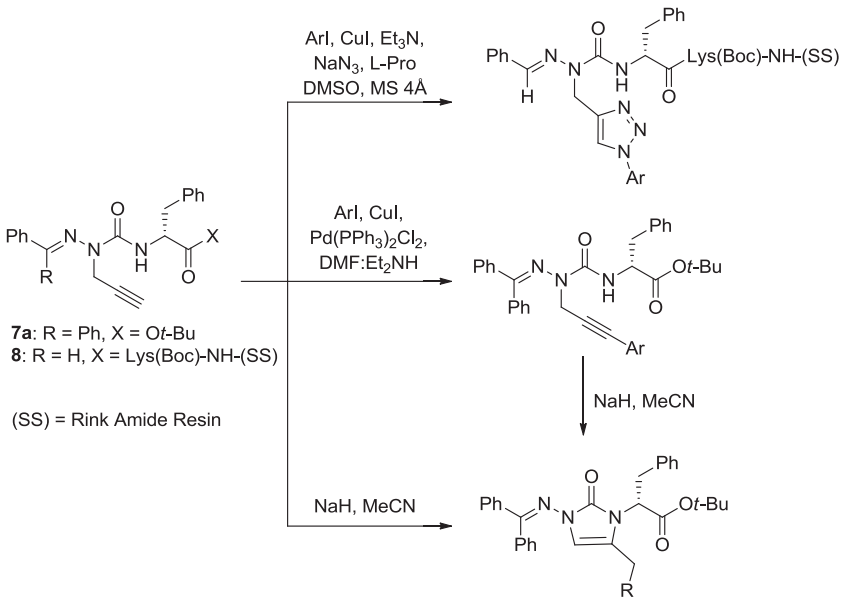


Scheme 1. Optimized azadipeptide synthesis.



Scheme 2. Applications of aza-propargylglycinyl dipeptides in Sonogashira coupling, 5-*exo-dig* cyclization and 1,3-dipolar cycloaddition reactions [14, 20, 21].

crystallography and NMR spectroscopy to mimic turn geometry in model peptide **4** [14,21].

In this protocol, two innovations are highlighted, which have improved the synthesis and alkylation of aza-glycinyl dipeptides. By employing *N,N'*-disuccinimidyl carbonate (DSC) instead of *p*-nitrophenyl chloroformate in the activation of benzophenone hydrazone, symmetric urea **5** and *p*-nitrophenol, both can be avoided such that higher yields of aza-glycinyl dipeptide ester are obtained after a simpler chromatography (Scheme 1). *O*-Succinimidyl carbamates have previously been used in the synthesis of oligoureas from *N*-protected β-amino alcohols, because of their stability and tendency to form crystalline solids [22,23]. Activation of the ε-amine of lysine residues in peptides with DSC has also provided stable *O*-succinimidyl carbamates, which reacted effectively to make peptide–protein conjugates [24]. The application of DSC in the activation of diphenyl hydrazone has now been shown to provide a useful activated aza-glycine intermediate, which has been used to acylate primary amino esters, including α-aminoisobutyric *tert*-butyl ester, to provide aza-glycinyl dipeptides in high yields.

In light of the value of aza-propargylglycinyl dipeptide **7a**, a procedure is also described that employs tetraethylammonium hydroxide as base and microwave heating for the racemisation-free alkylation of aza-glycinyl dipeptide ester **6a** with propargyl bromide. Furthermore, variations of the protocols are given for coupling of aza-glycine to *tert*-butyl glycinate and α-amino iso-butyrate to provide the respective aza-glycinyl dipeptides **6b** and **6c**, which were similarly alkylated to provide aza-propargylglycinyl dipeptides **7b** and **7c**. In light of the practical advantages of these innovations as well as the utility of aza-propargylglycinyl dipeptides **7**, which have previously proven effective in Sonogashira coupling, 5-*exo-dig* cyclization and 1,3-dipolar cycloadditions (Scheme 2), these protocols should be of general use for the effective synthesis of azapeptides.

Experimental Procedure

Benzhydrylidene aza-glycinyl-L-phenylalanine *tert*-butyl ester (6a)

In a flame-dried round-bottom flask, at 0 °C, a solution of DSC (7.18 g, 28.03 mmol, 1.1 eq) in dry CH_2Cl_2 (80 ml) and DMF (15 ml) was treated dropwise by cannula with a 0 °C solution of benzophenone hydrazone (5 g, 25.5 mmol, 1 eq) in dry CH_2Cl_2 (112 ml). The ice bath was removed, and the reaction mixture was allowed to warm to room temperature. After stirring for 1 h, the mixture was cooled to 0 °C and treated dropwise by cannula with a premixed 0 °C solution of L-Phe-O*t*-Bu·HCl (6.57 g, 25.48 mmol, 1 eq) and DIEA (8.4 ml, 50.96 mmol, 2 eq) in CH_2Cl_2 (32 ml). The ice bath was removed. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. The volatiles were evaporated, and the

residue was purified on a column of silica gel using flash chromatography with 10–50% EtOAc in hexane as solvent system. Ester **6a** was obtained as oil (8.60 g, 76% yield): the physical and spectroscopic properties of **6a** were identical to those reported in [19].

Benzhydrylidene aza-glycinylglycine *tert*-butyl ester (6b)

This was prepared using the protocol described earlier for phenylalanine dipeptide **6a** using benzophenone hydrazone (697 mg, 3.55 mmol, 1 eq), DSC (1 g, 3.90 mmol, 1.1 eq), DIEA (1.2 ml, 7.1 mmol, 2 eq) and glycine *tert*-butyl ester (595 mg, 3.55 mmol, 1 eq) in dry CH_2Cl_2 (28 ml) and DMF (5 ml). Flash chromatography, using 20–50% EtOAc in hexane as solvent system, and evaporation of the collected fractions gave aza-dipeptide **6b** (873 mg, 70% yield); mp 141–142, $R_f = 0.14$ (8 : 2 hexane/EtOAc); δ 7.66 (s, 1H), 7.56 (m, 5H), 7.37 (m, 3H), 7.28 (m, 2H), 6.79 (s, 1H), 4.07 (d, $J = 4.9$ Hz, 2H), 1.54 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 169.1, 155.0, 148.2, 136.5, 131.5, 129.5, 129.4, 129.0, 128.1, 127.9, 126.8, 81.7, 42.0, 27.7. IR (thin film) ν 2986, 1739, 1688, 1524, 1370, 1226, 1164, 1153, 1116, 692 cm^{-1}; HRMS (LC-ESI) *m/z* calcd for $C_{20}H_{24}N_3O_3$ $[MH]^+$ 354.1812, found 354.1814.

tert-Butyl benzhydrylidene aza-glycinylamino *iso*-butyrate (6c)

This was prepared using the protocol described earlier for phenylalanine dipeptide **6a** using benzophenone hydrazone (1.35 g, 6.90 mmol, 1 eq), DSC (1.94 g, 7.59 mmol, 1.1 eq), DIEA (2.3 ml, 13.8 mmol, 2 eq) and α-aminoisobutyric *tert*-butyl ester (1.35 g, 6.90 mmol, 1 eq) in dry CH_2Cl_2 (54 ml) and DMF (10 ml). Aza-dipeptide **6c** (2.42 g, 92% yield) was obtained after purification by flash chromatography with 15–40% EtOAc in hexane as solvent system: mp 141–142, $R_f = 0.28$ (8 : 2 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.50 (m, 6H), 7.36 (m, 3H), 7.26 (m, 2H), 7.09 (s, 1H), 1.66 (s, 6H), 1.53 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 174.3, 154.4, 147.8, 137.3, 132.1, 130.0, 129.5, 128.7, 128.6, 127.3, 81.7, 57.0, 28.1, 25.5. IR (thin film) ν 2984, 1723, 1677, 1512, 1461, 1449, 1302, 1154, 1105, 1068, 701, 693 cm^{-1}; HRMS (LC-ESI) *m/z* calcd for $C_{22}H_{27}N_3O_3Na$ $[MNa]^+$ 404.1945, found 404.1948.

Benzhydrylidene aza-propargylglycinyl-L-phenylalanine *tert*-butyl ester (7a)

A solution of benzhydrylidene aza-glycinyl-L-phenylalanine *t*-butyl ester (**6a**, 500 mg, 1.13 mmol, 1 eq) in THF (15 ml) at 0 °C was treated with 40% tetraethylammonium hydroxide in H_2O (502 µl, 3.37 mmol, 3 eq), stirred for 30 min, treated with 80% propargyl bromide in toluene (502 µl, 3.37 mmol, 3 eq), heated to 60 °C using microwave irradiation in a 300 MW Biotage (Uppsala, Sweden) apparatus on the high absorption level with automated temperature monitoring for 3 h and cooled to room temperature, and the solvent was reduced. The volume was diluted with CH_2Cl_2 (10 ml), and the organic phase was washed three times with H_2O, dried and evaporated. The residue was purified by flash chromatography eluting with 1 : 9 EtOAc/hexane. Evaporation of the collected fractions gave ester **7a** as colorless oil (394 mg, 73% yield): the physical and spectroscopic properties of **7a** were identical to those reported in [19].

Benzhydrylidene aza-propargylglycinyl-glycine *tert*-butyl ester (7b)

This was prepared using the protocol described earlier for phenylalanine dipeptide **7a** employing aza-dipeptide ester **6b** (400 mg, 1.13 mmol, 1 eq) in THF (8 ml), 40% tetraethylammonium hydroxide in H_2O (416 µl, 1.31 mmol, 1 eq) and 80% propargyl bromide in toluene (168 µl, 1.31 mmol, 1 eq). Purification by flash chromatography, using 50% EtOAc in hexane as solvent system, gave **7b** (369 mg, 83% yield). $R_f = 0.51$ (7 : 3 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (m, 2H), 7.47–7.41 (m, 4H), 7.39–7.34 (m, 4H), 7.0 (t, $J = 5.1$ Hz, 1H), 4.05 (m, 4H), 2.06 (t, $J = 2.2$ Hz, 1H), 1.49 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 169.8, 159.4, 158.5, 138.9, 135.8, 130.5, 130.1, 129.5, 129.1, 129.0, 128.5, 82.2, 78.9, 72.3, 43.6, 35.6, 28.4. IR (thin film) ν 2984, 1736, 1682, 1500, 1448, 1370, 1222, 1152, 737, 698 cm^{-1}; HRMS (LC-ESI) *m/z* calcd for $C_{23}H_{26}N_3O_3$ $[MH]^+$ 392.1969, found 392.1969.

tert-Butyl benzhydrylidene aza-propargylglycinylamino iso-butyrate

This was prepared using the protocol described earlier for phenylalanine dipeptide **7a** employing the aza-dipeptide ester **6c** (552 mg, 1.45 mmol, 1 eq) in THF (10 ml), 40% tetraethylammonium hydroxide in H_2O (1.60 ml, 4.35 mmol, 3 eq) and 80% propargyl bromide in toluene (647 µl, 4.35 mmol, 3 eq). Flash chromatography, using 15–50% EtOAc in hexane as solvent system, and evaporation of the collected fractions gave **7c** (609 mg, 91% yield); mp 125–127, $R_f = 0.62$ (7 : 3 hexane/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (m, 2H), 7.48–7.43 (m, 4H), 7.40–7.37 (m, 4H), 7.3 (s, 1H), 4.06 (d, $J = 1.8$ Hz 2H), 2.06 (t, $J = 2.2$ Hz, 1H), 1.62 (s, 6H), 1.50 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 174.4, 157.8, 157.6, 139.1, 135.9, 130.3, 130.0, 129.5, 128.9, 128.9, 128.5, 81.4, 79.1, 72.1, 57.4, 34.9, 28.2, 25.6. IR (thin film) ν 2985, 1726, 1687, 1498, 1461, 1301, 1155, 1094, 949, 697 cm^{-1}; HRMS (LC-ESI) *m/z* calcd for $C_{25}H_{29}N_3O_3Na$ $[MNa]^+$ 442.2101, found 442.2107.

The ^{1}H and ^{13}C NMR spectra for compounds **6b**, **6c**, **7b** and **7c** are presented in the Supporting information.

Limitations

Although activation with DSC has given high yields with primary amino esters, including the sterically bulky *tert*-butyl α-amino *iso*-butyrate, symmetric urea **5** was not completely avoided in reactions with proline *tert*-butyl ester. Aza-glycinyl-proline analogues have been made using *p*-nitrophenylchloroformate as activating agent, which remains the reagent of choice for their synthesis [12,13].

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Supporting Information

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