Organic LETTERS

Letter

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Azopeptides: Synthesis and Pericyclic Chemistry

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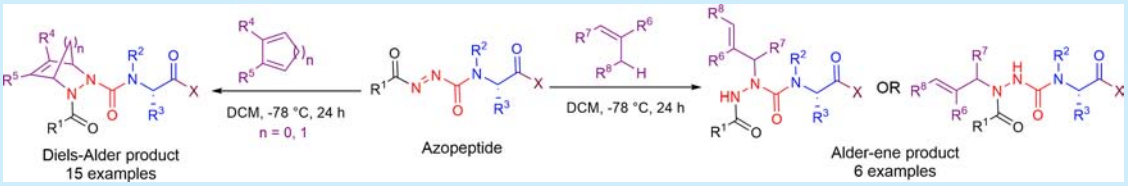
Supporting Information



ABSTRACT: Azopeptides possess an imino urea as an amino amide surrogate in the sequence. Azopeptides were synthesized by oxidation of aza-glycine residues and employed in pericyclic chemistry. Diels–Alder cyclizations and Alder–ene reactions on azopeptides enabled construction of constrained aza-pipecolyl and reactive aza-allylglycyl residues. X-ray crystallographic analyses of azopeptide **16a** and azapeptides **30a** and **35a** provided insight into imino urea configuration and conformational affects of cycloalkane side chains at the semicarbazide α- and β-nitrogen, respectively.

In the study of biologically active molecules, conformational constraint provides fundamental insight for designing enzyme inhibitors and receptor modulators.[1] In particular, the application of semicarbazides as amino acid surrogates in azapeptides (e.g., **1**, Figure 1) can restrict backbone geometry to enhance selectivity

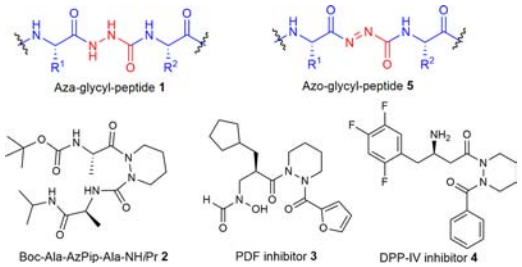


Figure 1. Aza- and azopeptides and aza-pipecolyl analogues.

and stability.[2] Azapeptides bearing cyclic and unsaturated residues are consequently important targets, respectively, because of their enhanced conformational rigidity and notable reactivity.[3,4] For example, aza-pipecolyl peptidomimetics (e.g., **2**–**4**) have displayed antibacterial and antidiabetic activities because of their inhibitory activity on peptide deformylase and dipeptidyl peptidase IV.[3b,5,6]

The introduction of cyclic and unsaturated aza-residues is, however, challenging, due to the need to selectively differentiate neighboring nitrogens, and accomplished typically by lengthy hydrazine chemistry using ionic intermediates.[2a] In contrast, pericyclic chemistry offers atom-economical access to cyclic and unsaturated systems. Azopeptides **5** possessing N=N bonds offer potential for pericyclic reactions with diene and ene systems, which have typically been performed using simple azodicarboxylates,[7] carbamoyl diazine carboxylates,[8] *N*-alkyltriazole diones,[9] and phthalazinedione.[10,11] Azo-bridges between aromatic peptide side chains have been made[12] and used to photoregulate dynamics of side-chain and backbone conformation.[13] To open potential for pericyclic chemistry in peptide frameworks, we have now created azopeptides **5** and studied their Diels–Alder and Alder–ene chemistry to synthesize aza-pipecolyl and aza-allylglycine residues.

Aza-glycine analogues **6**–**12** were synthesized using protocols featuring acylation of the peptide chain with activated methylidene or alkyl carbazates prior to deprotection and chain elongation (see the Supporting Information).[14−16] Azopeptides were then produced by oxidation of the aza-glycine residues using *N*-bromosuccinimide (NBS, Scheme 1) and pyridine in CH_2Cl_2 at −78 °C to room temperature.[3d,17]

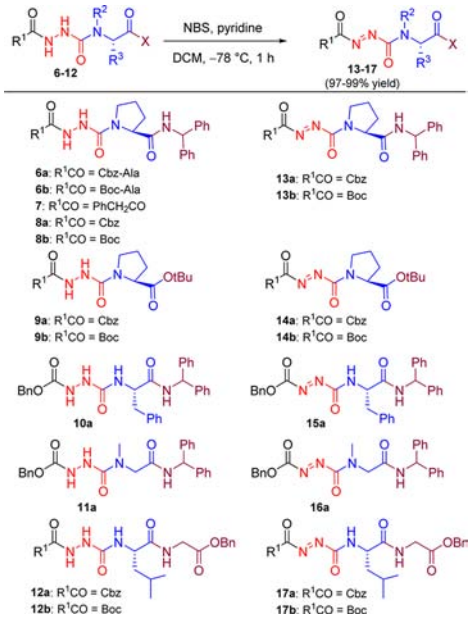
Carbamates **8**–**12** were converted, respectively, to azopeptides **13**–**17** using the NBS/pyridine conditions, as indicated by the change of the reaction solution color to pale yellow and observation of a new relatively nonpolar bright yellow spot on the TLC plate. Suitably pure azopeptides for subsequent chemistry were isolated in 97–99% yields after concentration of the reaction mixture, partitioning between aqueous sodium bicarbonate and ethyl acetate, and evaporation of the organic phase. In the IR spectra, azopeptides exhibited a band between 1700 to 1765 cm^{-1} indicative of the N=N stretching vibration.[18] Azopeptides **13**–**17** were used without further purification because they were relatively unstable and decomposed over time, as observed by the appearance of additional spots on TLC. Certain azopeptides (e.g., **14** and **16**) could be studied by NMR spectroscopy. Moreover, crystals of sarcosine analogue **16a** were isolated and characterized by X-ray diffraction (Figure 2).

A structure search of the Cambridge Crystallographic Data Center found only ethyl (*N*-phenylcarbamoyl)azoformate (**18**) as a simple diacyl diazine to compare with **16a**.[19] Azopeptide **16a** and azoformate **18** exhibited similar N=N bond lengths (~1.24

Received: September 22, 2015
Published: October 28, 2015

DOI: 10.1021/acs.orglett.5b02723
Org. Lett. 2015, 17, 5400−5403

Scheme 1. Synthesis of Azopeptides 13−17



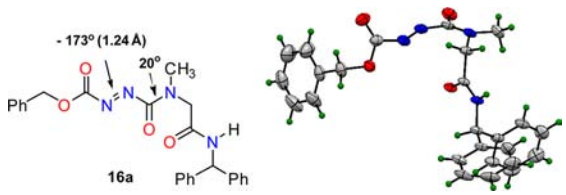


Figure 2. Crystal structure of azopeptide **16a** with bond lengths and dihedral angles (C = gray, H = green, N = blue, O = red).

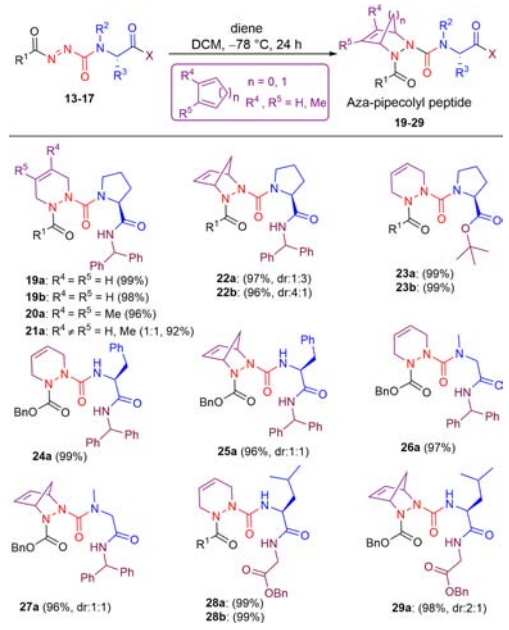
Å) and *E*-diazine configuration; the former was slightly distorted from planarity with a dihedral angle value of −173°. Ureas **16a** and **18** differed in their respective *E* and *Z* conformations. Torsion angles for **16a** and **18** were, respectively, 20° and −177°, indicative of greater twisting in the tertiary urea. Azopeptide **16a** adopted a bent conformation; however, in spite of their relative proximity, no seven-, eight-, or 10-membered hydrogen bonds were observed between the amide NH group and either diacyl diazine nitrogen or carbonyl groups. In the crystal lattice, azopeptides **16a** stacked antiparallel to each other forming intermolecular hydrogen bonds between the benzhydrylamide NH proton and sarcosine carbonyl oxygen.

In CD_3OD, the NMR spectrum of **16a** exhibited a 1:1 isomeric mixture. Isomer assignment to the diazine or tertiary urea was not possible, but evidence for isomerization of diazine **15a** was obtained by NMR spectroscopy, which indicated a 1:3 mixture of azo-isomers in $CDCl_3$.

Switching the *N*-terminal residue from carbamate to amide in aza-glycyl peptides **6** and **7** possessing alanyl and phenylacetyl residues, respectively, destabilized the oxidation product. Although the characteristic azopeptide yellow spot was observed by TLC analysis after treatment of **6b** with NBS at −78 °C for 10 min, the color was pale and other products were present. Attempts to trap the diazine with butadiene and isobutylene produced complex mixtures observed using LCMS, albeit minor peaks corresponded to the masses of the Diels−Alder and Alder−ene adducts. Attempts failed to make azopeptide using (diacetoxyiodo)benzene and lead tetraacetate to oxidize aza-glycines **6** and **7**.

Azopeptides **13−17** were examined as dienophiles in Diels−Alder reactions with butadiene, 2,3-dimethylbutadiene, cyclopentadiene, and cyclohexadiene. Butadiene was used in a sealed tube. Azopeptides **13−17** reacted smoothly with the butadienes and cyclopentadiene in dichloromethane at −78 °C to rt over 24 h to give the aza-pipecolyl analogues **19−29** in 92−99% yields after purification by silica gel chromatography (Scheme 2). 2-

Scheme 2. Aza-pipecolyl Peptide Synthesis from Diels−Alder Reactions of Azopeptides 13−17

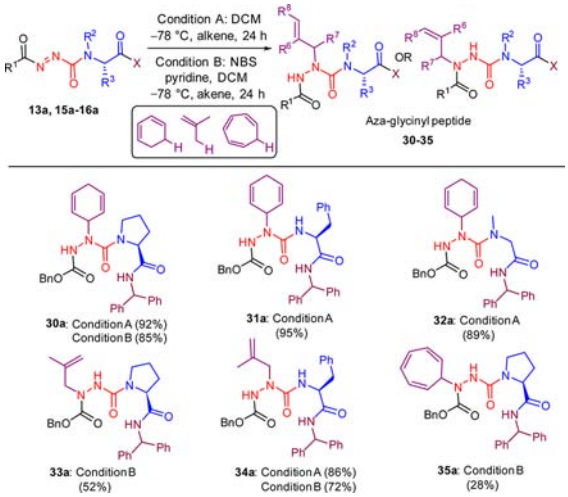


Methyl-1,3-butadiene reacted with azopeptide **13a** to give a 1:1 regioisomeric mixture of Diels−Alder adducts **21a** in 92% yield (see the SI). Cycloadditions with cyclopentadiene gave 1:1−4:1 diastereomeric mixtures measured using super critical fluid chromatography (SFC). The NMR spectra of Diels−Alder adducts from butadiene and 2,3-dimethylbutadiene were recorded at 100−120 °C to resolve broad signals due to tertiary urea isomers. Molecular ions characteristic of Diels−Alder adducts were observed from reactions between cyclohexadiene and azopeptide (e.g., **13a**); however, their NMR spectra exhibited an additional set of downfield vinyl protons indicative of Alder−ene reaction products (Scheme 3; see the SI).[20]

Alder−ene reactions were examined with two different conditions using azopeptides **13a** and **15a−16a** and three olefins: 1,3-cyclohexadiene, cycloheptatriene, and isobutylene. Aza-allylglycines **30a−32a** and **34a** were, respectively, obtained using cyclohexadiene and isobutylene in CH_2Cl_2 at −78 °C to rt overnight. Isobutylene was used in a sealed tube. Under these conditions, no product was isolated from the reaction of cycloheptatriene and azopeptide **13a**. In contrast, cycloheptatriene and isobutylene reacted, respectively, with azopeptide **13a** in the presence of NBS and pyridine at −78 °C in CH_2Cl_2 to give *β*-substituted analogues **35a** and **33a** in 28% and 52% yield. Aza-glycine **8a** was isolated with **35a** by chromatography of the reaction mixture of azopeptide **13a**, likely due to loss of cycloheptatrienyl cation and protonation on silica gel. The Alder−ene reaction typically provided *α*-substituted product under both conditions A and B (e.g., **30a** and **34a**); however, in cases where no reaction was observed with conditions A, *β*-substituted product was obtained using conditions B.

DOI: 10.1021/acs.orglett.5b02723
Org. Lett. 2015, 17, 5400−5403

Scheme 3. Aza-allylglycine Synthesis by Alder−Ene Reactions



Alder−ene regiochemistry was determined by a combination of NMR spectroscopy, MS experiments, and X-ray analyses (see the SI). Azapeptides **33a** and **34a** were examined by heteronuclear multiple-bond correlation (HMBC) NMR spectroscopy using nonuniform sampling at 120 °C, which resolved the proton signals at distinct chemical shifts (Figure 3; see the SI).

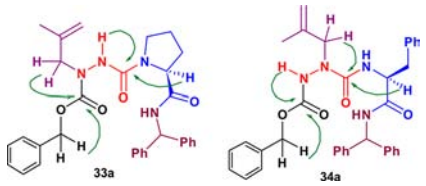


Figure 3. HMBC correlations used to assign the regiochemistry of the ene adducts **33a** and **34a**.

The α-position of the cyclohexadiene ring in azapeptides **31a** and **32a** was determined by hydrogenation with Raney nickel as catalyst, which cleaved the N−N bond giving products with molecular ion peaks of m/z $[M + H]^+$ 456 and 380, respectively, corresponding to fragments **36** and **37** (Scheme 4).

Scheme 4. Hydrogenolytic Cleavage and Mass Spectrometry To Confirm Regioselectivity of Aza-allylglycines 31a and 32a

Alder−ene products from reactions of azopeptide **13a** with cyclohexadiene and cycloheptatriene crystallized from EtOAc on diffusion of hexane vapors. X-ray diffraction indicated α- and β-substituted semicarbazides **30a** and **35a**, respectively (Figure 4). In the solid state, α-cyclohexadienyl azapeptide **30a** adopted dihedral angle values indicative of a type I β-turn with intramolecular 10-membered hydrogen bond between residues i and $i + 3$ (Table 1). On the other hand, the torsion angles of β-substituted azaglycine **35a** were indicative of a type VIa-like β-turn, albeit the urea ω-dihedral angle N-terminal to proline was

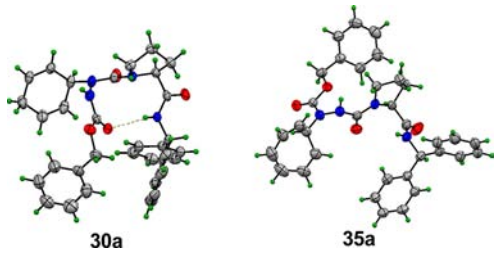
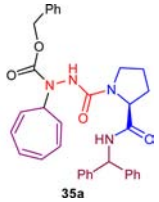


Figure 4. X-ray structures of **30a** and **35a**: broken lines represent inferred hydrogen bonds.

Table 1. Structures and ϕ and ψ Dihedral Angles (in degrees) from Crystal Analyses and Ideal Turns



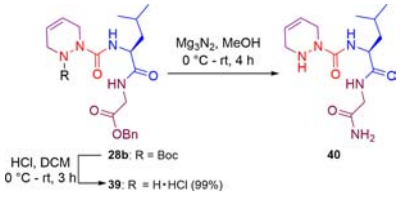
entry	ϕ^{i+1}	ψ^{i+1}	ϕ^{i+2}	ψ^{i+2}
type I β-turn[22b]	−60	−30	−90	0
30a	−60.9	−33.0	−79.0	−14.2
Boc-azaAla-Pro-NH*i*Pr (**38**)[21a]	−58.1	−24.7	−66.7	−17.7
type VIa β-turn[22b]	−60	120	−90	0
35a	−65.0	153.7	−81.9	−17.8

−169.7°. The ϕ and ψ dihedral angles of the X-ray structures for **30a** and **35a** are compared in Table 1 with ideal turn geometry and the crystal structure of Boc-azaAla-Pro-NH*i*Pr **38**, which also adopted a type-I β-turn geometry.[21,22] Aza-residue substituent position influenced turn geometry, offering intriguing potential as a design element for controlling peptide and peptoid conformation.

The practical utility of this method was demonstrated by the synthesis of aza-pipecolyl analogues of melanocyte-stimulating hormone release inhibiting factor-1 (MIF-1, Pro-Leu-Gly-NH_2).[23] A bioactive β-turn conformation has been proposed for the positive allosteric modulator activity of this endogenous neuropeptide on the D2 and D4 dopamine receptor subtypes,[24,25] inspiring MIF-1 analogue synthesis to develop therapeutics to treat Parkinson's disease and depression. Aza-pipecolyl MIF-1 analogue **40** was synthesized to study the influence of conformational restriction of the prolyl residue on biological activity by removal of the Boc group from **28b** with HCl gas in dichloromethane and aminolysis of ester **39** with magnesium nitride in methanol (Scheme 5).[26]

Pericyclic chemistry of azopeptides has provided effective entry to azapeptides bearing aza-pipecolyl and aza-allylglycyl residues without hydrazine chemistry using ionic intermediates. Oxidation of carbamate protected aza-glycyl peptides followed by

Scheme 5. Synthesis of Aza-pipecolylleucylglycinamide 40



DOI: 10.1021/acs.orglett.5b02723
Org. Lett. 2015, 17, 5400−5403

Diels–Alder and Alder–ene chemistry on the resulting diazine gave access to diverse azapeptides adopting type I and VI β-turns. The azopeptide approach offers thus a promising means for synthesizing restrained mimics to study the conformations of biologically active peptide sequences in pursuit of enzyme inhibitors and receptor modulators.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02723.

Experimental procedures, compound characterization data, and NMR spectra for all new compounds (PDF)
X-ray data for **16a** (CIF)
X-ray data for **28a** (CIF)
X-ray data for **33a** (CIF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). We thank M. C. Tang, Sylvie Bilodeau, and Francine Belanger of the Université de Montréal centers for HR-MS analysis, NMR spectroscopy, and X-ray crystallography, respectively.

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DOI: 10.1021/acs.orglett.5b02723
Org. Lett. 2015, 17, 5400–5403