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N-Aminoimidazolidin-2-one Peptidomimetics

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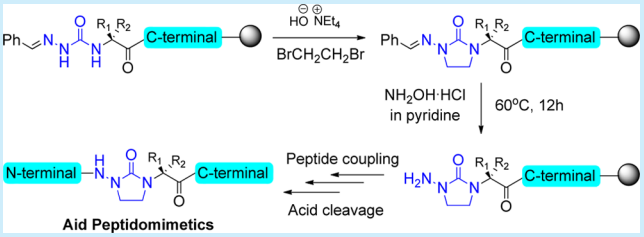
Supporting Information

ABSTRACT: The synthesis of *N*-aminoimidazolidin-2-one (Aid) peptidomimetics has been achieved by alkylation of the urea nitrogen of a semicarbazone residue using ethylene bromide. The Aid scaffold combines electronic and structural constraints to rigidify the peptide backbone in the equivalent of an aza variant of a Freidinger–Veber lactam. The syntheses and isolation of 25 Aid peptides, including eight GHRP-6 analogues, are reported to demonstrate the utility of this method for controlling conformation.



Constrained analogues are useful for characterizing biologically active peptide conformers. Optimal restraint can enhance affinity by preorganizing geometry that favors receptor binding surmounting the required loss of entropy for correct folding.[1] Moreover, rigid peptide analogues may avoid undesirable conformers prone to degradation and off-target interactions and may thus exhibit improved pharmacological properties, such as enhanced stability and bioavailability.[2]

Among approaches for rigidifying peptides, the utilization of α-amino-γ-lactam (Agl, so-called "Freidinger–Veber lactam", Figure 1) residues as backbone constraints has proven

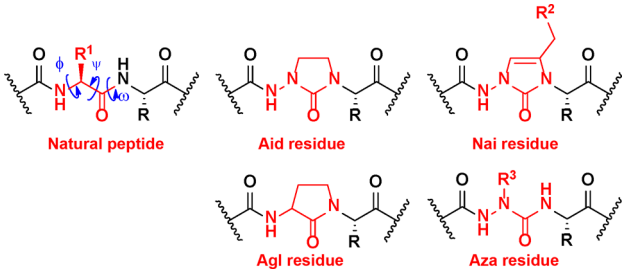


Figure 1. Peptide and Agl, Aza, Nai, and Aid mimics.

particularly effective in the design of ligands for therapeutic targets.[3−6] The Agl residue restrains rotation about the ψ- and ω-dihedral angles, forces the *C*-terminal amide to adopt a *trans*-conformation,[3] and predisposes the peptide backbone to mimic β-turn secondary structures,[7] which are commonly involved in molecular recognition events of biologically active peptides.[8,9]

In contrast to the structural restraints of Agl residues, azapeptides, in which the $C_\alpha H$ group is substituted for a N atom (Figure 1), utilize electronic forces to stabilize β-turn conformations in peptides.[10] As demonstrated by computational and X-ray analyses, the semicarbazide structure of the azapeptide rigidifies the ψ-dihedral angle, due to the planarity of the urea moiety, and the ϕ-dihedral angle, because of hydrazine N–N lone-pair repulsion.[11] Recently, the confluence of the amino lactam and azapeptide strategies for peptide mimicry has been achieved by the synthesis of *N*-aminoimidazolin-2-one (Nai) peptide mimics.[12] Designed to favor β-turn secondary structures, these dipeptide surrogates employ both electronic and structural constraints to hinder rotation about the backbone ϕ-, ψ- and ω-dihedral angles. X-ray crystallographic and NMR spectroscopic analyses have shown that the Nai residue prefers to sit in the central position of γ- and type II′ β-turns.[12,13] To study the importance of unsaturation for conformational control as well as to simplify the synthesis of such aza-variants of Freidinger–Veber lactams, we have pursued a method to produce the saturated version of the Nai residue. The *N*-aminoimidazolidin-2-one (Aid) residue has now been synthesized by double alkylation of a semicarbazone-protected azaglycinamide residue using 1,2-dibromoethane.

A solution-phase approach was first employed to synthesize Aid dipeptides. Benzhydrylidene azaglycinylphenylalanine *tert*-butyl ester[14] (**1**, 1 equiv) was treated with 1,2-dibromoethane (2.5 equiv) using different bases (5 equiv) in THF to alkylate first the acidic semicarbazone nitrogen followed by the urea nitrogen.[15,16] Tetraethylammonium hydroxide (TEAH, 53% yield) gave better results than its tetrabutylammonium counterpart and the nonionic phosphazene base *tert*-butyliminotri(pyrrolidino)phosphorane (BTPP, Table 1). No cyclic product was isolated using sodium hydride and potassium *tert*-butoxide, possibly due to β-elimination. Similarly, 1,2-diiodo- and 1,2-dichloroethanes failed to give Aid products. Although racemization (up to 30%) was detected using *tert*-butyl ester **1**, no loss of configuration was observed after similar alkylation of its isopropyl amide counterpart **3** (55% yield, Table 1, Supporting Information). In agreement with the alkylation of semicarbazones on solid phase,[15] benzylidene **6** gave a higher yield (87%) of Aid dipeptide than its benzhydrylidene counterpart **3**. Removal of the benzylidene group from **6** was effectively achieved using 1 N HCl in THF

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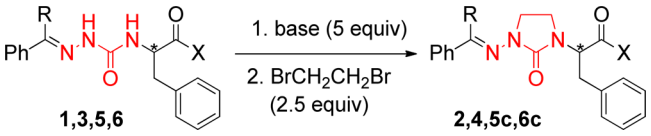
(1:2 v/v) at 40 °C to provide the corresponding semicarbazide,[17] which was acylated using 4-methoxybenzoyl chloride and DIEA, as well as with the symmetric anhydride prepared from Fmoc-Ala and DIC to give,[15] respectively, Aid peptides **7a** and **7b** in 89% and 68% overall yields (Scheme 1).

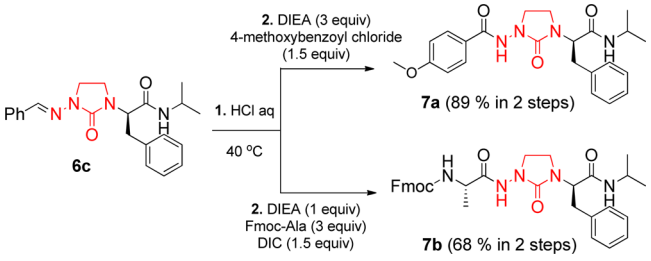
Table 1. Aid Dipeptide Solution-Phase Synthesis



substrate	R	X	product	base	yield[a] (%)
(*S*)-**1**	Ph	OtBu	(*S*)-**2**	TBAH	15 (71)
				TEAH	53 (41)
				BTPP	20 (37)
				KOtBu	trace (0)
				NaH	trace (54)
(*R*)-**1**	Ph	OtBu	(*R*)-**2**	TEAH	62 (15)
(*S*)-**3**	Ph	NHiPr	(*S*)-**4**	TEAH	55 (31)
				KOtBu	trace (78)
(*R*)-**3**	Ph	NHiPr	(*R*)-**4**	TEAH	51 (18)
5	H	OtBu	**5c**	TEAH	trace (0)
				BTPP	52 (41)
6	H	NHiPr	**6c**	TEAH	87 (−)

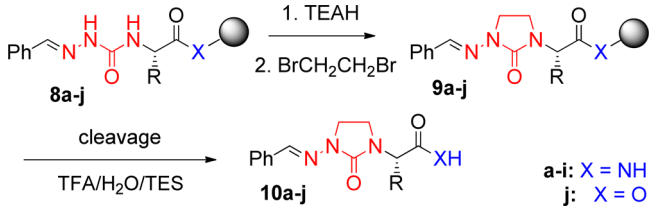
[a]% recovered starting material is indicated in parentheses.

Scheme 1. Aid Peptide Solution-Phase Synthesis



With a solution-phase method for the synthesis of *N*-aminoimidazolidin-2-one peptide mimics in hand, we turned our attention toward a solid-phase approach. On Rink amide resin, a set of benzylidene azaglycinyl dipeptides **8** were prepared using a diverse array of *C*-terminal amino acid residues (Table 2).[15] In order to verify the compatibility of this synthetic strategy, benzylidene azaglycinyl-D-phenylalanine **8j** was also synthesized on Wang resin. Semicarbazone alkylation was performed on resin **8** swollen in THF by treatment with TEAH (5 equiv) for 30 min, followed by 1,2-dibromoethane (2.5 equiv) and agitation overnight, after which time LC−MS analysis was performed on an aliquot of cleaved resin. For the most part, bis-alkylation of azaglycinyl dipeptide **8** linked to Rink amide resin was effectively achieved and provided a new peak with a molecular ion corresponding to the mass of the desired Aid dipeptide in high conversion (Table 2). For example, after cleavage using a freshly made solution of TFA/H_2O/TES (95:2.5:2.5, v/v/v), benzylidene *N*-aminoimidazolidinones **10a** and **10b** were isolated by preparative HPLC in 41% and 47% overall yields, respectively. On the other hand, multiple attempts failed to synthesize imidazolidinone from alkylation of azaglycinylcysteine **10c**; instead, HPLC analysis showed formation of multiple unidentifiable products. Using tetraethylammonium hydroxide as base, the benzyl ester side chain of aspartate **8e** was hydrolyzed during alkylation leading to undesired side products. Similar conditions were not fruitful using Wang ester resin, likely due to competitive hydrolytic resin cleavage. To avoid hydrolysis, BTPP was employed and provided respectively **10e** and **10j** in 63% and 82% conversion, as determined by HPLC analysis. Acid **10j** was cleaved from Wang resin **9j** using TFA/phenol (95:5), and isolated by preparative HPLC in 21% overall yield. Analysis of the cleavage product from an aliquot of resin **9d** by HPLC indicated two closely eluting peaks with the same mass and may be due to ring-opening of succinimide formed during alkylation to produce a mix of aspartamide and asparagine analogues.[18]

Table 2. Solid-Phase Aid Dipeptide Synthesis



R		
8	**10**	conv[a] (%)
CH_2Ph (**8a**)	CH_2Ph (**10a**)	quant
$(CH_2)_4NHBoc$ (**8b**)	$(CH_2)_4NH_2$ (**10b**)	quant
CH_2STrt (**8c**)	CH_2SH (**10c**)	*b*
CH_2CO_2tBu (**8d**)	CH_2CO_2H (**10d**)	quant
CH_2CO_2Bn (**8e**)	CH_2CO_2Bn (**10e**)	63[c]
$CH_2CONHTrt$ (**8f**)	CH_2CONH_2 (**10f**)	quant
H (**8g**)	H (**10g**)	quant
$(CH_2)_2SCH_3$ (**8h**)	$(CH_2)_2SCH_3$ (**10h**)	quant
CH_2OtBu (**8i**)	CH_2OH (**10i**)	quant
CH_2Ph (**8j**)	CH_2Ph (**10j**)	82[c,d]

[a]Conversion determined by HPLC of cleaved product (λ = 214 nm). [b]Multiple products observed. [c]BTPP as base. [d]from Wang resin.

Chemoselective benzylidene removal and liberation of semicarbazide **9** was effectively accomplished employing NH_2OH·HCl in pyridine at 60 °C for 12 h.[15] Acylation was subsequently performed with phenylacetyl chloride (3 equiv) and DIEA (5 equiv) in DCM or by coupling *N*-protected amino acids (Boc-Gly, Boc-Met, Fmoc-Cys(Trt), Boc-Ala, Boc-Tyr(OtBu), Fmoc-Pro and Boc-Pro) activated by way of their symmetric anhydrides, which were freshly prepared using DIC. Resin cleavage using TFA/H_2O/TES provided Aid peptides **12** in 17−47% overall yields after purification by preparative HPLC (Figure 2). Multiple attempts to couple different protected amino acids (Boc-Tyr(OtBu), Fmoc-Pro, and Boc-Ala) to the semicarbazide derived from aspartate resin **9f** were, however, unsuccessful using symmetric anhydrides.

An Aid scan of a peptide sequence was next performed. Growth hormone-releasing peptide-6 (GHRP-6, His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2) was selected for this purpose because of its interesting biological activity and challenging chemical structure. This synthetic hexapeptide exhibited affinity for the ghrelin receptor (GHS-R1a) and the cluster of differentiation 36 (CD36) scavenger receptor.[19,20] Activation of GHS-R1a stimulates growth hormone secretion, appetite, food intake, weight gain, and gastric emptying.[21−23] Recently, GHS-R1a

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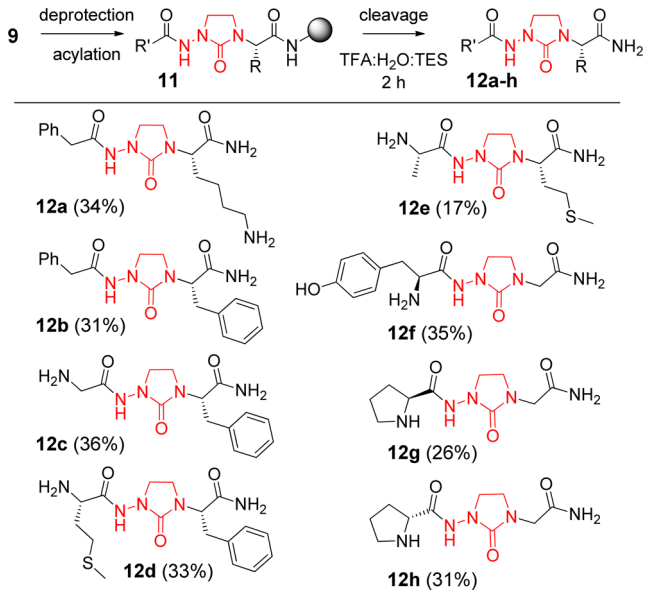
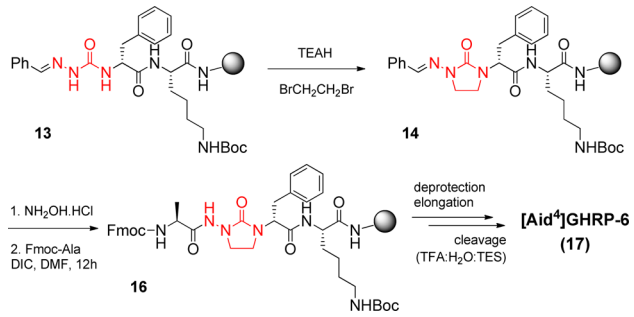


Figure 2. Aid peptide solid-phase synthesis.

activation has been recognized to regulate glucose-induced insulin secretion.[24−26] Consequently, GHS-R1a has been targeted to treat obesity and type 2 diabetes.[27] The CD36 receptor exhibited pleiotropic effects on immunity, metabolism, behavior, and angiogenesis.[28] Implicated in the clearance of subretinal deposits,[29] the CD36 receptor plays roles in facilitating fatty acid transport to the heart[30] and reversing cholesterol transport.[31−33] Selective CD36 receptor ligands are thus being pursued as potential treatments for age-related macular degeneration[29] and cardiovascular diseases.[34] Interest in receptor selective GHRP-6 analogues inspired earlier studies employing aza-amino acid residues, as well as α- and β-amino γ-lactams to control conformation.[7,16,35] Receptor selectivity was shown to be contingent on peptide conformation. For example, [aza-Tyr^4]GHRP-6 exhibited significantly reduced affinity toward the GHS-R1a yet retained affinity for the CD36 receptor.[36] Similarly, [*S*-Agl^3]GHRP-6 had CD36 receptor selectivity.[35]

Targeting [Aid^4]GHRP-6 initially, a general solid-phase protocol was developed for introducing *N*-aminoimidazolidin-2-one residues into the GHRP-6 sequence (Scheme 2). Azaglycine resin **13** was prepared by acylation of D-Phe-Lys(Boc)-Rink amide resin with an activated carbazate, which was formed in situ from benzaldehyde hydrazone and *p*-nitrophenyl chloroformate.[15] Alkylation of semicarbazone **13** with 1,2-dibromoethane (2.5 equiv) and TEAH (5 equiv) in THF gave imidazolidinone **14** in quantitative conversion as indicated by LC−MS analysis of a cleaved resin aliquot. Semicarbazone **14** was then treated with hydroxylamine hydrochloride in pyridine, and the resulting semicarbazide **15** was coupled to Fmoc-Ala by way of its symmetric anhydride, which was prepared using DIC. Removal of the Fmoc protection from the resulting tetrapeptide **16**, elongation of the sequence by standard Fmoc-based solid-phase peptide synthesis,[37] and resin cleavage using a cocktail of TFA/H_2O/TES gave [Aid^4]-GHRP-6 (**17**) in 44% crude purity (Table 3, entry 4). After purification by preparative HPLC, 99% pure Aid peptide **17** was obtained in 16% overall yield. For comparison, mimic **17** was also prepared from building block **2c** by a route involving *tert*-butyl ester removal and coupling of the resulting Aid dipeptide to Lys(Boc)-Rink amide resin using HBTU and DIEA. After benzylidene removal, peptide elongation, resin cleavage and HPLC purification, [Aid^4]-GHRP-6 (**17**) was obtained in 13% yield and exhibited identical retention time as material prepared by alkylation on resin. Employing the solid-phase method, four additional [Aid]-GHRP-6 analogues were successfully prepared in 6−13% yields and >99% purity (Table 3, entries 1−3 and 5). Notably, in the syntheses of [Aid^1]- and [Aid^2]-GHRP-6, the alkylation step was incomplete, consequently complicating HPLC purification of the final Aid peptides. Shorter [Aid]-GHRP-6 peptides were also prepared in 11−14% overall yield using this method (Table 3, entries 6−8).

Scheme 2. Solid-Phase Synthesis of [Aid^4]GHRP-6



In summary, we have developed an effective method for systematically introducing the *N*-aminoimidazolidin-2-one (Aid) scaffold into peptide sequences by alkylation of semicarbazones with 1,2-dibromoethane. Employing both electronic and structural constraints to induce conformational rigidity, the Aid residue offers interesting potential for exploring structure−activity relationships to elucidate active conformers of biologically relevant peptides. The Aid method was used to

Table 3. Yields and Purities of Aid-Containing Peptides

					HRMS	
entry	peptides	crude purity (%)	purity (%)	isolated yield (%)	*m/z* (cal)	*m/z* (obs)
1	**Aid**-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	35	>99	6.8	820.4259	820.4253
2	His-**Aid**-Ala-Trp-D-Phe-Lys-NH_2	51	>99	5.8	793.3872	793.3869
3	His-D-Trp-**Aid**-Trp-D-Phe-Lys-NH_2	51	>99	13.2	908.4318	908.4291
4	His-D-Trp-Ala-**Aid**-D-Phe-Lys-NH_2 (**17**)	44	>99	15.8	793.3867	793.3869
5	His-D-Trp-Ala-Trp-**Aid**-Lys-NH_2	44	>99	9.7	832.3995	832.3978
6	Ala-**Aid**-D-Phe-Lys-NH_2	72	>99	12.8	448.2685	448.2667
7	His-D-Trp-**Aid**-Trp-D-Phe-NH_2	68	>99	14.0	758.3521	758.3538
8	PhAc-**Aid**-D-Phe-Lys-NH_2	67	>99	11.1	495.2727	495.2714

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prepare short peptides and to scan the hexapeptide GHRP-6. The conformations and biological activities of these compounds are currently under evaluation and will be reported in due time.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization data, NMR spectra, and analytical HPLC profiles of representative peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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