Published in final edited form as:

J Am Chem Soc. 2009 November 11; 131(44): 16018–16020. doi:10.1021/ja907233q.

Stereospecific Synthesis of Conformationally Constrained γ -Amino Acids: New Foldamer Building Blocks that Support Helical Secondary Structure

Li Guo, Yonggui Chi, Aaron M. Almeida, Ilia A. Guzei, Brian K. Parker, and Samuel H. Gellman*

Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706

Abstract

A highly stereoselective synthesis of novel cyclically constrained γ -amino acid residues is presented. The key step involves organocatalytic Michael addition of an aldehyde to 1-nitrocyclohexene. After aldehyde reduction, this approach provides optically active β -substituted- δ -nitro alcohols (96–99% *e.e.*), which can be converted to γ -amino acid residues with a variety of substituents at the α -position. We have used these new building blocks to prepare α/γ -peptide foldamers that adopt a specific helical conformation in solution and in the solid state.

Oligomers constructed from β -amino acid residues (“ β -peptides”) or from combinations of α - and β -amino acid residues (“ α/β -peptides”) can adopt protein-like folding patterns.^{1,2} These conformational properties provide a basis for ongoing development of β - and α/β -peptides that display interesting functional properties. β -Amino acid residues can be endowed with higher intrinsic folding propensities than those of α residues by use of cyclic constraints to limit backbone torsional mobility, and this capacity for residue-based rigidification has proven to be important for both structure and function of β - and α/β -peptide foldamers.^{3–6} Analogous benefits should result from the use of constrained γ -amino acid residues in foldamers, but it is difficult to explore this hypothesis because only a few types of ring-containing γ -amino acids are known.^{7,8} The few cyclic γ residues examined to date have been found to promote sheet secondary structure,^{8b,c} which contrasts with the helix-favoring effects of the most common cyclic β residues.^{1,2,5,6}

Here we report a new synthetic approach that provides γ -amino acids containing a cyclohexyl constraint on the C_β - C_γ bond and a variable side chain at C_α . All three stereocenters of the γ -amino acid skeleton are generated from achiral precursors in a single process with high diastereo- and enantioselectivity. We show that the new type of γ -amino acid residue supports helix formation by an α/γ -peptide backbone.

Figure 1 shows our synthetic approach, the key step of which is the pyrrolidine-catalyzed Michael addition of an aldehyde to 1-nitrocyclohexene. Chiral pyrrolidines have been shown to catalyze the Michael addition of aldehydes to nitroalkenes with high stereoselectivity.^{9,10} Most precedents involve β -aryl nitroalkenes, such as β -nitrostyrene, which lead to $\gamma^{2,3}$ -amino acids.^{10a–d} We have reported that Michael addition of aldehydes to nitroethylene provides access to γ^2 -amino acids.^{10e} Use of pyrrolidine (*S*)-**A** along with acidic co-catalyst **B** proved

gellman@chem.wisc.edu.

Supporting Information Available: Experimental procedures and compound characterizations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

to be optimal in terms of efficiency and enantioselectivity. Wennemers et al.^{10f} concurrently devised an effective tripeptide catalyst for nitroethylene additions. In complementary work, List et al.^{10g} and Hayashi et al.^{10h} have found that (*S*)-**A** catalyzes highly enantioselective Michael additions of acetaldehyde to β -substituted nitroalkenes, which provides γ^3 -amino acids.

Our attention was drawn to 1-nitrocyclohexene as a Michael acceptor because the adducts could be easily converted to novel cyclically constrained γ -amino acid residues. Reaction of *n*-butanal and 1-nitrocyclohexene (2:1 molar ratio) in the presence of 20 mol % **A** in toluene provided only 25% of the Michael adduct after 24 hr, and the two major diastereomers (**2a** and **3a**) were produced in a ~1:1 ratio (Table 1). When 10 mol % **B** was employed as a co-catalyst, the Michael adduct yield rose to 44% yield, and **2a** was favored (6:1 dr); however, the major product was **4**, resulting from aldol condensation. Michael adduct yield was improved to 80% (7:1 dr) by using 5 equiv. of *n*-butanal. Under these conditions, replacing **B** with either benzoic acid or acetic acid caused a modest decline in diastereoselectivity, and replacing **B** with trifluoroacetic acid completely inhibited the reaction. We speculate that a key role of the acidic component is to facilitate catalyst turnover, perhaps by promoting hydrolysis of an iminium intermediate. The selectivity for **2a** relative to *trans* diastereomer **3a** may result from preferential equatorial protonation of the 2-substituted cyclohexane nitronate intermediate.¹¹

Solvent choice proved to have a substantial impact on Michael adduct yield and diastereoselectivity (Table 2). Both parameters were optimal when the reaction was conducted in CH_2Cl_2 and catalyzed by 20 mol % **A** and 10 mol % **B**, starting with 0.5 M 1-nitrocyclohexene. These conditions led to high selectivity for *cis* adduct **2a** (17:1 dr relative to **3a**).

Further exploration revealed that Michael additions catalyzed by **A** to 1-nitrocyclohexene are highly enantioselective and that many aldehydes are compatible with the catalytic process (Table 3). For these studies, *ee* was determined by HPLC after γ -nitro aldehydes had been reduced to the corresponding nitro alcohols, to avoid epimerization at the α -carbon. The absolute configuration of the major diastereomer generated with *n*-butanal and **A** was determined via derivatization (Scheme 1). Nitro alcohol **5** was oxidized to the corresponding nitro acid **6**, which was then coupled to L-phenylalanine methyl ester. The nitro group in the product was hydrogenated, and the resulting amino group was protected with a Boc group. A crystal structure of this α/γ -dipeptide revealed the (*S,S,S*) configuration for the γ -amino acid residue. The absolute configuration of other Michael adducts (Table 3) was assigned by analogy. γ -Nitro acid **6** could be easily converted to the Boc-protected γ -amino acid **7**. In terms of Michael addition scope (Table 3), it is noteworthy that aldehydes bearing a branch point adjacent to nucleophilic carbon (such as **1d** and **1h**) are tolerated, although these examples require > 2 days to produce good yields, perhaps because steric effects diminish reactivity. The success of the aldehyde with a protected lysine-like side chain (**1j**) will facilitate the synthesis of oligomers that can be subjected to conformational analysis in aqueous solution.

Overall, the results in Table 3 show that we can gain rapid access to stereochemically pure γ -amino acid building blocks with a *cis* cyclohexyl constraint in the backbone and a variety of

substituents adjacent to the carbonyl. The utility of the Michael addition-based approach is enhanced by the fact that the analogous *trans* diastereomers can be easily generated as well, as illustrated in Scheme 2. Thus, treating *cis* nitro alcohol **5** with NaHCO₃ in ethanol at reflux quantitatively induces epimerization at the nitro-bearing carbon. Subsequent oxidation yields nitro acid **9**, which is identical to the nitro acid obtained by oxidation of **3a** (the minor product of the Michael addition, which was characterized crystallographically). Boc-protected γ -amino acid **10** can be readily prepared from **9**.

The availability of cyclically constrained γ -amino acid building blocks in stereochemically pure form prompted us to begin to explore the conformational behavior of oligomers containing the corresponding subunits. Recent work suggests that oligomers constructed from α - and flexible γ -amino acid subunits can display a variety of discrete folding patterns.¹² We predict that α/γ -peptide foldamers will be conformationally stabilized by γ -residues with appropriate cyclic constraints.

Simulations from Hofmann et al.¹³ have identified a number of helical conformations that could be adopted by oligomers with a 1:1 alternation of α - and γ -residues. The helix containing 12-atom ring C=O(i)–H–N(i+3) H-bonds, which may be designated the α/γ -peptide “12-helix”, is predicted to have the *g*, *g* local conformation about the C $_{\alpha}$ –C $_{\beta}$ (ζ) and C $_{\beta}$ –C $_{\gamma}$ (θ) bonds. Fundamental principles lead one to expect that γ -residues derived from **7** (Figure 2) will favor this local conformation. We hypothesized that the α/γ -peptide 12-helix secondary structure would be favored by combining (*R,R,R*)-**7** (generated with (*S*)-**A**) with D- α -amino acid residues. This hypothesis was tested by preparation and analysis of tetramer **11** and hexamer **12**.

Crystal structures of both **11** and **12** reveal 12-helical conformations (Figure 3); in each case the maximum number of C=O(i)–H–N(i+3) H-bonds is formed. α/γ -Peptide **12** displayed sufficient proton resonance dispersion in CDCl₃ solution to enable NOESY analysis. Among the unambiguous NOEs involving backbone protons, four strong NOEs were observed between protons from different γ -residues: C $_{\gamma}$ H(2)–NH(4), C $_{\gamma}$ H(2)–C $_{\alpha}$ H(4), C $_{\gamma}$ H(4)–C $_{\alpha}$ H(6) and C $_{\gamma}$ H(4)–NH(6) (Figure 4). The C $_{\gamma}$ H(i)–NH(i+2) distances in the crystal structure of **12** are 2.5 and 2.7 Å, and the C $_{\gamma}$ H(i)–C $_{\alpha}$ H(i+2) distances are 2.4 and 2.4 Å, which suggests that these two NOE patterns should be characteristic of the α/γ -peptide 12-helix in solution. Balaram et al.^{12b} have recently suggested that 1:1 α/γ -peptides derived from exclusively achiral amino acids can adopt the 12-helix in chloroform, but in these cases only nearest neighbor NH (i)–NH(i+1) NOEs were observed.

We have developed a short and general route to γ -amino acids that feature a cyclohexyl constraint on the C $_{\beta}$ –C $_{\gamma}$ bond and a variety of side chains at C $_{\alpha}$. The key step is Michael addition of an aldehyde to 1-nitrocyclohexene, a process that is catalyzed by pyrrolidine **A** and that strongly favors just one of the eight possible stereoisomers. A second stereoisomer is available via epimerization at C $_{\gamma}$; absolute configuration is controlled by the enantiomer of catalyst **A** that is employed. α/γ -Peptides containing our constrained γ -residues favor a specific helical conformation. We anticipate that incorporation of these new γ -residues into other types of heterogeneous peptidic backbones will give rise to new families of foldamers, and that synthetic approaches related to those described here will provide access to γ -amino acids with complementary constraints that further broaden the foldamer realm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by NSF (CHE-0848847). NMR spectrometers were purchased with partial support from NIH and NS. We thank Prof. W. Seth Horne and Dr. Soo Hyuk Choi for helpful discussions, Prof. Shannon Stahl and Richard McDonald for assistance with chiral HPLC, and Galina Popova, Andrew Reidenbach, Weicheng Zhang for help with preparation of materials, and Prof. A. J. Andre Cobb for sharing unpublished results.

References

1. (a) Gellman SH. *Acc Chem Res* 1998;31:173. (b) Hecht, S.; Huc, I., editors. *Foldamers: Structure, Properties and Applications*. Wiley-VCH Weinheim; Germany: 2007. (c) Goodman CM, Choi S, Shandler S, DeGrado WF. *Nat Chem Biol* 2007;3:252. [PubMed: 17438550] (d) Seebach D, Beck AK, Bierbaum DJ. *Chem Biodivers* 2004;1:1111. [PubMed: 17191902]
2. Horne WS, Gellman SH. *Acc Chem Res* 2009;41:1399. [PubMed: 18590282]
3. (a) Huck BR, Fisk JD, Gellman SH. *Org Lett* 2000;2:2607. [PubMed: 10990408] (b) De Pol S, Zorn C, Klein CD, Zerbe O, Reiser O. *Angew Chem, Int Ed* 2004;43:511. (c) Baldauf C, Gunther R, Hofmann HJ. *Biopolymers* 2006;84:408. [PubMed: 16506208] (d) Sharma GVM, Nagendar P, Jayaprakash P, Krishna PR, Ramakrishna KVS, Kunwar AC. *Angew Chem, Int Ed* 2005;44:5878.
4. (a) Hayen A, Schmitt MA, Ngassa FN, Thomasson KA, Gellman SH. *Angew Chem, Int Ed* 2004;43:505. (b) Schmitt MA, Choi SH, Guzei IA, Gellman SH. *J Am Chem Soc* 2005;127:13130. [PubMed: 16173725] (c) Schmitt MA, Choi SH, Guzei IA, Gellman SH. *J Am Chem Soc* 2006;128:4538. [PubMed: 16594667] (d) Horne WS, Price JL, Keck JL, Gellman SH. *J Am Chem Soc* 2007;129:4178. [PubMed: 17362016] (e) Choi SH, Guzei IA, Gellman SH. *J Am Chem Soc* 2007;129:13780. [PubMed: 17949002]
5. (a) Sadowsky JD, Schmitt MA, Lee HS, Umezawa N, Wang S, Tomita Y, Gellman SH. *J Am Chem Soc* 2005;127:11966. [PubMed: 16117535] (b) Sadowsky JD, Fairlie WD, Hadley EB, Lee HS, Umezawa N, Nikolovska-Coleska Z, Wang SM, Huang DCS, Tomita Y, Gellman SH. *J Am Chem Soc* 2007;129:139. [PubMed: 17199293] (c) Horne WS, Price JL, Gellman SH. *Proc Natl Acad Sci USA* 2008;105:9151. [PubMed: 18587049] (d) Horne WS, Johnson LM, Ketas TJ, Klasse PJ, Lu M, Moore JP, Gellman SH. *Proc Natl Acad Sci USA* 2009;106:14751. [PubMed: 19706443]
6. (a) Appella DH, Barchi JJ, Durell SR, Gellman SH. *J Am Chem Soc* 1999;121:2309. (b) LePlae PR, Fisk JD, Porter EA, Weisblum B, Gellman SH. *J Am Chem Soc* 2002;124:6820. [PubMed: 12059191] (c) Lee M, Raguse TL, Schinnerl M, Pomerantz WC, Wang X, Wipf P, Gellman SH. *Org Lett* 2007;9:1801. [PubMed: 17394351]
7. (a) Hanessian S, Luo X, Schaum R, Michnick S. *J Am Chem Soc* 1998;120:8569. (b) Hintermann T, Gademann K, Jaun B, Seebach D. *Helv Chim Acta* 1998;81:983. (c) Farrera-Sinfreu J, Zaccaro L, Vidal D, Salvatella X, Giralt E, Pons M, Albericio F, Royo MA. *J Am Chem Soc* 2004;126:6048. [PubMed: 15137770] (d) Vasudev PG, Ananda K, Chatterjee S, Aravinda S, Shamala N, Balaram P. *J Am Chem Soc* 2007;129:4039. [PubMed: 17348653]
8. (a) Hagihara M, Anthony NJ, Stout TJ, Clardy J, Schreiber SL. *J Am Chem Soc* 1992;114:6568. (b) Woll MG, Lai JR, Guzei IA, Taylor SJC, Smith MEB, Gellman SH. *J Am Chem Soc* 2001;123:11077. [PubMed: 11686719] (c) Qureshi MKN, Smith M. *Chem Commun* 2006:5006.
9. For reviews, see: (a) Berner OM, Tedeschi L, Enders D. *Eur J Org Chem* 2002:1877. (b) Santanu M, Yang JW, Hoffmann S, List B. *Chem Rev* 2007;107:5471. [PubMed: 18072803]
10. For selected examples of organocatalytic Michael reactions of aldehydes to nitroalkenes, see: (a) Betancort JM, Barbas CF III. *Org Lett* 2001;3:3737. [PubMed: 11700126] (b) Alexakis A, Andrey O. *Org Lett* 2002;4:3611. [PubMed: 12375900] (c) Wang W, Wang J, Li H. *Angew Chem, Int Ed* 2005;44:1369. (d) Hayashi Y, Gotoh H, Hayashi T, Shoji M. *Angew Chem, Int Ed* 2005;44:4212. (e) Chi Y, Guo L, Kopf N, Gellman SH. *J Am Chem Soc* 2008;130:5608. [PubMed: 18386925] (f) Wiesner M, Revell JD, Tonazzi S, Wennemers H. *J Am Chem Soc* 2008;130:5610. [PubMed: 18386927] (g) Garcia-Garcia P, Ladepeche A, Halder R, List B. *Angew Chem, Int Ed* 2008;47:4719. (h) Hayashi Y, Itoh T, Ohkubo M, Ishikawa H. *Angew Chem, Int Ed* 2008;47:4722.
11. (a) Bordwell FG, Yee KC. *J Am Chem Soc* 1970;92:5939. (b) Hayashi T, Senda T, Ogasawara M. *J Am Chem Soc* 2000;122:10716. (c) List B, Pojarliev P, Martin HJ. *Org Lett* 2001;3:2423. [PubMed: 11483025]

12. (a) Vasudev PG, Chatterjee S, Shamala N, Balaram P. *Acc Chem Res.* ASAP. (b) Chatterjee S, Vasudev PG, Raghothama S, Ramakrishnan C, Shamala N, Balaram P. *J Am Chem Soc* 2009;131:5956. [PubMed: 19341285] (c) Chatterjee S, Vasudev PG, Raghothama S, Shamala N, Balaram P. *Biopolymers* 2008;90:759. [PubMed: 18767124] (d) Vasudev PG, Chatterjee S, Ananda K, Shamala N, Balaram P. *Angew Chem, Int Ed* 2008;47:6430. (e) Chatterjee S, Vasudev PG, Ananda K, Raghothama S, Shamala N, Balaram P. *J Org Chem* 2008;73:6595. [PubMed: 18662036]
13. Baldauf C, Gunther R, Hofmann HJ. *J Org Chem* 2006;71:2000. [PubMed: 16496986]

Figure 1.

Figure 2.
Intramolecular H-bonding patterns in the crystal structures of **11** and **12**.

Figure 3. Crystal structures of **11** (left) and **12** (right): (top) views perpendicular to helical axis; (bottom) views along the helical axis.

Figure 4. Characteristic NOEs patterns observed for the 1:1 α/γ -peptide hexamer **12** in CDCl_3 .

Scheme 1.

Scheme 2.

Table 1

Co-catalyst effects^a

entry	co-catalyst (mol%)	yield ^b (%)	dr ^{b,c} (2a:3a)	M:4 ^d
1	none	25	1:1	1:1
2	<i>m</i> -NO ₂ C ₆ H ₄ CO ₂ H (10)	44	6:1	1:2.2
3 ^e	<i>m</i> -NO ₂ C ₆ H ₄ CO ₂ H (10)	80	7:1	1:3.2
4 ^e	HOAc (100)	80	5:1	1:5.0
5 ^e	TFA (10)	0	n.d.	n.d.
6 ^e	Benzoic acid (10)	82	5:1	1:1.8

^a Reactions were performed with 1.0 M 1-nitrocyclohexene using 2 equiv. of aldehyde.^b Determined from ¹H NMR of the crude reaction mixture after 24 h.^c See supporting information for details.^d **M** stands for all Michael adduct diastereomers observed by NMR.^e 5 equiv. *n*-butanol was employed.

Table 2Solvent effects^a

entry	solvent	concentration of 1- nitrocyclohexene	Yield ^b (%)	dr ^b (2a:3a)
1	Toluene	1.0 M	80	7:1
2	Hexane	1.0 M	80	5:1
3	DMSO	1.0 M	26	1:1
4	DMF	1.0 M	80	6:1
5	i-PrOH	1.0 M	55	4:1
6	CHCl ₃	1.0 M	85	10:1
7	CH ₂ Cl ₂	1.0 M	86	10:1
8	CHCl ₃	0.5 M	81	15:1
9	CH ₂ Cl ₂	0.5 M	82	17:1

^aReactions were performed using 5 equiv. of aldehyde, see supporting information for details.^bDetermined from ¹H NMR of the crude reaction mixture after 24 h.

Table 3

Aldehydes variation

entry	product	R	t (h)	Yield ^a (%)	dr ^b	ee ^{c,d} (%)
1	2a	Et	38	87	17:1	99
2	2b	Me	36	84	8:1	97
3	2c	<i>n</i> -Pr	40	86	16:1	99
4	2d	<i>i</i> -Pr	54	79	10:1	>99
5	2e	<i>n</i> -Bu	40	85	16:1	99
6	2f	<i>n</i> -Hex	42	81	15:1	>99
7	2g	<i>c</i> -Hex	54	70	16:1	>99
8	2h	CH ₂ CH ₂ Ph	40	75	9:1	98
9	2i	(CH ₂) ₄ N(Boc) ₂	42	73	13:1	96

^aYield of isolated alcohol (major diastereomer) after reduction with NaBH₄.

^bDetermined from ¹H NMR of the crude reaction mixture.

^cDetermined by chiral HPLC analysis of the alcohol derived from **2a-i**.

^dAbsolute configuration of **2a** and **3a** was determined by the X-ray structure analysis, see Supporting Information for details.