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

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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 the 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 the 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 $C_\beta-C_\gamma$ 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.

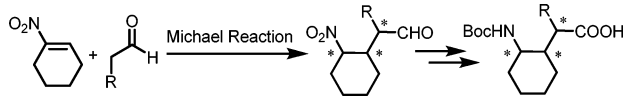
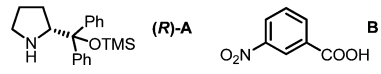


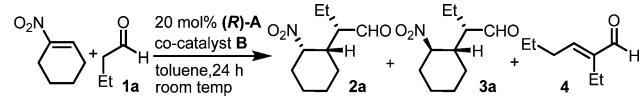
Figure 1

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 γ[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 cocatalyst **B** proved to be optimal in terms of efficiency and enantioselectivity. Wennemers and co-workers[10f] concurrently devised an effective tripeptide catalyst for nitroethylene additions. In complementary work, List and co-workers[10g] and Hayashi et al.[10h] found that (*S*)-**A** catalyzes highly enantioselective Michael additions of acetaldehyde to β-substituted nitroalkenes, providing 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 the Michael adduct in only 25% yield after 24 h, and the two major diastereomers (**2a** and **3a**) were produced in a ratio of ∼1:1 (Table 1). When 10 mol % **B** was employed as a cocatalyst, the Michael adduct yield rose to 44%, and **2a** was favored (6:1 dr); however, the major product was **4** resulting from aldol condensation. The 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 imminium intermediate. The selectivity for **2a** relative to the *trans* diastereomer **3a** may result from preferential equatorial protonation of the 2-substituted cyclohexane nitronate intermediate.[11]

Table 1. Cocatalyst Effects[a]



entry	cocatalyst (mol %)	yield[b] (%)	dr (**2a**/**3a**)[b,c]	**M**/**4**[d]
1	none	25	1:1	1:1
2	*m*-$NO_2C_6H_4CO_2H$ (10)	44	6:1	1:2.2
3[e]	*m*-$NO_2C_6H_4CO_2H$ (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 ^{1}H NMR analysis of the crude reaction mixture after 24 h. [c] See the Supporting Information for details. [d] **M** stands for all of the Michael adduct diastereomers observed by NMR spectroscopy. [e] Using 5 equiv of *n*-butanal.

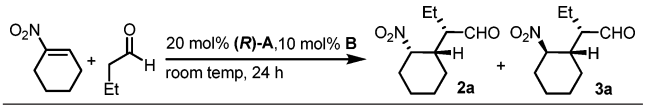
Solvent choice proved to have a substantial impact on the Michael adduct yield and diastereoselectivity (Table 2). Both parameters were optimal when the reaction was conducted in CH_2Cl_2 and catalyzed by 20 mol % **A** and 10 mol % **B**, starting with 0.5 M 1-nitrocyclohexene. These conditions led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

Further exploration revealed that Michael additions to 1-nitrocyclohexene catalyzed by **A** 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

10.1021/ja907233q CCC: $40.75

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 configurations of other Michael adducts (Table 3) were 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 the nucleophilic carbon (such as **1d** and **1g**) are tolerated, although these examples required >2 days to produce good yields, perhaps because steric effects diminished the reactivity. The success of the aldehyde with a protected lysine-like side chain (**1i**) will facilitate the synthesis of oligomers that can be subjected to conformational analysis in aqueous solution.

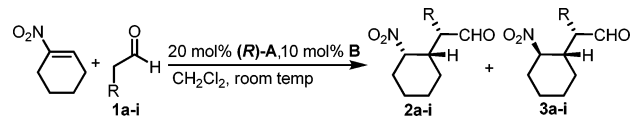
Table 2. Solvent Effects[a]



entry	solvent	concentration of 1-nitrocyclohexene (M)	yield (%)[b]	dr (**2a**/**3a**)[b]
1	toluene	1.0	80	7:1
2	hexane	1.0	80	5:1
3	DMSO	1.0	26	1:1
4	DMF	1.0	80	6:1
5	*i*-PrOH	1.0	55	4:1
6	$CHCl_3$	1.0	85	10:1
7	CH_2Cl_2	1.0	86	10:1
8	$CHCl_3$	0.5	81	15:1
9	CH_2Cl_2	0.5	82	17:1

[a] Reactions were performed using 5 equiv of aldehyde (see the Supporting Information for details). [b] Determined from ^{1}H NMR analysis of the crude reaction mixture after 24 h.

Table 3. Aldehyde Variation

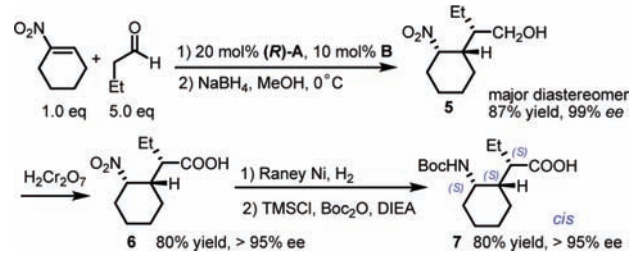


entry	product	R	time (h)	yield (%)[a]	dr[b]	ee (%)[c,d]
1	**2a**	Et	38	87	17:1	99
2	**2b**	Me	36	84	8:1	97
3	**2c**	*n*-Pr	40	86	16:1	99
4	**2d**	*i*-Pr	54	79	10:1	>99
5	**2e**	*n*-Bu	40	85	16:1	99
6	**2f**	*n*-Hex	42	81	15:1	>99
7	**2g**	*c*-Hex	54	70	16:1	>99
8	**2h**	CH_2CH_2Ph	40	75	9:1	98
9	**2i**	$(CH_2)_4N(Boc)_2$	42	73	13:1	96

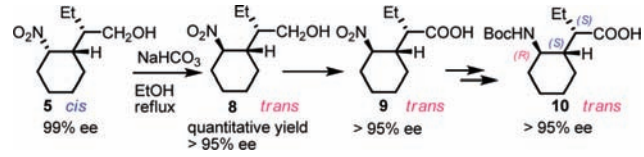
[a] Yield of isolated alcohol (major diastereomer) after reduction with $NaBH_4$. [b] Determined from ^{1}H NMR analysis of the crude reaction mixture. [c] Determined by chiral HPLC analysis of the alcohols derived from **2a**−**i**. [d] Absolute configurations of **2a** and **3a** were determined by X-ray structure analysis (see the Supporting Information for details).

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 $NaHCO_3$ 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**.

Scheme 1



Scheme 2



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 and co-workers[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 using (*S*)-**A**] with D-α-amino acid residues. This hypothesis was tested by preparation and analysis of tetramer **11** and hexamer **12**.

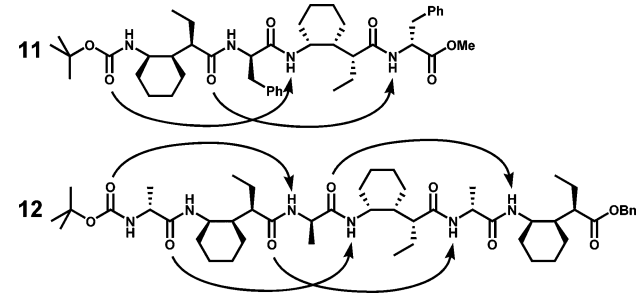


Figure 2. Intramolecular H-bonding patterns in the crystal structures of **11** and **12.**

The 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 $CDCl_3$ solution to enable nuclear Overhauser effect spectroscopy (NOESY) analysis. Among the unambiguous NOEs involving backbone protons, four strong NOEs were observed between protons from different γ residues: $C_\gamma H(2)\cdots NH(4)$, $C_\gamma H(2)\cdots C_\alpha H(4)$, $C_\gamma H(4)\cdots C_\alpha H(6)$, and $C_\gamma H(4)\cdots NH(6)$ (Figure 4). The $C_\gamma H(i)\cdots NH(i+2)$ distances in the crystal structure of **12** are 2.5 and 2.7 Å, and the $C_\gamma H(i)\cdots C_\alpha H(i+2)$ 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 and co-workers[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.

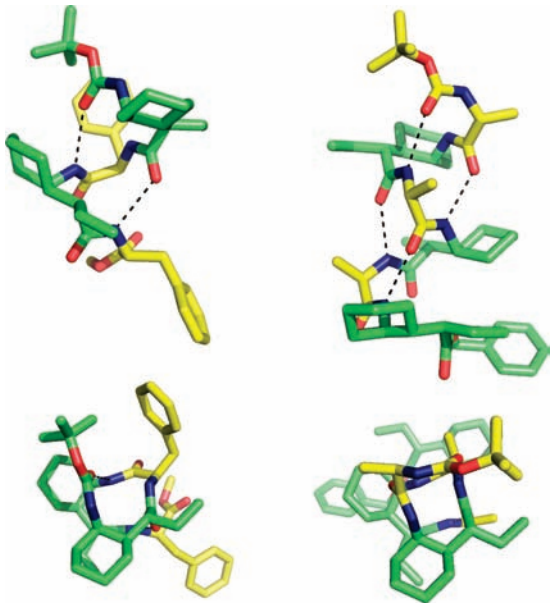


Figure 3. Crystal structures of (left) **11** and (right) **12**: (top) views perpendicular to the helical axis; (bottom) views along the helical axis.

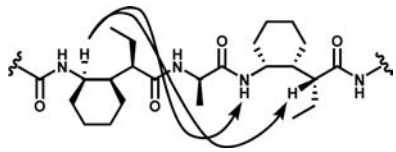


Figure 4. Characteristic NOEs patterns observed for the 1:1 α/γ-peptide hexamer **12** in $CDCl_3$.

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_α. The key step is Michael addition of an aldehyde to 1-nitrocyclohexene, a process that is catalyzed by pyrrolidine **A** and strongly favors just one of the eight possible stereoisomers. A second stereoisomer is available via epimerization at C_γ; the 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.

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Supporting Information Available: Experimental procedures, compound characterizations, and crystallographic data for **11** and **12** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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