

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_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.

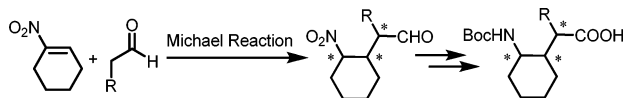
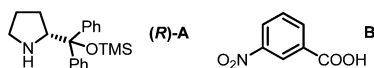


Figure 1

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 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 iminium 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	<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

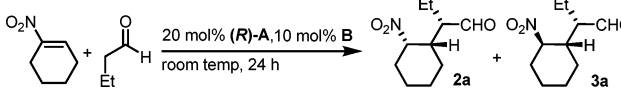
^a Reactions were performed with 1.0 M 1-nitrocyclohexene using 2 equiv of aldehyde. ^b Determined from ¹H NMR analysis of the crude reaction mixture after 24 h. ^c See the Supporting Information for details. ^d M stands for all of the Michael adduct diastereomers observed by NMR spectroscopy. ^e Using 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 CH₂Cl₂ 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

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.

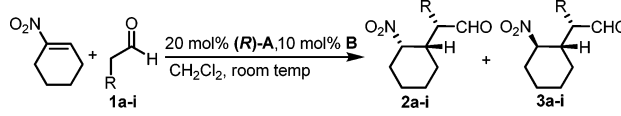
Table 2. Solvent Effects^a



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>i</i> -PrOH	1.0	55	4:1
6	CHCl ₃	1.0	85	10:1
7	CH ₂ Cl ₂	1.0	86	10:1
8	CHCl ₃	0.5	81	15:1
9	CH ₂ Cl ₂	0.5	82	17:1

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

Table 3. Aldehyde Variation

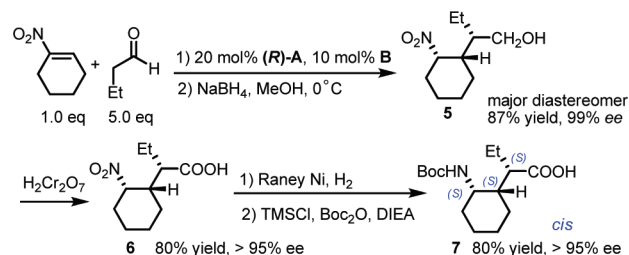


entry	product	R	time (h)	yield (%) ^a	dr ^b	ee (%) ^{c,d}
1	2a	Et	38	87	17:1	99
2	2b	Me	36	84	8:1	97
3	2c	<i>n</i> -Pr	40	86	16:1	99
4	2d	<i>i</i> -Pr	54	79	10:1	>99
5	2e	<i>n</i> -Bu	40	85	16:1	99
6	2f	<i>n</i> -Hex	42	81	15:1	>99
7	2g	<i>c</i> -Hex	54	70	16:1	>99
8	2h	CH ₂ CH ₂ Ph	40	75	9:1	98
9	2i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

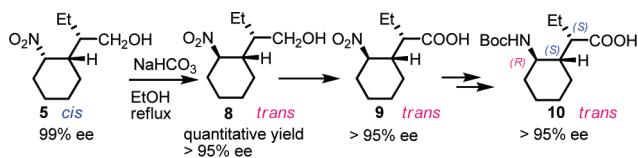
^a Yield of isolated alcohol (major diastereomer) after reduction with NaBH₄. ^b Determined from ¹H NMR analysis of the crude reaction mixture. ^c Determined by chiral HPLC analysis of the alcohols derived from **2a–i**. ^d Absolute configurations of **2a** and **3a** were determined by X-ray structure 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 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**.

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-workers¹³ 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**.

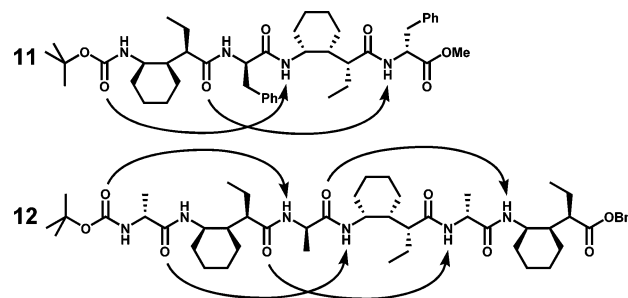


Figure 2. Intramolecular H-bonding patterns in the crystal structures of **11** and **12**.

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 CDCl₃ 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)···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 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 $\text{NH}(i)\cdots\text{NH}(i+1)$ NOEs were observed.

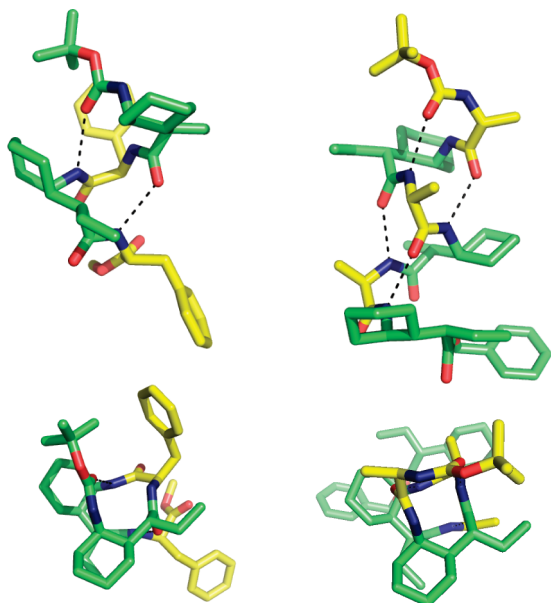


Figure 3. Crystal structures of (left) **11** and (right) **12**: (top) views perpendicular to the helical axis; (bottom) views along the helical axis.

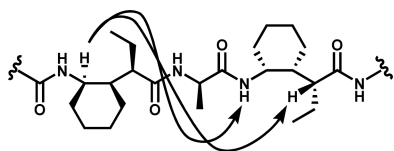


Figure 4. Characteristic NOEs patterns observed for the 1:1 α/γ -peptide hexamer **12** in CDCl_3 .

We have developed a short and general route to γ -amino acids that feature a cyclohexyl constraint on the $\text{C}_\beta\text{--C}_\gamma$ 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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Reidenbach, and Weicheng Zhang for help with preparation of materials; and Prof. A. J. Andre Cobb for sharing unpublished results.

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>.

References

- (1) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (b) *Foldamers: Structure, Properties and Applications*; Hecht, S., Huc, I., Eds.; Wiley-VCH: Weinheim, Germany, 2007. (c) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nat. Chem. Biol.* **2007**, *3*, 252. (d) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiversity* **2004**, *1*, 1111.
- (2) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399.
- (3) (a) Huck, B. R.; Fisk, J. D.; Gellman, S. H. *Org. Lett.* **2000**, *2*, 2607. (b) De Pol, S.; Zorn, C.; Klein, C. D.; Zerbe, O.; Reiser, O. *Angew. Chem., Int. Ed.* **2004**, *43*, 511. (c) Baldauf, C.; Gunther, R.; Hofmann, H. J. *Biopolymers* **2006**, *84*, 408. (d) Sharma, G. V. M.; Nagendar, P.; Jayaprakash, P.; Krishna, P. R.; Ramakrishna, K. V. S.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2005**, *44*, 5878.
- (4) (a) Hayen, A.; Schmitt, M. A.; Ngassa, F. N.; Thomasson, K. A.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 505. (b) Schmitt, M. A.; Choi, S. H.; Guzei, I. A.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 13130. (c) Schmitt, M. A.; Choi, S. H.; Guzei, I. A.; Gellman, S. H. *J. Am. Chem. Soc.* **2006**, *128*, 4538. (d) Horne, W. S.; Price, J. L.; Keck, J. L.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 4178. (e) Choi, S. H.; Guzei, I. A.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 13780.
- (5) (a) Sadowsky, J. D.; Schmitt, M. A.; Lee, H.-S.; Umezawa, N.; Wang, S.; Tomita, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11966. (b) Sadowsky, J. D.; Fairlie, W. D.; Hadley, E. B.; Lee, H. S.; Umezawa, N.; Nikolovska-Coleska, Z.; Wang, S. M.; Huang, D. C. S.; Tomita, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 139. (c) Horne, W. S.; Price, J. L.; Gellman, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 9151. (d) Horne, W. S.; Johnson, L. M.; Ketts, T. J.; Klasse, P. J.; Lu, M.; Moore, J. P.; Gellman, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 14751.
- (6) (a) Appella, D. H.; Barchi, J. J.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309. (b) LePlae, P. R.; Fisk, J. D.; Porter, E. A.; Weissblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 6820. (c) Lee, M.; Raguse, T. L.; Schinnerl, M.; Pomerantz, W. C.; Wang, X.; Wipf, P.; Gellman, S. H. *Org. Lett.* **2007**, *9*, 1801.
- (7) (a) Hanessian, S.; Luo, X.; Schaum, R.; Michnick, S. J. *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, M. A. *J. Am. Chem. Soc.* **2004**, *126*, 6048. (d) Vasudev, P. G.; Ananda, K.; Chatterjee, S.; Aravinda, S.; Shamala, N.; Balaram, P. *J. Am. Chem. Soc.* **2007**, *129*, 4039.
- (8) (a) Hagihara, M.; Anthony, N. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6568. (b) Woll, M. G.; Lai, J. R.; Guzei, I. A.; Taylor, S. J. C.; Smith, M. E. B.; Gellman, S. H. *J. Am. Chem. Soc.* **2001**, *123*, 11077. (c) Qureshi, M. K. N.; Smith, M. *Chem. Commun.* **2006**, 5006.
- (9) For reviews, see: (a) Berner, O. M.; Tedeschi, L.; Enders, D. *Eur. J. Org. Chem.* **2002**, 1877. (b) Santanu, M.; Yang, J. W.; Hoffmann, S.; List, B. *Chem. Rev.* **2007**, *107*, 5471.
- (10) For selected examples of organocatalytic Michael additions of aldehydes to nitroalkenes, see: (a) Betancort, J. M.; Barbas, C. F., III. *Org. Lett.* **2001**, *3*, 3737. (b) Alexakis, A.; Andrey, O. *Org. Lett.* **2002**, *4*, 3611. (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, S. H. *J. Am. Chem. Soc.* **2008**, *130*, 5608. (f) Wiesner, M.; Revell, J. D.; Tonazzi, S.; Wennemers, H. *J. Am. Chem. Soc.* **2008**, *130*, 5610. (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, F. G.; Yee, K. C. *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, H. J. *Org. Lett.* **2001**, *3*, 2423.
- (12) (a) Vasudev, P. G.; Chatterjee, S.; Shamala, N.; Balaram, P. *Acc. Chem. Res.* [Online early access]. DOI: 10.1021/ar9001153. Published Online: July 2, 2009. (b) Chatterjee, S.; Vasudev, P. G.; Raghothama, S.; Ramakrishnan, C.; Shamala, N.; Balaram, P. *J. Am. Chem. Soc.* **2009**, *131*, 5956. (c) Chatterjee, S.; Vasudev, P. G.; Raghothama, S.; Shamala, N.; Balaram, P. *Biopolymers* **2008**, *90*, 759. (d) Vasudev, P. G.; Chatterjee, S.; Ananda, K.; Shamala, N.; Balaram, P. *Angew. Chem., Int. Ed.* **2008**, *47*, 6430. (e) Chatterjee, S.; Vasudev, P. G.; Ananda, K.; Raghothama, S.; Shamala, N.; Balaram, P. *J. Org. Chem.* **2008**, *73*, 6595.
- (13) Baldauf, C.; Gunther, R.; Hofmann, H. J. *J. Org. Chem.* **2006**, *71*, 2000.

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