ORGANIC LETTERS

2004
Vol. 6, No. 24
4411−4414

Synthesis of 4,4-Disubstituted 2-Aminocyclopentanecarboxylic Acid Derivatives and Their Incorporation into 12-Helical β-Peptides

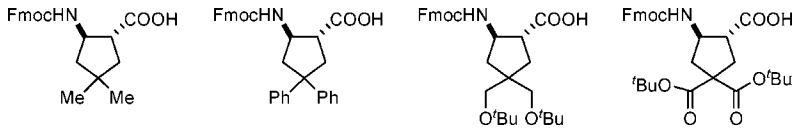
Timothy J. Peelen,[†] Yonggui Chi,[†] Emily Payne English, and Samuel H. Gellman*

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

gellman@chem.wisc.edu

Received August 20, 2004

ABSTRACT



An enantioselective synthetic route is reported for *trans*-2-aminocyclopentanecarboxylic acids (ACPC) bearing geminal side chain pairs at the 4-position. β-Peptides containing the 4,4-disubstituted ACPC residues adopt the 12-helical conformation, as demonstrated by 2D NMR analysis in aqueous solution. These 4,4-disubstituted ACPC residues display functional groups, including acidic and hydrogen bond donating groups, in a geometrically defined fashion, which should be useful for the design of β-peptides for specific applications.

Unnatural oligomers with discrete folding propensities ("foldamers") have received much attention in recent years.[1,2] Oligomers consisting of β-amino acids constrained with five-membered rings (Figure 1), such as *trans*-2-aminocyclopentanecarboxylic acid (ACPC) and *trans*-3-aminopyrrolidine-4-carboxylic acid (APC), have been shown to adopt a helical conformation defined by 12-membered-ring hydrogen bonds, C═O(i) → N-H(i + 3) ("12-helix").[3] A 12-helical structure can be observed with as few as six residues in aqueous solution.[4] Properly designed 12-helical β-peptides mimic the antimicrobial activity of natural host-defense peptides.[5] Biological applications of non-12-helical β-peptides have also been reported.[6]

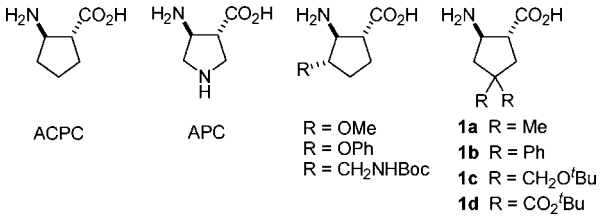


Figure 1. β-Amino acids with five-membered ring constraint.

The synthesis of a diverse set of β-amino acid residues is vital for developing β-peptides with useful functions.[7] Previously we showed that side chains can be introduced into 12-helical β-peptides by sulfonylation of the ring nitrogen of APC[8] or by introduction of a few acyclic β-amino acids among ACPC and/or APC residues.[9] Diversity can be achieved also by introducing side chains on the cyclopentane ring. Recently, we reported the synthesis of ACPC residues

[†] These authors contributed equally to the work.

(1) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219−3232.

(2) For a review, see: Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4011. For selected recent examples, see: Jiang, H.; Léger, J.-M.; Huc, I. *J. Am. Chem. Soc.* **2003**, *125*, 3448−3449. Levins, C. G.; Schafmeister, C. E. *J. Am. Chem. Soc.* **2003**, *125*, 4702−4703.

(3) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180. (b) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Richards, M. R.; Powell, D. R.; Gellman S. H. *J. Am. Chem. Soc.* **1999**, *121*, 7574−7581.

(4) Wang, X.; Espinosa, J. F.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 4821−4822.

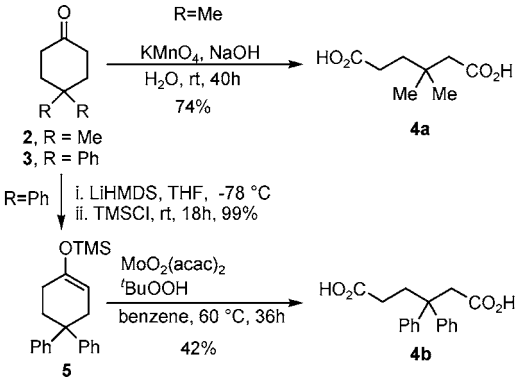
(5) (a) Porter, E. A.; Wang, X.; Lee, H.-S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565. (b) Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 7324−7330.

10.1021/ol0483293 CCC: $27.50
Published on Web 11/05/2004

containing side chains at the 3-position of ACPC.[10] Here we disclose a route to cyclopentane-based β-amino acids that are symmetrically disubstituted at the 4-position (Figure 1). We demonstrate that these side chains, including the bulky phenyl substituent, are compatible with folding into the 12-helical conformation. Thus, the new β-amino acids allow the incorporation of a variety of functional groups along the outside of the 12-helical scaffold, including acidic and hydrogen bond donating groups.

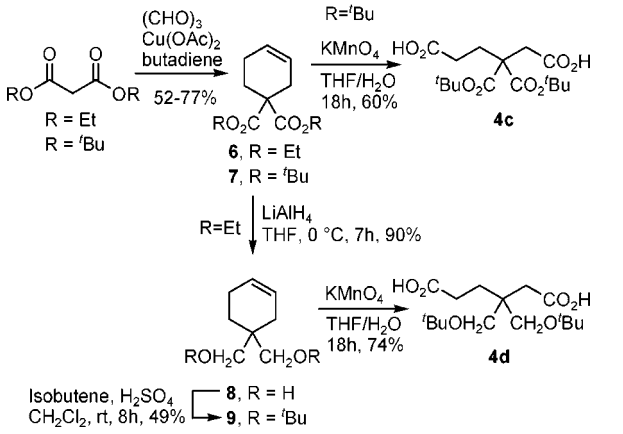
β-Amino acids **1a**−**d** were prepared enantioselectively from 3,3-disubstituted hexanedioic acids. The dicarboxylic acid precursors for monomers **1a** (R = Me) and **1b** (R = Ph) could be accessed by oxidation of known cyclohexanones (Scheme 1). 4,4-Dimethylcyclohexanone (**2**) readily underwent oxidative C−C bond cleavage in aqueous $KMnO_4$ to furnish the desired diacid **4a**.[11] The more hydrophobic 4,4-diphenylcyclohexanone could not be cleaved using aqueous conditions. Instead, the ketone was converted to TMS enol ether **5**,[12] which was oxidized to diacid **4b** in organic solvents.[13]

Scheme 1



Precursors for monomers **1c** and **1d** were prepared by the oxidation of disubstituted cyclohexenes (Scheme 2). Disubstituted cyclohexenes **6** and **7** were prepared from the Diels−Alder reaction between butadiene and methylidene malonate generated in situ from the corresponding malonate ester and formaldehyde.[14] Cyclohexene **6** was reduced to diol **8** and protected as the bis-*tert*-butyl ether **9**. Disubstituted cyclohexenes **7** and **9** were oxidized to the corresponding diacids **4c** and **4d** in aqueous $KMnO_4$.

Scheme 2



Diacids **4a**−**d** were converted to enantiopure aminoesters **12a**−**d** and **13a**−**d** through an auxiliary-based synthesis (Scheme 3). The diacids **4a**−**d** were first converted to the corresponding diesters **10a**−**d**, which were cyclized to the β-keto esters **11a**−**d**. The 4,4-disubstituted regioisomeric product was predominant in each case, presumably because of steric effects. The β-keto esters were converted to diastereomeric *trans*-amino esters **12a**−**d** and **13a**−**d**, in one-pot operations, by enamine formation with α-methylbenzylamine and subsequent reduction using $NaBH_3CN$.[15] In each case the reduction was highly *trans*-selective, albeit with low selectivity between the two *trans* diastereomers. These isomers could be readily separated from each other and from the minor *cis* isomers by column chromatography, allowing access to both enantiomers of the β-amino acids.[16] The stereochemistry of the diastereomeric β-amino esters was assigned by X-ray crystallography of salts derived from **12a**−**c**.[17,18] Saponification of the ester, hydogenolysis of the auxiliary, and Fmoc protection yielded the protected β-amino acids **14a**−**d** and ***ent*-14a**−**d**.[19]

To determine whether 4,4-disubstituted ACPC residues are compatible with the 12-helix, we prepared β-peptides **15** and

(6) (a) Werder, M.; Hauser, H.; Abele, S.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1774−1783. (b) Hamuro, Y.; Schneider, J. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1999**, *121*, 12200−12201. (c) Liu, D.; DeGrado, W. F. *J. Am. Chem. Soc.* **2001**, *123*, 7553−7559. (d) Gademann, K.; Kimmerlin, T.; Hoyer, D.; Seebach, D. *J. Med. Chem.* **2001**, *44*, 2460−2468. (e) Raguse, T. L.; Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 12774−12785. (f) Gelman, M. A.; Richter, S.; Cao, H.; Umezawa, N.; Gellman, S. H.; Rana, T. M. *Org. Lett.* **2003**, *5*, 3563−3565. (g) Arvidsson, P. I., Ryder, N. S., Weiss, H. M., Gross, G., Kretz, O., Woessner, R., Seebach, D. *ChemBioChem* **2003**, *4*, 1345−1347. (h) Potocky, T. B.; Menon, A. K.; Gellman, S. H. *J. Biol. Chem.* **2003**, *278*, 50188−50194.

(7) For a recent review on β-amino acid synthesis, see: (a) Liu, M.; Sibi, M. P. *Tetrahedron* **2002**, *58*, 7991−8035. For selected recent examples, see: (b) Tang, W.; Wu, S.; Zhang, X. *J. Am. Chem. Soc.* **2003**, *125*, 9570−9571. (c) Minter, A. R.; Fuller, A. A.; Mapp, A. K. *J. Am. Chem. Soc.* **2003**, *125*, 6846−6847. (d) Davis, F. A.; Prasad, K. R.; Nolt, M. B.; Wu, Y. *Org. Lett.* **2003**, *5*, 925−927.

(8) Lee, H.-S.; Syud, F. A.; Wang, X.; Gellman, S. H. *J. Am. Chem. Soc.* **2001**, *123*, 7721−7722.

(9) LePlae, P. R.; Fisk, J. D.; Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 6820−6821.

(10) (a) Woll, M. G.; Fisk, J. D.; LePlae, P. R.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 12447−12452. (b) Bunnage, M. E.; Chippindale, A. M.; Davies, S. G.; Parkin, R. M.; Smith, A. D.; Withey, J. M. *Org. Biomol. Chem.* **2003**, *1*, 3698−3707.

(11) Maryanoff, B. E.; Costanzo, M. J.; Nortey, S. O.; Greco, M. N.; Shank, R. P.; Schupsky, J. J.; Ortegon, M. P.; Vaught, J. L. *J. Med. Chem.* **1998**, *41*, 1315−1343

(12) Heathcock, C. H.; Buse, C. T.; Kleschick, W. A.; Pirrung, M. C.; Sohn, J. E.; Lampe, J. *J. Org. Chem.* **1980**, *45*, 1066−1081.

(13) Kaneda, K.; Kii, N.; Jitsukawa, K.; Teranishi, S. *Tetrahedron Lett.* **1981**, *22*, 2595−2598.

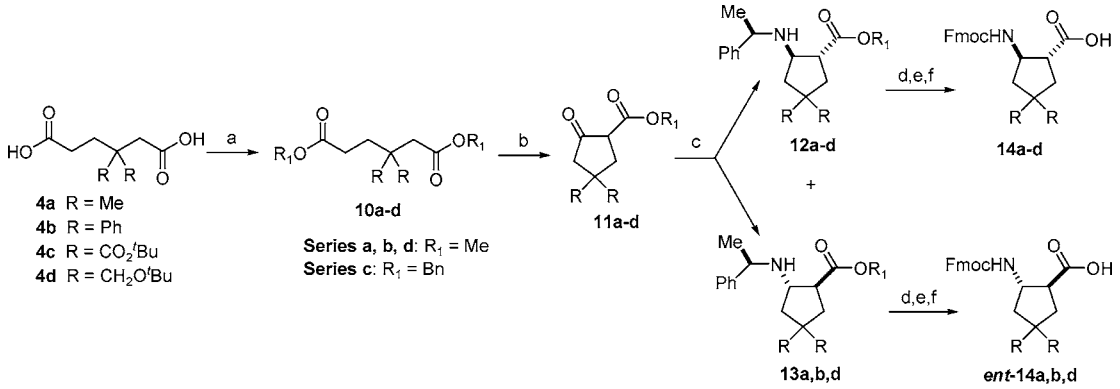
(14) (a) De Keyser, J. L.; De Cock, C. J. C.; Poupaert, J. H.; Dumont, P. *J. Org. Chem.* **1988**, *53*, 4859−4862. (b) Nenajdenko, V. G.; Statsuk, A. V.; Balenkova, E. S. *Tetrahedron* **2000**, *56*, 6549−6556.

(15) *S*)-α-Methylbenzylamine was used with β-ketoesters **11c** and **11d**, not the (*R*)-enantiomer as shown.

(16) Compound **12c** was purified from the other diastereomeric products by selective recrystallization of the amine **12c**·HCl salt, not column chromatography.

(17) See Supporting Information.

Scheme 3[a]



[a] Reagents and conditions: (a) MeOH, benzene, H_2SO_4 or MeI, K_2CO_3, DMF or BnBr, Cs_2CO_3, CH_3CN, 70−100%; (b) KO^{t}Bu, THF, 51−70%; (c) (i) (*R*)-(+)-α-methylbenzylamine, AcOH, MeOH, (ii) $NaBH_3CN$, 38−41% of **12a**−**d**, 10−19% of **13a**−**d**; (d) LiOH, 0 °C; (e) 10% Pd/C/NH_4HCO_2, MeOH, reflux, 16 h; (f) Fmoc-OSu/$NaHCO_3$, acetone/H_2O, rt, 12 h, 63−76% over 3 steps.

16.[20] Hexamers **15** and **16** were both suitable for two-dimensional NMR analysis (Figure 2). The proton resonances

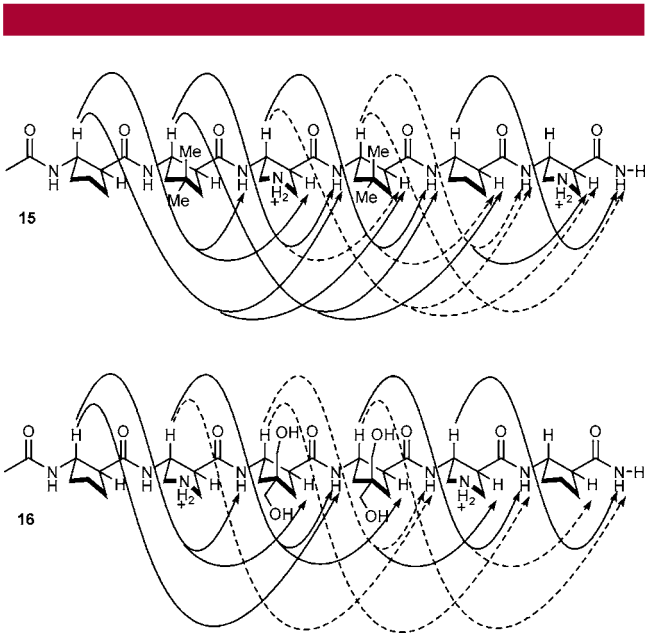


Figure 2. NOEs between nonadjacent residues observed for hexamers containing 4,4-disubstituted ACPC residues in 9:1 H_2O/D_2O at pH 3.8. Dashed lines indicate NOEs that are ambiguous because of resonance overlap. Data were obtained on a Varian 600 MHz spectrometer. Data for **15** was acquired at 14 °C at 5−10 mM concentration. Data for **16** was acquired at 4 °C at 5−10 mM concentration.

of these *β*-peptides were well resolved, both in CD_3OH and in aqueous solution. NOEs are indicated as ambiguous where resonance overlap precludes a definitive assignment (Figure 2).

Hexamer **15** displayed NOEs in methanol consistent with the 12-helical conformation. Three unambiguous $C_\beta H(i)$ to $C_\alpha H(i + 2)$ NOEs were assigned (of four possible NOEs of this type); four unambiguous $C_\beta H(i)$ to NH(i + 2) NOEs were assigned (of five possible NOEs), and two unambiguous $C_\beta H(i)$ to NH(i + 3) NOEs were assigned (of four possible NOEs). These observations are fully consistent with a 12-helical structure. Hexamer **15** displayed a similar set of NOEs in water. Two of four $C_\beta H(i)$ to $C_\alpha H(i + 2)$ NOEs, four of five $C_\beta H(i)$ to NH(i + 2) NOEs, and two of four $C_\beta H(i)$ to NH(i + 3) NOEs were unambiguously assigned.

Interestingly, a previously unobserved type of NOE was observed for hexamer **15** in water. Two NOEs from $C_\beta H(i)$ to $C_\alpha H(i + 3)$ were identified. Crystal structure data for 12-helical ACPC oligomers[21,3b] suggest that these two protons would be too far apart for an NOE between them to be observed.[22] This contradiction suggests that the "12-helix" may be a family of related conformers, perhaps in rapid exchange with one another, rather than a single, rigid conformation. The introduction of side chains at particular positions on the five-membered rings of individual residues may perturb the 12-helix conformational manifold in such a way that new NOEs are detected.

Hexamer **16** also displayed NOEs consistent with 12-helical structure in both methanol and water. In methanol, one of four $C_\beta H(i)$ to $C_\alpha H(i + 2)$ NOEs, four of five $C_\beta H(i)$

(18) The stereochemistry of **12d** and **13d** could be determined by incorporating **14d** and ***ent*-14d** into short *β*-peptides containing cyclic *β*-amino acid residues with known configuration, including (*S*,*S*)-ACPC. The matched *β*-peptide, containing **14d**, showed a 12-helical signature by CD, whereas the mismatched *β*-peptide, containing ***ent*-14d**, showed a very different CD signature.

(19) ***ent*-14c** could be incorporated into *β*-peptides and deprotected with no observed decarboxylation of the *β*-dicarboxylic acids; see Supporting Information.

(20) For CD characterization of these and other *β*-peptides containing 4,4-disubstituted ACPC residues, see Supporting Information.

(21) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J., Jr.; Gellman, S. H. *Nature* **1997**, *387*, 381−384.

(22) The $C_H\beta(i)$ to $C_H\alpha(i + 3)$ NOEs are unlikely to arise from spin diffusion because they were detected via ROESY. We attempted to probe directly for spin diffusion by examining NOE intensity buildup as a function of mixing time (NOESY), but poor signal-to-noise ratio hampered this approach, presumably because the molecular weight of the hexa-*β*-peptides falls in the range where the NOE is close to zero intensity. The origin and significance of $C_H\beta(i)$ to $C_H\alpha(i + 3)$ NOEs will be examined with larger *β*-peptides in the future.

to NH(i + 2) NOEs, and one of four $C_\beta H(i)$ to NH(i + 3) NOEs were unambiguously assigned. In water, the same types of NOEs were identified and unambiguously assigned: three of four $C_\beta H(i)$ to $C_\alpha H(i + 2)$ NOEs, four of five $C_\beta H(i)$ to NH(i + 2) NOEs, and one of four $C_\beta H(i)$ to NH(i + 3) NOEs. Only one fewer NOE was observed in water for this peptide than in methanol, perhaps suggesting that the structural differences between the two solvents are minimal.

Acknowledgment. This research was supported by the NIH (GM56414). T.J.P was supported by a NIH postdoctoral fellowship (GM065713). E.P.E. was supported by a NDSEG fellowship that is sponsored by the Department of Defense. NMR spectrometers were purchased with partial support from NIH and NSF. CD spectra were obtained in the NSF-supported Biophysics Instrumentation Facility. Crystallographic work was performed by Dr. Ilia A. Guzei.

Supporting Information Available: General procedures, characterization data, ^1H and ^{13}C spectra of all numbered intermediates in the schemes and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0483293