# Elevated Conformational Rigidity in Dipeptides Incorporating Piperazic Acid Derivatives

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**Abstract:** *N*-Acyl derivatives of piperazic acids display an unusual degree of conformational rigidity. As a consequence, α-N-coupling of a piperazic acid of given configuration (D or L) with an α-aminoacyl unit of opposite configuration (L or D) produces a "heterochiral" dipeptide that exists in a conformation conducive to the formation of a peptide turn. Piperazic acids and related compounds may thus be regarded as conformationally rigid analogues of proline, and because they are readily available in either antipodal form by synthesis, they should become of great interest in the study of peptide turns.

## Introduction

There is much current interest in the preparation of peptides and peptidomimetics with a well-defined conformation.[1] These conformationally constrained peptides are useful to probe issues of protein folding and to mimic peptide turns. The latter structural motifs are of special relevance as key features of *β*-sheet architectures (hairpin turns)[2] and because of their perceived role in ligand−receptor binding interactions and in other functional aspects of protein chemistry ("*β*-turns", "zinc fingers", etc.).[3] Turns frequently occur at the site of a proline, probably because unlike a secondary amide, which is rigidly held in an *s-Z* conformation, the flexibility of the tertiary amide unit of *N*-acylprolines may accommodate such turns more readily. Turns are also favored when the proline and the amino acid contributing its *N*-acyl group are of opposite configuration.[4] However, introduction of a proline within a peptide chain is insufficient, per se, to induce a turn, because *N*-acylprolines do not manifest a sufficiently great preference for that conformer which promotes turn formation. Indeed, peptide turns must be further stabilized by a number of intramolecular interactions, e.g., hydrogen bonds.[5] At least 20−30 amino acids seem to be necessary to stabilize a turn induced by a proline residue and to create a conformationally rigid construct.[6] We have found that 2,3,4,5-tetrahydropyridazine-3-carboxylic acids ("PCAs"), readily available in either the D or L configuration by synthesis, behave as conformationally fixed proline equivalents. In particular, N-2 acylation of PCAs with an α-amino acid of opposite configuration generates a dipeptide that possesses the vector properties of a peptide turn. Furthermore, PCAs are good mimics of proline; therefore, it is likely that PCA dipeptides may be used as building blocks for turn mimics that more closely resemble their naturally occurring originals.

## Results and Discussion

We have recently described dipeptide **1a** (Table 1),[7] a key subunit of the antitumor and anti-HIV antibiotics luzopeptins and quinoxapeptins ("peptin" antibiotics).[8] This material, together with the similar dipeptides **1b** (a precursor to luzopeptin E2) and **1c**, all related *N*-2-acyl derivatives of 4-hydroxy-2,3,4,5-tetrahydropyridazine-3-carboxylic acid or 2,3,4,5-tetrahydropyridazine-3-carboxylic acid **1d**−**1l**, as well as *N*-2-acetyl analogues **2** and **3** exist as a single rotamer about the *N*-2-acyl bond within the limits of detection of $^{1}H$ and $^{13}C$ NMR spectroscopy (5.87 T). Compounds **1b**−**1l**, **2**, and **3** were

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1997.

(1) Cf. (a) Regan, L.; DeGrado, W. F. *Science* **1988**, *241*, 976. (b) Hecht, M. H.; Richardson, J. S.; Richardson, D. C.; Ogden, R. C. *Science* **1990**, *249*, 884. (c) Osterhout, J. J., Jr.; Handel, T.; Na, G.; Arazdordi, T.; Long, R. C.; Connolly, P. J.; Hoch, J. C.; Johnson, W. C. Jr., Live, D.; DeGrado, W. F. *J. Am. Chem. Soc.* **1992**, *114*, 331.

(2) Cf. Smith, C. K.; Regan, L. *Acc. Chem. Res.* **1997**, *30*, 153.

(3) Cf. (a) Creighton, T. E. *Proteins: Structure and Molecular Properties;* Freeman: New York, 1983. (b) Rizo, J.; Gierasch, L. *Annu. Rev. Biochem.* **1992**, *61*, 387. (c) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1. (d) Ferguson, M. D.; Meara, J. P.; Nakanishi, H.; Lee, M. S.; Kahn, M. *Tetrahedron Lett.* **1997**, *38*, 6961 and references cited therein.

(4) One of the earliest examples of the turn-forming ability of *N*-acyl proline dipeptides composed of amino acids of opposite configurations ("heterochiral sequences") may be found in the structure of gramicidin: Hull, S. E.; Karlsson, R.; Main, P.; Woolfson, M. M.; Dodson, E. J. *Nature* **1978**, *275*, 206. An excellent discussion of the turn-forming ability of heterochiral sequences appears in ref 3c, p 22 ff.

(5) Alternative techniques used to stabilize turns may include formation of disulfide bridges (cf. Quinn, T. P.; Tweedy, N. B.; Williams, R. W.; Richardson, J. S.; Richardson, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8747) or metal ligation (cf. Handel, T. M.; Williams, S. A.; DeGrado, W. F. *Science* **1993**, *261*, 879). In extreme cases, a rigid scaffolding resembling the environment of turns has been used to enforce a desired conformation: e.g., Kim, K.; Germanas, J. P. *J. Org. Chem.* **1997**, *62*, 2847, 2853. Lombart, H. G.; Lubell, W. D. *J. Org. Chem.* **1996**, *61*, 9437.

(6) Struthers, M. D.; Cheng, R. P.; Imperiali, B. *Science* **1996**, *271*, 342.

(7) Ciufolini, M. A.; Xi, N. *J. Org. Chem.* **1997**, *62*, 2320.

(8) Luzopeptins: (a) Konishi, M.; Ohkuma, H.; Sakai, F.; Tsuno, T.; Koshiyama, H.; Naito, T.; Kawaguchi, H. *J. Am. Chem. Soc.* **1981**, *103*, 1241. (b) Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 1243 and references therein. Quinoxapeptins: (c) Lingham, R. B.; Hsu, A. H. M.; O'Brien, J. A.; Sigmund, J. M.; Sanchez, M.; Gagliardi, M. M.; Heimbuch, B. K.; Genilloud, O.; Martin, I.; Diez, M. T.; Hirsch, C. F.; Zink, D. L.; Liesch, J. M.; Koch, G. E.; Gartner, S. E.; Garrity, G. M.; Tsou, N. N.; Salituro, G. M. *J. Antibiot.* **1996**, *49*, 253. We propose the convenient descriptor "peptins" to refer to the entire class of these antibiotics.

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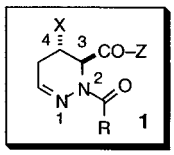
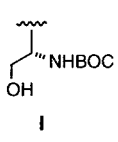
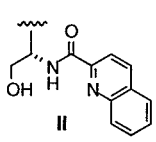
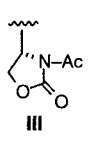
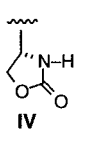
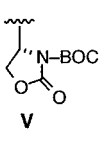
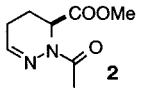
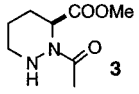
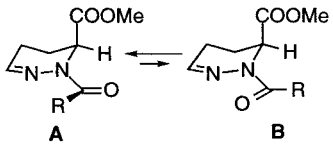
**Table 1.** *N*-2-Serinyl Derivatives of 2,3,4,5-Tetrahydropyridazine-3-carboxylic Acids (Compounds **1**) and *N*-2-Acetyl Analogues (Compounds **2** and **3**) That Exist as Single Rotamers about the Tertiary Amide Structure ($^1$H and $^{13}$C NMR Spectroscopy)[a]



R :  I  II  III  IV  V

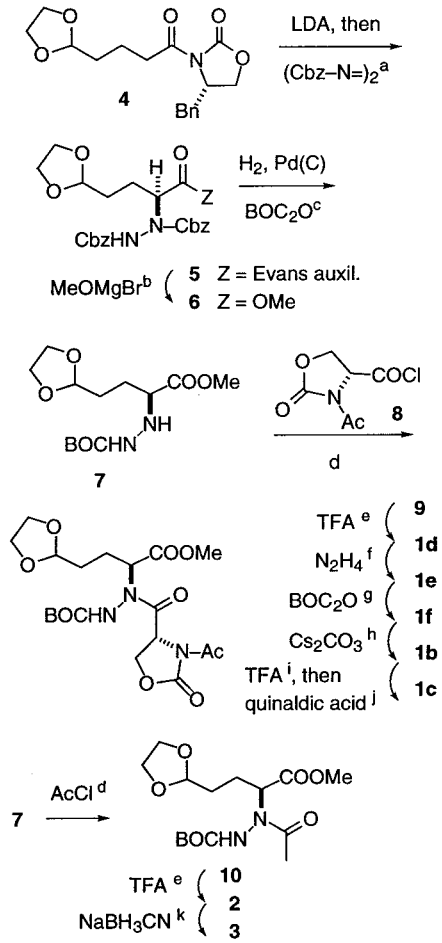
| Entry | 1a | 1b | 1c | 1d | 1e | 1f | 1g | 1h | 1i | 1j | 1k | 1l |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | I | I | II | III | IV | V | III | IV | IV | IV | IV | V |
| X | OH | H | H | H | H | H | OAc | OAc | OH | OH | OAc | OAc |
| Z | NHBu-*n* | OMe | OMe | OMe | OMe | OMe | OBu-*i* | OBu-*i* | OBu-*i* | NHBu-*n* | NHBu-*n* | NHBu-*n* |

 2  3  A B

[a] All compounds were observed in $CDCl_3$ solutions at rt, except **1c**, which was observed in $CDCl_3$, $CD_3CN$, $(CD_3)_2CO$, and $(CD_3)_2SO$ solutions. Different solvents had virtually no effect on the conformation of **1c**.

prepared from **4** as shown in Scheme 1.[9] The degree of conformational rigidity observed for **1**−**3** is unusual, and it is clearly produced by the PCA residue. Indeed, compounds similar to **1**−**3**, but displaying a proline or a pipecolinic acid in place of PCA, exist as mixtures of rotamers about the peptide bond (compounds **11**−**13**, Table 2).[10] Experimental ($^1$H NMR) and computational[11] evidence indicates that **1**−**3** and **11**−**13** favor a conformation in which the proline/pipecolinic acid COOR unit is essentially axial or pseudoaxial, while the *N*-acyl carbonyl group points toward the ring atom bearing the COOR unit and away from the imino linkage. This is described in Tables 1 and 2 as conformation **A**, while conformation **B**, wherein the *N*-acyl unit has rotated by approximately 180°, is disfavored. Molecular mechanics (MM+ force field)[11b] and semiempirical (PM3)[12] calculations estimate the conformational energy difference between the two limiting conformers **A** and **B** ($\Delta E_{AB}$) of compounds **11**−**13** to be generally less than 1 kcal/mol, in line with experiment (rotamer ratio ≤4:1). In sharp contrast, the $\Delta E_{AB}$ between the two states of several molecules of type **1**−**3** may be between about 3.5 (PM3)[12] and 5.7 kcal/mol (MM+), corresponding to a rotamer ratio of 370−15600:1 in favor of **A**.

**Scheme 1.**[a]




[a] Reagents and conditions: (a) THF, −78 °C, 65%; (b) MeOH, 0 °C, 80%; (c) MeOH, rt, 93%; (d) *sym*-collidine, $CH_2Cl_2$, 0 °C, 74%; (e) 90% in $H_2O$, rt, 96%; (f) MeCN, rt, 90%; (g) $Et_3N$, cat. DMAP, $CH_2Cl_2$, 0 °C, 99%; (h) MeOH, 88%; (i) 50% in $CH_2Cl_2$; (j) DCC, 70% i−j; (k) TFA, THF, rt, 99%.

Experimental evidence in support of the calculated conformational preferences of *N*-acyl-PCAs may be found in work by Blount.[13] Specifically, the X-ray crystal structure of

(9) Compound **4** was obtained by Schreiber ozonolysis of cyclopentene to methyl 5-oxopentanoate (Claus, R. E.; Schreiber, S. L. *Organic Syntheses;* Wiley: New York, 1990; Collect. Vol. VII, p 168) followed by ketalization, hydrolysis, and coupling to the Evans auxiliary. Relevant references for key steps: (step a) Gennari, C.; Colombo, L.; Bertolini, G. *J. Am. Chem. Soc.* **1986**, *108*, 6394. Evans, D. A.; Britton, T. C.; Dorow, R. L.; Dellaria, J. F. *J. Am. Chem. Soc.* **1986**, *108*, 6395. Trimble, L. A.; Vederas, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 6397. (Step b) Evans, D. A.; Britton, T. C.; Dorow, R. L.; Dellaria, J. F. *Tetrahedron* **1988**, *44*, 5525. (Steps c and d) see refs 7 and 10. (Steps g and h) Ishizuka, T.; Kunieda, T. *Tetrahedron Lett.* **1987**, *28*, 4185.

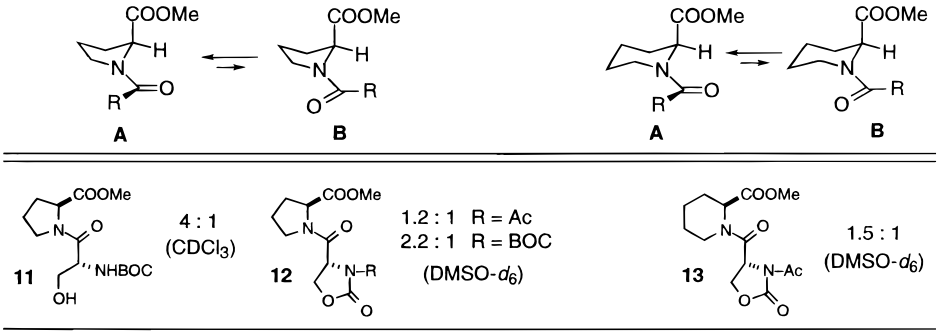
(10) Cf. Ciufolini, M. A.; Xi, N. *Tetrahedron Lett.* **1995**, *36*, 6595.

(11) (a) All calculations were carried out with the Hyperchem 4.0 package, available from Hypercube, Inc., Ontario, Canada. (b) No parameters exist for cyclic hydrazones of the type found in PCA in the $MM^+$ method implemented in this package. The program will automatically use approximate parameters in such cases; consequently, the results of $MM^+$ calculations are of qualitative, rather than quantitative, significance. We relied primarily on PM3 semiempirical methods, because this technique seems to reproduce experimental conformational population ratios rather well.

(12) Repulsive forces are not as strong in PM3, a reparametrized form of AM1, as they are in other semiempirical methods (Hyperchem literature and manuals). Therefore the conformational energy differences calculated by this method are likely to represent lower-end estimates.

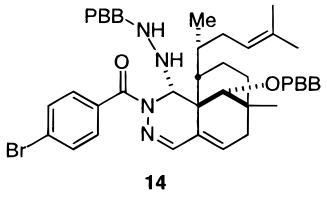
(13) Blount, J. F. *Tetrahedron Lett.* **1975**, 913.

**Table 2.** Rotamer Ratio ($^1$H NMR, 25 °C) for Proline (**11**, **12**) and Pipecolinic Acid (**13**) Analogs of **1b** and **1d**[a]




[a] Rotamer **A** is always favored over **B**.

compound **14**, a derivative of a diformyl terpenoid, reveals that




**14**

PBB = 4-bromobenzoyl

the 4-bromobenzoyl group connected to the tetrahydropyridazine unit exists in a conformation of type **A** (the carbonyl unit points away from the imino linkage). This observation is noteworthy, in that compound **14** is not encumbered by a ring structure that might perturb the innate conformational preferences of the *N*-acyltetrahydropyridazine.[14]

The energy barrier for rotation about the tertiary amide C−N bond serves perhaps as a more useful parameter to gauge the conformational rigidity of *N*-acyl-PCAs, and since only one conformer is readily detected by NMR, rotation about the C−N bond in **1**−**3** may be either very fast or extremely slow. It seems unlikely that fast rotation is operative here, since the bond in question is part of an amide, and indeed, the VTNMR spectrum of **1c** in $(CD_3)_2CO$ shows no evidence for two rotamers down to −89 °C. Indeed, little change is observed in the NMR spectrum at −89 °C relative to room temperature, except for the expected downfield shifts of NH and OH resonances. This suggests that the spectral properties of **1c** and related structures are a consequence of hindered rotation. In an effort to determine the magnitude of the rotational barrier, a VTNMR study of **1c** was conducted at temperatures up to 55 °C in $CDCl_3$, up to 85 °C in $CD_3CN$, up to 93 °C in $C_6D_5CD_3$, and up to 107 °C in DMSO-$d_6$. Again, no significant changes other than expected shifts of NH and OH resonances were apparent in this temperature range. Similarly, VTNMR studies of **2** up to 120 °C in DMSO-$d_6$ revealed no evidence of thermally induced conformational motion, suggesting that the activation barrier for internal rotation of PCA dipeptides is very high.

The NMR experiments on **2** were carried out at a digital resolution of 0.0305 Hz. The spectral resolution increased steadily with increasing temperature. The half-height line width of the central line of the DMSO-*h*-$d_5$ quintet steadily narrowed from 1.30 Hz at room temperature (rt) to 0.40 Hz at >100 °C. The half-height line width of each of the methyl signals (methyl ester and acetyl) of **2** narrowed from 0.67 Hz at rt to 0.46 Hz at 79 °C. At 86 °C or higher, each methyl group appeared as a doublet, $J = 0.18 \pm 0.03$ Hz. Between 86 and 93 °C, the total half-height line width of each methyl resonance was 0.43 Hz. At temperatures between 100 and 121 °C, the total half-height line width narrowed to 0.40 Hz. Selective irradiation of the C-3 methine proton at $\delta$ 5.07 induced collapse of each pair of methyl signals into a singlet, thus demonstrating $^5J_{HH}$ between the methine H and the methyl hydrogens. Such five-bond *J* couplings of ca. 0.2 Hz are well documented in very similar molecular environments.[15] We presume that these *J* couplings became visible at higher temperatures due to the diminished viscosity of the DMSO-$d_6$ solvent and the attendant improvement in spectral resolution. Selective irradiation of the C-6 imino proton at $\delta$ 6.93 did not cause collapse of either pair of methyl signals, the only detectable effects being the expected upfield Bloch−Siegert shifts[16] of 0.33 Hz for the ester methyl group and just 0.20 Hz for the more upfield *N*-acetyl methyl group. As expected, irradiation of the C-3 methine proton at $\delta$ 5.07 resulted in larger upfield Bloch−Siegert shifts of 0.85 Hz for the ester Me group and 0.39 Hz for the *N*-acetyl Me group.

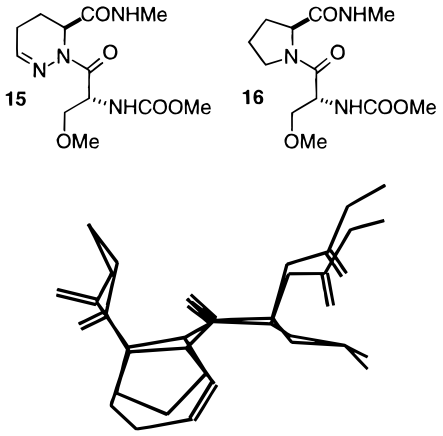



**Figure 1.** Overlay of the PM3-optimized structures of compounds **15** and **16**. dPCA may form tighter turns than proline.

The above results suggest that PCAs might act as conformationally rigid analogues of proline, and as such they might force peptide turns. Figure 1 shows the overlays of the PM3-optimized structures of dipeptide **15**, a computationally better

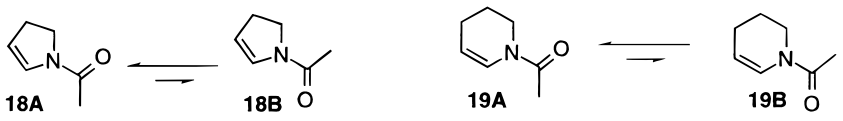
(13) Blount, J. F.; Dunlop, R. W.; Erickson, K. L.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 5, 145.

(14) We are grateful to one of the reviewers for kindly bringing to our attention the work cited in refs 13, 17, and 20.

(15) (a) Davies, D. B.; Abu Khaled, M.; Urry, D. W. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1294. (b) Hayamizu, K.; Yamamoto, O. *J. Mol. Spectrosc.* **1967**, *22*, 119. (c) Riggs, N. V.; Verma, S. M. *Tetrahedron Lett.* **1968**, *19*, 3767.

(16) (a) Mersh, J. D.; Sanders, J. K. M. *J. Magn. Reson.* **1982**, *50*, 289. (b) Hosur, R. V.; Ernst, R. R.; Wüthrich, K. *J. Magn. Reson.* **1983**, *54*, 142. (c) Freeman, R. *A Handbook of Nuclear Magnetic Resonance*; John Wiley & Sons: New York, 1988; pp 14−16.

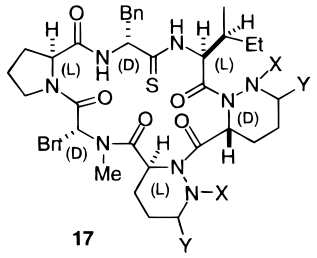
**Table 3.** Experimental[19] and Calculated (PM3) Rotamer Ratios for *N*-Acyl-2-pyrrolideine **18** and *N*-Acyl-2-piperideine **19**[b]




| ΔΔH$_f$, PM3 (kcal/mol) | Est. **A** : **B** ratio[a] | Experimental ratio (ref. 16) | ΔΔH$_f$, PM3 (kcal/mol) | Est. **A** : **B** ratio[a] | Experimental ratio (ref. 16) |
|---|---|---|---|---|---|
| + 0.3 | 1.7 : 1 | 1.9 : 1 | + 0.4 | 2.0 : 1 | 2.3 : 1 |

[a] Estimated (25 °C) from an expression of the type $\Delta\Delta H_f \approx \Delta G^\circ = -RT \ln(K)$. [b] The presence of a $\pi$ system in the ring is insufficient to induce significant bias in favor of conformer **A**.

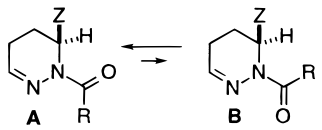
tractable congener of molecule **1b**, and its proline analogue **16**. These compounds display the popular D−L amino acid pair that promotes turn formation.[4,6] It is apparent that a great deal of shape similarity exists between proline and PCA peptides; furthermore, PCAs seem to favor a tighter turn than proline. Therefore, it seems quite plausible that *N*-acyl-PCAs could be utilized to engineer peptides with enforced turns. Some evidence in support of this surmise may be gathered from the work of Bock.[14,17] The X-ray crystal structure of oxytocin antagonist **17** reveals turn motifs at the level of the PCA-type




subunits, which interestingly are each part of a heterochiral sequence and exist in a conformation of type **A**. A motif similar to a $\beta$-turn, though not a $\beta$-turn proper, is also apparent at the level of the proline. Similar conclusions may be drawn also from the X-ray crystal structure of luzopeptin A.[8b] Whereas the turn feature present in this antibiotic is not produced by the *N*-2-(D-serinyl)-PCA amide (the serine N atom carries a quinaldoyl unit), but it rather occurs in proximity of the depsi bond between the D-serine and the carboxy group of L-*N*-methyl-3-hydroxyvaline,[18] it is apparent that the serine N atom and the PCA amide group are arranged in a conformation that would promote turn formation. Moreover, the constraints imposed by the macrocyclic ring cause only slight deviation from the computed (PM3) global minimum for the serine−PCA subunits.

**Proposed Origin of the Conformational Properties of PCAs.** It is unlikely that the presence of a $\pi$ system in the PCA ring suffices, per se, to induce the observed bias in favor of conformer **A**. This is apparent from the $^1$H NMR spectra of enamides **18** and **19**: while the $\pi$ bond does induce a preference for conformers **18A** and **19A** over their corresponding **B** types, this preference is a modest 1.9:1 and 2.3:1, respectively[19] (Table 3). Calculations (PM3) reproduce these experimental values rather well.

**Table 4.** Conformational Energy Differences (PM3) in *N*-2-Acyl-PCAs and Analogs




| Entry | R | Z | ΔΔH$_f$, PM3 (kcal/mol) | Est. **A** : **B** ratio[a] |
|---|---|---|---|---|
| **2** | $CH_3$ | COOMe | + 3.8 | 626 : 1 |
| **20** | H | COOMe | + 3.8 | 626 : 1 |
| **21** | $CH_3$ | H | + 3.7 | 529 : 1 |
| **22** | $CH_3$ | CONHMe | + 4.8 | 3412 : 1 |
| **23** | $OCH_3$ | COOMe | + 1.3 | 9 : 1 |

[a] Estimated (25 °C) from an expression of the type $\Delta\Delta H_f \approx \Delta G^\circ = -RT \ln(K)$.

Intramolecular hydrogen bonding interactions, or steric effects, are also unlikely to be primary determinants of the strong conformational preferences of *N*-2-acyl-PCAs. All compounds of type **1**−**3** display identical conformational behavior, regardless of potential intramolecular H bonding interactions (not possible with **1d**, **1f**, **1g**, **1l**, and **2**) and regardless of the steric bulk of the *N*-2-acyl groups present on the PCA. Furthermore, PM3 calculations on compound **2**, on its *N*-2-formyl analogue **20**, and on structure **21**, wherein the ester group is missing altogether, suggest that conformational energy differences between the **A** and **B** states ($\Delta\Delta H_f$) are large (ca. 3.8 kcal/mol)[12] and practically identical in all three molecules (Table 4). Thus, the steric demand of the *N*-2-acyl group or the presence of the ester unit seems to be largely an insignificant parameter. It is noteworthy, however, that the preference for conformer **A** becomes even stronger in PCA amides (cf. **22**, Table 4), which correspond to the form of PCA found in peptin antibiotics. Conversely, the preference for conformation **A** is calculated to be significantly attenuated in PCA *N*-2-carbamates (cf. **23**).

These computational results suggest that an electrostatic phenomenon may be at the root of the conformational behavior of *N*-acyl-PCAs. We propose that the primary interaction that determines conformational preferences in such structures is the electrostatic repulsion between the carbonyl oxygen and the imino nitrogen atoms in conformers of type **B** and the attendant dipolar effects. Further details of PM3 calculations on compound **2** appear in Figure 2. We stress that the following data probably are useful only in a qualitative, or at most a semiquantitative, sense.[12] It seems that a redistribution of charge occurs in the molecule in going from conformer **2A** to **2B**.

(17) Bock, M. G.; DiPardo, R. M.; Williams, P. D.; Pettibone, D. J.; Clineschmidt, B. V.; Ball, R. G.; Veber, D. F.; Freidiger, R. M. *J. Med. Chem.* **1990**, *33*, 2321.

(18) For a discussion of the ability of depsipeptides to establish turn motifs, see: Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6975.

(19) Cf. Stille, J. K.; Becker, Y. *J. Org. Chem.* **1980**, *45*, 2139.

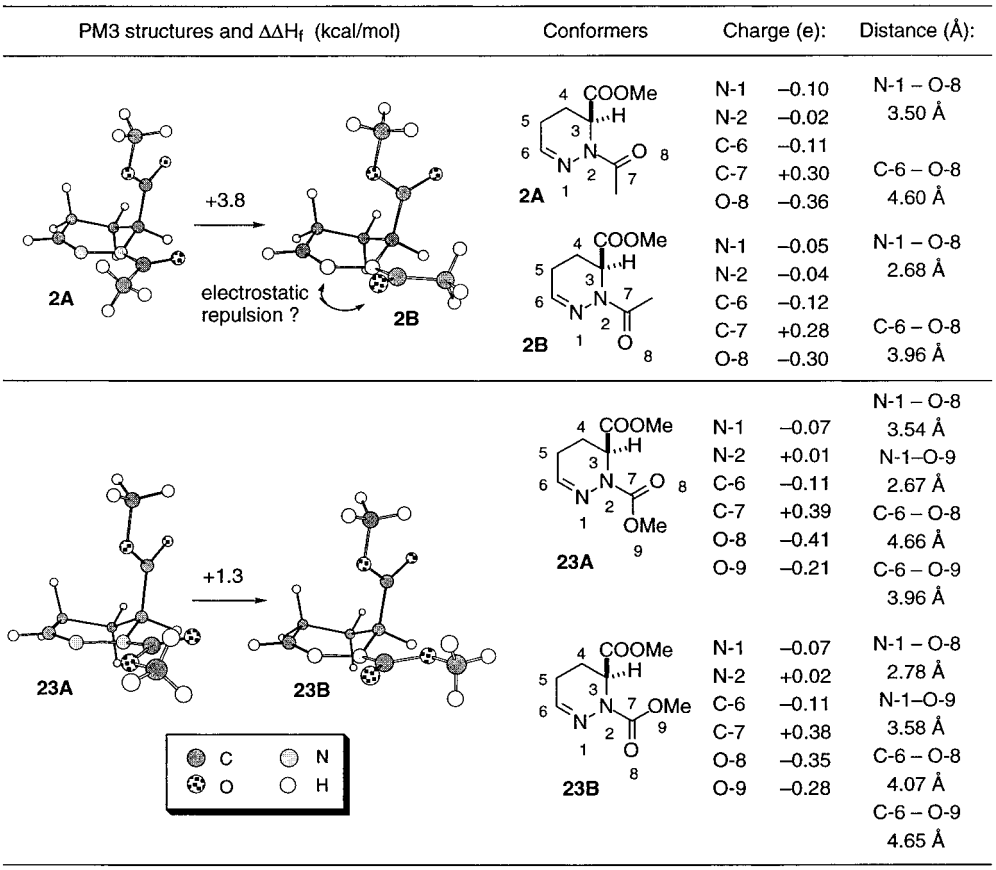



**Figure 2.** Electrostatic repulsion between the carbonyl O and imino N atoms as the proposed origin of the conformational preference in *N*-2-acyl derivatives of PCAs (PM3 calculations).

Specifically, the density of negative charge on N-1 is halved in conformer **2B** (the more energetic one) relative to conformer **2A**. The negative charge density on O-8 is also slightly less in **2B** than it is in **2A**, whereas the negative charge density on the imino and carbonyl carbons, C-6 and C-7, is very similar in the two conformers. Seemingly, electrostatic repulsion between the N and O atoms pushes electronic density away from N-1 in conformer **2B**, resulting in polarization of the imino linkage toward the carbon atom. However, N is more electronegative than C; therefore, this electronic redistribution is probably unfavorable. Likewise, bond polarization in the amide carbonyl is reduced considerably in **2B**, signifying that electrostatic interactions within the molecule must work against the electronegativity of the O atom. Such effects would be expected to be less pronounced in carbamate analogue **23**, because an O atom is forced in close proximity to the imino N in either limiting conformer. This is indeed the case: the energy difference between conformers **A** and **B** is now estimated to be 1.3 kcal/mol. Most of this energy difference may be due to repolarization of the carbamate carbonyl, but notice that no charge redistribution occurs within the imino linkage when the molecule switches between the two conformations.[14,20]

A consequence of the above hypotheses is that reduction of the imino linkage in PCA peptides would be expected to diminish the conformational bias toward conformers of type **A**. Whereas this is not readily apparent from the spectra of our compound **3**, Bock and co-workers[14,17] observed that the solution conformation (NMR) of substances related to **17** differs depending on whether the PCA units are present as such or in reduced form. Presumably, the constraints imposed upon these molecules by a relatively small 18-membered ring suffice to promote a conformational change upon removal of the important control element represented by the imino subunit. It is also noteworthy that biological activity was greatly affected by the precise oxidation state of the PCA units (cf. **17**, X, Y = H or $\pi$ bond).

Finally, PCA-like behavior is seen in the interesting proline analogue **24**, a lower ring homologue of **2** (Figure 3).[21] In this case, though, charge redistribution effects are less pronounced than in **2**, probably because atoms N-1 and O-7 are not as close in conformation **24B** as the corresponding N-1 and O-8 are in conformer **2B**. Indeed, the calculated energy difference between the two conformational states of **24** is reduced to 2.0 kcal/mol.

In summary, *N*-2-acyl-PCAs display an elevated degree of conformational rigidity that strongly favors rotamer **A** (Table 1 and Figure 2). This preference is probably due to electrostatic repulsion between the ring imino N atom and the carbonyl oxygen on the PCA *N*-2-acyl unit. Calculations also suggest that *N*-2-acyl PCAs are similar in shape to *N*-acylprolines. As a result, it is likely that PCAs may be utilized to replace proline within peptides whenever and wherever a turn motif is desired. Further ramifications of these ideas are currently under investigation, and results in this area will be the subject of future papers.

(20) Results of more sophisticated *ab initio* calculations concerning charge distribution and rotational barriers in various carbonyl compounds may be found in the following: (a) Wiberg, K. B.; Laidig, K. E. *J. Am. Chem. Soc.* **1987**, *109*, 5935. (b) Laidig, K. E.; Cameron, L. M. *Can. J. Chem.* **1993**, *71*, 872. These methods may permit calculation of rotational barriers in PCA peptides; however, we have not yet carried out such studies.

(21) After the submission of this paper the preparation of the remarkable ring system **24** was described: Mish, M. R.; Guerra, F. M.; Carreira, E. M. *J. Am. Chem. Soc.* **1997**, *119*, 8379.

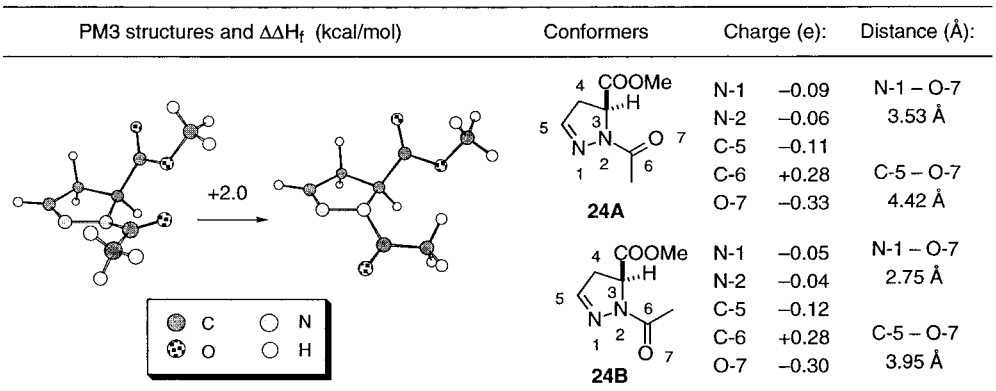



**Figure 3.** Summary of computational data for proline analogue **24** (PM3 calculations).

## Experimental Section[22]

**Hydrazine 5**. A solution of **4**[9] (6.0 g, 18.8 mmol, 1 equiv) in THF (40 mL) was added slowly to a cold (−78 °C) solution of LDA [22.6 mmol, 1.2 equiv, from *i*-$Pr_2NH$ (4.0 mL, 1.5 equiv) and BuLi in hexane (2.5 M, 9.0 mL, 22.6 mmol, 1.2 equiv); 1,10-phenanthroline indicator]. The mixture was stirred at −78 °C for 30 min, and then (Cbz−N=)$_2$ (11.2 g, 37.6 mmol, 2.0 equiv) in THF (40 mL) was added. After 2 min, the reaction was quenched (glacial AcOH, 5 mL) and partitioned between $CH_2Cl_2$ and pH 7 phosphate buffer. The combined extracts were washed (saturated aqueous $NaHCO_3$), dried ($Na_2SO_4$), and concentrated. Chromatography of the residue (40% EtOAc/hexane) yielded 7.5 g (65%) of **5** as a white waxy solid. The NMR spectra of this compound were broad at rt (slow conformational motion of tertiary carbamate). $[\alpha]^{25}_D = +35.1°$ ($c$ = 0.073 g/mL). $^1$H ($C_6D_6$, 70 °C): $\delta$ 6.9−7.3 (15H, m), 6.2−6.3 (1H, m), 5.13 (2H, s), 5.16 (1H, d, $J$ = 12.4), 5.06 (1H, d, $J$ = 12.4), 4.9−5.0 (1H, m), 4.21 (1H, m), 3.3−3.7 (4H, m), 3.06 (1H, dd, $J_1$ = 2.9, $J_2$ = 13.67), 2.08−2.46 (6H, m). $^{13}$C ($C_6D_6$, 70 °C): $\delta$ 173.7, 157.4, 156.8, 153.3, 137.3, 137.1, 136.4, 130.0, 129.5, 129.4, 129.0, 128.8, 128.5, 128.4, 128.0, 127.7, 127.5, 104.9, 69.5, 69.0, 68.1, 66.9, 65.3, 62.2, 56.1, 41.8, 38.0, 31.4, 24.4. IR: 3316, 1787, 1759, 1721 cm1$^{-1}$. MS (FAB): *m/z* 618 ($M^+$ + 1), 91 (100). HRMS (EI): calcd for $C_{33}H_{35}N_3O_9$ 617.2373, obsd 617.2373.

**Ester 6**. Methanolic MeOMgBr was prepared by dropwise addition of 8.9 mL of MeMgBr (26.8 mmol of 3 M solution in $Et_2O$, 1.5 equiv) into cold (0 °C) MeOH (50 mL). A solution of **5** (11.0 g, 17.8 mmol, 1.0 equiv) in $CH_2Cl_2$ (50 mL) was slowly added, and the mixture was stirred at 0 °C for 30 min before being quenched with saturated aqueous $NH_4Cl$ (30 mL). The organic phase was separated, and the aqueous layer was extracted with $CH_2Cl_2$. The combined organic phases were washed (brine), dried ($Na_2SO_4$), and concentrated. Chromatography of the residue (40% → 70% EtOAc/hexane) retrieved the chiral auxiliary and furnished the desired **6** (6.7 g, 80%) as a white waxy solid. $[\alpha]^{25}_D = -7.4°$ (0.084 g/mL). The NMR spectra of this compound were broad at rt (slow conformational motion of tertiary carbamate). $^1$H (50 °C): $\delta$ 7.28 (10H, br), 6.93 ($^1$H, br), 5.14 (2H, s), 5.12 (2H, s), 4.80−4.96 (2H, m), 3.70−3.90 (4H, m), 3.62 (3H, s), 1.70−2.10 (4H, m). $^{13}$C (50 °C): $\delta$ 171.4, 156.2, 155.9, 135.7, 135.6, 128.2, 128.0, 127.9, 127.8, 127.5, 103.8, 68.2, 67.4, 65.6, 60.8, 51.9, 30.0, 22.8. IR: 3301, 1740, 1716 cm$^{-1}$. MS (CI): *m/z* 473 ($M^+$ + 1), 429, 395, 367 (100). HRMS (EI): calcd for $C_{24}H_{28}N_2O_8$ 472.1845, obsd 472.1849.

**Monoprotected Hydrazine 7**. A mixture of **6** (6.0 g, 12.7 mmol, 1.0 equiv), $BOC_2O$ (3.3 g, 15.3 mmol, 1.2 equiv), 10% Pd/C catalyst (0.6 g, 10 wt % **6**), and MeOH (200 mL) was stirred under an $H_2$ atmosphere (balloon) until all the starting material was consumed (TLC, ca. 12 h). The mixture was filtered (Celite), the filtrate was concentrated, and the residue was chromatographed (40% EtOAc/hexane) to yield **7** (3.6 g, 93%) as a light yellow oil. $[\alpha]^{25}_D = -15.0°$ (0.306 g/mL). $^1$H: $\delta$ 6.32 (1H, br), 4.86 (1H, br t, $J$ = 4.0), 3.76−3.95 (4H, m), 3.70 (3H, s), 3.62 (1H, br), 1.69−1.84 (4H, m), 1.40 (9H, s). $^{13}$C: $\delta$ 173.5, 156.2, 103.7, 80.4, 64.8, 62.7, 51.9, 29.6, 28.1, 24.3. IR: 3316, 1732 cm$^{-1}$. MS (EI): *m/z* 304 ($M^+$ + H) 248, 232, 204, 57 (100). HRMS (EI): calcd for $C_{13}H_{24}N_2O_6$ ($M^+$ + H): 304.1634, obsd 304.1628.

**Protected Dipeptide 9**. A solution of acid chloride **8** (2.5 g, 13.2 mmol, 1.5 equiv) in $CH_2Cl_2$ (15 mL) was slowly added to a cold (0 °C) solution of **7** (2.7 g, 8.8 mmol, 1.0 equiv) and collidine (3.5 mL, 26.4 mmol, 3 equiv) in $CH_2Cl_2$ (10 mL) under Ar. The mixture was stirred at 0 °C for 20 min; then it was directly applied to a short silica gel column and eluted with EtOAc. The filtrate, containing **9** and collidine, was concentrated, and the residue was chromatographed (30 → 60% EtOAc/hexane) to yield 3.0 g (74%) of **9** as a waxy solid. $[\alpha]^{25}_D = +17°$ (0.055 g/mL). $^1$H: $\delta$ 7.50 (1H, br), 5.2−5.3 (2H, m), 4.83 (1H, br t, $J$ = 3.8), 4.3−4.4 (2H, m), 3.8−4.0 (4H, m), 3.71 (3H, s), 2.47 (3H, m), 1.8−2.0 (2H, m), 1.6−1.8 (2H, m), 1.46 (9H, s). $^{13}$C: $\delta$ 172.1, 171.2, 170.1, 154.9, 152.9, 103.4, 94.9, 82.6, 64.8, 58.2, 52.5, 52.4, 30.0, 27.8, 27.5, 22.8, 22.6. IR (film): 3297, 1792, 1740, 1699 cm$^{-1}$. MS (EI): *m/z* 459 ($M^+$), 458, 403, 386, 359, 57 (100). HRMS (EI): calcd for $C_{19}H_{29}N_3O_{10}$ 459.1853, obsd 459.1858.

**Compound 1d**. A solution of **9** (2.4 g, 5.2 mmol) in 25 mL of 90% aqueous TFA was stirred at rt for 30 min; then the solvent was evaporated, and the residue was partitioned between $CH_2Cl_2$ and saturated aqueous $NaHCO_3$ (extra solid $NaHCO_3$ was added to neutralize all the TFA). The aqueous layer was extracted with more $CH_2Cl_2$; the combined extracts were washed (brine), dried ($Na_2SO_4$), and concentrated to afford 1.6 g (100%) of **1d**. Recrystallization from 30% EtOAc/hexanes gave white prisms (1.5 g, 96%), mp = 152−153 °C. $[\alpha]^{25}_D = +37.1°$ (0.062 g/mL). $^1$H: $\delta$ 6.92 (1H, m), 5.68 (1H, dd, $J_1$ = 3.91, $J_2$ = 9.52), 5.13 (1H, m), 4.56 (1H, t, $J$ = 9.52), 4.21 (1H, dd, $J_1$ = 3.91, $J_2$ = 9.52), 3.71 (3H, s), 2.52 (3H, s), 1.78−2.45 (4H, m). $^{13}$C: $\delta$ 169.6, 169.3, 168.3, 153.3, 144.0, 65.0, 54.8, 52.8, 51.2, 23.1, 20.4, 18.1. IR (KBr): 3021, 1782, 1748, 1708, 1689, 1641 cm$^{-1}$. MS (EI): *m/z* 297 ($M^+$), 255, 237, 231, 83 (100). HRMS (EI): calcd for $C_{12}H_{15}N_3O_6$ 297.0961, obsd 297.0962.

**Compound 1e**. The preparation of this compound is fully detailed in the Supporting Information. Colorless oil. $[\alpha]^{25}_D = -9.54°$ (0.022 g/mL). $^1$H: $\delta$ 6.95 (1H, t, $J$ = 2.07), 5.60 (1H, br), 5.17 (1H, m), 4.99 (1H, dd, $J_1$ = 9.3, $J_2$ = 6.1), 4.72 (1H, t, $J$ = 9.3), 4.46 (1H, dd, $J_1$ = 9.3, $J_2$ = 6.1), 3.75 (3H, s), 1.87−2.45 (4H, m). $^{13}$C: $\delta$ 170.0, 169.4, 158.6, 143.9, 67.0, 53.8, 52.8, 51.2, 20.4, 18.2. IR: 3289, 1754, 1702, 1639 cm$^{-1}$. MS (EI): *m/z* 255 ($M^+$), 211, 196, 170, 142, 83 (100). HRMS (EI): calcd for $C_{10}H_{13}N_3O_5$ 255.0855, obsd 255.0855.

**Compound 1f**. The preparation of this compound is fully detailed in the Supporting Information. White crystals, mp = 166−167 °C. $[\alpha]^{25}_D = +32.3°$ (0.048 g/mL). $^1$H: $\delta$ 6.87 (1H, br d, $J$ = 4.1), 5.45 (1H, dd, $J_1$ = 9.5, $J_2$ = 3.9), 5.04 (1H, m), 4.45 (1H, t, $J$ = 9.5), 4.06 (1H, dd, $J_1$ = 9.5, $J_2$ = 3.9), 3.64 (3H, s), 1.77−2.33 (4H, m), 1.37 (9H, s). $^{13}$C: $\delta$ 169.3, 168.5, 152.0, 148.2, 144.1, 83.3, 64.3, 55.8, 52.4, 51.1, 27.2, 20.1, 18.0. IR (KBr): 3039, 1819, 1746, 1724, 1691, 1634 cm$^{-1}$. MS (EI): *m/z* 356 ($M^+$ + 1), 340, 311, 282, 255, 83, 57 (100). HRMS (EI): calcd for $C_{15}H_{21}N_3O_7$ 355.1379, obsd 355.1378.

(22) Melting points (mp) are uncorrected. Unless otherwise stated (a) $^1$H (250 MHz) and $^{13}$C (62.9 MHz) NMR spectra were recorded at 25 °C in $CDCl_3$, (b) FTIR spectra were obtained from films on NaCl plates, and (c) optical rotations were measured in $CH_2Cl_2$.

**Compound 1b**. The preparation of this compound is fully detailed in the Supporting Information. Colorless oil. $[\alpha]^{25}_{D} = -45°$ (0.110 g/mL). $^1$H: $\delta$ 6.93 (1H, d, $J = 3.9$), 5.76 (1H, d, $J = 6.8$), 5.24 (1H, X part of ABX system, m), 5.12 (1H, m), 3.84 (2H, AB part of ABX system), 3.69 (3H, s), 2.97 (1H, br), 1.81−2.38 (4H, m), 1.40 (9H, s). $^{13}$C: $\delta$ 171.1, 169.9, 143.1, 79.7, 64.4, 54.1, 52.6, 51.3, 28.2, 20.3, 18.6. IR: 3434, 1748, 1713, 1691, 1631 cm$^{-1}$. MS (EI): *m/z* 330 ($M^+ + 1$), 311, 299, 279, 256, 243, 83 (100). HRMS (EI): calcd for $C_{14}H_{24}N_3O_6$ ($M^+ + H$): 330.1665, obsd 330.1668.

**Compound 1c**. The preparation of this compound is fully detailed in the Supporting Information. Pale yellow oil. $^1$H: $\delta$ 9.29 (1H, d, $J = 7.3$), 8.29 (2H, br, s), 8.19 (1H, d, $J = 8.5$), 7.81 (1H, d, $J = 8.5$), 7.75 (1H, t, $J = 8.5$), 7.59 (1H, t, $J = 7.5$), 7.03 (1H, m), 5.80 (1H, X part of ABX system, m), 5.28 (1H, br, d, $J = 5.5$), 4.18 (1H, dd, B part of ABX, $J = 11.2$, 3.5), 4.04 (1H, dd, A part of ABX, $J = 11.2$, 4.9), 3.72 (3H, s), 1.9−2.5 (4H, m). $^{13}$C: $\delta$ 170.6, 169.9, 154.9, 149.3, 146.4, 143.3, 137.4, 130.1, 129.9, 129.3, 128.0, 127.6, 118.8, 64.7, 54.1, 52.9, 51.5, 20.5, 18.7.

**Compound 2**. The preparation of this compound is fully detailed in the Supporting Information. Pale yellow oil. $^1$H: $\delta$ 6.83 (1H, m), 5.20 (1H, m), 3.67 (3H, s), 2.30 (3H, s), 1.78−2.31 (4H, m). $^{13}$C: $\delta$ 172.3, 170.3, 141.4, 52.5, 50.5, 20.9, 20.2, 18.6.

**Compound 3**. The preparation of this compound is fully detailed in the Supporting Information. Colorless oil. $^1$H: $\delta$ 5.32 (1H, X part of ABX system, br dd, $J_1 = 6.0$, $J_2 = 1.9$), 3.77 (3H, s), 3.05 (1H, br, B part of ABX, $J_1 = 13.9$ Hz, $J_2 = 2.0$ Hz), 2.75 (1H, br, ddd, A part of ABX, $J_1 = J_2 = 13.9$, $J_3 = 3.0$), 2.19 (3H, s), 1.8−2.3 (2H, m), 1.3−1.6 (2H, m). $^{13}$C (DEPT): $\delta$ 52.4, 50.6, 47.1 ($CH_2$), 25.6 ($CH_2$), 21.9 ($CH_2$), 20.7.

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**Supporting Information Available:** Hardcopy spectra of compounds **1b**, **1c**, **2**, and **3**, calculated vs experimental energy differences for compounds **18** and **19**, tabulation of calculated energy differences between conformers **A** and **B** of some PCA derivatives/analogues, and detailed experimental procedures for the preparation of compounds **1b**, **1c**, **1f**, **1g**, **2**, and **3** (7 pages). See any current masthead page for ordering and Internet access instructions.

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