C–H···O Hydrogen Bond Mediated Chain Reversal in a Peptide Containing a γ-Amino Acid Residue, Determined Directly from Powder X-ray Diffraction Data**

Eugene Y. Cheung, Emma E. McCabe, Kenneth D. M. Harris,* Roy L. Johnston, Emilio Tedesco, K. Muruga Poopathi Raja, and Padmanabhan Balaram*

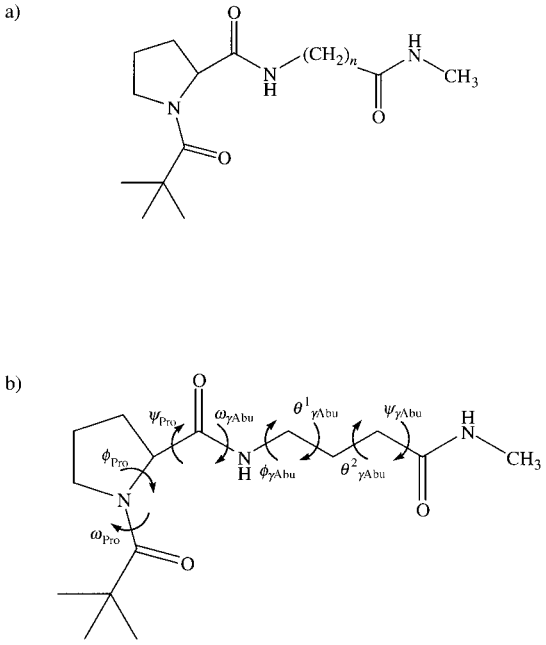
The finding that peptides containing β-amino acid residues give rise to folding patterns hitherto unobserved in α-amino acid peptides[1] has stimulated considerable interest in the conformational properties of peptides built from β, γ, and δ residues,[2] as the introduction of additional methylene (CH_2) units into peptide chains provides further degrees of conformational freedom. Studies of the influence of introducing ω-amino acids into regular polypeptide structures derived from α residues have demonstrated that extra methylene groups can be inserted into helical backbones and into the strand and turn segments of β hairpins.[3] In regard to the influence of CH_2 group insertion into the $i+2$ position of isolated peptide β turns, we are investigating the conformational properties of a series of model sequences Piv-^{L}Pro-Xxx-NHMe (defined in Scheme 1 and reference [4]).

The parent peptide Piv-^{L}Pro-Gly-NHMe has been shown, through structure determination directly from powder X-ray diffraction data,[5] to adopt a classical Type II β turn, previous studies having established the sequence as one that forms a canonical β turn.[6] Preliminary CD spectroscopic analysis of the peptides with Xxx = β-Gly, γ-Abu, δ-Ava, and ε-Acp showed that all four peptides give distinctly different spectral bands compared to the parent sequence with Xxx = Gly. Attempts to obtain single crystals of these materials appropriate for single-crystal X-ray diffraction have been unsuccessful, and we have instead exploited the opportunities that now exist for determining crystal structures directly from powder X-ray diffraction data.[7] Herein we report the structure of Piv-^{L}Pro-γ-Abu-NHMe determined by ab initio structure solution from powder X-ray diffraction data using the Genetic Algorithm technique[8] followed by Rietveld refinement. As discussed below, the structure of Piv-^{L}Pro-γ-Abu-NHMe reveals a novel chain reversal motif that is> stabilized by an intramolecular C–H···O interaction.

[*] Prof. Dr. K. D. M. Harris, Dr. E. Y. Cheung, E. E. McCabe, Dr. R. L. Johnston, Dr. E. Tedesco
School of Chemistry
University of Birmingham
Edgbaston, Birmingham, B15 2TT (UK)
Fax: (+44) 121-414-7473
E-mail: K.D.M.Harris@bham.ac.uk

Prof. Dr. P. Balaram, K. M. P. Raja
Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560012 (India)
Fax: (+91) 80-3600683
E-mail: pb@mbu.iisc.ernet.in

[**] We are grateful to the EPSRC, the University of Birmingham, Purdue Pharma, Wyeth Ayerst, and the Department of Biotechnology of the Government of India (under the Drug and Molecular Design Programme) for financial support.



Scheme 1. a) Piv-^{L}Pro-Xxx-NHMe, where Xxx = -NH-$(CH_2)_n$-CO- ($n=1$, Gly; $n=2$, β-Gly; $n=3$, γ-Abu; $n=4$, δ-Ava; $n=5$, ε-Acp). b) Definition of the backbone torsional angles in Piv-^{L}Pro-γ-Abu-NHMe.

Figure 1 (top) shows the molecular conformation of Piv-^{L}Pro-γ-Abu-NHMe in the crystal structure determined from powder X-ray diffraction data. The relevant backbone torsion angles (see references [9] and [3b] for the definition of the

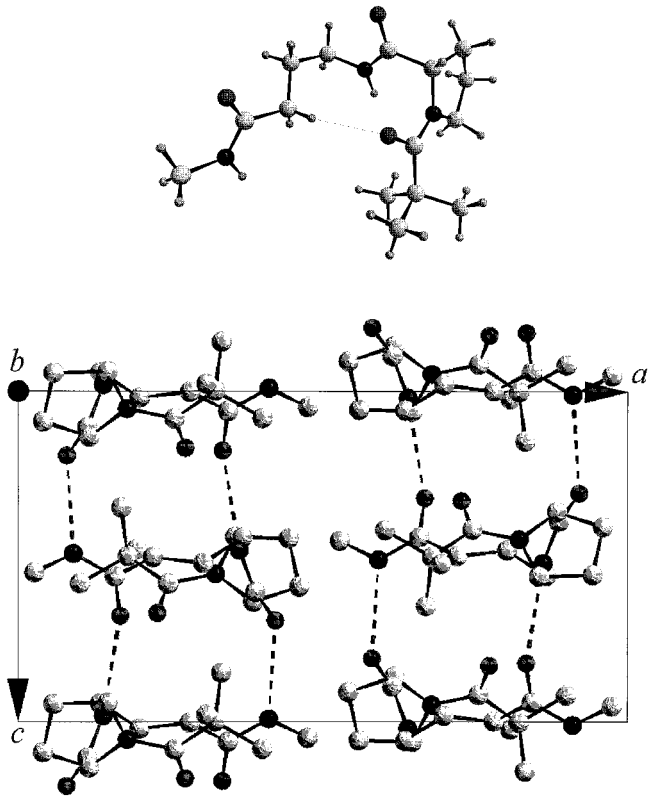


Figure 1. Top: Conformation of Piv-^{L}Pro-γ-Abu-NHMe in the crystal structure. Bottom: Crystal structure of Piv-^{L}Pro-γ-Abu-NHMe illustrating the one-dimensional columns of hydrogen-bonded molecules along the *c* axis (hydrogen atoms omitted for clarity).

 © WILEY-VCH Verlag GmbH, 69451 Weinheim, Germany, 2002 1433-7851/02/4103-0494 $ 17.50+.50/0

backbone torsion angles of peptides containing ω-amino acids) are: ϕ_{Pro} −71.0, ψ_{Pro} −26.1, $\phi_{\gamma Abu}$ −77.2, $\theta^1{}_{\gamma Abu}$ −50.2, $\theta^2{}_{\gamma Abu}$ −172.2, and $\psi_{\gamma Abu}$ +140.0°. The internal torsion angles of the pyrrolidine ring of the Pro residue are: θ (C^δ-N-C^α-C^β) −10.4, χ^1 (N-C^α-C^β-C^γ) +20.9, χ^2 (C^α-C^β-C^γ-C^δ) −23.5, χ^3 (C^β-C^γ-C^δ-N) +17.4, and χ^4 (C^γ-C^δ-N-C^α) −4.4°. The observed conformation is clearly folded, and is reminiscent of chain reversals found in α-peptide structures. Interestingly, a short C−H···O interaction is observed between one of the α-methylene hydrogen atoms of γ-Abu and the C=O group of Piv. The observed geometry of the hydrogen bond (with the hydrogen atom position normalized according to standard geometries from neutron diffraction) is: C^α–$H_{\gamma Abu}$···O 2.51 Å, $C^\alpha_{\gamma Abu}$···O 3.59 Å, C^α–$H_{\gamma Abu}$···O 172.4°. Recently, there has been considerable interest[10] in the role of C−H···O interactions in influencing molecular conformation and molecular organization in crystals. The observed geometry of the intramolecular C−H···O interaction in Piv-L-Pro-γ-Abu-NHMe is within the limits generally accepted for significant C−H···O hydrogen bonds.[10a] A notable feature of the molecular conformation of Piv-L-Pro-γ-Abu-NHMe is that this C−H···O interaction defines an intramolecular cyclic 10-atom motif, similar to that observed in the classical β turn.[11] Indeed, the observed motif can be considered as a mimetic of the conformation observed[5] in the parent peptide Piv-L-Pro-Gly-NHMe, with the hydrogen bonding function of the C-terminal amide group now taken by an ethylene moiety. Clearly the formation of the folded structure of Piv-L-Pro-γ-Abu-NHMe is facilitated by the *gauche* conformation adopted about the C^β–C^γ bond of the γ-Abu residue. Indeed, there is growing evidence[1–3] that the formation of compact, folded structures by peptides containing β-, γ-, and δ-amino acid residues, by adopting *gauche* conformations in polymethylene chains, is fairly widespread. In the cyclic molecular conformation adopted by Piv-L-Pro-γ-Abu-NHMe, all the C=O groups point outwards from one face of the molecule and all the N−H groups point outwards from the other face, in a manner that is conducive to the formation of columns of hydrogen-bonded molecules along the *c* axis in the crystal (Figure 1 (bottom)). Adjacent molecules along these columns interact through two intermolecular N−H···O hydrogen bonds (N−H(methylamide)···O=C(Pro): N···O 2.84 Å, N−H···O 162.5°; N−H(γ-Abu)···O=C(methylamide): N···O 2.95 Å, N−H···O 139.1°).

Our reported ab initio structure determination of Piv-L-Pro-γ-Abu-NHMe from powder X-ray diffraction data not only reveals a novel structural motif, but in more general terms emphasizes the considerable potential of this approach for revealing structural features of materials that are recalcitrant to the formation of single crystals of suitable size and quality for single-crystal X-ray diffraction studies. Extension of this approach to peptides and other molecular crystals with significantly greater conformational complexity is clearly possible.

Experimental Section

The synthesis of Piv-L-Pro-γ-Abu-NHMe was carried out by coupling Piv-L-Pro-OH to H_2N-(CH_2)-$COOCH_3$ using isobutylchloroformate and triethylamine. The isolated dipeptide ester Piv-L-Pro-γ-Abu-OMe was converted into Piv-L-Pro-γ-Abu-NHMe by saturating the methanolic solution with methylamine gas and allowing the mixture to stand at room temperature for 72 h. The peptide was purified by medium pressure liquid chromatography over a reverse-phase C_{18} column (40–60 μm). The structural identity was established by ^{1}H NMR spectroscopy (500 MHz) and electrospray mass spectrometry (calcd *m*/*z*: 297 [M^+]; found: 298 [M+H], 320 [M+Na]). The peptide was obtained as a white crystalline solid.

High-quality powder X-ray diffraction data for this sample of Piv-L-Pro-γ-Abu-NHMe were recorded at ambient temperature in transmission mode on a Siemens D5000 diffractometer ($Cu_{K\alpha 1}$ (Ge monochromated); linear position-sensitive detector covering 8° in 2θ; 2θ range: 5–70°; step size: 0.019°; data collection time: 10 h).

The unit cell and space group were determined prior to structure solution directly from the powder X-ray diffraction pattern.[12] Structure solution from the powder X-ray diffraction data was carried out using our Genetic Algorithm (GA) technique[8] within the program EAGER.[22] In this method, a population of trial structures is allowed to evolve subject to rules and operations (mating, mutation, and natural selection) analogous to those that govern evolution in biological systems. Each structure is specified by the position {x, y, z}, orientation {θ, ϕ, ψ}, and conformation (defined by variable torsion angles {τ_1, τ_2, …, τ_n}) of each molecule in the asymmetric unit. New structures are generated by the mating and mutation operations, and in the implementation used here,[8d] each new structure is subjected to local minimization of the powder profile R factor R_{wp}. In natural selection, only the structures of highest "fitness" (lowest R_{wp}) are allowed to pass from one generation to the next generation. Examples of the application of this method are given in references [5, 8, and 23].

In the structure solution calculation for Piv-L-Pro-γ-Abu-NHMe, the "structural fragment" comprised one complete molecule with hydrogen atoms omitted, and each structure was defined by 13 variables (7 variable torsion angles). The torsion angle (ω_{Pro}) of the peptide bond of L-proline was restricted to either 0 or 180°. The other two amide linkages CO−NH were maintained as planar units with a O-C-N-H torsion angle fixed at 180°. All other torsion angles were treated as variables. The GA calculation involved the evolution of a population of 100 structures, with convergence on the correct structure solution achieved in 13 generations. 50 mating operations (to produce 100 offspring) and 20 mutation operations were carried out in each generation.

The best structure in the final generation of the GA structure solution calculation was used as the starting model for Rietveld refinement[24] (Figure 2), which was carried out using the GSAS program.[21] Initially, bond lengths and angles were restrained to standard values and planar restraints were applied to the carbonyl groups. These restraints were gradually relaxed as the refinement progressed. The isotropic displacement parameters of the non-hydrogen atoms were fixed at $B_{iso} = 0.05$ Å^2. Hydrogen atoms were introduced in calculated positions towards the end of the refinement, with $B_{iso} = 0.06$ Å^2. Refinement of a preferred orientation parameter (in the [010] direction) gave a slight improvement to the

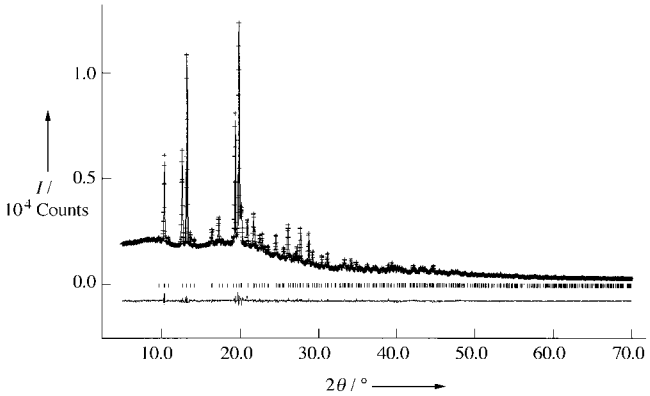


Figure 2. Experimental (+), calculated (solid line), and difference (lower line) powder X-ray diffraction profiles for the Rietveld refinement of Piv-L-Pro-γ-Abu-NHMe. Reflection positions are marked. The calculated powder-diffraction profile is for the final refined crystal structure.

 © WILEY-VCH Verlag GmbH, 69451 Weinheim, Germany, 2002 1433-7851/02/4103-0495 $ 17.50+.50/0

Table 1. Fractional coordinates of Piv-^{L}Pro-γ-Abu-NHMe.

C1	0.0263(6)	0.721(1)	0.548(1)
C3	0.1576(4)	0.6379(6)	0.5598(7)
C5	0.2199(6)	0.5547(7)	0.502(1)
C6	0.3019(6)	0.6098(9)	0.519(1)
C7	0.3659(6)	0.5343(7)	0.442(1)
C9	0.3871(4)	0.3567(5)	0.6077(6)
C11	0.3839(3)	0.2173(6)	0.6369(5)
C12	0.4595(4)	0.1554(8)	0.591(1)
C13	0.4422(4)	0.0982(7)	0.445(1)
C14	0.3540(4)	0.0801(9)	0.440(1)
C16	0.2447(3)	0.1742(6)	0.5821(7)
C18	0.1785(3)	0.1108(5)	0.4982(7)
C19	0.1929(6)	−0.0291(8)	0.477(1)
C20	0.1011(5)	0.125(1)	0.581(1)
C21	0.1683(6)	0.174(1)	0.350(1)
N2	0.0902(5)	0.650(1)	0.4903(8)
N8	0.3612(5)	0.4027(6)	0.480(1)
N15	0.3221(3)	0.1577(7)	0.5524(6)
O4	0.1664(5)	0.6846(9)	0.6784(9)
O10	0.4204(6)	0.4230(7)	0.6929(9)
O17	0.2270(4)	0.2531(8)	0.6703(9)

overall fit. The final refined structure is shown in Figure 1. Fractional atomic coordinates are given in Table 1.

Received: September 24, 2001 [Z17952]

[1] a) D. Seebach, J. L. Matthews, *Chem. Commun*. **1997**, *21*, 2015; b) D. Seebach, S. Abele, K. Gademann, B. Jaun, *Angew. Chem.* **1999**, *111*, 1700; *Angew. Chem. Int. Ed.* **1999**, *38*, 1595; c) D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913; d) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173; e) D. H. Apella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc*. **1996**, *118*, 13071; f) D. H. Apella, L. A. Christianson, D. A. Klein, D. R. Powell, X. Huang, J. J. Barchi, S. H. Gellman, *Nature* **1997**, *387*, 381.

[2] a) D. Liu, W. F. DeGrado, J. P. Schneider, Y. Hamuro, *J. Am. Chem. Soc*. **2001**, *123*, 7553; b) S. Hanessian, X. Luo, R. Schaum, S. Michnick, *J. Am. Chem. Soc*. **1998**, *120*, 8569; c) L. Szabo, B. L. Smith, K. D. McReynolds, A. L. Parill, E. R. Morris, J. Gervay, *J. Org. Chem.* **1998**, *63*, 1074; d) D. Seebach, M. Brenner, M. Rueping, B. Schweizer, B. Jaun, *Chem. Commun*. **2001**, 207; e) G. P. Dado, S. H. Gellman, *J. Am. Chem. Soc*. **1994**, *116*, 1054; f) R. P. Cheng, W. F. DeGrado, *J. Am. Chem. Soc*. **2001**, *123*, 5162; g) M. D. Smith, T. D. W. Claridge, G. E. Tranter, M. S. P. Sansom, G. W. J. Fleet, *Chem. Commun.* **1998**, 2041.

[3] a) A. Banerjee, A. Pramanik, S. Bhattacharjya, P. Balaram, *Biopolymers* **1996**, *39*, 769; b) I. L. Karle, A. Pramanik, A. Banerjee, S. Bhattacharjya, P. Balaram, *J. Am. Chem. Soc*. **1997**, *119*, 9087; c) S. C. Shankaramma, S. K. Singh, A. Sathyamurthy, P. Balaram, *J. Am. Chem. Soc*. **1999**, *121*, 5360; d) I. L. Karle, H. N. Gopi, P. Balaram, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3716.

[4] Abbreviations used: Piv: Pivaloyl; β-Gly: β-amino propionic acid (also referred to as β-alanine in the early literature); γ-Abu: γ-aminobutyric acid; δ-Ava: δ-aminovaleric acid; ε-Acp: ε-aminocaproic acid.

[5] E. Tedesco, K. D. M. Harris, R. L. Johnston, G. W. Turner, K. M. P. Raja, P. Balaram, *Chem. Commun*. **2001**, 1460.

[6] a) P. A. Aubry, J. Protas, G. Boussard, M. Marraud, *Acta Crystallogr. Sect. B* **1980**, *107*, 2822; b) B. N. N. Rao, A. Kumar, H. Balaram, A. Ravi, P. Balaram, *J. Am. Chem. Soc.* **1983**, *105*, 7423; c) B. V. V. Prasad, H. Balaram, P. Balaram, *Biopolymers* **1982**, *21*, 1261.

[7] a) K. D. M. Harris, B. M. Kariuki, M. Tremayne, *Angew. Chem.* **2001**, *113*, 1674; *Angew. Chem. Int. Ed.* **2001**, *40*, 1626; b) K. D. M. Harris, *J. Chin. Chem. Soc.* **1999**, *46*, 23; c) K. D. M. Harris, M. Tremayne, *Chem. Mater*. **1996**, *8*, 2554; d) K. D. M. Harris, M. Tremayne, P. Lightfoot, P. G. Bruce, *J. Am. Chem. Soc.* **1994**, *116*, 3543; e) A. K. Cheetham, A. P. Wilkinson, *Angew. Chem.* **1992**, *104*, 1594; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1557; f) D. M. Poojary, A. Clearfield, *Acc. Chem. Res.* **1997**, *30*, 414; g) A. Meden, *Croat. Chem. Acta* **1998**, *71*, 615.

[8] a) B. M. Kariuki, H. Serrano-González, R. L. Johnston, K. D. M. Harris, *Chem. Phys. Lett.* **1997**, *280*, 189; b) K. D. M. Harris, R. L. Johnston, B. M. Kariuki, M. Tremayne, *J. Chem. Res. (S)* **1998**, 390; c) K. D. M. Harris, R. L. Johnston, B. M. Kariuki, *Acta Crystallogr. Sect. A* **1998**, *54*, 632; d) G. W. Turner, E. Tedesco, K. D. M. Harris, R. L. Johnston, B. M. Kariuki, *Chem. Phys. Lett.* **2000**, *321*, 183; e) K. D. M. Harris, R. L. Johnston, B. M. Kariuki, in *Evolutionary Algorithms in Computer-Aided Molecular Design* (Ed.: D. E. Clark), Wiley-VCH, Weinheim, **2000**, chap. 9, p. 159.

[9] A. Banerjee, P. Balaram, *Curr. Sci*. **1997**, *73*, 1067.

[10] a) G. R. Desiraju, T. Steiner, *The Weak Hydrogen Bond in Structural Chemistry and Biology*, International Union of Crystallography and Oxford Science Publications, New York, **1999**; b) Z. Berkovitch-Yellin, L. Leiserowitz, *Acta Crystallogr. Sect. B* **1984**, *40*, 159; c) P. Seiler, J. D. Dunitz, *Helv. Chim. Acta* **1989**, *72*, 1125; d) Z. S. Derewenda, L. Lee, U. Derewenda, *J. Mol. Biol.* **1995**, *252*, 248; e) G. R. Desiraju, *Acc. Chem. Res.* **1996**, *29*, 441; f) T. Steiner, *Chem. Commun.* **1997**, 727; g) B. M. Kariuki, K. D. M. Harris, D. Philp, J. M. A. Robinson, *J. Am. Chem. Soc.* **1997**, *119*, 12679; h) Y. Gu, T. Kar, S. Scheiner, *J. Am. Chem. Soc.* **1999**, *121*, 9411; i) R. Vargas, J. Garza, D. A. Dixon, B. P. Hay, *J. Am. Chem. Soc.* **2000**, *122*, 4750.

[11] a) C. M. Venkatachalam, *Biopolymers* **1968**, *6*, 1425; b) G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Protein Chem.* **1985**, *37*, 1.

[12] The powder X-ray diffraction pattern was indexed using the programs ITO,[13] TREOR,[14] DICVOL,[15] and GAIN,[16] which all gave the following unit cell dimensions with orthorhombic metric symmetry: $a = 16.96$, $b = 10.77$, $c = 9.15$ Å. Both Pawley fitting[17] (using the PowderFit program[18] in Materials Studio[19]) and LeBail fitting[20] (using the GSAS program[21]) gave very good fits ($R_{wp} = 0.018$ and $R_{wp} = 0.020$, respectively) to the complete powder diffraction profile using this unit cell. The space group was assigned from systematic absences as $P2_12_12_1$. Density considerations indicate that there is one molecule in the asymmetric unit (with four molecules in the unit cell, the predicted density is 1.18 g cm^{-3}, consistent with typical densities of organic materials).

[13] J. W. Visser, *J. Appl. Crystallogr*. **1969**, *2*, 89.

[14] P.-E. Werner, L. Eriksson, M. Westdahl, *J. Appl. Crystallogr.* **1985**, *18*, 367.

[15] A. Boultif, D. Louër, *J. Appl. Crystallogr.* **1991**, *24*, 987.

[16] K. D. M. Harris, R. L. Johnston, M. H. Chao, B. M. Kariuki, E. Tedesco, G. W. Turner, *Genetic Algorithm for Indexing Powder Diffraction Data*, University of Birmingham, UK, **2000**.

[17] G. S. Pawley, *J. Appl. Crystallogr.* **1981**, *14*, 357.

[18] G. E. Engel, S. Wilke, O. König, K. D. M. Harris, F. J. J. Leusen, *J. Appl. Crystallogr.* **1999**, *32*, 1169.

[19] *Molecular Simulations Inc.*, 9685 Scranton Road, San Diego, CA, 9212103752, USA.

[20] A. Le Bail, H. Duroy, J. L. Fourquet, *Mater. Res. Bull.* **1988**, *23*, 447.

[21] A. C. Larson, R. B. Von Dreele, *Los Alamos Natl. Lab. Rep.* **1987**, LA-UR-86-748.

[22] K. D. M. Harris, R. L. Johnston, D. Albesa Jové, M. H. Chao, E. Y. Cheung, S. Habershon, B. M. Kariuki, O. J. Lanning, E. Tedesco, G. W. Turner, *Evolutionary Algorithm Generalized for Energy and R-factor*, University of Birmingham, Birmingham, UK, **2001** (an extended version of the program *GAPSS*, K. D. M. Harris, R. L. Johnston, B. M. Kariuki, University of Birmingham, **1997**).

[23] a) B. M. Kariuki, P. Calcagno, K. D. M. Harris, D. Philp, R. L. Johnston, *Angew. Chem.* **1999**, *111*, 860; *Angew. Chem. Int. Ed.* **1999**, *38*, 831; b) B. M. Kariuki, K. Psallidas, K. D. M. Harris, R. L. Johnston, R. W. Lancaster, S. E. Staniforth, S. M. Cooper, *Chem. Commun.* **1999**, 1677; c) E. Tedesco, G. W. Turner, K. D. M. Harris, R. L. Johnston, B. M. Kariuki, *Angew. Chem.* **2000**, *112*, 4662; *Angew. Chem. Int. Ed.* **2000**, *39*, 4488; d) E. Tedesco, B. M. Kariuki, K. D. M. Harris, R. L. Johnston, O. Pudova, G. Barbarella, E. A. Marseglia, G. Gigli, R. Cingolani, *J. Solid State Chem.* **2001**, *161*, 121.

[24] Summary of final Rietveld refinement: $a = 16.9433(6)$, $b = 10.7282(3)$, $c = 9.15073(3)$ Å; $V = 1663.3(1)$ Å^3; $R_{wp} = 0.026$, $R_p = 0.019$; 64 refined variables, 3397 profile points, 920 reflections; $2\theta_{max} = 70^\circ$ (1.3-Å resolution).

 © WILEY-VCH Verlag GmbH, 69451 Weinheim, Germany, 2002 1433-7851/02/4103-0496 $ 17.50+.50/0