Designing amino acids to determine the local conformations of peptides

(α -helix/asymmetry/polymers)

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ABSTRACT The local conformations of proteins and peptides are determined by the amino acid sequence. However, the 20 amino acids encoded by the genome allow the peptide backbone to fold into many conformations, so that even for a small peptide it becomes very difficult to predict the three-dimensional structure. By using empirical conformational energy calculations, a set of amino acids has been designed that would be expected to constrain the conformation of a peptide or a protein to one or two local minima. Most of these amino acids are based on asymmetric substitutions at the C α atom of each residue. The H α atom of alanine was replaced by various groups: -OCH $_3$, -NCH $_3$, -SCH $_3$, -CONH $_2$, -CONHCH $_3$, -CON(CH $_3$) $_2$, -NH-CO-CH $_3$, -phenyl, or -*o*-(OCH $_3$)phenyl. Several of these new amino acids are predicted to fold into unique peptide conformations such as right-handed α -helical, left-handed α -helical, or extended. In an attempt to produce an amino acid that favored the C γ^1 conformation (torsion angles: $\phi = -70^\circ$ and $\psi = +70^\circ$), an extra amide group was added to the C β atom of the asparagine side chain. Conformationally restricted amino acids of this type could prove useful for developing new peptide pharmaceuticals, catalysts, or polymers.

The prediction of the three-dimensional structures of peptides and proteins is not yet possible (1, 2). In part, the difficulties stem from the intrinsic conformational freedom associated with each amino acid in the polypeptide chain (3, 4). If we assume that the conformational freedom is determined essentially by the ϕ and ψ rotations about the N—C α and C α —C' bonds, respectively, almost all of the amino acids except proline and glycine are able to access $\approx 30\%$ of the two-dimensional (ϕ , ψ) conformational space (5). Proline restricts the conformational freedom considerably (6), whereas glycine allows access to $>60\%$ of the total (ϕ , ψ) rotational space (7). When additional degrees of freedom are considered (e.g., bond angle bending or bond length stretching), the accessible conformational space is even larger (8, 9). As a consequence, unless there are unusual amino acids in a peptide sequence (10, 11) and/or the peptide is cyclic (12), most small peptides will have multiple conformations in solution. In proteins, long-range interactions lead to cooperative folding (13) and low-energy conformations which include structures such as β -sheets and helices (α or 3_{10}). However, constraints on the local conformation of the polypeptide chain are relatively weak, thus predicting *a priori* that the preferred local conformation of a peptide requires an accurate comparison of the enormous numbers of possible conformations. Even the relatively simple task of redesigning peptide loops by homology modeling requires approximations that hinder the reliability of these calculations (2, 14, 15), and when there is no highly homologous structure, the

semi-empirical calculations are not yet sufficiently powerful to generate a reliable model of the three-dimensional structure.

Small peptides are ubiquitous regulators in many biological systems, and in many of these control systems (e.g., peptide hormones or antigens) much of the conformational specificity appears to reside with the protein receptor (16). However, there are peptides that have evolved to restrict the number of accessible conformations by incorporating unusual amino acids into the peptide backbone (17). Although these unusual amino acids can provide specific binding motifs (e.g., the bmt side chain in cyclosporin) (18), it is clear that amino acids such as *N*-methylvaline (18) and α -aminoisobutyric acid (Aib) (19) have been incorporated to restrict the conformational space available to the peptide. In the laboratory, unusual amino acids can now be incorporated into peptides or proteins by chemical synthesis or by coupling them to tRNA and using the ribosomal machinery (20).

When Aib is incorporated into polypeptides, the local conformation is restricted to the left- or right-handed 3_{10} - or α -helical regions (21, 22). The lack of stereospecificity of Aib still limits our ability to produce small peptides of known conformation; however, in longer peptides it can be used to stabilize α -helices. Some chiral α -alkyl α' -methyl amino acids have been used to direct the local conformation of peptide hormones (23). However, the groups attached to the C β atom of an amino acid side chain have little influence on the local folding of a peptide backbone (7). Since substitution at the C α atom has a strong effect on the conformational distribution for an amino acid in a peptide (21, 22), in this study the effect of different substituents at C α on the local conformation of a peptide chain has been investigated with ECEPP/2 conformational energy calculations (24). In particular, the predicted effects of replacing the H α atom of an Ala residue with a number of different substituents such as -NCH $_3$, -OCH $_3$, -SCH $_3$, -CONH $_2$, or an aromatic group have been investigated. The results indicate that some of these amino acids are predicted to have such restricted conformational preferences that they can be expected to specify the local fold of a polypeptide chain.

METHODS

Nomenclature, Geometry, and Conformational Energy Calculations. The standard conventions for the nomenclature of side-chain torsional angles for polypeptide conformations have been used (25). Except for the bond angles associated with the C α atom, all bond angles and bond lengths were derived from the standard ECEPP/2 algorithm (24). The C α bond angles were estimated by using the AM1 quantum mechanical algorithm (26) for the *N*-acetyl *N'*-methylamide derivatives of each residue. The partial Mulliken charges on all atoms were derived from the appropriate ECEPP/2 parameters or, where necessary, the AM1 calculations on low-energy conformations for each molecule. The ECEPP/2 Fortran program and residue data were modified to allow the

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FIG. 1. Schematic structures for the amino acids investigated in this study. The R configuration was used for the initial (ϕ, ψ) conformational energy calculations. The amino, carboxyl, and the two groups attached to the C^α atom are shown. The chiral center on the Luds side chain was also in the R configuration.

FIG. 2. Conformational energy maps for the *N*-acetyl *N'*-methylamide derivatives of the amino acid residues shown in Fig. 1. The lowest energy region is depicted in red and the different color contours represent 1-kcal/mol increments in conformational energy. The values of the local energy minima for each residue are documented in Tables 1–3.

calculations to be performed for these unusual amino acids. The nomenclature and structures of the amino acids designed in this study are shown in Fig. 1.

The (ϕ, ψ) energy maps for the *N*-acetyl *N'*-methylamide derivatives of each amino acid were obtained by computing the conformational energy at 10° grid points on the ϕ (rotation around the $N-C^\alpha$ bond), ψ (rotation around the $C^\alpha-C'$ bond) surface with ω (rotation around the peptide bond) fixed at 180° . At each (ϕ, ψ) grid point all side-chain dihedral angles were varied systematically in 10° steps through their full range and the lowest energy conformation was used for generating the contour map. The contour maps were generated by using Mathematica (Wolfram Research, Champaign, IL) on a NeXT workstation. The minimum energy conformations on the (ϕ, ψ) surfaces were identified by minimizing the conformational energy for all local minima with respect to backbone and side-chain dihedral angles. The conjugate gradient method of Powell (27) was used for the energy minimizations.

RESULTS

The (ϕ, ψ) conformational energy map for the *N*-methyl *N'*-methylamide Aib derivative, generated with the ECEPP/2 algorithm (Fig. 2a), indicates that the $\alpha^R/3_{10}^R$ and $\alpha^L/3_{10}^L$

Table 1. Conformational energy minima for *N*-acetyl *N'*-methylamide derivatives of amino acids substituted with α -Me and α' -Me, -NMe, -OMe, -SMe, -Phe, or -*o*-OMePhe moieties

Residue	Angle size, degrees						E_{rel} , kcal/mol
	ϕ	ψ	$\chi^{1,2}$	$\chi^{1,1}$	χ^2	χ^3	
Aib	-58	-34	-16	-29			0.00
	58	34	29	16			0.00
	-173	49	0	38			1.56
	173	-49	-38	0			1.56
	-173	-172	33	33			2.44
	173	172	-33	-33			2.44
	62	-170	37	3			2.48
	-62	170	-3	-37			2.48
Menala	-169	157	55	-52	101		0.00
	-168	163	56	-61	24		0.03
	52	47	53	174	40		0.81
	-64	88	64	64	82		1.95
	63	177	77	-69	149		2.86
	-59	-34	67	66	80		2.91
Meoala	-63	122	69	-112	36		0.00
	-65	-33	70	-112	36		2.29
	64	34	76	-107	34		2.36
	-176	156	55	139	31		2.50
	73	159	83	-110	39		3.13
Mashala	65	22	69	102	50		0.00
	-52	-43	62	-147	43		0.26
	175	-48	49	130	69		0.79
	179	166	56	118	58		2.01
Pheala	68	174	76	103	46		2.69
	178	-48	35	155			0.00
	-69	73	57	25			0.35
	179	60	42	157			1.67
	-44	-49	49	0			1.90
Mpheala	-174	171	41	141			1.93
	56	27	79	86			3.66
	52	43	-171	-151	107	60	0.00
	172	-45	-80	147	-103	60	2.36
	165	74	33	-7	112	60	7.39

$E_{rel} = E_{tot} - E_0$, where E_0 is the conformational energy of the minimum derived from the lowest energy conformation on the appropriate (ϕ, ψ) map (see Fig. 2). E_0 (kcal/mol) for Aib, 1.92; Menala, -2.25; Meoala, 6.58; Mashala, -0.05; Pheala, -0.83; Mpheala, 2.55.

FIG. 3. Stereodiagrams of the lowest energy conformations for each of the *N*-acetyl *N'*-methylamide amino acid residues. The corresponding values for the ϕ , ψ , and χ torsional rotations are listed in Tables 1–3.

regions are the lowest energy areas (21, 22). Indeed, the lowest energy conformations correspond to the two 3_{10} -helix regions (-58° , -34° or 58° , 34°) (Table 1, Fig. 3). The (ϕ, ψ) map for the Aib residue is symmetrical (see Fig. 2a), so both conformations are likely to occur with equal frequency. The other regions of low energy at $\phi = -173^\circ$, $\psi = 49^\circ$; $\phi = -62^\circ$, $\psi = -170^\circ$; and $\phi = -173^\circ$, $\psi = -172^\circ$ were almost 2 kcal/mol higher in energy and unlikely to be observed in the crystal or solution structures of peptides.

The first α, α' -disubstituted amino acid peptide to be considered in this study consisted of α -methyl- α' -methyl-amido glycine (Menala; see Fig. 1). The (ϕ, ψ) map for the Menala residue indicated three major regions of low energy (extended, α^L , and C_7) (see Fig. 2b). Upon minimization the fully extended conformer (-168° , $+163^\circ$) remains the most stable; however, the α^L conformer is predicted to be <1 kcal/mol higher in energy, so this conformation would also be expected to be adopted in some peptides.

When one of the Aib methyl groups is replaced by a methoxy moiety (Meoala; Fig. 1), the $\alpha/3_{10}$ -helical regions are still accessible conformations (Fig. 2c); however, the low-energy conformation in the extended region (the γ -turn at -60° , $+130^\circ$) is the global energy minimum. Indeed, after minimization this conformation is predicted to be 2 kcal/mol more stable than any other conformation for this peptide. To my knowledge, this is the first amino acid known for which only a single peptide conformation is likely to occur. Of course, the *S* optical isomer of Meoala would also have a single preferred conformation at $(-63^\circ, +122^\circ)$ and would be expected to provide a second option for constraining the peptide backbone to a particular local conformation.

Replacing one of the Aib methyl groups with a thiomethyl moiety (Mashala; Fig. 1) increases the size of this side chain and reduces the conformational stability of the $\alpha^R/3_{10}$ region (Fig. 2d). Four regions of low energy [$\alpha^L/3_{10}^L$; $\alpha^R/3_{10}^R$; $(+170^\circ, -50^\circ)$; and the fully extended conformations] are all within 2 kcal/mol (Fig. 2d). After minimization of the energies in these four regions, the fully extended conformer was not as favorable as the other three conformations (Table 1).

Enlarging one of the α methyl groups of Aib to a phenyl ring (Pheala; Fig. 1) destabilizes both the $\alpha^R/3_{10}^R$ and $\alpha^L/3_{10}^L$ conformers and several other conformations, including $(+180^\circ, -50^\circ)$ and C_5^q , are more likely to form (Fig. 2e). After

minimization, these two conformers appear to be the most stable; however, two other conformers, $(+179^\circ, +60^\circ)$ and $(-174^\circ, +171^\circ)$, are <2 kcal/mol higher in energy, so these conformers would also be expected to be accessible during peptide folding.

Interestingly, when a methoxy group is added to the ortho position of the phenyl ring (Mpheala; Fig. 1), the predicted conformational (ϕ, ψ) distribution is greatly restricted (Fig. 2f). Only two conformers appear to be possible, $(+60^\circ, +40^\circ)$ and $(+170^\circ, -50^\circ)$. After minimization the lowest energy conformation occurs at $(+52^\circ, +43^\circ)$, and this is >2 kcal/mol lower in energy than the semi-extended conformer. This low-energy conformation is much closer to the α^L than the 3_{10}^L conformation. Thus, Mpheala is the second amino acid predicted to constrain a peptide chain to one conformer. Thus the *S* optical isomer of Mpheala could be used to direct a peptide to the α^R ($-52^\circ, -43^\circ$) conformation.

When one of the C^α methyl groups of Aib is replaced by a peptide moiety (*N*-acetylamide, Nub; Fig. 1) the C_5^q and the fully extended conformations appear to be accessible (Fig. 2g). After minimization the fully extended conformation and 3_{10} conformers appear to be the most likely to form; however, the energy of the C_5^q ($-73^\circ, +84^\circ$) is <2 kcal/mol above the global minimum (Table 2). When an amide group ($-\text{CONH}_2$) replaces a methyl group of Aib (Bun; Fig. 1) favorable side-chain interactions stabilize the fully extended conformer (Fig. 2h; Table 2). Neither the $\alpha^R/3_{10}^R$ nor the $\alpha^L/3_{10}^L$ conformer is likely to be occupied for this amino acid. When a methyl group is added to the amide side chain of Bun (trans to C^α , Mbun; Fig. 2i) the fully extended conformation is destabilized and the 3_{10}^L and $(-176^\circ, -56^\circ)$ conformers (Table 2) are the lowest in energy. However, five other local minima are within 2 kcal/mol of the 3_{10}^L (Table 2), so this amino acid might be expected to allow the peptide chain to fold in multiple directions. When both of the Bun side-chain amide protons are replaced with methyl groups (Dmbun; Fig. 1) the side chain becomes quite bulky and only two possible conformers are likely to form (Fig. 2j; Table 2). The fully extended structure appears to be the most likely conformation to be adopted by a Dmbun residue. Even after minimization, the $\alpha^R/3_{10}^R$ and $\alpha^L/3_{10}^L$ conformers were >3 kcal/mol higher in energy than the global minimum (Table 2).

Table 2. Conformational energy minima for *N*-acetyl *N'*-methylamide derivatives of amino acids substituted with α -methyl and α' -amide moieties

Residue	Angle size, degrees							E_{rel} , kcal/mol
	ϕ	ψ	$\chi^{1,2}$	$\chi^{1,1}$	χ^2	$\chi^{3,1}$	$\chi^{3,2}$	
Nub	-174	156	-63	120	179	60		0.00
	-65	-35	-178	-127	180	60		0.48
	-73	84	-68	6	180	60		1.55
	-69	164	-173	-122	179	60		2.04
	-176	86	-72	4	-178	60		2.41
	178	-47	170	125	180	60		2.58
	52	43	-173	128	-177	60		2.69
	-177	165	57	137	-179			0.00
	178	-48	49	137	-179			0.49
	-46	-41	58	106	179			1.96
Bun	55	38	69	-124	-179			2.08
	-71	74	55	-135	179			2.37
	-64	169	65	-136	179			2.38
	-179	49	58	147	-177			2.93
	58	35	70	-129	-178	60		0.00
	-176	-50	52	139	179	60		0.26
	-71	69	60	-138	-179	60		0.71
	-44	-41	61	110	178	60		0.72
	-174	166	56	140	179	60		0.95
	-174	46	58	145	-178	60		1.91
Dmbun	-69	-12	63	-132	179	60		2.12
	-172	169	58	121	-159	60	60	0.00
	-175	-48	52	122	-160	60	60	0.35
	62	33	82	-134	-147	60	60	3.00
	-73	70	65	-141	-145	60	60	3.51

E_{rel} is defined in the legend to Table 1. E_o (kcal/mol) for Nub, -18.86; Bun, -12.92; Mbun, -7.44; Dmbun, 3.18.

Table 3. Conformational energy minima for *N*-acetyl *N'*-methylamide derivatives of Luds and Ludam2

Residue	Angle size, degrees							E_{rel} , kcal/mol
	ϕ	ψ	χ^1	$\chi^{2,2}$	$\chi^{2,1}$	$\chi^{3,1}$	$\chi^{3,2}$	
Luds	-80	70	51	-1	-166	59		0.00
	-74	-38	58	68	-71	62		0.21
	-140	-60	50	121	-72	60		0.77
	-140	39	50	122	-72	60		0.81
	-158	169	-166	86	-76	58		1.17
	57	41	-47	-78	-157	57		1.87
	55	58	-48	105	-1	180	180	0.00
	-81	78	-54	103	0	180	180	-0.60
Ludam2	-84	-46	-51	-65	0	180	180	1.09
	-159	-49	166	82	0	180	180	1.40
	66	160	-41	113	-2	180	180	2.75

E_{rel} is defined in the legend to Table 1. E_o (kcal/mol) for Luds, -3.55; Ludam2, -49.11.

Attempts to design side chains which might preferentially stabilize the $C^{\gamma a}$ conformation through specific hydrogen bonding have not been as successful yet. An amino acid residue with amino and acetyl groups attached to form an S chiral center at C^{β} of an alanine residue (Luds; Fig. 1) yielded a global energy minimum at $C^{\gamma a}$ (Table 3); however, the accessible conformational space includes multiple minima within 2 kcal/mol (Fig. 2*k*; Table 3) of the global minimum. This indicates that Luds could adopt a number of other conformations, including 3^1_{10} and the fully extended conformers. The other attempt to direct the folding to the $C^{\gamma a}$ region was made by adding two amide moieties to C^{β} of alanine (Ludam2; Fig. 1). The conformational space accessible to the Ludam2 dipeptide is more restricted than that for alanine,

FIG. 4. Predicted lowest energy left-handed helical conformation for [(*R*)-Ludam2]₁₂; the average ϕ angle is close to +52° and the ψ angle alternates between +65° and +47°. Both side-chain-side-chain and backbone-backbone hydrogen bonds stabilize this α -helix.

and C_7^a is indeed a local minimum (Fig. 2f). Even though the (ϕ, ψ) grid search indicated that the α^L conformer was the lowest energy region, after energy minimization C_7^a was slightly more stable (Table 3).

The unusual amino acids designed in this study not only offer the possibility of strong local conformational constraints for small peptides or protein loops but also can be used as monomer units for stabilizing the three-dimensional structure of helical polymers. For example, a homopolymer of Mbun is able to form a 3_{10} -helix which is stabilized by backbone van der Waals interactions and side-chain backbone hydrogen bonds (Fig. 4).

DISCUSSION

Improvements to the chemistry of coupling amino acid analogues to tRNA (20) allows the exploration of the effects of new amino acids on protein structure and function (28, 29). These amino acid substitutions have usually been chosen to alter the functional properties of particular side chains (28) without attempting to direct the conformation of the polypeptide backbone. Indeed, apart from Aib (21, 22), the conformational properties of most of the "non-genetic" amino acids have yet to be explored in detail.

The constraints introduced by disubstitution at the C^α atom of amino acid residues sterically restrict the conformational freedom of the peptide chain at that amino acid. The Aib residue has been studied in detail (10, 19), and the experimental and theoretical studies are in excellent agreement. This residue is essentially constrained to the $\alpha^R/3_0^R$ or $\alpha^L/3_{10}^L$ conformation. Extension of the methyl side chains of Aib to ethyl or benzyl groups leads to the fully extended conformation being strongly preferred (11, 30). These asymmetrical C^α -, C^β -substituted amino acids are conformationally restricted and can now be incorporated into proteins and peptides by biosynthetic or chemical methods (20, 23, 28, 31). However, no attempt has been made to design analogues of this class of amino acids which would preferentially fold to a unique conformer.

The predicted conformational properties of the amino acids designed in this report indicate that it might be possible to derive an amino acid set for synthesizing peptides which are conformationally determined. Since substitutions beyond the side-chain β position should have minimal effects on these conformational preferences, an array of functionalities can be added to manipulate the solubility and catalytic potentials of peptides constructed from variations on the amino acids which are asymmetrically substituted by heavy atoms at the C^α moiety. Indeed, replacement of H^α by the groups described in this report should be feasible for all amino acids, yielding a large set of conformationally defined residues with a variety of hydrophobic, hydrophilic, or aromatic properties. A number of reports indicate that amino acids of the type described in this study can be synthesized (32) and incorporated into peptides (31). Although the local conformational minima would be determined, peptides synthesized with these amino acids would still be expected to have some (ϕ, ψ) flexibility, and consequently, long-range forces should be capable of inducing uniquely folded, compact structures which can mimic enzyme or ligand binding sites.

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1. Némethy, G. & Scheraga, H. A. (1990) *FASEB J.* **4**, 3189–3197.
2. Bowie, J. U., Reidhaar-Olsen, J. F., Lim, W. A. & Sauer, R. T. (1990) *Science* **247**, 1306–1310.
3. Ramachandran, G. N., Ramakrishnan, C. & Sasisekharan, V. (1963) *J. Mol. Biol.* **7**, 95–99.
4. Schellman, J. A. & Schellman, C. (1964) *The Proteins*, ed. Neurath, H. (Academic, New York), Vol. 2, pp. 1–137.
5. Roterman, I. K., Lambert, M. H., Gibson, K. O. & Scheraga, H. A. (1989) *J. Biomol. Struct. Dyn.* **7**, 421–453.
6. Zimmerman, S. S., Pottle, M. S., Némethy, G. & Scheraga, H. A. (1977) *Macromolecules* **10**, 1–9.
7. Ramachandran, G. N. & Sasisekharan, V. (1968) *Adv. Protein Chem.* **23**, 283–437.
8. Pettit, G. M. & Karplus, M. (1985) *Chem. Phys. Lett.* **121**, 194–201.
9. Anderson, A. G. & Hermans, J. (1988) *Proteins Struct. Funct. Genet.* **3**, 262–265.
10. Iijima, N., Dunbar, J. B. & Marshall, G. R. (1987) *Proteins Struct. Funct. Genet.* **2**, 330–339.
11. Toniolo, C. (1989) *Biopolymers* **28**, 247–257.
12. Gilon, C., Halle, D., Chorev, M., Selinger, Z. & Byk, G. (1991) *Biopolymers* **31**, 745–750.
13. Creighton, T. E. (1991) *Curr. Opin. Struct. Biol.* **1**, 5–16.
14. Jones, D. T., Taylor, W. R. & Thornton, J. M. (1992) *Nature (London)* **358**, 86–89.
15. Bowie, J. U., Lüthy, R. & Eisenberg, C. (1991) *Science* **253**, 164–170.
16. Madden, D. R. & Wiley, D. C. (1992) *Curr. Opin. Struct. Biol.* **2**, 300–304.
17. Kleinkauf, H. & Döhren, H. (1990) *Eur. J. Biochem.* **192**, 1–15.
18. Rosen, M. K. & Schreiber, S. L. (1992) *Angew. Chem. Int. Ed. Eng.* **31**, 384–400.
19. Prasad, B. V. V. & Balaram, P. (1984) *CRC Crit. Rev. Biochem.* **16**, 307–348.
20. Noren, C. J., Anthony-Cahill, S. J., Griffith, M. C. & Schultz, P. G. (1989) *Science* **244**, 182–188.
21. Marshall, G. R. & Bosshard, H. E. (1972) *Circ. Res.* **30/31 Suppl. II**, 143.
22. Burgess, A. W. & Leach, S. J. (1973) *Biopolymers* **12**, 2599–2605.
23. Altmann, E., Altmann, K.-H., Nebel, K. & Mutter, M. (1988) *Int. J. Pept. Protein Res.* **32**, 344–351.
24. Némethy, G., Pottle, M. S. & Scheraga, H. A. (1983) *J. Phys. Chem.* **87**, 1883–1887.
25. IUPAC-IUB Commission on Biochemical Nomenclature (1970) *Biochemistry* **18**, 3471–3479.
26. Zebisch, E. G., Healy, E. F. & Stewart, J. J. P. (1985) *J. Am. Chem. Soc.* **107**, 3902–3909.
27. Powell, M. J. D. (1964) *Comput. J.* **7**, 155–162.
28. Ellman, J. A., Mendel, D. & Schultz, P. G. (1992) *Science* **255**, 197–200.
29. Chung, H.-H., Benson, D. R. & Schultz, P. G. (1993) *Science* **259**, 806–809.
30. Valle, G., Crisma, M., Bonora, G. M., Toniolo, C., Lelj, F., Barone, V., Fraternali, F., Hardy, P. M., Langran-Goldsmith, A. & Maia, H. L. S. (1990) *J. Chem. Soc. Perkins Trans. 2*, 1481–1487.
31. Lucente, G., Lucente, G. M., Pantanella, F. & Romeo, A. (1969) *Chem. Ind. (Berlin)* **39**, 696–697.
32. Poisel, H. (1978) *Chem. Ber.* **111**, 3136–3139.