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SHORT COMMUNICATION

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Helical Oligomers with a Changeable Chiral Acetal Moiety

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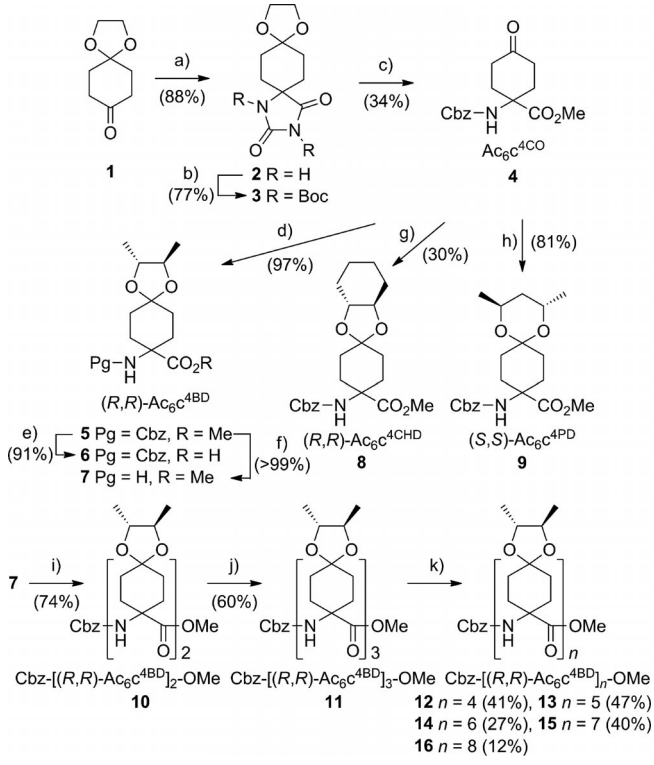
(R,R)-Ac_6c^{4BD} homopeptides form helical structures with slight control of the helical screw sense to the right-handed form. The chiral acetal moieties in (R,R)-Ac_6c^{4BD} are changeable in the peptide state.

Introduction

The precise control of the secondary structure in peptides and proteins is one of the most challenging assignments for bioorganic, peptide, and medicinal chemists. α,α-Disubstituted α-amino acids (dAAs) are particularly promising candidates for this purpose because of their characteristic secondary structural preferences, such as β turns,[1] 3_{10} helices,[2] and extended planar C_5 conformations.[3] Therefore, much effort has been concentrated on the design of new dAAs[4] and the conformational analysis of their oligopeptides. We recently reported that chiral cyclic dAAs bearing only side-chain chiral centers could control the helical screw sense of their oligopeptides.[5] α-Helices and 3_{10} helices in natural proteins almost always adopt a right-handed (*P*) helical screw sense owing to the chiral centers at the α-position of L-α-amino acids. Thus, chiral centers not only at the α-position but also in the side chain of amino acids could contribute to the regulation of the helical screw sense of peptides. In the present study, we designed a chiral acetal dAA {(2*R*,3*R*)-8-amino-1,4-dioxo-2,3-dimethylspiro[4.5]-decane-8-carboxylic acid, (R,R)-Ac_6c^{4BD}}, which possesses two chiral centers that are remote from the α-position, and achieved conformational analysis of its homopeptides. A feature of the dAA designed here is that the acetal moiety in the side chain is changeable. We also demonstrated the synthesis of other chiral acetal dAAs and the facile conversion of the chiral acetal moieties in the (R,R)-Ac_6c^{4BD} peptide.

Results and Discussion

Chiral acetal dAAs were synthesized from cyclohexane-1,4-dione monoethylene acetal (**1**; Scheme 1). Monoethylene acetal **1** was converted into hydantoin **2** by the Buch-



Scheme 1. Synthesis of chiral acetal dAAs and their peptides. Yields are based on recovered materials. Reagents and conditions: (a) KCN, $(NH_4)_2CO_3$; (b) Boc_2O; (c) 1. $LiOH·H_2O$; 2. HCl; 3. CbzCl; 4. MeOH, HCl; 5. AcOH; (d) (*R*,*R*)-butane-2,3-diol, chlorotrimethylsilane (TMSCl), TMSOTf; (e) NaOH; (f) H_2, Pd/C; (g) (*R*,*R*)-cyclohexane-1,2-diol, TMSCl, trimethylsilyl trifluoromethanesulfonate (TMSOTf); (h) (*S*,*S*)-pentane-2,4-diol, TMSCl, TMSOTf; (i) **6**, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl), hydroxybenzotriazole (HOBt); (j) 1. NaOH; 2. **7**, 1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), HOBt; (k) 1. H_2, Pd/C; 2. **6**, HATU, HOAt.

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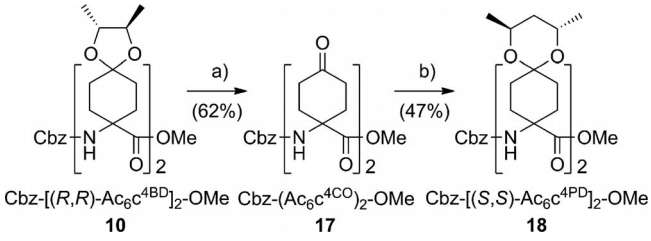
erer–Bergs procedure,[6] followed by *tert*-butoxycarbonyl (Boc) protection to give di-Boc-hydantoin **3**. After alkaline hydrolysis of di-Boc-hydantoin **3**, carboxybenzyl (Cbz) protection and subsequent methyl esterification afforded a mixture of Cbz-Ac_6c^{4CO}-OMe (**4**) and acetal-**4**. The direct alkaline hydrolysis of hydantoin was performed by the treatment of **2** at 150 °C in a sealed tube without di-Boc-hydantoin **3**. Deprotection of the acetal moiety in acetal-**4** was completed by acidic hydrolysis to give carbonyl dAA **4** in a yield of 34% (based on **3**). The acetalization of **4** with (*R*,*R*)-butane-2,3-diol, (*R*,*R*)-cyclohexane-1,2-diol, and (*S*,*S*)-pentane-2,4-diol afforded chiral acetal dAAs (*R*,*R*)-Ac_6c^{4BD} (**5**, 97%), (*R*,*R*)-Ac_6c^{4CHD} (**8**, 30%), and (*S*,*S*)-Ac_6c^{4PD} (**9**, 81%), respectively. The hydrolysis of **5** under alkaline conditions gave the C-terminal-free Cbz-(*R*,*R*)-Ac_6c^{4BD}-OH (**6**, 91%), and the hydrogenolysis of **5** resulted in the N-terminal-free H-(*R*,*R*)-Ac_6c^{4BD}-OMe (**7**, >99%). (*R*,*R*)-Ac_6c^{4BD} dipeptide **10** was synthesized from **6** and **7** by solution-phase methods. (*R*,*R*)-Ac_6c^{4BD} tripeptide **11** was synthesized from the dipeptide acid and **7**. The (*R*,*R*)-Ac_6c^{4BD} homopeptides Cbz-[(*R*,*R*)-Ac_6c^{4BD}]$_n$-OMe [*n* = 4 (**12**), 5 (**13**), 6 (**14**), 7 (**15**), 8 (**16**)] were prepared by the coupling of the N-terminal-free oligomers and *N*-protected **6** by using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and 1-hydroxy-7-azabenzotriazole (HOAt) as coupling reagents. We demonstrated that the acetal moieties in the peptide were changeable (Scheme 2). Briefly, the treatment of (*R*,*R*)-Ac_6c^{4BD} dipeptide **10** with trifluoroacetic acid resulted in acetal deprotection to give dipeptide **17**, which possesses carbonyl functions. The re-acetalization of **17** by using (*S*,*S*)-pentane-2,4-diol afforded dipeptide Cbz-[(*S*,*S*)-Ac_6c^{4PD}]$_2$-OMe (**18**) in a yield of 47%.



Scheme 2. Conversion of the chiral acetal moiety of the dipeptide. Yields are based on recovered materials. Reagents and conditions: (a) trifluoroacetic acid; (b) (*S*,*S*)-pentane-2,4-diol, TMSCl, TMSOTf.

We studied the preferred secondary structure of the homopeptides in $CDCl_3$ solution by FTIR absorption spectroscopy (Figure 1). The IR spectra of Cbz-[(*R*,*R*)-Ac_6c^{4BD}]$_n$-OMe (*n* = 1–8) showed weak bands in the 3420–3440 cm^{-1} region [free (solvated) peptide NH groups] and strong bands in the 3320–3380 cm^{-1} region (intramolecularly H-bonded peptide NH groups). The low-frequency band observed at 3380 cm^{-1} in tripeptide **11** shifted to a lower wavelength of 3350 cm^{-1} in octapeptide **16**, and its intensity steadily increased with elongation of the peptide length. These results are very similar to those of Ac_nc homopeptides, which adopt a helical secondary structure in solution.[7] It should be noted that we observed a shoulder band at 3415 cm^{-1} for the dipeptide to octapeptide (**10**–**16**). The homooligopeptides based on α-hydroxymethylserine with an acetal group were reported to prefer β-bend/3_{10}-helical structures and showed intramolecular H bonds (3410–3415 cm^{-1} in the FTIR spectra) between the peptide NH groups and the ethereal oxygen atoms of the side chain.[8] Thus, the peptide NH groups in homopeptides **10**–**16** may partly form weak H bonds with the ethereal oxygen atoms of the acetal moieties.

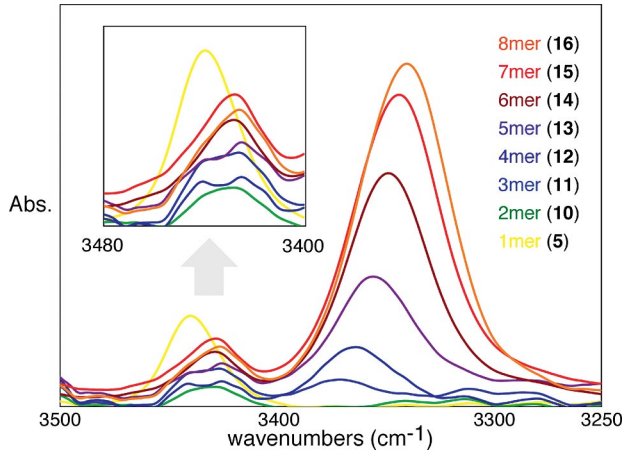


Figure 1. IR absorption spectra of Cbz-[(*R*,*R*)-Ac_6c^{4BD}]$_m$-OMe [*m* = 1 (**5**), 2 (**10**), 3 (**11**), 4 (**12**), 5 (**13**), 6 (**14**), 7 (**15**), 8 (**16**)] in $CDCl_3$ solution. Peptide concentration: 3.0 mM.

The ROESY ^{1}H NMR spectrum of hexapeptide **14** showed a complete series of sequential $d_{N,N}$ cross-peaks of NOEs from the N-terminal NH(1) to the C-terminal NH(6) group (Figure S1). These correlations are characteristic of a helical secondary structure.[9] ^{1}H NMR experiments were also performed with the addition of dimethyl sulfoxide (DMSO) or free-radical 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO; Figure S2). Free NH groups without intramolecular H bonds are affected by the addition of DMSO or TEMPO.[10] In the case of hexapeptide **14**, the NH(1) signal was very sensitive. The two NH groups [NH(1) and NH(2)] in the 3_{10}-helical peptides were usually free (solvated), and their signals were sensitive. Therefore, hexapeptide **14** may adopt a helical secondary structure in $CDCl_3$ solution, whereas the NH(2) group may not be completely free of H bonding, which is consistent with the results of the FTIR measurements.

We measured the CD spectra of (*R*,*R*)-Ac_6c^{4BD} hexapeptide **14**, heptapeptide **15**, and octapeptide **16** in 2,2,2-trifluoroethanol (TFE) solution (Figure 2). The negative and positive maxima of three bands (192, 208, and 222 nm) and the relative intensity of two bands (208 and 222 nm) indicate the screw sense of helicity and the 3_{10}- or α-helical structure of peptides, respectively.[11] The CD spectra of **14**, **15**, and **16** showed negative maxima at 215 and 230 nm and a positive maximum at 205 nm (for **16** only), which suggests that right-handed (*P*) helices might be slightly predominant. Interestingly, the intensities of maxima increased with the elongation of peptide lengths. However, the intensities

of these maxima were weak and shifted from the ideal values of the helix. These results may be attributed to the fact that the secondary structures of **14**, **15**, and **16** were not ideal helical structures in TFE solution, maybe because of H bonds between the NH groups and the ethereal oxygen atoms of the acetal moieties. Furthermore, the coexistence of right-handed (*P*) and left-handed (*M*) helices may induce a decrease in the intensities of the maxima. The CD spectra of the homochiral octapeptide (*S*,*S*)-Ac$_5$c^{dOMe} showed clear positive maxima at 208 and 225 nm and a negative maximum at 200 nm, which indicates that its secondary structure is a left-handed α helix. Compared to the (*S*,*S*)-Ac$_5$c^{dOMe}

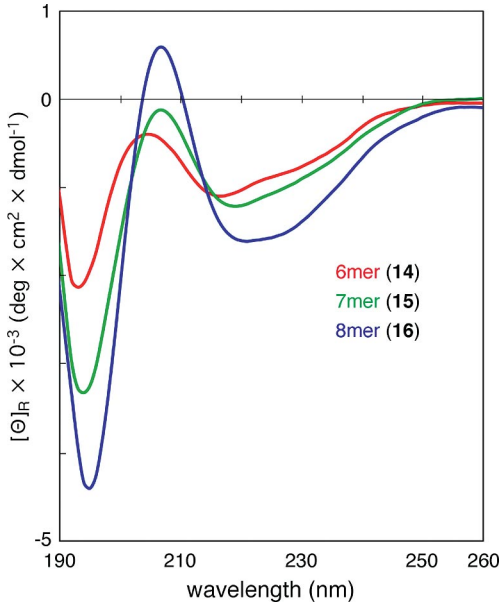


Figure 2. CD spectra of Cbz-[(*R*,*R*)-Ac$_6$c^{4BD}]$_m$-OMe [*m* = 6 (**14**), 7 (**15**), 8 (**16**)] in TFE solution. Peptide concentration: 0.3 mM.

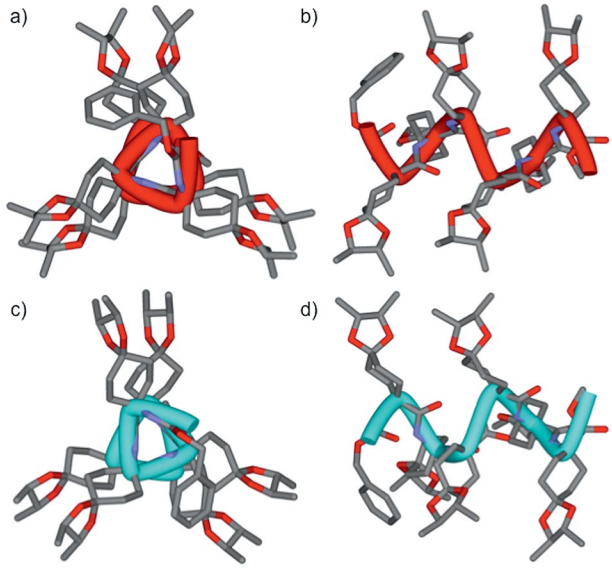


Figure 3. Calculated minimum-energy conformation of (*R*,*R*)-Ac$_6$c^{4BD} hexapeptide **14**: (a, b) (*P*) 3$_{10}$-helical structure and (c, d) (*M*) 3$_{10}$-helical structure.

homopeptides, it seems to be difficult for the (*R*,*R*)-Ac$_6$c^{4BD} homopeptides to control the helical screw sense.[5b]

The conformational search calculation of hexapeptide **14** was performed by using the Monte Carlo multiple minimum (MCMM) method of the MacroModel program (version 9.1 Schrodinger, Inc.) with the AMBER* force field to find several local minimum-energy conformations (Figure 3). The (*P*) 3$_{10}$-helical structure was produced as the global minimum-energy conformation (0 kcal/mol), and the (*M*) 3$_{10}$-helical structure was obtained as a local minimum-energy conformation (+0.67 kcal/mol). The (*P*) 3$_{10}$-helical structure was slightly more stable than the (*M*) 3$_{10}$-helical structure, but the difference in energies between them was not sufficient to control the helical screw sense.

Conclusions

We synthesized cyclic dAAs with a chiral acetal moiety. The IR, ^{1}H NMR, and CD spectra revealed that the (*R*,*R*)-Ac$_6$c^{4BD} homopeptides formed helical structures with a slight control of the helical screw sense to right-handed. These findings imply that side-chain chiral centers at remote positions from the α carbon atom can control the helical screw sense of their peptides. The acetal moieties in (*R*,*R*)-Ac$_6$c^{4BD} were changeable in the peptide state. Thus, we could control the helical screw sense of peptides to right-handed or left-handed by changing the chirality of the acetal moiety. This strategy can be applied not only to the dAA peptides described here but also to other foldamers. The use of a new helical peptide with an acetal moiety as a chiral organocatalyst is being investigated by our group.

Experimental Section

The chiral acetal amino acids (*R*,*R*)-Ac$_6$c^{4BD} **5**, (*R*,*R*)-Ac$_6$c^{4CHD} **8**, and (*S*,*S*)-Ac$_6$c^{4PD} **9** were synthesized from cyclohexane-1,4-dione monoethylene acetal (**1**). The syntheses of the (*R*,*R*)-Ac$_6$c^{4BD} homopeptides were performed by solution-phase segment condensation methods by using EDC·HCl and HOBt, HBTU and HOBt, or HATU and HOAt, as coupling reagents. All compounds were purified by column chromatography on silica gel.

Supporting Information (see footnote on the first page of this article): Full experimental details.

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[1] a) P. K. C. Paul, M. Sukumar, R. Bardi, A. M. Piazzesi, G. Valle, C. Toniolo, P. Bardi, *J. Am. Chem. Soc.* **1986**, *108*, 6363–6370; b) E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, A.

Santini, M. Crisma, G. Valle, C. Toniolo, *Biopolymers* **1989**, *28*, 175–184; c) G. Valle, M. Pantano, F. Formaggio, M. Crisma, C. Toniolo, G. Precigoux, G. Sulzenbacher, W. H. J. Boesten, Q. B. Broxterman, H. E. Schoemaker, J. Kamphuis, *Biopolymers* **1993**, *33*, 1617–1625.

[2] a) Y. Paterson, S. M. Rumsey, E. Benedetti, G. Nemethy, H. A. Scheraga, *J. Am. Chem. Soc.* **1981**, *103*, 2047–2955; b) C. Toniolo, M. Bonora, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, *Macromolecules* **1986**, *19*, 472–479; c) I. L. Karle, P. Balaram, *Biochemistry* **1990**, *29*, 6747–6756; d) F. Formaggio, M. Crisma, G. M. Bonora, M. Pantano, G. Valle, C. Toniolo, A. Aubry, D. Bayeul, J. Kamphuis, *Pept. Res.* **1995**, *8*, 6–15; e) A. Polese, F. Formaggio, M. Crisma, G. Valle, C. Toniolo, G. M. Bonora, Q. B. Broxterman, J. Kamphuis, *Chem. Eur. J.* **1996**, *2*, 1104–1111; f) B. Jaun, M. Tanaka, P. Seiler, F. N. M. Kuhnle, C. Baraun, D. Seebach, *Liebigs Ann./Recueil* **1997**, 1697–1710.

[3] a) E. Benedetti, C. Toniolo, P. Hardy, V. Barone, A. Bavoso, B. Di Blasio, P. Grimaldi, F. Lelj, V. Pavone, C. Pedone, G. M. Bonora, I. Lingham, *J. Am. Chem. Soc.* **1984**, *106*, 8146–8152; b) E. Benedetti, V. Barone, A. Bavoso, B. Di Blasio, F. Lelj, V. Pavone, C. Pedone, G. M. Bonora, C. Toniolo, M. T. Leplawy, K. Kaczmarek, A. Redlinski, *Biopolymers* **1988**, *27*, 357–371; c) C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, V. Barone, F. Lelj, M. T. Leplawy, K. Kaczmarek, A. Redlinski, *Biopolymers* **1988**, *27*, 373–379; d) M. Tanaka, N. Imawaka, M. Kurihara, H. Suemune, *Helv. Chim. Acta* **1999**, *82*, 494–510; e) N. Imawaka, M. Tanaka, H. Suemune, *Helv. Chim. Acta* **2000**, *83*, 2823–2835; f) M. Crisma, A. Moretto, C. Peggion, L. Panella, B. Kaptein, Q. B. Broxterman, F. Formaggio, C. Toniolo, *Amino Acids* **2011**, *41*, 629–641.

[4] a) M. Tanaka, M. Oba, K. Tamai, H. Suemune, *J. Org. Chem.* **2001**, *66*, 2667–2673; b) M. Oba, M. Tanaka, Y. Takano, H. Suemune, *Tetrahedron* **2005**, *61*, 593–598; c) C. Cativiela, M. D. Diaz-de-Villegas, *Tetrahedron: Asymmetry* **2007**, *18*, 569–623; d) C. Cativiela, M. Ordonez, *Tetrahedron: Asymmetry* **2009**, *20*, 1–63; e) M. Oba, A. Shimabukuro, M. Ono, M. Doi, M. Tanaka, *Tetrahedron: Asymmetry* **2013**, *24*, 464–467.

[5] a) M. Tanaka, *Chem. Pharm. Bull.* **2007**, *55*, 349–358; b) Y. Demizu, M. Doi, M. Kurihara, T. Maruyama, H. Suemune, M. Tanaka, *Chem. Eur. J.* **2012**, *18*, 2430–2439; c) K. Anan, Y. Demizu, M. Oba, M. Kurihara, M. Doi, H. Suemune, M. Tanaka, *Helv. Chim. Acta* **2012**, *95*, 1694–1713.

[6] Y. Chu, V. Lynch, B. L. Iverson, *Tetrahedron* **2006**, *62*, 5536–5548.

[7] a) M. Gatos, F. Formaggio, M. Crisma, C. Toniolo, G. M. Bonora, Z. Benedetti, B. Di Blasio, R. Iacovino, A. Santini, M. Saviano, J. Kamphuis, *J. Pept. Sci.* **1997**, *3*, 110–122; b) E. Benedetti, B. Di Blasio, R. Iacovino, V. Menchise, M. Saviano, C. Pedone, G. M. Bonora, A. Ettore, L. Graci, F. Formaggio, M. Crisma, G. Valle, C. Toniolo, *J. Chem. Soc. Perkin Trans. 2* **1997**, 2023–2032; c) M. Nagano, M. Tanaka, M. Doi, Y. Demizu, M. Kurihara, H. Suemune, *Org. Lett.* **2009**, *11*, 1135–1137.

[8] W. M. Wolf, M. Stasiak, M. T. Leplawy, A. Bianco, F. Formaggio, M. Crisma, C. Toniolo, *J. Am. Chem. Soc.* **1998**, *120*, 11558–11566.

[9] a) K. Wuthrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, **1986**; b) G. Wagner, D. Neuhaus, E. Worgotter, M. Vasak, J. H. R. Kagi, K. Wuthrich, *J. Mol. Biol.* **1986**, *187*, 131–135.

[10] a) G. M. Bonora, C. Toniolo, B. Di Blasio, V. Pavone, C. Pedone, E. Benedetti, I. Lingham, P. Hardy, *J. Am. Chem. Soc.* **1984**, *106*, 8152–8156; b) C. Toniolo, G. M. Bonora, V. Barone, A. Bavoso, E. Benedetti, B. Di Blasio, P. Grimaldi, F. Lelj, V. Pavone, C. Pedone, *Macromolecules* **1985**, *18*, 895–902.

[11] a) C. Toniolo, A. Polese, F. Formaggio, M. Crisma, J. Kamphuis, *J. Am. Chem. Soc.* **1996**, *118*, 2744–2745; b) G. Yoder, A. Polese, R. A. G. D. Silva, F. Formaggio, M. Crisma, Q. B. Broxterman, J. Kamphuis, C. Toniolo, T. A. Keiderling, *J. Am. Chem. Soc.* **1997**, *119*, 10278–10285; c) S. Mammi, M. Rainaldi, M. Bellanda, E. Schievano, E. Peggion, Q. B. Broxterman, F. Formaggio, M. Crisma, C. Toniolo, *J. Am. Chem. Soc.* **2000**, *122*, 11735–11736; d) P. Pengo, L. Pasquato, S. Moro, A. Brigo, F. Fogolari, Q. B. Broxterman, B. Kaptein, P. Scrimin, *Angew. Chem.* **2003**, *115*, 3510–3514; *Angew. Chem. Int. Ed.* **2003**, *42*, 3388–3392.

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