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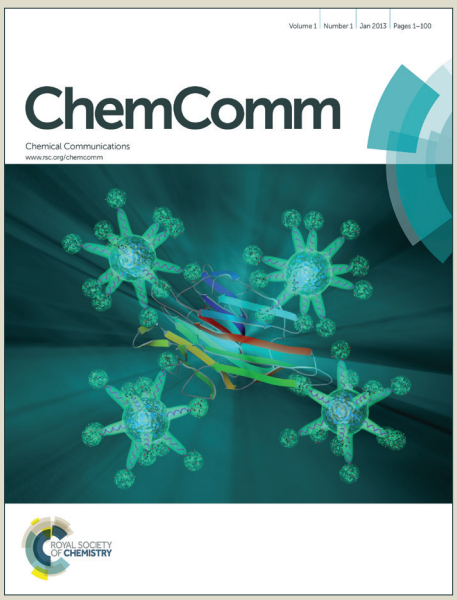

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Controlling the sign and magnitude of screw-sense preference from the C-terminus of an achiral helical foldamer†

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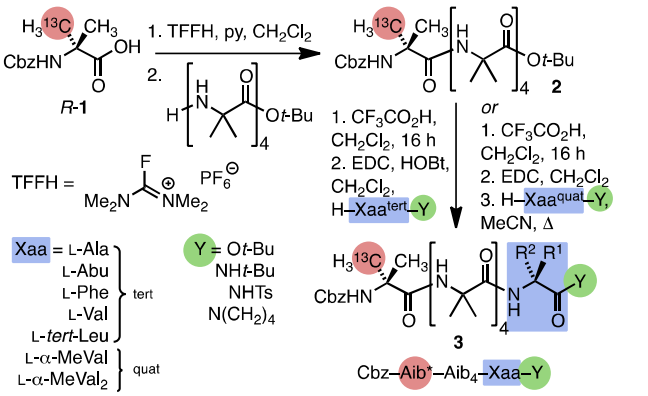
The global screw-sense preference of an achiral helical oligomer may be controlled by a single chiral monomer located at one terminus. Remarkably, maximal control is induced in oligomers of the achiral quaternary amino acid Aib by a single C-terminal alaninamide residue, probably because the Ala side chain, though small, is compatible with a 3_{10} helical conformation. The presence or absence of a C-terminal hydrogen bond donor determines the screw sense of the entire oligomer.

The adoption of well-defined conformations is a characteristic feature of many classes of biomolecules, and understanding the encoding of conformational features within the primary structure of proteins is an important challenge.[1] Foldamers are synthetic oligomers and polymers that likewise adopt well-defined conformations,[2] and their utility depends on using simple structural features (dipole orientation, hydrogen bonding ability, stereochemical configuration) to induce global conformational, and hence ultimately functional, consequences.[3] We[4] and others[5,6] have shown that helical oligomers containing the achiral quaternary amino acid Aib (α-aminoisobutyric acid)[7] adopt preferentially a left- or a right-handed[4a] global screw sense as a result of the local stereochemical influence of a chiral N-terminal amino acid residue,[4a-j,5a] a chiral diol bound to an N-terminal boronate binding site,[4k] or a chiral carboxylic acid ion-paired with an N-terminal amino group.[5b] These N-terminal controllers of global conformation function by providing appropriately orientated hydrogen-bond acceptors that organise the N-terminal NH groups of the overall 3_{10} helical structure[7] of the oligomer into a left- or a right-handed β-turn.[8]

Little is known about the propensity of achiral peptide helices to be induced from the C terminus. Chiral C-terminal residues induce some degree of screw-sense preference in short achiral helices,[5c,6] but a comparison[9] between an N- and a C-terminal controller suggested that C-terminal control was subordinate to N-terminal control.

We now report a quantitative analysis of the role played by a chiral C-terminal residue in determining the global screw-sense preference of a series of otherwise achiral Aib-based oligomers **3**. The compounds in question were synthesised by ligating a range of amino acid derivatives H–Xaa–Y (esters Y = OR or amides Y = NHR or NR_2) to the C terminus of an Aib pentamer **2**, as shown in Scheme 1. Derivatives of tertiary amino acids (H–Xaa^{tert}–Y) coupled cleanly using the coupling agent EDC in the presence of HOBt. Derivatives of quaternary amino acids (H–Xaa^{quat}–Y) failed to couple under these conditions, but nonetheless cleanly opened the azlactone derivative of **2** (generated using EDC) on reflux in acetonitrile.



Scheme 1: Synthesis of the ^{13}C labelled oligomers **3**.

In order to quantify the screw-sense preference induced by the C-terminal residues, the N-terminal Aib residue of the pentamer **2** was isotopically labelled in an enantioselective manner with ^{13}C. The required protected amino acid *R*-**1** was synthesised from L-Ala by a method[10] that enriches the ^{13}C abundance in the pro-*R* methyl group to ca. 80% and the pro-*S* methyl group to ca. 20%.

The ^{13}C NMR spectrum of each oligomer **3** was acquired at +23 °C in CD_3OD, a solvent in which Aib oligomers show no concentration-dependent effects.[11] The chemical shift separation $\Delta\delta_{fast}$ between the minor and major ^{13}C NMR signals of each oligomer is reported in Table 1. For a number of oligomers, the ^{13}C NMR spectrum was also acquired

at –70 °C in CD_3OD. Variable temperature NMR over the range –70 to +40 °C (Figure 1b) showed that spectra at the upper and lower limits of this range provided suitable values for chemical shift separation at slow and fast exchange $\Delta\delta_{slow}$ and $\Delta\delta_{fast}$,[4c] and the values obtained at –70 °C for $\Delta\delta_{slow}$ are also reported in Table 1.

The measured values of $\Delta\delta_{slow}$ are constant within 1% across the compounds studied, consistent with the assumption[4c] that the spatial separation between the ^{13}C NMR reporter and the chiral C-terminal residue ensures that the anisochronicity of the ^{13}C labels at low temperature results entirely from their local interaction with the slowly inverting helix. Thus, at fast exchange, the value of $\Delta\delta_{fast}$ is dependent only on the equilibrium ratio of the *M* and *P* helices,[4c,e] and the value $\Delta\delta_{fast}$ / $|\Delta\delta_{slow}|$ may be interpreted as helical excess (h.e.), as reported in Table 1.

Table 1: Conformational preferences in Aib oligomers **3** carrying C-terminal controllers Xaa-Y

entry	compound	residue Xaa	R^1	R^2	Y	$\Delta\delta_{fast}$ [a]	$\lvert\Delta\delta_{slow}\rvert$ [b]	h.e. [c]	h.r. [d]
1	**3**-Ala-NH*t*-Bu	Ala	Me	H	NH*t*-Bu	+1807	2415	+75	88:12
2	**3**-AlaNHMe	Ala	Me	H	NHMe	+1883	–	+78	89:11
3	**3**-Ala-N$(CH_2)_4$	Ala	Me	H		–800	–	–33	33:67
4	**3**-Abu-NH*t*-Bu	Abu[e]	Et	H	NH*t*-Bu	+1857	–	+77	88:12
5	**3**-Ser(O*t*-Bu)[f]-NH*t*-Bu	Ser(O-*t*-Bu)[f]	CH_2O*t*-Bu	H	NH*t*-Bu	+668	–	+28	64:36
6	**3**-Pro-NH*t*-Bu	Pro	-$(CH_2)_3$CH-	H	NH*t*-Bu	+376	–	+16	58:42
7	**3**-Phe-NH*t*-Bu	Phe	Bn	H	NH*t*-Bu	+1676	2410	+70	85:15
8	**3**-Phe-NHTs	Phe	Bn	H	NHTs	+1057	–	+36	68:32
9	**3**-Val-NH*t*-Bu	Val	*i*-Pr	H	NH*t*-Bu	+1726	2420	+71	86:14
10	**3**-Tle-N*t*-Bu	*tert*-Leu	*t*-Bu	H	NH*t*-Bu	+1575	–	+65	83:17
11	**3**-αMv-NH*t*-Bu	α-MeVal	*i*-Pr	Me	NH*t*-Bu	+1710	–	+70	85:15
12	**3**-αMv_2-NH*t*-Bu	α-$MeVal_2$	*i*-Pr	Me	NH*t*-Bu	+1943	–	+80	90:10
13	**3**-Ala-O*t*-Bu	Ala	Me	H	O*t*-Bu	–1336	–	–55	22:78
14	**3**-Phe-O*t*-Bu	Phe	Bn	H	O*t*-Bu	–816	2427	–34	33:67
15	**3**-Val-O*t*-Bu	Val	*i*-Pr	H	O*t*-Bu	–1112	2441	–46	27:73
16	**3**-Tle-O*t*-Bu	*tert*-Leu	*t*-Bu	H	O*t*-Bu	–838	–	–35	32:68
17	**3**-αMv-O*t*-Bu	α-MeVal	*i*-Pr	Me	O*t*-Bu	–1037	–	–43	28:72

[a] Chemical shift separation between minor and major labelled peaks [$\delta_{fast}^{minor} - \delta_{fast}^{major}$] in the ^{13}C NMR spectrum in CD_3OD at +23 °C; [b] Modulus of the chemical shift separation between labelled peaks in the ^{13}C NMR spectrum in CD_3OD at –40 °C. Where no value for $\Delta\delta_{slow}$ was measured, an average value of $\Delta\delta_{slow}$ = 2420 was employed; [c] helical excess = $\Delta\delta_{fast}$ / $|\Delta\delta_{slow}|$ interpreted as {[*P*]–[*M*]}/{[*P*]+[*M*]}. Positive values indicate right-handed screw sense predominates; [d] helical ratio = [*P*]:[*M*]; [e] Abu = (*S*)-(+)-2-aminobutyric acid = L-(+)-butyrine; [f] Serine side-chain protected as its *t*-butyl ether.

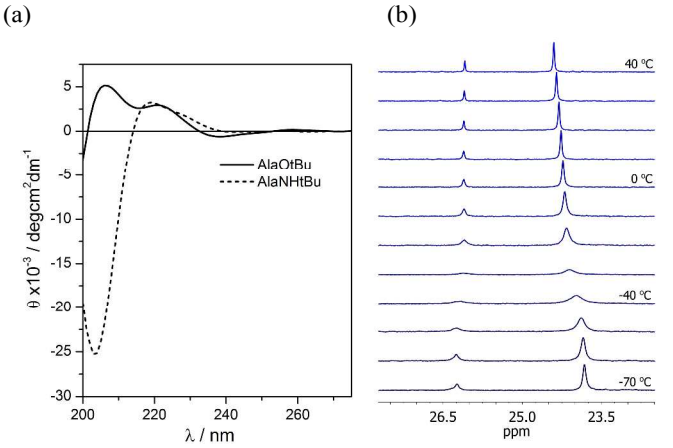


Figure 1: (a) Circular dichroism spectra of **3**-Ala-NH*t*-Bu and **3**-Ala-O*t*-Bu at +20 °C in MeOH, measured at 7x10^{-4} mol dm^{-3}. The signs of the bands at 205 nm[4e,13] indicate a *P* (right-handed) screw-sense preference in **3**-Ala-NH*t*-Bu and an *M* (left-handed) screw-sense preference in **3**-Ala-O*t*-Bu; (b) Variable temperature ^{13}C NMR spectra of **3**-Ala-NH*t*-Bu at 10 °C intervals from –70 °C (bottom) to +40 °C (top). Coalescence occurs between –50 and –20 °C.

The sign of the helical excess was deduced from the location of the major signal arising from the ^{13}C-labelled Aib residue. The pro-*R* methyl group of an Aib residue resonates upfield of the pro-*S* methyl group in a right handed (*P*) 3_{10} helix and downfield in a left handed (*M*) 3_{10} helix.[4a,12] Positive values of $\Delta\delta_{fast}$ thus correspond to *P* helicity, a deduction confirmed by circular dichroism (Figure 1a): the negative diagnostic band at 205 nm[4e,13] for **3**-Ala-NH*t*-Bu confirms *P* helicity, and the positive band at 205 nm for **3**-Ala-O*t*-Bu confirms *M* helicity.

The first conclusion that can be drawn from the results in Table 1 is that C-terminal secondary amides of L-amino acids, whether tertiary (entries 1-2, 4-10) or quaternary (entries 11,12), induce *P* helicity, while C-terminal esters (entries 13-17) and a tertiary amide (entry 3) induce *M* helicity. We deduce that the divergent behaviour of these two groups of compounds is due to the ability of the secondary amides to use their NH group to form an additional C-terminal hydrogen bond, promoting continuation of a 3_{10} helical structure, as illustrated in Figure 2a. It is well established that L-residues located within a 3_{10} helix promote right-handed screw-sense preference,[4a,14] and Figure 2c illustrates the mechanism by which a bulky R^1 substituent exerts this effect by lying perpendicular to the plane of the adjacent amide group. The weak preference induced by ProNH*t*-Bu (entry 6) is likely to be due to the bend induced by a Pro residue,[15] preventing or weakening this C-terminal hydrogen bond. By contrast, the inability of C-terminal esters to continue a hydrogen-bonded network gives rise to the well-known 'Schellman motif',[16] in which dipole repulsion leads to a local helical inversion. Such a motif is evident in the X-ray crystal structure of **3**-Ala-O*t*-Bu (Figure 3). A single L residue adopting this motif has been noted before to induce a left-handed screw sense in an otherwise achiral oligomer,[5c] and Figure 2d illustrates the origin of the effect. The tertiary amide **3**-Ala-N$(CH_2)_4$ also exhibits *M* screw-sense, presumably also the result of a corresponding '*tert*-amide Schellman' motif.[17]

The conformational constraint imposed by their additional hydrogen bond means that the secondary amides generally control screw-sense preference to a greater degree than the esters, irrespective of the size of the amide N-substituent (compare entries 1 and 2). Within each series there are however some surprising features. Acidification of the C-terminal NH group by the tosyl group in **3**-Phe-NHTs (entry 8) fails to increase conformational preference over the *tert*-butyl amide (entry 7), and in both the amide and the ester series the greatest degree of screw-sense preference (75% h.e. for **3**-Ala-NH*t*-Bu) induced by a single chiral residue results from Ala (entries 1, 2, 13), rather than the more bulky chiral amino acids. Only the α-methylvaline dimer of **3**-αMv_2-NH*t*-Bu (entry 12; a motif which induces comparably high screw-sense control when located at the N terminus[4d,e]) exerts more powerful control (80% h.e.) than AlaNH*t*-Bu.

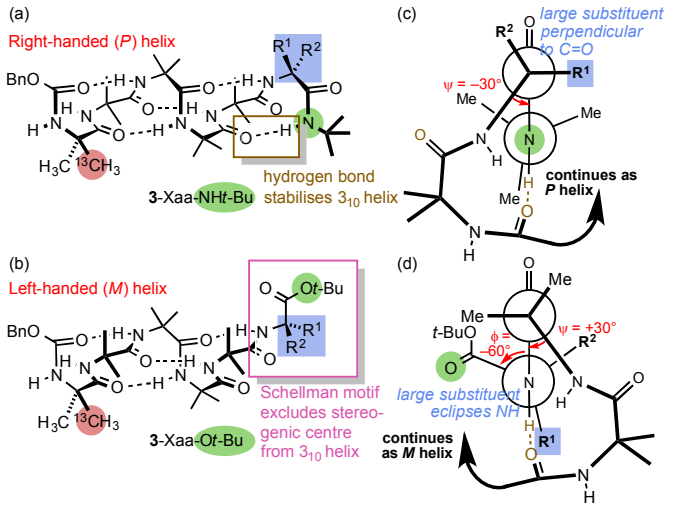


Figure 2: Conformations of Aib oligomers **3** bearing (a) a C-terminal secondary amide function and (b) C-terminal ester (or tertiary amide) function, with (c) and (d) showing Newman projections of their C-termini viewed from N-terminal direction to illustrate the origin of the conformational preference.

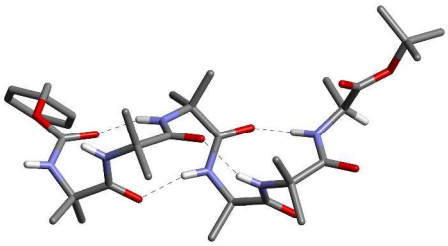


Figure 3: X-ray crystal structure[18] of **3**-Ala-O*t*-Bu, showing a C-terminal Schellman motif and left-handed (*M*) helicity.

The more powerful control exerted by residues Ala or Abu (entries 1-3) with smaller side chains is consistent with screw-sense control being greatest in a conformationally uniform helix that can adopt through its whole length a 3_{10} helical structure,[7] since Ala and Abu are readily incorporated into the 3_{10} helical structure.[19] Although the steric differentiation at the stereogenic centre of Val, Phe and *tert*-Leu (entries 6-9) is greater, these residues have a lower propensity for helix formation,[20] and presumably favour alternative conformations with lower screw-sense preferences. The lower screw-sense control induced by Ser(O*t*-Bu)NH*t*-Bu (entry 4) suggests that more remote steric bulk is likewise not well tolerated by a 3_{10}-helical structure. α-MeVal, being quaternary, is compatible with a 3_{10} helix,[4d] the greater selectivity observed with Ala vs α-MeVal being simply due to the steric differentiation between Me vs. H and *i*-Pr vs Me.

In conclusion, C-terminal L amino acid residues induce a preferred right-handed screw sense as their amide derivatives and left-handed screw sense as their ester derivatives in a series of helical Aib oligomers. Screw-sense control is maximised by residues that can participate in a 3_{10} helical structure, namely L-Ala and the dimer of L-α-MeVal.

Notes and references

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1 Zhang, Y. *Curr. Opin. Struc. Biol.* **2008**, *17*, 342; Lesk, A. M. *Introduction to Bioinformatics*, 3rd edn., OUP (Oxford) 2008

2 (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173; (b) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893

3 (a) Hecht, S.; Huc, I. *Foldamers*, Wiley-VCH, Weinheim, **2007**; (b) Gong, B. *Acc. Chem. Res.* **2008**, *41*, 1376; (c) Saraogi, I.; Hamilton, A. D. *Chem. Soc. Rev.*, **2009**, *38*, 1726; (d) Juwarker, J.-m. Suk, H.; Jeong, K.-S. *Chem. Soc. Rev.* **2009**, *38*, 3316; (e) Zhang, D.-W.; Zhao, X.; Hou, J.-L.; Li, Z.-T. *Chem. Rev.*, **2012**, *112*, 5271; (f) Fu, H. L.; Liu, Y.; Zeng, H. Q. *Chem. Commun.*, **2013**, *49*, 4127.

4 (a) Brown, R. A.; Marcelli, T. ; De Poli, M ; Solà, J. ; Clayden, J. *Angew. Chem. Int. Ed.*, **2012**, *51,* 1395; (b) Clayden, J.; Castellanos, A.; Solà, J.; Morris, G. A. *Angew. Chem., Int. Ed.,* **2009**, *48*, 5962; (c) Solà, J.; Morris, G. A.; Clayden, J. *J. Am. Chem. Soc.,* **2011**, *133*, 3712; (d) Byrne, L.; Solà, J.; Boddaert, T.; Marcelli, T.; Adams, R. W.; Morris, G. A.; Clayden, J. *Angew. Chem. Int. Ed.* **2014**, *53*, 151; (e) De Poli, M.; Byrne, L.; Brown, R. A.; Solà, J.; Castellanos, A.; Boddaert, T.; Wechsel, R.; Beadle, J. D.; J. Clayden *J. Org. Chem.* in press; (f) Orcel, U.; De Poli, M.; De Zotti, M.; Clayden, J. *Chem. Eur. J.,* **2013**, *19*, 16357; (g) Boddaert, T.; Clayden, J. *Chem. Commun.* **2012**, *48*, 3397; (h) Pike, S. J.; De Poli, M.; Zawodny, W.; Raftery, J. Webb, S. J.; Clayden, J. *Org. Biomol. Chem.* **2013**, *11*, 3168; (i) De Poli, M.; Clayden, J. *Org. Biomol. Chem.* **2014**,*12*, 836; (j) Solà, J.; Fletcher, S. P.; Castellanos, A.; Clayden, J. *Angew. Chem. Int. Ed.* **2010**, *49*, 6836; (k) Brown, R. A.; Diemer, V.; Webb, S. J.; Clayden, J. *Nature Chem.,* **2013**, *5,* 835

5 (a) Inai, Y.; Kurokawa, Y.; Ida, A.; Hirabayashi, T *Bull. Chem. Soc. Jap.* **1999**, *72*, 55; (b) Ousaka, N.; Inai, Y. *J. Org. Chem.* **2009**, *74*, 1429 and references therein.; (c) Inai, Y.; Kurokawa, Y.; Hirabayashi, T. *Biopolymers* **1999**, *49*, 551.

6 Pengo, B.; Formaggio, F.; Crisma, M.; Toniolo, C.; Bonora, G. M.; Broxterman, Q. B.; Kamphuis, J.; Saviano, M.; Iacovino, R.; Rossi, F.; Benedetti E. *J. Chem. Soc. Perkin Trans. 2* **1998**, 1651

7 Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rev.* **2001**, *101*, 3131

8 De Poli, M.; De Zotti, M.; Raftery, J.; Aguilar, J. A.; Morris, G. A.; Clayden, J. *J. Org. Chem.* **2013**, *78*, 2248.

9 (a) Solà, J.; Helliwell, M.; Clayden, J. *J. Am. Chem. Soc.* **2010**, *132*, 4548; (b) Inai, Y.; Ishida, Y.; Tagawa, K.; Takasu, A.; Hirabayashi, T, *J. Am. Chem. Soc.* **2002**, *124*, 2466; (c) Pengo, B; Formaggio, F; Crisma, M.; Toniolo, C.; Bonora, G. M.; Broxterman, Q. B.; Kamphuis, J.; Saviano, M.; Iacovino, R.; Rossi, F.; Benedetti, E. *J. Chem. Soc., Perkin Trans. 2*, **1998**, 1651. See also 4(f).

10 Fletcher, S. P.; Solà, J.; Holt, D.; Brown, R. A.; Clayden, J. *Beilstein J. Org. Chem.* **2011**, *7*, 1304

11 Clayden, J.; Diemer, V.; Pike, S. J.; Raftery, J.; Webb, S. J. manuscript in preparation; see also ref 4(b).

12 Hummel, R.-P.; Toniolo, C.; Jung, G. *Angew. Chem. Int. Ed.* **1987**, *26*, 1150.

13 (a) Toniolo, C.; Polese, A.; Formaggio, F.; Crisma, M.; Kamphuis, J. *J. Am. Chem. Soc.*, **1996**, *118*, 2744; (b) Yoder, G.; Polese, A.; Silva, R. A. G. D.; Formaggio, F.; Crisma, M.; Broxterman, Q. B.; Kamphuis, J.; Toniolo, C.; Keiderling, T.A. *J. Am. Chem. Soc.*, **1997**, *119*, 10278.

14 Dunitz, J. D. *Angew. Chem. Int. Ed.* **2001**, *40*, 4167.

15 Di Blasio, B.; Pavone, V.; Saviano, M; Lombardi, A.; Nastri, F.; Pedone, C.; Benedetti, E.; Crisma, M.; Anzolin, M.; Toniolo, C. *J. Am. Chem. Soc.* **1992**, *114*, 6273.

16 (a) Schellman, C. in *Protein Folding*, ed. Jaenicke, R. Elsevier, Amsterdam, 1980; pp 53-54; (b) Pike, S. J.; Raftery, J.; Webb, S. J.; Clayden, J. *Org. Biomol. Chem.* in press

17 The adoption of such motifs by tertiary amides may be related to the termination of helices by Pro (ref 15).

18 The X-ray crystal structure of **3**-Ala-O*t*-Bu has been deposited with the CCDC, deposition number 994389.

19 Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. *Biopolymers*, **2002**, *60*, 396. Alamethicin for example contains an AlaAibAlaAib sequence that adopts the 3_{10} conformation: Bosch, R.; Jung, G.; Schmitt, H.; Sheldrick, G. M.; Winter, W. *Angew. Chem. Int. Ed.* **1984**, *23*, 450.

20 Pace, C. N.; Scholtz, J. M. *Biophys. J.* **1998**, *75,* 1, 422

21 Polese, A.; Formaggio, F.; Crisma, M.; Valle, G.; Toniolo, C.; Bonora, G. M.; Broxterman, Q. B.; Kamphuis, J. *Chem. Eur. J.* **1996**, *2*, 1104.

