Bioorganic & Medicinal Chemistry Letters 19 (2009) 6931–6934

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Effect of chirality of small molecule organofluorine inhibitors of amyloid self-assembly on inhibitor potency

Abha Sood, Mohammed Abid, Samson Hailemichael, Michelle Foster, Béla Török, Marianna Török *

Department of Chemistry, University of Massachusetts Boston, 100 Morrissey Blvd. Boston, MA 02125-3393, United States

ARTICLE INFO

Article history:
Received 14 September 2009
Revised 14 October 2009
Accepted 15 October 2009
Available online 21 October 2009

Keywords:
Amyloid-beta peptide
Fibrillogenesis
Small molecule inhibitor
Chiral
Organofluorine

ABSTRACT

The effect of enantiomeric trifluoromethyl-indolyl-acetic acid ethyl esters on the fibrillogenesis of Alzheimer's amyloid β (Aβ) peptide is described. These compounds have been previously identified as effective inhibitors of the Aβ self-assembly in their racemic form. Thioflavin-T Fluorescence Spectroscopy and Atomic Force Microscopy were applied to assess the potency of the chiral target compounds. Both enantiomers showed significant inhibition in the in vitro assays. The potency of the enantiomeric inhibitors appeared to be very similar to each other suggesting the lack of the stereospecific binding interactions between these small molecule inhibitors and the Aβ peptide.

© 2009 Elsevier Ltd. All rights reserved.

Amyloid formation, either beneficial or harmful, is central to many life processes. Such protein deposits are associated with several human diseases, including the Alzheimer's disease.[1–6] As a possible therapeutic option, the theory of self-assembly inhibition of Alzheimer's amyloid-beta (Aβ) peptide has been widely tested and many effective inhibitors have been described, usually in two broadly defined categories: small molecule and peptide-based inhibitors.[6–12] Among these inhibitors there are several compounds, either natural or synthetic, that are chiral. However, the role of molecular chirality during the self-assembly is poorly understood and only sporadically investigated. There are many reasons to broaden these investigations. First, if such molecules ever reach the clinical trial phase, data regarding both enantiomers of a drug candidate are required. Aside from this practical reason, the role of chirality in the design and action of Aβ inhibitors is still unclear. The literature appears to be very limited on this issue. A recent study on amyloid type fibrils, including Aβ, reported the formation of specific amyloid suprastructures of helical chirality indicating that Aβ is sensitive to a chiral environment.[13] Regarding inhibition-related investigations similar conclusions were drawn by Chalifour et al. using peptide-based inhibitors.[14] The authors observed that peptide inhibitors assembled from the unnatural D-enantiomer of the amino acids exhibited significantly stronger potency in inhibition than that of the peptides synthesized from L-amino acids.[14] In contrast, using both enantiomers of nicotine, an alkaloid, in Aβ self-assembly and neurotoxicity inhibition Allsop et al. found that the absolute configuration did not play a significant role in determining potency.[15] The two nicotine enantiomers showed consistent activity within ~10% range. Based on their studies the authors concluded that the effect of nicotine cannot be due to a stereospecific binding between the alkaloid and the peptide.[15]

Organofluorine compounds are of exceptional interest in medical applications;[16] in fact, approximately 20% of all currently approved drugs contain at least one fluorine atom.[17] Several organofluorine compounds have already been shown to inhibit Aβ self-assembly.[18] In an earlier study, we introduced a new class of organofluorine inhibitor compounds. We have designed and

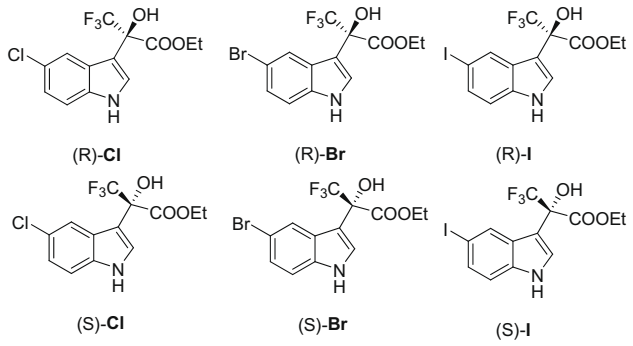

Figure 1. Structure of the enantiomeric indolyl-trifluoromethyl-hydroxypropanoic acid esters used in this study.

* Corresponding author.
E-mail address: marianna.torok@umb.edu (M. Török).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.10.066

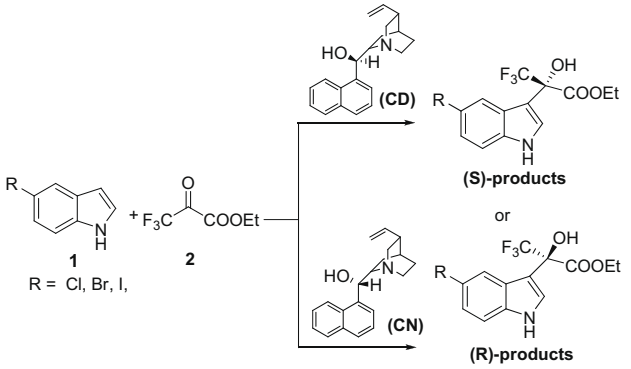

Figure 2. Schematic synthesis of the chiral inhibitors.

synthesized several indolyl-trifluoromethyl-hydroxypropionic acid esters and based on the structure–activity relationship we have identified three lead compounds.[19]

Herein, continuing these investigations, we describe the specific effect of the chirality of our lead compounds on the inhibition of Aβ self-assembly. We demonstrate the efficacy of the enantiomeric compounds in the in vitro assays and place our data in context with literature findings on the enantiospecificity of the inhibition.

The structures of the enantiomeric inhibitor lead compounds are shown in Figure 1. These compounds are Cl, Br, and I derivatives of the core structure. We have also examined the F-containing derivative, and found that its inhibition potential was only ~40%.[19] Thus, we decided not to include that compound in further studies.

The synthesis of the compounds has been carried out based on our earlier work using cinchonidine (CD) and cinchonine (CN) organocatalysts.[20,†] While CD provided the (*S*)-products, CN resulted in the formation of the (*R*)-enantiomers (Fig. 2). The enantiomeric excesses of the products were determined by chiral HPLC or ^{19}F NMR spectroscopy.[21]

The efficacy of the inhibitors has been evaluated by the commonly applied Thioflavin-T (THT) assay.[22,‡] The calculated intensity (I_{THT}) values were based on maximum fluorescence intensities in the 480–490 nm region (emission spectra) after subtracting the background fluorescence of the starting solutions (0 h). The samples were incubated for up to 140 h, and the increase in the fluorescence intensities were periodically measured (Fig. 3).

The results indicate, that as expected on the basis of the data obtained with the racemic samples earlier[19] the chiral inhibitors actively block the self-assembly of the Aβ peptide, however, the difference between the two enantiomers appears to be minor.

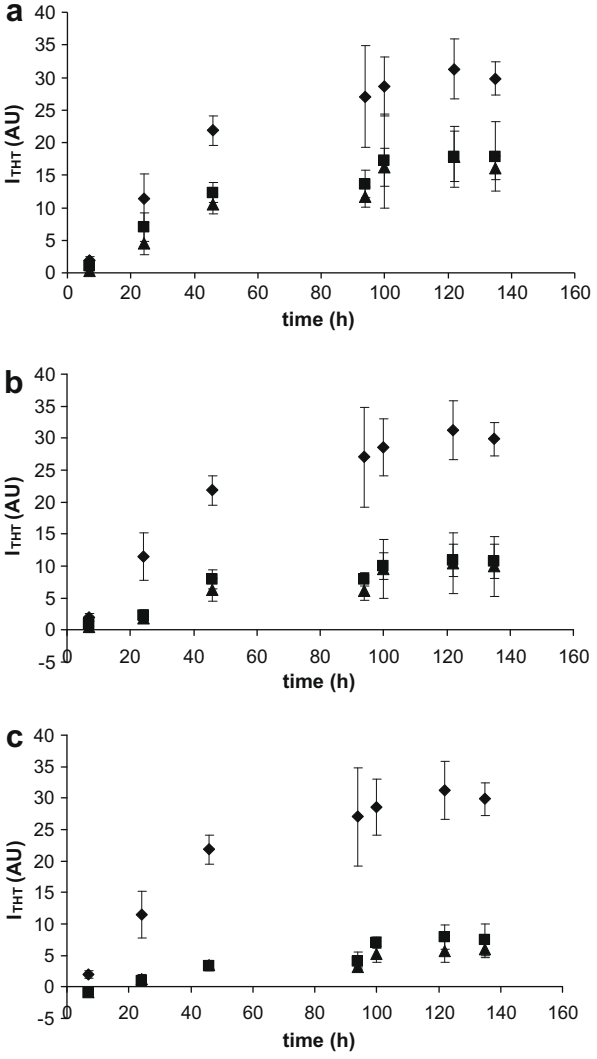

Figure 3. Time dependence of the THT fluorescence values (I_{THT}) of the inhibition of Aβ fibrillogenesis by (*R*)- (■) and (*S*)- (▲) enantiomers of **Cl** (a), **Br** (b) and **I** (c), compared to the inhibitor-free control sample (♦). The data are average of parallel experiments, the values represent means ± standard deviation ($n = 4 - 5$).

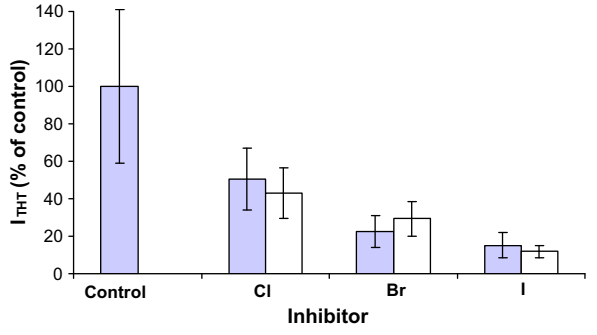

Figure 4. Inhibition of $A\beta_{1-40}$ fibrillogenesis by enantiomeric trifluoro-hydroxy-lindolyl-propionic acid esters at $mol_{inhibitor}/mol_{peptide} = 10$ stoichiometry. THT fluorescence intensities (I_{THT}) are normalized to that of the inhibitor-free $A\beta_{1-40}$ sample (control); (*R*)-enantiomers are shown in blue, and (*S*)-enantiomers in white. The data are average of parallel experiments, the values represent mean ± standard deviation ($n = 4 - 5$).

The 94 h data were used to calculate the percentile inhibition compared to the control samples. The signal of the control samples

† Indoles (**1**) and ethyl trifluoropyruvate (**2**) were reacted in a glass reaction vessel in diethylether at −8 °C. Cinchonidine (CD) and cinchonine (CN) were used as catalysts. The progress of the reaction was monitored by TLC. After the reaction was completed, the solvent and excess **2** were removed by evaporation. The catalyst was removed by a treatment with 500 mg of K-10 montmorillonite, and then the solvent was evaporated. The crude products were purified by flash chromatography.

‡ The synthetic lyophilized $A\beta_{1-40}$ peptide was dissolved in 100 mM NaOH to a concentration of 40 mg/ml and diluted in 10 mM HEPES,100 mM NaCl, 0.02% NaN_3 (pH 7.4) buffer to a final peptide concentration of 100 μM. The inhibitors were dissolved in DMSO and added to the Aβ samples (inhibitor/Aβ = 10). After 30 s of vigorous vortexing the solutions were incubated at 37 °C with gentle shaking (77 rpm) and the increase in fibril amount in each sample was followed by Thioflavin-T fluorescence, and atomic force microscopy (AFM). The fluorescence measurements have been carried out using a Hitachi F-2500 fluorescence spectrophotometer. The incubated peptide solutions were briefly vortexed before each measurement, and then 3.5 μl aliquots of the suspended fibrils were withdrawn and added into 700 μl of 5 μM Thioflavin-T prepared freshly in 50 mM glycine–NaOH (pH 8.5) buffer. The fluorescence spectra of these mixtures have been measured at 430 nm (excitation) and 484 nm (emission) wavelengths, respectively. None of the inhibitor compounds showed fluorescence intensity in this region.

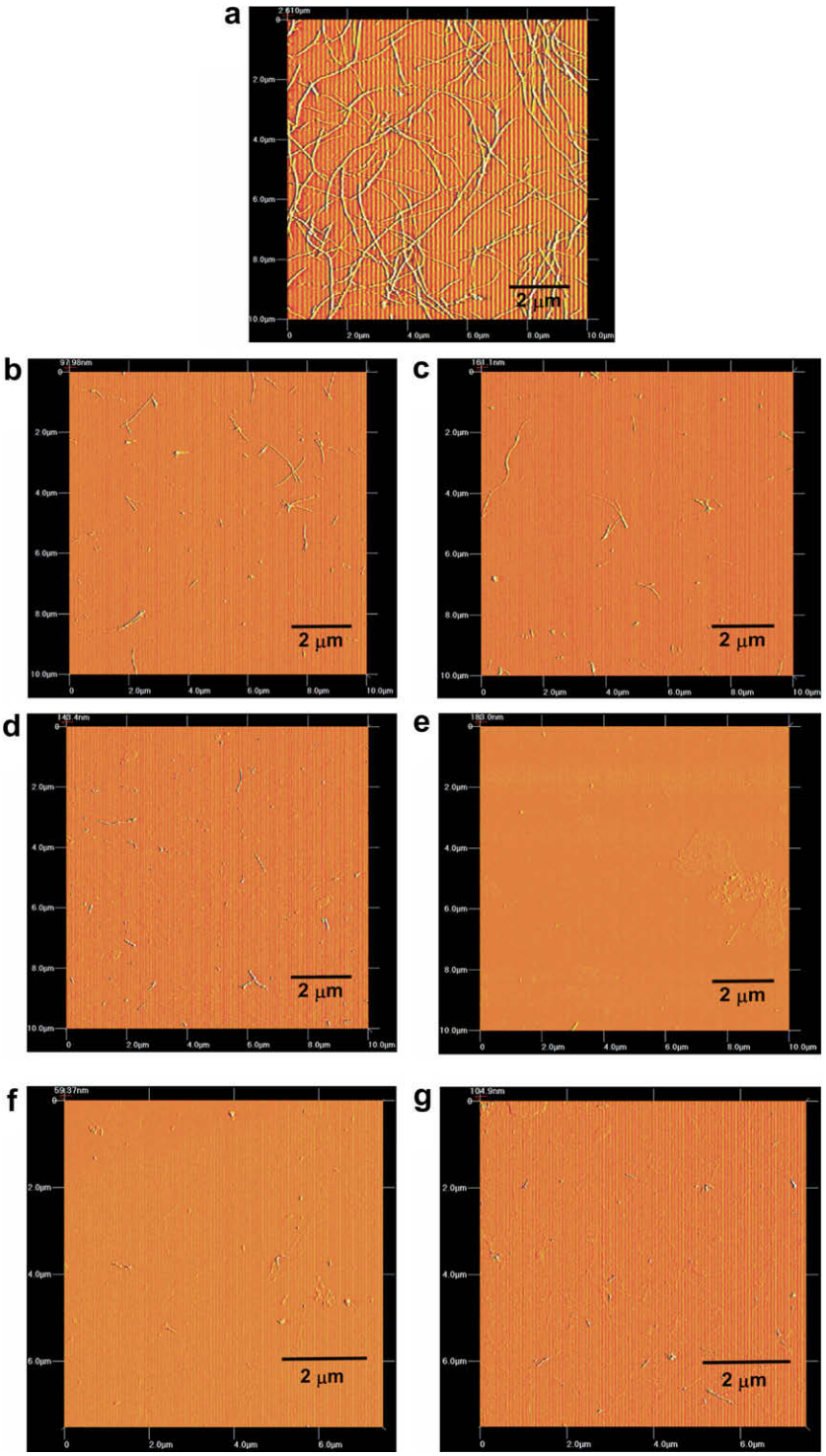

Figure 5. Atomic Force Microscopy images of $A\beta_{1-40}$ samples incubated in the presence of (a) control, 10 μm^2 scan with *z*-axis of 2.6 μm, (b) (*R*)-**Cl**, 10 μm^2 scan with *z*-axis of 97.98 nm, (c) (*S*)-**Cl**, 10 μm^2 scan with *z*-axis of 161 nm, (d) (*R*)-**Br**, 10 μm^2 scan with *z*-axis of 143 nm, (e) (*S*)-**Br**, 10 μm^2 scan with *z*-axis of 183 nm, (f) (*R*)-**I**, 6 μm^2 scan with *z*-axis of 59.37 nm, (g) (*S*)-**I**, 6 μm^2 scan with *z*-axis of 105 nm.

reaches saturation at 94 h, and the measurements are not significantly affected by the solvent evaporation yet, as it is a frequent problem with longer incubation times.

According to the comparative data presented in Figure 4 the enantiomers show similar potency. Based on the statistical analysis of the data (Student's *t*-test) it can be concluded with 95% confidence that there is no significant difference between the mean values of the enantiomeric compounds.

Further morphological characterization of the samples was carried out by Atomic Force Microscopy, using a Quesant Q-Probe 360 microscope in non-contact mode (Fig. 5).[23,§]

§ Aliquots from controls and inhibition assays were diluted with a 10 mM HEPES, 100 mM NaCl, 0.02% NaN_3 (pH 7.4) buffer and 2–5 μL samples were placed onto freshly cleaved mica. The samples were allowed to sit for 30–60 s. The excess peptide and buffer salts were carefully rinsed with de-ionized water and the specimen were air dried and subjected to analysis.

The AFM images corroborate with the findings of the fluorescence spectroscopic assays. The image of the control shows well-developed fibrils as expected (Fig. 5a). Such extended network of fibrils did not form in the presence of inhibitors. The comparison of the images of samples incubated with inhibitors shows a small amount of fibril in Figure 5b–d, where according to Figure 4 the inhibition is 60–80%. The images obtained with (*R*)- and (*S*)-**I** inhibitors show certain protein deposits, possibly amorphous aggregates and protofibrils, rather than fibrils (Fig. 5f and g), while the presence of (*S*)-**Br** resulted in the complete lack of protein deposits (Fig. 5e). While the AFM cannot give quantitative information, the visual analysis of the images is in good correlation with the data in Figure 4.

These results confirm that similar to their racemic mixtures the enantiomers of our lead compounds can also significantly inhibit the fibrillogenesis of Aβ. The individual enantiomers studied in this work demonstrated similar inhibition potency. Our data are in a close agreement with results from Allsop's group, whom described the behavior of nicotine enantiomers. The authors reported only ~10% difference in the respective efficacy of the enantiomers. While the data at present are limited to basic nicotine enantiomers and acidic trifluoromethyl-indolyl-hydroxypropionic acid esters from this work, it appears that when using small molecule inhibitors the chemical nature, substituents, steric demand and electronic character of the molecule determines the inhibitor-like characteristics. The role of the absolute configuration or exact 3D structure is rather minor. This suggests that the inhibitory effect of these compounds is not the result of highly stereospecific binding between these molecules and the Aβ peptide, but rather non-stereospecific binding forces. In the case of the present compounds the role of the halogens is not quite clear yet. As the potency increases from F to I significantly (40% to ~97%)[19] it appears that the size plays a role rather than their distance from the chiral center. On the contrary, when using a peptide-based inhibitor the individual chirality of the amino acids appears to be of paramount importance, which indicates the highly stereospecific nature of the small peptide–Aβ interaction, probably due to peptide–peptide interactions via β-strands.

In conclusion, the present work demonstrates that the individual enantiomers of substituted trifluoromethyl-indolyl-hydroxypropionic acid esters are potent inhibitors of the Aβ self-assembly. Furthermore, the data indicated that these (*R*)- and (*S*)-optical isomers exhibit comparable in vitro inhibition activity to each other. Our results present further evidence and confirmation of the lack of stereospecific binding interactions between small molecule inhibitors and the Aβ peptide providing important details for the future design of effective inhibitors.

Acknowledgments

Financial support provided by the University of Massachusetts Boston, and National Institute of Health(R-15 AG025777-03A1) are gratefully acknowledged.

References and notes

1. Chiti, F.; Dobson, C. M. *Annu. Rev. Biochem.* **2006**, *75*, 333.
2. Pearson, H. A.; Peers, C. *J. Physiol.* **2006**, *575*, 5.
3. Otzen, D.; Nielsen, P. H. *Cell. Mol. Life. Sci.* **2008**, *65*, 910.
4. Rochet, J. C.; Lansbury, P. T., Jr. *Curr. Opin. Sruct. Biol.* **2000**, *10*, 60.
5. Hamley, I. W. *Angew. Chem., Int. Ed.* **2007**, *46*, 8128.
6. Morozova-Roche, L.; Malisauskas, M. *Curr. Med. Chem.* **2007**, *14*, 1221.
7. Xia, W. *Curr. Opin. Invest. Drugs* **2003**, *4*, 55.
8. Wolfe, M. S. *Nat. Rev. Drug Disc.* **2002**, *1*, 859.
9. (a) Gerrard, A.; Hutton, C. A.; Perugini, M. A. *Mini-Rev. Med. Chem* **2007**, *7*, 151; (b) Estrada, L. D.; Soto, C. *Curr. Top. Med. Chem.* **2007**, *7*, 115.
10. (a) LeVine, H., III *Amyloid* **2007**, *14*, 185; (b) Török, B.; Dasgupta, S.; Török, M. *Curr. Bioact. Comp.* **2008**, *4*, 159.
11. Sciarretta, K. L.; Gordon, D. J.; Meredith, S. C. Peptide-Based Inhibitors of Amyloid Assembly. In *Methods in Enzymology*; Kheterpal, I., Wetzel, R., Eds., 2006; Vol. 413, p 273.
12. Takahashi, T.; Mihara, H. *Acc. Chem. Res.* **2008**, *41*, 1309.
13. Rubin, N.; Perugia, E.; Goldschmidt, M.; Fridkin, M.; Addadi, L. *J. Am. Chem. Soc.* **2008**, *130*, 4602.
14. Chalifour, R. J.; McLaughlin, R. W.; Lavoie, L.; Morisette, C.; Tremblay, N.; Boule, M.; Sarazin, P.; Stea, D.; Tremblay, P. *J. Biol. Chem.* **2003**, *278*, 34874.
15. Moore, S. A.; Huckerby, T. N.; Gibson, G. L.; Fullwood, N. J.; Turnbull, S. T.; Tabner, B. J.; El-Agnaf, O. M. A.; Allsop, D. *Biochemistry* **2004**, *43*, 819.
16. (a) Olah, G. A.; Chambers, R. D.; Prakash, G. K. S. *Synthetic Fluorine Chemistry*; Wiley: New York, 1992; (b) *Enantiocontrolled Synthesis of Fluoroorganic Compounds: Stereochemical Challenges and Biomedicinal Targets*; Soloshonok, V. A., Ed.; Wiley: New York, 1999; (c) *Asymmetric Fluoroorganic Chemistry*; Ramachandran, P. V. Ed.; ACS Symp. Series, ACS: Washington DC, 2000.; (d) *Organofluorine Compounds*; Hiyama, T., Ed.; Springer: Berlin, 2001; (e) Kirsch, P. *Modern Fluoroorganic Chemistry: Synthesis, Reactivity, Applications*; Wiley: New York, 2004; (f) *Fluorine Containing Synthons*; Soloshonok, V. A. Ed.; ACS Symp. Series, ACS: Washington, DC, 2005.
17. Swinson, J. *Chem. Today* **2005**, *23*, 14.
18. (a) Vieira, E. P.; Hermel, H.; Möhwald, H. *Biochim. Biophys. Acta* **2003**, *1645*, 6; (b) Adamski-Werner, S. L.; Palaninathan, S. K.; Sacchettini, J. C.; Kelly, J. W. *J. Med. Chem.* **2004**, *47*, 355; (c) Rocha, S.; Thueneman, A. F.; Pereira, M. C.; Coelho, M.; Möhwald, H.; Brezesinski, G. *Biophys. Chem.* **2008**, *137*, 35.
19. Török, M.; Abid, M.; Mhadgut, S. C.; Török, B. *Biochemistry* **2006**, *45*, 5377.
20. Török, B.; Abid, M.; London, G.; Esquibel, J.; Török, M.; Mhadgut, S. C.; Yan, P.; Prakash, G. K. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 3086.
21. Abid, M.; Török, B. *Tetrahedron: Asymmetry* **2005**, *16*, 1547.
22. (a) Naiki, H.; Higuchi, K.; Hosokawa, M.; Takeda, T. *Anal. Biochem.* **1989**, *177*, 244; (b) LeVine, H., III *Protein Sci.* **1993**, *2*, 404; (c) Biancalana, M.; Makabe, K.; Koide, A.; Koide, S. *J. Mol. Biol.* **2009**, *385*, 1052.
23. (a) Ding, T. T.; Harper, J. D. *Methods Enzymol.* **1999**, *309*, 510; (b) Antzutkin, O. N. *Magn. Reson. Chem.* **2004**, *42*, 231.