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Photoinduced Intramolecular Macrocyclization Reaction between a Bpa and a Met Residue in a Helical Peptide: 3D Structures of the Diastereomeric Products

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The 3-(4-benzoylphenyl)alanyl (Bpa) residue has been extensively used as a probe in the photoaffinity scanning approach for studies of intermolecular labelled (peptide) ligand–receptor (protein) interactions,[1a–d] despite the significant rotational freedom of some of its side-chain bonds which may encompass a radius for cross-linking[2] as large as 10 Å. Photoexcitation of the benzophenone chromophore at 350–360 nm results in the efficient formation of its n,π* triplet state, which is able to abstract a hydrogen atom from a geometrically accessible C–H σ bond.[3,4] In the second step of this process, the resulting ketyl radical combines with the newly generated carbon radical, thus covalently linking the two moieties. Using a set of simple model compounds (terminally-blocked protein amino acids), it was demonstrated that the excited benzophenone preferentially alkylates the Gly α carbon and the two (γ and ε) carbon atoms adjacent to sulfur in the Met side chain.[5] All reactions generate two or multiple diastereomeric products. In actual ligand–receptor studies the preferential selectivity of Bpa for Met over all other amino acids in the target protein is even more pronounced, so that terms and methodologies such as "Met-magnetic effect"[6a] and "Met-proximity assay"[6b] have been introduced and designed. In a related perspective, Bpa has been biosynthetically incorporated into proteins, allowing photocross-linking to other proteins in their vicinity.[7] If the hydrogen atom is abstracted from a remote carbon of the same molecule, the resulting biradical can react intramolecularly (Yang photocyclization) to yield an annular system.

Intramolecular side-chain to side-chain ring formation is an approach currently extensively exploited to achieve more robust peptide conformations, reduce the barrier to membrane penetration, and improve resistance towards proteolytic attack.[8] More specifically, stapled helices may overcome some of the drawbacks that have hampered the development of peptide drugs.

In connection with our investigations of intramolecular macrocyclization reactions in helical peptides, we are currently carrying out a first detailed study of the Yang photocyclization reaction in a set of four, backbone rigidified, terminally protected, potentially 3_{10}-helical[9] hexapeptides of general sequence Boc-(Aib)$_x$-L-Bpa-(Aib)$_y$-L-Met-(Aib)$_z$-OMe, where Boc is *tert*-butyloxycarbonyl, Aib is α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine, OMe is methoxy, and $x+y+z=4$. In this investigation we aim to determine the effects induced by the length of the peptide spacer, which is entirely based on the strongly helicogenic Aib residue,[10] on the extent of formation of the regio- and stereoselective reaction products and the rate of the intramolecular excited state reaction. In this article, we describe the results of our study on Boc-Aib-L-Bpa-(Aib)$_2$-L-Met-Aib-OMe (hereafter called starting hexapeptide, **SH**), which, amongst other things, has allowed for the first time the unambiguous, detailed chemical and configurational characterization of the diastereomeric peptides arising from the intramolecular photoreaction of the Bpa and Met residues. A preliminary account of part of this work has already been reported.[11]

The synthesis of **SH** was performed step-by-step by solution methods (see the Supporting Information). The results of a concentration-dependent FT-IR absorption/^{1}H NMR analysis (see below and Figure S1 and S2 in the Supporting Information) clearly indicate that this hexapeptide is essentially monomeric and overwhelmingly folded in a 3_{10}-helical

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structure in a solvent of relatively low polarity, such as $CDCl_3$, as expected from a variety of investigations published on closely related, Aib-rich, short peptides.[10b,c]

Laser flash photolysis experiments (Figures S3 and S4) were carried out to characterize the triplet-state behavior of **SH** and establish the reaction conditions necessary to maximize the yield of the desired intramolecular coupling product(s) in a preparative experiment, and minimize intermolecular coupling reactions. Pulsed laser excitation (248 nm; $\approx$100 mJ; $\approx$20 ns) of a deoxygenated 58 μM acetonitrile solution of **SH** led to strong transient absorptions centered at $\lambda_{max} \approx 525$ nm, assignable to the triplet state of the L-Bpa moiety based on the similarity of the spectrum to that obtained from the model compound Boc-L-Bpa-OMe ($\lambda_{max} \approx 525$ nm; $\tau \approx 6.3$ μs) under similar conditions; both spectra are quite similar to that of the 4-methylbenzophenone triplet in acetonitrile.[12] The **SH**-triplet absorption decayed with biexponential kinetics, characterized by an initial fast decay component that comprised about 75% of the signal ($\tau \approx 70$ ns), superimposed on a longer-lived component ($\tau \approx 3.3$ μs) with the same absorption maximum. The lifetime of the Boc-L-Bpa-OMe triplet was quenched efficiently by adding (0–1.5 mM) Boc-L-Met-OMe [$k_Q = (1.02 \pm 0.05) \times 10^9\,M^{-1}s^{-1}$]. We thus assign the short-lived component of the **SH-**triplet decay to Bpa triplet moieties that are quenched intramolecularly by the Met residue in the molecule, and the long-lived component to Bpa moieties residing in a minor proportion of non-quenching conformations. From the various lifetimes and rate constants obtained in these experiments, we estimate that at a concentration of 1 mM, $\geq$80% of Bpa triplets produced on photoexcitation of **SH** will be quenched intramolecularly by interaction with the remote L-Met moiety in the molecule.

The Yang-photocyclization reaction was thus conducted in a deoxygenated 0.8 mM acetonitrile solution of **SH** in a 12 mm diameter quartz tube, by irradiating it with 12 (350 nm each) lamps. The course of the reaction was followed by HPLC-MS analysis. As the reaction proceeds, two equally intense new peaks progressively emerge in the HPLC profile at the expense of **SH**. After 40 min, the residual amount of **SH** is almost negligible (Figure 1). The photoproducts (**A** and **B**) corresponding to the two intense peaks were chromatographically purified (Figure 1) and their chemical and three-dimensional structures were separately analyzed by mass spectrometry, UV/Vis, CD, FT-IR, and NMR techniques, and in addition, for one of them (**A**) by X-ray diffraction.

ESI-TOF mass spectrometry data unequivocally show that **SH** and products **A** and **B** are isomeric compounds: m/z calcd for $[M+H]^+$: 855.4248; found: 855.4139 (**SH**), 855.4424 (product **A**), and 855.4427 (product **B**).

The near-UV spectra of **A** and **B** lack the band at 338 nm in acetonitrile solution that is shown by the Bpa-containing **SH** and due to the $n \rightarrow \pi^*$ transition of its benzophenone chromophore[3] (Figure S5), clearly indicating the absence of this moiety in both products. The CD spectra in 2,2,2-trifluoroethanol solution (Figure S6) further highlight the close

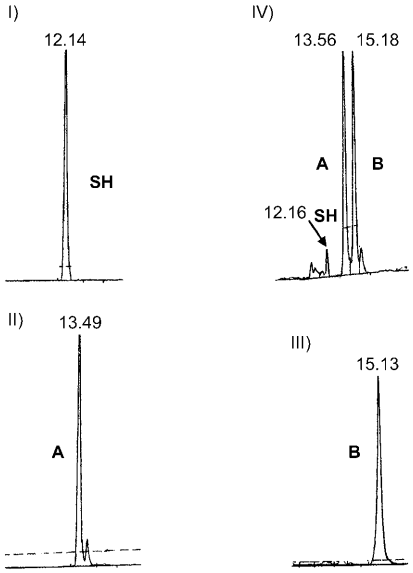

Figure 1. HPLC profiles and retention times of **SH** (I), the final photoreaction mixture (after 40 min) showing **SH** and products **A** and **B** (IV), and the purified products **A** (II) and **B** (III). Conditions: Vydac C_{18} column; 2.5 mL min^{-1}; 20–80% of solvent mixture B in 25 min [A = H_2O/CH_3CN 9:1; B = H_2O/CH_3CN 9:1 (TFA 0.05%)]; UV detector at 226 nm.

analogy of the chromophores in products **A** and **B** and their diverging characteristics relative to those in **SH** (we interpret this finding as mainly due to the onset of a new chiral carbon atom in the vicinity of the aromatic groups of **A** and **B**; see below). Moreover, the Cotton effects of **A** and **B** are: i) quasi-mirror images, suggesting a diastereomeric relationship between the two compounds; and ii) very intense and their shapes are quite unusual for short peptide molecules, thus preventing any reliable conformational assignment based only on this spectroscopic technique.

In the N–H stretching region of the FT-IR spectra of **A** and **B** in $CDCl_3$ solution (Figure S7), the relative intensities of the two bands (weak at about 3425 cm^{-1}, associated with free NH groups,[13] and strong at about 3315 cm^{-1}, associated with intramolecularly H-bonded NH groups) are quite close, concentration independent (in the range 1–0.1 mM), and strictly comparable to that of **SH** (Figure S1). This finding strongly supports the view that the three hexapeptides exhibit similar helical propensities. In the C=O stretching region the marked shoulder at 1677 cm^{-1} shown by **SH** (Figure S8) is assigned to the contribution of the Bpa benzophenone carbonyl chromophore,[14] which appears to be absent in both products **A** and **B**.

The 600 MHz NMR spectra of **SH** and the two products **A** and **B** were recorded in $CDCl_3$. In the 1D spectra the proton signals are reasonably dispersed and produce minimal overlaps. For the assignment of all proton resonances a combination of NOESY and TOCSY experiments[15] was used. It is evident that the Met ε-CH_3 proton signal at $\delta =$ 2.1 ppm in the spectrum of **SH** is missing in those of the two products (Figure 2). Concomitantly, new signals, assigned to

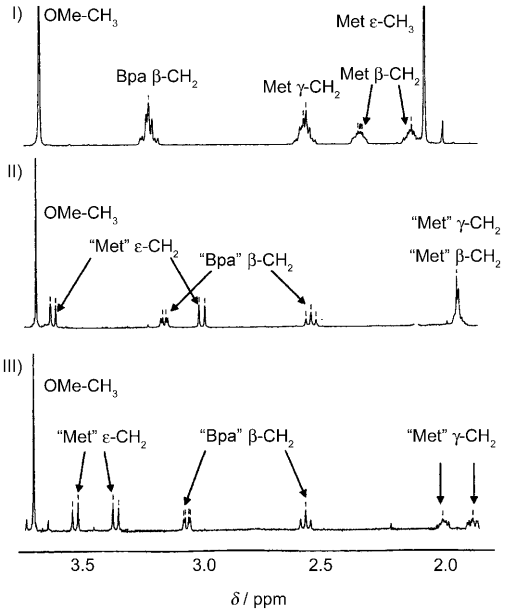

Figure 2. Portions of the 600 MHz ^{1}H NMR spectra for **SH** (I) and the photoproducts **A** (II) and **B** (III) in $CDCl_3$ solution. Peptide concentration: 1 mM.

the "Met" ε-CH_2 protons, are seen in the 3.7–3.0 ppm region of both products. The better resolved peaks for the "Bpa" β-CH_2 protons in **A** and **B** as compared to those in **SH** should be associated with a reduced local flexibility of the two products as compared to that of the peptide reagent. The splitting of the "Met" ε-CH_2 proton peaks is more marked in compound **A** than in **B**, suggesting that the former molecule is more conformationally constrained in that region. We attribute the differences observed in the separation and chemical shifts of the "Met" β-CH_2 and γ-CH_2 proton peaks of **SH**, **A**, and **B** to a combination of diverging local conformational constraints and neighborhoods to the "Bpa" aromatic rings. However, similar sets of $NH_i \rightarrow NH_{i+1}$ sequential NOE connectivities[15a] in the three compounds (see Figure S9 for **B**) confirm our view that macrocyclization did not alter significantly the helical structure of **SH**.

Remarkably, after a number of attempts, we succeeded in growing a single crystal, suitable for an X-ray diffraction analysis, from slow evaporation of a methanol solution of compound **A** (Table S1) (a reasonable motivation for the unavailability of a single crystal from the strictly related compound **B** might be associated with an overall enhanced flexibility of the macrocycle, as discussed above). This first crystallographic study of a Bpa photoproduct with any amino acid in a peptide or protein conjugate allowed us to unravel the configuration of the novel side-chain chiral center and the backbone conformation of this diastereomeric hexamer (and, by inference, of those of photoproduct **B** as well) (Figure S10). The backbone of peptide **A** is folded in a regular, right-handed 3_{10}-helix from residue 1 to 5 (Figure 3 and Table S2). The average ϕ,ψ torsion angles are −58,−31°. These values should be compared with those of a classical peptide 3_{10}-helix (−57,−30°).[9] The separation between the α-carbons of the "Bpa" (*i*) and "Met" (*i*+3) residues is 5.841(11) Å, close to that expected for a type-III β-turn[16] and the related pitch of the 3_{10}-helix.[9] Also, the C-terminal Aib residue adopts a helical conformation, but it has a handedness opposite to that exhibited by the preceding residues (a common observation for 3_{10}-helical peptide esters[9,10]). Four, consecutive $i \leftarrow i+3$ C=O···H−N intramolecular H-bonds stabilize the helical structure (Table S3). All O···N separations are below the upper limit (3.2 Å) for a C=O···H−N H-bond.[17] This 3D-structural analysis confirms that the Yang photocyclization of this L-Bpa/L-Met hexapeptide involves the Met side-chain ε-CH_3, not its γ-CH_2, group. Moreover, it turns out that in this diastereomeric product (**A**) the configuration of the newly formed chiral center (the carbon atom between the two phenyl rings) is *R*. Finally, the occurrence of two, almost equally populated, side-chain conformers in the molecule (Figure S11) underscores the flexibility of a portion of its 20-membered ring system.

In summary, we have performed the first photoinduced intramolecular Yang-macrocyclization reaction by exploiting the side chains of a helical peptide with Bpa and Met residues incorporated at positions *i*, *i*+3 in the sequence, that is, one on top of the other after a complete turn of the 3_{10}-helix. The regularity of the 3_{10}-helix backbone is not disturbed by this type of bridging. Remarkably, this combined chromatographic, spectrophotometric, spectrometric, and X-ray crystallographic investigation has allowed us to separate and characterize in great detail the two diastereomeric photoproducts that are formed for the first time. In particular, the photochemical remote functionalization and subsequent intramolecular cross-linking on this peptide substrate is strictly regioselective, involving exclusively the original Met side-chain ε-CH_3 carbon and generating two diastereomeric macrocyclized peptides. A simple molecular modeling analysis shed light on the observed regioselectivity in that it shows that the conformational restraints imposed by the

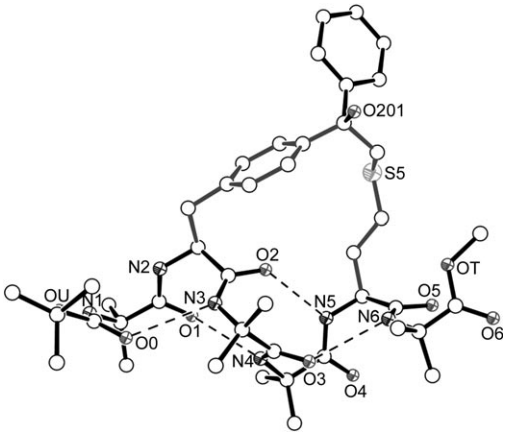

Figure 3. X-ray diffraction structure of the major side-chain conformer of the macrocyclized hexapeptide diastereomer (product **A**) arising from the Yang photocyclization of **SH**. The configuration of the side-chain chiral center is *R*. Hydrogen atoms have been omitted. Only the N, O, and S atoms are numbered. H-bonds are represented by dashed lines.

3_{10}-helical peptide backbone, combined with the lengths and flexibilities of the *i*,*i*+3 Bpa and Met side chains, should result in a more efficient hydrogen abstraction from the Met ε-CH_3 than from the γ-CH_2 carbon. This conclusion, however, by no means excludes the possibility of a hydrogen abstraction from the Met side-chain γ-CH_2 carbon in other peptide or protein substrates with different geometrical characteristics. It is worth noting that in this latter case a complex mixture of four diastereomeric photoproducts is expected, originating from two novel chiral carbons. As stated above, to analyze whether the Yang photocyclization would overpower the conformational preferences, detailed results describing its applications to additional hexapeptide substrates with larger or shorter spacings between the Bpa and Met residues will be forthcoming. Finally, in the reaction described here we did not find any evidence in favor of a significant diastereoselectivity.

Experimental Section

The synthesis of the terminally protected, linear hexapeptide Boc-Aib-L-Bpa-$(Aib)_2$-L-Met-Aib-OMe (**SH**) was performed step-by-step by solution methods starting with the C-terminal H-Aib-OMe derivative. The L-Met, Aib, and L-Bpa residues were introduced using the N^{α}-protected 9-fluorenylmethoxycarbonyl (Fmoc) derivatives, whereas the N-terminal Aib was inserted by use of Boc-Aib-OH. Good to excellent (74–93%) yields were obtained in each of these difficult coupling steps by C-activation with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC)/7-aza-1-hydroxy-1,2,3-benzotriazole (HOAt). Details of peptide characterization, laser flash photolysis, preparative photolysis, and X-ray diffraction may be found in the Supporting Information.

CCDC 698562 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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[1] a) J. C. Kauer, S. Erickson-Viitanen, H. R. Wolfe, Jr., W. F. De Grado, *J. Biol. Chem.* **1986**, *261*, 10695–10700; b) D. Fillion, M. Deraët, B. J. Holleran, E. Escher, *J. Med. Chem.* **2006**, *49*, 2200–2209; c) L.-Y. Huang, G. Umanah, M. Hauser, C. Son, B. Arshava, F. Naider, J. M. Becker, *Biochemistry* **2008**, *47*, 5638–5648; d) D. Monaghan, B. E. Thomas, I. Woznica, A. Wittelsberger, D. F. Mierke, M. Rosenblatt, *Biochemistry* **2008**, *47*, 5889–5895.

[2] a) M. Sarmiento, Y. A. Puius, S. W. Vetter, Y.-F. Keng, L. Wu, Y. Zhao, D. S. Lawrence, S. C. Almo, Z.-Y. Zhang, *Biochemistry* **2000**, *39*, 8171–8179; b) M. Saviano, R. Improta, E. Benedetti, B. Carrozzini, G. L. Cascarano, C. Didierjean, C. Toniolo, M. Crisma, *ChemBioChem* **2004**, *5*, 541–544; c) E. Sartori, A. Toffoletti, F. Rastrelli, C. Corvaja, A. Bettio, F. Formaggio, S. Oancea, C. Toniolo, *J. Phys. Chem. A* **2003**, *107*, 6905–6912.

[3] a) R. Breslow, M. A. Winnik, *J. Am. Chem. Soc.* **1969**, *91*, 3083–3084; b) R. Breslow, S. W. Baldwin, *J. Am. Chem. Soc.* **1970**, *92*, 732–734.

[4] a) G. Dorman, G. D. Prestwich, *Biochemistry* **1994**, *33*, 5661–5673; b) S. A. Fleming, *Tetrahedron* **1995**, *51*, 12479–12520; c) V. Pham, P. M. Sexton, *J. Pept. Sci.* **2004**, *10*, 179–203.

[5] E. Deseke, Y. Nakatani, G. Ourisson, *Eur. J. Org. Chem.* **1998**, 243–251.

[6] a) A. Wittelsberger, B. E. Thomas, D. F. Mierke, M. Rosenblatt, *FEBS Lett.* **2006**, *580*, 1872–1876; b) M. Clement, S. S. Martin, M. Beaulieu, C. Chamberland, P. Lavigne, R. Leduc, G. Guillemette, E. Escher, *J. Biol. Chem.* **2005**, *280*, 27121–27129.

[7] a) T. Hohsaka, *Bull. Chem. Soc. Jpn.* **2004**, *77*, 1041–1049; b) S. Chen, P. G. Schultz, A. Brock, *J. Mol. Biol.* **2007**, *371*, 112–122.

[8] a) C. Drahl, *Chem. Eng. News* **2008**, *86*, 18–23; b) C. Toniolo, *Int. J. Pept. Protein Res.* **1990**, *35*, 287–300; c) M. J. I. Andrews, A. B. Tabor, *Tetrahedron* **1999**, *55*, 11711–11743; d) D. Y. Jackson, D. S. King, J. Chmielewski, S. Singh, P. G. Schultz, *J. Am. Chem. Soc.* **1991**, *113*, 9391–9392; e) G. Osapay, J. W. Taylor, *J. Am. Chem. Soc.* **1990**, *112*, 6046–6051; f) E. Blackwell, J. D. Sadowski, R. J. Howard, J. N. Sampson, J. A. Chao, W. E. Steinmetz, D. J. O'Leary, R. H. Grubbs, *J. Org. Chem.* **2001**, *66*, 5291–5302; g) A. K. Boal, I. Guryanov, A. Moretto, M. Crisma, E. L. Lanni, C. Toniolo, R. H. Grubbs, D. J. O'Leary, *J. Am. Chem. Soc.* **2007**, *129*, 6986–6987; h) F. Bernal, A. F. Tyler, S. J. Korsmeyer, L. D. Walensky, G. L. Verdine, *J. Am. Chem. Soc.* **2007**, *129*, 2456–2457; i) H. Zhang, Q. Zhao, S. Bhattacharya, A. A. Waheed, X. Tong, A. Hong, S. Heck, F. Curreli, M. Goger, D. Cowburn, E. O. Freed, A. K. Debneth, *J. Mol. Biol.* **2008**, *378*, 565–580; j) S. Cantel, A. Le Chevalier-Isaad, M. Scrima, J. J. Levy, R. D. Di Marchi, P. Rovero, J. A. Helperin, A. M. D'Ursi, A. M. Papini, M. Chorev, *J. Org. Chem.* **2008**, *73*, 5663–5674.

[9] a) C. Toniolo, E. Benedetti, *Trends Biochem. Sci.* **1991**, *16*, 350–353; b) K. A. Bolin, G. L. Millhauser, *Acc. Chem. Res.* **1999**, *32*, 1027–1033.

[10] a) G. R. Marshall in *Intra-Science Chemistry Reports* (Ed.: N. Kharasch), Gordon and Breach, New York, **1971**, pp. 305–316; b) I. L. Karle, P. Balaram, *Biochemistry* **1990**, *29*, 6747–6756; c) C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, *Biopolymers* **2001**, *60*, 396–419; d) V. Pavone, B. Di Blasio, A. Santini, E. Benedetti, C. Pedone, C. Toniolo, M. Crisma, *J. Mol. Biol.* **1990**, *214*, 633–635; e) C. Toniolo, M. Crisma, G. M. Bonora, E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, A. Santini, *Biopolymers* **1991**, *31*, 129–138; f) R. Gessmann, H. Brückner, K. Petratos, *J. Pept. Sci.* **2003**, *9*, 753–762.

[11] A. Moretto, S. Oancea, F. Formaggio, C. Toniolo, L. A. Huck, D. Mangion, W. J. Leigh, in *Peptides for Youth* (Eds.: S. Del Valle, E. Escher, W. D. Lubell), Springer, Secaucus, **2008**; in press.

[12] E. C. Lathioor, W. J. Leigh, *Photochem. Photobiol.* **2006**, *82*, 291–300.

[13] a) G. M. Bonora, C. Mapelli, C. Toniolo, R. R. Wilkening, E. S. Stevens, *Int. J. Biol. Macromol.* **1984**, *6*, 179–188; b) S. C. Yasui, T. A. Keiderling, F. Formaggio, G. M. Bonora, C. Toniolo, *J. Am. Chem. Soc.* **1986**, *108*, 4988–4993.

[14] L. J. Bellamy in *The Infrared Spectra of Complex Molecules*, Methuen, London, **1966**.

[15] a) K. Wüthrich in *NMR of Proteins and Nucleic Acids*, Wiley, New York, **1986**; b) L. Braunschweiler, R. R. Ernst, *J. Magn. Reson.* **1983**, *53*, 521–528; c) A. Bax, D. G. Davis, *J. Magn. Reson.* **1985**, *65*, 355–360.

[16] a) C. M. Venkatachalam, *Biopolymers* **1968**, *6*, 1425–1436; b) G. D. Rose, L. M. Gierasch, P. J. Smith, *Adv. Protein Chem.* **1985**, *38*, 1–109.

[17] C. H. Görbitz, *Acta Crystallogr. Sect. B* **1989**, *45*, 390–395.

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