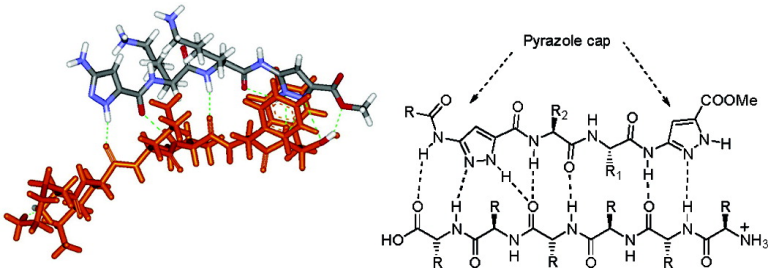
Article

β-Sheet Ligands in Action: KLVFF Recognition by Aminopyrazole Hybrid Receptors in Water

Petra Rzepecki, and Thomas Schrader

J. Am. Chem. Soc., **2005**, 127 (9), 3016-3025• DOI: 10.1021/ja045558b • Publication Date (Web): 11 February 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 7 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 02/11/2005

β-Sheet Ligands in Action: KLVFF Recognition by Aminopyrazole Hybrid Receptors in Water

Petra Rzepecki and Thomas Schrader*

Contribution from the Department of Chemistry, Philipps-Universität Marburg, Hans-Meerwein-Strasse, 35032 Marburg, Germany

Received July 23, 2004; E-mail: schradet@staff.uni-marburg.de

Abstract: Little is known about the precise mechanism of action of β-sheet ligands, hampered by the notorious solubility problems involved with protein misfolding and amyloid formation. Recently the nucleation site for the pathogenic aggregation of the Alzheimer's peptide was identified as the KLVFF sequence in the central region of Aβ. A combination of two aminopyrazole ligands with di- or tripeptides taken from this key fragment now furnished water-soluble Aβ-specific ligands which allow model investigations in water. A detailed conformational analysis provides experimental evidence for an increased β-sheet content induced in the peptide. Strong indications were also found for the peptide backbone recognition via hydrogen bonds plus hydrophobic contributions between aminopyrazole nuclei and Phe residues. The affinity of these new ligands toward the KKLVFF fragment is highly dependent on their sequence and composition from natural and artificial amino acids. Thus, for the first time, detailed insight is gained into the complexation of β-sheet ligands with model peptides taken directly from Aβ.

Introduction

A series of pathological processes is associated with the formation of a β-sheet structure and consecutive protein aggregation in the form of β-amyloid deposition. Those amyloids are discussed as the primary cause for Alzheimer's disease; the conversion of α-helices to larger β-sheet aggregates is also found with Creutzfeldt−Jakob disease, BSE, and other protein-folding diseases.[1] Therefore, a better understanding of the mechanism of aggregation and the development of possible β-sheet binders which can slow or even prevent the pathological process is of high interest from both a mechanistic and a therapeutic view.[2]

About a decade ago a worldwide search was begun for small molecules to treat protein-folding diseases with an ever increasing intensity. Several new ligands or ligand classes have been identified which intervene with fibril or plaque formation.[3] Early reports involved β-sheet ligands such as Congo Red,[4] which are remarkably potent inhibitors of amyloid formation.[5] However, very few ligands demonstrate a rational design, and those which do are modified peptides derived from key recognition elements of pathological proteins: Tjernberg was the first to discover that small peptides with the peptide fragment KLVFF taken directly from the central region of Aβ were able to block Aβ aggregation.[6] Soto then developed peptides with the LPFFD sequence which could be used as β-sheet breakers against Aβ.[7] Others have modified these sequences, e.g., by inserting disrupting elements such as KKKKKK or EEEEE[8] or adding amino acids DD.[9] In a very interesting approach the groups of Meredith and Kapurniotu introduced N-methylated amino acids in every second position of a key peptide NH_2-K(Me-L)V(Me-F)F(Me-A)ECONH_2 and obtained inhibitors for Aβ aggregation with ester instead of amide bonds.[10]

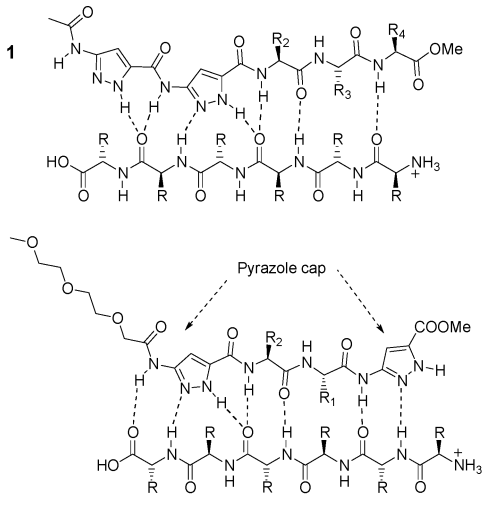
However, nonpeptidic inhibitors with a rational design are unknown to date. Little is also known about the precise mechanism of action of β-sheet ligands, hampered by the notorious solubility problems involved with protein misfolding and amyloid formation. In recent years considerable effort has been invested in the synthesis of small soluble β-sheet structures.[11] Simple β-sheet templates have also been incorporated into drugs which bind to a peptide region with β-sheet conformation.[12] However, external templates, which force a peptide strand into the β-sheet conformation, are very rare.[13]

(1) (a) Wisniewski, T.; Aucouturier, P.; Soto, C.; Frangione, B. *Amyloid: Int. J. Exp. Clin. Invest.* **1998**, *5*, 212. (b) Carrell, R. W.; Lomas, D. A. *Lancet* **1997**, *350*, 134−138.
(2) (a) Lansbury, P. T., Jr. *Acc. Chem. Res.* **1996**, *29*, 317−321. (b) Riesner, D.; Kellings, K.; Post, K.; Wille, H.; Serban, H.; Baldwin, M.; Groth, D. *J. Virol.* **1996**, *70*, 1714−1722. (c) Pitschke, M.; Prior, R.; Haupt, M.; Riesner, D. *Nat. Med.* **1998**, *4*, 832−834.
(3) Some representative examples: (a) Papolla, M.; Bozner, P.; Soto, C.; Shao, H.; Robakis, N. K.; Zagorski, M.; Frangione, B.; Ghiso, J. *J. Biol. Chem.* **1998**, *273*, 7185. (b) Reixach, N.; Crooks, E.; Ostresh, J. M.; Houghten, R. A.; Blondelle, S. E. *J. Struct. Biol.* **2000**, *130*, 247−258. (c) Hammarström, P.; Wiseman, R. L.; Powers, E. T.; Kelly, J. W. *Science* **2003**, *299*, 713−716. (d) Ono, K.; Hasegawa, K.; Yoshiike, Y.; Takashima, A.; Yamada, M.; Naiki, H. *J. Neurochem.* **2002**, *81*, 434−440. (e) Harris, J. R. *Micron* **2002**, *33*, 609−626.
(4) Khurana, R.; Uversky, V. N.; Nielsen, L.; Fink, A. L. *J. Biol. Chem.* **2001**, *276*, 22715−22721.
(5) Lorenzo, A.; Yankner, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12243−12247.
(6) Tjernberg, L. O.; Näslund, J.; Lindqvist, F.; Johansson, J.; Karlström, A. R.; Thyberg, J.; Terenius, L.; Nordstedt, C. *J. Biol. Chem.* **1996**, *271*, 8545.
(7) Soto, C.; Sigurdsson, E. M.; Morelli, L.; Kumar, R. A.; Castano, E. M.; Frangione, B. *Nat. Med.* **1998**, *4*, 822−826.
(8) Lowe, T. L.; Strzelec, A.; Kiessling, L. L.; Murphy, R. M. *Biochemistry* **2001**, *40*, 7882−7889.
(9) Watanabe, K.; Nakamura, K.; Akikusa, S.; Okada, T.; Kodaka, M.; Konakahara, T.; Okuno, H. *Biochem. Biophys. Res. Commun.* **2002**, *290*, 121−124.
(10) (a) Gordon, D. J.; Sciarretta, K. L.; Meredith, S. C. *Biochemistry* **2001**, *40*, 8237−8245. (b) Gordon, D. J.; Meredith, S. C. *Biochemistry* **2003**, *42*, 475−485. (c) Kapurniotu, A. et al. U.S. Patent 6,359,112. (d) Kapurniotu, A.; Schmauder, A.; Tenidis, K. *J. Mol. Biol.* **2002**, *315*, 339−350.

10.1021/ja045558b CCC: $30.25

The Hamilton group truncated the diaminoquinolone motif used by Kemp et al. in their epindolidione receptors[14] for the intramolecular stabilization of short peptides in the β-sheet conformation.[15] Bartlett recently presented the azacyclohexenone fragment for incorporation in drugs targeting β-sheets.[16] Meredith et al. very recently demonstrated the paramount importance of backbone hydrogen bonding for the formation of β-amyloid fibrils: replacing the amide bonds by ester linkages completely eliminated any aggregation propensity of the respective model peptide taken from the Aβ sequence.[17]

Some time ago we introduced 3-aminopyrazoles as simple heterocyclic building blocks with a DAD hydrogen-bond pattern perfectly complementary to that of a β-sheet.[18] In organic solution even glycine-containing dipeptides can be locked in the β-sheet conformation on complexation with acylated aminopyrazoles due to the formation of all three hydrogen bonds with the top face of the peptidic guest.[19] Multiplication of these effects by oligomerization leads to much stronger binding with larger peptides.[20] Thus, dimeric to tetrameric aminopyrazole ligands have been synthesized[21] and tested in aggregation assays with $A\beta_{(1-42)}$.[22] However, most of these structures are prone to extensive self-association via hydrogen bonds and therefore pose severe solubility problems. To increase the water solubility we recently designed hybrid ligands combining natural and unnatural amino acids, the latter derived from 3-aminopyrazole-5-carboxylic acid.[23] Recently we found that the amino acid lysine especially renders these ligands water soluble. Since the nucleation site for the pathogenic aggregation of the Alzheimer's peptide could be identified as the KLVFF sequence in the central region of Aβ,[24] we now combined two aminopyrazole ligands with di- or tripeptides taken from this key fragment[25] and obtained water-soluble Aβ-specific ligands which allow model investigations in water. For the first time detailed insight is gained into the complexation of β-sheet ligands with model peptides taken directly from Aβ.



2: $R_1 = (CH_2)_4\text{-}NH_3^+$, $R_2 = \text{-}(CH_2)_4\text{-}NH_3^+$, **3**: $R_1 = CH_3$, $R_2 = \text{-}(CH_2)_4\text{-}NH_3^+$

Figure 1. Two general classes of hybrid ligands displaying the structural features necessary for perfect complementarity toward naturally occurring β-sheets, with representatives **1**−**3** in their complexes with a hexapeptide.

Results

Design and Synthesis. In preliminary force-field calculations we optimized the tentative 1:1 complexes formed between the new hybrid ligands and a hexapeptide.[26] From these it became immediately evident that only two combinations fulfill the geometrical requirements of a perfect β-sheet complementarity: Either an aminopyrazole−carboxylic acid block must be followed by a natural amino acid block or an even number of α-amino acids must be inserted between two aminopyrazoles to achieve matching hydrogen-bond donor and acceptor patterns (Figure 1).

Three selected examples of the above-described general structure were synthesized by convergent protocols: The C-terminal KVF tripeptide building block **7** was coupled to the N-terminal pyrazole amino acid dimer **6** in its PMB-protected form with Mukaiyama's reagent,[27] followed by simultaneous removal of all protecting groups with hot trifluoroacetic acid. Two hybrid molecules with a central peptide core (**2**, **3**) and flanking aminopyrazole units were prepared by sequential attachment of amino acids to the C-terminal PMB-protected aminopyrazole with HCTU/HOBt (**8**, **9**),[28] followed by linking with the N-terminal aminopyrazole **10**, which had an oligoethyleneglycol acyl group (using 2-chloro-1-methylpyridinium iodide).[27] Final deprotection was again effected with hot trifluoroacetic acid, without any racemization (Figure 2).[23]

The KLVFF fragment was elongated with an additional N-terminal lysine for solubility reasons[29] and conventionally assembled by an automated solid-phase protocol using the Fmoc strategy[30] on a peptide synthesizer from Applied Biosystems.[31] Unfortunately, both an N-terminal Ac-Ala fragment (our own

(11) (a) Review: Nowick, J. S. *Acc. Chem. Res.* **1999**, *32*, 287−296. (b) Tsai, J. H.; Waldman, A. S.; Nowick, J. S. *Bioorg. Med. Chem.* **1999**, *7*, 29−38. (c) Chitnumsub, P.; Fiori, W. R.; Lashuel, H. A.; Diaz, H.; Kelly, J. W. *Bioorg. Med. Chem.* **1999**, *7*, 39−59. (d) Schenck, H. L.; Gellman, S. *J. Am. Chem. Soc.* **1998**, *120*, 4869.
(12) Smith, A. B., III; Hirschmann, R.; Pasternak, A.; Akaishi, R.; Guzman, M. C.; Jones, D. R.; Keenan, T. P.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Holloway, M. K.; Schleif, W. A. *J. Med. Chem.* **1994**, *37*, 215.
(13) (a) Schrader, T.; Kirsten, C. *J. Chem. Soc., Chem. Commun.* **1996**, 2089. (b) Schrader, T. H.; Kirsten, C. N. *J. Am. Chem. Soc.* **1997**, *119*, 12061. (c) Weiner, W. S.; Hamilton, A. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 681−686.
(14) Kemp, D. S.; Bowen, B. R.; Muendel, C. C. *J. Org. Chem.* **1990**, *55*, 4650.
(15) Weiner, W. S.; Hamilton, A. D. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 681−686.
(16) Phillips, S. T.; Rezac, M.; Abel, U.; Kossenjans, M.; Bartlett, P. A. *J. Am. Chem. Soc.* **2002**, *124*, 58−66.
(17) Gordon, D. J.; Meredith, S. C. *Biochemistry* **2003**, *42*, 475−485.
(18) Schrader, T.; Kirsten, C. *J. Chem. Soc., Chem. Commun.* **1996**, 2089.
(19) Schrader, T. H.; Kirsten, C. N. *J. Am. Chem. Soc.* **1997**, *119*, 12061.
(20) Cernovská, K.; Kemter, M.; Gallmeier, H.-C.; Rzepecki, P.; Schrader, T.; König, B. *Org. Biomol. Chem.* **2004**, *2*, 1603−1611.
(21) Rzepecki, P.; Wehner, M.; Molt, O.; Zadmard, R.; Schrader, T. *Synthesis* **2003**, 1815−1826.
(22) Schrader, T.; Riesner, D.; Nagel-Steger, L.; Aschermann, K.; Kirsten, C.; Rzepecki, P.; Molt, O.; Zadmard, R.; Wehner, M. Patent application DE 102 21 052.7, May 10, 2002.
(23) Rzepecki, P.; Gallmeier, H.; Geib, N.; Cernovska, K.; König, B.; Schrader, T. *J. Org. Chem.* **2004**, *69*, 5168−5178.
(24) Tjernberg, L. O.; Lilliehook, C.; Callaway, D. J. E.; Naslund, J.; Hahne, S.; Thyberg, J.; Terenius, L.; Nordstedt, C. *J. Biol. Chem.* **1997**, *272*, 17894.
(25) Tjernberg demonstrated that even tripeptides with parts of the KLVFF sequence influenced the aggregation behavior of the amyloid peptide: (a) Tjernberg, L. O.; Lilliehook, C.; Callaway, D. J. E.; Naslund, J.; Hahne, S.; Thyberg, J.; Terenius, L.; Nordstedt, C. *J. Biol. Chem.* **1997**, *272*, 12601. (b) Tjernberg, L. O.; Callaway, D. J. E.; Tjernberg, A.; Hahne, S.; Lilliehook, C.; Terenius, L.; Thyberg, J.; Nordstedt, C. *J. Biol. Chem.* **1999**, *274*, 12619.
(26) MacroModel 7.0, Schrödinger, Inc. 3000, force-field: Amber*. Molecular mechanics calculations were carried out for 2000 steps treating solvents in a continuum model characterized by its dielectric constant.
(27) (a) Bald, E.; Saigo, K.; Mukayima, T. *Chem. Lett.* **1975**, 1163. (b) Mukaiyama, T. *Angew. Chem.* **1979**, *91*, 798. (c) Li, P.; Xu, J. C. *Tetrahedron* **2000**, *56*, 8119.
(28) (a) Davis, A. M.; Teague, S. J. *Angew. Chem., Int. Ed* **1999**, *38*, 737. (b) Sabatino, G.; Mulinacci, B.; Alcaro, M. C.; Chelli, M.; Rovero, P.; Papini, A. M. *Lett. Pept. Sc.* **2002**, *9*, 119. (c) Marder, O.; Shvo, Y.; Albericio, F. *Chim. Oggi-Chem. Today* **2002**, *20*, 37.
(29) K(15)KLVFFA is offered by Bachem as a "labelled probe for screening defined sequences in the full length amyloid beta" (see ref 25a).

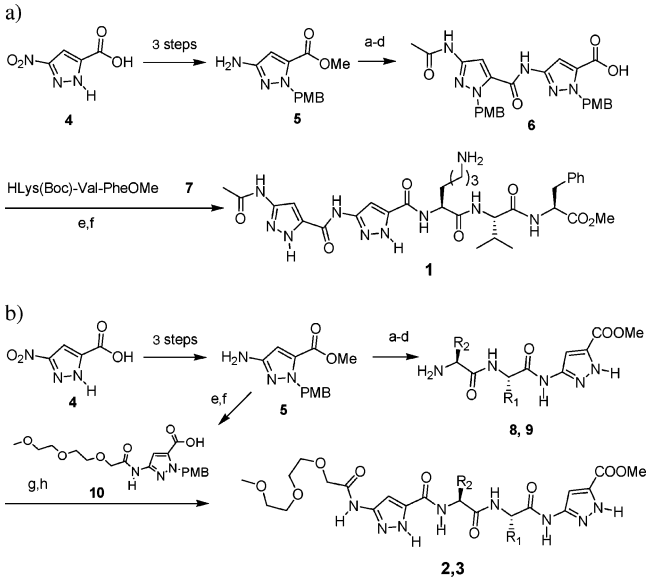


Figure 2. Synthesis scheme for the new hybrid ligands: convergent synthetic approaches using modular building blocks: (a) 1; (b) 2 ($R_1 = R_2 = (CH_2)_4NH_3^+$) and 3 ($R_1 = i$-Pr, $R_2 = (CH_2)_4NH_3^+$). (a) **a**: AcCl, DIEA, DCM; **b**: LiOH, MeOH, THF, H_2O; **c**: PyClop, DIEA + **5**, DCM; **d**: LiOH, MeOH, THF, H_2O; **e**: +**7**, 1-chloro-3-methylpyridinium iodide, DIEA, DCM; **f**: TFA, Δ. (b) **a**: +*N*-(and side chain) protected amino acid, HCTU, Cl-HOBt, 2,6-lutidine, DCM, DMF; **b**: deprotection (Boc: TFA, DCM; Z = Pd/C, H_2, MeOH) **c**: Z-Lys(Boc)-OH, HCTU, Cl-HOBt, 2,6-lutidine, DCM, DMF; **d**: Pd/C, H_2, MeOH; **e**: $+C_7H_{14}O_5$, 1-chloro-3-methylpyridinium iodide, DIEA, DCM; **f**: LiOH, MeOH, THF, H_2O; **g**: 2-chloro-1-methylpyridinium iodide, DIEA, DCM; **h**: TFA, Δ.

synthesis) as well as the Ac-Gln moiety introduced by Tjernberg[6] (Bachem) did not impart sufficient solubility to the KLVFF peptide for NMR titrations. Introduction of the 2-[2-(2-methoxyethoxy)ethoxy]acetyl group markedly increased the solubility of the respective ligand; however, the presence of a single lysine residue was even more effective, leading to solubilities for the whole ligand of >10 mM in water.[32] The ligands were initially examined in dilution experiments in order to detect any potential self-association. Spectra were recorded in H_2O using the Watergate suppression[33] and a D_2O/TMS-filled capillary as a reference. However, between 2 mM and 75 μM no shifts were found in the ^{1}H NMR spectrum even in pure H_2O, indicating the complete absence of aggregates. This is not true for the model peptide KKLVFF, which produced significant NH upfield shifts on dilution from 2 to 1 mM (~0.1 ppm), revealing a substantial amount of self-association via intermolecular hydrogen bonding. A K_{self} value of ~100 M^{-1} could be calculated from an NMR dilution titration.[34] Subsequent measurement of NOESY, DQF−COSY, and TOCSY spectra allowed the assignment of all peptide signals with some ambiguity left for the Phe amide protons. A final Karplus analysis[35] of all $^3J_{\alpha-CH,NH}$ values demonstrated that the hydrophobic C-terminal VFF part was indeed roughly preoriented in

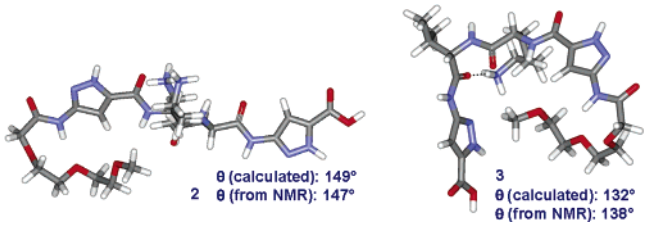


Figure 3. Monte Carlo simulation and corresponding torsion angles θ (top, calculation; below, ^{1}H NMR) for the free new hybrid ligands **2** and **3** (note the kink induced in the lysine residue of **3** (MacroModel 7.2, Amber*, 3000 steps, water)).

a β-sheetlike geometry ($^3J_{H,H}$ = 8.1−8.8 Hz) while the polar N-terminal KK fragment was best described by a random coil conformation ($^3J_{H,H}$ = 6.6−7.0 Hz). Ligands **2** and **3**, however, with an almost identical sequence, differ in the ^3J coupling constants for their respective valine and lysine residue: while the dilysine core in **2** adopts an extended conformation, the KV sequence in **3** produced a marked kink in the backbone arrangement. This divergent behavior is confirmed by Monte Carlo simulations of both ligands; even when starting with a perfect extended conformation, ligand **3** always ends up in a kinked geometry at valine (Figure 3). This kink was later also found in the ligand's crystal structure (not shown).

Binding Experiments. To establish the peptide affinities of the new hybrid ligands, we performed NMR titrations with **1**−**3**, which were added in increasing amounts to KKLVFF. The resulting binding isotherms were analyzed by nonlinear regression methods and furnished association constants for the complexes.[36] Interestingly enough, these covered a broad range from 0 to 130 to 1700 M^{-1}. While the block hybrid **1** reaches a moderate K_a of 130 M^{-1}, the Pz-AA-AA-Pz structure seems to be very effective, producing a more than 10-fold higher association constant for **2** (Figure 4). However, it also turned out to be very sensitive toward small structural changes: exchange of a single lysine for valine from **2** to **3** leads to a total loss of affinity! The drastic effect coincides with the ligand's altered overall conformation—its kinked structure, which is not able to form multiple hydrogen bonds to the top face of the peptide, does not bind at all. This is a first piece of experimental evidence for the postulated β-sheet recognition. Job plots[37] contribute another interesting piece of information: While the expected 1:1 stoichiometry is found for the complex between KKLVFF and the most effective hybrid ligand **2** (Figure 4), the moderately strong β-sheet binder **1** forms a 2:1 complex, even more effective for β-sheet capping.[38]

A thorough chemical shift analysis shows numerous complexation-induced shifts in both host and guest, producing the same overall shape of a saturation curve. Since the NMR measurements were carried out in H_2O, marked downfield shifts for all amide protons in the extended hydrophobic region of the guest peptide as well as the corresponding upfield shifts for selected amide protons in the added aminopyrazole hybrid

(30) Final cleavage from the polymer was effected with a 95:2.5:2.5 mixture of trifluoracetic acid, triisopropylsilane (TIS). and water.
(31) The synthesizer model 433A and all chemicals were supplied to us for a test period by Applied Biosystems.
(32) In some cases a clear solution was reached only after sonication of the aqueous suspension at ambient temperature.
(33) (a) Piotto, M.; Saudek, V.; Sklenar, V. *J. Biomol. NMR* **1992**, *2*, 661−665. (b) Sklenar, V.; Piotto, M.; Leppik, R.; Saudek, V. *J. Magn. Reson. (A)* **1993**, *102*, 241−245.
(34) Dilution titration to determine self-association constants: Wilcox, C. S. *Frontiers in Supramolecular Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds.; VCH: New York, 1991; p 123.
(35) (a) Delepierre, M.; Dobson, C. M.; Poulsen, F. M. *Biochemistry* **1982**, *21*, 4756. (b) Bystrov, V. F. *Prog. Nucl. Magn. Reson. Spectrosc.* **1976**, *10*, 41.
(36) (a) Conners, K. A. *Binding Constants-The Measurement of Molecular Complex Stability;* Wiley: New York, 1987. (b) Schneider, H. J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442.
(37) (a) Job, P. *Compt. Rend.* **1925**, *180*, 928. (b) Blanda, M. T.; Horner, J. H.; Newcomb, M. *J. Org. Chem.* **1989**, *54*, 4626.
(38) In a 2:1 complex both the peptide's top and bottom face can be simultaneously blocked by β-sheet ligands.

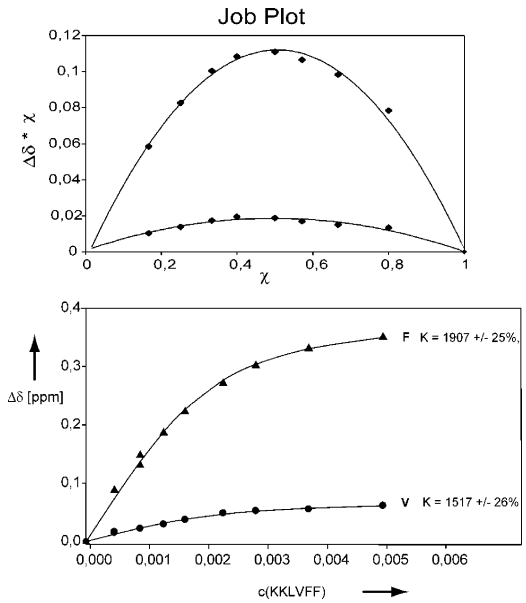


Figure 4. Job plot and NMR titration curves for complex formation between KLVFF and hybrid ligand **2**.

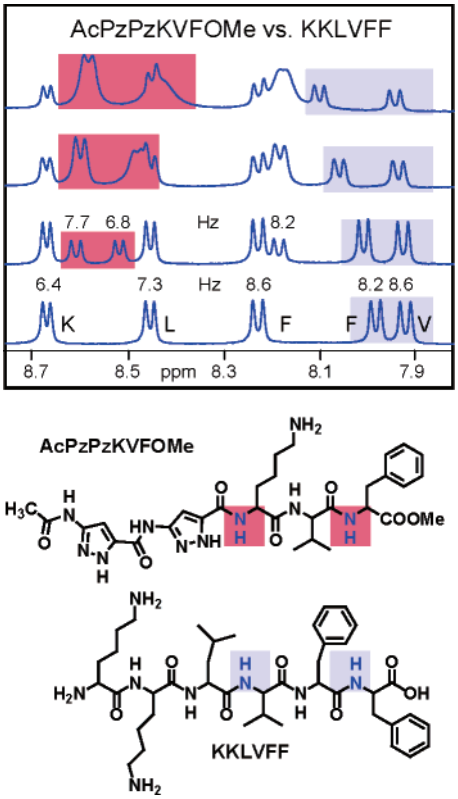


Figure 5. Complexation-induced chemical shift changes in host **1** and KKLVFF guest molecule during the NMR titration. The corresponding $^3J_{\alpha-CH,NH}$ values are depicted next to their NMR signals.

host molecule become clearly visible—a second strong indication of hydrogen bonds between the respective backbones. In addition, several CH protons undergo distinct upfield shifts, especially in the aromatic regions of both host and guest—a hint toward hydrophobic contacts (Figure 5).

Modeling. Monte Carlo simulations for both complexes reach preferred geometries, which make use of extensive hydrogen bonding. In both complexes, however, these attractive forces are also combined with additional hydrophobic interactions

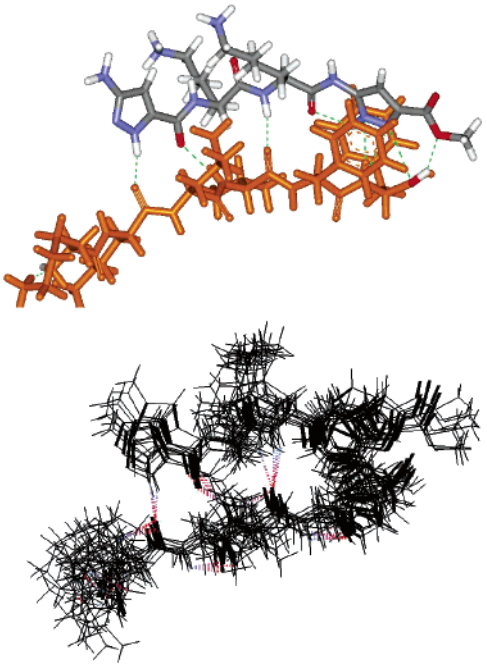


Figure 6. Monte Carlo simulation (top) and subsequent molecular dynamics calculation (bottom) of the complex formed between KKLVFF and hybrid ligand **2** (MacroModel 7.2, Amber*, 3000 steps, water). The ethyleneglycol tail has been omitted for clarity.

between Phe and pyrazole nuclei. A quasi-aromatic cleft between KKLVFF's Phe residues and the pyrazole nuclei in **2** offers a possible explanation for its efficient binding in water despite the small free energy from pure hydrogen bonds (Figure 6). Such a combination of hydrogen bonds reinforced by hydrophobic contacts is also found in natural examples for the recognition of small peptides, e.g., in the thermodynamically extremely stable complex of the D-Ala-D-Ala fragment with the naturally occurring antibiotic vancomycin.[39] Recent observations about the self-assembly of the central hydrophobic Phe-Phe element into peptide nanotubes suggests a potential application of our aminopyrazole templates as controlling elements in this process.[40]

Spectroscopic Complex Analysis. For the most stable complex between KKLVFF and hybrid ligand **2**, a detailed Karplus analysis was carried out.[35] In three amino acid residues the $^3J_{H,H}$ coupling constants increased by 0.6−0.9 Hz and reached values of up to 9.1 Hz. In protein β-sheets ^3J values are usually around 9 Hz, corresponding to torsion angles θ of ∼160°.[41] The reader should also bear in mind that at a K_a value of 1700 M^{-1} only ∼50% of the analytes are involved in the complex.[42] Although starting already from an extended geometry, the peptide's backbone is clearly twisted even more toward a perfect β-sheet conformation as the hybrid ligand approaches (induced fit).[43] Close inspection of changes in coupling constants reveals another detail: one of the benzylic Phe protons is

(39) Williams, D. H.; Searle, M. S.; Mackay, J. P.; Gerhard, U.; Maplestone, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1172−8. Williams, D. H.; Bardsley, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1173−1193.
(40) (a) Reches, M.; Gazit, E. *Science* **2003**, *300*, 625−627. (b) Song, Y.; Challa, S. R.; Medforth, C. J.; Qiu, Y.; Watt, R. K.; Peña, D.; Miller, J. E.; van Swol, F.; Shelnutt, J. A. *J. Chem. Soc., Chem. Commun.* **2004**, 1044−1045.
(41) Pardi, A.; Billeter, M.; Wüthrich, K. *J. Mol. Biol.* **1984**, *180*, 741.
(42) In addition, even extended peptides in a β-sheet are always conformationally much more flexible than proteins: Nesloney, C. L.; Kelly, J. W. *Biorg. Med. Chem.* **1996**, *4*, 739.
(43) Schneider, H. J. *Angew. Chem.* **1991**, *103*, 1419.

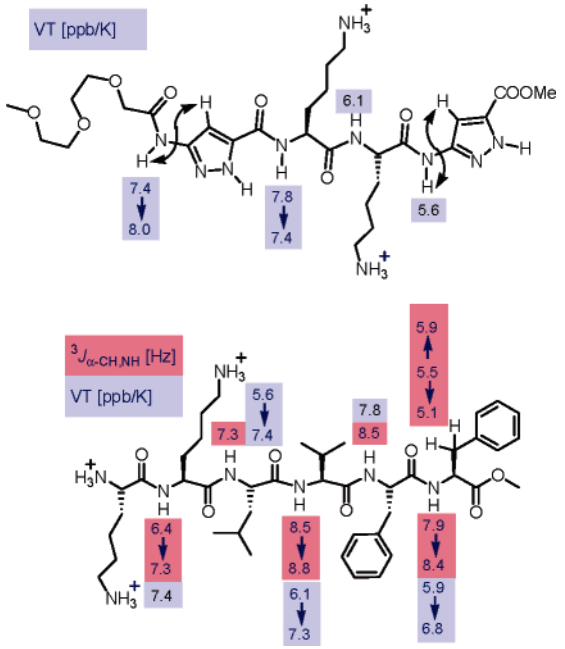


Figure 7. NMR-spectroscopic analysis of the complex formation between ligand **2** and KKLVFF. Changes in coupling constants are depicted in red and VT slopes in blue. Reciprocal NOESY cross-peaks are represented by double arrows.

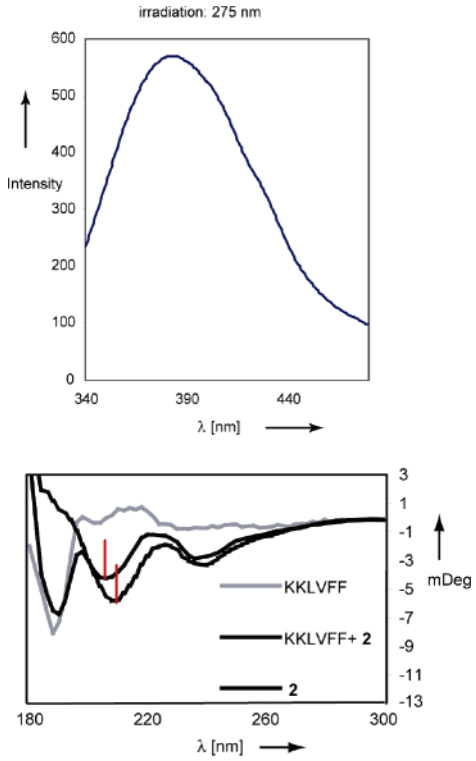


Figure 8. (Left) Fluorescence emission spectrum for ligand **2** in water. (Right) CD spectra for free KKLVFF (50 μM), ligand **2** (250 μM), and their 1:1 mixture.

distinctly shifted upfield with concomitant changes in the respective $^3J_{\alpha-\mathrm{CH},\beta-\mathrm{CH}}$ values.[35] This may indicate a partial conformational lock for the Phe aromatic residues and is in excellent agreement with the arrangement proposed by Monte Carlo simulations (Figure 5). In addition, no shift was observed for the pyrazole amide protons, coinciding with the 180° twist found in the calculated structure. ROESY experiments[44] (Watergate) confirmed strong intramolecular cross-peaks between the pyrazole amide and CH protons.

Subsequent VT (variable-temperature) experiments were carried out in order to discern between hydrogen-bonded and solvent-exposed NH protons. Identical concentrations of ligand **2**, hexapeptide, as well as their 1:1 complex were heated in 5° steps from 300 to 350 K. However, only unspectacular changes in the ppb/K dependencies were observed in the guest peptide (increasing by less than 2 ppb/K to values between 6.8 and 7.8 ppb/K, Figure 7). Unfortunately, besides the expected strong intramolecular cross-peaks, NOESY and ROESY measurements did not produce any intermolecular contacts which could have shed new light on the mutual orientation of both complex partners.

Another promising feature of our new hybrid molecules is their strong fluorescence emission of the pyrazole nucleus around 390 nm on irradiation at 275 nm (Figure 8). We assumed that formation of hydrogen bonds between the pyrazole nucleus and peptidic amide protons would influence the electronic nature of the pyrazole chromophore and hence lead to changes in their emission spectra. During a titration with KKLVFF a marked fluorescence quenching could be observed; however, in our hands this effect was not reproducible, perhaps due to a beginning self-aggregation of the guest peptide. Contrary to the peptide itself, our new hybrid ligands have characteristic CD curves with negative minima between 208 and 214 nm and, in the case of **1**, a maximum at 196 nm. These values are not far away from the usual Cotton effect found in natural β-sheets (minimum at 215 nm, maximum at 195 nm).[45] On addition of the KKLVFF peptide, the minimum of **2** shifted by 2−3 nm toward lower wavenumbers.[46] Although existent, this effect is too small for an experimental basis of a conformational change (Figure 8b).

Finally, a neutral tetramethylammonium acetate buffer was explored which allows detection of Aβ and its fragments by ESI-MS at near physiological conditions (pH 6.8).[47] Very small molecular-ion peaks were found for homodimers between two ligands or two KLVFF peptides as well as for the peptide ligand complex. However, incubation of ligand **2** with the KLVFF recognition element and free Aβ(1−40) furnished a clear molecular-ion peak for the respective 1:1 complex.[48] Thus, a certain confirmation of self-recognition was indeed found by ESI-MS.

Summary and Conclusion

In conclusion, two classes of designed aminopyrazole hybrid ligands were shown to undergo a specific interaction with

(44) Williamson, M. P.; Waltho, J. P. *Chem. Soc. Rev.* **1992**, 227.

(45) Lottspeich, F.; Zorbs, H. *Bioanalytik*; Spektrum Akademischer Verlag GmbH: Heidelberg, Berlin, 1998.

(46) Small peptides in a β-sheet often show a slightly divergent behavior, in some cases without a maximum at 195 nm or with a minimum shift of ±5 nm around 215 nm: (a) Diaz, H.; Tsang, K. Y.; Choo, D.; Espina, J. R.; Kelly, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 370. (b) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988. (c) Choo, D. W.; Schneider, J. P.; Graciani, N. R.; Kelly, J. W. *Macromolecules* **1996**, *29*, 355.

(47) Related experiments with the Aβ monomer have hitherto been restricted to acidic buffers (pH = 4.2): Chen, X. G.; Brining, S. K.; Nguyen, V. Q.; Yergey, A. L. *FASEB J.* **1997**, *11*, 817−823.

(48) Recently, it was shown that complexes of the well-known β-peptide aggregation inhibitor melatonin with Aβ can be detected by ESI-MS: Skribanek, Z.; Balázspiri, L.; Mák, M. *J. Mass Spectrom.* **2001**, *36*, 1226−1229.

KKLVFF, a key fragment from the nucleation site of Aβ in water. A detailed conformational analysis provides experimental evidence for an increased β-sheet content induced in the peptide. Strong indications were also found for the peptide backbone recognition via hydrogen bonds plus hydrophobic contributions between aminopyrazole nuclei and Phe residues. The affinity of these new ligands toward the critical KKLVFF fragment is highly dependent on their sequence and composition from natural and artificial amino acids.[49]

The search for *the* optimal amyloid binder can only be conducted in a test with a broad range of divergent ligand structures. In the future, we will carry out a lead optimization with a combinatorial assay—solid-phase peptide synthesis creates a library of immobilized aminopyrazole hybrid molecules which is screened with fluorometric detection of the binding event. Our best ligands, **2** and **3**, have already produced very promising effects both in antiaggregation experiments with isolated Aβ in vitro[50] and also in whole cell culture assays with neuronal cells;[51] animal tests are currently being carried out on transgenic mice. These results will be presented in a more biochemical journal. All these experiments generally serve a dual purpose: we want to identify potent β-sheet binders for a potential Alzheimer therapy and on our way strive to gain a deeper understanding of the mechanism of protein misfolding in Nature.

Experimental Section

Fluorescence and Circular Dichroism Spectra. Fluorescence and circular dichroism spectra were recorded on a FP-750 spectrofluorometer (Jasco) and a J-810 spectropolarimeter (Jasco), respectively. For circular dichroism spectra a 50 μM solution of KKLVFF in bidest water and 250 μM solutions of the ligands were used.

Molecular Modeling. Force-field calculations were initially carried out with MacroModel 7.2 using the force fields AMBER* or OPLS-AA and GB/SA. Monte Carlo conformational searches were carried out with 3000−5000 steps without any constraints. Molecular dynamics were calculated for 100 ps at 300 K. Snapshots were taken every 10 ps.

Karplus Analysis. For the peptide KKLVFF as well as for each amino acid in the hybrid ligands **1**−**3** the respective dihedral angles θ were calculated from their $^3J_{NH,\alpha-CH}$ coupling constants by means of the Karplus equation, modified for peptides.

NMR Titrations and VT Experiments. NMR titrations and VT experiments were recorded at 400 or 500 MHz in H_2O with a D_2O/TMS capillary as internal standard (and water suppression by watergate). For the titration a NMR tube was filled with 0.80 mL of the guest compound (KKLVFF). The host compound was dissolved in 0.80 mL of the guest compound (using ultrasonic), and the resulting solution was added in amounts increasing from 0 to 5 equiv. Volume and concentration changes of the probes and upfield shifts of the peptide in dilution experiments were taken into account. The association constants were calculated by nonlinear regression methods. Variable-temperature experiments: a 1:1 mixture of KKLVFF and ligand **2** was warmed in 5° steps from 300 to 350K (water). The resulting chemical shift changes of the amidic NH protons were recorded; their ppb/K values result from the pitch of the straight line δ [ppm] = $a \cdot T$[K] + b.

ESI-MS Spectra. For ESI-MS experiments an acidic buffer (buffer A, NH_4Ac/HCOOH; pH 4.2) or a neutral buffer (buffer B, Me_4NAc; pH 6.8) was used. Samples containing 25 μM Aβ(1−40) (Bachem Biochemica, Heidelberg, Germany) with or without ligands **1**−**3** (250 μM) in 10 mM buffer with 4% DMSO were freshly prepared. At last the stock solution of Aβ(1−40) (2.50 mM in DMSO) was added to the different samples, and the mixtures were subsequently incubated at 25 °C. Masses were measured between 700 and 2000 amu. As transport solvents, buffer A or buffer B was used at a flow rate of 50 μL/min. For ESI-MS experiments in the positive-ion mode the following settings were applied: declustering potential (DP1) 85, focusing potential (FP) 225, declustering potential two (DP2) 15, ion energy (IE1) 2.0, nebulizer gas (GS1) 40, curtain gas (CUR) 25 and ion spray voltage (IS) 5400. Monomeric Aβ was detetcted as M^{3+} = 1443.383 m/z, M^{4+} = 1082.787 m/z, and M^{5+} = 866.430 m/z peaks.

General Procedure A (HCTU). Under an inert atmosphere the N-terminal and side-chain-protected amino acid or peptide (1.00 equiv) were dissolved in DCM/DMF (3:1). Cl-HOBt (2.50 equiv), HCTU (1.00 equiv), and 2,6-lutidine (3.00 equiv) were added and stirred for 10 min at 0 °C. The C-terminal and PMB-protected aminopyrazolecarbonyl monomer (1.00 equiv) or the C-protected amino acid (1.00 equiv) were dissolved in DCM/DMF (3:1) and added to the reaction mixture. If the C-protected amino acid was applied as ammonium salt, 2,6-lutidine (1.00 equiv) was added to this solution before adding it to the reaction mixture. The reaction mixture was stirred afterward for 2−3 h. The mixture was washed with 1 M HCl, saturated $NaHCO_3$, and saturated NaCl solution and dried over Na_2SO_4. After filtration the solvent was removed in vacuo. Further workup procedures (if necessary) are described for each compound in detail.

General Procedure B (Mukayiama's Reagent). Under an inert atmosphere the C-terminal and PMB-protected aminopyrazole carbonyl monomer (1.00 equiv), the N-terminal and side-chain-protected amino acid (1.00 equiv), 2-chloro-1-methyl pyridinium iodide (1.20 equiv), and diisopropylethylamine (3.00 equiv) were dissolved in DCM and stirred overnight at room temperature under an inert atmosphere. Unless otherwise noted, the mixture was washed with 1 M HCl, saturated $NaHCO_3$, and saturated NaCl solution and dried over Na_2SO_4. After filtration the solvent was removed in vacuo.

***N-tert*-Butyloxycarbonyl-(*S*)-valinyl-(*S*)-phenylalanine Methyl Ester [Boc-VF-OMe].** A 1.70 g (7.88 mmol, 1.00 equiv) amount of (*S*)-phenylalanine methyl ester hydrochloride and 1.71 g (7.88 mmol, 1.00 equiv) of *N-tert*-butyloxycarbonyl-(*S*)-valine were reacted with 3.26 g (7.88 mmol, 1.00 equiv) of HCTU, 3.34 g (19.7 mmol, 2.50 equiv) of Cl-HOBt, and 3.7 mL (31.5 mmol, 4.00 equiv) of 2,6-lutidine according to general procedure A to yield the compound as a colorless solid. Yield: 2.89 g (7.66 mmol, 97%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (d, ^3J = 6.6 Hz, 3H, CH_3-Val), 0.91 (d, ^3J = 7.0 Hz, 3H, CH_3-Val), 1.44 (s, 9H, CH_3-*t*Bu), 2.03−2.13 (m, 1H, CH-*i*prop), 3.09−3.13 (m, 2H, CH_2), 3.70 (s, 3H, $OOCH_3$), 3.89 (dd, ^3J = 6.3 Hz, ^3J = 8.3 Hz, 1H, α-CH), 4.83−4.97 (m, 1H, α-CH), 4.99 (d, ^3J = 7.6 Hz, 1H, NH), 6.28 (d, ^3J = 6.0 Hz, 1H, NH), 7.08−7.27 (m, 5H, CH-arom). ^{13}C NMR (75 MHz, DMSO-d_6): δ 17.0, 19.1, 28.2, 37.9, 38.6, 52.3, 53.1, 59.9, 127.1, 128.6, 135.6, 162.5, 171.2, 171.6. MS (ESI): m/z 417 $(M + K)^+$, 401 $(M + Na)^+$. HRMS: calcd for $C_{20}H_{30}N_2O_5$-Na, 401.2052; found, 401.2053. Mp: 101 °C.

(*S*)-Valinyl-(*S*)-phenylalanine Methyl Ester Trifluoroacetate [H-VF-OMe]. A 1.50 g (3.96 mmol, 1.00 equiv) amount of *N-tert*-butyloxycarbonyl-(*S*)-valinyl-(*S*)-phenylalanine methyl ester [Boc-VF-OMe] was stirred in a mixture of dichloromethane and trifluoroacetic acid (10:1) at 0 °C until the starting material disappeared on TLC. The dichloromethane was removed in vacuo, and an excess amount of ice-cold diethyl ether was added to the remaining trifluoroacetic acid. The product precipitated as colorless solid, which was filtered off, washed with diethyl ether, and dried in vacuo to yield the compound as a

(49) It might be argued that earlier attempts to disrupt assembly via this region have not resulted in an improvement of biological function. However, it should be kept in mind that these compounds, derived from the KLVFF sequence, had a peptidic origin and were hence labile to hydrolysis by peptidases or proteases in vivo. Our aminopyrazole ligands contain no natural peptide bond and are hence completely resistant toward proteolytic digestion. In addition, they recognize primarily the β-sheet conformation of the Aβ peptide's backbone and are thus not restricted to attack at the KLVFF site; in fact, they may as well bind to the other critical hydrophobic region between residue 38−42 at the *C*-terminus of Aβ.
(50) Cooperation with D. Riesner and L. Nagel-Steger, Institute of Physical Biology, University of Düsseldorf, Germany.
(51) Collaboration with JSW Research GmbH, Graz, Austria.

colorless solid. Yield: 1.50 g (3.82 mmol, 96%). ^{1}H NMR (400 MHz, MeOH-d_3): δ 1.01 (d, ^3J = 7.0 Hz, 3H, CH_3-Val), 1.05 (d, ^3J = 7.0 Hz, 3H, CH_3-Val), 2.14−2.24 (m, 1H, CH-*i*prop), 3.01 (dd, ^3J = 8.7 Hz, ^3J = 14.1 Hz, 1H, CH_2-Benzyl), 3.18 (dd, ^3J = 5.7 Hz, ^3J = 14.0 Hz, 1H, CH_2-Benzyl), 3.66−3.68 (m, 4H, $OOCH_3$, α-CH), 4.67 (1H, α-CH), 7.19−7.30 (m, 5H, CH-arom.), 8.70 (d, ^3J = 7.2 Hz, 1H, NH. ^{13}C NMR (75 MHz, MeOH-d_3): δ 17.3, 18.7, 31.5, 37.9, 52.6, 55.5, 59.5, 127.8, 129.5, 129.9, 137.8, 169.7, 172.8. MS (ESI): *m*/*z* 301 (M + Na)$^+$, 279 (M + H)$^+$. HRMS: calcd for $C_{15}H_{23}N_2O_8$, 279.1709; found, 279.1704. Anal. Calcd for $C_{15}H_{22}N_2O_8$: C, 52,04; H, 5,91; N, 7,14. Found: C, 51,50; H, 5,77; N, 7,14. Mp: 163 °C. $[\alpha]_{Na}$: + 19.2, T = 21 °C, c = 1.00 in methanol. R_f: 0.71 in dichloromethane/methanol (30:1).

***N*-(Benzyloxycarbonyl)-*N*$^\epsilon$-(*tert*-butyloxycarbonyl)-(*S*)-lysinyl-(*S*)-valinyl-(*S*)-phenylalanine Methyl Ester [Z-K(Boc)VF-OMe].** A 1.15 g (2.92 mmol, 1.00 equiv) amount of (*S*)-valinyl-(*S*)-phenylalanine methyl ester trifluoracetate [H-VF-OMe] and 1.11 g (2.92 mmol, 1.00 equiv) of *N*-(benzyloxycarbonyl)-*N*$^\epsilon$-(*tert*-butyloxycarbonyl)-(*S*)-lysine were reacted with 1.21 g (2.92 mmol, 1.00 equiv) of HCTU, 1.24 g of Cl-HOBt (7.30 mmol, 2.50 equiv), and 1.36 mL (11.68 mmol, 4.00 equiv) of 2,6-lutidine according to general procedure A to yield the compound as a colorless solid. Yield: 1.39 g (2.17 mmol, 75%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (d, ^3J = 6.8 Hz, 3H, CH_3-Val), 0.87 (d, ^3J = 6.8 Hz, 3H, CH_3-Val), 1.32−1.38 (m, 2H, CH_2-Lys), 1.41 (s, 9H, H-23), 1.45−1.47 (m, 2H, CH_2-Lys), 1.58−67 (m, 2H, CH_2-Lys), 1.79−1.82 (m, 1H, CH_2-Lys), 2.08−2.15 (m, 1H, H-12), 3.07−3.10 (m, 4H, CH_2-Benz, CH_2-Lys), 3.69 (s, 3H, $OOCH_3$), 4.10−4.13 (m, 1H, α-CH), 4.20 (dd, ^3J = 6.2 Hz, ^3J = 8.5 Hz, 1H, α-CH), 4.84 (m, 1H, α-CH), 5.05−5.12 (m, 2H, CH_2-Benz), 5.52 (brs, 1H, NH), 6.41 (d, ^3J = 6.4 Hz, 1H, NH), 6.57 (d, ^3J = 8.9 Hz, 1H, NH), 7.08−7.25 (m, 11H, CH-arom). ^{13}C NMR (125 MHz, DMSO-d_6): δ 17.9, 18.6, 19.2, 22.5, 28.5, 29.7, 30.7, 31.7, 37.9, 52.5, 53.3, 55.2, 58.7, 67.3, 127.3, 128.3, 128.4, 128.7, 128.8, 129.4, 135.8, 136.3, 156.4, 170.6, 171.8, 171.9. MS (ESI): *m*/*z* 679 (M + K, 8)$^+$ 641 (M + H, 7)$^+$, 663 (M + Na, 100)$^+$. HRMS (ESI): calcd for $C_{34}H_{48}N_4O_8Na$, 663.3370; found, 663.3362. Anal. Calcd for $C_{34}H_{48}N_4O_8$: C, 63.73; H, 7.55; N, 8.74. Found: C, 63.41; H, 7.64; N, 8.77. Mp: 143 °C. R_f: 0.26 in dichlormethane/methanol (30:1).

***N*$^\epsilon$-(*tert*-Butyloxycarbonyl)-(*S*)-lysinyl-(*S*)-valinyl-(*S*)-phenylalanine Methyl Ester [H-K(Boc)VF-OMe], 7.** A 820 mg (1.28 mmol, 1.00 equiv) amount of *N*-(benzyloxycarbonyl)-*N*$^\epsilon$-(*tert*-butyloxycarbonyl)-(*S*)-lysinyl-(*S*)-valinyl-(*S*)-phenylalanine methyl ester [Z-K(Boc)-VF-OMe] was dissolved in 50 mL of methanol (HPLC grade). To this solution was added 5 mol % Pd/C (Degussa Typ E101 NE/W), and the mixture was stirred in a H_2 atmosphere until the starting material disappeared on TLC. The catalyst was filtered off using kieselguhr, and the solvent was evaporated to yield compound **7** as a pale yellow solid. Yield: 650 mg (1.28 mmol, quant.). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (d, ^3J = 6.9 Hz, 3H, CH_3-Val), 0.91 (d, ^3J = 6.9 Hz, 3H, CH_3-Val), 1.39−1.60 (m, 13H, CH_2-Lys, NH_2, CH_3-*t*Bu), 1.77−1.88 (m, 1H, CH_2-Lys), 2.07−2.19 (m, 1H, CH-*i*prop), 3.02−3.17 (m, 4H, CH_2-Benz, CH_2-Lys), 3.32−3.36 (m, 1H, α-CH), 3.71 (s, 3H, $OOCH_3$), 4.16 (dd, $^3J_{H(11,12)}$ = 6.9 Hz, $^3J_{H(11,30)}$ = 8.8 Hz, α-CH), 4.57 (brs, 1H, NH), 4.82−4.89 (m, 1H, α-CH), 6.38 (d, ^3J = 7.9 Hz, 1H, NH), 7.09−7.30 (m, 5H, CH-arom.), 7.79 (d, ^3J = 8.7 Hz, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.3, 19.6, 23.2, 28.8, 30.2, 30.8, 34.9, 38.3, 40.5, 52.7, 53.4, 55.5, 58.5, 127.5, 128.9, 129.6, 136.1, 156.2, 160.4, 171.3, 172.1, 175.5. MS (ESI): *m*/*z* 507 (M + H)$^+$, 529 (M + Na)$^+$. HRMS: calcd for $C_{26}H_{42}N_4O_6Na$, 529.3002; found, 529.3015. Anal. Calcd for $C_{26}H_{42}N_4O_6$: C, 61.64; H, 8.36; N, 11.06. Found: C, 61.20; H, 8.43; N, 11.05. R_f: 0.15 in dichlormethane/methanol (50:1). Mp: 156 °C.

5-{[5-Acetylamino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carbonyl]-amino}-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid Methyl Ester [Ac-Pz(PMB)Pz(PMB)-OMe]. A 650 mg (2.25 mg, 1.00 equiv) amount of 5-acetylamino-2-(4-methoxy-benzyl)-2*H*-pyrazole-3-carboxylic acid[52] and 646 mg (2.47 mmol, 1.10 equiv) of *N*-*p*-methoxybenzyl-5-aminopyrazole-3-carboxylic acid methyl ester **5**[52] were reacted according to general procedure B. The residue was purified by column chromatography on silica gel using ethyl acetate/*n*-pentane (1:1). Yield: 553 mg (1.04 mmol, 46%), colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (s, 3H, CH_3-Acetyl), 3.79, 3.81 (2s, 6H, CH_3-PMB), 3.91 (s, 3H, $OOCH_3$), 5.64, 5.66 (2s, 4H, CH_2-PMB), 6.82−6.88 (m, 4H, CH-arom.), 7.24−7.37 (m, 6H, CH-arom., CH-pyrazole), 7.93 (s, 1H, NH), 8.68 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.0, 52.2, 52.4, 53.9, 54.1, 55.4, 102.9, 114.1, 114.3, 114.7, 128.7, 129.0, 129.2, 129.4, 132.3, 134.7, 145.1, 145.8, 156.7, 159.4, 159.5, 160.1, 167.8. MS (EI): *m*/*z* 532 (M)$^+$, 411 (M − PMB)$^+$. HRMS (ESI) calcd for $C_{27}H_{28}N_6ONa$, 555.1968; found, 555.1958. Mp: 98 °C. R_f: 0.10 ethyl acetate/*n*-pentane (1:1).

5-{[5-Acetylamino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carbonyl]-amino}-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid [Ac-Pz(PMB)Pz(PMB)-OH], 6. A 500 mg (940 μmol, 1.00 equiv) amount of 5-{[5-acetylamino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carbonyl]-amino}-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic acid [Ac-Pz-(PMB)Pz(PMB)-OMe] and 25 mg (1.03 mmol, 1.10 equiv) of lithium hydroxide were stirred in a mixture of methanol/THF/water (2:2:1) for 16 h. The solvent was evaporated, the remaining residue dissolved in water, and the solution acidified with 1 M HCl. The product precipitated as a colorless solid, which was filtered off, washed with water, and dried in vacuo to yield compound **6**. Yield: 462 mg (893 μmol, 95%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.98 (s, 3H, CH_3-acetyl), 3.69, 3.71 (2s, 6H, CH_3-PMB), 5.57, 5.62 (2s, 4H, CH_2-PMB), 6.48−7.16 (2m, 10H, CH-pyrazole, CH-arom.), 7.47 (s, 1H, OOH), 10.57, 11.27 (2s, 2H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.0, 52.9, 53.0, 55.0, 99.8, 103.1, 113.8, 113.9, 128.7, 128.8, 129.4, 132.5, 133.9, 145.5, 146.0, 157.3, 156.6, 158.7, 160.4, 167.5. MS (ESI): *m*/*z*541 (M + Na)$^+$, 519 (M + H)$^+$. HRMS: calcd for $C_{26}H_{27}N_6O_6$, 519.1992; found, 519.2016. Mp: 110 °C.

5-Acetylamino-2-[4-methoxybenzyl]-2*H*-pyrazole-3-carbonylamino-2-[4-methoxybenzyl]-2*H*-pyrazole-3-carbonyl-*N*$^\epsilon$-*tert*-butyloxycarbonyl-(*S*)-lysinyl-(*S*)-valinyl-(*S*)-phenylalanine Methyl Ester [Ac-Pz(PMB)Pz(PMB)K(Boc)VF-OMe]. Under an inert atmosphere 154 mg (296 μmol, 1.00 equiv) of 5-acetylamino-2-(4-methoxybenzyl)-2*H*-pyrazol-3-carbonylamino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carbonylic acid **6** and 150 mg (296 μmol, 1.00 equiv) of *N*$^\epsilon$-*tert*-butyloxycarbonyl-(*S*)-lysinyl-(*S*)-valinyl-(*S*)-phenylalanine methyl ester **7** were reacted with 91 mg (356 μmol, 1.20 equiv) of 2-chloro-1-methylpyridinium iodide and 116 of μg/155 μL (900 μmol, 3.00 equiv) of diisopropylethylamine in a mixture of dry dichloromethane and dry THF (5:1). The reaction mixture was stirred overnight at room temperature. Afterward the solvent was evaporated and the remaining residue dissolved in dichloromethane. The organic solvent was washed with 1 M HCl, saturated $NaHCO_3$, and saturated NaCl solution and dried over Na_2SO_4. After filtration the solvent was removed in vacuo. The remaining residue was purified over silica gel column, eluting chloroform/methanol (30:1) to yield the compound as a colorless solid. Yield: 170 mg (169 μmol, 57%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.80−0.83 (m, 6H, CH_3-Val), 1.18−1.35 (m, 4H, CH_2-Lys), 1.38 (s, 9H, CH_3-*t*Bu), 1.61−1.68 (m, 2H, CH_2-Lys) 1.89−1.96 (m, 1H, CH-*i*prop), 1.99 (s, 1H, CH_3-Acetyl), 2.90−2.96 (m, 3H, CH_2-Lys, CH_2-Phe), 3.02 (dd, ^2J = 13.8 Hz, ^3J = 6.0 Hz, 1H, CH_2-Phe), 3.55 3.69, 3.70 (3s, 9H, $OOCH_3$, CH_3-PMB), 4.20−4.24 (m, 1H, α-CH), 4.38−4.47 (m, 1H, α-CH), 4.49−4.52 (m, 1H, α-CH), 5.51−5.59 (brs, 4H, CH_2-PMB), 6.74 (brs, 1H, NH), 6.82−6.86 (m, 4H, CH-arom) 7.12−7.26 (m, 9H, CH-arom.), 7.40 (s, 1H, CH-pyrazole), 7.48 (s, 1H, CH-pyrazole), 7.73 (d, ^3J = 8.9 Hz, NH), 8.43 (d, ^3J = 7.2 Hz, NH), 8.69 (d, ^3J = 7.9 Hz, NH), 10.53 (s, 1H, NH), 11.20 (s, 1H, NH). ^{13}C NMR

(52) For a detailed description of the synthesis of this building block, see: (a) Rzepecki, P.; Wehner, M.; Molt, O.; Zadmard, R.; Schrader, T. *Synthesis* **2003**, 1815−1826. (b) Rzepecki, P.; Gallmeier, H.; Geib, N.; Cernovska, K.; König, B.; Schrader, T. *J. Org. Chem.* **2004,** ASAP.

(100 MHz, DMSO-d_6): δ 17.8, 19.9, 23.0, 23.2, 28.2, 29.2, 30.9, 31.0, 36.4, 51.6, 52.7, 52.8, 53.3, 53.4, 55.0, 57.0, 77.2, 99.8, 100.0, 113.6, 113.8, 126.4, 129.1, 128.6, 128.9, 129.1, 129.7, 129.8, 134.0, 134.6, 136.9, 145.2, 146.1, 155.5, 157.2, 158.6, 158.7, 159.2, 167.5, 170.9, 171.3, 171.7. MS (ESI): *m*/*z* 1029 $(M + Na)^+$, 1007 $(M + H)^+$. HRMS: calcd for $C_{52}H_{66}N_{10}O_{11}Na$, 1029.4810; found, 1029.4944. Mp: >240 °C. R_f: 0.22 in chloroform/methanol (30:1).

5-Acetylamino-2*H*-pyrazole-3-carbonylamino-2*H*-pyrazole-3-carbonyl-(*S*)-lysinyl-(*S*)-valinyl-(*S*)-phenylalanine Methyl Ester Trifluoroacetate [Ac-PzPzKVF-OMe], 1. A 100 mg (99.3 μmol, 1.00 equiv) amount of 5-acetylamino-2-[4-methoxybenzyl]-2*H*-pyrazole-3-carbonylamino-2-[4-methoxybenzyl]-2*H*-pyrazole-3-carbonyl-*N*$^{\epsilon}$-*tert*-butyloxyarbonyl-(*S*)-lysinyl-(*S*)-valinyl -(*S*)-phenylalanine methyl ester [Ac-Pz(PMB)Pz(PMB)K(Boc)VF-OMe] was stirred under an inert atmosphere in dry trifluoroacidic acid for 2−3 h at 70 °C. After cooling to room temperature, ice-cold diethyl ether was added. The product precipitated as a colorless solid. This was centrifuged off, washed several times with ice-cold diethyl ether, and dried in vacuo to yield compound **1**. Yield: 90 mg (89 mmol, 90%). ^{1}H NMR (500 MHz, MeOH-d_3): δ 0.80 (d, ^3J = 6.9 Hz, 3H, CH_3-Val), 0.83 (d, ^3J = 6.9 Hz, 3H, CH_3-Val), 1.35−1.47 (m, 2H, CH_2-Lys), 1.63−1.69 (m, 2H, CH_2-Lys), 1.74−1.70 (m, 2H, CH_2-Lys), 1.99−2.05 (m, 1H, CH-*i*prop), 2.14 (s, 3H, CH_3-Acetyl), 2.84−2.91 (2H, CH_2-Lys), 2.98 (dd, ^2J = 13.9 Hz, ^3J = 8.4 Hz, 1H, CH_2-Phe), 3.12 (dd, ^2J = 13.9 Hz, ^3J = 5.8 Hz, 1H, CH_2-Phe), 3.64 (s, 1H, $OOCH_3$), 4.22 (dd, ^3J = 8.3 Hz, ^3J = 7.6 Hz, 1H, α-CH), 4.57−4.66 (m, 2H, α-CH), 6.81, 6.88 (2s, 2H, CH-pyrazole), 7.17−7.27 (m, 5H, CH-arom.), 7.75 (brs, 3H, NH_3^+), 8.04 (d, ^3J = 8.4 Hz, 1H, NH), 8.34 (d, ^3J = 7.8 Hz, 1H, NH), 8.53 (d, ^3J = 7.6 Hz, 1H, NH), 10.68, 10.84 (s, 2H, NH). The pyrazole NHs are broadened. ^{13}C NMR (125 MHz, MeOH-d_3): δ 18.5, 19.4, 22.8, 23.6, 28.1, 32.0, 32.5, 38.3, 40.7, 52.5, 54.1, 55.3, 60.0, 95.9, 96.4, 127.7, 129.4, 130.1, 134.3, 134.5, 137.9, 144.2, 144.9, 160.6, 162.9, 171.2, 173.1, 173.5, 173.7. MS (ESI): *m*/*z* 667 $(M + H)^+$. HRMS (ESI): calcd for $C_{31}H_{43}N_{10}O_7$, 667.3316; found, 667.3332. Mp: 165 °C.

***N*-Benzyloxycarbonyl-*N*$^{\epsilon}$-*tert*-butyloxycarbonyl-(*S*)-lysinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid Methyl Ester [Z-Lys(Boc)-Pz(PMB)-OMe].** A 1.04 g (4.00 mmol, 1.00 equiv) amount of 5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic acid methyl ester **5**[52] and 1.67 g (4.40 mmol, 1.10 equiv) of *N*-benzyloxycarbonyl-*N*$^{\epsilon}$-*tert*-butyloxycarbonyl-(*S*)-lysine were reacted with 1.82 g (4.40 mmol, 1.10 equiv) of HCTU, 1.86 g (11.0 mmol, 2.75 equiv) of Cl-HOBt, and 1.50 mL (13.2 mmol, 3.30 equiv) of 2,6-lutidine in a mixture of dry dichloromethane and dry DMF (3:1) according to general procedure A. The crude product was purified by column chromatography on silica gel using chloroform/methanol (25:1) to yield the compound as a yellow solid. Yield: 1.50 mg (2.41 mmol, 60%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.39 (s, 9H, CH_3-*t*Bu), 1.39−1.45 (m, 4H, CH_2-Lys), 1.67−1.71 (m, 1H, CH_2-Lys) 1.86−1.92 (m, 1H, CH_2-Lys), 3.05 (brs, 2H, CH_2-Lys), 3.96 (s, 3H, CH_3-PMB) 4.05 (s, 3H, $OOCH_3$), 4.35−4.39 (m, 1H, α-CH), 4.69 (brs, 1H, NH), 5.05 (d, ^2J = 20.8 Hz, 2H, CH_2-PMB), 5.13 (d, ^2J = 20.8 Hz, 2H, CH_2-PMB), 5.77 (s, 2H, CH_2-Z), 5.81 (brs, 1H, NH), 6.03 (d, ^3J = 8.0 Hz, 2H, CH-arom.), 7.15 (d, ^3J = 8.0 Hz, 2H, CH-arom.), 7.25−7.31 (m, 6H, CH-pyrazole, CH-arom.), 9.20 (brs, 1H, NH). ^{13}C NMR (500 MHz, $CDCl_3$): δ 22.3, 28.3, 29.4, 31.8, 31.9, 38.6, 39.6, 51.9, 53.8, 55.2, 67.2, 102.6, 109.5, 113.8, 120.1, 126.0, 128.0, 128.1, 128.4, 128.8, 128.9, 131.9, 136.0, 145.4, 156.4, 159.1, 159.9, 169.8. MS (ESI): *m*/*z* 646 $(M + Na, 70)^+$. HRMS: calcd for $C_{32}H_{41}N_5O_8Na$, 646.2853; found, 646.2862. R_f: 0.18 in chloroform/methanol (25:1). $[\alpha]_{Na}$: −6.2, *T* = 20 °C, *c* = 1.00 in chloroform. Mp: 115 °C.

***N*$^{\epsilon}$-*tert*-Butyloxycarbonyl-(*S*)-lysinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid Methyl Ester [H-Lys(Boc)-Pz(PMB)-OMe].** A 600 mg (963 μmol, 1.00 equiv) amount of *N*-benzyloxycarbonyl-*N*$^{\epsilon}$-*tert*-butyloxycarbonyl-(*S*)-lysinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic acid methyl ester [Z-Lys(Boc)-Pz(PMB)-OMe] was stirred together with 5 mol % Pd/C (Degussa Typ E101 NE/W) in methanol under a H_2 atmosphere until the starting material disappeared on TLC. The catalyst was removed by filtration over kieselguhr, and the solvent was evaporated. The residue was purified by column chromatography on silica gel using dichloromethane/methanol (10:1). Yield: 350 mg (714 μmol, 74%), colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H, CH_3-*t*Bu), 1.41−1.45 (m, 4H, CH_2-Lys), 1.62−1.70 (m, 1H, CH_2-Lys), 1.86−1.95 (m, 1H, CH_2-Lys), 3.08−3.11 (m, 3H, CH_2-Lys, NH), 3.56−3.63 (s, 2H, NH_2), 3.77 (s, 3H, CH_3-PMB), 3.84 (s, 3H, $OOCH_3$), 4.62 (brs, 1H, α-CH), 5.59 (s, 2H, CH_2-PMB), 6.82 (d, ^3J = 8.6 Hz, 2H, CH-arom.), 7.19 (d, ^3J = 8.6 Hz, 2H, CH-arom.), 7.26 (s, 1H, CH-pyrazole), 9.99 (brs, 1H, NH). MS (ESI): 490 $(M + K)^+$, 512 $(M + Na)^+$. HRMS: calcd for $C_{24}H_{35}N_5O_{56}Na$, 512.2485 found, 512.2507. R_f: 0.41 in dichloromethane/methanol (10:1). $[\alpha]_{Na}$: +8.3, *T* = 21 °C, *c* = 1.00 in methanol. Mp: 52 °C.

***N*-Benzyloxycarbonyl-*N*$^{\epsilon}$-*tert*-butyloxycarbonyl-(*S*)-lysinyl-*N*$^{\epsilon}$-*tert*-butyloxycarbonyl-(*S*)-lysinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid Methyl Ester [Z-Lys(Boc)-Lys(Boc)-Pz-(PMB)-OMe].** A 300 mg (675 μmol, 1.00 equiv) amount of *N*$^{\epsilon}$-*tert*-butyloxycarbonyl-(*S*)-lysinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazol-3-carboxylic acid methyl ester [H-Lys(Boc)-Pz(PMB)-OMe] and 308 mg (810 μmol, 1.20 equiv) of *N*-benzyloxycarbonyl-*N*$^{\epsilon}$*tert*-butyloxycarbonyl-(*S*)-lysine were reacted with 335 mg (810 μmol, 1.20 equiv) of HCTU, 344 mg (2.02 mmol, 3.00 equiv) of Cl-HOBT, and 284 μL (2.43 mmol, 3.60 equiv) of 2,6-lutidine according to general procedure A. Yield: 431 mg (506 μmol, 72%), pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33−1.99 (m, 12H, CH_2-Lys), 1.40, 1.41 (2s, 18H, CH_3-*t*Bu), 3.01−3.08 (m, 4H, CH_2-Lys), 3.73 (s, 3H, CH_3-PMB), 3.86 (s, 3H, $OOCH_3$), 4.14−4.12 (m, 1H, α-CH), 4.62−4.75 (m, 3H, α-CH, NH-Boc), 5.11 (s, 2H, CH_2-PMB), 5.49 (d, ^2J = 14.7 Hz, 1H, CH_2-Z), 5.61 (d, ^2J = 14.7 Hz, 1H, CH_2-Z), 6.07 (brs, 1H, NH), 6.70 (d, ^3J = 8.3 Hz, 1H, NH), 6.76 (d, ^3J = 8.6 Hz, 2H, CH-arom.), 7.14 (d, ^3J = 8.6 Hz, 2H, CH-arom.), 7.25 (s, 1H, CH-pyrazole), 7.31 (brs, 5H, CH-arom.), 9.18 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.7, 22.8, 28.5, 29.5. 29.7, 32.5, 39.9, 40.3, 52.1, 53.4, 53.9, 55.2, 55.3, 67.2, 79.2, 102.8, 114.1, 128.3, 128.6, 128.8, 129.1, 129.3, 132.1, 136.4, 145.6, 156.3, 156.4, 159.2, 160.0, 169.5, 172.5. MS (ESI): *m*/*z* 875 $(M + Na)^+$. HRMS (ESI): calcd for $C_{43}H_{61}N_7O_{11}Na$, 874.4327; found, 874.4322. Mp: 101 °C. R_f: 0.57 in dichloromethane/methanol (10:1). $[\alpha]_{Na}$: −10.1, *T* = 20 °C, *c* = 1.00 in chloroform.

***N*$^{\epsilon}$-*tert*-Butyloxycarbonyl-(*S*)-lysinyl-*N*$^{\epsilon}$-*tert*-butyloxycarbonyl-(*S*)-lysinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid Methyl Ester [H-Lys(Boc)-Lys(Boc)-Pz(PMB)-OMe], 8.** To a solution of 400 mg (469 μmol, 1.00 equiv) of *N*-benzyloxycarbonyl-*N*$^{\epsilon}$-*tert*-butyloxycarbonyl-(*S*)-lysinyl-*N*$^{\epsilon}$-*tert*-butyloxycarbonyl-(*S*)-lysinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic acid methyl ester [Z-Lys(Boc)-Lys(Boc)-Pz(PMB)-OMe] in methanol was added 5 mol % Pd/C (Degussa Typ E101 NE/W). The reaction mixture was stirred under a H_2 atmosphere until the starting material disappeared on TLC. Then the mixture was filtrated over kieselguhr, and the solvent was evaporated. The residue was purified by column chromatography on silica gel using dichloromethane/methanol (10:1) to yield compound **8** as a colorless solid. Yield: 305 mg (425 μmol, 91%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.34, 1.54 (2s, 18H, CH_3-*t*Bu), 1.15−1.87 (m, 14H, CH_2-Lys, NH_2), 2.84−2.88 (m, 4H, CH_2-Lys), 3.11−3.14 (m, 1H, α-CH), 3.71 (s, 3H, CH_3-PMB), 3.82 (s, 3H, $OOCH_3$), 4.39 (brs, 1H, α-CH), 5.51 (d, ^2J = 14.9 Hz, 1H, CH_2-PMB), 5.55 (d, ^2J = 14.9 Hz, 1H, CH_2-PMB), 6.71 (brs, 2H, 2NH), 6.87 (d, ^3J = 8.7 Hz, CH-arom.), 7.06 (s, 1H, CH-prazole), 7.15 (d, ^3J = 8.7 Hz, CH-arom.), 8.03, (brs, 1H, NH), 10.78 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.4, 22.5, 28.2, 28.5, 29.1, 29.4, 31.9, 34.6, 52.1, 52.3, 53.1, 54.5, 55.0, 77.2, 99.3, 101.9, 113.8, 128.7, 129.1, 131.1, 145.9, 155.5, 158.7, 159.3, 170.3, 175.1. MS (ESI): *m*/*z* 741 $(M + Na)^+$. HRMS: calcd for $C_{35}H_{55}N_7O_9Na$, 740.3959; found, 740.3941. Mp: 56 °C.

2-(2-{2-[2-(2-Methoxyethoxy)ethoxy]acetyl-*N*-2-(4-methoxybenzyl)-2*H*-pyrazolecarboxyl-N^{ϵ}-*tert*-butyloxycarbonyl-(*S*)-lysinyl-N^{ϵ}-*tert*-butyloxycarbonyl-(*S*)-lysinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid Methyl Ester [Glycol-Pz(PMB)-Lys(Boc)-Lys(Boc)-Pz(PMB)-OMe]. A 260 mg (362 μmol, 1.00 equiv) amount of N^{ϵ}-*tert*-butyloxycarbonyl-(*S*)-lysinyl-N^{ϵ}-*tert*-butyloxycarbonyl-(*S*)-lysinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazol-3-carboxylic acid methyl ester, **8**, 220 mg (267 μmol,1.00 equiv) of 2-(4-methoxybenzyl)-5-{2-[2-(2-methoxyethoxy)ethoxy]acetylamino}-2*H*-pyrazole-3-carboxylic acid, **10**,[52] 102 mg (401 μmol, 1.50 equiv) of 2-chloro-1-methylpyridinium iodide, and 142 μL (801 μmol, 3.00 equiv) of diisopropylethylamine were reacted overnight according to general procedure B. The residue was purified by column chromatography on silica gel using dichloromethane/methanol (50:1) to yield the compound as a colorless solid. Yield: 179 mg (162 μmol, 61%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 (s, 18 H, CH_3-*t*Bu), 1.40−2.16 (m, 12H, CH_2-Lys), 2.95−3.05 (m, 4H, CH_2-Lys), 3.32 (s, 1H, CH_3-glycole), 3.56−3.65 (m, 10H, CH_2-glycole, 2NH), 3.66, 3.67 (2s, 6H, CH_3-PMB), 3.87 (s, 3H, $OOCH_3$), 4.11 (d, ^2J = 15.9 Hz, 1H, CH_2-glycole), 4.15 (d, ^2J = 15.9 Hz, 1H, CH_2-Lys), 4.49−4.63 (2m, 2H, α-CH), 5.50−5.65 (m, 4H, CH_2-PMB), 6.78 (d, ^3J = 8.3 Hz, 4H, CH-arom.), 6.84 (d, ^3J = 7.8 Hz, 1H, NH), 7.12, 7.15 (2d, ^3J = 8.6 Hz, 4H, CH-arom.), 7.28 (brs, 2H, CH-pyrazole), 7.42 (brs, 1H, NH), 9.22, 9.33 (2s, 2H, NH). ^{13}C NMR (500 MHz, $CDCl_3$): δ 22.7, 23.0, 28.5, 29.6, 31.3, 31.8, 40.2, 52.1, 53.5, 53.7, 53.9, 55.3, 70.1, 70.4, 70,5, 70.6, 70.8, 99.1, 102.9, 114.0, 114.1, 129.0, 129.1, 129.3, 129.7, 130.1, 131.1, 145.5, 160.1. MS (ESI): 1130 $(M + Na)^+$, 1146 $(M + K)^+$. HRMS: calcd for $C_{54}H_{78}N_{16}O_{15}Na$, 1129.5546; found, 1129.5560. R_f: 0.28 in dichloromethane/methanol (50:1). Mp: 85 °C.

2-(2-{2-[2-(2-Methoxyethoxy)ethoxy]acetyl-aminopyrazole-carboxyl-(*S*)-lysinyl-(*S*)-lysinyl-aminopyrazole Carboxylic Acid Methyl Ester Trifluoroacetate [Glycol-Pz-Lys-Lys-Pz-OMe], 2. A solution of 170 mg (154 μmol, 1.00 equiv) of 2-(2-{2-[2-(2-methoxyethoxy)-ethoxy]-acetyl-*N*-2-(4-methoxybenzyl)-2*H*-pyrazolecarboxyl-(*S*)-N^{ϵ}-*tert*-butyloxycarbonyl-(*S*)-lysinyl-(*S*)-N^{ϵ}-*tert*-butyloxycarbonyl-lysinyl-2-(4-methoxybenzyl)-2*H*-pyrazole carboxylic acid methyl ester in trifluoroacetic acid [Glycol-Pz(PMB)-Lys(Boc)-Lys(Boc)-Pz(PMB)-OMe] was stirred for 3 h at 70 °C under an inert atmosphere. The solution was cooled to toom temperature, and an excess amount of ice-cold diethyl ether was added. The product precipitated as a colorless solid. This was centrifuged off, washed several times with ice-cold diethyl ether, and dried in vacuo to yield compound **2**. Yield: 100 mg (88 μg, 57%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.29−1.77 (m, 12H, CH_2-Lys), 2.73−2.79 (m, 4H, CH_2-Lys), 3.23 (s, 3H, CH_3-glycole), 3.44−3.66 (m, 8H, CH_2-glycole), 3.82 (s, 3H, $OOCH_3$), 4.10 (s, 2H, CH_2-Lys), 4.41−4.50 (m, 2H, α-CH), 6.92 (s, 1H, CH-pyrazole), 7.16 (brs, 1H, CH-pyrazole), 7.69 (brs, 6H, NH_2), 8.23 (d, ^3J = 8.0 Hz, 1H, NH), 8.45 (brs, 1H, NH), 10.13 (s, 1H, NH), 10.79 (s, 1H, NH). The pyrazole NHs are broadened. ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.3, 22.5, 26.5, 26.6, 31.3, 51.8, 52.2, 52.3, 58.0, 69.5, 69.6, 69.7, 70.4, 71.2, 100.0, 167.6, 170.1, 171.4. Only the most intense signals are given. MS (ESI): *m*/*z* 667 $(M + H)^+$. HRMS (ESI): calcd for $C_{28}H_{46}N_{10}O_9H$, 667.3527; found, 667.3545. Mp: 115 °C.

***N*-*tert*-Butyloxycarbonyl-(*S*)-valinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid Methyl Ester [Boc-VPz(PMB)-OMe].** A 820 mg (3.15 mmol, 1.00 equiv) amount of 5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic acid methyl ester **5**[52] and 685 mg (3.15 mmol, 1.00 equiv) of *N*-*tert*-butyloxycarbonyl-(*S*)-valine were reacted with 1.30 g (3.15 mmol, 1.00 equiv) of HCTU, 1.34 g (7.87 mmol, 2.50 equiv) of Cl-HOBt, and 1.10 mL (9.45 mmol, 3.00 equiv) of 2,6-lutidine in a mixture of dry dichloromethane/dry DMF (3:1) according to general procedure A. The residue was purified over silica gel using *n*-pentane/ethyl acetate (1:1) as eluent to yield the compound as a colorless solid. Yield: 750 mg (1.63 mmol, 52%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.92 (d, ^3J = 7.1 Hz, 3H, CH_3-Val), 0.99 (d, ^3J = 6.9 Hz, 3H, CH_3-Val), 1.34 (s, 9H, CH_3-*t*Bu), 2.24−2.31 (m, 1H, CH-*i*prop), 3.76 (s, 3H, CH_3-PMB), 3.85 (s, 3H, $OOCH_3$), 4.12 (brs, 1H, α-CH), 5.03 (brs, 1H, NH), 5.57 (s, 2H, CH_2-PMB), 6.82 (d, ^3J = 8.6 Hz, 2H, CH-arom.), 7.21 (d, ^3J = 8.6 Hz, 2H, CH-arom.), 7.27 (s, 1H, CH-pyrazole), 8.42 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.7, 19.6, 28.5, 30.8, 52.2, 54.1, 55.4, 60.5, 102.8, 114.2, 129.2, 129.3, 132.2, 145.4, 156.1, 159.5 160.2, 169.6. MS (ESI): *m*/*z* 483 $(M^+ + Na)$. MS (FD): *m*/*z* 460 $(M)^+$. HRMS (ESI): calcd for $C_{23}H_{32}N_4O_6Na$, 483.2220; found, 483.2232. Anal. Calcd for $C_{23}H_{32}N_4O_6$: C, 59.99; H, 7.00; N, 12.17. Found: C, 59.93; H, 7.03; N, 12.10. Mp: 130 °C. R_f: 0.28 in *n*-pentane/diethyl ether (1:1), 0.43 in *n*-pentane/ethyl acetate (1:1). $[\alpha]_{Na}$:−11.4, T = 20 °C, c = 0.50 in chloroform.

(*S*)-Valinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid Methyl Ester Trifluoracetate [H-VPz(PMB)-OMe]. A 700 mg (1.52 mmol, 1.00 equiv) amount of *N*-*tert*-butyloxycarbonyl-(*S*)-valinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic acid methyl ester [Boc-VPz(PMB)-OMe] was stirred in a mixture of dry dichloromethane and trifluoroacetic acid (3:1) until the starting material disappeared on TLC (2 h). The solvent was evaporated, the residue was three times dissolved in toluene, and the solvent was again evaporated. Finally, the residue was dissolved in acetone, the solvent evaporated, and the residue dried in vacuo to yield the compound as a solid. Yield: 720 mg (1.52 mmol, quant.). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82 (d, ^3J = 6.0 Hz, 6H, CH_3-Val), 1.95−2.07 (m, 1H, H-*i*prop), 3.61 (s, 3H, CH_3-PMB), 3.71 (s, 3H, $OOCH_3$), 3.96−3.98 (m, 1H, α-CH), 5.43 (d, ^2J = 14.9 Hz, 1H, CH_2-PMB), 5.54 (d, ^2J = 14.6 Hz, 1H, CH_2-PMB), 6.65 (d, ^3J = 8.6 Hz, 2H, CH-arom.), 7.00 (d, ^3J = 8.3 Hz, 2H, CH-arom.), 7.03 (s, 1H, CH-pyrazole), 8.06 (brs, 3H, NH_3^+), 10.17 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.3, 18.1, 30.4, 52.2, 54.0, 55.3, 59.2, 103.3, 114.1, 128.8, 129.0, 132.6, 144.7, 159.4, 159.7, 166.8. MS (ESI): *m*/*z* 383 $(M + Na)^+$, 45. HRMS (ESI): calcd for $C_{18}H_{24}N_4O_4Na$, 383.1695; found, 383.1711. Mp: 75 °C. $[\alpha]_{Na}$: +18.0, T = 20 °C, c = 1.00 in methanol.

***N*-Benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxycarbonyl-(*S*)-lysinyl-(*S*)-valinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid Methyl Ester [Z-K(Boc)VPz(PMB)-OMe].** A 720 mg (1.52 mmol, 1.00 equiv) amount of (*S*)-valinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic acid trifluoroacetate [H-VPz(PMB)-OMe], 645 mg (1.52 mmol, 1.00 equiv) of HCTU, 629 mg (3.80 mmol, 2.50 equiv) of Cl-HOBt, and 532 mL (4.56 mmol, 3.00 equiv) of 2,6-lutidine were reacted with 175 μL (1.52 mmol, 1.00 equiv) of 2,6-lutidine and 577 mg (1.52 mmol, 1.00 equiv) of *N*-benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxy-carbonyl-(*S*)-lysine in DMF/dichloromethane (1:3) according to general procedure A. The remaining brown solid was dissolved in a small amount of dichloromethane. To this solution was added an excess amount of *n*-pentane. The product precipitated, was filtered off and dried in vacuo. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (d, ^3J = 8.8 Hz, CH_3-Val), 0.95 (d, ^3J = 7.0 Hz, CH_3-Val), 1.41 (s, 9H, CH_3-*t*Bu), 1.19−1.70 (m, 6H, CH_2-Lys), 2.13−2.90 (m, 1H, CH-*i*prop), 2.92 (m, 2H, CH_2-Lys), 3.70 (s, 3H, CH_3-PMB), 3.80 (s, 3H, $OOCH_3$), 4.27 (brs, 1H, α-CH), 4.80 (dd, ^3J = 7.0 Hz, ^3J = 8.6 Hz, 1H, α-CH), 5.08 (s, 2H, CH_2-PMB), 5.49 (d, ^2J = 14.9 Hz, CH_2-Z), (d, ^2J = 14.9 Hz, CH_2-Z), 6.68−6.71, (m, 4H, CH-arom.), 6.73 (brs, 1H, NH), 7.01 (d, ^3J = 9.0 Hz, CH-arom.), 7.24−7.29 (m, 6H, CH-arom), 10.16 (brs, 1H, NH). ^{13}C NMR (MHz, $CDCl_3$): δ 18.2, 19.2, 22.6, 28.4, 29.4, 31.6, 32.3, 39.8, 51.9, 53.6, 55.2, 58.6, 67.2, 77.2, 79.1, 101.2, 113.8, 128.1, 128.4, 128.5, 129.1, 132.0, 136.1, 145.5, 156.2, 156.6, 159.0, 159.8, 169.2, 172.8. MS (ESI): 746 $(M + Na)^+$. HRMS: calcd for $C_{37}H_{50}N_6O_9Na$, 745.3537; found, 745.3539. Mp: 33 °C. $[\alpha]_{Na}$: −15.5, T = 20 °C, c = 0.75 in chloroform.

***N*$^{\epsilon}$-*tert*-Butyloxycarbonyl-(*S*)-lysinyl-(*S*)-valinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic Acid Methyl Ester [H-K(Boc)VPz(PMB)-OMe], 9.** A 5.50 g (7.11 mmol, 1.00 equiv) amount of *N*-benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxycarbonyl-(*S*)-lysinyl-(*S*)-valinyl-5-amino-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carboxylic acid methyl ester [Z-K(Boc)VPz(PMB)-OMe] was dissolved in a small amount of THF and diluted with methanol (THF:methanol, 1:20) To this

solution was added 5 mol % Pd/C (Degussa Typ E101 NE/W), and the reaction mixture was stirred overnight under a H_2 atmosphere. The catalyst was filtered off using kieselguhr, and the solvent was evaporated. The remaining residue was purified on a silica gel column using dichloromethane/methanol (10:1) as eluent to yield compound **9** as a colorless solid. Yield: 3.35 g (5.68 mmol, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (d, ^3J = 7.0 Hz, 3H, CH_3-Val), 0.98 (d, ^3J = 7.0 Hz, 3H, CH_3-Val), 1.52 (s, 9H, CH_3-*t*Bu), 1.53−1.86 (m, 8H, NH_2, CH_2-Lys), 2.22−2.29 (m, 1H, CH-*i*prop), 3.05−3.13 (m, 2H, CH_2-Lys), 3.44 (dd, ^3J = 4.3 Hz, ^3J = 3.6 Hz, 1H, α-CH), 3.75 (s, 3H, CH_3-PMB), 3.83 (s, 3H, $OOCH_3$), 4.43−4.48 (m, 1H, α-CH), 4.56−4.60 (m, 1H, α-CH), 5.56 (s, 2H, CH_2-PMB), 6.81 (d, ^3J = 8.6 Hz, 2H, CH-arom.), 7.17 (d, ^3J = 8.6 Hz, 2H, CH-arom.), 7.26 (s, 1H, CH-pyrazole), 7.97 (d, ^3J = 8.6 Hz, 1H, NH), 8.98 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.1, 19.6, 23.0, 28.5, 29.9, 30.7, 34.6, 34.7, 38.7, 40.3, 52.1, 54.0, 55.2, 55.4, 58.5, 102.9, 114.1, 129.1, 132.0, 145.5, 156.2, 159.3, 160.1, 169.3, 175.9. MS (ESI): 589 $(M)^+$, 611 $(M + Na)^+$. HRMS: calcd for $C_{29}H_{45}N_6O_7$, 589.3350; found, 589.3351. Mp: 47 °C. R_f: 0.11 in dichloromethane/methanol (10:1).

2-(2-{2-[2-(2-methoxyethoxy)ethoxy]acetyl-2-(4-methoxybenzyl)-2*H*-pyrazole-carboxyl-(*S*)-N^ϵ-*tert*-butyloxycarbonyl-lysinyl-(*S*)-valinyl-2-(4-methoxybenzyl)-2*H*-pyrazolecarboxylic Acid Methyl Ester [Glycol-Pz(PMB)K(Boc)VPz(PMB)-OMe]. A 100 mg (169 μmol, 1.00 equiv) amount of N^ϵ-*tert*-butyloxycarbonyl-(*S*)-lysinyl-(*S*)-valinyl-5-amino-2-(4-methoxybenzyl)-2 *H*-pyrazole-3-carboxylic acid methyl ester, **9**, and 104 mg (254 μmol, 1.50 equiv) of 2-(4-methoxybenzyl)-5-{2-[2-(2-methoxyethoxy)ethoxy]acetylamino}-2*H*-pyrazole-3-carboxylic acid, **10**,[52] 56 mg (204 μmol, 1.80 equiv) of 2-chloro-1-methylpyridinium iodide, and 133 μL (760 μmol, 4.50 equiv) of diisopropylethylamine were reacted according to general procedure B. The crude product was purified on a silica gel column using a gradient of *n*-pentane/ethyl acetate (5:1) to pure ethyl acetate as eluent. Yield: 110 mg (112 μmol, 66%), colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.72 (d, 3H, ^3J = 6.7 Hz, CH_3-Val), 0.74 (d, 3H, ^3J = 6.7 Hz, CH_3-Val), 1.22−1.35 (m, 3H, CH_2-Lys, CH-*i*prop), 1.34 (s, 9H, CH_3-*t*Bu), 1.73−1.97 (m, 4H, CH_2-Lys), 2.98 (brs, 2H, CH_2-Lys), 3.30 (s, 3H, CH_3-glycole), 3.54−3.72 (m, 8H, CH_2-glycole), 3.72 (s, 6H, CH_3-PMB), 3.82 (s, 3H, $OOCH_3$), 4.13 (d, ^2J = 16.1 Hz, 1H, CH_2-glycole), 4.19 (d, ^2J = 16.1 Hz, 1H, CH_2-glycole), 4.50−4.55 (m, 1H, α-CH), 4.61−4.70 (m, 1H, α-CH), 5.48−5.66 (m, 4H, CH_2-PMB), 6.75−6.77 (m, 4H, CH-arom.), 6.94 (d, ^3J = 9.1 Hz, 1H, NH), 7.07 (d, ^3J = 8.4 Hz, 2H, CH-arom.), 7.18 (d, ^3J = 8.7 Hz, 2H, CH-arom.), 7.28 (s, 1H, CH-pyrazole), 7.33 (s, 1H, CH-pyrazole), 7.90 (d, ^3J = 8.1 Hz, 1H, NH), 9.22, 9.98 (2s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.0, 19.1, 20.7, 23.2, 28.5, 19.7, 30.7, 31.7, 40.4, 52.1, 53.6, 53.8, 53.9, 55.3, 58.3, 59.1, 70.3, 70.5, 70.8, 71.5, 72, 99.2, 102.9, 113.7, 113.9, 128.8, 129.2, 129.6, 132.1, 134.7, 144.8, 145.7, 156.2, 159.2, 159.3, 160.1, 168.6, 169.5, 171.7. MS (ESI): *m*/*z* 1001 $(M + Na)^+$. HRMS: calcd for $C_{48}H_{67}N_9O_{13}Na$, 1000.4756; found, 1000.4768. Mp: 52 °C. R_f: 0.42 in ethyl acetate.

2-(2-{2-[2-(2-Methoxyethoxy)ethoxy]acetyl-aminopyrazolecarboxyl-(*S*)-lysinyl-(*S*)-valinyl-aminopyrazole Carboxylic Acid Methyl Ester Trifluoroacetate [Glycol-PzKVPz-OMe], 3. A 100 mg (112 μmol, 1.00 equiv) amount of 2-(2-{2-[2-(2-methoxyethoxy)ethoxy]acetyl-2-(4-methoxybenzyl)-2*H*-pyrazole carboxyl-N^ϵ-*tert*-butyloxycarbonyl-(*S*)-lysinyl-(*S*)-valinyl-2-(4-methoxybenzyl)-2*H*-pyrazole carboxylic acid methyl ester [Gly-Pz(PMB)K(Boc)VPz(PMB)-OMe] was stirred under an inert atmosphere in dry trifluoroacetic acid for 3 h at 70 °C. The solution was cooled to room temperature, and an excess amount of ice-cold diethyl ether was added. The mixture was stored overnight at 0 °C. A small amount of the product precipitated as colorless crystals. These were filtered off, and the remaining solution was evaporated. The residue was several times dissolved in toluene, and the solvent was again evaporated. Finally, the residue was dissolved in dichloromethane and an excess amount of *n*-pentane was added. The product precipitated as a colorless solid, which was filtered off, washed with *n*-pentane, and dried in vacuo to yield compound **3**. Yield: 15 mg (17 μmol, 15%).[53] ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.87 (d, ^3J = 6.6 Hz, 3H, CH_3-Val), 0.88 (d, ^3J = 6.6 Hz, 3H, CH_3-Val), 1.30−1.39 (m, 2H, CH_2-Lys), 1.50−1.59 (m, 2H, CH_2-Lys), 1.67−1.76 (m, 2H, CH_2-Lys), 2.01 (qd, ^3J = 6.9 Hz, ^3J = 13.6 Hz, 1H, CH-*i*prop), 2.72−2.81 (m, 2H, CH_2-Lys), 3.23 (s, 3H, CH_3-glycole), 3.45−3.47, 3.55−3.59, 3.64−3.66 (3m, 8H, CH_2-glycole), 3.82 (s, 3H, $OOCH_3$), 4.10 (s, 2H, CH_2-glycole), 4.36 (dd, ^3J = 7.2 Hz, ^3J = 8.4 Hz, 1H, α-CH), 4.46−4.54 (m, 1H, α-CH), 6.91 (s, 1H, CH-pyrazole), 7.16 (s, 1H, CH-pyrazole), 7.66 (brs, 3H, NH_3^+), 7.99 (d, ^3J = 8.7 Hz, 1H, NH), 8.49 (d, 1H, ^3J = 6.9 Hz, 1H, NH), 10.10 (s, 1H, NH), 10.78 (s, 1H, NH). The pyrazole NHs are broadened. ^{13}C NMR (125 MHz, DMSO-d_6): δ 18.1, 19.1, 22.5, 26.6, 30.7, 30.9, 38.7, 51.8, 52.5, 57.8, 57.9, 69.5, 69.6, 69.7, 70.4,71.2, 96.3, 99.0, 114.4, 116.7, 158.1, 158.4, 159.1, 159.6, 167.6, 169.5, 171.6. Mp: 134 °C. MS (ESI): 683 $(M + H)^+$. MS (ESI): *m*/*z* 638 $(M + H)^+$. HRMS: calcd for $C_{27}H_{44}N_9O_9$, 638.3262; found, 638.3264.

(*S*)-Lysinyl-(*S*)-lysinyl-(*S*)-leucinyl-(*S*)-valinyl-(*S*)-phenylalaninyl-(*S*)-phenylalanine Trifluoroacetate [KKLVFF]. The peptide was synthesized using solid-phase techniques on an Applied Biosystems 433A peptide synthesizer. All chemicals were supplied for a test period by Applied Biosystems. Fmoc-Phe preloaded Wang resin was used. The coupling cycles were carried out using 5 equiv of Fmoc-protected amino acid, 4.95 equiv of HBTU, 15.0 equiv of HOBt, and 15.0 equiv of DIEA. The deprotection was performed using 20% piperidine in DMF (2 × 10 min). Cleavage from the resin was carried out with 95% trifluoroacetic acid, 2.5% water, and 2.5% triisopropylsilane. The peptides were subsequently filtered from the resin and precipitated using ice-cold diethyl ether. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.70 (d, ^3J = 5.8 Hz, 6H, CH_3-Val), 0.79 (d, ^3J = 6.6 Hz, 3H, CH_3-Lys), 0.85 (d, ^3J = 6.8 Hz, 3H, CH_3-Lys), 1.30−1.68 (m, 14H, CH_2-Lys, CH2-Leu, i-Prop-H), 1.81−1.90 (m, 2H, CH_2-Lys), 2.71−2.76 (m, 5H, CH_2-Lys, Benzyl-CH), 2.91 (dd, ^3J = 8.6 Hz, ^2J = 14.0 Hz, 1H, Benzyl-CH), 2.97 (dd, ^3J = 4.2 Hz, ^2J = 14.3 Hz, 1H, Benzyl-CH), 3.05 (dd, ^3J = 5.4 Hz, ^2J = 14.0 Hz, 1H, Benzyl-CH), 3.78 (brs, 1H, α-CH), 4.10 (dd, ^3J = 6.8 Hz, ^3J = 8.6 Hz, 1H, α-CH), 4.28−4.34 (m, 2H, α-CH), 4.27−4.48 (m, 1H, α-CH), 4.52−4.58 (m, 1H, α-CH), 7.13−7.28 (m, 10H, H-arom.), 7.61 (d, ^3J = 8.9 Hz, 1H, NH), 7.81 (brs, 6H, NH_3-Lys), 7.98 (d, ^3J = 8.6 Hz, 1H, NH), 8.17−8.19 (m, 4H, NH_3-Lys, NH), 8.22 (d, ^3J = 7.5 Hz, 1H, NH), 8.56 (d, ^3J = 7.9 Hz, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.8, 19.1, 20.9, 21.4, 22.1, 23.1, 24.1, 26.4, 26.6, 30.5, 30.8, 31.4, 38.4, 38.6, 50.9, 51.8, 52.3, 53.4, 57.3, 115.6, 118.6, 126.2, 126.4, 127.9, 128.2, 129.1, 137.3, 137.5, 168.2, 170.4, 170.8, 170.9, 171.5, 172.6. MS (ESI): *m*/*z* 782 $(M + H)^+$. HRMS: calcd for $C_{41}H_{64}N_8O_7H$, 781.4976; found, 781.5001. Mp: 165−170 °C. $[\alpha]_{Na}$: −23.3, T = 20 °C, c = 1.00 in methanol.

Acknowledgment. This work was supported by the Volkswagen foundation, priority area: conformational control of biomolecular function. We express our thanks to Gerhard Schäfer and Kamila Szczepanska for their valuable preparative work. We are also indebted to Uwe Linne for his ESI-MS measurements of Aβ peptide/ligand complexes.

Supporting Information Available: NMR titration curves, maximum CIS and derived binding constants, Job plots, NOESY, DQF−COSY and TOCSY of the KKLVFF peptide, Karplus analysis of the $^3J_{H,H}$-coupling constants of the KLVFF peptide, VT experiments and calculated ppb/K values, CD spectra of hosts, guest, and their complexes, ESI-MS of ligand **2** with KKLVFF and Aβ(1−40). This material is available free of charge via the Internet at http://pubs.acs.org.

JA045558B

(53) The yield for the crystals was not determined.