JOC Articles

New Heterocyclic β-Sheet Ligands with Peptidic Recognition Elements

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A detailed and comprehensive overview is presented about the design, modeling, and synthesis, as well as spectroscopic characterization, of a new class of β-sheet ligands. The characteristic feature of these compounds is a peptidic chimeric structure formed from a specific combination of aminopyrazolecarboxylic acids with naturally occurring α-amino acids. These hybrid peptides are designed with the aid of molecular modeling to exist mainly in an extended conformation. All their hydrogen bond donors and acceptors can be aligned at the bottom face in such a way that a perfect complementarity toward β-sheets is obtained. Thus the aminopyrazoles impart rigidity and a highly efficient DAD sequence for the recognition of whole dipeptide fragments, whereas the natural α-amino acids are designed to mimick recognition sites in proteins, ultimately leading to sequence-selective protein recognition. The synthetic protocols either rely upon solution phase peptide coupling with a PMB protecting group strategy or solid-phase peptide coupling based on the Fmoc strategy, using the same protecting group. In solution, a key building block was prepared by catalytic reduction of a nitropyrazolecarboxylic acid precursor. Subsequently, it was (*N*-1)-protected with a PMB group, and elongated by HCTU- or T3P-assisted peptide coupling with dipeptide fragments, followed by PyClop-assisted coupling with another nitropyrazolecarboxylic acid building block. Final simultaneous deprotection of all PMB groups with hot TFA completed the high-yield protocol, which works racemization-free. After preparing a similar key building block with an Fmoc protection at *N*-3, we developed a strategy suitable for automated synthesis of larger hybrid ligands on a peptide synthesizer. Attachment of the first amino acid to a polystyrene resin over the Sieber amide linker is followed by an iterative sequence consisting of Fmoc deprotection with piperidine and subsequent coupling with natural α-amino acid via HATU/HOAt. High yields of free hybrid peptides are obtained after mild acidic cleavage from the resin, followed by deprotection of the PMB groups with hot TFA. The new aminopyrazole peptide hybrid compounds were characterized by various spectroscopic measurements including CD spectra, VT, and ROESY NMR experiments. All these accumulated data indicate the absence of any intramolecular hydrogen bonds and strongly support an extended conformation in solution, ideal for docking on to solvent-exposed β-sheets in proteins. Initial results from aggregation tests of pathological proteins with these and related ligands look extremely promising.

Introduction

A series of pathological processes is connected 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] Despite their abundance and dramatic progression there is virtually no therapy for protein misfolding diseases such as BSE/Creutzfeldt−Jakob or Alzheimer's. One of the most promising approaches aims at the prevention of amyloid aggregation by designed β-sheet ligands;[3] this process is today considered the basic

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(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, M.; Baldwin, D.; Groth, D. *J. Virol.* **1996**, *70*, 1714−1722. (c) Pitschke, M.; Prior, R.; Haupt, M.; Riesner, D. *Nat. Med.* **1998**, *4*, 832−834.

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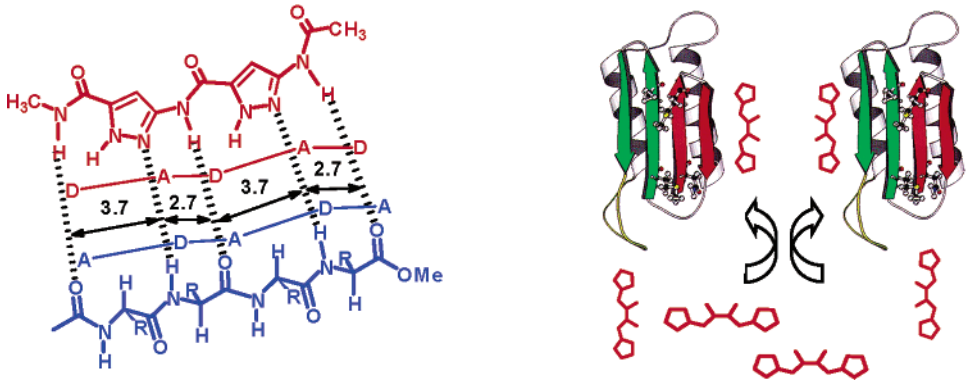


FIGURE 1. Capping the β-sheets of critical proteins with dimeric aminopyrazoles. Left: Geometrical constraints for β-sheet ligands with a DAD pattern. Right: Schematic illustrating the interaction of the new ligands with solvent-exposed β-sheets on the prion protein.[8]

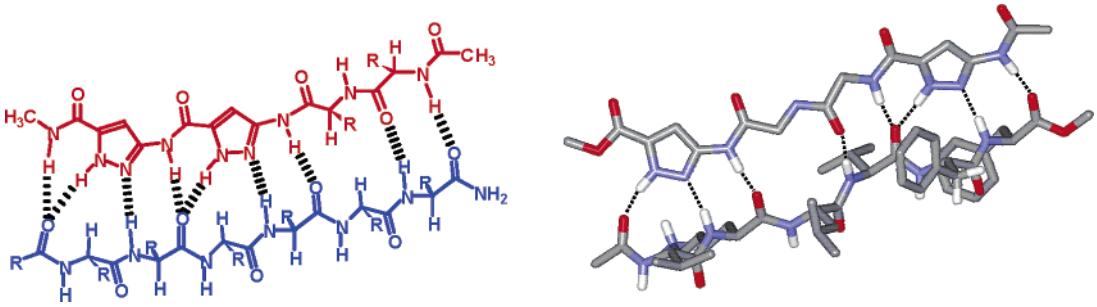


FIGURE 2. Left: Attachment of a dipeptide at the *C*-terminus of a dimeric aminopyrazole leads to an enhanced hydrogen bond network with a hexapeptide. Right: Monte Carlo simulation of a complex of a tetrameric ligand carrying two amino acids between *N*- and *C*-terminal aminopyrazoles with a hexapeptide (VKLVFF) taken from the putative nucleation site of Aβ (MacroModel 7.2, Schrödinger 2002, Amber*, water, 3000 steps).

pathological event in Alzheimer's disease and is also discussed as the major cause for related diseases.[4]

Several years ago, we introduced acylated 3-aminopyrazoles as effective β-sheet ligands, which are able to stabilize an extended conformation in small peptides.[5] However, the monomeric ligands turned out to be biologically inactive toward recombinant prion protein in simple aggregation assays. By a very straightforward synthetic protocol, we obtained dimeric and oligomeric covalently fused aminopyrazole ligands, which were fixed in a planar arrangement and retained the essential DAD hydrogen bond Donor and Acceptor pattern necessary for complex formation with peptides in their β-sheet conformation. These dimeric ligands gave very promising preliminary results in biophysical experiments with the prion protein and Aβ, where the aggregation kinetics were monitored by Fluorescence Correlation Spectroscopy (FCS) and density gradient centrifugation (Figure 1).[6] A very urgent question, however, remained unanswered, as to how the pathologic proteins could be selectively addressed, without harming other well-behaved native proteins. This left us with the task of building into our ligand structure a recognition element for a certain protein. With Alzheimer's disease, the general strategy seemed obvious: in the future, we have to incorporate in our ligands at least a truncated version of one of the self-complementary pentapeptide sequences thought to be the nucleation sites in Alzheimer's aggregation: KLVFF in the middle or VGGVV at the *C*-terminus.[7] As an additional advantage, the notorious solubility problem, connected with the high tendency of aminopyrazoles to undergo self-association, might be circumvented by introducing amino acids into the ligand structure, which cannot form π-stacks. To this end, we developed new synthetic protocols for the regioselective incorporation of proteinogenic (preferentially hydrophobic) amino acids into the sequence of oligomeric aminopyrazoles. These protocols rely either upon solution-phase peptide coupling with a special protecting group strategy or solid-phase peptide coupling based on the Fmoc strategy, using the same protecting group. Herein we disclose the synthetic strategies employed in a flexible general access to the new class of peptide aminopyrazole hybrid compounds. Results from biophysical and cell culture experiments will be reported elsewhere.

Modeling. Extensive modeling experiments including Monte Carlo simulations in water[9] suggest that a perfect

(3) Another important approach is the immune-mediated Aβ-clearance by active or passive immunization: Solomon, B. *Curr. Med. Chem.* **2002**, *9*, 1737−1750.

(4) Selkoe, D. J. *Science* **1997**, *275*, 630−631.

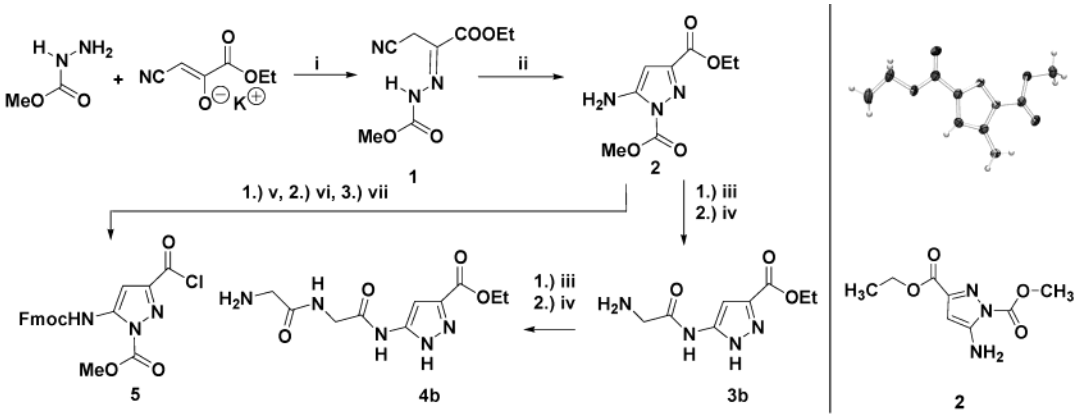
(5) (a) Schrader, T.; Kirsten, C. *J. Chem. Soc., Chem. Commun.* **1996**, 2089. (b) Schrader, T.; Kirsten, C. N. *J. Am. Chem. Soc.* **1997**, *119*, 12061−12068.

(6) (a) 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, 5/10/2002. (b) Rzepecki, P.; Wehner, M.; Molt, O.; Zadmard, R.; Schrader, T. *Synthesis* **2003**, 1815−1826.

(7) (a) Tjernberg, L. O.; Näslund, J.; Lindqvist, F.; Johansson, J.; Karlström, A. R.; Thyberg, J.; Terenius, L.; Nordstedt, C. *J. Biol. Chem.* **1996**, *271*, 8545. (b) Soto, C.; Sigurdsson, E. M.; Morelli, L.; Kumar, R. A.; Castano, E. M.; Frangione, B. *Nat. Med.* **1998**, *4*, 822−826.

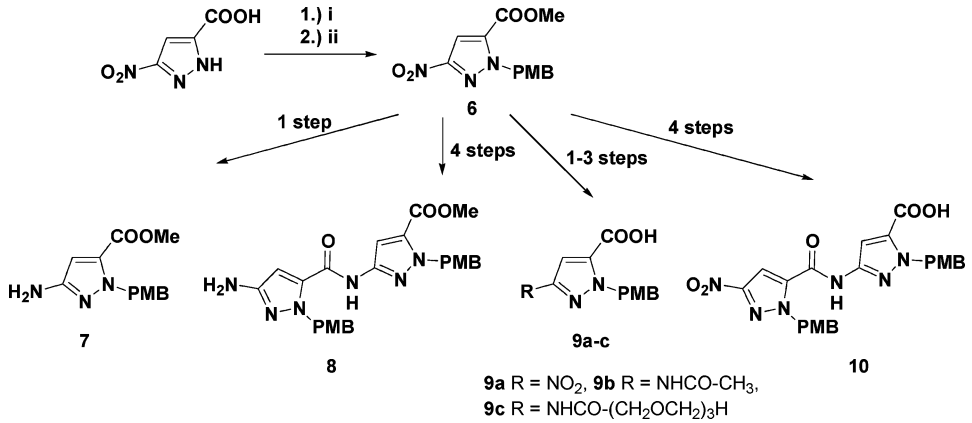
(8) Scrapie protein model: Mestel, R. *Science* **1996**, *273*, 184−189.

SCHEME 1. Preparation of the New Pyrazole 3-Amino Acid by a Cycloaddition Route (left) and Crystal Structure of 2 (right)[a]



[a] Reagents and conditions: (i) $CHCl_3/EA_{HCLsatd}$, rt, 39%; (ii) CH_3CN, NEt_3, rt, 98%; (iii) Fmoc-Gly-Cl, THF, 70 °C; (iv) 20% piperidine, DMF; (v) Fmoc-Cl, THF, 70 °C, 96%; (vi) HCl, HOAc, EtOH; (vii) $SOCl_2$, DMF.

SCHEME 2. Examples for Synthesized Pyrazole Building Blocks[a]



[a] Reagents and conditions: (i) HCl, MeOH, Δ, quant.; (ii) PMB-Br, K_2CO_3, DMF, Δ.

β-sheet complementarity can be retained not only in hybrid compounds with successive aminopyrazole and peptide blocks but also in those with an even number of amino acids between two aminopyrazole moieties (Figure 2).

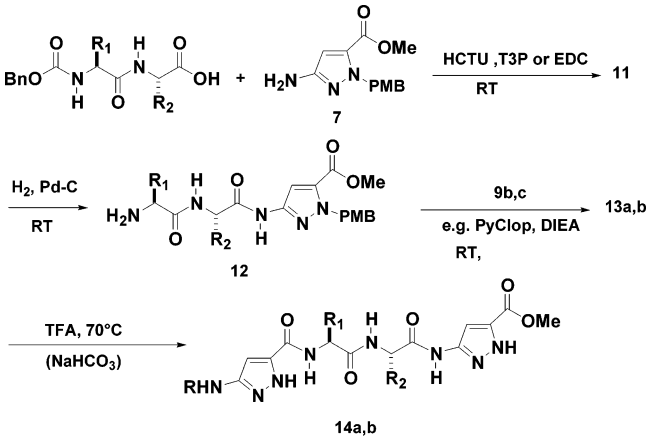
Synthesis: (a) Synthesis of the New Amino Acid by Cycloaddition of a Hydrazide with a β-Cyanopyruvate. Condensation of acetonitrile's potassium salt with diethyl oxalate in ethanol/diethyl ether leads in quantitative yield to the potassium salt of ethyl cyanopyruvate (Scheme 1).[10] This is reacted with hydrazinomethylformate in a mixture of chloroform and ethyl acetate, saturated with hydrogen chloride. In situ protonation of the potassium salt is followed by hydrazone formation of the liberated α-keto group (**1**, 39%). Subsequent treatment with triethylamine in acetonitrile triggers base-catalyzed cyclization of the intermediate and furnishes the new pyrazole amino acid ester with a carbamate protecting group on pyrazole's $N-2$ (**2**, 98%). This key compound could be characterized by an X-ray crystal structure confirming its 3,5-substitution pattern as well as the location of the urethane at the ring nitrogen.[11] Reaction of this building block with the acid chloride of Fmoc-protected glycine led to peptide coupling with concomitant urethane cleavage at the pyrazole nucleus (**3a**). Fmoc deprotection with piperidine yielded **3b**; a second coupling step can subsequently be performed with Fmoc-protected glycine acid chloride to give **4a**, which affords, after final Fmoc removal, the diglycylpyrazole trimer **4b** (Scheme 4). Unfortunately, its reaction with the acid chloride of Fmoc-protected aminopyrazole carboxylic acid **5** did not give the desired tetramer, but rather transferred the Fmoc group on to the *N*-terminal glycine of the trimer. Further attempts to effect *C*-terminal peptide coupling of the new pyrazole-based amino acid were hampered by competing acylation of the softer ring nitrogen, so that a special protecting group strategy became inevitable.

(b) Solution-Phase Elongation with PMB Protection. In an alternative strategy, the new heterocyclic amino acid was made accessible by way of catalytic reduction of its nitro predecessor **6**. The introduction of the *p*-methoxybenzyl (PMB) protecting group at *N*-2

(9) MacroModel 7.2, Schrödinger 2002, Amber*, water, 3000 steps.
(10) Borsche, W.; Manteuffel, R. *Liebigs Ann. Chem.* **1934**, *512*, 102−106.

(11) For detailed crystallographic information, refer to the Cambridge Crystallographic Database, where structure **2** has been deposited with full data. Crystal structures of related aminopyrazole derivatives: Saweczko, P.; Enright, G. D.; Kraatz, H. B. *Inorg. Chem.* **2001**, *40*, 4409−4419.

SCHEME 3. Stepwise Coupling Protocol for the Combination of Pyrazole-Based and Proteinogenic Amino Acids in Hybrid Ligands



allowed subsequent peptide coupling. Starting with compound **6** several pyrazol intermediates could be synthesized (**7**−**10**, for synthetic details see ref 6b).

With these building blocks (Scheme 2) subsequent coupling with various amino acids and peptide esters (Scheme 4) could be carried out. However, to avoid racemization, higher oligomers were always prepared from *C*- to *N*-terminus (Scheme 3). To this end, PMB-protected ester **7** was coupled with *N*-Z-protected amino acids or peptides with benztriazoles (HCTU or HATU), phosphonate anhydrides (T3P), or EDC/HOBt.[12] After hydrogenolytic Z-removal, the PMB-protected nitropyrazol carboxylic acid (**9a)** or an *N*-acylated aminopyrazole carboxylic acid (**9b**,**c)** could be attached either with PyClop or Mukaiyama's reagent, since no racemization can occur at the heterocylic amino acid.[13] In principle coupling with T3P is also possible; however, the received yields were much lower. The final coupling step was followed by complete removal of all PMB protecting groups with hot trifluoroacetic acid, which leads to the desired ligands (**14a**,**b**). This procedure is racemization-free despite its rather harsh acidic conditions.

The synthetic route outlined above proved to be extremely flexible with respect to the number and order of all coupling partners. In conclusion dimeric aminopyrazoles could be coupled at their free *N*-terminus with *N*-acylated dipeptides by HCTU or T3P (**17**). If two amino acids are to be inserted between flanking aminopyrazoles, Z-protected dipeptides are the building blocks of choice: The series of model compounds includes tetramers with diglycyl bridges, either carrying an *N*-terminal acetylamino (**14a**) or an ethyleneglycol-elongated acetylamino functionality (**14b**). In addition the trimeric ligand **16** was assembled by using the same strategy as described for **14a**,**b**. Finally, the aminopyrazole monomers or dimers can also be placed at the *N*-terminus: coupling is most conveniently accomplished by way of their free carboxylic acid with peptide esters, using PyClop or Mukaiyama's reagent (**15**, **18**). All the tetramers carry eight hydrogen bond donors and acceptors capable in principle of stabilizing a hexapeptide strand in its β-sheet conformation by a double attack from the top and bottom face. Although the five-membered pyrazole ring confers to the ligand an overall shape similar to a halfmoon, self-association seems to increase proportional to the number of incorporated aminopyrazoles. Its solubility is, however, markedly enhanced by the presence of two or more amino acids, and especially with an oligomeric ethylene glycole elongation.

(c) Preparation of the Fmoc Building Block. The facile and highly variable access to β-sheet hybrid ligands described above prompted us to develop a strategy suitable for automated synthesis of larger hybrid ligands on a peptide synthesizer. Starting from the PMB-protected 3-nitropyrazole-5-carboxylic acid **9a**, key compound **19** was assembled through a two-step route: reduction of the nitro group with H_2/Pd-C and following treatment with Fmoc-Cl yields in the corresponding Fmoc protected amino acid.

(d) Automated Iterative Solid-Phase Peptide Synthesis with the New Building Block. Two representative tetramers (**20**, **21**) were prepared on an ACT Model 90 automated peptide synthesizer.[14]

To introduce an additional *C*-terminal amide group as hydrogen bond donor, the solid-phase protocol starts from a polystyrene resin, carrying the Sieber amide linker.[15] After Fmoc deprotection, the first pyrazole amino acid is attached by HOBt and diisopropylcarbodiimide (DIPCDI) in *N*-methylpyrrolidone (NMP).[16] All coupling steps were carried out twice, to ensure complete conversion. Each step was monitored by analytic HPLC or HPLC-MS, since the low nucleophilicity of the pyrazole free amine group does not guarantee positive color tests with NF-31.[17] A loading of ∼0.2 mmol/g of resin is achieved (gravimetric determination; due to the low amount of free amino groups a quantitative Kaiser test was not sufficiently sensitive). An iterative sequence consisting of Fmoc deprotection (piperidine) and coupling (HATU/HOAt, collidine) follows.[18] Aliphatic amino acids can also be coupled by TBTU, HOBt, and diisopropylethylamine (DIEA). Final Fmoc deprotection and acetylation of the free *N*-terminus with acetic acid anhydride completes the solid-phase synthesis. The PMB-protected tetramer is cleaved off the Sieber amide resin by 2% TFA in dichloromethane, whereas the following PMB deprotection requires short heating to 70 °C. Semipreparative purification affords the analytically pure ligands as triflate salts, which can eventually be converted to the free

(12) (a) HCTU is the "cheap" modification of HATU or HBTU; recent findings distinguish between the guanidinium and the much more potent uronium form: Carpino, L.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. *Angew. Chem.* **2002**, *114*, 457−461. (b) T3P: Wissmann, H.; Kleiner, H.-J. *Angew. Chem.* **1980**, *92*, 129−130. Klose, J.; Bienert, M.; Mollenkopf, C.; Wehle, D.; Zhang, C.; Carpino, L.; Henklein, P. *Chem. Commun.* **1999**, 1847−1848. EDC: Sheehan, J. C.; Cruickshank, P. A.; Boshart, G. L. *J. Org. Chem.* **1961**, *26*, 2525.

(13) PyClop: Coste, J.; Frerot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437−2446. Mukaiyama's reagent: Mukaiyama, T. *Angew. Chem.* **1979**, *91*, 798−812.

(14) Automated Synthesizer, Model 90, Advanced ChemTech.

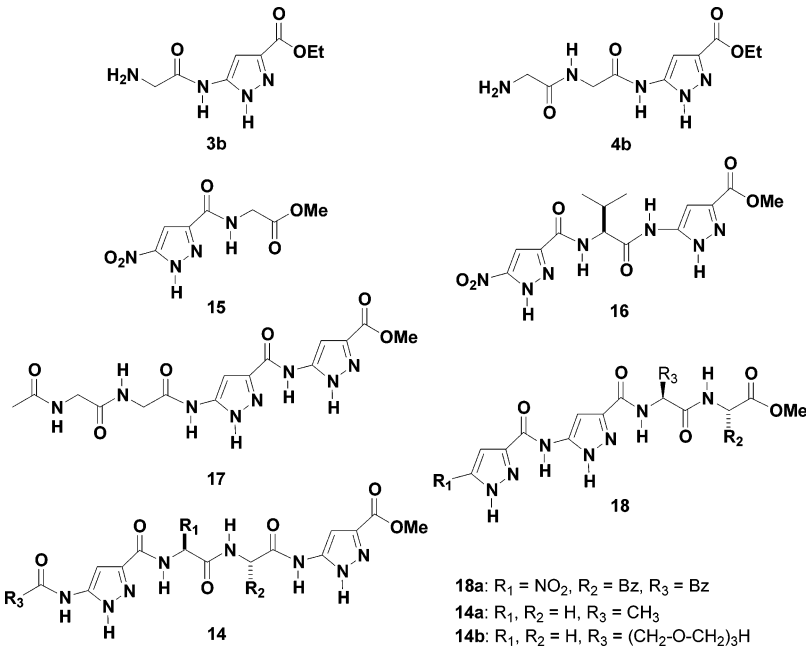
(15) Sieber, P. *Tetrahedron Lett.* **1987**, *28*, 2107−2110.

(16) HOBt, DIPCDI: Tartar, A.; Gesquiere, J. C. *J. Org. Chem.* **1979**, *44*, 5000.

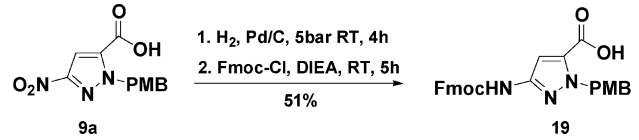
(17) Madder, A.; Farcy, N.; Hosten, N. G. C.; DeMuynck, H.; DeClercq, P. J.; Barry, J.; Davis, A. P. *Eur. J. Org. Chem.* **1946**, *11*, 2787−2791.

(18) HATU, HOAt: Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1995**, *60*, 3561−3564.

SCHEME 4. Overview of All New Aminopyrazole Peptide Hybrid Ligands Synthesized in Solution



SCHEME 5. Synthesis of the Fmoc Building Block



pyrazoles by neutralization with saturated $NaHCO_3$ solution. No fluorine signals are found in the ^{19}F NMR spectrum.

Conformation. The new aminopyrazole peptide hybrid compounds were characterized by various spectroscopic measurements including CD spectra, VT, and ROESY NMR experiments. Contrary to oligopeptides from proteinogenic amino acids, the hybrid compounds displayed pronounced CD couplets in their CD spectra (e.g., for **21**: Cotton effect at 242 nm, $\Delta\epsilon = +17.1$ mol^{-1} cm^{-1} and at 209 nm, $\Delta\epsilon = -16.9$ mol^{-1} cm^{-1}).[19] Obviously, the flanking pyrazole chromophores interact with the stereogenic environment of the dipeptide core unit.

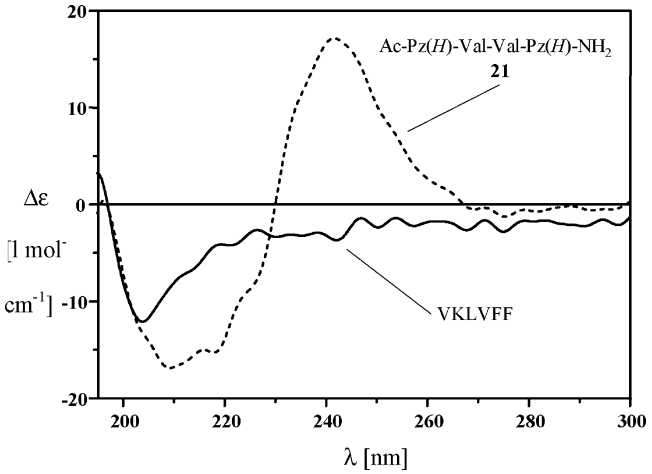


FIGURE 3. CD spectrum of tetramer **21** in acetonitrile (dotted lines) and the CD curve (solid line) for a hexapeptide derived from the putative nucleation site of Aβ (VKLVFF).

This might be due to a folded conformation in the flexible peptide unit, acting as a β-turn. A ROESY spectrum in connection with COSY and HMQC spectra revealed the true conformation along the central peptide chain: numerous cross-peaks were observed, which are all consistent with an extended conformation of the peptide hybrid molecule. Even at highest resolution no cross-peak appeared, which would indicate a folded conformation. Considering valine's high propensity to induce a β-sheet conformation in peptide strands, this result is not surprising.[20]

Final proof came from variable temperature (VT) experiments. If one conformation dominates in solution, intramolecular hydrogen bonds only produce a small temperature gradient for the corresponding NH signals of $\Delta\delta/\Delta T < 2-3$ ppb/K in DMSO. Temperature gradients of > 4 ppb/K, on the other hand, indicate intermolecular strong hydrogen bonds with other peptide molecules.[21] In the temperature range between 302 and 343 K the NH protons 2 and 16 correspond to a gradient of 5.6 ppb/K, those of NH-4 to 6.7 ppb/K, those of NH-18 to 6.1 ppb/K, and those of NH-11 to 7.8 ppb/K. Thus, no intramolecular hydrogen bonds are found in this ligand, which must adopt an extended conformation in solution, ideal for docking on to solvent-exposed β-sheets in proteins.

Outlook

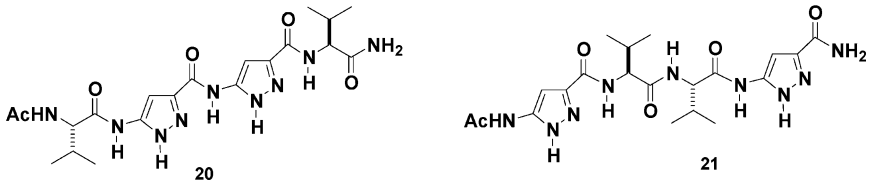
Although the new generation of β-sheet ligands displays a pronounced increase in solubility in organic solvents, it is still only sparingly soluble in water. Further optimization may be effected by releasing ionic groups at the carboxy or amino terminus of the peptide

(19) Note that none of the molar ellipticity maximums corresponds to formation of a β-sheet-like structure by self-association (218 nm).

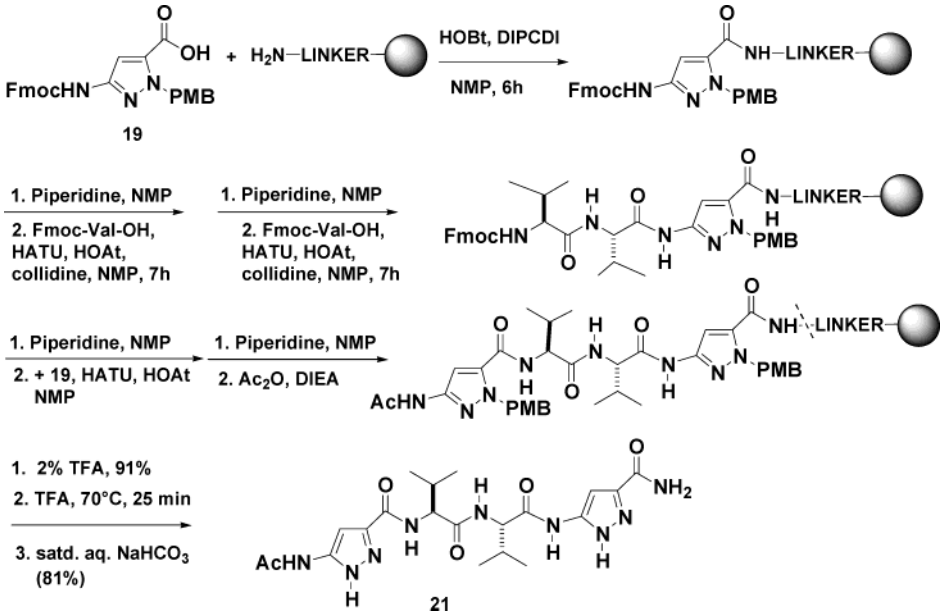
(20) Kim, C. A.; Berg, J. M. *Nature* **1993**, *362*, 267. Nowick, J. S.; Insaf, S. *J. Am. Chem. Soc.* **1997**, *119*, 10903.

(21) (a) Ribeiro, A. A.; Goodman, M.; Naider, F. *Int. J. Peptide Protein Res.* **1979**, *14*, 414. (b) Kessler, H. *Angew. Chem.* **1982**, *94*. 509−520.

SCHEME 6. The New Aminopyrazole Peptide Hybrid Ligands Synthesized with Fmoc-SPPS



SCHEME 7. Solid-Phase Protocol for the Automated Synthesis of a Tetrameric Aminopyrazole Peptide Hybrid Based on the Fmoc Strategy



hybrids, or by introducing ionic amino acid residues. Alternatively, solubility-conferring/enhancing protecting groups may be attached at either terminus. Aggregation tests of pathological proteins with these and related ligands are underway *in vitro* in biophysical aggregation experiments as well as in cell culture assays.[22] Screening is carried out for efficiency and selectivity toward Aβ aggregation as well as toxicity for the cells. The preliminary results look extremely promising and will be published in due course.

Experimental Section

X-ray Structure of *N*-Methoxycarbonyl-3-aminopyrazole-5-carboxylic Acid Ethyl Ester (2). $C_8H_{11}N_3O_4$; M = 213.20 g/mol, triclinic, space group $P1$, a = 6.3241(7) Å, α = 93.687(14)°, b = 6.4956(8) Å, β = 96.857(13)°, c = 12.4132(15) Å, γ = 95.235(14)°, V = 0.5027 nm^3, Z = 2, D_x = 1.408 Mg/m^3, λ(MoKα) = 71.073 pm, μ = 0.114 mm^{-1}, F(000) = 224, T = 173 (1) K. A colorless needle-shaped crystal (0.70 × 0.12 × 0.06 mm^3) was used to collect on a STOE-IPDS diffractometer 4238 reflections (1567 independent signals), R_{int} = 0.0380 from 3.16° to 25.73°. The structure was solved by direct methods (SIR97)[23] and the F^2-value was optimized with the program SHELXL-97[24] for all non-hydrogen atoms. The postion of hydrogen atoms was calculated. The final $\omega R(F^2)$-Wert for all reflections was 0.0754; $R(F)$ = 0.0560, S = 0.905, max $\Delta\rho$ = 158 e·nm^{-3}.

(22) Cooperation with biophysicist Detlev Riesner at the University of Düsseldorf, Germany, and JSW research, Graz, Austria.

(23) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. *J. Appl. Crystallogr.* **1993**, *26*, 343−350.

(24) Sheldrick, G. M. Programm für Kristallstrukturverfeinerung, Universität Göttingen, 1997.

2-(Methoxycarbonylhydrazono)cyanopyruvate (1). Potassium (50.0 g, 1.28 mol) was dissolved in a mixture of dry EtOH (320 mL) and dry Et_2O (225 mL) under nitrogen. The mixture was cooled to 0 °C in ice, diethyl oxalate (93.5 g, 0.64 mol), disolved in Et_2O (30 mL), was added dropwise, and the reaction mixture was stirred for 30 min. A solution of CH_3CN (26.3 g, 0.64 mol) in Et_2O (30 mL) was added, and the mixture was allowed to warm to room temperature and stirred for 1 h. The precipitated solid was collected by filtration to give 115 g (quantitative yield). The product was used without further purification. Mp 105 °C dec; IR (KBr) $\bar{\nu}$ [cm^{-1}] 3393, 2170, 1626, 1558, 1363, 1310, 1112, 594, 526; ^{1}H NMR (250 MHz, D_2O) δ [ppm] 1.19 (t, 3 H, ^3J = 7.1 Hz, CH_3), 4.13 (q, 2 H, ^3J = 7.1 Hz, CH_2), 4.80 (s, 1H, CH); MS (ESI, H_2O) *m*/*z* (%) 140 (100) [M]$^-$.

To a suspension of the potassium cyano pyruvate (895 mg, 5 mmol) in chloroform (50 mL) was added HCl saturated ethyl ester (3 mL). Methyl hydrazino formate (450 mg, 5 mmol) was added, the mixture was stirred for 24 h at room temperature, any precipitated solid was removed by filtration, and the filtrate was concentrated to give an oil. The crude product was purified by CC (ethyl acetate, R_f = 0.72) to yield **1** (1.95 mmol, 39%) as a colorless solid. Mp 131 °C; IR (KBr) $\bar{\nu}$ [cm^{-1}] 3250 , 2250, 1700; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ [ppm] 1.40 (t, 3 H, ^3J = 7.1 Hz), 3.61 (s, 2 H), 3.89 (s, 3 H), 4.38 (q, ^3J = 7.1 Hz, 2 H), 11.93 (br s, 1 H, NH); MS (CI, CH_2Cl_2, NH_3) *m*/*z* (%) 214 (34) [MH]$^+$, 231 (100) [M + NH_4]$^+$. Anal. Calcd for $C_8H_{11}N_3O_4$: C 45.07; H 5.20; N 19.71. Found: C 44.91; H 5.10; N 19.50.

***N*-Methoxycarbonyl-3-aminopyrazole-5-carboxylic Acid Ethyl Ester (2).** To a solution of **1** (107 mg, 0.5 mmol) in CH_3CN (5 mL) was added triethylamine (0.14 mL, 1 mmol) and the mixture was stirred for 10 min at room temperature. The solvent was removed in vacuo and the solid residue was recrystallized from ethyl acetate to give **2** (104 mg, 98%; R_f

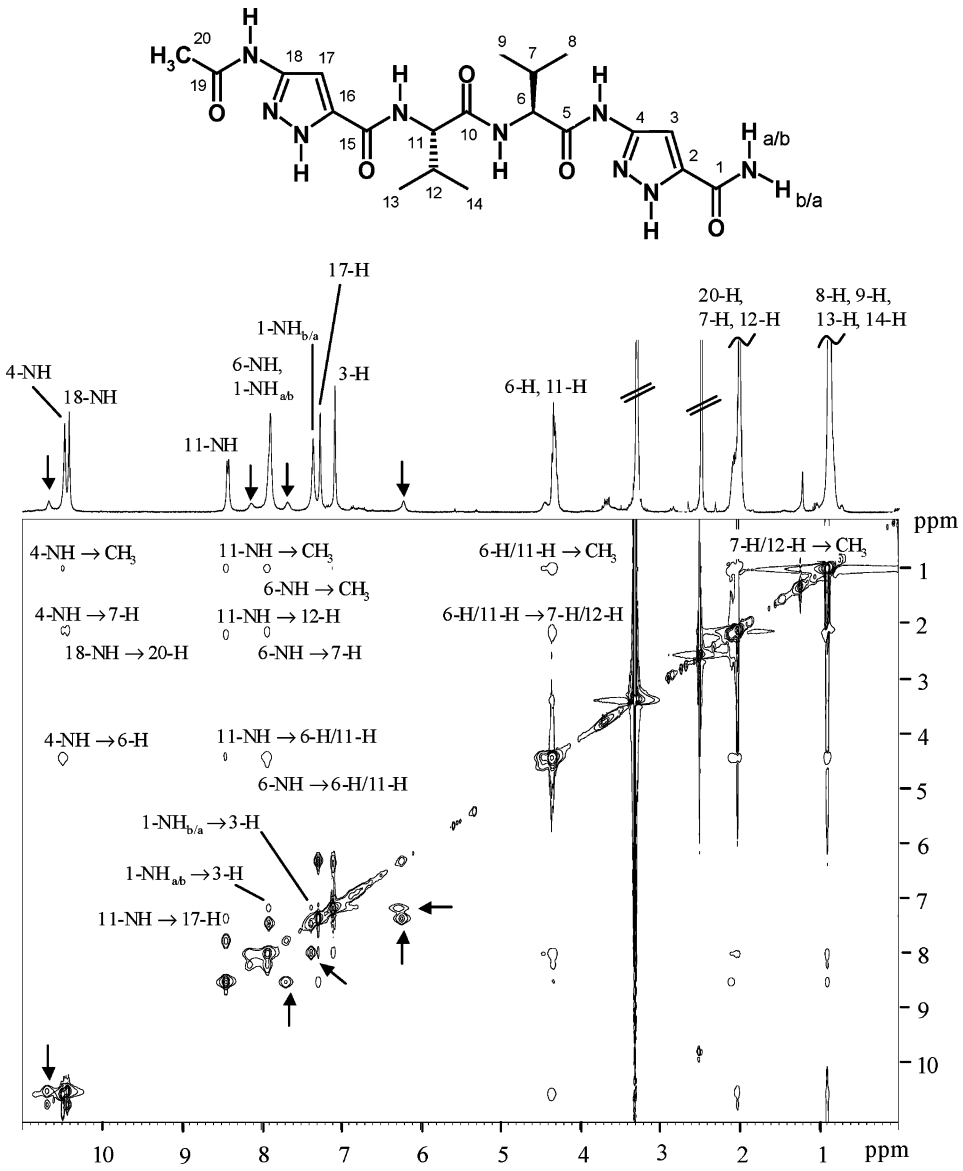


FIGURE 4. Phase-sensitive ROESY spectrum of tetramer **21** in DMSO-d_6 (positive cross-peaks, exchange-effects; negative cross-peaks, NOE effects); the signals of both pyrazole ring NH groups at 12.97 and 13.01 are not shown; the methyl groups H-8, H-9, H-13, and H-14 are abbreviated by CH_3 for clarity; smaller signals without assignment (arrows): minor diastereomer.

0.52) as colorless crystals. Mp 163 °C; IR (KBr) $\bar{\nu}$ [cm^{-1}] 3490, 3287, 3157, 1744, 1729, 1614, 1457, 1243; UV/vis (CH_3CN) λ_{max} [nm] (log ϵ) 216 (4.37), 292 (3.58); ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ [ppm] 1.38 (t, ^3J = 7.1 Hz, 3 H), 4.06 (s, 3 H), 4.39 (q, ^3J = 7.1 Hz, 2 H), 5.50 (br s, 2 H), 5.90 (s, 1 H); ^{13}C NMR (62 MHz, $CDCl_3$, TMS) δ [ppm] 14.3 (+), 54.8 (+), 61.5 (−), 89.8 (+), 147.3 (C_{quat}), 151.3 (C_{quat}), 152.2 (C_{quat}), 161.9 (C_{quat}); MS (EI, CH_2Cl_2) *m*/*z* (%) 213 (36) [$M^{+\bullet}$], 169 (24) [$M^{+\bullet} - C_2H_4O$], 141 (100) [$M^{+\bullet} - C_3H_4O_2$]; HRMS ($C_8H_{11}N_3O_4$) calcd 213.0750, found 213.0752 ± 0.56 ppm. Anal. Calcd for $C_8H_{11}N_3O_4$: C 45.07; H 5.20, N 19.72. Found: C 44.87; H 5.04, N 19.44.

Fmoc-Gly-Pz(*H*)-OEt (3a). Compound **2** (3.80 g, 17.82 mmol) was dissolved in anhydrous THF (120 mL) and the solution was treated with 6.19 g (19.61 mmol) of *N*-(9-fluorenylmethoxycarbonyl)glycine chloride and stirred for 24 h at 70 °C. After evaporation of the solvent the yellowish crude product was absorbed onto silica gel and chromatographed over silica gel eluting with ethyl acetate/petrol ether (4:1). Compound **3a** (7.50 g, 17.26 mmol, 97%) was isolated as a colorless solid (R_f 0.32, EE:PE = 2:1). Mp 132 °C; IR (KBr) $\bar{\nu}$ [cm^{-1}] 3307, 3173, 1704, 1591, 1548, 1506, 1254; UV/vis (CH_3CN) λ_{max} [nm] (log ϵ) 208 (4.73), 220 (sh, 4.47), 266 (4.27), 276 (sh, 4.12), 289 (3.73), 300 (3.74); ^{1}H NMR (250 MHz, DMSO-d_6, TMS) δ [ppm] 1.30 (t, ^3J = 7.1 Hz, 3 H, CH_3), 3.81 (d, 2 H, ^3J = 5.7 Hz, CH_2-glycine), 4.15−4.41 (m, 5 H, CH_2-ester, CH_2-Fmoc, CH-Fmoc), 6.96 (br s, 1 H, CH-pyrazole), 7.29−7.47 (m, 4H, CH-arom Fmoc), 7.62 (t, 1 H, ^3J = 5.7 Hz, NH), 7.73 (d, 2 H, ^3J = 5.9 Hz, CH-arom Fmoc), 7.90 (d, 2 H, ^3J = 7.1 Hz, CH-arom Fmoc), 10.67 (br s, 1 H, NH), 13.55 (br s, 1 H, NH-pyrazole); ^{13}C NMR (62 MHz, DMSO-d_6) δ [ppm] 14.1 (+), 43.4 (−), 46.6 (+), 60.7 (−), 65.7 (−), 99.2 (+), 120.1 (+), 125.2 (+), 127.0 (+), 127.6 (+), 133.0 (C_{quart}), 140.7 (C_{quart}), 143.8 (C_{quart}), 147.5 (C_{quart}), 156.5 (C_{quart}), 158.8 (C_{quart}), 167.5 (C_{quart}); MS (FAB, CH_2Cl_2, glycerin) *m*/*z* (%) 435 (100) [MH]$^+$; HRMS ($C_{23}H_{22}N_4O_5$) calcd 435.1668, found 435.1662.

H-Gly-Pz(*H*)-OEt (3b). Fmoc-protected **3a** (200 mg, 0.46 mmol) was dissolved in 2 mL of 20% piperidine in DMF and the solution was stirred for 15 min at ambient temperature. The solvent was removed in vacuo and the resulting solid was absorbed onto silica gel. After chromatographic purification over silica gel eluting with CH_2Cl_2:MeOH:NH_3 80:20:1, 91 mg (0.43 mmol, 93%) of the free amine **3b** was obtained as a

colorless solid (R_f 0.54). Mp 188 °C dec; IR (KBr) $\bar{\nu}$ [cm^{-1}] 3374, 3236, 1722, 1654, 1528, 1472; UV/vis (CH_3CN) λ_{max} [nm] (log ϵ) 219 nm (3.79); ^{1}H NMR (250 MHz, DMSO-d_6, TMS) δ [ppm] 1.31 (t, 3 H, ^3J = 7.1 Hz, CH_3), 3.32 (s, 2 H, CH_2-glycine), 4.30 (q, 2 H, ^3J = 7.1 Hz CH_2), 6.94 (s, 1 H, CH); ^{13}C NMR (62 MHz, DMSO-d_6, TMS) δ [ppm] 14.1 (+), 44.5 (−), 60.6 (−), 98.2 (+), 134.7 (C_{quart}), 145.8 (C_{quart}), 159.3 (C_{quart}), 170.9 (C_{quart}); MS (EI, 70 eV) *m*/*z* (%) 212 (10) [M]$^{\bullet+}$, 155 (12) [M − $NHCOCH_2NH_2$]$^{\bullet+}$; HRMS ($C_8H_{12}N_4O_3$) calcd 212.0909, found 212.0908.

Fmoc-Gly-Gly-Pz(*H*)-OEt (4a). Free amine **3b** (100 mg, 0.23 mmol) and 145 mg (0.46 mmol) of *N*-(9-fluorenylmethoxycarbonyl)glycine chloride were dissolved in 4 mL of anhydrous DMF. By addition of *N*,*N*-diisopropylethylamine the pH of the solution was brought to 8−9. After the mixture was stirred for 30 min at room temperature, the solvent was stripped off in vacuo. The oily residue was suspended in CH_2Cl_2 and sonicated for several minutes, and the solvent was subsequently removed again. The resulting solid was absorbed onto silica gel and chromatographed over silica gel eluting with CH_2Cl_2:MeOH:NH_3 100:10:1 (R_f 0.20). Trimer **4a** (46 mg, 0.09 mmol, 41%) was isolated as a colorless solid. Mp 198 °C dec; IR (KBr) $\bar{\nu}$ [cm^{-1}] 3325, 3140, 1701, 1640, 1571, 1211; UV/vis (CH_3CN) λ_{max} [nm] (log ϵ) 208 (4.73), 220 (sh, 4.49), 265 (4.28), 275 (sh, 4.12), 289 (3.69), 300 (3.70); ^{1}H NMR (250 MHz, DMSO-d_6, TMS) δ [ppm] 1.30 (t, 3 H, ^3J = 7.1 Hz, CH_3), 3.69 (d, 2 H, ^3J = 6.0 Hz, CH_2-glycine), 3.91 (d, 2 H, ^3J = 5.6 Hz CH_2-glycine), 4.19−4.36 (m, 5 H, 2 CH_2-ester, CH_2-Fmoc, CH-Fmoc), 6.92 (br s, 1 H, CH-pyrazole), 7.29−7.47 (m, 4 H, CH-arom Fmoc), 7.60 (t, 1 H, ^3J = 6.0 Hz, 1 H, NH), 7.72 (d, 2 H, ^3J = 7.1 Hz, CH-arom Fmoc), 7.89 (d, 2 H, ^3J = 7.1 Hz, CH-arom Fmoc), 8.19 (t, 1 H, ^3J = 5.6 Hz, NH), 10.63 (s, 1 H, NH), 13.55 (br s, 1 H, NH-pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ [ppm] 14.1 (+), 42.1 (−), 43.4 (−), 46.6 (+), 60.7 (−), 65.7 (−), 99.0 (+), 120.1 (+), 125.2 (+), 127.0 (+), 127.6 (+), 133.1 (C_{quart}), 140.7 (C_{quart}), 143.8 (C_{quart}), 147.3 (C_{quart}), 156.5 (C_{quart}), 159.0 (C_{quart}), 167.2 (C_{quart}), 169.5 (C_{quart}); MS (FAB, DMSO/glycerin) *m*/*z* (%) 492 (100) [MH]$^+$; HRMS ($C_{25}H_{25}N_5O_6$) calcd 492.1883 [MH]$^+$, found 492.1879 [MH]$^+$.

H-Gly-Gly-Pz(*H*)-OEt (4b). The Fmoc-protected **4a** (360 mg, 0.73 mmol) was dissolved in 2 mL of 20% piperidine in DMF and the solution was stirred for 15 min at room temperature. The solvent was removed and the resulting solid was suspended in CH_2Cl_2. After sonication the solution was carefully removed by means of a pipet. This procedure was repeated twice; the remaining solid was filtered off. After purification over silica gel (R_f 0.16, CH_2Cl_2:MeOH:NH_3 80:20:1), free amine **4b** was obtained in the form of a colorless solid (193 mg, 0.72 mmol, 98%). Mp 190 °C; IR (KBr) $\bar{\nu}$ [cm^{-1}] 3363, 3249, 1723, 1663, 1547, 1473; UV/vis (CH_3CN) λ_{max} [nm] (log ϵ) 223 (3.60); ^{1}H NMR (250 MHz, DMSO-d_6, TMS) δ [ppm] 1.30 (t, 3 H, ^3J = 7.1 Hz CH_3), 3.19 (s, 2 H, CH_2-glycine), 3.93 (s, 2 H, CH_2-glycine), 4.29 (q, 2 H, ^3J = 7.1 Hz, CH_2-ester), 6.90 (s, 1 H, CH), 8.24 (br s, 1 H, NH), 10.67 (br s, 1 H, NH); ^{13}C NMR (62 MHz, DMSO-d_6, TMS) δ [ppm] 14.1 (+), 41.9 (−), 44.2 (−), 60.6 (−), 98.8 (+), 134.1 (C_{quart}), 146.3 (C_{quart}), 159.2 (C_{quart}), 167.3 (C_{quart}), 172.8 (C_{quart}); MS (CI, NH_3) *m*/*z* (%) 270 (58) [MH]$^+$, 156 (100) [M − $C_4H_5N_2O_2$]$^+$; HRMS ($C_{10}H_{15}N_5O_4$) calcd 270.1202 [MH]$^+$, found 270.1204 [MH]$^+$.

Z-Gly-Gly-Pz(PMB)-OMe (11). Under inert atmosphere a mixture of 150 g of *N*-*p*-Methoxybenzyl-3-aminopyrazolee-5-carbonylic acid methyl ester (**7**)[6b] (570 μmol, 1.00 equiv), 152 mg of *N*-benzyloxycarbonyl-glycinyl-glycin (570 μmol, 1.00 equiv), 136 mg of EDC (710 μmol, 1.25 equiv), 219 mg of 1-hydroxybenztriazol (HOBt) (1.43 mmol, 2.50 equiv), and 250 mL of DIEA (1.43 mmol, 2.50 equiv) were stirred for 1 h at 0 °C and for another 15 h at room temperature. The organic layer was washed with satd aq $NaHCO_3$, 1 M HCl, and satd aq NaCl and dried over Na_2SO_4. After filtration the solution was concentrated and crystallized from EtOAc. The colorless solid was washed with EtOAc and dried in vacuo. Yield 42% (120 mg, 240 μmol); colorless solid. ^{1}H NMR (200 MHz, DMSO) δ [ppm] 3.67 (d, ^3J = 6.3 Hz, 2H, CH_2-Gly), 3.71 (s, 3H, OCH_3-PMB), 3.83 (s, 3H, OCH_3), 3.87 (d, ^3J = 5.7 Hz, 2H, CH_2-Gly), 5.03 (s, 2H, CH_2-Z), 5.54 (s, 2H, CH_2-Bz), 6.87 (d, ^3J = 8.7 Hz, 2H, CH-arom), 7.06 (s, 1H, CH-pyrazole), 7.14 (d, ^3J = 8.7 Hz, 2H, CH-arom), 7.35 (m, 5H, CH-arom), 7.50 (t, ^3J = 6.2 Hz, 1H, NH-Gly), 8.18 (t, ^3J = 5.7 Hz, 1H, NH-Gly), 10.71 (s, 1H, NH-pyrazole); ^{13}C NMR (125 MHz, DMSO) δ [ppm] 42.2, 43.5, 52.2, 53.2, 55.0, 65.5, 101.7, 113.9, 127.7, 128.3, 128.7, 129.1, 131.2, 137.0, 145.9, 158.7, 159.3, 167.3, 169.5; MS (ESI) *m*/*z* (%) 532 (M^+ + Na, 100), 548 (M^+ + K, 20), 510 (M^+, 25); HRMS calcd 532.181, found 532.180; R_f 0.10 in EtOAc/*n*-pentane 2:1; mp 165−170 °C. Anal. Calcd for $C_{25}H_{27}N_5O_7$: C, 58.93; H, 5.34; N, 13.75. Found: C, 58.93; H, 5.50; N, 13.48.

H-Gly-Gly-Pz(PMB)-OMe (12). Under inert atmosphere 700 mg of Z-Gly-Gly-Pz(PMB)-OMe **(11)** (1.37 mmol, 1.00 equiv) was dissolved in 2 mL of dichloromethan and the solution was diluted with 25 mL of methanol. Next, 5 mol % of Pd/C was added. The flask was then evacuated and filled with H_2. The reaction mixture was stirred for 16−52 h, then the catalyst was removed by filtration over Celite and the solution concentrated in vacuo. Yield 91% (470 μg, 1.25 μmol); colorless solid. ^{1}H NMR (200 MHz, DMSO-d_6) δ [ppm] 3.61 (br s, 2H, CH_2-Gly), 3.71 (s, 3H, OCH_3-PMB), 3.83 (s, 3H, OCH_3), 3.97 (d, ^3J = 5.5 Hz, 2H, CH_2-Gly), 5.54 (s, 2H, CH_2-Bz), 6.88 (d, ^3J = 8.7 Hz, 2H, CH-arom), 7.05 (s, 1H, CH-pyrazole), 7.17 (d, ^3J = 8.7 Hz, 2H, CH-arom), 8.17 (br s, 2H, NH_2), 8.77 (t, ^3J = 5.6 Hz, 1H, NH), 10.86 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO) δ [ppm] 42.2, 52.2, 52.9, 53.2, 55.1, 101.7, 113.9, 128.8, 129.1, 131.2, 145.8, 158.7, 159.3, 166.4, 166.9; MS (ESI) *m*/*z* (%) 398 (M^+ + Na), 121 (PMB^+); HRMS calcd 398.144, found 398.143; R_f 0 in EtOAc/*n*-pentane 2:1; mp 219 °C.

Ac-Pz(PMB)-Gly-Gly-Pz(PMB)-OMe (13a). In an argon atmosphere a mixture of 158 mg of H-Gly-Gly-Pz(PMB)-OMe (420 μmol, 1.00 equiv) **(12)**, 120 mg (420 μmol, 1.00 equiv) of 5-Acetylamino-2-(4-methoxy-benzyl)-2*H*-pyrazole-3-carbonsäure **(9b)**,[6b] 260 μL of DIEA (1.52 mmol, 3.60 equiv), and 232 mg of chlortripyrolidinophosphoniumhexafluorphosphat (PyClop) (550 μmol, 1.30 equiv) was stirred in dry dichloromethane for 16 h. During the reaction a yellow solid precipitated, which was filtrated, washed with *n*-pentane, and dried in vacuo. Yield 61% (165 mg, 260 μmol), pale yellow solid. ^{1}H NMR (300 MHz, DMSO) δ [ppm] 1.98 (s, 3H, CH_3-acetyl), 3.68 (s, 2H, CH_3-PMB), 3.70 (s, 3H, CH_3-PMB), 3.82 (s, 3H, CH_3), 3.83−3.85 (m, 4H, CH_2-glycine), 5.53 (s, 2H, CH_2-Bz), 5.56 (m 2H, CH_2-Bz), 6.82 (d, ^3J = 8.7 Hz, 2H, CH-arom), 6.85 (d, ^3J = 8.6 Hz, 2H, CH-arom), 7.06−7.22 (m, 6H, CH-arom, pyrazole), 8.29 (br s, 1H, NH), 8.93 (br s, 1H, NH), 10.56 (s, 1H, NH), 10.64 (s, 1H, NH); MS (ESI) *m*/*z* (%) 669 (M^+ + Na); HRMS calcd 669.2397, found 669.2370; R_f 0.10 in CH_2Cl_2/MeOH 10:1; mp 156 °C.

Ac-Pz(*H*)-Gly-Gly-Pz(*H*)-OMe 2**TFA (14a).** In an inert atmosphere 64 mg (100 μmol, 1.00 equiv) of Ac-Pz(PMB)-Gly-Gly-Pz(PMB)-OMe **(13a)** was heated in 10 mL of anhydrous TFA for 4 h to 70 °C. The solution was cooled to room temperature and an excess of ice-cold diethyl ether was added. The product precipitated as a colorless solid, which was filtered off, washed several times with diethyl ether, and dried in vacuo. Yield 47% (30 mg, 47 μmol); colorless solid. ^{1}H NMR (300 MHz, DMSO) δ [ppm] 2.02 (s, 3H, CH_3-acetyl), 3.82 (s, 3H, CH_3), 3.88−3.91 (m, 4H, CH_2-glycine), 6.91 (s, 1H, CH-pyrazole), 7.03 (s, 1H, CH-pyrazole), 8.25−8.28 (t, ^3J = 5.6 Hz, 1H, NH glycine), 8.73 (br s, 1H, NH glycine), 10.50 (s, 1H, amide pyrazole), 10.63 (s, 1H, amide pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6.) δ [ppm] 22.4, 52.18, 53.2, 55.1, 113.5, 113.8, 128.7, 128.8, 129.6, 145.6, 157.2, 159.7, 159.3, 168.5, 169.5; mp >250 °C.

Glycol-Pz(PMB)-Gly-Gly-Pz(PMB)-OMe (13b). In an argon atmosphere a mixture of 184 mg of Gly-Pz(PMB)-OH **(9c)**[6b] (490 μmol, 1.00 equiv), 200 mg (490 μmol, 1.00 equiv) of H-Gly-Gly-Pz(PMB)-OMe **(14d)**, 310 μL (1.77 mmol, 3.60 equiv) of DIEA, and 270 mg (640 μmol, 1.30 equiv) of PyClop

was stirred for 16 h at room temperature. The solvent was evaporated and the residue was purified by chromatography on silica gel column (gradient: CH_2Cl_2/CH_3OH 30:1 and 10:1). Finally the product was recrystallized from $CHCl_3$ and diethyl ether. Yield 69 mg (98 μmol, 20%); colorless solid. ^{1}H NMR (300 MHz, $CDCL_3$) δ [ppm] 3.31 (s, 3H, CH_3-glycol), 3.52−3.73 (m, 10H, CH_2-glycol), 3.67 (s, 3H, CH_3-PMB), 3.73 (s, 3H, CH_3-PMB), 3.81 (s, 3H, CH_3), 4.05 (d, ^3J = 5.4 Hz, 2H, CH_2-Gly), 4.12−4.17 (m, 4H, CH_2-Gly, OCH_2), 5.50 (s, 2H, CH_2-Bz), 5.61 (s, 2H, CH_2-Bz), 6.74−6.77 (m, 4H, CH_2-arom), 7.19−7.26 (m, 6H, H-CH_2-arom, CH-pyrazole), 7.51 (t, ^3J = 5.4 Hz, 1H, NH), 8.16 (t, ^3J = 5.4 Hz, 1H, NH), 9.27, 9.80 (2s, 2H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm] 46.2, 46.3, 51.9, 53.8, 54.6, 55.2, 55.3, 59.0, 70.2, 70.4, 70.6, 71.4, 71.9, 98.9, 102.8, 113.8, 113.9, 128.9, 129.2, 129.3, 129.5, 131.8, 134.5, 144.7, 145.5, 159.1, 159.2, 160.0, 160.3, 167.0, 168.6, 170.2; MS (ESI) *m*/*z* (%) 787 (M^+ + Na); HRMS calcd 707.3027, found 787.3069; mp 126 °C.

Glycol-Pz(*H*)-Gly-Gly-Pz(*H*)-OMe·2TFA (14b). In an argon atmosphere 50 mg (71 μmol) of Glycol-Pz(PMB)-Gly-Gly-Pz(PMB)-OMe **(13b)** was heated in 10 mL of anhydrous TFA for 4 h to 70 °C. The solution was cooled to room temperature and an excess of ice-cold diethyl ether was added. The product precipitated as a colorless solid, which was filtrated, washed several times with diethyl ether, and dried in vacuo. Yield 13 mg (25%,17.5 μmol); colorless solid. ^{1}H NMR (300 MHz, DMSO) δ [ppm] 3.23 (s, 3H, CH_3-glycole), 3.44−3.47 (m, 2H, CH_2), 3.54−3.65 (m, 6H, CH2), 3.82 (s, 3H, CH_3), 3.90−3.91 (m, 4H, CH_2-glycine), 4.09 (s, 2H, CH_2), 6.91 (s, 1H, CH-pyrazole), 7.07 (s, 1H, CH-pyrazole), 8.27 (t, ^3J = 5.6 Hz, NH-glycine), 8.75 (br s, 1H, NH-glycine), 10.11 (s, 1H, NH), 10.63 (s, 1H, NH); MS (ESI) *m*/*z* (%) 547 (M^+ + Na); HRMS calcd for $C_{20}H_{28}N_8NaO_9$ 547.1877, found 547.1872.

NO_2-Pz(PMB)-Gly-OMe (15a). In an inert atmosphere 63 mg (500 μmol, 1.00 equiv) of glycin-methyl ester hydrochloride, 139 mg (500 μmol, 1.00 equiv) of 5-nitro-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carbonylic acid **(9a)**, 273 mg (650 μmol, 1.30 equiv) of PyClop, and 427 μL (2.45 mmol, 4.90 equiv) of diisopropylethylamine were dissolved in dry dichloromethane and the solution was stirred at room temperature for 16 h. The solvent was evaporated and the residue was purified by chromatography on silica gel column (eluent EtOAc/*n*-pentane 1:2). Yield 170 mg (495 μmol, 99%); colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 3.67 (s, 3H, CH_3-PMB), 3.72 (s, 3H, CH_3-glycine), 4.05 (d, ^3J = 5.3 Hz, 2H, CH_2-glycine), 5.75 (s, 2H, CH_2-Bz), 6.89 (d, ^3J = 8.3 Hz, 2H, CH_2-arom), 7.25 (d, ^3J = 8.3 Hz, 2H, CH_2-arom), 7.67 (s, 1H, CH_2-pyrazole), 9.38 (t, ^3J = 5.3 Hz, 1H, NH); MS (ESI) *m*/*z* 349 (M^+ + H); R_f 0.19 in ETOAc/*n*-pentane 1:2.

NO_2-Pz(*H*)-Gly-OMe (15). In an argon atmosphere 50 mg (146 μmol, 1.00 equiv) of NO_2-Pz(PMB)-Gly-OMe **(15a)** were heated in 10 mL of anhydrous TFA for 4 h to 70 °C. The solvent was evaporated and the residue was purified by chromatography on silica gel column (eluent CH_2Cl_2/CH_3OH 10:1) Yield 20 mg (88 μmol, 60%); colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 3.67 (s, 3H, CH_3), 4.07 (d, ^3J = 5.9 Hz, 2H, CH_2), 7.62 (s, 1H, CH), 9.29 (t, ^3J = 5.4 Hz, 1H, NH), 14.87 (s, 1H, NH-pyrazole); MS (EI) *m*/*z* 228 (M^+); R_f 0.55 in dichlormethan/methanol 10:1; mp 167 °C.

Boc-L-Phe-L-Phe-OMe. *N-tert*-Butyloxycarbonyl-(*S*)-phenylalanine (1.22 g, 4.60 mmol, 1.00 equiv) and 1.00 g (4.60 mmol, 1.00 equiv) of *(S)*-Phenylalanine-methyl ester were stirred together with 1.90 g (4.60 mmol, 1.00 equiv) of HCTU, 11.5 mmol (2.50 equiv) of Cl-HOBt, and 2.14 mL (17.6 mmol, 4.00 equiv) of 4,6-lutidin. The organic layer was washed with satd aq $NaHCO_3$, 1 M HCl, and satd aq NaCl and dried over Na_2SO_4. After filtration the solution was evaporated and the residue dried in vacuo. Yield 1.57 g (3.68 mmol, 80%); colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] 1.39 (s, 9H, CH_3-Boc), 2.95−3.04 (m, 4H, CH_2-phe), 3.67 (s, 3H, CH_3), 4.31−4.33 (m, 1H, α-CH), 4.76−4.79 (m, 1H, α-CH), 4.91 (br s, 1H, NH), 6.25 (d, J = 6.6 Hz, 1H, NH), 6.95−7.29 (2m, 10H, CH-arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm] 28.4, 37.6, 38.1, 38.4, 52.4, 53.4, 55.8, 127.1, 127.3, 128.6, 128.8, 129.3, 129.5, 170.9, 171.5; MS (ESI) *m*/*z* (%) 465 (M^+ + K), 449 (M^+ + Na); HRMS calcd for $C_{24}H_{30}N_2O_5Na$ 449.2052, found 449.2050; mp 132 °C; R_f 0.85 in CH_2Cl_2/MeOH 30:1; $[\alpha]_{Na}$ +23.2 at T = 21 °C, *c* 1.00 in $CHCl_3$.

H-Phe-Phe-OMe·TFA. Boc-L-Phe-L-Phe-OMe (2.50 g, 7.36 mmol, 1.00 equiv) was stirred in a mixture of dichloromethane and trifluoroacidic acid (5:1) at 0 °C until complete conversion. The dichloromethane was evaporated and ice-cold diethyl ether was added to the remaining TFA solution. The precipitating solid was filtered off, washed several times with diethyl ether, and dried in vacuo. Yield 1.42 g (3.22 mmol, 44%); colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ [ppm] 2.87−3.13 (m, 4H, CH_2-Phe), 3.61 (s, 3H, CH_3), 4.02 (br s, 1H, α-CH), 4.54−4.61 (m, 1H, α-CH), 7.21−7.35 (m, 10H, CH-arom), 8.10 (br s, 3H, NH_3), 8.98 (d, J = 7.63 Hz, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ [ppm] 36.6, 36.9, 52.0, 53.1, 53.8, 126.7, 127.2, 128,3, 128.4, 129.0, 129.5, 133.9, 136.6, 168.2, 171.1; MS (ESI) *m*/*z* (%) 349 (M + Na)$^+$, 327 (M^+ + H)$^+$; HRMS calcd for $C_{19}H_{23}N_2O$: 327.1709, found 327.1706; m: 135 °C; $[\alpha]_{Na}$ +4.9 at T = 20 °C, *c* 1.00 in MeOH; R_f 0.13 in dichloromethane/methanol 30:1.

Boc-L-Val-Pz(PMB)-OMe (16a). In an inert atmosphere a mixture of 820 mg (3.15 mmol, 1.00 equiv) of H-Pz(PMB)-OMe (**7**), 685 mg (3.15 mmol, 1.00 equiv) of *N-tert*-butyloxycarbonyl-(*S*)-valine, 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 lutidin was stirred in a mixture of dry CH_2Cl_2:dry DMF 3:1**.** After 2 h the organic layer was washed with satd aq $NaHCO_3$, 1 M HCl, and satd aq NaCl and dried over Na_2SO_4. After filtration the solution was concentrated and purified by chromatography over silica gel (eluent:EtOAc/*n*-pentane 1:1). Yield 750 mg (1.63 mmol, 52%); colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm] 0.92 (d, ^3J = 7.1 Hz, 3H, CH_3-Valα), 0.99 (d, ^3J = 6.9 Hz, 3H, CH_3-Valβ), 1.34 (s, 9H, *t*-Bu), 2.24−2.31 (m,1H, CH-*i*-Pr), 3.76 (s, 3H, CH_3-PMB), 3.85 (s, 3H, CH_3), 4.12 (br s, 1H, α-H), 5.03 (br s, 1H, NH-Boc), 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 [ppm] 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. R_f 0.28 in *n*-pentane/diethyl ether 1:1; $[\alpha]_{Na}$ −11.4, T = 20 °C, *c* 0.50 in $CHCl_3$; mp 130 °C.

H-L-Val-Pz(PMB)-OMe·TFA (16b). Boc-L-Val-Pz(PMB)-OMe (**16a**) (700 mg, 1.52 mmol) was stirred at room temperature in a mixture of CH_2Cl_2 and trifluoroacidic acid (3:1) until the starting material disappeared on TLC. The solvent was evaporated and the residue was dissolved two times in toluene and again evaporated. Finally the residue was dissoved in acetone, the solvent was again evaporated, and the solid was dried in vacuo. Yield 725 mg (1.52 mmol, quant.); solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] 0.82 (d, ^3J = 6.0 Hz, 6H, CH_3), 1.95−2.07 (m, 1H, CH-*i*-Pr), 3.61 (s, 3H, CH_3-PMB), 3.71 (s, 3H, CH_3), 3.96−3.98 (m, 1H, αH), 5.43 (d, ^2J = 14.9 Hz, 1H, CH-benz.α), 5.54 (d, ^2J = 14.6 Hz, 1H, CH-benz.β), 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 (br s, 3H, NH_3), 10.17 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm] 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; mp 75 °C; $[\alpha]_{Na}$ +18.0, T = 20 °C, *c* 1.00 in MeOH; MS (ESI) *m*/*z* (%) 383 (M^+ + Na).

NO_2-Pz(PMB)-L-Val-Pz(PMB)-OMe (16c). A mixture of 167 mg (604 μmol, 1.00 equiv) of 5-nitro-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carbonylic acid (**9a**),[6b] H-L-Val-Pz(PMB)-OMe·TFA (**16b**) (549 μmol, 1.00 equiv), 699 mg (1.10 mmol, 1.10 equiv) of T3P, and 845 μL (3.84 mmol, 7.00 equiv) of *N*-methylmorpholin was stirred under argon for 24 h in dry

dichloromethane. The solvent was evaporated and the residue was purified by chromatography on a silica gel column (eluent EtOAc/*n*-pentane 1:2). Yield 50% (165 mg, 275 μmol); colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] 089 (d, ^3J = 6.6 Hz, 3H, CH_3-Valα), 0.93 (d, ^3J = 5.6 Hz, 3H, CH_3-Val β), 2.10−2.17 (m, 1H, CH-*i*-Pr), 3.66 (s, 3H, OCH_3-PMB), 3.71 (s, 3H, OCH_3-PMB), 3.79 (s, 3H, OCH_3), 4.43 (dd, ^3J = 6.6 Hz, ^3J = 8.3 Hz, 3H, α-H-Val), 5.50−5.68 (m, 4H, CH_2-Bz), 6.65−6.80, 7.13−7.73 (2m, 7H, H-arom), 8.21 (s, 1H, H-NH-pyrazole); MS (FD) *m*/*z* (%) 619 (M^+); R_f 0.31 in *n*-pentane/EtOAc 2:1.

NO_2-Pz(*H*)-L-Val-Pz(*H*)-OMe (16). Under inert atmosphere 45 mg (73 μmol, 1.00 equiv) of NO_2-Pz(PMB)-L-Val-Pz-(PMB)-OMe (**16c**) was heated in 10 mL of anhydrous TFA for 4 h to 70 °C. The solvent was evaporated and the residue was purified by chromatography on silica gel column (eluent CH_2Cl_2/CH_3OH 30:2). Yield 90% (26 mg, 66 μmol); colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 1.03 (d, ^3J = 6.3 Hz, 3H, CH_3-Val α), 1.05 (d, ^3J = 6.3 Hz, 3H, CH_3-Val β), 2.25−2.35 (m, 1H, CH-*i*-Pr), 3.92 (s, 3H, OCH_3), 4.19 (m, 1H, α-H-Val), 7.09 (s, 1H, CH-pyrazole), 8.00 (s, 1H, H-CH-pyrazole), 8.93 (d, ^3J = 8.6 Hz, 1H, NH-Val), 10.98 (s, 1H, amid NH-pyrazole), 13.72, 14.86 (2s, 2H, H-NH-pyrazole; ^{13}C NMR (75 MHz, DMSO-d_6) δ [ppm] 18.6, 18.9, 30.1, 51.8, 59.7, 99.4, 102.2, 113.9, 129.5, 139.7, 129.5, 138.7, 155.7, 157.3, 169.1; MS (FD) *m*/*z* 379 (M^+); HRMS (ESI) calcd for $C_{14}H_{18}N_7O_6$ 380.1319, found 380.1349; mp 182 °C.

Ac-Gly-Gly-Pz(PMB)-Pz(PMB)-OMe (17a). In an argon atmosphere 130 mg (265 μmol, 1.00 equiv) of 3-{[5-Amino-2-(4-methoxy-benzyl)-2*H*-pyrazole-3-carbonyl]amino}-2-(4-methoxybenzyl)-2*H*-pyrazole-3-carbonylic acid methylester (**8**),[6b] 92 mg (530 μmol, 2.00 equiv) of *N*-Acetyl-glycinyl-glycine, 138 mL (795 μmol, 3.00 equiv) of DIEA, and 75 mg (291 μmol, 1.10 equiv) of 2-chlor-1-methylpyridiniumiodid were stirred for 2 h. The organic layer was washed with satd aq $NaHCO_3$, 1 M HCl, and satd aq NaCl and dried over Na_2SO_4. After filtration the solution was concentrated and purified by chromatography over silica gel (eluent CH_2Cl_2/CH_3OH 20:1). Yield 64% (110 mg, 170 mmol); colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] 1.82 (s, 3H, CH_3-acetyl), 3.69, 3.72 (2s, 6H, OCH_3-PMB), 3.86 (br s, 5H, OCH_3, CH_2-Gly), 4.03 (s, 2H, CH_2-Gly), 5.56, 5.59 (2s, 4H, CH_2-Bz), 6.75 (d, ^3J = 8.6 Hz, 2H, CH-arom), 6.75 (d, ^3J = 8.6 Hz, 2H, CH-arom), 6.75 (d, ^3J = 7.9 Hz, 2H, CH-arom), 7.22 (br s, 1H, NH), 7.34 (s, 1H, NH), 9.27, 9.58 (2s, 2H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm] 22.1, 43.5, 43.6, 52.2, 54.1, 55.3, 103.6, 114.1, 114.2, 128.7, 129.2, 129.6, 129.7, 132.3, 145.2, 159.4, 159.5, 159.5, 159.6; MS (FD) *m*/*z* (%) 669 (M^+ + Na); mp 122 °C.

Ac-Gly-Gly-Pz(*H*)-Pz(*H*)-OMe (17). In an argon atmosphere 100 mg (154 μmol, 1.00 equiv) of Ac-Gly-Gly-Pz(PMB)-Pz(PMB)-OMe (**17a**) was heated in 10 mL of anhydrous TFA for 4 h to 70 °C. The solvent was evaporated and the residue was washed several times with a mixture of *n*-pentane/diethyl ether (1:1) and finally with satd aq $NaHCO_3$. The product was dried in vacuo. Yield 60% (50 mg, 92 μmol), pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 1.87 (s, 3H, CH_3-acetyl), 3.73 (m, 2H, CH_2-Gly), 3.84 (s, 3H, CH_3), 3.89 (d, 2H, CH_2-Gly), 7.02 (s, 1H, CH-pyrazole), 7.13 (s, 1H, CH-pyrazole), 8.14−8.20 (m, 2H, NH-gly), 10.48, 11.12 (2brs, 2H, amidNH), 13.31, 13.77 (2 br s, 2H, NH-pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ [ppm] 22.4, 52.18, 53.2, 55.1, 113.5, 113.8, 128.7, 128.8, 129.6, 145.6, 157.2, 159.7, 159.3, 168.5, 169.5; MS (FD) *m*/*z* (%) 429 (M^+ + Na); mp 200 °C dec.

NO_2-Pz(PMB)-Pz(PMB)-L-Phe-L-Phe-OMe (18a). NO_2-Pz(PMB)-Pz(PMB)-OH (**10**) (50.6 mg,100 μmol, 1.00 equiv), 48 mg (110 μmol, 1.10 equiv) of H-Phe-Phe-OMe·TFA, 31 mg (120 μmol, 1.20 equiv) of 3-chloro-1-methylpyridinium iodide, and 55 μL (300 μmol, 3.00 equiv) of DIEA were stirred in dry dichloromethane overnight. The organic layer was washed with satd aq $NaHCO_3$, 1 M HCl, and satd aq NaCl and dried over Na_2SO_4. After filtration the solution was evaporated and the residue dried in vacuo. The residue was purified by chromatography on silica gel column (eluent CH_2Cl_2/2-propanol 50:1). Yield 77 mg (95 μmol, 95%); colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ [ppm] 2.87−3.10 (m, 4H, CH_2-Phe), 3.59 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 3.71 (s, 3H, CH_3), 4.51−4.57 (m, 1H, αH), 4.74−4.79 (m, 1H, αH), 5.40 (d, 1H, ^2J = 14.4 Hz, CHα-PMB), 5.54 (d, 1H, ^2J = 14.4 Hz, CHβ-PMB), 5.82 (s, 2H, CH_2), 6.79 (d, ^3J = 8.5 Hz, 2H, CH-PMB), 6.89 (d, ^3J = 8.8 Hz, 2H, CH-PMB), 6.91 (d, ^3J = 8.5 Hz, 2H, CH-PMB), 7.15−7.32 (m, 12H, H-arom), 7.38 (s, 1H, CH-pyrazole), 7.91 (s, 1H, CH-pyrazole), 8.56 (d, ^3J = 7.5 Hz, 1H, NH), 8.65 (d, ^3J = 8.8 Hz, 1H, NH), 11.45 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ [ppm] 36.6, 36.7, 51.9, 52.7, 53.7, 53.9, 55.0, 55.1, 55.2, 99.9, 105.1, 133.7, 114.1, 126.3, 126.5, 126.6, 128.0, 128.1, 128.3, 129.1, 129.3, 129.5, 134.8, 136.8, 137.1, 138.1, 144.8, 153.6, 155.5, 158.6, 158.8, 159.1, 171.2, 171.8; MS (ESI) *m*/*z* (%) 837 (M^+ + Na); mp 215 °C.

NO_2-Pz-Pz-L-Phe-L-Phe-OMe (18). In an argon atmosphere 20 mg (24.5 μmol, equiv) of NO_2-Pz(PMB)-Pz(PMB)-L-Phe-L-Phe-OMe (**18a**) was heated in 2 mL of anhydrous TFA for 2 h to 70 °C. The solvent was cooled to room temperature and an excess of an ice-cold mixture of diethyl ether and *n*-pentane (1:1) was added. The product precipitated as a colorless solid, which was filtered off, washed several times with *n*-pentane, and dried in vacuo. Yield 10 mg (12.4 μmol, 51%); colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] 2.92−3.10 (m, 4H, CH_2-Phe), 3.59 (s, 3H, CH_3), 4.49−4.56 (m, 1H, αCH), 4.71−4.75 (m, 1H, αCH), 7.15−7.37 (m, 11H, CH-arom), 7.93 (s, 1H, CH-pyrazole), 8.61 (d, ^3J = 7.3 Hz, 1H, NH), 8.80 (d, ^3J = 8.9 Hz, 1H, NH), 11.38 (s, 1H, NH), 13.2 (s, 1H, NH), 14.9 (s, 1H, NH); mp 140 °C; MS (ESI) *m*/*z* (%) 613 (M^+ + Na).

***N*-4-Methoxybenzyl-3-(9-fluorenylmethoxycarbonyl)-aminopyrazole-5-carboxylic Acid (Fmoc-Pz(PMB)-OH) (19).** In a Schlenck tube a solution of 910 mg (3.28 mmol) of *N*-4-methoxybenzyl-3-nitropyrazole-5-carboxylic acid methyl ester in 4 mL of anhydrous DMF was treated with 65 mg of Pd/C (10%). The mixture was subsequently transferred into an autoclave and stirred at a hydrogen pressure of 5 bar for 4 h at room temperature. After filtration over Celite at 0 °C, 1.26 mL (959 mg, 7.42 mmol) of *N*,*N*-diisopropylethylamine and 891 mg (3.44 mmol) of 9-(fluorenylmethyl)chloroformiate were added. The resulting solution was stirred for 5 h at room temperature, diluted with 4 mL of water, acidified with 1 N HCl (pH ∼1) and extracted with Et_2O (3 × 5 mL). The combined organic layers were washed with water and dried over Na_2SO_4. The solvent was removed in vacuo and the crude product was purified over silica gel (eluent CH_2Cl_2:MeOH:NH_3 100:10:1, R_f 0.2), furnishing pure **5** as a colorless solid (784 mg, 1.67 mmol, 51%). Mp 236 °C; IR (KBr) $\bar{\nu}$ [cm^{-1}] 3424, 3263, 3100, 2930, 1733, 1586, 1513, 1249; UV/vis (CH_3CN) λ_{max} [nm] (log ε) 208 (4.51), 265 (4.03), 275 (sh, 3.88), 289 (3.45), 300 (3.44); ^{1}H NMR (200 MHz, DMSO-d_6, TMS) δ [ppm] 3.71 (s, 3 H, CH_3), 4.20−4.36 (m, 3 H, CH_2-Fmoc, CH_{Fmoc}), 5.63 (s, 2 H, CH_{2Bzl}), 6.62 (br s, 1 H, $CH_{Pyraz.}$), 6.82−6.89 (m, 2 H, CH_{arom}), 7.14−7.22 (m, 2 H, CH_{arom}), 7.28−7.47 (m, 4 H, CH_{Fmoc}), 7.78 (d, 2 H, ^3J = 7.1 Hz, CH_{arom}), 7.90 (d, 2 H, ^3J = 7.1 Hz, CH_{arom}), 10.19 (br s, 1 H, NH); ^{13}C NMR (62 MHz, DMSO-d_6, TMS) δ [ppm] 46.4 (+), 52.0 (−), 55.0 (+), 66.0 (−), 99.4 (+), 113.6 (+), 120.0 (+), 125.4 (+), 127.0 (+), 127.6 (+), 128.8 (+), 130.6 (C_{quart}), 138.6 (C_{quart}), 140.6 (C_{quart}), 143.7 (C_{quart}), 145.4 (C_{quart}), 153.4 (C_{quart}), 158.4 (C_{quart}), 162.1 (C_{quart}); MS (FAB, glycerin/MeOH) *m*/*z* (%) 470 (100) $[MH]^+$; HRMS ($C_{27}H_{23}N_3O_5$) calcd 470.1716 $[MH]^+$, found 470.1708 $[MH]^+$ ±1.32 ppm. Anal. Calcd for $C_{27}H_{23}N_3O_5$: C 69.07; H 4.94, N 8.95. Found: C 68.93; H 4.88; N 8.64.

General Procedure for the Solid-Phase Peptide Synthesis (Fmoc Strategy). In the first step, the Fmoc-protected amino acid was coupled to the Sieber amide resin. To this end, the Fmoc group was cleaved off the resin by 20% piperidine in NMP (30 min, 2 runs). The first coupling step was performed twice with 5 equiv of Fmoc-protected amino acid, 5 equiv of HOBt, and 5 equiv of DIPCDI in NMP (6 h, maximum loading ∼0.2 mmol/g of resin, determined by gravimetry). All reagents

were added in quantities of 8 mL/g of resin. Deprotection of the resin-bound first amino acid was again effected with 20% piperidine in NMP (20 min, 2 runs). The next Fmoc-protected amino acid (3 equiv) was coupled twice with 3.3 equiv of HATU, 3.3 equiv of HOAt, and 30 equiv of collidine in NMP or alternatively 3.3 equiv of TBTU, 3.3 equiv of HOBt, and 8.4 equiv of DIEA in NMP (7 h each run), followed by a double deprotection step with 20% piperidine in NMP (30 min, 2 runs).[25] After the last amino acid was attached, the free *N*-terminal amino group was acetylated by treating 1.00 g of the resin (loading ~0.2 mmol/g) twice with a solution of 190 μL (2.0 mmol, 10 equiv) of Ac_2O and 68 μL (0.2 mmol, 1 equiv) of DIEA in 3 mL of CH_2Cl_2 (30 min, 2 runs). Finally the solvent was evaporated and the resin-bound peptide was triply washed with CH_2Cl_2. The Sieber amide resin was subsequently transferred into a PE syringe with integrated filterplate and swollen for 10 min in CH_2Cl_2. Then TFA in CH_2Cl_2 (2% v/v, 8 mL/g of resin) was added and the solution shaken for 5 min. The peptide cleavage procedure was repeated 8−10 times and the combined filtrates were evaporated to dryness on a rotatory evaporater. The resulting solid was dissolved in a little CH_2Cl_2, sonicated intensively, and precipitated again by addition of Et_2O. After filtration the crude product was dried in vacuo and purified by column chromatography or semipreparative HPLC. For the final PMB removal, the PMB-protected oligoamide was dissolved in a small amount of dry TFA and heated under argon within 20 min to 70−72 °C. The reaction mixture was kept for 5 min at this temperature; then the solvent was removed in vacuo and the remaining solid was dissolved in a small amount of dichloromethane, sonicated, and precipitated again by addition of ether. The crude product was collected by filtration, dried in high vacuum, and purified by HPLC. The combined fractions were evaporated to dryness and lyophilized. To liberate the free pyrazole amines, the resulting solid was suspended in saturated aqueous $NaHCO_3$, sonicated for 10 min, filtered, and dried. The colorless solids were usually spectroscopically pure.

Ac-Val-Pz(PMB)-Pz(PMB)-Val-NH$_2$ (20a). Fmoc-*Sieber* amide resin: 1.00 g (0.20 mmol/g); first loading, Fmoc-Pz-(PMB)-*Sieber* amide resin: 0.19 mmol/g. The product was purified by column chromatography over silica gel (eluent CH_2Cl_2:MeOH 10:1, R_f 0.34). Yield 125 mg (0.18 mmol, 92%) of a colorless solid. Mp 251 °C; $[\alpha]^{20}_D$ +26.7 (*c* 0.06 in DMSO); IR (KBr) $\bar{\nu}$ [cm^{-1}] 3423, 2965, 1669, 1576, 1514, 1470, 1250; UV/vis (CH_3CN) λ_{max} [nm] (log ϵ) 227 (4.37), 271 (sh, 4.05); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ [ppm] 0.84−0.93 (m, 12 H, CH_3-*i*-Pr), 1.88 (s, 3 H, CH_3-acetyl), 1.95−2.02 (m, 1 H, CH-*i*-Pr), 2.05−2.12 (m,1 H, CH-*i*-Pr), 3.70 (s, 3 H, CH_3), 3.71 (s, 3 H, CH_3), 4.21 (dd, 1 H, ^3J = 8.6 Hz, ^3J = 7.0 Hz, α-CH), 4.29 (dd, 1 H, ^3J = 8.6 Hz, ^3J = 7.2 Hz, α-CH), 5.50−5.67 (m, 4 H, CH_2-PMB), 6.83−6.89 (m, 4 H, CH-arom PMB), 6.83−6.89 (m, 4 H, CH-arom PMB), 7.09 (br s, 1 H, NH), 7.13−7.20 (m, 4 H, CH-arom PMB), 7.41 (s, 1 H, CH-pyrazole), 7.45 (br s, 1 H, NH), 7.45 (br s, 1 H, NH), 7.46 (s, 1 H, CH-pyrazole), 7.96 (d, 1 H, ^3J = 8.6 Hz, NH), 8.47 (d, 1 H, ^3J = 8.6 Hz, NH), 10.59 (s, 1 H, NH), 11.18 (s, 1 H, NH); ^{13}C NMR (62 MHz, DMSO-d_6, TMS) δ [ppm] 18.2 (+), 18.7 (+), 19.1 (+), 19.3 (+), 22.4 (+), 30.2 (+), 31.2 (+), 52.7 (−), 52.8 (−), 55.0 (+), 55.0 (+), 58.1 (+), 58.5 (+), 100.0 (+), 100.1 (+), 113.7 (+), 113.8 (+), 128.7 (+), 129.0 (+), 129.7 (C_{quart}), 129.7 (C_{quart}), 134.1 (C_{quart}), 134.8 (C_{quart}), 145.1 (C_{quart}), 145.5 (C_{quart}), 157.1 (C_{quart}), 158.6 (C_{quart}), 158.6 (C_{quart}), 159.1 (C_{quart}), 169.3 (C_{quart}), 169.8 (C_{quart}), 172.6 (C_{quart}); MS (FAB, glycerin/DMSO) *m*/*z* (%) 716 (100) [MH]$^+$; HRMS ($C_{36}H_{45}N_9O_7$) calcd 716.3520 [MH]$^+$, found 716.3509 [MH]$^+$ ±1.21 ppm.

Ac-Pz(PMB)-Val-Val-Pz(PMB)-NH$_2$ (20). Fmoc-*Sieber* amide resin: 1.00 g (0.20 mmol/g); first loading, Fmoc-Pz-(PMB)-*Sieber* amide resin: 0.19 mmol/g. The product was purified by column chromatography over silica gel (eluent CH_2Cl_2:MeOH 10:1, R_f 0.32). Yield 124 mg (0.17 mmol, 91%) of a colorless solid. Mp 185 °C; $[\alpha]^{20}_D$ +19.4 (*c* 0.07 in DMSO); IR (KBr) $\bar{\nu}$ [cm^{-1}] 3414, 3298, 2963, 2930, 1668, 1577, 1514, 1250; UV/vis (CH_3CN) λ_{max} [nm] (log ϵ) 222 (4.72), 271 (sh, 4.13); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ [ppm] 0.85−0.93 (m, 12 H, CH_3-*i*-Pr), 2.00 (s, 3 H, CH_3-acetyl), 2.02−2.20 (m, 2 H, CH-*i*-Pr), 3.69 (s, 3 H, CH_3), 3.71 (s, 3 H, CH_3), 4.28 (dd, 1 H, ^3J = 8.7 Hz, ^3J = 7.3 Hz, α-CH), 4.34 (dd, 1 H, ^3J = 8.5 Hz, ^3J = 7.3 Hz, α-CH), 5.45−5.65 (m, 4 H, CH_2-PMB), 6.79−6.89 (m, 4 H, CH-arom PMB), 7.12−7.18 (m, 5 H, CH-arom pMB, CH-pyrazole), 7.26 (s, 1 H, CH-pyrazole), 7.51 (br s, 1 H, NH_2), 7.97 (d, 1 H, ^3J = 8.5 Hz, NH), 8.06 (br s, 1 H, NH), 8.63 (d, 1 H, ^3J = 8.7 Hz, NH), 10.58 (s, 1 H, NH), 10.67 (s, 1 H, NH); ^{13}C NMR (62 MHz, DMSO-d_6, TMS) δ [ppm] 18.1 (+), 19.0 (+), 19.2 (+), 23.0 (+), 29.5 (+), 30.6 (+), 52.5 (−), 52.5 (−), 55.0 (+), 55.0 (+), 57.8 (+), 59.0 (+), 98.7 (+), 99.1 (+), 113.6 (+), 113.7 (+), 128.9 (+), 129.0 (+), 129.7 (C_{quart}), 129.9 (C_{quart}), 134.6 (C_{quart}), 134.7 (C_{quart}), 145.3 (C_{quart}), 145.9 (C_{quart}), 158.5 (C_{quart}), 158.6 (C_{quart}), 159.2 (C_{quart}), 160.8 (C_{quart}), 167.5 (C_{quart}), 169.2 (C_{quart}), 170.7 (C_{quart}); MS (FAB, glycerol/DMSO) *m*/*z* (%) 716 (100) [MH]$^+$; HRMS ($C_{36}H_{45}N_9O_7$) calcd 716.3520 [MH]$^+$, found 716.3528 [MH]$^+$ ±1.23 ppm.

Ac-Val-Pz(H)-Pz(H)-Val-NH$_2$ (21a). From Ac-Val-Pz(PMB)-Pz(PMB)-Val-NH_2 (**13a**): 100 mg (0.14 mmol). The crude product was purified by semipreparative HPLC. A colorless solid (47 mg) was obtained (0.11 mmol, 71%). Mp:188 °C; $[\alpha]^{20}_D$ +38.8 (*c* 0.07 in DMSO); IR (KBr) $\bar{\nu}$ [cm^{-1}] 3402, 2967, 1664, 1600, 1541; UV/vis (CH_3CN) λ_{max} [nm] (log ϵ) 222 (4.56); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ [ppm] 0.87−0.94 (m, 12 H, CH_3-*i*-Pr), 1.90 (s, 3 H, CH_3-acetyl), 1.96−2.12 (m, 2 H, CH-*i*-Pr), 4.25−4.35 (m, 2 H, α-CH), 7.07−7.36 (br, 3 H, 2 NH, CH-pyrazole), 7.52 (br s, 1 H, CH-pyrazole), 8.01 (d, 1 H,^3J = 8.5 Hz, NH), 8.05−8.27 (br s, 1 H, NH), 10.60 (br s, 1 H, NH), 10.92 (s, 1 H, NH), 12.60−13.80 (br, 2 H, 2 NH-pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ [ppm] 18.3 (+), 19.1 (+), 19.3 (+), 22.4 (+), 30.3 (+), 57.6 (+), 58.0 (+), 97.0 (+), 137.0 (C_{quart}), 145.9 (C_{quart}), 156.9 (C_{quart}), 156.2 (C_{quart}), 169.4 (C_{quart}), 169.8 (C_{quart}), 172.7 (C_{quart}); MS (FAB, glycerin/DMSO) *m*/*z* (%) 476 (38) [MH]$^+$; HRMS ($C_{20}H_{29}N_9O_5$) calcd 476.2370 [MH]$^+$, found 476.2366 [MH]$^+$ ±1.48 ppm.

Ac-Pz(*H*)-Val-Val-Pz(*H*)-NH$_2$ (21). From Ac-Pz(PMB)-Val-Val-Pz(PMB)-NH_2 (**14a**): 100 mg (0.14 mmol). The oligoamide **14b** was obtained as a colorless solid (54 mg, 0.17 mmol, 81%). Mp 201 °C; $[\alpha]^{20}_D$ +34.3 (*c* 0.07 in DMSO); IR (KBr) $\bar{\nu}$ [cm^{-1}] 3264, 2967, 1665, 1594, 1526; UV/vis (CH_3CN) λ_{max} [nm] (log ϵ) 222 (4.62); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ [ppm] 0.86−0.94 (m, 12 H, CH_3-Val), 1.98−2.13 (m, 5 H, 2H-*i*-Pr, CH_3), 4.29−4.41 (m, 2H, 2αH), 7.10 (s, 1 H, CH-pyrazole), 7.28 (s, 1 H, CH-pyrazol), 7.37 (s, NH), 7.90 (br, 2 H, 2NH), 8.44 (d, 1 H, *J* = 8.1 Hz, NH), 10.43 (s, 1 H, NH), 10.49 (s, 1 H, NH), 12.97 (s, 1 H, NH-pyrazole), 13.01 (s, 1 H, NH-pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6, TMS) δ [ppm] 18.2 (+), 18.7 (+), 19.0 (+), 19.2 (+), 23.0 (+), 30.1 (+), 30.5 (+), 57.8 (+), 57.9 (+), 95.9 (+), 96.5 (+), 137.4 (C_{quart}), 146.3 (C_{quart}), 158.8 (C_{quart}), 160.4 (C_{quart}), 167.5 (C_{quart}), 169.1 (C_{quart}), 170.8 (C_{quart}); MS (ESI, MeOH + 10 mmol of NH_4Ac) *m*/*z* (%) 474 (100) [M]$^-$; HRMS ($C_{20}H_{29}N_9O_5$) calcd 476.2370 [MH]$^+$, found 476.2362 [MH]$^+$ ±0.90 ppm.

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(25) This method is used for the direct coupling of pyrazole-based and proteinogenic amino acids. In the case of two aliphatic amino acids, coupling times can be considerably shortened.