

# PEG-supported synthesis of pyrazole oligoamides with peptide $\beta$ -sheet affinity †

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Pyrazole amino acid oligoamides were prepared on polyethylene glycol starting from nitro pyrazole carboxylic acids or protected pyrazole amino acids. The polymer support facilitates product isolation during synthesis and makes the target oligoamides soluble in chloroform and water. This allows the determination of their binding properties towards peptides. Moderate affinity, which increases with the number of pyrazole units, is observed in chloroform and water.

## Introduction

$\beta$ -Sheets are prominent structural features of peptides and proteins.<sup>1</sup> They ensure protein function, but at the same time they can be the source of serious protein misfunction, as in the amyloid plaque formation<sup>2–5</sup> connected with Alzheimer's disease.<sup>6–9</sup> Synthetic receptors with  $\beta$ -sheet affinity may be useful for clinical diagnostic, medicinal chemistry and biotechnology, and they are therefore the aim of ongoing research. In most cases structures that closely resemble a  $\beta$ -sheet motif have been used for molecular recognition. Heterocycles with a complementary hydrogen bonding pattern have been identified as particularly useful due to their rigid structure providing sufficient preorganization. Hydrazino benzoic acids<sup>10–16</sup> and pyrazole amino acids<sup>17,18</sup> are established binding motifs, but so far their peptide affinity is mainly restricted to non polar organic solvents. In sequence-selective DNA binding the concept of modular oligomerization was successfully applied by Dervan<sup>19</sup> and others<sup>20</sup> to obtain highly selective binders with up to nanomolar affinity. However, the well known rapid decrease of solubility of heterocyclic oligoamides limits the adaptation of this strategy to peptide binding. Nevertheless, extension of the  $\beta$ -sheet peptide binding motif seems necessary to obtain synthetic receptors with higher performance. A first step has recently been taken in this direction by dimerization and oligomerization of aminopyrazoles.<sup>21</sup> However, the synthetic procedures are complicated by the excessive use of protecting groups; in addition, some of the higher oligomers turned out to be only sparingly soluble in organic solvents. Nevertheless, in cooperation with biophysicists, several highly promising candidates were identified among those oligomers which are capable of preventing the aggregation of the Prion protein as well as the Alzheimer's protein A $\beta$  (1–42).<sup>22</sup> Further tests in cell cultures are in progress. These encouraging results call for the development of a convenient assembly of oligomeric  $\beta$ -sheet ligands with improved solubility. We report here liquid phase procedures for the synthesis of pyrazole amino acid oligoamides on a polyethylene glycol support. The PEG support facilitates oligoamide synthesis and provides high solubility in organic and aqueous solvents.

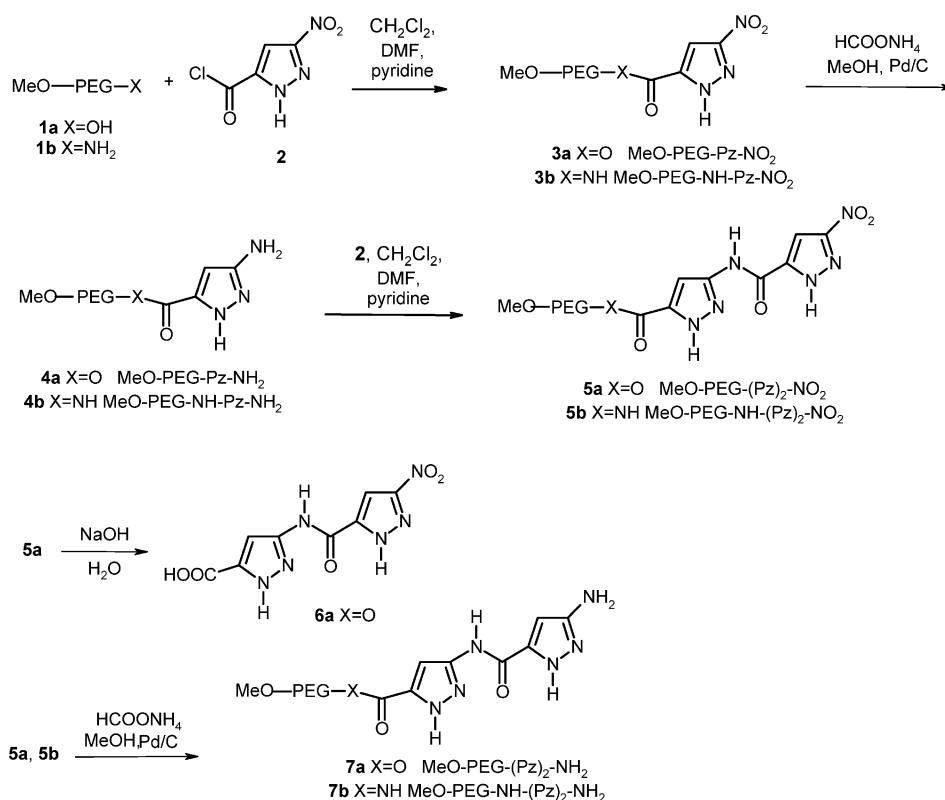
## Results and discussion

Peptide synthesis on polyethylene glycol (PEG) as polymer support is an established method. First introduced by Bayer and Mutter,<sup>23</sup> the method was more recently used by Janda<sup>24</sup> and others in combinatorial approaches. The main advantages of the method are reaction on support in homogeneous phase which can be monitored by solution NMR and the good availability of the polymer. Disadvantages are the lower loading capacity of the polymer if compared to resins and a more difficult automatization of synthesis procedures. For the synthesis of oligoamides with poor solubility (which counts for most peptides derived from heteroaromatic amino acids), however, PEG support is of particular benefit to ensure good solubility during the synthesis and for applications.<sup>25</sup> Bound to PEG support the target oligoamides become soluble both in water and chloroform.

### Synthesis of pyrazole oligoamides on PEG starting from pyrazole nitro carboxylic acid

Methoxypoly(ethylene glycol) (MeO-PEG-X) **1a** (X = OH), **1b** (X = NH<sub>2</sub>) with an average molecular weight of 5000 g mol<sup>-1</sup> was used as polymer support. 5-Nitro-3-pyrazolecarbonyl chloride (**2**) is bound to MeO-PEG-OH (**1a**) or MeO-PEG-NH<sub>2</sub> (**1b**) via an ester or amide linkage using standard conditions (Scheme 1). For purification the polymer bound products **3** are precipitated by addition of Et<sub>2</sub>O. Excess of acid chloride **2** is removed with ether. The nitro group is reduced by transfer hydrogenation with HCOONH<sub>4</sub>-Pd/C in homogeneous methanol solution at room temperature, the catalyst is removed by filtration and Et<sub>2</sub>O is added to precipitate both the amine and excess HCOONH<sub>4</sub>. The solid is dissolved in dichloromethane leaving behind excess HCOONH<sub>4</sub> and amines **4** are precipitated with Et<sub>2</sub>O. The second heterocyclic amino acid is introduced by yet another reaction of **4** with **2**. The reaction proceeds quantitatively, as confirmed by NMR spectroscopy. Oligoamide **5a** was cleaved from the polymer support by treatment with a base providing the corresponding carboxylic acid **6a** in good yield. Reduction of **5** gives amines **7**, but the subsequent reaction with **2** is not a clean process. A mixture of the desired coupling product, unreacted **7** and not identified byproducts is obtained, which prohibits any further transformation. The loading of the polymer significantly decreases. Therefore the synthesis of extended pyrazole oligoamides requires protected building blocks.

† Electronic supplementary information (ESI) available: NMR and IR spectra of synthesized compounds. See <http://www.rsc.org/suppdata/ob/b4/b401968g/>



Scheme 1 Synthesis of amides 5, 6, and 7 from pyrazole 2.

### Synthesis of pyrazole oligoamides on PEG from a N-protected nitro pyrazole acid

The limitation of the protecting group-free procedure to two pyrazolic fragments made it necessary to use the protected nitro pyrazole acid **8**<sup>21,26</sup> for the preparation of more extended oligoamides. Compound **8** was activated with DCC, HOBT and DIEA as base in DMF yielding **9** with quantitative loading of the polymer (Scheme 2). Reduction of the nitro group with HCOONH<sub>4</sub> and Pd/C in methanol gave the corresponding amines **10**. For the introduction of the second pyrazolic fragment a different method of activation, using HATU, HOAt and collidine as a base in DMF, was employed. Oligoamides **11**, with two pyrazolic fragments, were obtained quantitatively. Repetition of the reduction and coupling step gave compounds **12**, with three pyrazolic fragments, and **13**, with four pyrazolic fragments. The coupling and loading of the polymer was monitored by <sup>1</sup>H NMR for each step and found to be quantitative. To obtain the desired peptide  $\beta$ -sheet binding motif, the PMB protecting groups have to be removed by treatment with TFA.<sup>27</sup> Surprisingly the PEG ester linkage remains intact under the rather harsh conditions of deprotection. PEG-bound deprotected pyrazole oligoamides **14** and **15** were obtained in high yield as their TFA salts.<sup>28</sup>

This procedure is a highly flexible protocol. Thus, it also allows the synthesis of peptides that contain natural amino acids in combination with pyrazole amino acids, such as in oligoamide **20**. Starting from compound **10a** Fmoc protected valine was introduced in excellent yield using HATU, HOAt, DIEA in DMF and CH<sub>2</sub>Cl<sub>2</sub> to give **16** (Scheme 3). After deprotection with 20% piperidine in DMF the second Fmoc valine is introduced and again deprotected to give **18**. Coupling to nitro pyrazole acid **8** and reduction completes the synthesis of **19**, which is deprotected to yield compound **20**.

### Determination of binding properties

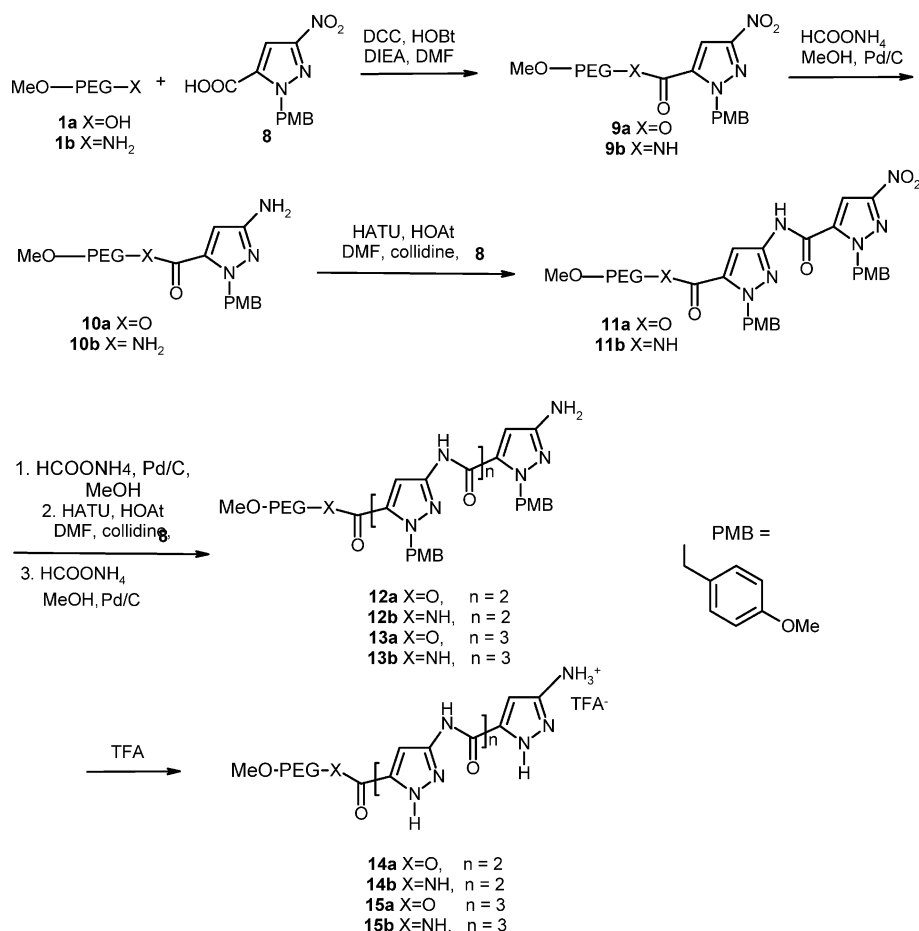
The binding affinities of the PEG-pyrazoles **5**, **7**, **14** and **15** to small peptides<sup>29</sup> of natural amino acids were determined by <sup>1</sup>H NMR titration in CDCl<sub>3</sub>. To avoid multiple binding equilibria

Table 1 *K<sub>a</sub>* values determined from NMR titrations of Ac-Val-Val-OMe with ligands **4a**, **4b**, Boc-Phe-Ala-Val-Leu-OMe with ligands **7a**, **7b** and Ac-Lys-Leu-Lys-Leu-Lys-Leu-OEt with ligands **14a**, **14b** in CDCl<sub>3</sub>

Compound	Binding constant <sup>a</sup>
<b>4a</b>	25 M <sup>-1</sup>
<b>4b</b>	39 M <sup>-1</sup>
<b>7a</b>	100 M <sup>-1</sup>
<b>7b</b>	160 M <sup>-1</sup>
<b>14a</b>	790 M <sup>-1</sup>
<b>14b</b>	950 M <sup>-1</sup>

<sup>a</sup> All derived binding constants have errors of approx.  $\pm 10\%$ .

only those peptides were used which match in length the oligoamide under investigation. In addition, the amino acid residues chosen for these studies show a high propensity for  $\beta$ -sheet formation. Binding constants were determined by non linear fitting of chemically induced shifts (CIS) of several protons to a 1 : 1 binding model (see Experimental section for details).<sup>30</sup> Self-association of the peptides was determined by dilution experiments and taken into account.<sup>31</sup> Addition of PEG **1** to the peptides does not induce any changes in chemical shifts. The results of binding studies are summarized in Table 1. Fig. 1 shows the titration curves of compounds **4a,b** and **7a,b**. The affinity increases, as expected, with increasing length of the binding motif leading to more hydrogen bond contacts. Since hydrogen bonds are strong in non-polar solution such as chloroform (1–2 kcal mol<sup>-1</sup> for each C=O  $\cdots$  HN<sub>amide</sub> interaction), and typical downfield-shifts of the NH protons accompany the complexation, the affinity increase with the growing aminopyrazole oligomer length provides experimental evidence for the multipoint binding mode, already found in monomeric and dimeric systems. The differences in binding affinity between pyrazole oligoamides connected to PEG by amide or ester linkage are small. The additional hydrogen bond in amide derivatives **4b**, **7b** and **14b** leads to a small increase in affinity (Table 1). For compound **15**, although still soluble



Scheme 2 Synthesis of PEG-pyrazole oligoamides **14** and **15**.

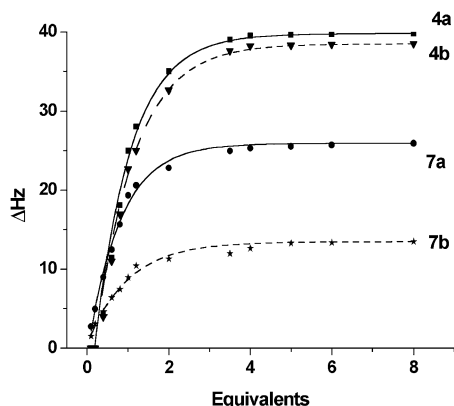


Fig. 1 NMR titration curves for binding experiments of Ac-Val-Val-OEt with ligands **4a**, **4b** and Boc-Phe-Ala-Val-Leu-OMe with ligands **7a**, **7b** in CDCl<sub>3</sub>,  $c_0 = 2.6 \times 10^{-2} \text{ mol l}^{-1}$ . CIS of pyrazole C-H and peptide amide N-H is observed.

due to the PEG support, no chemical induced shift was observed in the titration with a hexapeptide. The increasing curvature of the oligoamide, its growing tendency to form aggregates<sup>32</sup> or internal hydrogen bonds<sup>33</sup> may prohibit peptide binding.

The PEG-support makes the pyrazole oligoamides soluble in water. Their affinity to model peptides of natural amino acids in buffered aqueous solution (phosphate buffer : D<sub>2</sub>O = 9 : 1; pH = 7, for **4a,b**, **7a,b**; or pH = 5.2, for **14a,b**) was again determined by <sup>1</sup>H NMR titration. The chemically induced shift of the 4-pyrazole C-H protons was observed. Table 2, Figs. 3 and 4 summarize the results for compounds **4a,4b**, **7a,7b**, **14a** and **14b**. The observed affinities are in the same order of magnitude as in chloroform.<sup>34</sup> This is very interesting, because for a binding mechanism relying solely on hydrogen bonds, water is

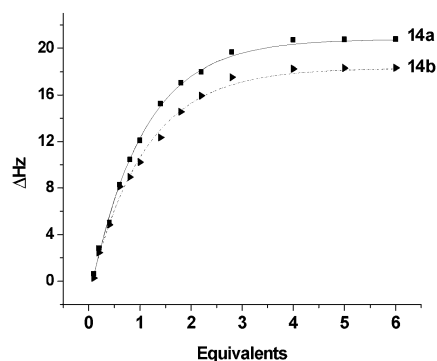


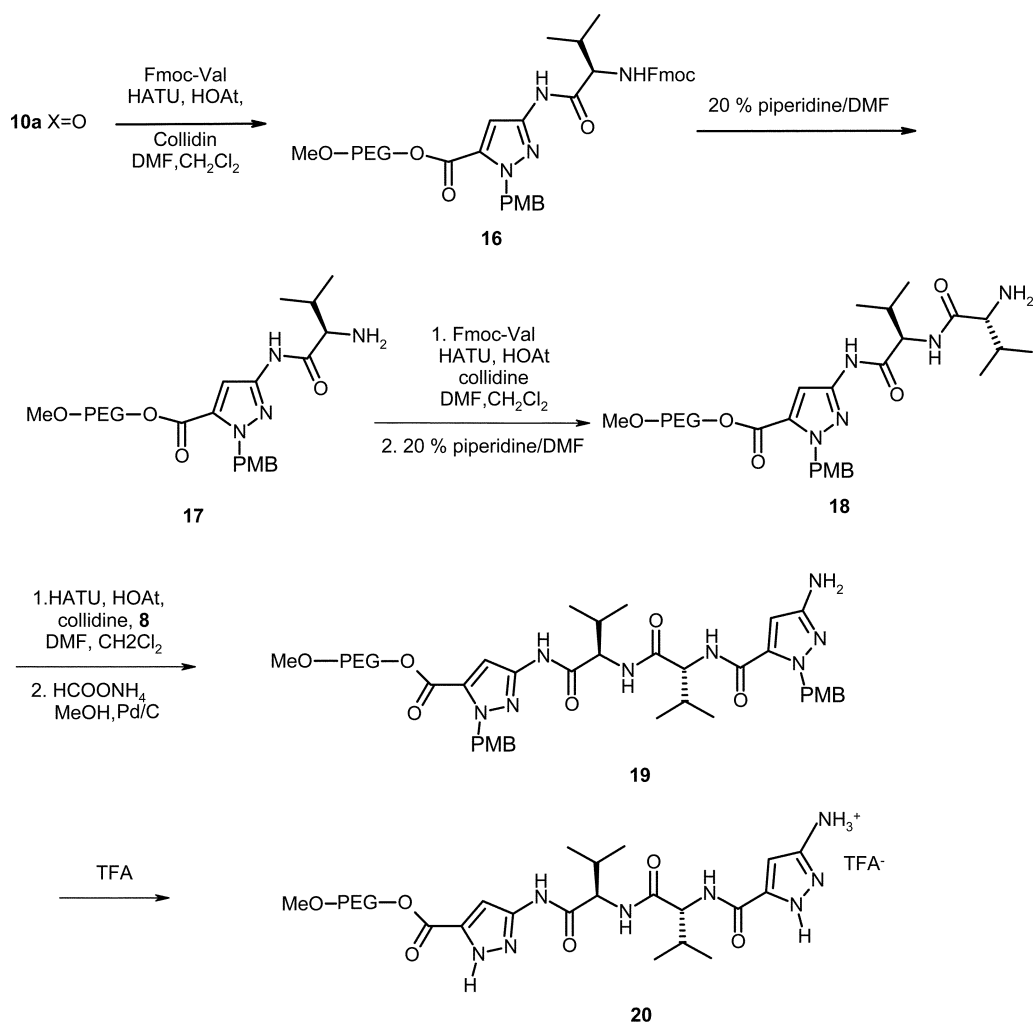
Fig. 2 Binding isotherms from NMR titration experiments of Ac-Lys-Leu-Lys-Leu-OEt with ligands **14a**, **14b** in CDCl<sub>3</sub>,  $c_0 = 2.6 \times 10^{-2} \text{ mol l}^{-1}$ . CIS of pyrazole C-H and peptide amide N-H is observed.

Table 2  $K_a$  values determined from NMR titrations of H-His-Gly-Gly-OH with ligands **4a**, **4b**, **7a**, **7b** (pH 7) and Ac-Lys-Leu-Lys-Leu-OEt with ligands **14a**, **14b** (pH 5.2) in phosphate buffer : D<sub>2</sub>O = 9 : 1

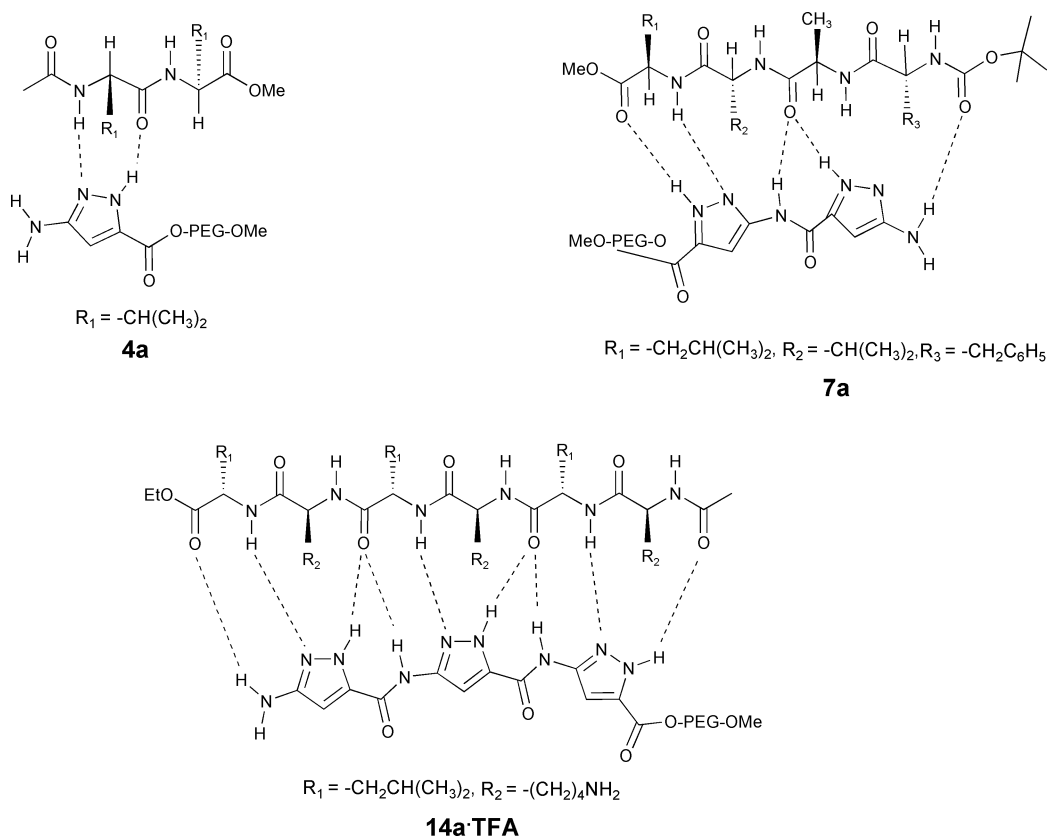
Compound	Binding constant <sup>a</sup>
<b>4a</b>	6 M <sup>-1</sup>
<b>4b</b>	13 M <sup>-1</sup>
<b>7a</b>	32 M <sup>-1</sup>
<b>7b</b>	79 M <sup>-1</sup>
<b>14a</b>	400 M <sup>-1</sup>
<b>14b</b>	450 M <sup>-1</sup>

<sup>a</sup> All derived binding constants have errors of approx. ±10%.

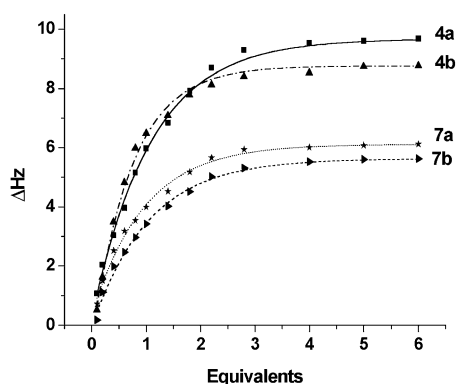
not the appropriate solvent. Thus, the relatively low decrease in free binding enthalpy between complexation in chloroform and in water proves the existence of additional attractive forces.



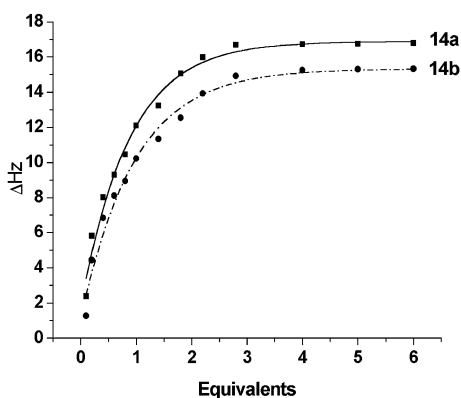
**Scheme 3** Synthesis of oligoamide **20**.



**Scheme 4** Proposed hydrogen bond motifs of PEG-oligoamides and model peptides in  $\text{CDCl}_3$ .



**Fig. 3** NMR titration curves from binding experiments of free H-His-Gly-OH with ligands **4a**, **4b** and **7a**, **7b** in phosphate buffer : D<sub>2</sub>O = 9 : 1, pH 7,  $c_0 = 2.6 \times 10^{-2} \text{ mol l}^{-1}$ . CIS of pyrazole ring C-H and peptide amide N-H resonances is observed.



**Fig. 4** Binding isotherms from NMR titration experiments of Ac-Lys-Leu-Lys-Leu-Lys-Leu-OEt with ligands **14a**, **14b** in phosphate buffer: D<sub>2</sub>O = 9 : 1, pH = 5.2;  $c_0 = 2.6 \times 10^{-2} \text{ mol l}^{-1}$ . CIS of pyrazole C-H resonances is observed.

It seems very likely that hydrophobic interactions between aromatic or non polar amino acid residues in the peptides and aminopyrazole-based oligomers substantially contribute to the host-guest interaction in water. This may also explain the efficiency of some of these  $\beta$ -sheet binders in preventing the pathological aggregation of PrP<sup>Sc</sup> and A $\beta$  protein. Compounds **15** and **20** showed no significant chemically induced shifts of proton resonances upon titration with a hexapeptide. Aggregation or folding may eliminate their ability for intermolecular binding, but spectroscopic measurements did not provide conclusive evidence for this assumption.

## Modeling

Force-field calculations were initially carried out with MacroModel 7.2 (Amber\*, chloroform or water, 1000 steps) to optimize the host and guest conformations prior to complexation (Fig. 5). Then the formation of 1 : 1 complexes was calculated by molecular mechanics, followed by a Monte-Carlo conformational search in chloroform and water, respectively.<sup>35</sup> The three-point binding mode between each aminopyrazole, clamping together two amino acid residues, was found in both solvents. In spite of the experimental failure to prove affinity of **15** and **20** towards the hexapeptide Ac-(Lys-Leu)<sub>3</sub>-OEt, the modeling experiments suggested formation of a 1 : 1-complex with the peptide in its extended conformation.

## Conclusion

Oligoamides of pyrazole amino acids are conveniently synthesized on PEG as soluble polymer support. Dipeptides are

prepared from pyrazole nitro carboxylic acids without the use of protecting groups, while more extended oligoamides require the use of PMB-protected pyrazole amino acid building blocks. All transformations on PEG support are quantitative as confirmed by NMR monitoring. The PEG support facilitates workup during synthesis and makes the target oligoamides soluble in chloroform and water. This allows the determination of binding affinities of otherwise insoluble pyrazole oligoamides to model peptides from natural amino acids. NMR titrations reveal moderate affinities, increasing up to three pyrazole units due to the multipoint binding mode. Interestingly, only slightly lower association constants are measured for the complexation of peptides in aqueous buffer, probably because the repertoire of noncovalent interactions is complemented by hydrophobic attraction. This is encouraging for the development of  $\beta$ -sheet binders for aggregation prevention under physiological conditions. Tetraamides **15** and **20** did not show affinity to model peptides of natural amino acids in chloroform or aqueous buffer. It may be speculated that the lysine residues of the hexapeptide guest could be captured by the oligoethylene tail in a crown-ether-like fashion, and compete with the backbone recognition by the aminopyrazole moieties.

In summary, the reported synthetic procedure allows the preparation of sparingly soluble heterocyclic oligoamides and the investigation of their binding properties to peptide structures in chloroform and water. The concept is general and can be applied to prepare water soluble extended synthetic receptors in a sequential modular way from known building blocks with affinity to peptide structures.

## Experimental

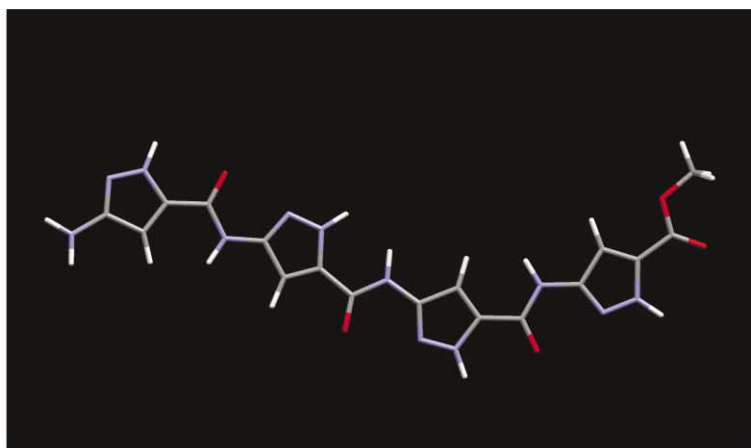
### General

<sup>1</sup>H NMR Spectra were recorded at 300 MHz in DMSO-d<sub>6</sub> unless otherwise stated. The conversion of all reactions on PEG support and the loading of the polymer was monitored by NMR. To calculate elemental compositions the following average MeO-PEG 5000 structures have been assumed: H<sub>3</sub>C-O-(C<sub>2</sub>H<sub>4</sub>O)<sub>110</sub>-C<sub>2</sub>H<sub>4</sub>OH (4922 g mol<sup>-1</sup>) or H<sub>3</sub>C-O-(C<sub>2</sub>H<sub>4</sub>O)<sub>110</sub>-C<sub>2</sub>H<sub>4</sub>NH<sub>2</sub> (4921 g mol<sup>-1</sup>). Abbreviations used: Et, ethyl; AcO, acetate; Pz, pyrazole, MeO-PEG, methoxypolyethylene(glycol); DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; DCC, *N,N*-dicyclohexylcarbodiimide; HOBT, 1-hydroxybenzotriazole; DIEA, *N,N*-diisopropylethylamine; HATU, *O*-(7-azabenzotriazol-1-yl)-*N,N,N'*-tetramethyluroniumhexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; PMB, *para*-methoxybenzyl; TFA, trifluoroacetic acid.

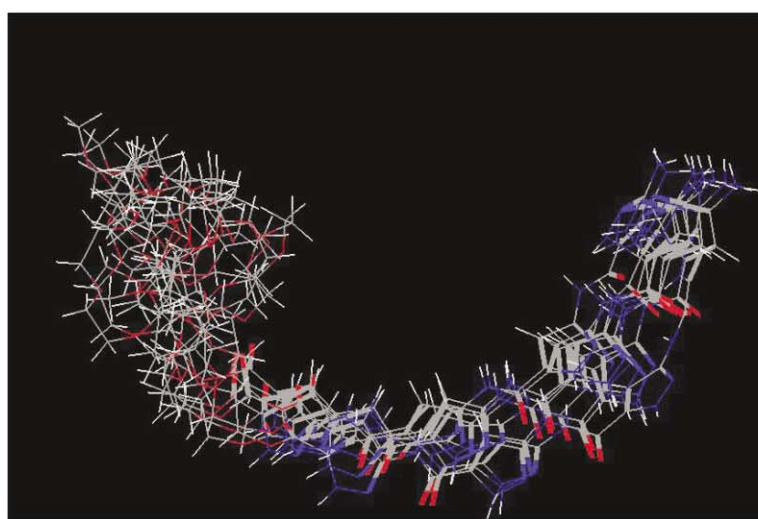
### General procedure 1 (GP 1) for the reduction of PEG-bound nitro pyrazoles

The MeO-PEG-bound nitro compound (1 g, 0.2 mmol) was dissolved in methanol (not more than 100 ml). For large-scale reactions a minimum amount of dichloromethane had to be added until the entire solid was dissolved. Excess NH<sub>4</sub>HCO<sub>2</sub> (0.5 g, 8.3 mmol) and a catalytic amount Pd/C (10%, 100 mg) were added, and the reaction mixture was stirred for 1 h at room temp. The start of the reaction was indicated by the evolution of gas. In cases where this could not be observed the reaction mixture was heated with a heat gun for a short period. After 1 h at room temp. the catalyst was removed by filtration over Celite and Et<sub>2</sub>O was added (250–1000 ml depending on the scale of the reaction) to precipitate the MeO-PEG-bound amine and excess NH<sub>4</sub>HCO<sub>2</sub>. The solid was collected by suction and dissolved in dichloromethane leaving behind excess NH<sub>4</sub>HCO<sub>2</sub> that was filtered off. The obtained solution was once more precipitated with Et<sub>2</sub>O (200 ml), filtered and dried *in vacuo*.

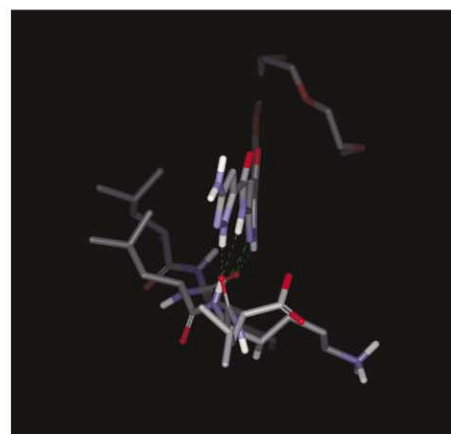
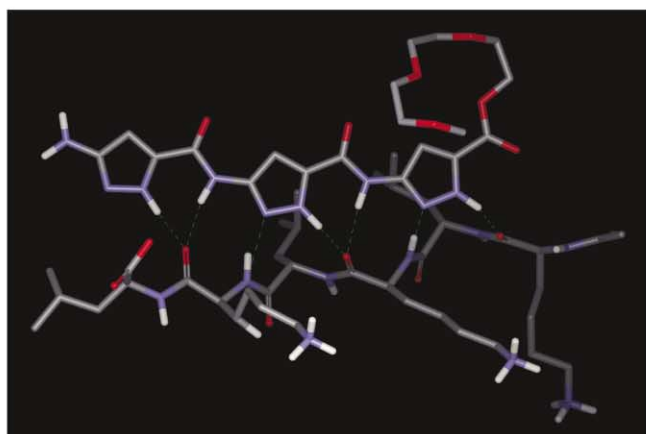
a)



b)



c)



**Fig. 5** a) Two minimum conformations for the core unit of tetrameric ligand **19**, found by a Monte-Carlo conformational search (MacroModel 7.2, Amber\*, water, 5000 steps). b) Subsequent molecular dynamics calculation of the whole ligand including the ethylene glycol tail (100 ps, superimposed snapshots every 10 ps). c) Proposed structure for the complex between trimeric host **18** [ $\text{H}_3\text{N}^+\text{-Pz}_3\text{-(C=O)O-PEG}$ ] and the hexapeptide Ac-(Lys-Leu)<sub>3</sub>-OMe in water (MacroModel 7.2, OPLS-AA, Monte-Carlo simulation, 2000 steps). Note leucine's isobutyl groups bending towards the aminopyrazole nuclei for hydrophobic interactions.

#### General procedure 2 (GP 2) for coupling of PEG-bound pyrazole amines with acid chloride 2

To a solution of the polymer-bound amino compound (0.85 g, 0.0017 mmol) from **GP 1** in dry  $\text{CH}_2\text{Cl}_2$  (25 ml) an excess of pyridine (13 mmol) and 3–5 equivalents of the appropriate

acid chloride were added and the reaction mixture was stirred for 12 h. The product was precipitated by addition of  $\text{Et}_2\text{O}$  and collected by filtration. The polymer was redissolved, precipitated twice for purification and dried *in vacuo*.

### General procedure 3 (GP 3) for the coupling of PEG-bound pyrazole amines with activated acids I

3–5 Equivalents of the appropriate amino acid were activated with DCC and HOBT·H<sub>2</sub>O (1.0 equivalent of each, corresponding to the amount of acid) in DMF (25 ml) for 4 h. To this reaction mixture the PEG-bound amino compound from **GP 1** (1 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> was added followed by an excess of DIEA. After 12 h of stirring at room temp. the reaction mixture was filtered and the product was precipitated by addition of Et<sub>2</sub>O, and collected by filtration. The polymer was redissolved, precipitated twice and dried *in vacuo*.

### General procedure 4 (GP 4) for the coupling of PEG-bound pyrazole amines with activated amino acids I

3–5 Equivalents of the appropriate acid were activated with HATU and HOAt·H<sub>2</sub>O (1.1 equivalent of each, corresponding to the amount of acid) in DMF (25 ml) for 4 h. To this reaction mixture the PEG-bound amino compound (1 mmol) from **GP 1** in dry CH<sub>2</sub>Cl<sub>2</sub> was added followed by an excess of collidine. After 12 h of stirring at room temp. the reaction mixture was filtered and the product was precipitated by addition of Et<sub>2</sub>O, and collected by filtration. The polymer was redissolved, precipitated twice and dried *in vacuo*.

### General procedure 5 (GP 5) for the cleavage of the oligoamide from the polymer

The MeO-PEG-bound oligoamide was dissolved in 10 ml of aqueous 2 M NaOH and stirred at room temp. for 12 h. The solution was acidified with HCl, the precipitated oligoamide was collected by filtration and dried *in vacuo*.

### General procedure 6 (GP 6) for PMB deprotection

The PMB protected PEG-bound oligoamide (0.2 g) was dissolved in a small amount TFA (3 ml) and heated to 72 °C. The reaction conversion was monitored by TLC (EtOAc) detecting the cleaved arene. The PEG-bound product of the reaction was precipitated by the addition of Et<sub>2</sub>O and filtered off.

### General procedure 7 (GP 7) for Fmoc deprotection of PEG-bound oligoamides

The Fmoc-protected PEG-bound compound was dissolved in 20% piperidine in DMF. The solution was stirred for 2 h at room temp. and the product was precipitated by the addition of Et<sub>2</sub>O and collected by filtration. The polymer was redissolved and precipitated twice for purification, and dried *in vacuo*.

**4-Nitro-3-pyrazolecarbonyl chloride (2).** 5-Nitro-3-pyrazolecarboxylic acid (5 g, 32 mmol) was dissolved in 10 ml of thionyl chloride, 5 drops of DMF were added and the solution was refluxed for 3 h at 85 °C. After cooling to room temp. the excess of thionyl chloride was evaporated, the crude product was suspended in benzene (10 ml), evaporated and dried *in vacuo*. Yield 5.6 g (94%) of 4-nitro-3-pyrazolecarbonyl chloride, as a yellow solid, mp.: >340°C. For analytical characterisation a small sample was converted into the methyl ester<sup>36</sup> by heating it in dry methanol. IR (CHCl<sub>3</sub>):  $\tilde{\nu}$  = 3620 cm<sup>-1</sup>, 3019, 1744, 1516, 1320, 1215. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  = 3.44 (s, 3H), 8.47 (s, 1H).

**MeO-PEG-X-Pz-NO<sub>2</sub> (X = O 3a, X = NH 3b).** MeO-PEG-X (X = OH **1a**, X = NH<sub>2</sub> **1b**) (1.0 g, 0.2 mmol) was allowed to react with **2** (0.2 g, 1.1 mmol) following **GP 2** (pyridine: 0.3 ml) to yield **3a** (0.94 g, 94%), **3b** (0.93 g, 93%). The NMR spectra confirm the complete loading of the PEG polymer support. **3a**: <sup>1</sup>H-NMR:  $\delta$  = 3.24 (s, 3 H), 7.49 (s, 1 H), 15.24 (s, 1H). IR (KBr):  $\tilde{\nu}$  = 3500 (w) cm<sup>-1</sup>, 2886 (s), 1705 (w), 1465, 1108 (s).

Anal. (%) C<sub>227</sub>H<sub>449</sub>N<sub>3</sub>O<sub>115</sub> (5061): calcd.: C 53.87, H 8.94, N 0.83; found: C 53.76, H 8.81, N 0.85. **3b**: <sup>1</sup>H-NMR:  $\delta$  = 3.24 (s, 3 H), 7.50, 7.64 (s, 1 H), 8.89 (s, 1H), 14.80 (s, 1H).

**MeO-PEG-X-Pz-NH<sub>2</sub> (X = O 4a, X = NH 4b).** MeO-PEG-X-Pz-NO<sub>2</sub> (X = O **3a**, X = NH **3b**) (1.0 g, 0.2 mmol) was reduced according to **GP 1** (NH<sub>4</sub>HCO<sub>2</sub>: 0.5 g, 7.9 mmol; Pd/C: 0.1 g) to yield **4a** (0.85 g, 85%), **4b** (0.80 g, 80%). The NMR spectra confirm the complete loading of the PEG polymer support. **4a**: <sup>1</sup>H-NMR (CDCl<sub>3</sub>):  $\delta$  = 3.31 (s, 3 H), 6.06 (s, 1 H). IR (KBr):  $\tilde{\nu}$  = 3500 (w) cm<sup>-1</sup>, 2887 (s), 1719 (w), 1467, 1342, 1108 (s). Anal. (%) C<sub>227</sub>H<sub>451</sub>N<sub>3</sub>O<sub>113</sub> (5031): calcd.: C 54.19, H 9.04 N 0.84; found: C 53.86, H 8.57, N 0.79. **4b**: <sup>1</sup>H-NMR (CDCl<sub>3</sub>):  $\delta$  = 3.15 (s, 3 H), 5.87 (s, 1 H). IR (KBr):  $\tilde{\nu}$  = 3500 (w) cm<sup>-1</sup>, 2887 (s), 1719 (w), 1467, 1342, 1108 (s). Anal. (%) C<sub>227</sub>H<sub>452</sub>N<sub>4</sub>O<sub>112</sub> (5030): calcd.: C 54.20, H 9.06, N 1.11; found: C 53.87, H 8.82, N 1.01.

**MeO-PEG-X-(Pz)<sub>2</sub>-NO<sub>2</sub> (X = O 5a, X = NH 5b).** MeO-PEG-X-Pz-NH<sub>2</sub> (X = O **4a**, X = NH **4b**) (1.0 g, 0.2 mmol) was allowed to react with **2** (0.2 g, 1.1 mmol) following **GP 2** (pyridine: 0.3 ml) to yield **5a** (0.97 g, 97%) and **5b** (0.94 g, 94%). The NMR spectra confirm the complete loading of the PEG polymer support. **5a**: <sup>1</sup>H-NMR:  $\delta$  = 3.25 (s, 3H), 7.14 (s, 1H, CH), 7.94 (s, 1H), 11.52 (s, 1H), 13.84 (s, 1H), 14.99 (s, 1H). **5b**: <sup>1</sup>H-NMR:  $\delta$  = 3.31 (s, 3H), 7.35 (s, 1H), 7.90 (s, 1H), 8.48 (s, 1H), 11.32 (s, 1H), 13.28 (s, 1H), 14.97 (s, 1H).

**O<sub>2</sub>N-Pz(H)-Pz(H)-OH (6a).** Compound **5a** (820 mg, 0.16 mmol) was treated according to **GP 5** and gave 23 mg of **6a** (54%), as a yellow solid, mp 214–216 °C. IR (KBr):  $\nu$  = 3212 cm<sup>-1</sup>, 2925, 1665, 1542, 1480, 1320, 1251, 991. <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 7.04 (s, 1 H), 8.91 (s, 1 H), 11.24 (s, 1 H), 13.52 (s, 1 H), 14.16 (s, 1 H). <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 99.9 (+), 102.2 (+), 134.6 (C<sub>quat</sub>), 138.5 (C<sub>quat</sub>), 146.2 (C<sub>quat</sub>), 155.0 (C<sub>quat</sub>), 155.7 (C<sub>quat</sub>), 160.2 (C<sub>quat</sub>). MS (EI, 70 eV): *m/z* (%): 266 (68) [M]<sup>+</sup>. HRMS (C<sub>8</sub>H<sub>6</sub>N<sub>6</sub>O<sub>5</sub>) calc. 266.0399 [M]<sup>+</sup>, found 266.0397 [M]<sup>+</sup>.

**MeO-PEG-X-(Pz)<sub>2</sub>-NH<sub>2</sub> (X = O 7a, X = NH 7b).** MeO-PEG-X-(Pz)<sub>2</sub>-NO<sub>2</sub> (X = O **5a**, X = NH **5b**) (1.0 g, 0.2 mmol) was reduced according to **GP 1** (NH<sub>4</sub>HCO<sub>2</sub>: 0.5 g, 7.9 mmol; Pd/C: 0.1 g) to yield **7a** (0.84 g, 84%), resp. **7b** (0.79 g, 79%). The NMR spectra confirm the complete loading of the PEG polymer support. **7a**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 3.37 (s, 3H), 6.01, 6.07 (s, 1H), 7.08, 7.29 (s, 1H), 8.38, 8.42 (s, 1H), 8.59, 8.72 (s, 1H). **7b**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 3.38 (s, 3H), 6.01 (s, 1H), 6.83 (s, 1H), 7.71 (s, 1H), 8.59 (s, 1H), 10.14 (s, 1H). IR (KBr):  $\tilde{\nu}$  = 3433 (w) cm<sup>-1</sup>, 2888 (s), 1671 (m), 1467, 1343, 1109 (s), 842. Anal. (%) C<sub>230</sub>H<sub>455</sub>N<sub>7</sub>O<sub>112</sub> (5111): calcd.: C 54.05, H 8.97, N 1.92; found: C 53.96, H 8.55, N 1.72.

**MeO-PEG-X-PzPMB-NO<sub>2</sub> (X = O 9a, X = NH 9b).** MeO-PEG-X (X = O **1a**, X = NH<sub>2</sub> **1b**) (5.0 g, 1.0 mmol) was allowed to react with **8** (1 g, 3.6 mmol) following **GP 3** (DCC: 750 mg, 3.6 mmol; HOBT: 500 mg, 3.7 mmol; DIEA: 1.2 ml) to yield **9a** (4.9 g, 98%), resp. **9b** (4.8 g, 96%). The NMR spectra confirm the complete loading of the PEG polymer support. **9a**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 3.31 (s, 3 H), 3.71 (s, 3 H), 5.69, 5.73 (s, 2H), 6.76 (d, *J* = 11.5 Hz, 2 H), 7.26 (d, <sup>3</sup>*J* = 11.5 Hz, 2 H), 7.37 (s, 1 H), 7.95 (s, 1H). IR (KBr):  $\tilde{\nu}$  = 3442 (w) cm<sup>-1</sup>, 2887 (s), 1730 (w), 1467, 1342, 1108 (s); Anal. (%) C<sub>235</sub>H<sub>457</sub>N<sub>3</sub>O<sub>116</sub> (5181): calcd.: C 54.48, H 8.89 N 0.81; found: C 54.17, H 8.48, N 0.82. **9b**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 3.31 (s, 3 H), 3.70 (s, 3 H), 5.76 (s, 2H), 6.74 (d, *J* = 11.4 Hz, 2 H), 7.29 (d, *J* = 11.4 Hz, 2 H), 7.36 (s, 1 H), 7.95 (s, 1H). IR (KBr):  $\tilde{\nu}$  = 3358 (w) cm<sup>-1</sup>, 2887 (s), 1702 (w), 1466, 1343, 1110 (s). Anal. (%) C<sub>235</sub>H<sub>458</sub>N<sub>4</sub>O<sub>115</sub> (5180): calcd.: C 54.49, H 8.91, N 1.08; found: C 54.05, H 8.58, N 0.90.

**MeO-PEG-X-PzPMB-NH<sub>2</sub>** (X = O **10a**, X = NH **10b**). MeO-PEG-X-PzPMB-NO<sub>2</sub> (X = O **9a**, X = NH **9b**) (5.0 g, 1.0 mmol) was reduced corresponding to **GP 1** (NH<sub>4</sub>HCO<sub>2</sub>: 2.5 g, 39.6 mmol; Pd/C: 250 mg) to yield **10a** (4.6 g, 92%), resp. **10b** (4.5 g, 89%). The NMR spectra confirm the complete loading of the PEG polymer support. **10a**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.31 (s, 3 H), 3.70 (s, 3 H), 5.44, 5.51 (s, 1 H), 6.24 (s, 1H), 6.73 (d, *J* = 11.5 Hz, 2H), 7.13 (d, *J* = 11.5 Hz, 2H). IR (KBr): ν̄ = 2887 (s) cm<sup>-1</sup>, 1717 (w), 1465, 1343, 1107 (s); Anal. (%) C<sub>235</sub>H<sub>459</sub>N<sub>3</sub>O<sub>114</sub> (5151): calcd.: C 54.79, H 8.98, N 0.82; found: C 54.51, H 8.61, N 0.78. **10b**: <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ = 3.33 (s, 3 H), 3.72 (s, 3 H), 5.46 (s, 1 H), 6.26 (s, 1H), 6.76 (d, *J* = 11.4 Hz, 2H), 7.16 (d, *J* = 11.4 Hz, 2H).

**MeO-PEG-X-(PzPMB)<sub>2</sub>-NH<sub>2</sub>** (X = O **11a**, X = NH **11b**). MeO-PEG-X-PzPMB-NH<sub>2</sub> (X = O **10a**, X = NH **10b**) (5 g, 1 mmol) was reacted with **8** (1.00 g, 3.6 mmol) according to **GP 4** (HATU: 500 mg, 1.3 mmol; HOAt: 1.4 g, 10.3 mmol; collidine: 1.4 ml) and was subsequently reduced corresponding to **GP 1** (NH<sub>4</sub>HCO<sub>2</sub>: 2.5 g, 39.6 mmol; Pd/C: 250 mg) to yield **11a** (4.70 g, 94%), resp. **11b** (4.60 g, 92%). The NMR spectra confirm the complete loading of the PEG polymer support. **11a**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.31 (s, 3H), 3.69 (s, 3H), 3.70 (s, 3H), 5.50 (s, 2H), 5.53 (s, 2H), 6.29 (s, 1H), 6.76 (m, 4H), 7.14 (s, 1H), 7.19 (m, 4H), 7.29 (s, 1H). IR (KBr): ν̄ = 3442 (b) cm<sup>-1</sup>, 2886 (s), 1719 (w), 1467, 1343, 1111 (s). Anal. (%) C<sub>247</sub>H<sub>468</sub>N<sub>6</sub>O<sub>115</sub> (5362): calcd.: C 55.32, H 8.80, N 1.57; found: C 55.27, H 8.45, N 1.21. **11b**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.31 (s, 3H), 3.69 (s, 3H), 3.71 (s, 3H), 5.46 (s, 2H), 5.55 (s, 2H), 6.02 (s, 1H), 6.75 (m, 4H), 7.67 (s, 1H), 7.19 (m, 4H), 8.29 (s, 1H), 8.54 (s, 1H). IR (KBr): ν̄ = 3442 (b) cm<sup>-1</sup>, 2885 (s), 1655 (w), 1465, 1343, 1111 (s). Anal. (%) C<sub>247</sub>H<sub>469</sub>N<sub>7</sub>O<sub>114</sub> (5361): calcd.: C 55.33, H 8.82, N 1.83; found: C 55.10, H 8.24, N 1.46.

**MeO-PEG-X-(PzPMB)<sub>3</sub>-NH<sub>2</sub>** (X = O **12a**, X = NH **12b**). MeO-PEG-X-(PzPMB)<sub>2</sub>-NH<sub>2</sub> (X = O **11a**, X = NH **11b**) (5 g, 1 mmol) was reacted with **8** (1 g, 3.6 mmol) according to **GP 4** (HATU: 500 mg, 1.3 mmol; HOAt: 1.4 g, 10.3 mmol; collidine: 1.4 ml) and subsequently reduced in CH<sub>2</sub>Cl<sub>2</sub> solution according to **GP 1** (NH<sub>4</sub>HCO<sub>2</sub>: 2.5 g, 39.6 mmol; Pd/C: 250 mg) to yield **12a** (4.30 g, 86%) and **12b** (4.20 g, 84%), resp. The NMR spectra confirm the complete loading of the PEG polymer support. **12a**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.31 (s, 3H), 3.69 (m, 9H), 5.46 (s, 2H), 5.54 (s, 2H), 5.59 (s, 2H), 6.00 (s, 1H), 6.76 (m, 6H), 7.20 (m, 6H), 7.24 (s, 1H), 8.48 (s, 1H), 8.55 (s, 1H). IR (KBr): ν̄ = 3433 (b) cm<sup>-1</sup>, 2886 (s), 1719 (w), 1467, 1343, 1111 (s). **12b**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.31 (s, 3H), 3.69 (m, 9H), 6.46 (s, 2H), 5.57 (m, 4H), 6.05 (s, 1H), 6.75 (m, 6H), 7.06 (s, 1H), 7.21 (m, 6H), 8.57 (s, 1H), 8.63 (s, 1H). IR (KBr): ν̄ = 3436 (b) cm<sup>-1</sup>, 2887 (s), 1672 (w), 1467, 1343, 1111 (s).

**MeO-PEG-X-(PzPMB)<sub>4</sub>-NH<sub>2</sub>** (X = O **13a**, X = NH **13b**). MeO-PEG-X-PzPMB-NH<sub>2</sub> (X = O **12a**, X = NH **12b**) (5 g, 1 mmol) was reacted with **8** (1.00 g, 3.6 mmol) following **GP 4** (HATU: 500 mg, 1.3 mmol; HOAt: 1.4 g, 10.3 mmol; collidine: 1.4 ml) and subsequently reduced according to **GP 1** (NH<sub>4</sub>HCO<sub>2</sub>: 2.5 g, 39.6 mmol; Pd/C: 250 mg) to yield **13a** (3.90 g, 78%) and **13b** (3.65 g, 73%), resp. The NMR spectra confirm the complete loading of the PEG polymer support. **13a**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.31 (s, 3H), 3.68 (m, 12H), 5.57 (m, 6H), 6.01 (s, 1H), 6.75 (s, 2H), 6.76 (m, 8H), 7.26 (m, 9H), 7.52 (s, 1H), 8.63 (s, 1H), 8.72 (s, 1H), 9.34 (s, 1H). IR (KBr): ν̄ = 3433 (b) cm<sup>-1</sup>, 2887 (s), 1721 (w), 1466, 1343, 1110 (s). **13b**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.31 (s, 3H), 3.68 (m, 12H), 5.44 (s, 2H), 5.56 (m, 6H), 6.01 (s, 1H), 6.75 (m, 8H), 7.20 (m, 8H), 7.31 (s, 1H), 8.54 (s, 1H), 8.60 (s, 1H). IR (KBr): ν̄ = 3433 (b) cm<sup>-1</sup>, 2887 (s), 1721 (w), 1466, 1343, 1110 (s).

**MeO-PEG-X-(Pz)<sub>3</sub>-NH<sub>3</sub><sup>+</sup> TFA<sup>-</sup>** (X = O **14a**, X = NH **14b**). MeO-PEG-X-(PzPMB)<sub>3</sub>-NH<sub>2</sub> (X = O **12a**, X = NH **12b**) (0.8 g, 1.6 mmol) was deprotected following **GP 7** (TFA: 4 ml) to yield

**14a** (0.46 g, 58%) and **14b** (0.43 g, 54%). **14a**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.34 (s, 3H), 5.58 (s, 1H), 5.62 (s, 1H), 5.78 (s, 1H), 7.20 (s, 1H), 7.28 (s, 1H), 7.53 (s, 1H), 8.65 (s, 1H), 9.22 (s, 1H). **14b**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.31 (s, 3H), 5.57 (m, 2H), 5.75 (s, 1H), 7.07 (s, 1H), 7.27 (s, 1H), 7.54 (s, 1H), 8.71 (s, 1H), 9.33 (s, 1H).

**MeO-PEG-X-(Pz)<sub>4</sub>-NH<sub>3</sub><sup>+</sup> TFA<sup>-</sup>** (X = O **15a**, X = NH **15b**). MeO-PEG-X-(PzPMB)<sub>3</sub>-NH<sub>2</sub> (X = O **13a**, X = NH **13b**) (0.8 g, 1.6 mmol) was reduced according to **GP 7** (TFA: 4 ml) to yield **15a** (0.45 mg, 56%) and **15b** (0.43 mg, 54%), resp. **15a**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.31 (s, 3H), 5.44 (s, 1H), 5.56 (m, 3H), 7.17 (s, 1H), 7.24 (s, 1H), 7.30 (s, 1H), 8.54 (s, 1H), 8.60 (s, 1H). **15b**: <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 3.34 (s, 3H), 5.50 (m, 3H), 5.78 (s, 1H), 7.09 (s, 1H), 7.53 (s, 1H), 7.98 (s, 1H), 8.69 (s, 1H), 8.75 (s, 1H), 9.34 (s, 1H).

**MeO-PEG-PzPMB-Val-Fmoc (16)**. MeO-PEG-PzPMB-NH<sub>2</sub> (**10a**) (7.89 g, 1.5 mmol) was reacted with Fmoc-valine (2.05 g, 6 mmol) following **GP 4** (HATU: 2.53 g, 6.64 mmol; HOAt: 0.90 g, 6.64 mmol; collidine: 8.0 ml) to yield **16** (7.82 g, 93%). The NMR spectra confirm the complete loading of the PEG polymer support. <sup>1</sup>H-NMR: δ = 0.87–0.91 (m, 6 H), 1.97–2.04 (m, 1 H), 3.24 (s, 3 H), 3.71 (s, 3 H), 4.19–4.28 (m, 3 H), 4.37–4.42 (m, 1 H), 5.53 (s, 1 H), 5.57 (s, 1 H), 6.88 (d, 2 H, *J* = 8.6 Hz), 7.10 (s, 1 H), 7.17 (d, 2 H, *J* = 8.6 Hz), 7.28–7.43 (m, 4 H), 7.53 (d, 1 H, *J* = 8.51), 7.72–7.76 (m, 2 H), 7.88 (d, 2 H, *J* = 7.41 Hz), 10.78 (s, 1 H).

**MeO-PEG-PzPMB-Val-NH<sub>2</sub> (17)**. MeO-PEG-PzPMB-Val-Fmoc (**16**) (7.82 g, 1.41 mmol) was treated with 20% piperidine in DMF (130 ml) according to **GP 7** to give **17** (6.6 g, 88%). <sup>1</sup>H-NMR: δ = 0.85 (d, 3 H, *J* = 6.86 Hz), 0.91 (d, 3 H, *J* = 6.86 Hz), 1.87–1.98 (m, 1 H), 3.27 (s, 3 H), 3.74 (s, 3 H), 4.35–4.42 (m, 1 H), 5.57 (s, 2 H), 6.90 (d, 2 H, *J* = 8.51 Hz), 7.14 (s, 1 H), 7.19 (d, 2 H), 10.76 (s, 1H).

**MeO-PEG-PzPMB-Val-Val-NH<sub>2</sub> (18)**. MeO-PEG-PzPMB-Val-NH<sub>2</sub> (**17**) (6.6 g, 1.24 mmol) was reacted with Fmoc-valine (1.7 g, 4.9 mmol) following **GP 4** (HATU: 2.08 g, 5.46 mmol; HOAt: 0.74 g, 5.46 mmol; collidine: 6.5 ml) and subsequently deprotected following **GP 7** (120 ml 20% piperidine in DMF) to yield **18** (5.7 g, 89%). <sup>1</sup>H-NMR: δ = 0.77–0.90 (m, 12 H), 1.91–2.08 (m, 2 H), 3.23 (s, 3 H), 3.70 (s, 3 H), 4.32–4.39 (m, 2 H), 5.45–5.54 (m, 2 H), 6.88 (d, 2 H, *J* = 8.6 Hz), 7.08 (s, 1 H), 7.16 (d, 2 H, *J* = 8.6 Hz), 8.10 (d, 1 H, *J* = 7.1 Hz), 10.87 (s, 1 H).

**MeO-PEG-PzPMB-Val-Val-PzPMB-NH<sub>2</sub> (19)**. MeO-PEG-PzPMB-Val-Val-NH<sub>2</sub> (**18**) (5.7 g, 1 mmol) was reacted with **8** (1.16 g, 4.2 mmol) following **GP 4** (HATU: 1.75 g, 4.61 mmol; HOAt: 0.63 g, 4.61 mmol; collidine: 5.5 ml) and subsequently reduced according to **GP 1** (NH<sub>4</sub>HCO<sub>2</sub>: 2.8 g, 44.4 mmol; Pd/C: 0.56 g) to yield **19** (4.26 g, 77%). <sup>1</sup>H-NMR: δ = 0.86–0.88 (m, 12 H), 1.95–2.13 (m, 2 H), 3.24 (s, 3 H), 3.71 (s, 6 H), 4.18–4.39 (m, 2 H), 5.24–5.60 (m, 4 H), 6.06 (s, 1 H), 6.75–6.86 (m, 4 H), 7.04–7.15 (m, 5 H), 7.94 (d, 1 H, *J* = 8.6 Hz), 8.21 (d, 1 H, *J* = 8.6 Hz), 10.81 (s, 1 H).

**MeO-PEG-Pz-Val-Val-Pz-NH<sub>3</sub><sup>+</sup> TFA<sup>-</sup> (20)**. MeO-PEG-PzPMB-Val-Val-PzPMB-NH<sub>2</sub> (**19**) (1 g, 0.18 mmol) was deprotected following **GP 7** (TFA: 6 ml) to yield **20** (0.82 g, 86%). <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 0.89–0.90 (m, 12 H), 2.15–2.24 (m, 2 H), 3.29 (s, 3 H), 4.31–4.43 (m, 2 H), 6.87 (s, 1 H), 7.06 (s, 1 H), 7.35 (d, 1 H, *J* = 8.2 Hz), 7.70 (d, 1 H, *J* = 8.0 Hz), 9.83 (s, 1 H), 10.67 (br, 1 H), 11.09 (br, 1 H).

#### <sup>1</sup>H NMR titration

Model peptides for the titration experiments were prepared by standard peptide coupling reactions. For the titration of **4a**, **4b**



in CDCl<sub>3</sub>, Ac-Val-Val-OMe was used. A self-association constant of  $K = 2.5 \text{ M}^{-1}$  was derived from the CIS of the N-H resonance of the central amide in dilution experiments. For the titration of **7a**, **7b** in CDCl<sub>3</sub>, Boc-Phe-Ala-Val-Leu-OMe was used. A self-association of  $K = 6.3 \text{ M}^{-1}$  was determined from dilution experiments following the CIS of N-H resonances of Phe. For the titration of **14a**, **14b** in CDCl<sub>3</sub>, Ac-Lys-Leu-Lys-Leu-Lys-Leu-OEt was used. Dilution experiments showed no CIS. It was concluded that there is no self-association of the peptide under the experimental conditions. The titration of **4a**, **4b**, **7a**, **7b** in aqueous phosphate buffer : D<sub>2</sub>O = 9 : 1, pH 7, was carried out with H-His-Gly-Gly-OH. A self-association of the peptide of  $K = 2.5 \text{ M}^{-1}$  was determined by dilution experiments following the CIS of the N-H resonance of His. For the titration of **14a**, **14b** in phosphate buffer : D<sub>2</sub>O = 9 : 1, pH 5.2, Ac-(Lys-Leu)<sub>3</sub>-OEt was used. No self-association of the peptide was observed in dilution experiments under the experimental conditions. A pH of 5.2 was necessary for experiments using peptide Ac-(Lys-Leu)<sub>3</sub>-OEt to ensure its sufficient solubility in aqueous solution. All PEG pyrazole oligoamides were tested for self-association in CDCl<sub>3</sub> and water solution, but no CIS were observed under the experimental conditions. NMR spectra were recorded at 300 K. For aqueous samples the Watergate experiment was used to suppress the water resonance.

For the determination of binding affinities in CDCl<sub>3</sub> solution the CIS of the pyrazole C-H resonance and the peptide N-H resonance (same as used to determine the peptide self-association) was used. Pyrazole N-H signals show significant CIS, but the signals become broad during titration and were not included in calculations. The association constants were derived using the CIS of several resonances and peptide self-association was taken into account.

For the determination of binding affinities in buffered aqueous solution the CIS of the pyrazole C-H resonance and the peptide N-H resonance (same as used to determine the peptide self-association) was used. For compound **14** only the CIS of all three pyrazole C-H resonances was used, due to line broadening and overlap of the peptides signals. The association constants were derived using the CIS of several resonances and peptide self-association was taken into account.

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