DOI: 10.1002/ejoc.200500393

Sequence-Selective Peptide Recognition with Designed Modules

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Keywords: Amino acids / Molecular recognition / Peptides / Rational design / Receptors / Sequence selectivity / Noncovalent interactions

A concept for the rational design of sequence-selective peptide receptors has been extended: in addition to recognition modules for polar, aromatic and basic amino acids, the series has now been completed with new receptor units for apolar and acidic amino acids. The underlying strategy uses the intermolecular β-sheet stabilization of a dipeptide as a prerequisite to bind its N-terminal amino acid side chain through a strategically placed recognition tip at the end of a U-turn protruding from the receptor moiety. Thus, a diaminopyrazole has been covalently attached to Kemp's triacid by way of a cyclic imide, while a *meta*-substituted aniline was coupled as an amide to the pendant third carboxylate arm, bringing the two aromatic units into a sub-van der Waals distance in a tight conformational lock. NMR titrations, Karplus analyses and Monte-Carlo simulations demonstrate the effective sequence-selective recognition of alanine-containing dipeptides. No example of such a rationally designed set of peptide receptors had existed previously.

Introduction

Although considerable progress has been achieved in designing artificial receptors for small biomolecules, including most amino acids, the task of sequence-specific amino acid recognition in peptides still poses a severe problem: an efficient host for oligopeptides must be able to distinguish the consecutive order of side chains. Human efforts to mimic nature's potent peptide hosts (mainly high molecular weight proteins) with artificial structures are hampered by the complexity of three-dimensional protein structures and our lack of knowledge for prediction of protein folding. In the early stages (and well beyond), success came through serendipity: Rebek created dimeric host molecules based on Kemp's triacid, featuring a chiral cleft adorned with convergent imide and amide groups, their mutual arrangement enforcing a steric constraint on sterically demanding dipeptides, which were only bound if the second amino acid had a sterically undemanding side chain.[1] Early work by Still produced macrocyclic, tricyclic and even polycyclic receptor structures, with unsymmetric arrangements of hydrogen bond donors and acceptors with remarkable selectivities. These artificial receptor molecules were developed for apolar solvents, and bound to di- and tripeptides in an impressively, albeit unpredictably, sequence- or stereoselective fashion.[2]

Subsequent approaches used a more efficient avenue through the employment of combinatorial methods. The general concept introduced by the Still group starts from a rigid scaffold bearing two parallel peptide strands, the sequence of which is varied statistically.[3] From the resulting combinatorial receptor library, the best binder for a given small peptide is identified by an efficient high-throughput screening method. Alternatively, the binding profile for a given host can be established by varying the sequence of its guest, which is often fluorescence-labelled. In recent years the groups of Wennemers,[4] Kilburn[5] and Schmuck[6] have developed their own optimized systems used for, for example, recognition of the Alzheimer's peptide, the D-Ala-D-Ala sequence and many more related small peptides. Very few attempts to create sequence-selective peptide receptors by rational design have been made. Schneider et al. (Figure 1) recently reported that a zwitterionic peptide will be especially tightly bound if its N terminus is inserted into a pendant crown ether while the C terminus is drawn into the centre of the positive charges of a triply pyridinium-substituted porphyrin.[7] π-Stacking interactions make these receptors selective for aromatic residues; with an additional Zn^{2+} central ion, histidine-containing peptides are bound preferentially (Mizutani).[8] An elegant extension is the construction of mixed receptor lipid monolayers at the air/water interface, introduced by Kunitake for distinction between dipeptides with very similar amino acid compositions.[9]

Prominent examples of the development of specific receptor units for small peptides are also found in nature: vancomycin provides a binding pocket as an apolar environment for very strong hydrogen bonds directed towards the backbone of its target peptide. The typical D-Ala-D-Ala sequence essential for most bacterial cell walls is thus ster-

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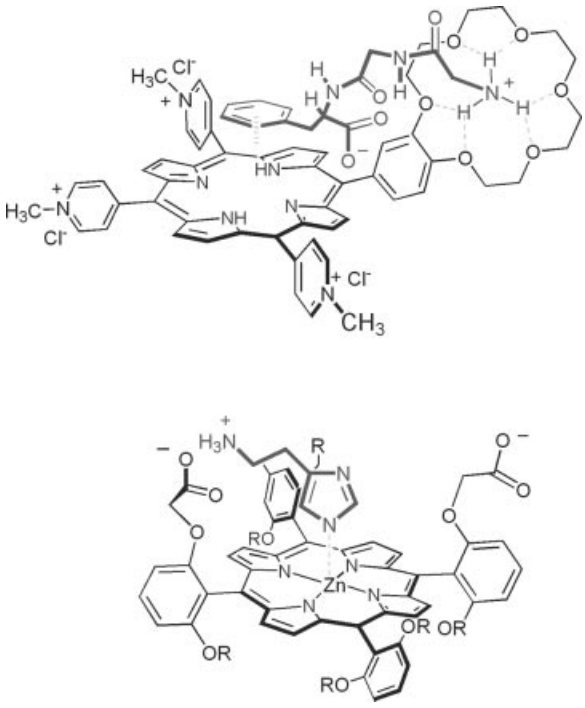


Figure 1. Rational design of sequence-selective peptide receptors. Left: Schneider's porphyrin-based tripeptide receptor fills its crown ether with Gly-Gly-Phe's N-terminal ammonium group, while the C-terminal Phe residue π-stacks with the porphyrin. Right: Mizutani's receptor uses a coordinative bond with the central Zn^{2+} ion for superior affinity towards histidine-containing peptides, reinforced by ion pairing with additional carboxyalkyloxy groups at the periphery.

eoselectively recognized under physiological conditions.[10] The RGD–integrine interaction is responsible for a variety of biological events governing the correct molecular function of proteins such as fibrinogen[11] (blood coagulation), fibronectine[12,13] (cell matrix binding), osteopontine[14,15] (bone formation) or certain growth factors[16] (cell differentiation and angiogenesis). All these proteins carry the critical RGD sequence on a solvent-exposed loop; mutagenesis studies showed that this is indeed the primary binding site for interaction with their biological counterparts (e.g., cell surface-bound receptors such as the integrines).[17–20] Many proteases bind their peptidic substrates in a shallow groove, where it is locked in an extended β-sheet-like conformation that produces a horizontal presentation of the guest's amino acid side chains favourable for lateral recognition. β-Sheet mimics make use of this principle: they replace the flexible peptide backbone with heterocyclic recognition elements possessing a much higher degree of rigidity, while simultaneously retaining the hydrogen bond donor and acceptor pattern of a β-sheet.[21] A logical extension affords the first soluble β-sheet models, in which combinations of these artificial (Nowick[22]) and natural (Kemp,[23] Feigel,[24] Kelly,[25] Gellman[26]) peptide strands are attached to common scaffolds and interact along their inner faces like zippers to form two- or three-stranded parallel or antiparallel β-sheets. Very recently the first intermolecular versions of these β-sheet models were created (Schrader,[27] Hamilton,[28] Bartlett[29]), albeit in organic solvents. Aminopyrazoles, diaminoquinolones and azacyclohexenones (Figure 2) are thus all able to form the maximum number of possible hydrogen bonds to the top and bottom faces of an extended peptide strand backbone.

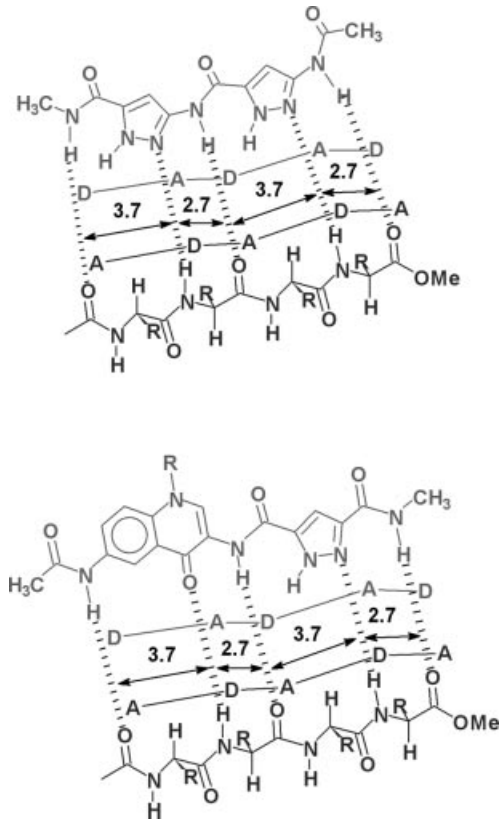


Figure 2. Intermolecular β-sheet recognition by aminopyrazoles and diaminoquinolones requires perfect three-dimensional complementarity in their D-A-D arrangements with exact alternating distances of 2.7 and 3.7 Å.

Starting from the efficient conformational lock represented by aminopyrazole β-sheet templates we recently put forward a new concept for the rational design of sequence-selective peptide receptors, using the intermolecular β-sheet stabilization of a dipeptide as a prerequisite to bind one of its amino acid side chains through a strategically placed recognition tip at the end of a U-turn protruding from the receptor moiety.[30] To the best of our knowledge, no example of such a rationally designed set of peptide receptors had existed previously. The U-turn was constructed from the very well known, highly preoriented skeleton of Kemp's triacid.[31] Several elegant applications in the area of peptide recognition and stereocontrolled photochemistry have used this remarkable platform,[32] which allows the insertion of an amino-substituted heterocycle between two of its carboxylic acid functionalities, whilst still leaving a third binding site for an interchangeable recognition tip (Figure 3). This sets the stage for the synthetic procedure: condensation of the protected aminopyrazole unit with Kemp's triacid through the imide derivative must be followed by attachment of the additional binding site, preferentially again

by amide formation. This sounds simple, and indeed offers a very short, straightforward route to a number of closely related recognition modules, but it also poses the nontrivial problem of creating a new Kemp's acid derivative with two parallel aromatic moieties at sub-van der Waals distances (2.6 Å and less; vide infra). The amide coupling will thus pass through a sterically extremely demanding tetrahedral intermediate stage. After extensive screening of various coupling reagents we found that PyClop activation of the corresponding monoacid could be carried out effectively at elevated temperatures and provided moderate yields of the desired sterically congested coupling products. Only one other report relating to the synthesis of doubly aromatic substituted derivatives of Kemp's triacid has been published, and this group also pointed out the synthetic difficulties of the critical coupling step.[33]

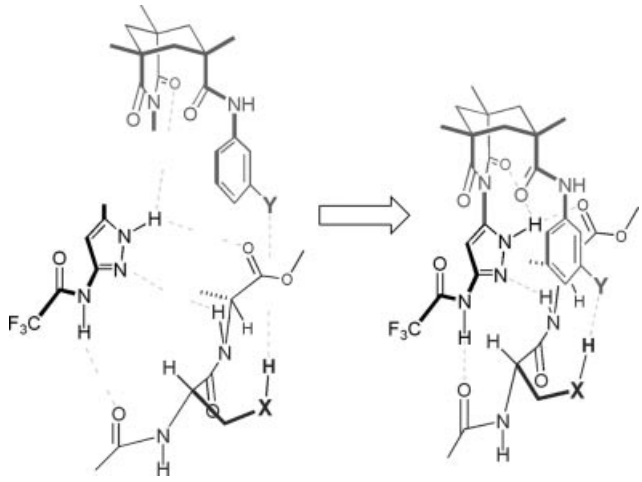


Figure 3. Design of the new peptide recognition modules with amino acid specificity. XH = representative amino acid side chain functionality; Y = recognition tip of each module.

Results and Discussion

In order to retain the DAD hydrogen bond donor and acceptor pattern of the heterocycle we chose 3,5-diaminopyrazole as core unit. After its selective monotrifluoroacetylation with TFAA (**1**; chemoselectivity follows the HSAB principle), it was condensed with the simple anhydride of Kemp's triacid, which is accessible from the parent molecule by sublimation (Figure 4).[34] If the condensation procedure is carried out in toluene under dehydrating conditions, the analytically pure product precipitates almost quantitatively on cooling to room temperature. The two subsequent steps are carried out in one-pot fashion and require exact control of reaction conditions: ring protection of the deactivated pyrazole nucleus takes place at 90 ± 5 °C, whilst above this temperature the labile Boc group is cleaved off.[35] Careful removal of excess Boc_2O is followed by preactivation of the remaining carboxylic acid with PyClop/DIEA and subsequent addition of the aniline derivative.[36] Only on prolonged heating at 50 ± 5 °C in chloroform (>12 h) does amide formation take place without decomposition, and the crude product is finally stirred with silica gel for a convenient and quantitative Boc deprotection. To our great pleasure, the purified products are extremely soluble in solvents of low polarity; despite their trifluoroacetylated aminopyrazole components, known to self-associate strongly through hydrogen bonds, these Kemp's acid adducts are very soluble in chloroform.

Monte-Carlo simulations carried out for various potential host–guest complexes in chloroform suggested: a) formation of an intramolecular hydrogen bond between the pyrazole NH group and one of the imide carbonyls, which would produce a favourable conformational lock for the aminopyrazole ring, and b) an optimal tip orientation over the horizontal amino acid side chain, if the aniline derivative is *meta*-substituted. Indeed, in the imide intermediate, a distinct NMR signal was observed for the pyrazole NH proton, shifted downfield to 12.5 ppm ([D_6]DMSO), indicating the postulated intramolecular hydrogen bond. By the synthetic approach outlined above, *m*-ethylaniline,[37] *m*-aminoacetophenone, benzyl *m*-aminobenzoate[38] and even *m*-aminonitrobenzene could be coupled to the imide intermediate **2**. The benzyl ester was finally cleaved by hydrogenolysis and the resulting free acid was converted into the tetrabutylammonium benzoate **8** – the binding site for basic, cationic amino acid residues. *m*-Aminoacetophenone derivative **4** constitutes a host molecule for OH- and SH-containing amino acids, *m*-aminonitrobenzene **5** should pick electron-rich aromatic amino acids, and *m*-ethylaniline **3** serves as a reference compound with a comparable substituent size, but suitable only for recognition of the peptide backbone.

As already stated above, **3**–**9** are among the few Kemp's triacid derivatives containing two closely stacked aromatic rings. Modelling experiments predict a high degree of bond-angle strain and repulsive van der Waals interactions; in fact, the two aromatic planes are not completely parallel, but diverge at a dihedral angle of ca. 15°. From the unsymmetric geometries of the two aromatic groups, the existence of two conformers must be assumed, and these should be distinguishable through their spectroscopic properties. Only single sets of signals appear, however, in both the ^1H and the ^{13}C NMR spectra of **3**–**9**. In addition, although the two aromatic planes should be in close proximity, NOESY spectra obtained for **3** find only one cross-peak between them, as opposed to numerous contacts between alkyl substituents of the cyclohexyl skeleton. Close inspection of the calculated H,H distances in the productive conformation confirms that only one distance is calculated to be less than 4.5 Å, and this is precisely the one observed in the NOESY experiment (3.2 Å). From Monte-Carlo simulations and molecular dynamics calculations[39] we obtained indications of greater flexibility of these aromatic moieties within the Kemp's triacid scaffold than would be expected from the Lewis structures. The best explanation seems to be a concerted rotation of the whole *trans*-amide bond, carrying the aniline derivative further away from the aminopyrazole and also allowing the ring-flip necessary for a fast interconversion of the two conformers on the NMR timescale.

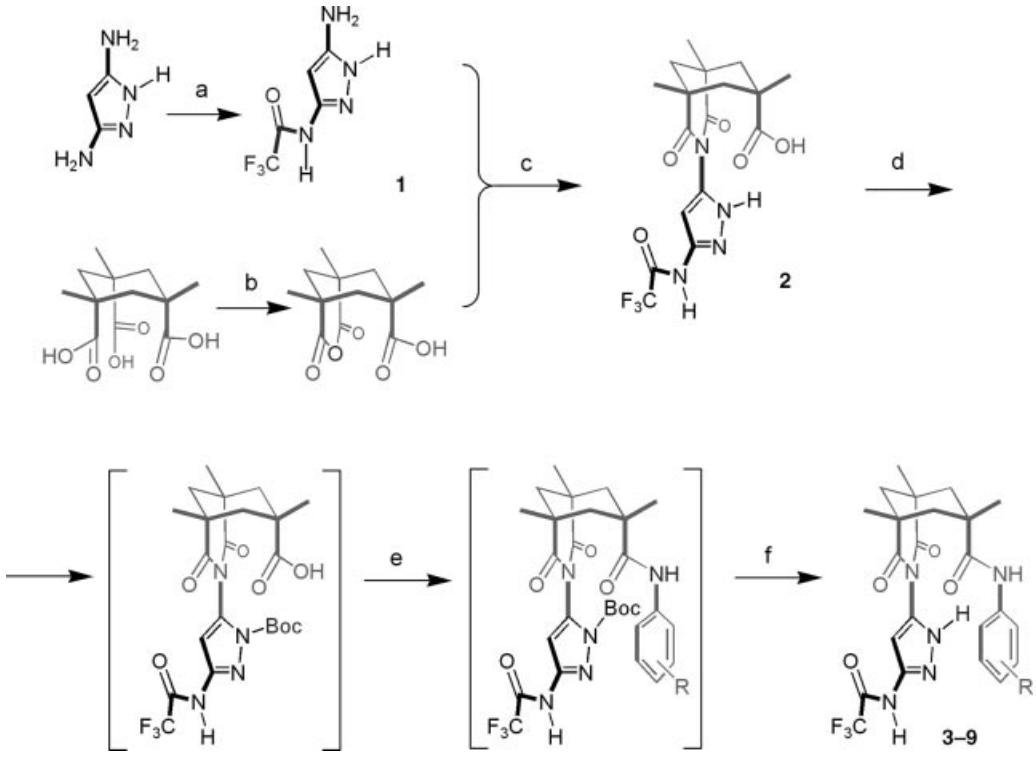


Figure 4. Synthetic route to all new Kemp's acid-based host compounds. a) TFAA. b) Sublimation. c) Toluene, 110 °C (–H_2O), Et_3N. d) Boc_2O, 90 °C. e) *m*-Aminobenzene derivative, PyCloP, 50 °C, $CHCl_3$. f) Silica gel, (–Boc). a–f) 17–22% overall yield. R = *m*-C_2H_5 (**3**), *m*-(C=O)CH_3 (**4**), *m*-NO_2 (**5**), *m*-CO_2^- (**6**), *p*-CH_2–C_6H_5 (**7**), *m*-CH_2–C_6H_5 (**8**), *p*-CH_2–NH_3^+ (**9**).

The first important question to answer is that concerning potential self-association of the new host compounds, a critical point with the simple monomeric and dimeric linear acylated aminopyrazoles. In the concentration range between 10^{-2} M and 10^{-3} M later used in the binding experiments, no chemically induced shift of any receptor proton could be observed. Obviously, the sterically demanding framework of the Kemp's triacid effectively prevents the aggregation of host molecules with one another.

The binding profiles of the different receptor modules next had to be examined, the challenge being to create modules for each category among the five major classes of amino acids: polar, aromatic, basic, acidic and nonpolar. Binding experiments were generally carried out through NMR titrations,[40] whereas stoichiometries were determined by Job plots.[41] Preliminary investigations of the steric tolerance in the guest peptide structure were informative, since it became clear that neither a bulky N-terminal acyl group (such as Boc) nor two sterically demanding amino acids (Ac-Val-Val-OMe) were tolerated in the complex. It must be assumed that the sterically congested chemical environment around the aminopyrazole renders the Kemp's acid-based peptide hosts selective for relatively slim guests. This view is also supported by force-field calculations. We thus replaced the C-terminal valine with alanine, and observed the restoration of the expected downfield shifts of the top face NH ($\Delta\delta_{max}$ = 0.1–0.8 ppm). Accordingly, we can now write the general structure of the preferred guests as Ac-NH-AA1-Ala-OMe. Such peptides present only one side chain (the one at the N terminus) to the receptor. Since

Table 1. Overview of all association constants K_a [M^{-1}] for complex formations between hosts **3**–**9** and representative dipeptides, determined by NMR titrations in $CDCl_3$. All dipeptides were protected as *N*-acetyl methyl esters. Stoichiometries were always 1:1, except when stated otherwise.

Host	Standard	Selective binding	Special	Special
TriflAMP[a]	Ala-Ala: 50[b]	–	Phe-Ala: 40	
Reference **3**	Orn-Ala: 280	Ala-Ala: 70	Phe-Ala: ≤ 40	Val-Ala: 50
XH-binder **4**	Ala-Ala: 80	Ser-Ala: 900	Ser-Val: no shifts	Val-Val: no shifts
Arene-binder **5**	–	Phe-Ala: 350	Ala-Phe: no shifts	–
Cation-binder **6**	propylamine: 490	Orn-Ala: 2360	Gly-Lys: 130	–
Alkyl-binder **7**	–	Val-Ala: 110	Ala-Val: 130	Leu-Val: no shifts
Alkyl-binder **8**	–	Val-Ala: 70	Leu-Val: no shifts	–
Anion-binder **9**	acetate: < 10	Glu-Ala: 1300	–	–

[a] TriflAMP = 3-trifluoroacetylamino-5-methylpyrazole. [b] Errors were determined as standard deviations from the nonlinear regressions; they usually varied between 10% and 40% (exceptions: very weak binding).

alanine has a much higher propensity to adopt an α-helical conformation than to form a β-sheet,[42] the intended stabilization of the extended conformation in the guest peptide had to be expected to become more difficult – lower K_a values being the consequence. After a perfect 1:1 stoichiometry had been established by means of a Job plot, nonlinear regression analysis of the binding isotherms between reference compound **3** and alanine-containing apolar peptides in $CDCl_3$ confirmed this assumption: K_a values remained below 100 M^{-1} (Table 1). Moreover, identical values of around 50 M^{-1} were obtained in titrations of these peptides with the best monomeric aminopyrazole peptide binders previously developed by our group.

Polar Amino Acids

A host molecule to serve this purpose must form strong hydrogen bonds to the OH or SH groups in serine, threonine and cysteine. As already described in our first report, Ac-Ser-Ala-OMe furnished a high association constant of ca. 900 M^{-1} when it was titrated with **4**, as opposed to control experiments with the dialanine derivative or the reference compound **3** (K_a = 80 M^{-1}, Table 1).

Aromatic Amino Acids

π-Stacking interactions are often used in protein recognition events, especially within extended hydrophobic domains. For the most efficient attraction of Phe, Tyr or Trp residues a *m*-aminonitroarene was introduced in the host molecule. Comparison of their associated EPS colour codes demonstrates that the electron-rich aromatics among the proteinogenic amino acids should all form favourable complexes with the electron-deficient nitroarene.[43] The nitroaniline-functionalized host **5** (Figure 5) showes an affinity towards Ac-Phe-Ala-OMe ($K_{1:1}$ = 350 M^{-1}) nine times higher than toward the reference host **3** ($K_a < 40$ M^{-1}). In a molecular modelling study of the complex the nitroarene is reproducibly flipped out to receive the phenylalanine aromatic in a parallel orientation at the ideal van der Waals distance of 3.4 Å.

Basic Amino Acids

A biomimetic approach towards the recognition of ionic amino acid residues involves the coulombic attraction between those in the guest and oppositely charged residues in the host compound. Basic amino acids are thus often bound by aspartate or glutamate anions. A short basic amino acid, ornithine, was chosen because molecular mechanics calculations in this case suggested a perfect alignment of the two ionic groups. In summary, a marked preference of 9:1 was found for the selective recognition of Ac-Orn-Ala-OMe by the corresponding aminopyrazole receptor unit **6** (K_a = 2400 M^{-1}) over the closely related reference host **3** (280 M^{-1}). A single ion pair-reinforced hydrogen bond between the host module's benzoate tip and the guest's ammonium cation was thus designed and was experimentally found to increase the affinity towards basic peptide by roughly one order of magnitude.

In order to examine the sequence selectivity of this ionic recognition process, we carried out a synthesis of the inverted sequence Ac-Ala-Orn-OMe, but failed in the last step, which involved hydrogenolytic cleavage of the δ-Z group: even under acidic conditions we could not avoid intramolecular cyclization of ornithine's free amino group onto its *C*-terminal methyl ester to furnish the stable preferred piperidone. As an alternative we prepared the related lysine derivative Ac-Gly-Lys-OMe. Its tosylate did not cyclize and could be titrated with host **6** to give a low association constant of 130 M^{-1}. In spite of the small structural differences this result clearly again indicates a strong preference of host **6** for peptides with N-terminal basic residues.

Nonpolar Amino Acids

To achieve efficient recognition of nonpolar amino acids is a difficult task, especially in apolar chloroform, in which

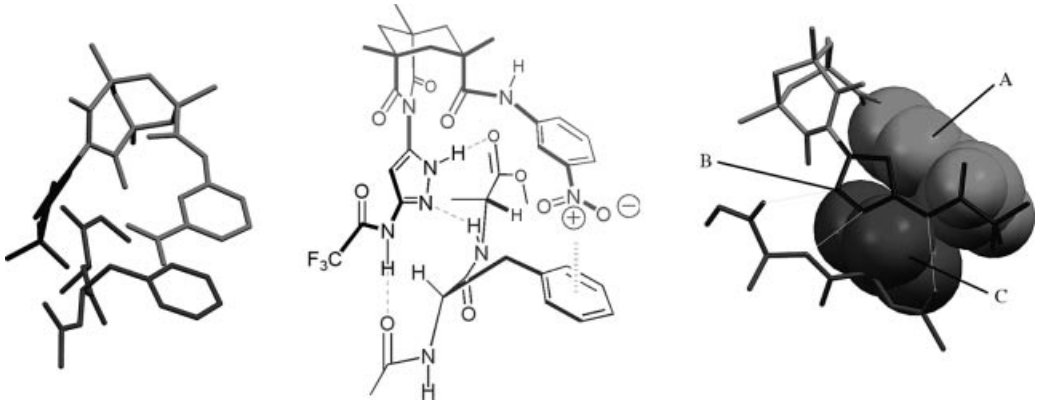


Figure 5. Phenylalanine recognition within Ac-Phe-Ala-OMe by nitroarene-modified aminopyrazole host **5**. Left and middle: front view. Right: side view. A: Kemp's triacid with arene tip. B: aminopyrazole. C: aromatic guest dipeptide. Note the perfect π-stacking between the nitroarene and the electron-rich Phe benzene ring.

hydrophobic interactions are intrinsically weak. Furthermore, most aliphatic amino acids carry bulky side chains, so a compromise must be found between steric repulsion and maximum van der Waals contact. Since protein–protein complexes often use phenylalanine units to provide large nonpolar π-faces, we chose the diphenylmethane unit as such a compromise: this offers a large π-face, and simultaneously, because of its CH_2 hinge group, forms a shallow groove for maximum van der Waals contact. Modelling experiments suggest that this binding site is easily accessible for secondary alkyl groups, so that most apolar proteinogenic amino acids should be able to insert their alkyl side chains into the shallow groove of the corresponding receptor moiety. According to the optimized procedure described above, both the *m*- and the *p*-benzylaniline derivatives of Kemp's acid with a pendant aminopyrazole imide could be prepared in a straightforward synthesis. Both new hosts turned out still to be very soluble in chloroform. Binding experiments with ethyl reference host **3** and the new diphenylmethane-modified **7** and **8** demonstrate the validity of both the assumptions detailed above: leucine-containing dipeptides are rejected by both **3** and **7** (*para* derivative), due to steric restrictions. However, valine-containing dipeptides again show pronounced downfield shifts in their NH protons, demonstrating that backbone recognition takes place. This time, comparison of the affinity towards Ac-Val-Ala-OMe with the reference host **3** ($55\ M^{-1}$) reveals a moderate, but distinct twofold increase in K_a for the *p*-diphenylmethane-modified host **7** ($105\ M^{-1}$). The *meta*-substituted host **8** binds the guest peptide somewhat less tightly ($70\ M^{-1}$). Although steric restrictions are far more important in these cases, surprisingly, no sequence selectivity is found this time: the inverted peptide sequence Ac-Ala-Val-OMe binds equally well to **7** ($134\ M^{-1}$). We attribute this divergent behaviour to the facile rotation of the flexible diphenylmethane linker around its benzylic bond. Subsequent Karplus analysis also produced interesting results: from the preoriented dipeptide (Ac-Val-Ala-OMe) to the complex the coupling constants dropped markedly, suggesting that the two amino acids avoid a perfect β-sheet conformation to optimize their hydrogen bonding to the peptide backbone. The effect is most pronounced in the case of the *meta* derivative **8** [Val: ^3J(H,H) from 8.8 to 7.8 Hz; Ala: ^3J(H,H) from 7.1 to 6.2 Hz]. It lowers the free energy gain on complex formation and might thus explain the lower affinity of **8** towards aliphatic dipeptides. As a further improvement of this concept, we intend in the future to extend the diphenylmethane unit into a dihydromethanoanthracene moiety, which could form a much more extended cleft for increased hydrophobic contacts (Figure 6).

Acidic Amino Acids

The simplest case of such a receptor based on our Kemp's triacid aminopyrazole module would be the recognition of an aspartate or glutamate side chain by a permanently charged cationic binding site attached to the *m*-aniline arm. Force field calculations and synthetic considerations suggested aminomethylaniline as a good candidate for glutamate recognition (Figure 7). Its synthesis was achieved through Z-protection of the more basic benzylamine, amide coupling with PyClop at elevated temperatures and Boc deprotection on the column. Final removal of the Z group was effected by hydrogenolysis and subsequent treatment with 1 equiv. of HCl. Binding experiments with Ac-Glu-Ala-OMe tetrabutylammonium salt resulted in chemical shift changes exclusively at the top-face NH proton, whereas the bottom face NH remained untouched, indicating the desired 1:1 complex formation with concomitant backbone recognition. That strong ion pairs were formed simultaneously is well documented by the elevated binding constant of $1300\ M^{-1}$. A final titration with tetrabutylammonium acetate did not furnish any appreciable chemical shift change.

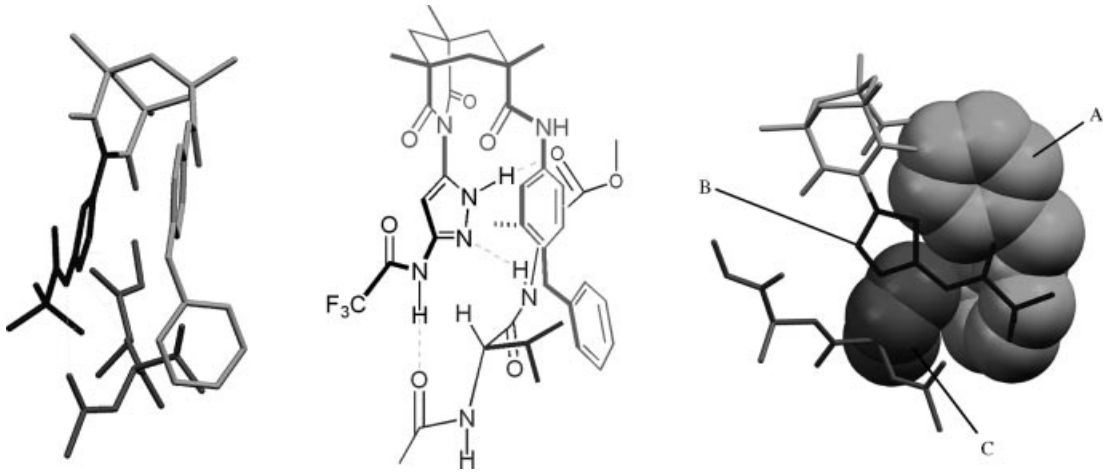


Figure 6. Valine recognition within Ac-Val-Ala-OMe by diphenylmethane-modified aminopyrazole host **7**. Left and middle: front view. Right: side view. A: Kemp's triacid with arene tip. B: aminopyrazole. C: aliphatic guest dipeptide. Note the close fit between valine's isopropyl group and the diphenylmethane "roof".

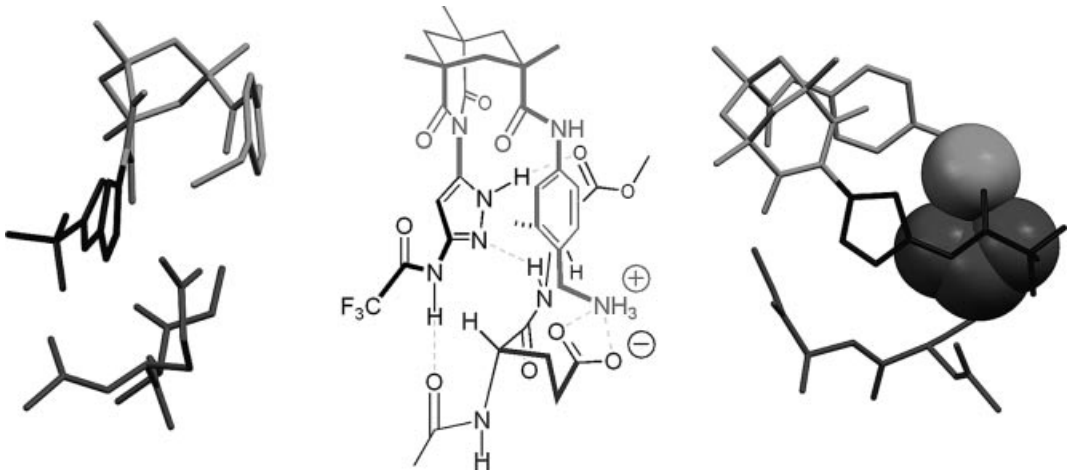


Figure 7. Glutamate recognition by benzylammonium-modified aminopyrazole host **9**.

Karplus Analysis

Direct information relating to the peptide conformation can be drawn from Karplus analyses of the *NH-a-CH* coupling constants, which correlate with the characteristic torsion angle θ (Figure 8).[44] For convenient comparison, we examined complexes of three hosts carrying additional binding sites, each with their best peptide binding partners. If hosts **3**–**8** were added to the peptide solution, signals were constantly sharpened and in most cases ^3J values increased relative to the free peptides. Both the peptide's top and bottom side showed this effect. However, the experimentally obtained values did not reach the calculated coupling constants derived from the calculated complex geometries (MacroModel 7.0), see Table 2. This makes sense, because at the relatively low concentrations used for the NMR titration experiments, only a certain fraction of all molecules was fixed in the complex.

The host modules **7** and **9** for nonpolar and acidic amino acids constitute exceptions to this rule: most probably for steric reasons, the peptide backbones avoided the adoption of perfect β-sheet conformations in order to maximize hydrogen bond interactions with the receptors, and thus produced lower ^3J values in the complexes as opposed to the free peptides.

$$\theta = \arccos\left[\frac{1.1}{18.8} - \sqrt{\left(\frac{1.1}{18.8}\right)^2 - \left(\frac{0.4 - 1.09^3J}{9.4}\right)}\right]$$

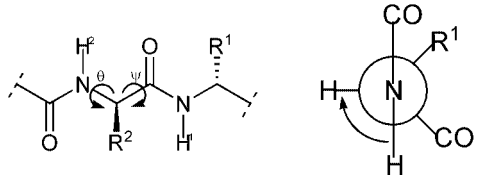


Figure 8. Top: Karplus equation, modified for peptides. Bottom: assignment of NH^1 and NH^2 and corresponding torsion angles θ and ψ in an *N*/*C*-protected dipeptide.

Conclusion

In the experiments described above we have shown that peptide receptors selective for some of the main classes of amino acids in a peptidic environment can be developed by rational design. Further work towards improved efficiency and selectivity of all receptor modules is in progress in our laboratory.

Table 2. Experimentally measured and calculated ^3J coupling constants [Hz] of free dipeptides and their complexes with the corresponding best host molecules discussed above (MacroModel 7.2, Amber*, $CHCl_3$, 1000 steps).

Host	Dipeptide	Experiment			Calculation		
		free		solution	complex		
		H^1	H^2	H^1	H^2	H^1	H^2
4	Ac-Ser-Ala-OMe	6.3	6.3	6.6	7.6	8.7	9.7
5	Ac-Phe-Ala-OMe	7.3	7.6	6.6	8.3	5.4	6.6
6	Ac-Orn-Ala-OMe·HOAc	5.3	6.0	7.0	6.4	6.5	6.7
7	Ac-Val-Ala-OMe	8.8	7.1	8.6	6.7	8.0	5.0
9	Ac-Glu-Ala-OMe·LiOH	7.0	6.8	6.4	5.0	9.0	6.7

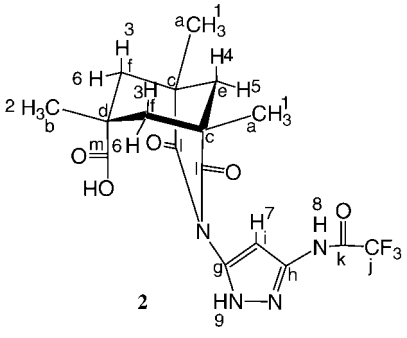
If the dipeptides carry their hydroxy, aromatic or cationic amino acid residues at the C terminus, binding constants drop drastically. Thus, the serine- or phenylalanine-containing dipeptides **11** and **14** are not complexed at all. Even a highly flexible lysine residue at the C terminus is bound with a K_a value of only 130 M^{-1} as opposed to N-terminal ornithine with 2400 M^{-1}. These examples show that a high simple sequence selectivity is achieved with our new receptor modules, which we attribute mainly to steric reasons. In some cases, complex formation is so sensitive towards steric factors that the same receptor module completely rejects the same dipeptide with an inverted sequence. Modelling studies indicate that only one mutual orientation of both binding partners is productive, whilst the other one seems to be sterically hindered.

Outlook

In the future we intend to connect two or more peptide receptor modules covalently for the five major classes of amino acids and to achieve sequence-selective recognition of larger peptides, hopefully also in more polar solutions. Dimeric aminopyrazoles and aminopyrazole/peptide hybrid receptor molecules have recently been shown to bind a hexapeptidic key sequence taken from Aβ (1–40) – in water! A detailed spectroscopic investigation indicates that an increased number of hydrogen bonds *plus* hydrophobic stacking interactions are responsible for this possible drastic solvent change. Recognition of larger peptide sequences in peptides and proteins might then become feasible with constructs of several peptide mimics attached to a central scaffold, as introduced by Nowick's group. We thus propose a tridentate fork, made up of a central peptide chain and peripheral aminopyrazole oligomers, as an inhibitor of HIV-protease dimerization for a potential application in AIDS therapy.

Experimental Section

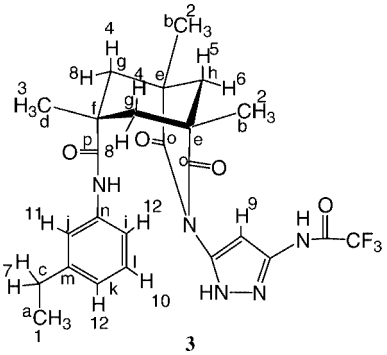
Receptor Molecules



2

1,5,7-Trimethyl-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonane-7-carboxylic Acid (2): A suspension of 1,3,5-trimethyl-1,3,5-cyclohexanetricarboxylic acid anhydride (**1**) (114 g, 4.8 mmol), (0.94 g, 4.8 mmol) and triethylamine (0.1 mL, 0.7 mmol) were heated at reflux in dry toluene (150 mL) in a Dean–Stark trap, until no water was produced any longer. The solvent was removed by distillation, until precipitation started at the boiling point. After the system had been cooled to –24 °C the white precipitate was filtered off and dried in vacuo (R_F = 0.45 in CH_2Cl_2/methanol, 5:1). Yield 1.88 g (4.5 mmol, 94%); m. p. 221 °C. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 1.25 (s, 6 H, 1), 1.26 (s, 3 H, 2), 1.43 (d, ^2J(H,H) = 13.9 Hz, 2 H, 3), 1.66 (d, ^2J(H,H) = 12.9 Hz, 1 H, 4), 2.10 (d, ^2J(H,H) = 12.9 Hz, 1 H, 5), 2.53 (d, ^2J(H,H) = 13.9 Hz, 2 H, 6), 6.55 (br., 1 H, 7), 12.02 (br., 1 H, 8), 12.48 (br., 1 H, 9) ppm. ^{13}C NMR (126 MHz, [D$_6$]DMSO, 25 °C): δ = 25.1 (a), 30.2 (b), 39.9 (c), 40.9 (d), 41.7 (e), 42.9 (f), 59.7 (g), 89.2 (h), 96,5 (i), 115.7 [q, ^2J(C, F) = 287.0 Hz; j], 154.0 [q, ^3J(C, F) = 36.9 Hz]; (k), 175.4 (l), 176.9 (m) ppm. MS (CI, NH_3, 200 °C): *m*/*z* = 417 [*M* + H]$^+$. HRMS (EI): *m*/*z*, found 416.1304; calcd. 416.1308. $C_{17}H_{19}F_3N_4O_5$: found C 49.04, H 4.60, N 13.46; calcd. C 49.56, H 4.38, N 12.94.

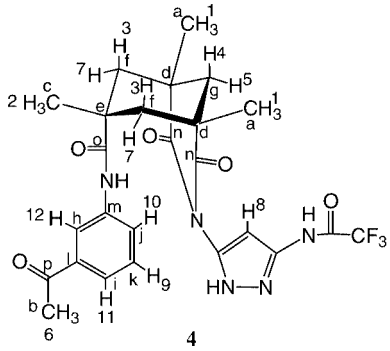
General Procedure for the Amidation of 2 with Aniline Derivatives: A suspension of **2** (250 mg, 0.6 mmol) and Boc_2O (10 mL, 46.7 mmol) was heated to 90 °C for 30 min, after which the starting material had disappeared in the TLC. The excess of Boc_2O was condensed off at a water bath temperature of >70 °C. The remaining solid was dissolved in chloroform (40 mL) and treated with diisopropylethylamine (0.84 mL, 4.8 mmol) and PyCloP (0.89 g, 2.1 mmol). Stirring was continued overnight. The solution was warmed to 50 °C and a suspension of chloroform (25 mL) and the corresponding aniline derivative (2.5 equiv., 1.5 mmol) was added dropwise over 3 h. After the addition heating was continued for another 7 hr, and the solution was then stirred at room temperature overnight and at 50 °C for another 6 h. The solution was concentrated to dryness and the remaining yellow oil was suspended in a little ethyl acetate/hexane (1:2). It was then placed on a chromatography column filled with silica gel 60. After standing overnight, elution was carried out until no substance was eluted any longer; the product was then eluted with ethyl acetate. The crude product was chromatographed a second time over silica gel 60 with ethyl acetate/hexane (2:1).



3

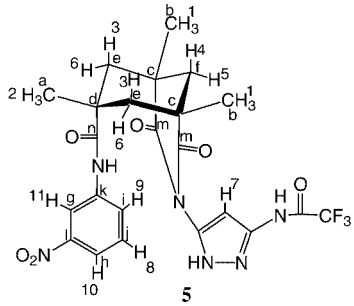
***N*-(3-Ethylphenyl)-1,5,7-trimethyl-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonane-7-carboxamide (3):** The preparation followed the general procedure outlined above. R_F = 0.64 (ethyl acetate): Yield 0.1 g (0.1 mmol, 23%); m. p. 152 °C. ^{1}H NMR (300 MHz, [D$_4$]methanol, 28 °C): δ = 1.37 (t, ^3J(H,H) = 7.6 Hz, 3 H, 1), 1.56 (s, 6 H, 2), 1.58 (s, 3 H, 3), 1.68 (d, ^2J(H,H) = 14.3 Hz, 2 H, 4), 1.83 (d, ^2J(H,H) = 12.9 Hz, 1 H, 5), 2.40 (d, ^2J(H,H) = 12.9 Hz, 1 H, 6), 2.78 (q, ^3J(H,H) = 7.6 Hz, 2 H, 7), 3.07 (d, ^2J(H,H) = 14.3 Hz, 2 H, 8), 6.19 (br., 1 H, 9), 7.15 (t, ^3J(H,H) = 4.0 Hz, 1 H, 10), 7.32 (s, 1 H, 11), 7.37 (m, 2 H, 12) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C): δ = 15.9 (a), 26.0 (b), 29.2 (c), 32.2 (d), 41.4 (e), 43.5 (f), 45.6 (g), 45.7 (h), 110.4 (i), 122.7 (j), 126.4 (k), 129.5 (l), 136.3 (m), 146.1 (n), 176.0 (o),

178.3 (p) ppm. MS (FD): *m*/*z* = 519 [*M*]$^+$ (100%). $C_{25}H_{28}F_3N_5O_4$: calcd. C 57.80, H 5.43, N 13.48; found C 57.87, H 5.82, N 13.75.



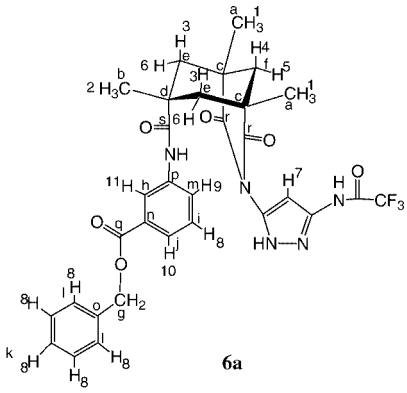
4

***N*-(3-Acetylphenyl)-1,5,7-trimethyl-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonane-7-carboxamide (4):** The preparation followed the general procedure outlined above. R_F = 0.49 (ethyl acetate); Yield 54 mg (0.1 mmol, 18%); m. p. 185 °C. ^{1}H NMR (500 MHz, [D$_4$]MeOH, 25 °C): δ = 1.32 (s, 6 H, 1), 1.35 (s, 3 H, 2), 1.49 (d, ^2J(H,H) = 14.2 Hz, 2 H, 3), 1.64 (d, ^2J(H,H) = 13.6 Hz, 1 H, 4), 2.14 (d, ^2J(H,H) = 13.6 Hz, 1 H, 5), 2.52 (s, 3 H, 6), 2.83 (d, ^2J(H,H) = 14.2 Hz, 2 H, 7), 5.93 (br., 1 H, 8), 7.34 (t, ^3J(H,H) = 8.2 Hz, 1 H, 9), 7.51 (d, ^3J(H,H) = 7.6 Hz, 1 H, 10), 7.67 (d, ^3J(H,H) = 7.6 Hz, 1 H, 11), 7.99 (s, 1 H, 12) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C); δ = 25.9 (a), 26.8 (b), 31.4 (c), 42.0 (d), 43.7 (e), 44.2 (f), 44.9 (g), 123.7 (h), 125.8 (i), 128.5 (j), 130.0 (k), 138.8 (l), 139.6 (m), 176.5 (n), 178.3 (o), 200.6 (p) ppm. MS (FD) *m*/*z* = 533 [*M*]$^+$ (100%). HRMS (EI) *m*/*z*, found 533.1876; calcd. 533.1886.



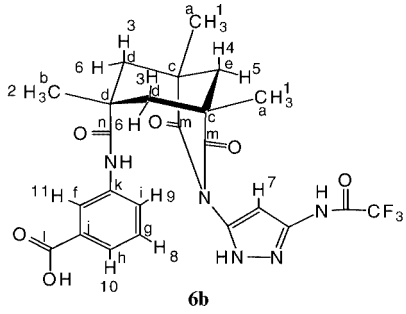
5

1,5,7-Trimethyl-*N*-(3-nitrophenyl)-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonan-7-carboxamide (5): The preparation followed the general procedure outlined above; R_F = 0.58 (ethyl acetate); Yield 85 mg (0.1 mmol, 18%); m. p. 208 °C. ^{1}H NMR (300 MHz, [D$_4$]MeOH, 28 °C): δ = 1.31 (s, 6 H, 1), 1.34 (s, 3 H, 2), 1.47 (d, ^2J(H,H) = 14.6 Hz, 2 H, 3), 1.61 (d, ^2J(H,H) = 13.2 Hz, 1 H, 4), 2.14 (d, ^2J(H,H) = 13.2 Hz, 1 H, 5), 2.84 (d, ^2J(H,H) = 14.6 Hz, 2 H, 6), 5.93 (br., 1 H, 7), 7.42 (t, ^3J(H,H) = 8.3 Hz, 1 H, 8), 7.66 (d, ^3J(H,H) = 9.0 Hz, 1 H, 9), 7.88 (d, ^3J(H,H) = 9.3 Hz, 1 H, 10), 8.37 (s, 1 H, 11) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C): δ = 26.0 (a), 31.3 (b), 42.0 (c), 43.8 (d), 44.3 (e), 44.8 (f), 117.9 (g), 120.1 (h), 128.9 (i), 130.5 (j), 140.6 (k), 149.8 (l), 176.3 (m), 177.7 (n) ppm. MS (FD): *m*/*z* = 536 [*M*]$^+$ (100%). HRMS (EI): *m*/*z*, found 536.1633; calcd. 533.1631.



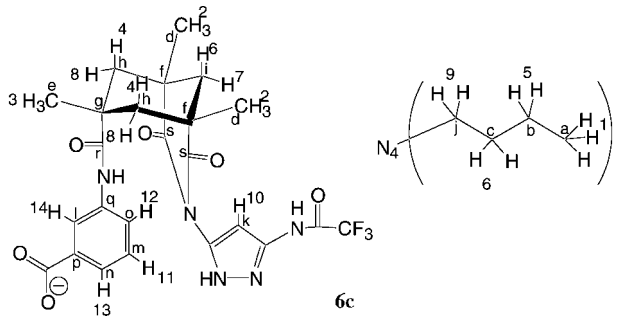
6a

Benzyl 3-({1,5,7-Trimethyl-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonane-7-carbonyl}amino)-benzoate (6a): The preparation followed the general procedure outlined above. R_F = 0.37 (ethyl acetate/hexane, 2:1); Yield 26 mg (0.04 mmol, 7%); m. p. 159 °C. ^{1}H NMR (300 MHz, [D$_6$]acetone, 28 °C): δ = 1.24 (s, 6 H, 1), 1.28 (s, 3 H, 2), 1.46 (d, ^2J(H,H) = 14.2 Hz, 2 H, 3), 1.59 (d, ^2J(H,H) = 13.3 Hz, 1 H, 4), 2.18 (d, ^2J(H,H) = 13.3 Hz, 1 H, 5), 2.82 (d, ^2J(H,H) = 14.2 Hz, 2 H, 6), 6.29 (s, 1 H, 7), 7.33 (m, 6 H, 8), 7.69 (d, ^3J(H,H) = 7.8 Hz, 1 H, 9), 7.80 (d, ^3J(H,H) = 8.3 Hz, 1 H, 10), 7.97 (s, 1 H, 11) ppm. ^{13}C NMR (50.4 MHz, [D$_6$]acetone, 25 °C): δ = 25.9 (a), 31.2 (b), 41.4 (c), 43.1 (d), 43.8 (e), 44.7 (f), 67.8 (g), 122.9 (h), 126.0 (i), 126.8 (j), 128.8 (k), 129.3 (l), 129.8 (m), 131.5 (n), 137.4 (o), 139.4 (p), 166.4 (q), 175.6 (r), 176.4 (s) ppm. MS (FD): *m*/*z* = 625 [*M*]$^+$ (100%). HRMS (EI): *m*/*z*, found 625.2156; calcd. 625.2148.



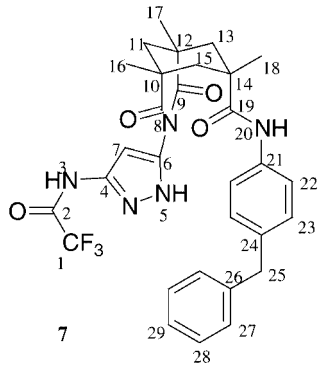
6b

3-({1,5,7-Trimethyl-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonane-7-carbonyl}amino)benzoic Acid (6b): Compound **6a** (50 mg, 0.08 mmol) was dissolved in methanol (50 mL) and treated with Pd/C (55 mg). The flask was connected to a hydrogen-filled balloon and the mixture was stirred for 6 h. The reaction mixture was filtered through silica gel and the filtrate was concentrated to dryness. The remaining solid was washed twice with ethyl acetate and twice with dichloromethane. Yield 16 mg (0.03 mmol, 72%); m. p. 230 °C. ^{1}H NMR (300 MHz, [D$_4$]MeOH, 28 °C): δ = 1.31 (s, 6 H, 1), 1.34 (s, 3 H, 2), 1.44 (d, ^2J(H,H) = 14.3 Hz, 2 H, 3), 1.59 (d, ^2J(H,H) = 12.9 Hz, 1 H, 4), 2.15 (d, ^2J(H,H) = 12.9 Hz, 1 H, 5), 2.84 (d, ^2J(H,H) = 14.3 Hz, 2 H, 6), 5.93 (br., 1 H, 7), 7.28 (t, ^3J(H,H) = 8.3 Hz, 1 H, 8), 7.57 (d, ^3J(H,H) = 8.0 Hz, 1 H, 9), 7.68 (d, ^3J(H,H) = 7.6 Hz, 1 H, 10), 7.87 (s, 1 H, 11) ppm. ^{13}C NMR (50.4 MHz, [D$_6$]acetone, 25 °C): δ = 26.0 (a), 31.5 (b), 41.9 (c), 44.1 (d), 45.0 (e), 124.5 (f), 126.8 (g), 126.9 (h), 129.5 (i), 129.5 (j), 139.2 (k), 173.9 (l), 176.3 (m), 178.1 (n) ppm. MS (ESI negative): *m*/*z* = 534 [*M*]$^+$.



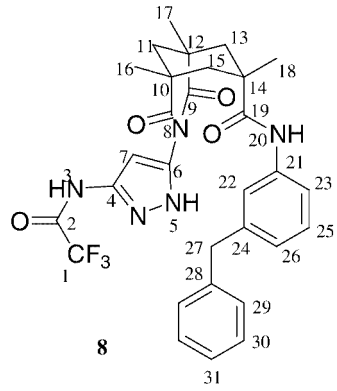
6c

Tetrabutylammonium 3-({1,5,7-Trimethyl-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonane-7-carbonyl}amino)benzoate (6c): Compound **6b** (20 mg, 0.037 mmol) was dissolved in methanol (10 mL) and treated with NBu_4OH (1 M, 37 μL), and the solution was concentrated to dryness. Yield 28.7 mg (0.037 mmol, 100%); m. p. >260 °C. ^1H NMR (300 MHz, [D$_4$]MeOH, 28 °C): δ = 0.99 (t, ^3J(H,H) = 7.3 Hz, 12 H, 1), 1.30 (s, 6 H, 2), 1.35 (s, 3 H, 3), 1.38 (d, ^2J(H,H) = 14.1 Hz, 2 H, 4), 1.38 (br., 8 H, 5), 1.59 (m, 9 H, 6), 2.16 (d, ^2J(H,H) = 13.3 Hz, 1 H, 7), 2.83 (d, ^2J(H,H) = 14.1 Hz, 2 H, 8), 3.18 (m, 8 H, 9), 5.87 (br., 1 H, 10), 7.25 (t, ^3J(H,H) = 7.6 Hz, 1 H, 11), 7.64 (d, ^3J(H,H) = 7.6 Hz, 1 H, 12), 7.67 (d, ^3J(H,H) = 10.3 Hz, 1 H, 10), 7.70 (s, 1 H, 11) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C): δ = 14.0 (a), 20.7 (b), 24.8 (c), 26.2 (d), 28.6 (e), 31.6 (f), 42.0 (g), 44.2 (h), 45.2 (i), 59.6 (j), 97.4 (k), 124.0 (l), 125.5 (m), 126.6 (n), 129.2 (o), 139.0 (p), 139.2 (q), 176.2 (r), 178.1 (s) ppm. MS (ESI negative): m/z = 776 $[M]^+$ (18%), 534 $[M - H]^+$, Carboxylic acid anion without $NBu_4]^+$ (100%). HRMS for $[M - NBu_4]^+$ (ESI negative): m/z, found 534.1595; calcd. 534.1600.



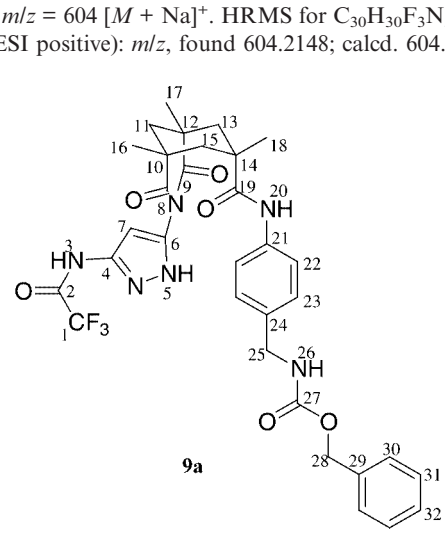
7

***N*-(4-Benzylphenyl)-1,5,7-trimethyl-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonane-7-carboxamide (7):** The preparation followed the general procedure outlined above except for the use of a less polar column chromatography gradient, with ethyl acetate/hexane, 1:2, first and subsequently with ethyl acetate/hexane, 3:2. Yield 19 mg (0.03 mmol, 13%); m. p. 207 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.34 (s, 6 H, H-16, H-17), 1.35 (s, 3 H, H-18), 1.50 (d, ^2J = 14.4 Hz, 2 H, H-13, H-15), 1.65 (d, ^2J = 13.4 Hz, 1 H, H-11), 2.22 (d, ^2J = 13.4 Hz, 1 H, H-11), 2.88 (d, ^2J = 14.4 Hz, 2 H, H-13, H-15), 3.94 (s, 2 H, H-25), 7.12 (d, ^3J = 8.3 Hz, 2 H, H-23), 7.21 (m, 3 H, H-27, H-28, H-29), 7.25 (m, 2 H, H-27, H-28, H-29), 7.27 (d, ^3J = 8.3 Hz, 2 H, H-22) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ = 22.8 (C-18), 25.6 (C-16, C-17), 29.5 (C-14), 29.9 (C-10, C-12), 40.9 (C-13, C-15), 41.3 (C-11), 43.2 (C-26), 95.9 (C-7), 123.4 (C-1), 126.1 (C-29), 128.5 (C-Ar), 129.1 (C-Ar), 129.6 (C-Ar), 133.8 (C-Ar), 139.6 (C-21, C-24), 141.2 (C-28), 175.1 (C-19), 175.7 (C-8, C-9) ppm. MS (ESI negative): m/z = 582 $[M - H]^-$.



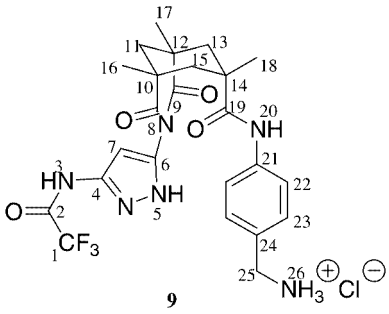
8

***N*-(3-Benzylphenyl)-1,5,7-trimethyl-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonane-7-carboxamide (8):** The preparation followed the general procedure outlined above except for the use of a less polar column chromatography gradient, first with ethyl acetate/hexane, 1:2 and subsequently with ethyl acetate/hexane, 3:2. Yield 16 mg (0.03 mmol, 13%); m. p. 224 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.34 (s, 6 H, H-16, H-17), 1.34 (s, 3 H, H-18), 1.46 (d, ^2J = 14.6 Hz, 2 H, H-13, H-15), 1.63 (d, ^2J = 12.4 Hz, 1 H, H-11), 2.18 (d, ^2J = 12.4 Hz, 1 H, H-11), 2.84 (d, ^2J = 14.6 Hz, 2 H, H-13, H-15), 3.89 (s, 2 H, H-27), 6.89 (b, 1 H, H-7), 7.16 (m, 9 H, H-aryl) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ = 25.3 (C-16, C-17), 27.6 (C-18), 40.6 (C-10. C-12), 41.4 (C-14), 42.8 (C-11), 44.2 (C-27), 44.8 (C-13, C-15), 95.7 (C-7), 120.2 (C-23), 120.5 (C-22), 123.1 (C-1), 125.9 (C-25), 126.0 (C-31), 128.2 (C-Ar), 128.7 (C-Ar), 128.9 (C-Ar), 137.7 (C-21), 142.1 (C-26, C-28), 174.5 (C-19), 175.3 (C-8, C-9) ppm. MS (ESI positive): m/z = 604 $[M + Na]^+$. HRMS for $C_{30}H_{30}F_3N_5O_4Na^+$ $[M + Na]^+$ (ESI positive): m/z, found 604.2148; calcd. 604.2155.



9a

Benzyl [4-({1,5,7-Trimethyl-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonane-7-carbonyl}amino)-benzyl]carbamate (9a): The preparation followed the general procedure outlined above except for the use of a different column chromatography gradient, finally using dichloromethane/methanol, 40:1. Yield 9 mg (0.02 mmol, 10%); m. p. 207 °C; ^1H NMR (200 MHz, [D$_4$]MeOH): δ = 1.30–1.50 (m, 11 H), 2.23 (d, ^2J = 13.6 Hz, 1 H, H-11), 2.66 (d, ^2J = 13.6 Hz, 1 H, H-11), 2.86 (d, ^2J = 13.9 Hz, 2 H, H-13, H-15), 4.23 (s, 2 H, H-25), 5.07 (s, 2 H, H-28), 6.34 (s, 1 H, H-7), 6.88 (d, ^3J = 8.3 Hz, 2 H, H-23), 7.14 (d, ^3J = 8.0 Hz, 2 H, H-22), 7.33 (m, 5 H, H-30, H-31, H-32) ppm. MS (ESI positive): m/z = 655 $[M + H]^+$. HRMS for $C_{32}H_{33}F_3N_6O_6H^+$ $[M + H]^+$ (ESI positive, MeOH): m/z, found 655.2498; calcd. 655.2492.



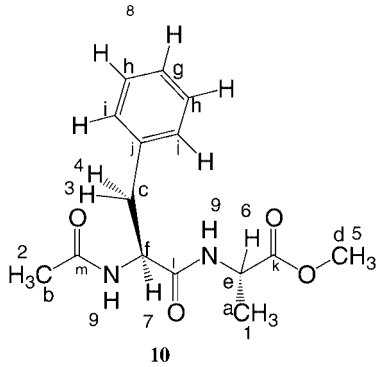
9

[4-({1,5,7-Trimethyl-2,4-dioxo-3-[5-(2,2,2-trifluoroacetylamino)-2*H*-pyrazol-3-yl]-3-azabicyclo[3.3.1]nonane-7-carbonyl}amino)benzylamine Hydrochloride (9): Compound **9a** (13.5 mg, 2 mmol) was dissolved in methanol (10 mL), treated with $Pd(OH)_2$ (20% on C, 20 mg) and quantitatively hydrogenated under normal pressure for 2 d. After filtration and evaporation of the solvent the free amine **9** was obtained as a colourless oil.

The free amine (0.4 mg, 2 mmol) was subsequently dissolved in dry methanol (10 mL) and cooled to 10 °C. Under pH control (glass electrode) a solution of HCl (1 M) in diethyl ether was carefully added dropwise, until a pH of 7.0–7.5 was reached. Stirring was continued at ambient temperature for another half hour, and the solvent was then concentrated to dryness and the resulting hydrochloride salt was dried in vacuo. Colourless oil, which solidifies after several days. Yield 11 mg (99%).

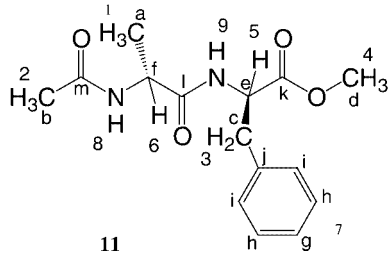
Compound 9: ^{1}H NMR (300 MHz, [D$_4$]MeOH): δ = 1.44 (d, ^2J = 14.4 Hz, 2 H, H-13,15), 1.50 (s, 6 H, H-16,17), 1.52 (s, 3 H, H-18), 2.06 (d, ^2J = 11.0 Hz, 1 H, H-11), 2.28 (d, ^2J = 11.0 Hz, 1 H, H-11′), 2.84 (d, ^2J = 14.4 Hz, 2 H, H-13′,15′), 3.73 (s, 2 H, H-25), 7.08 (d, ^3J = 8.4 Hz, 2 H, H-23), 7.21 (d, ^3J = 8.4 Hz, 2 H, H-22). HRMS for $C_{24}H_{22}D_5F_3N_6O_4^+$ + Li [M + Li]$^+$ (ESI positive, CD_3OH): *m*/*z*, found 532.2580; calcd. 532.2515. $C_{24}H_{22}D_5F_3N_6O_4^+$ + Na [M + Na]$^+$: found 548.2324; calcd. 548.2252.

Peptides



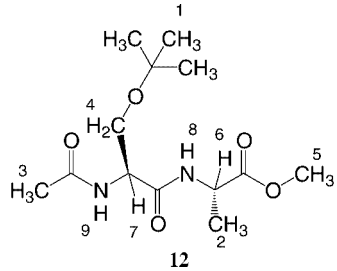
10

***N*-Acetyl-L-phenylalaninyl-L-alanine Methyl Ester (10):** L-Alanine methyl ester hydrochloride (0.1 g, 0.7 mmol), *N*-acetyl-L-phenylalanine (0.2 g, 1 mmol) and *N*-methylmorpholine (0.55 mL, 5 mmol) were dissolved in dry dichloromethane (50 mL) and cooled to –10 °C. T3P (0.5 mL, 3.1 mmol) was then added slowly, and stirring was continued for 3 h in the cold and afterwards for 3 d at room temperature. The solution was concentrated and subsequently washed with HCl (1 N), satd. aqueous $NaHCO_3$, satd. aqueous NaCl and water. The organic layer was dried with magnesium sulfate, filtered and concentrated to dryness. R_F = 0.16 (ethyl acetate); Yield 0.18 g (0.6 mmol, 88%); m. p. 149–155 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 28 °C): δ = 1.20 (d, ^3J(H,H) = 7.3 Hz, 3 H, 1), 1.63 (s, 3 H, 2), 2.60 (dd, ^2J(H,H) = 10.3 Hz, 1 H, 3), 2.89 (dd, ^2J(H,H) = 4.3 Hz, 1 H, 4), 3.52 (s, 3 H, 5), 4.18 (ddd, ^3J(H,H) = 7.3 Hz, 1 H, 6), 4.43 (m, 1 H, 7), 7.98 (d, ^3J(H,H) = 8.6 Hz, 1 H, 8), 8.40 (d, ^3J(H,H) = 7.0 Hz, 1 H, 9) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C): δ = 17.4 (a), 22.4 (b), 39.0 (c), 52.8 (d), 55.8 + 55.9 (e,f), 127.7 (g), 129.4 (h), 130.3 (i), 138.5 (j), 173.1 + 173.6 (k, l), 174.4 (m) ppm. MS (EI): *m*/*z* = 292 [M]$^+$ (15%), 233 [M – $CH_3C(O)NH$]$^+$ (25%), 162 (22%), 159 (38%), 131 [C(O)NH-Ala-OMe]$^+$ (29%), 120 (100%). HRMS (EI): *m*/*z*, found 292.1422; calcd. 292.1423. $C_{15}H_{20}N_2O_4$: calcd. C 61.63, H 6.90, N 9.58; found C 61.53, H 7.39, N 10.14.



11

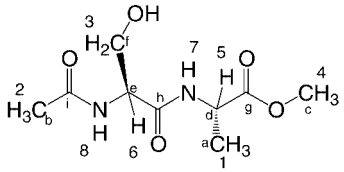
***N*-Acetyl-L-alaninyl-L-phenylalanine Methyl Ester (11):** *N*-Acetyl-L-alanine (0.48 g, 3.7 mmol), L-phenyalanine methyl ester hydrochloride (0.78 g, 3.6 mmol) and *N*-methylmorpholine (2.5 mL, 23 mmol) were dissolved in dry dichloromethane (50 mL) and cooled down to –10 °C. T3P (2,6 mL, 16 mmol) was added slowly and the reaction mixture was stirred for 3 h in the cold and afterwards for 3 d at room temperature. The solution was concentrated and the residue was subsequently washed with ethyl acetate and HCl (1 N), satd. aqueous $NaHCO_3$, satd. aqueous NaCl and water. The organic layer was dried with magnesium sulfate and filtered, and the solvents were removed. The residue was chromatographed over silica gel with elution with CH_2Cl_2/methanol (20:1); R_F = 0.23; Yield 0.65 g (2.2 mmol, 61%); m. p. 122 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 28 °C): δ = 0.94 (d, ^3J(H,H) = 2.0 Hz, 3 H, 1), 1.60 (s, 3 H, 2), 2.80 (m, 2 H, 3), 3.39 (s, 3 H, 4), 4.10 (m, 1 H, 5), 4.26 (m, 1 H, 6), 7.02 (m, 5 H, 7), 7.78 (d, ^3J(H,H) = 8.0 Hz, 1 H, 8), 8.07 (d, ^3J(H,H) = 7.6 Hz, 1 H, 9) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]-MeOH, 25 °C): δ = 18.1 (a), 22.6 (b), 50.2 (c), 52.8 (d), 54.9 + 55.2 (e, f), 128.0 (g), 129.6 (h), 130.4 (i), 138.0 (j), 173.0 + 173.3 + 175.0 (k, l, m) ppm. MS (FD): *m*/*z* = 292 [M]$^+$. HRMS (EI): *m*/*z*, found 292.1421; calcd 292.1423.



12

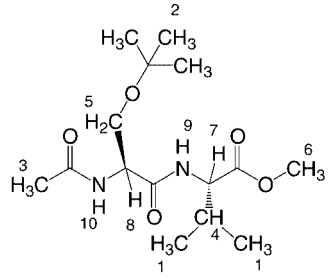
***N*-Acetyl-(*O*-*tert*-butyl)-L-serinyl-L-alanine Methyl Ester (12):** *N*-Acetyl-L-serine *tert*-butyl ether (0.61 g, 3 mmol), L-alanine methyl ester hydrochloride (0.43 g, 3 mmol) and *N*-methylmorpholine (1.8 mL, 16.4 mmol) were dissolved in dry dichloromethane (50 mL) and cooled down to –10 °C. T3P (2.1 mL, 13 mmol) was added slowly and stirring was continued for 3 h in the cold and another 3 d at room temperature. The solution was concentrated and washed with ethyl acetate and HCl (1 N), satd. aqueous $NaHCO_3$, satd. aqueous NaCl and water. The organic layer was dried with magnesium sulfate, filtered and concentrated to dryness. The resulting crude product was NMR-pure and was used without further purification. Yield 0.32 g (1.1 mmol, 37%); m. p. 108 °C.

^{1}H NMR (300 MHz, [D$_6$]DMSO, 28 °C): δ = 1.17 (s, 9 H, 1), 1.34 (d, ^3J(H,H) =Hz, 3 H, 2), 1.91 (s, 3 H, 3), 2.60 (dd, ^2J(H,H) = 10.3 Hz, 1 H, 3), 2.89 (dd, ^2J(H,H) = 4.3 Hz, 1 H, 4), 3.52 (s, 3 H, 5), 4.18 (ddd, ^3J(H,H) = 7.3 Hz, 1 H, 6), 4.43 (m, 1 H, 7), 7.98 (d, ^3J(H,H) = 8.6 Hz, 1 H, 8), 8.40 (d, ^3J(H,H) = 7.0 Hz, 1 H, 9).



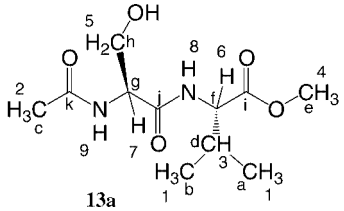
12a

***N*-Acetyl-L-serinyl-L-alanine Methyl Ester (12a):** Compound **12** (0.23 g, 0.8 mmol) was dissolved in formic acid (p. a., 40 mL) and kept overnight at room temperature. The solution was concentrated to dryness and the residue was purified by chromatography (CH_2Cl_2/methanol, 20:1, R_F = 0.1). Yield: 0.12 g (0.5 mmol, 65%); m. p. 135 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 28 °C): δ = 1.28 (d, ^3J(H,H) =Hz, 3 H, 1), 1.86 (s, 3 H, 2), 3.54 (m, 2 H, 3), 3.62 (s, 3 H, 4), 4.30 (m, 1 H, 5), 7.91 (d, ^3J(H,H) = 7.0 Hz, 1 H, 6), 8.28 (d, ^3J(H,H) = 7.3 Hz, 1 H, 7) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C): δ = 17.5 (a), 22.6 (b), 52.8 (c), 56.6 + 56.7 (d, e), 63.2 (f), 172.3 + 173.5 (g, h), 174.6 (i) ppm. MS (FD): *m*/*z* = 233 [*M* + H]$^+$. HRMS (ESI, for $C_9H_{16}O_5N_2Na$): *m*/*z*, found 255.09478; calcd. 255.09569. $C_9H_{16}O_5N_2$: calcd. C 46.55, H 6.94, N 12.06; found C 45.59, H 6.48, N 12.00.



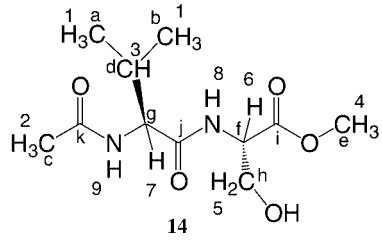
13

***N*-Acetyl-*O*-*tert*-butyl-L-serinyl-L-valine Methyl Ester (13):** *N*-Acetyl-L-serine *tert*-butyl ether (0.51 g, 2.5 mmol), L-valine methyl ester hydrochloride (0.39 g, 2.3 mmol), PyCloP (1.1 g, 2.6 mmol) and DIEA (1.6 mL, 9 mmol) were stirred in dry dichloromethane (50 mL) for 2 days at room temperature. The solution was concentrated to dryness and the residue was chromatographed over silica gel (ethyl acetate, R_F = 0.44). The crude product was NMR spectroscopically pure and was used for the next step without further purification. Yield 0.3 g (1.1 mmol, 48%); m. p. 107 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 28 °C): δ = 0.85 (m, 6 H, 1), 1.08 (s, 9 H, 2), 1.83 (s, 3 H, 3), 2.00 (m, 1 H, 4), 3.40 (m, 2 H, 5), 3.60 (s, 3 H, 5), 4.17 (dd, ^3J(H,H) = 6.6 Hz, 1 H, 7), 4.41 (dd, ^3J(H,H) = 8.0 Hz, 1 H, 8), 7.89 (d, ^3J(H,H) = 8.3 Hz, 1 H, 9), 8.00 (d, ^3J(H,H) = 8.6 Hz, 1 H, 10) ppm.



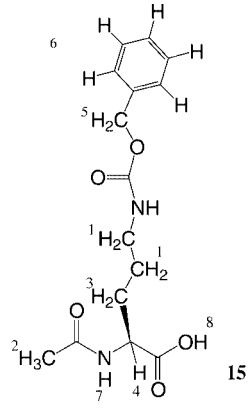
13a

***N*-Acetyl-L-serinyl-L-valine Methyl Ester (13a):** Compound **13** (0.2 g, 0.63 mmol) was dissolved in dry formic acid (p. a., 40 mL) and kept overnight at room temperature. The solution was concentrated to dryness and was subsequently chromatographed over silica gel (ethyl acetate, R_F = 0.11). Yield 84 mg (0.3 mmol, 51%); m. p. 129 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 28 °C): δ = 0.86 (m, 6 H, 1), 1.85 (s, 3 H, 2), 2.03 (m, 1 H, 3), 3.62 (s, 3 H, 4), 4.20 (m, 3 H, 5 + 6), 4.72 (m, 1 H, 7), 8.22 (d, ^3J(H,H) = 11.0 Hz, 1 H, 8), 8.31 (d, ^3J(H,H) = 8.0 Hz, 1 H, 9) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C): δ = 18.4 + 19.5 (a + b), 22.5 (c), 32.0 (d), 52.6 (e), 56.6 + 59.3 (f, g), 63.0 (h), 172.7 + 173.3 + 173.5 (i, j, k) ppm. HRMS (ESI for $C_{11}H_{20}O_5N_2Na$): *m*/*z*, found 283.12586; calcd. 283.12699. $C_{11}H_{20}O_5N_2$: calcd. C 50.76, H 7.74, N 10.76; found C 50.47, H 7.68, N 10.22.



14

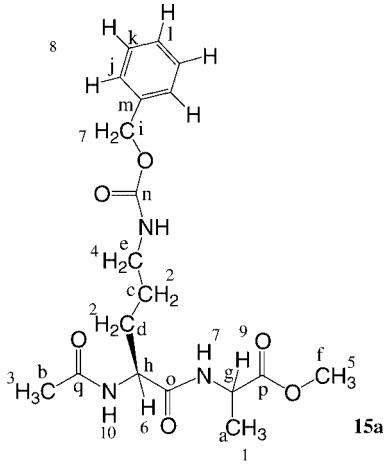
***N*-Acetyl-L-valinyl-L-serine Methyl Ester (14):** *N*-Acetyl-L-valine (0.67 g, 4.2 mmol), L-serine methyl ester hydrochloride (0.61 g, 3.9 mmol), PyCloP (2.1 g, 5 mmol) and DIEA (2.6 mL, 15 mmol) were dissolved in dry dichloromethane (50 mL) and were stirred for 2 days at room temperature. The solution was concentrated to dryness and the residue was purified by chromatography over silica gel (CH_2Cl_2/methanol, 20:1, R_F = 0.49). Yield 0.1 g (0.4 mmol, 9%); m. p. 212 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 28 °C): δ = 0.99 (dd, ^3J(H,H) = 7.0 Hz, 6 H, 1), 2.00 (s, 3 H, 2), 2.09 (m, 1 H, 3), 3.76 (s, 3 H, 4), 3.81 (m, 3 H, 5), 4.43 (m, 2 H, 6 +7), 8.03 (d, ^3J(H,H) = 9.0 Hz, 1 H, 8), 8.41 (d, ^3J(H,H) = 7.3 Hz, 1 H, 9) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C): δ = 17.3 + 18.6 (a + b), 22.4 (c), 32.0 (d), 52.7 (e), 56.2 + 60.2 (f, g), 62.8 (h), 172.1 + 173.4 (i, j), 173.9 (k) ppm. MS (CI, NH$_3$, 200 °C): *m*/*z* = 261 [*M* + H]$^+$ (59%), 278 [*M* + NH$_3$]$^+$ (100%). HRMS (ESI calcd. for $C_{11}H_{20}O_5N_2Na$): *m*/*z*, found 283.12599; calcd. 283.12699. $C_{11}H_{20}O_5N_2$: calcd. C 50.76, H 7.74, N 10.76; found C 50.47, H 7.68, N 10.22.



15

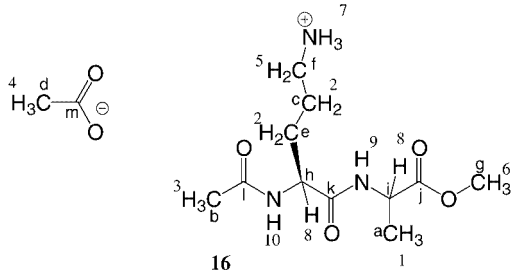
***N*-Acetyl-*N*$^\delta$-benzyloxycarbonyl-L-ornithine (15):** *N*$^\delta$-Benzyloxycarbonyl-L-ornithine (0.5 g, 1.9 mmol) was suspended in water (100 mL) and treated with acetic acid anhydride (1 mL). The reaction mixture was sonicated for 15 min, treated once more with acetic acid anhydride (1 mL) and sonicated again. This procedure was repeated six times. The solvent was evaporated and completely eliminated by threefold azeotropic distillation with small amounts

of toluene. The crude product was pure according to NMR and was used for the next step without further purification. Yield 0.58 g (1.9 mmol, 100%); m. p. 96 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 28 °C): δ = 1.58–2.08 (m, 4 H, 1), 1.63 (s, 3 H, 2), 3.16 (q, ^3J(H,H) = 6.3 Hz, 2 H, 3), 4.31 (m, 1 H, 4), 5.17 (s, 2 H, 5), 7.51 (m, 5 H, 6): δ = 8.27 (d, ^3J(H,H) = 8.0 Hz, 1 H, 7), 12.48 (br., 1 H, 8) ppm.



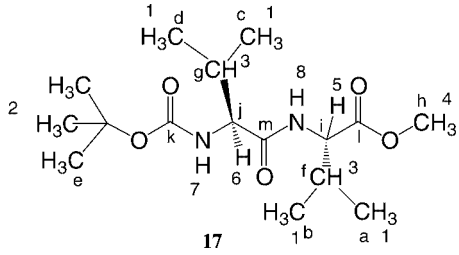
15a

***N*-Acetyl-N^{δ}-benzyloxycarbonyl-L-ornithinyl-L-alanine Methyl Ester (15a):** Compound **15** (1.36 g, 4.4 mmol), L-alanine methyl ester hydrochloride (0.58 g, 4.1 mmol), DIEA (4 mL, 23 mmol) and PyCloP (2.1 g, 5 mmol) were stirred in dry dichloromethane (50 mL) for three days at room temperature. The solution was concentrated and the residue was purified by chromatography over silica gel (CH_2Cl_2/methanol, 20:1, R_F = 0.08). The remaining oil was recrystallized from methanol/diethyl ether. Yield 1.21 g (3.1 mmol, 75%); m. p. 176 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 28 °C): δ = 1.28 (d, ^3J(H,H) = 7.3 Hz, 3 H, 1), 1.42–1.67 (m, 4 H, 2), 1.83 (s, 3 H, 3), 2.98 (q, ^3J(H,H) = 6.0 Hz, 2 H, 4), 3.60 (s, 3 H, 5), 4.25 (m, 2 H, 6), 5.01 (s, 2 H, 7), 7.35 (m, 5 H, 8), 7.97 (d, ^3J(H,H) = 8.0 Hz, 1 H, 9), 8.35 (d, ^3J(H,H) = 7.0 Hz, 1 H, 10) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C): δ = 17.4 (a), 22.5 (b), 27.4 (c), 30.6 (d), 41.3 (e), 52.9 (f), 54.2 + 56.0 (g, h), 70.9 (i), 128.9 + 129.1 + 129.6 (j, k, l), 138.5 (m), 159.1 (n), 173.3 + 174.2 + 174.6 (o, p, q) ppm. MS (EI): *m*/*z* = 393 [*M*]$^+$ (5%), 291 [Ac-Orn(Z)]$^+$ (6%), 104 [Ala-OMe + H]$^+$ (16%), 91 [C_6H_5–CH_2]$^+$ (100%). HRMS (EI): *m*/*z*, found 393.1900; calcd. 393.1894.



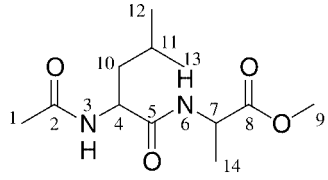
16

***N*-Acetyl-L-ornithinyl-L-alanine Methyl Ester Hydroacetate (16):** Compound **15a** (0.2 g, 0.5 mmol) was dissolved in methanol (40 mL) and treated with acetic acid (10 mL) and Pd/C (100 mg). Hydrogenolysis was carried out under hydrogen at normal pressure overnight. The catalyst was filtered off over silica gel and the residue was concentrated to dryness. A colourless solid remained. Yield 0.13 g (0.4 mmol, 81%); m. p. 40 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 28 °C): δ = 1.28 (d, ^3J(H,H) = 7.3 Hz, 3 H, 1), 1.45–1.70 (m, 4 H, 2), 1.82 (s, 3 H, 3), 1.83 (s, 3 H, 4), 2.61 (m, 1 H, 5), 3.61 (s, 3 H, 6), 4.14 (br., 3 H, 7), 4.26 (m, 2 H, 8), 8.05 (d, ^3J(H,H) = 8.0 Hz, 1 H, 9), 8.47 (d, ^3J(H,H) = 6.6 Hz, 1 H, 10) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C): δ = 17.3 (a), 22.5 (b), 24.1 + 25.0 (c, d), 30.2 (e), 40.2 (f), 52.8 (g), 53.7 + 53.9 (h, i), 173.3 + 173.7 + 174.5 + 174.6 (j, k, l, m). HRMS (ESI for $C_{11}H_{23}O_4N_3$ [*M* – Ac]$^+$): *m*/*z*, found 260.16110; calcd. 260.16103. $C_{13}H_{25}N_3O_6$: calcd. C 48.89, H 7.89, N 13.16; found C 48.80, H 7.96, N 12.64.



17

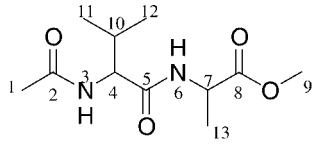
***N*-*tert*-Butoxycarbonyl-L-valinyl-L-valine Methyl Ester (17):** *N*-*tert*-Butoxycarbonyl-L-valine (0.3 g, 1.1 mmol), L-valine methyl ester (0.23 g, 1.4 mmol), PyCloP (0.58 g, 1.4 mmol) and DIEA (0.9 mL, 5.2 mmol) were dissolved in dry dichloromethane (50 mL) and stirred overnight. The solution was concentrated and the residue was purified by chromatography over silica gel to give a colourless solid (R_F = 0.50 with ethyl acetate/*n*-hexane, 1:2). Yield 0.23 g (0.7 mmol, 65%); m. p. 167 °C. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 0.86 (m, 3 H, 1), 1.40 (s, 9 H, 2), 2.10 (m, 2 H, 3), 3.67 (s, 3 H, 4), 3.83 (ddd, ^3J(H,H) = 8.9 Hz, 1 H, 5), 4.48 (ddd, ^3J(H,H) = 8.6 Hz, 1 H, 6), 4.95 (d, ^3J(H,H) = 6.0 Hz, 1 H, 7), 6.25 (d, ^3J(H,H) = 7.6 Hz, 1 H, 7) ppm. ^{13}C NMR (50.4 MHz, [D$_4$]MeOH, 25 °C): δ = 17.7 + 17.8 + 18.8 + 19.2 (a, b, c, d), 28.2 (e), 30.5 + 31.2 (f, g), 52.0 (h), 57.0 (i, j), 155.7 (k), 171.5 + 172.0 (l, m) ppm. MS (FD): *m*/*z* = 348 [*M* + NH$_4$]$^+$ (100%), 331 [*M* + H]$^+$ (90%). $C_{16}H_{30}N_2O_5$: calcd. C 58.16, H 9.15, N 8.48; found C 57.94, H 9.19, N 8.49.



18

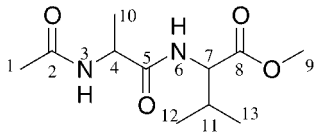
***N*-Acetyl-L-leucinyl-L-alanine Methyl Ester (18):** Ac-Leu-OH (173.2 mg, 1 mmol), *O*-(6-chloro-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU, 414 mg, 1.0 mmol), 6-chloro-1-hydroxybenzotriazole (Cl-HOBt, 424 mg, 2.5 mmol) and 2,6-lutidine (0.35 mL, 3.0 mmol) were dissolved in dry dichloromethane and DMF (3:1, 40 mL). The solution was cooled in an ice bath and treated with a solution of H-Ala-OMe hydrochloride (140 mg, 1.0 mmol) and 2,6-lutidine (0.12 mL, 1.0 mmol) in dichloromethane (1 mL). The reaction mixture was stirred for 2 h at 0 °C. Subsequently, the solution was washed thrice with satd. aqueous $NaHCO_3$, followed by HCl (1 M) and once with satd. NaCl. The aqueous layer was extracted with dichloromethane. After drying over Na_2SO_4, the combined organic phases were finally concentrated to dryness. Yield 189 mg (0.73 mmol, 73%); m. p. 114 °C. ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 0.84 (d, ^3J = 6.5 Hz, 3 H, H-12/13), 0.88 (d, ^3J = 6.5 Hz, 3 H, H-12/13), 1.27 (d, ^3J = 7.5 Hz, 3 H, H-14), 1.42 (d, ^3J = 6.0 Hz, 2 H, H-10), 1.57 (m, 1 H, H-11), 1.81 (s, 3 H, H-1), 3.30 (s, 3 H, H-9), 4.23 (m, 1 H, H-4), 4.33 (q, ^3J = 6.3 Hz, 1 H, H-7), 7.98 (d, ^3J = 8.5 Hz, 1 H, H-3/

6), 8.39 (d, ^3J = 7.0 Hz, 1 H, H-3/6) ppm. ^{13}C NMR (200 MHz, [D$_6$]DMSO): δ = 16.7 (C-14), 21.7 (C-12/13), 22.4 (C-11), 22.9 (C-12/13), 24.0 (C-1), 41.0 (C-10), 47.4 (C-9), 50.5 (C-7), 51.7 (C-4), 168.9 (C-2), 172.1 (C-8), 172.8 (C-5) ppm. MS (ESI positive): m/z = 281 [M + Na]$^+$. HRMS for $C_{12}H_{22}N_2O_4Na^+$ [M + Na$^+$]$^+$ (ESI positive): calcd. 281.1477; found 281.1479.



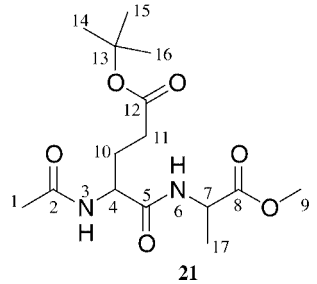
19

Acetyl-L-valinyl-L-alanine Methyl Ester (19): The preparation followed the procedure outlined above, with Ac-Val-OH and H-Ala-OMe hydrochloride. Yield 190 mg (0.78 mmol, 78%); m. p. 207 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 0.83 (d, ^3J = 7.0 Hz, 3 H, H-11/12), 0.87 (d, ^3J = 7.3 Hz, 3 H, H-11/12), 1.27 (d, ^3J = 7.5 Hz, 3 H, H-13), 1.85 (s, 3 H, H-1), 1.91 (m, 1 H, H-10), 3.60 (s, 3 H, H-9), 4.20 (m, 2 H, H-4, H-7), 7.88 (d, ^3J = 9.5 Hz, 1 H, H-3/6), 8.42 (d, ^3J = 6.3 Hz, 1 H, H-3/6) ppm. ^{13}C NMR (300 MHz, [D$_6$]-DMSO): δ = 16.7 (C11, C-12), 18.1 (C-13), 18.9 (C-1), 30.5 (C-10), 47.4 (C-9), 51.6 (C-7), 57.1 (C-4), 169.0 (C-2), 171.0 (C-8), 185.1 (C-5) ppm. MS (ESI positive): m/z = 267 [M + Na]$^+$. HRMS for $C_{11}H_{20}N_2O_4Na^+$ [M + Na]$^+$ (ESI positive): calcd. 267.1321; found 267.1324.



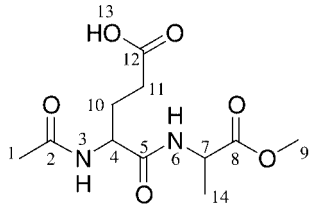
20

***N*-Acetyl-L-alaninyl-L-valine Methyl Ester (20):** The preparation followed the procedure outlined above, with Ac-Ala-OH and H-Val-OMe hydrochloride. Yield 166 mg (0.68 mmol, 68%); m. p. 105 °C; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 0.84 (d, ^3J = 3.5 Hz, 3 H, H-12/13), 0.89 (d, ^3J = 3.3 Hz, 3 H, H-12/13), 1.16 (d, ^3J = 7.0 Hz, 3 H, H-10), 1.81 (s, 3 H, H-1), 2.02 (m, 1 H, H-11), 3.62 (s, 3 H, H-9), 4.15 (dd, ^3J = 6.3 Hz, 1 H, H-7), 4.38 (q, ^3J = 7.3 Hz, 1 H, H-4), 8.03 (d, ^3J = 7.7 Hz, 1 H, H-3/6), 8.09 (d, ^3J = 8.3 Hz, 1 H, H-3/6) ppm. ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ = 18.0 (C-12/13), 18.1 (C-12/13), 18.8 (C-10), 22.3 (C-1), 29.8 (C-11), 47.7 (C-9), 51.5 (C-4), 57.2 (C-7), 168.9 (C-2), 171.8 (C-8), 172.7 (C-5) ppm. MS (ESI positive): m/z = 267 [M + Na]$^+$. HRMS for $C_{11}H_{20}N_2O_4Na^+$ [M + Na]$^+$ (ESI positive): calcd. 267.1321; found 267.1313.



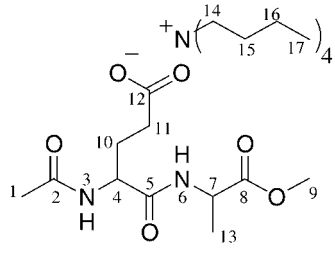
21

***N*-Acetyl-(*O*-*tert*-butyl)-L-glutamyl-L-alanine Methyl Ester (21):** The preparation followed the procedure outlined above, with Ac-Glu(O*t*Bu)-OH and H-Ala-OMe hydrochloride. Yield 270 mg (0.82 mmol, 82%); m. p. 103 °C; ^{1}H NMR: (300 MHz, [D$_6$]-DMSO): δ = 1.28 (d, ^3J = 7.3 Hz, 3 H, H-17), 1.39 (s, 9 H, H-14, H-15, H-16), 1.73 (m, 2 H, H-10), 1.82 (s, 3 H, H-1), 2.23 (m, 2 H, H-11), 3.61 (s, 3 H, H-9), 4.25 (m, 2 H, H-4, H-7), 8.01 (d, ^3J = 8.0 Hz, 1 H, H-3/6), 8.40 (d, ^3J = 6.8 Hz, 1 H, H-3/6) ppm. ^{13}C NMR: (300 MHz, DMSO): δ = 16.7 (C-17), 22.4 (C-1), 27.6 (C-14, C-15, C-16), 27.7 (C-10), 31.1 (C-11), 47.5 (C-9), 51.2 (C-7), 51.7 (C-4), 79.6 (C-13), 169.0 (C-2), 171.1 (C-12), 171.6 (C-8), 172.8 (C-5) ppm. MS (ESI positive): m/z = 331 [M + H]$^{++}$.



21a

***N*-Acetyl-L-glutamyl-L-alanine Methyl Ester (21a):** Compound **21** (50 mg, 0.15 mmol) was stirred in a mixture of dichloromethane/trifluoroacetic acid (DCM/TFA, 10 mL, 1:1) for 2 h with subsequent evaporation to dryness. Yield 41 mg (0.15 mmol, 99%); m. p. 64 °C; ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 1.28 (d, ^3J = 7.3 Hz, 3 H, H-14), 1.76 (m, 2 H, H-10), 1.83 (s, 3 H, H-1), 2.25 (m, 2 H, H-11), 3.61 (s, 3 H, H-9), 4.28 (m, 2 H, H-4, H-7), 8.02 (d, ^3J = 8.0 Hz, 1 H, H-3/6), 8.41 (d, ^3J = 7.0 Hz, 1 H, H-3/6), 12.13 (b, 1 H, H-13) ppm. ^{13}C NMR: (300 MHz, DMSO): δ = 16.6 (C-14), 22.3 (C-1), 27.5 (C-10), 29.9 (C-11), 47.4 (C-9), 51.3 (C-7), 51.7 (C-4), 169.0 (C-2), 171.2 (C-8), 173.8 (C-5), 195.4 (C-12) ppm. MS (ESI positive): m/z = 275 [M + Na]$^+$. HRMS for $C_{11}H_{18}N_2O_6H^+$ [M + Na]$^+$ (ESI positive): calcd. 275.1243; found 275.1233.



21b

Methyl Ester and Tetrabutylammonium Salt of *N*-Acetyl-L-glutamyl-L-alanine (21b): Compound **21a** (20 mg, 0.07 mmol) was dissolved in methanol (5 mL). A solution of HONBu$_4$ in methanol (1 M, 0.07 mmol, 70 µL) was added dropwise. The solution was concentrated to dryness and gave a viscous oil. Yield 36 mg (0.07 mmol, 99%); ^{1}H NMR: (300 MHz, [D$_6$]DMSO): δ = 0.92 (t, ^3J = 7.3 Hz, 12 H, H-17), 1.29 (m, 11 H, H-13, H-16), 1.56 (m, 8 H, H-15), 1.68 (m, 2 H, H-10), 1.77 (s, 3 H, H-1), 1.99 (m, 2 H, H-11), 3.17 (t, ^3J = 8.3 Hz, 8 H, H-14), 3.59 (s, 3 H, H-9), 4.15 (m, 2 H, H-4, H-7), 9.09 (d, ^3J = 6.6 Hz, 1 H, H-3/6), 9.29 (d, ^3J = 6.8 Hz, 1 H, H-3/6) ppm. ^{13}C NMR: (300 MHz, DMSO): δ = 13.3 (C-17), 16.8 (C-13), 19.1 (C-16), 22.5 (C-1), 23.0 (C-15), 28.9 (C-10), 35.3 (C-11), 47.5 (C-9), 51.5 (C-7), 53.0 (C-4), 57.5 (C-14), 168.6 (C-2), 172.1 (C-8), 173.1 (C-5), 175.1 (C-12) ppm. MS (ESI negative): m/z = 272 [M – NBu$_4$ – H]$^{2-}$.

Host–Guest Titrations: Two parent solutions were prepared; one contained the indicated amount of guest found in the tables, dissolved in CDCl$_3$ (7 mL). This solution was evenly distributed among 10 NMR tubes (0.7 mL per tube). For the host parent solu-

tion the corresponding amount of host was dissolved in the indicated amount of solvent (in general 1.5 mL). Defined amounts of the host parent solution were now added successively to the 10 samples (usually in steps of 40 μL up to 400 μL). Only sharp guest signals observable across the whole range of concentrations were selected for the evaluation. Association constants were determined from the $\Delta\delta$ values by nonlinear regression with the PC program SigmaPlot 2000. Volume changes were taken into account. The complete set of data is contained in the tables in the Supporting Information; for Supporting Information see also the footnote on the first page of this article

Job Plots: The indicated amounts of host and guest compound were dissolved in chloroform. Both solutions were combined in defined varying ratios, ranging from molar fractions of 0.1 to 0.9. All selected signals could be assigned unambiguously throughout the entire titration experiment. The solvent volumes and the complete set of complexation-induced shifts are contained in the appropriate tables in the Supporting Information (for details see the footnote on the first page of this article).

Self-Association Experiments: A self-association experiment with serine binder **4** over a concentration range of 2×10^{-2} to 2×10^{-3} M produced no change in the chemical shift (less than 0.03 ppm) of any NMR signal. Obviously the bulky substituted cyclohexane moiety of Kemp's triacid prevents any close approach of two host molecules to interact through their aminopyrazole binding sites. Considerable self-association might be expected for the ornithine-containing dipeptide. Although a self-association experiment in $CDCl_3$ over the concentration range of 3×10^{-2} to 3×10^{-3} M produced a large upfield shift of the C-terminal NH ($\Delta\delta$ = 1.2 ppm), the binding curve remained almost completely linear, indicating a negligible extent of self-association. We explain this upfield shift in terms of an intermolecular hydrogen bond between the appropriate amide proton and the acetate counterion of another peptide molecule.

Molecular Modelling Experiments: Force-field calculations were performed on a Silicon Graphics O2 workstation with MacroModel 7.0. The MM3 and OPLS-AA force-fields were both used for most calculations and produced similar results in chloroform. Monte-Carlo conformational searches were conducted over 1000 steps.

NOESY Experiments: The serine binder **4** was dissolved at a concentration of 6.1 mg mL^{-1} = 11.8 mM in a mixture of CD_2Cl_2 and CD_3CN (2:1); most NMR signals were sharp including the amidic NH protons of aminopyrazole and aminoacetophenone. A standard NOESY experiment was performed at 400 MHz and room temperature. Only positive cross-peaks were produced. All observed cross-peaks were reciprocal; they were strong in the Kemp's triacid part of the host molecule and weaker in the aromatic region. However, a distinct reciprocal NOE was found between two specific aromatic CH protons of the aminopyrazole and the aminoacetophenone moiety (see also Figure 6).

Karplus Analysis: ^3J coupling constants between the *NH* proton and the corresponding α-protons were measured by NMR in the free and complexed states of three selected peptides: Ac-Ser-Ala-OMe, Ac-Phe-Ala-OMe and Ac-Orn-Ala-OMe were each complexed with the host molecule designed for the specific additional noncovalent interaction. These values were compared with the calculated ^3J coupling constants obtained from Monte-Carlo simulations of the optimized complex geometries. In most cases, the experimental value found for the complex in solution was in-between the experimentally measured value for the free peptide and the calculated value for the pure complex. This correlates with the results from the nonlinear regressions, and indicates that saturation had not yet been reached by the end of each titration.

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Received: June 1, 2005
Published Online: November 9, 2005

