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Influence of ring substitution on the conformation and β-turn mimicry of 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one peptide mimetics

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ABSTRACT

Analogs of 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-ones, containing a methyl substituent at the 4- or 5-position, or a phenyl substituent at C-1, were prepared. Conformational analysis of tetrapeptide models containing these analogs indicated different conformations of the benzazepinone ring, and extended backbone conformations, except for the 4-methyl-substituted analog. The latter was shown to have a strong preference for a turn conformation. Incorporation into the N-terminal tetrapeptide sequence of dermorphin resulted in potent opioid analogs and an indication that the receptor-bound conformation might not adopt a turn structure.

1. Introduction

It is estimated that almost half of the current drugs targets G-protein coupled receptors (GPCRs). Designing ligands for these receptors therefore constitutes a major method toward new pharmaceuticals. Many of their natural ligands are peptides. Tyndall et al. demonstrated that most peptide-activated GPCRs bind to their ligands through a turn structure.[1] Also, turns usually occur on the exposed surface of proteins and are likely to be involved in many of the molecular recognition events, such as interactions between antibodies and antigens, and regulatory enzymes and their corresponding substrates.[2] Therefore, to better understand the molecular mechanism of peptide–protein or protein–protein interactions, and to provide potent therapeutic agents, much effort has been devoted to the design and synthesis of small mimetics of turn structures.[3–7]

Different types of tight turns have been defined, depending on the number of amino acid residues in the turn: δ-, γ-, β-, α-, and π-turns, involving 2–6 residues, respectively.[2] A general definition of a β-turn stated that any tetrapeptide chain in which the distance between the $C^{\alpha}(i)$ and the $C^{\alpha}(i+3)$ was <7 Å and which occurs in a non-helical region constitutes a β-turn.[8,9] The presence of an intramolecular H-bond between the CO of the first residue (i) and the amide NH of the fourth residue ($i+3$) to form a pseudo 10-membered ring is often, though not always, observed.[10]

One of the first examples of β-turn mimicry was reported by Freidinger who introduced a γ-lactam **1** into the luteinizing hormone-releasing hormone sequence (Fig. 1). The modified peptide turned out to be more potent than the parent hormone.[11] Freidinger also developed six- and seven-membered δ- and ε-lactam **2** and **3** as molecular scaffolds,[12] which were proposed to stabilize β-turns of type II.[13] Dehydro-Freidinger lactams **4**, synthesized by ring-closing metathesis reactions, were also claimed to be β-turn mimetics.[14,15] The application of these lactams and of their hetero- or benzo-fused analogs has been very successful for the design of inhibitors of angiotensin converting enzyme, neutral endopeptidase, renin, thrombin, and others, as well as for analogs of the dopamine modulator PLG, CCK and gastrin, bradykinin, neurokinin A, and growth hormone-releasing hormone peptides.[16]

Abbreviations: Aba, 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one; Ac, acetyl; Boc, *tert*-butyloxycarbonyl; CCK, cholecystokinin; DAMGO, [D-Ala^2,NMePhe^4,Gly^5-ol]enkephalin; DIPEA, diisopropylethylamine; Dmt, 2′,6′-dimethyl-L-tyrosine; DCC, *N*,*N*′-dicyclohexylcarbodiimide; EDC, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide; GPCR, G-protein coupled receptor; Hba, 4-amino-1,2,4,5-tetrahydro-8-hydroxy-3-oxo-2*H*-2-benzazepine; Moc, methyloxycarbonyl; MSB, methyl-2-((succinimidooxy)carbonyl)benzoate; NOE, Nuclear Overhauser Effect; SUMM, Systematic Unbounded Multiple Minimum search; TBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; TFMSA, trifluoromethanesulfonic acid.

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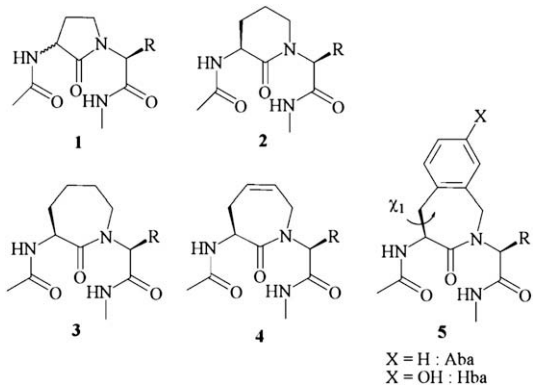


Figure 1. Examples of Freidinger lactams proposed as β-turn mimics.

The structural resemblance of the dehydro-Freidinger lactam **4** with the 4-amino-1,2,3,4-tetrahydro-2-benzazepin-3-one (Aba, Hba) dipeptidic motif **5** has stimulated us to investigate its potential to induce a β-turn conformation. The Aba and Hba structures have been applied successfully as conformational constrained Phe and Tyr analogs, respectively. The C^{α}–C^{β} dihedral angle (χ_1) is fixed to +60° or 180°, which corresponds to a *gauche*(+) and a trans conformation, respectively.[17] It has proven to influence receptor selectivity in opioid peptides,[17–19] and to provide selective enzyme inhibitors[20–23] and antigenic peptides.[24]

In an earlier report, the synthesis and evaluation of the β-turn properties of the tetrapeptide models Ac-Aba-Gly/Ala-NHMe **6**–**8**, Ac-MeAba-Gly-NHMe **9**, and of their spirocyclic derivative **10** (Fig. 2) were investigated by NMR spectroscopy and molecular modeling. Interestingly, all lactams **6**–**9** adopt extended conformations, only the spiro-benzazepinone-containing peptide analog **10** showed a strong preference for the formation of a type II′ β-turn.[25] It was noticed that the azepinone ring conformation was different in lactams **6**–**9** where it adopts a chair-like conformation, from that in spiro-analog **10** where it adopts a boat-like conformation. This suggested that the propensity of these 2-benzazepinones for adopting a turn conformation might depend on the ring conformation. The ring conformation, on the other hand, might be influenced by the introduction of substituents at position 1, 4 or 5.

In this paper, we report on the synthesis and conformational analysis by NMR spectroscopy and molecular modeling for the evaluation of the β-turn mimicry of the ring substituted *N*-acetyl dipeptide *N*′-methyl amides **11**–**15** tetrapeptide models.

2. Results and discussion

2.1. Synthesis of the tetrapeptide mimetics

The synthesis of the racemic *erythro*- and *threo*-5-Me-Aba-Gly building blocks for derivatives **12** and **13** was performed using an acyliminium ion cyclization pathway as was previously published for the unsubstituted analog **6** (Scheme 1).[17] The required (±) *erythro*-β-Me-Phe-OH **16** was obtained by crystallization from the *erythro*/*threo* mixture according to the procedure reported by Kataoka.[26] Catalytic hydrogenation of *Z*-2-benzamido-3-phenyl-2-butenoic acid methyl ester, which was obtained by Erlenmeyer condensation between acetophenone and hippuric acid, followed by isomerization of the *E*-isomer in pyridine, provided the precursor of (±) *threo*-β-Me-Phe-OH **17**.[27,28]

erythro-β-Me-Phe-OH **16** and *threo*-β-Me-Phe-OH **17** were phthaloyl protected (Scheme 1) by means of methyl-2-((succinimidooxy)carbonyl)benzoate (MSB) to afford **19** and **20**, respectively.[29] *threo*-isomer **20** was purified by flash column chromatography to remove the open derivative **18**. This side product was not observed during phthaloyl protection of the *erythro* derivative.

Coupling of Phth-*erythro*-β-Me-Phe-OH **19** and Phth-*threo*-β-Me-Phe-OH **20** with methyl or ethyl glycinate, respectively, using TBTU as a coupling reagent yielded **21** and **22**, which were subsequently hydrolyzed to **23** and **24**, respectively. Following formation of oxazolidinone **25** and **26** by reaction with formaldehyde using an azeotropic distillation, ring closure was effected by formation of an acyliminium ion intermediate using trifluoromethanesulfonic acid (TFMSA) to provide compounds **27** and **28**.[30,31] Phthaloyl deprotection by hydrazinolysis[17] led to *erythro*- and *threo*-5-Me-Aba-Gly-OH, which were acetylated with acetic anhydride in water,[32] and further transformed into methyl amides **12** and **13** using *N*,*N*-dicyclohexylamine (DCC) as a coupling reagent. The compounds were purified by preparative HPLC. Any epimerization at C-4 during these reaction steps would result in the formation of the *threo*-isomer from the *erythro*-isomer and vice versa. Since these stereoisomers were well separated in the HPLC chromatogram (see Experimental section), epimerization could be excluded.

The starting material for Ac-4-Me-Aba-Gly-NHMe **11**, (*R*,*S*)-2-amino-3-(2-cyanophenyl)-2-methylpropanoic acid hydrochloride **33** (Scheme 2), was prepared via a phase transfer catalyzed alkylation of *N*-benzylidene alanine ethyl ester **29**[33,34] with *o*-cyanobenzyl bromide, benzyltriethylammonium chloride as a catalyst and KOH/K_2CO_3 as a base, resulting in compound **30**.[35,36] Hydrolysis of

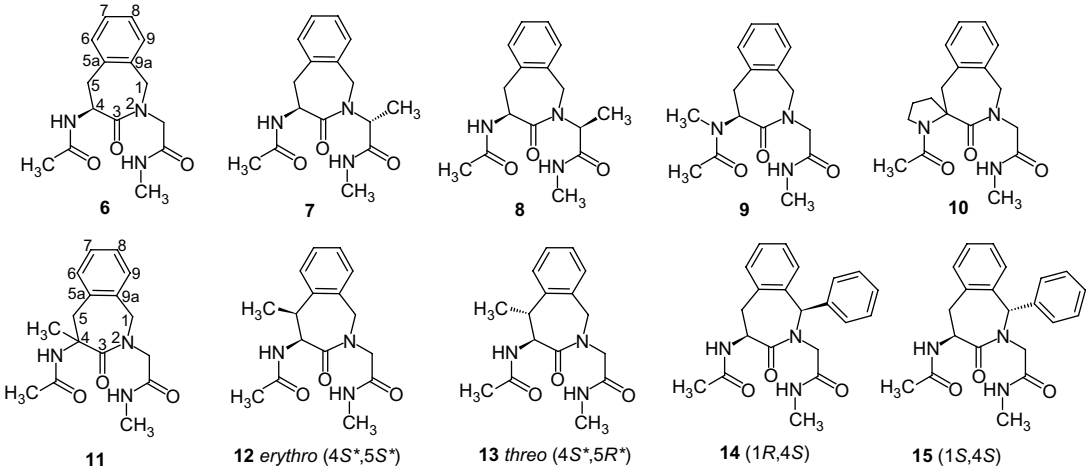
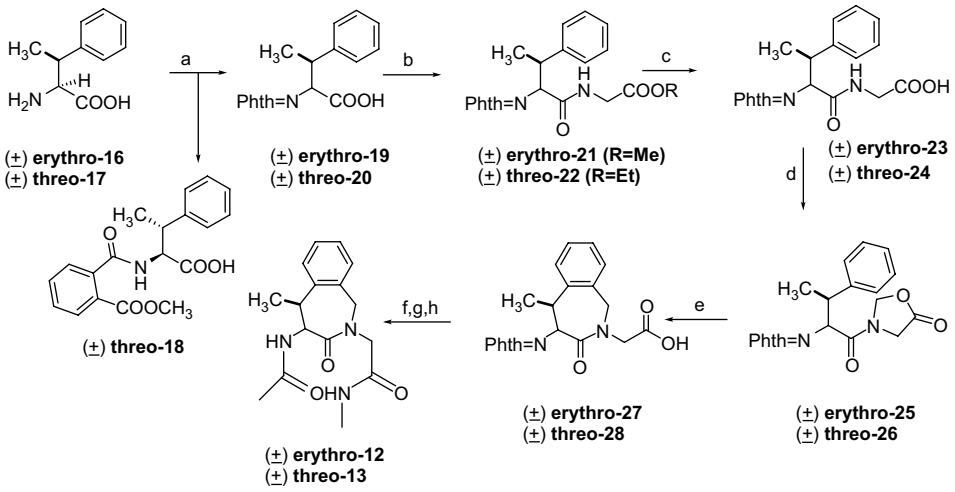


Figure 2. Tetrahydro-2-benzazepinones as potential β-turn mimetics.



Scheme 1. Synthesis of Ac-*erythro*- and *threo*-5-Me-Aba-Gly-NHMe **12** and **13**. (a) MSB, H_2O, CH_3CN, overnight, rt, **19**: 65%, **20**: 72%; (b) HCl·Gly-OMe/OEt, TBTU, Et_3N, CH_2Cl_2, 1 h, rt, **21**: 64%, **22**: 30%; (c) 1 N HCl, acetone, 19.5 h, Δ, **23**: 100%, **24**: 100%; (d) benzene, 5 h reflux, $(CH_2O)_n$, *p*-Tos-OH, **25**: 89%, **26**: 89%; (e) CF_3SO_3H, CH_2Cl_2, overnight, rt, **27**: 100%, **28**: 100%; (f) NH_2-NH_2·H_2O, EtOH; 1,5 h reflux; (g) H_2O, Et_3N, Ac_2O; (h) CH_3CN/H_2O (3:1), pyridine, DCC, CH_3-NH_2·HCl.

the imine protecting group using a 2 N HCl solution provided the aminoester, which was hydrolyzed with a 6 N HCl solution at 65 °C to obtain (*R*,*S*)-2-amino-3-(2-cyanophenyl)-2-methylpropanoic acid hydrochloride **33**. Other acidolytic conditions (6 N HCl/acetone, reflux) resulted in the formation of the side product 1-imino-3-methyl-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid **31**.

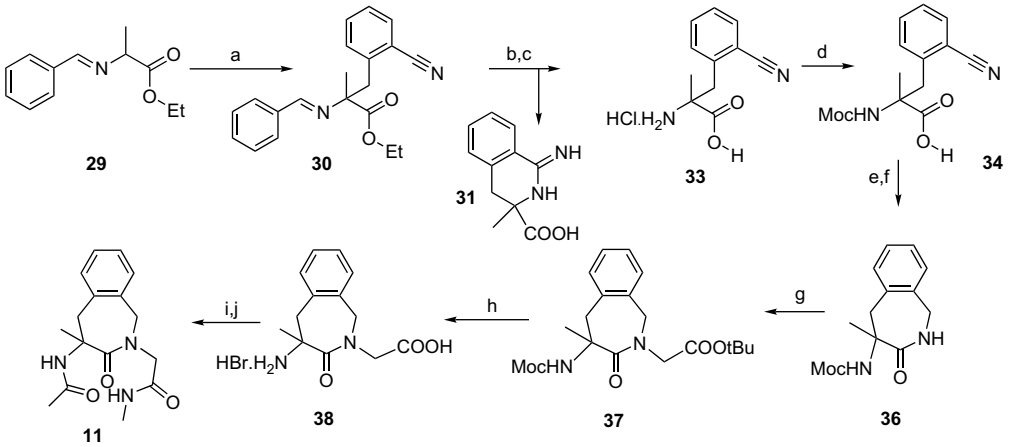
Compound **33** was methyloxycarbonyl (Moc) protected by means of MocCl. The use of 4.0 equiv of MocCl and a reaction temperature of 0 °C were necessary to avoid the formation of the side product **31**. Reduction of the nitrile in **34** with H_2/10% Pd on C provided the primary amine. Intramolecular cyclization was performed using 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDC) as a coupling reagent. Alkylation of (*R*,*S*)-Moc-4-Me-Aba **36** with ^{t}Bu-bromoacetate and NaH as a base provided ester **37**. Moc deprotection and ester hydrolysis were performed using a 33% solution of HBr in AcOH to provide compound **38**, which was further transformed into *N*-acetyl methyl amide **11**, as described above for **12** and **13**.

The 1-phenyl-Aba-Gly isomers for the tetrapeptide models **14** and **15** were synthesized by reaction of *N*-phthaloyl-phenylalanine chloride with *N*-benzylideneglycinate ethyl ester, as reported earlier.[37] The resulting trans and cis isomers **41** and **40** were separated using silica gel chromatography (Scheme 3). Further transformation of the *N*-phthaloyl-protected ethyl esters into pseudotetrapeptides **14** and **15** was performed as described above.

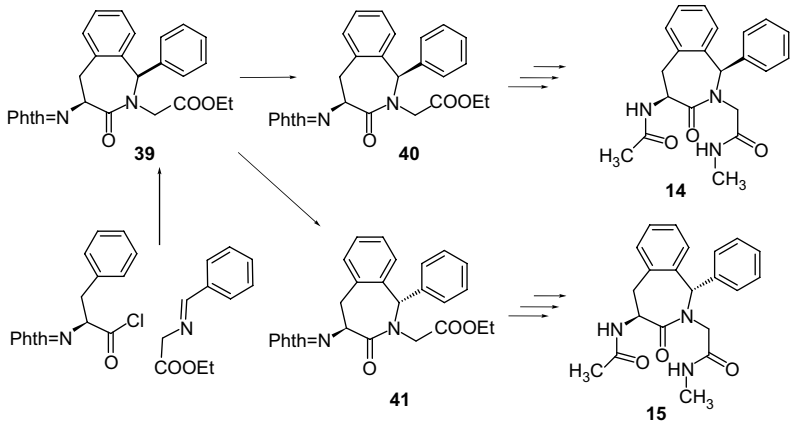
2.2. Molecular modeling results

The conformational preferences for the different tetrapeptide models **11**–**15** were investigated using an identical procedure as the one described for the non-ring substituted analogs **6**–**10**.[25] In order to find the most relevant minimum-energy conformations of the ring structure in the five substituted 4-amino-2-benzazepin-3-ones **11**–**15** (Fig. 2), a random pure low mode search[38] and energy minimization in vacuo with the Macromodel 5.0[39] MM3*[40] force field were carried out on the different 4-formylamino-2-*N*-Me-2-benzazepinones (4-Me-, *erythro*/*threo*-5-Me-, 1-phenyl-Aba). Secondly, the exocylic functions were added in each of the low energy ring conformations obtained to yield the pseudotetrapeptide structures Ac-4-Me-Aba-Gly-NHMe **11**, Ac-*erythro*-5-Me-Aba-Gly-NHMe **12** and *threo*-5-Me-Aba-Gly-NHMe **13**, (1*R*,4*S*)-4-acetamido-1-phenyl-Aba-Gly-NHMe **14**, and (1*S*,4*S*)-4-acetamido-1-phenyl-Aba-Gly-NHMe **15**. Subsequently, the peptide backbone was subjected to a systematic unbounded multiple minimum (SUMM) search[41] and energy minimization in combination with the GB/SA solvatation model (water).[42] All conformations within 50 kJ/mol of the global minimum were clustered into families based on geometrical similarity.

The β-turn mimicry of the different tetrapeptide mimetics was evaluated using various criteria (Fig. 3): (1) the presence of



Scheme 2. Synthesis of Ac-4-(*R*,*S*)-Me-Aba-Gly-NHMe **11**. (a) KOH, K_2CO_3, $(Et)_3BnNCl$ *o*-CN-BnBr; (b) 2 N HCl, 63% (four steps); (c) 6 N HCl, 65 °C, 81%; (d) 1 N NaOH, MocCl, 69%; (e) Pd/C, H_2, 74%; (f) Py, EDC, 97%; (g) NaH, $BrCH_2COO^tBu$, 79%; (h) 33% HBr/AcOH; (i) Et_3N, Ac_2O; (j) Py, DCC, CH_3-NH_2·HCl, 60% (three steps).



Scheme 3. Synthesis of tetrapeptide models **14** and **15**.[37]

a hydrogen bond in type I and II β-turns is indicated by a distance between the *N*-acetyl carbonyl oxygen atom and the methyl carboxamide proton smaller than 2.5 Å and an NH–O angle greater than 120°,[43] (2) an interatomic distance between $C^{\alpha}1$ and $C^{\alpha}4$ less than 7 Å,[8,9] (3) the virtual torsion angle β, defined by C1,$C^{\alpha}2$,$C^{\alpha}3$, and N4 of the tetrapeptide model defines the β-turn class (Table 1),[44] (4) the characteristic φ- and ψ-angle of the central residues, which define the turn type.[2]

In Table 2 and Figure 4 the lowest-energy conformers together with the numerical values for the various criteria are given.

The lowest-energy conformer **11a** of Ac-4-Me-Aba-Gly-NHMe **11** structure corresponds to a β-turn of type I, with a *gauche*(+) ring conformation. The second lower energy conformer **11b** is only 2.34 kJ/mol (0.56 kcal/mol) above the absolute minimum and adopts a trans1 ring conformation. A β-turn of type II′ is induced. For both Ac-*erythro*-5-Me-Aba-Gly-NHMe **12** and *threo*-5-Me-Aba-Gly-NHMe **13** none of the lowest-energy conformers show a β-turn structure. In both cases the ring adopts the same trans2 conformation, irrespective of the orientation of the 5-methyl substituent. The first conformation of Ac-*erythro*-5-Me-Aba-Gly-NHMe **12**, which adopts a turn (type I) is 17.87 kJ/mol above the minimum and adopts a *gauche*(+) ring conformation. For *threo*-isomer **13**, the first turn conformation is found to be 10.54 kJ/mol above the global minimum.

The lowest-energy conformer of (1*R*,4*S*)-4-acetamido-1-phenyl-Aba-Gly-NHMe **14** adopts a trans1 ring conformation, with a pseudo-axial phenyl orientation. No turn formation is observed for the low energy conformers. The conformer of **14**, which adopts a turn (type II′) is 29.44 kJ/mol above the minimum and adopts also a trans1 ring conformation. The lowest-energy conformation of 1(*S*)-isomer **15** adopts a trans2 conformation, without turn properties. A turn (type II′) conformation is found to be 25.38 kJ/mol above the minimum in a *gauche*(+) ring conformation. Interestingly, the preferred ring conformations calculated for **14** and **15** correspond to those found in the crystal structure of the phthaloyl-protected precursors.[37]

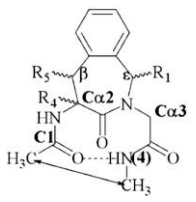


Figure 3. Criteria used to evaluate β-turn mimicry.

2.3. NMR analysis

The structural confirmation and the conformational analysis of the tetrapeptide models were accomplished by ^{1}H NMR and ^{13}C NMR. The chemical shift assignments were supported by heteronuclear ^{1}H–^{13}C HMQC[45] and HMBC[46] spectra. The conformation of the benzazepinone ring was examined by 2D ^{1}H NOESY NMR.[47]

As shown in Table 3, the conformation of the amino-2-benzazepinone ring in **11**–**13** was clearly identified by a set of NOE data. The conformations are characterized by the value of χ_1 angle of the Aba amino acid structure. All compounds were shown to adopt a trans conformation. For the 4-methyl-substituted isomer **11**, evidence (Table 3) is given by the NOE cross-peaks between the β-H atom and the methyl hydrogens, and the strong NOE between Hβ and the aromatic H6. In **11**, a strong NOE between Hβ′ and Hε′ and between Hε and the aromatic H9 indicates a preferred boat-like trans1 conformation. This ring conformation is not the lowest-energy conformer that was found by the molecular modeling (Table 2), but corresponds to that found as the second lowest one **11b** (2.34 kJ/mol above minimum). The 5-methyl-substituted isomers **12** and **13** both show a different ring conformation compared to **11b**. Indeed, in **12** a strong NOE is observed between Hα and Hε, and between Hε′ and H9, which corresponds to the calculated chair-like trans2 conformation with a pseudo-axial methyl group. A similar trans2 conformation was found for *threo*-isomer **13**, which also corresponds to the calculated preferred structure.

Evidence for the presence of an intramolecular hydrogen bond between the carbonyl oxygen atom of the first residue and the amide NH proton of the fourth residue, which is present in most of the β-turns, was evaluated in scaffolds **11**–**15**. Intramolecular hydrogen bonds are very little influenced by external factors such as temperature and solvent in comparison with intermolecular hydrogen bonds. Therefore, to evaluate the effect of switching from a non-hydrogen bond forming solvent to a strong hydrogen bond forming solvent on the chemical shift of the N*H*-Me and Ac-N*H* protons, the ^{1}H NMR spectra of the benzazepinone compounds were measured in $CDCl_3$ and DMSO-d_6. In a second

Table 1
Virtual torsion angle β defining β-turn classes[44]

Type of β-turn	Virtual torsion angle β
I	$10<\beta<80$
I′	$-79<\beta<-10$, $20<\beta<40$
II	$-69<\beta<-60$, $-49<\beta<-40$, $-29<\beta<40$
II′	$-59<\beta<-50$, $-29<\beta<30$

Table 2
The lowest-energy conformers and values for the various criteria

Structure	Ac-4-Me-Aba-Gly-NHMe **11a**	Ac-4-Me-Aba-Gly-NHMe **11b**	Ac-*erythro*-(4*S**,5*S**)-5-Me-Aba-Gly-NH-Me **12**	Ac-*threo*-(4*S**,5*R**)-5-Me-Aba-Gly-NH-Me **13**	(1*S*,4*S*)-Ac-phenyl-Aba-Gly-NHMe **15**	(1*R*,4*S*)-Ac-phenyl-Aba-Gly-NHMe **14**
Ring conformation[a]	*gauche* (+)	trans1	trans2	trans2	trans2	trans1
Turn	I	II′	—	—	—	—
d(NH-CO) (Å)	2.1	2.1 [2.08(4)][b]	6.6	6.6	6.5	6.3
NHO angle (°)	163	163 [161(3)][b]	147	140	149	157
d(Cα1-Cα4) (Å)	5.1	5.4 [5.505(4)][b]	9.6	9.3	9.3	9.6
β (°)	17	−10 [−17.4(2)][b]	−150	−147	−137	146
*Φ*2 (°)	−52	60 [50.7(4)][b]	−163	−131	−163	−153
*Ψ*2 (°)	−51	−121 [-131.5(3)][b]	161	159	164	−120
*Φ*3 (°)	−100	−98 [−93.6(3)][b]	−114	−100	−107	102
Ψ3 (°)	21	17 [14.3(4)][b]	24	26	27	−22
E of first turn (kJ/mol)	2.34, t1 (=**11b**), type II′		17.87, *g*(+), type I	10.54, *g*(+), type I	25.38, *g*(+), type II′	29.44, t1, type II′

[a] The ring conformations are classified by the χ_1 torsion angle of the Aba amino acid, which can be either *gauche*(+) or trans. For each of these conformations, two orientations of Cε are possible. In the trans1 conformation, the Cβ–H is in close proximity of Cε–H, whereas in the trans2 conformation the Cα–H is in close proximity of Cε'-H.
[b] Experimental values found in the x-ray structure (Fig. 5).

experiment, samples in DMSO-d_6 were heated to examine the effect of the temperature increase on the chemical shift of amide protons.

For the 4-Me-Aba derivative **11**, the N*H*-Me amide resonance is only very little influenced by switching from $CDCl_3$ to DMSO (−0.08 ppm), in contrast to that of the N*H*-Ac (−2.15 ppm). Moreover, its temperature coefficient is −3.7 ppb/K, indicating the presence of an intramolecular hydrogen bond for this compound. Further evidence of the intramolecular hydrogen bond was given by the presence of NOE correlations between the Aba ring protons and those of the glycine C^{α}. The observation of a strong NOE between one of the glycine C^{α} hydrogens and C–Hε, and not between the other pair, indicates a limited rotational freedom in the exocyclic part of the molecule, which supports the presence a pseudo 10-membered ring. The NMR data are therefore in complete accordance with the second lowest-energy conformer of the Ac-4-Me-Aba-Gly-NHMe tetrapeptide **11b**, which was obtained through molecular modeling.

For the 5-methyl-substituted isomers **12** and **13**, the NOE data in Table 3 indicate the presence of a chair-like trans2 conformation of the 5-methyl-Aba ring, in agreement with the molecular modeling results. The large shifts of the NH signal found in the solvent study and in the temperature study for compounds **12**–**15** (Table 4) do not support the presence of an intramolecular hydrogen bond. These data indicate that, in agreement with the theoretical prediction, compounds **12**–**15** do not adopt a β-turn conformation.

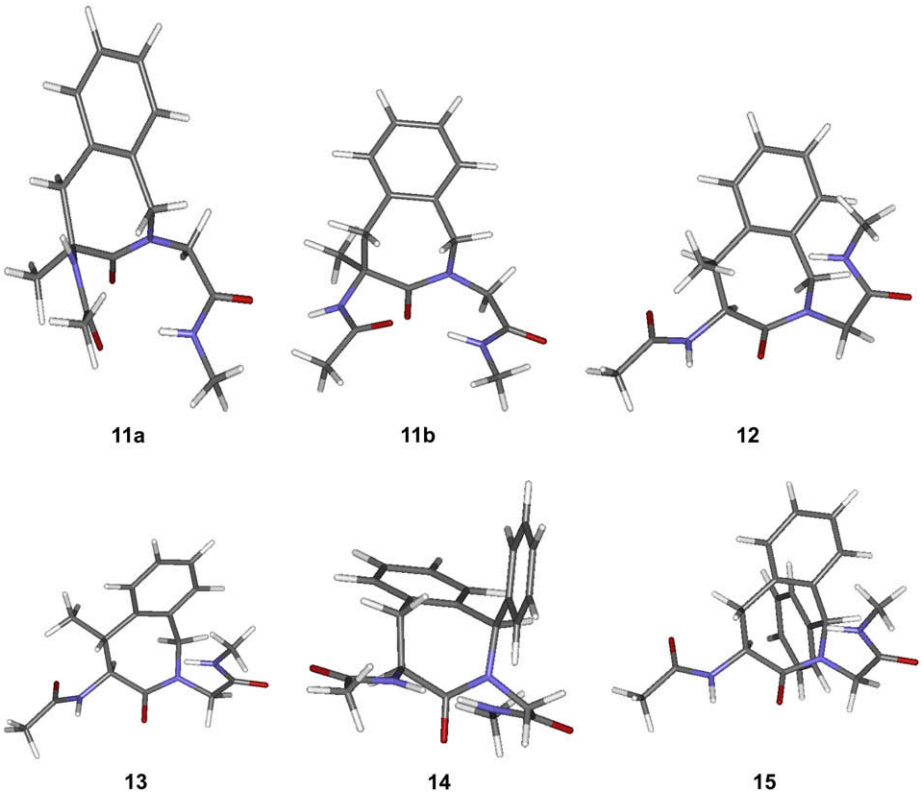
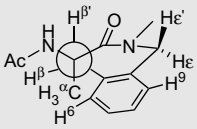
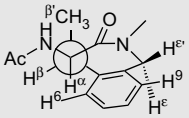
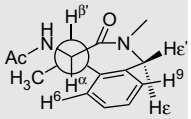


Figure 4. 3D representation of the lowest-energy conformers.

Table 3
NMR data for determination of the conformation of the Aba ring

	 **11b** Ac-4-Me-Aba-Gly -NHMe	 **12** Ac-*erythro*-(4*S**,5*S**)-5-Me-Aba-Gly-NH-Me	 **13** Ac-*threo*-(4*S**,5*R**)-5-Me-Aba-Gly-NH-Me
^3J (Hα,Hβ)	—	2.6 Hz	—
^3J (Hα,Hβ′)	—	—	11.0 Hz
NOE			
$C^{\alpha}(CH_3)$–$C^{\beta}(H)$	Strong	—	—
$C^{\alpha}(H)$–$C^{\beta}(H/CH_3)$	—	Strong	Strong
NOE			
$C^{\alpha}(CH_3)$–$C^{\beta'}(H)$	Absent	—	—
$C^{\alpha}(H)$–$C^{\beta'}(H/CH_3)$	—	Absent	Absent
NOE			
$NH_{Ac}/C^{\beta}(H/CH_3)$	Strong	Weak	Weak
NOE			
$NH_{Ac}/C^{\beta'}(H/CH_3)$	Weak	Strong	Strong
NOE			
Hβ/H6	Strong	Strong	—
Hβ′/H6	Absent	—	Strong
NOE			
$C^{\alpha}(H/CH_3)$/Hε	Absent	Strong	Strong
NOE			
$C^{\alpha}(H/CH_3)$/Hε′	Absent	Absent	Absent
NOE			
$C^{\beta'}(H/CH_3)$/Hε′	Strong	Absent	Weak
NOE			
Hε/H9	Strong	Absent	Weak
Hε′/H9	Absent	Strong	Strong

2.4. X-ray diffraction

Crystals suitable for X-ray diffraction were obtained for Ac-4-Me-Aba-Gly-NHMe **11**. Experimental values of the torsion angles and intramolecular H-bond geometry shown in Table 2 confirm that the crystal structure (Fig. 5) is completely identical with that of conformation **11b**, which was observed in solution, and which corresponds to the calculated second lowest-energy one. The seven-membered ring conformation is an ideal boat.

2.5. Biological application

The heptapeptide dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) was isolated from the skin of the South American frog *Phyllomedusa sauvagei*.[48] It is one of the most potent and selective μ-opioid receptor agonists among the naturally occurring opioids.[49] The minimal sequence required for opiate-like activity in vivo has been shown to be the N-terminal tetrapeptide.[50,51] Moreover, replacement of Tyr[1] in opioid peptides with the unnatural amino acid

Table 4
Solvent and temperature effect on amide protons in compounds **11**–**15**

	$\Delta\delta/\Delta T$ (ppb/K)	Δ $CDCl_3$ (ppm)	δ DMSO (ppm)	$\Delta\delta$
(R,S)-4-Me-Aba-Gly ***11***				
Ac-N*H*	−7.0	6.62	8.77	−2.15
N*H*-Me	−3.7	7.60	7.68	−0.08
Ac-erythro-(4S,5S*)-5-Me-Aba-Gly-NH-Me* ***12***				
Ac-N*H*	−7.0	6.94	7.87	−0.93
N*H*-Me	−6.5	6.08	7.71	−1.63
Ac-threo-(4R,5S*)-5-Me-Aba-Gly-NH-Me* ***13***				
Ac-N*H*	−5.4	6.40	8.18	−1.78
N*H*-Me	−6.1	5.95	7.75	−1.80
(1S,4S)-Ac-1-phenyl-Aba-Gly-NHMe ***15***				
Ac-N*H*	−7.0	6.86	7.84	−0.98
N*H*-Me	−7.0	6.38	7.83	−1.45
(1R,4S)-Ac-1-phenyl-Aba-Gly-NHMe] ***14***				
Ac-N*H*	−7.2	6.60	8.45	−1.85
N*H*-Me	−6.1	6.28	7.75	−1.47

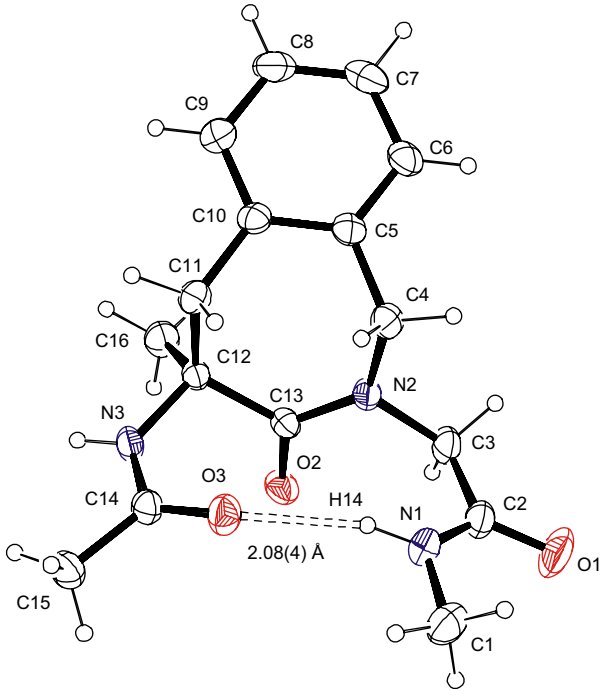


Figure 5. Crystal structure of **11** showing thermal ellipsoids at 30% probability level and intramolecular H-bond: O3···N1; O3···H14; angle 161; *d*(C1···C15) 5.505(5) Å.

Table 5
Afinities for the μ- and δ-opioid receptors[65]

Peptide sequence	K_i[a] (nM)	K_i[b] (nM)
H-Dmt-D-Ala-(*S*)-Aba-Gly-NH_2 **42**	0.047±0.0012	2.4±0.6
H-Dmt-D-Ala-(4*S*)-Me-Aba-Gly-NH_2 **43**	4.6±1.7	859±107
H-Dmt-D-Ala-(*S*)-spiroAba-Gly-NH_2 **44**	3.2±0.4	308±21

[a] Displacement of [^{3}*H*]DAMGO in rat brain homogenate.
[b] Displacement of [^{3}H] [Ile^{5,6}]deltorphin 2 in rat brain homogenate.

2′,6′-dimethyltyrosine (Dmt) resulted in compounds with increased agonist potency.[52–54] We therefore prepared analogs of H-Dmt-D-Ala-Phe-Gly-NH_2 in which we have substituted the Phe-Gly dipeptide by the constrained Aba-Gly (as in **6**), 4-Me-Aba-Gly (as in **11**) and by the previously reported spiro-Aba-Gly[25,55] (as in **10**). The peptides were prepared by standard solid phase peptide synthesis as described before[55] and their affinities for the μ- and δ-opioid receptors were determined. As shown in Table 5, analog **42** containing the Aba-Gly scaffold displayed the highest affinity for both receptors. Whereas analog **43** containing 4-Me-Aba-Gly showed a much reduced affinity compared to **42**, it still has low nanomolar affinity for the μ-opioid receptor, which is comparable to that of the spiro-Aba-Gly containing analog **44**. The latter scaffold was shown to induce a β-turn in the opioid peptide endomorphin-2.[55] This is consistent with the fact that the (4*S*)-Me-Aba-Gly scaffold induces a similar turn conformation in tetrapeptide **43**. The lower affinities of the two analogs having a turn conformation compared to that of the analog having the Aba-Gly scaffold, which adopts an extended conformation, might point out that the latter is more able to adopt the optimal binding conformation. This also suggests that the turn conformation induced by the (4*S*)-Me-Aba-Gly and spiro-Aba-Gly scaffolds in these tetrapeptides would not be the bioactive one for binding to the μ-opioid receptor. Further application of all stereoisomers of the scaffolds reported here in opioid peptides is currently ongoing and will be reported separately.

3. Conclusions

The attempts to influence the ring conformation of the 1,2,4,5-tetrahydro-4-amino-2-benzazepin-3-one scaffold by the introduction of ring substituents were only successful for the 4-methyl analog **11**. In this case the preferred chair-like trans2 conformation found for the unsubstituted Aba-Gly in **6** shifted to a boat-like trans1 conformation, as was observed for spiro-analog **10**.[25] At the same time the tetrapeptide model **11b** adopted a β-turn conformation. Methyl substitution at position 5 was not able to shift the ring conformation from trans2 to trans1, and no β-turn conformation was observed. Whereas the 1(*R*)-phenyl-substituted analog **14** adopts a trans1 conformation with a pseudo-axial phenyl substituent, 1(*S*)-isomer **15** adopts a trans2 conformation. However, neither the molecular modeling nor the solution conformational study was able to indicate the formation of a turn structure. Therefore no correlation between the ring conformation and the propensity to adopt a turn conformation could be demonstrated. This study identified the α-MeAba scaffold as a strong turn inducer, which is in agreement with the finding that α-MePro is a stronger turn inducer than Pro.[57,58] An asymmetric synthesis of this scaffold and its application in peptide mimetic design are currently in progress.

4. Experimental section

4.1. General methods

RP-HPLC was performed using an RP C-18 column (Supelco Discovery ®BIO Wide Pore C18, *l*=25 cm, *d*=0.45 cm, PS=5 μm) with a mobile phase consisting of water/acetonitrile containing 0.1% TFA. Products were eluted using the gradient: *t*=0 min, 3% CH_3CN, *t*=20 min, 97% CH_3CN; flow rate: 1.0 mL/min, λ=215 nm. Preparative HPLC was performed using an RP C-18 column (Supelco Discovery ®BIO Wide Pore C18, *l*=25 cm, *d*=2.12 cm, PS=10 μm) with the above mentioned gradient at a flow rate of 20.0 mL/min. TLC analysis was performed on plastic sheets precoated with silica gel 60F_{254} (Merck). Silica gel 60 (0.040–0.063 mm) from Merck was used for flash column chromatography (w/w, 60:1). Melting points were measured with a Büchi B 540 melting point apparatus, with a temperature increment of 1 °C/min. ^{1}H and ^{13}C NMR spectra were recorded at 250.13 and 62.90 MHz, respectively, with a Bruker Avance DRX250 spectrometer, using TMS or the residual solvent signal as internal reference. For some advanced NMR analyses samples were measured with the Bruker AMX500 spectrometer, using pulse sequences of the Bruker program library. The temperature study was performed in DMSO-d_6, with a temperature increment of 5 K between 298 K and 343 K. Mass spectra were recorded on a VG Quattro II spectrometer using electrospray ionization (positive ion mode).

4.2. Molecular modeling

The calculations were carried out with Macromodel 5.0[39] with Maestro 8.0 as graphic interface. The MM3* force field[40] was used in vacuo for the energy minimizations on the formyl-Xxx-NMe rings (Xxx=4-Me-, *erythro*/*threo*-5-Me-, 1-Ph-Aba). The conformational analyses of the ring structures were carried out with the Pure Low Mode search;[38] 1000 structures were generated and minimized by means of the Polak–Ribière conjugate gradient method as implemented in Macromodel, using a gradient convergence criterion of 0.02 kJ/mol Å. For the tetrapeptide mimetics, the MM3* force field was used as well, but this time in combination with the GB/SA solvation model of Still et al.,[42] using Macromodel's default parameters for an aqueous medium. The conformational space was sampled by use of a systematic unbounded multiple mimimum search[41] in which three internal coordinates (φ2, φ3, and ψ3) were varied. Here the generation of 2000 structures was conducted and the conformers were minimized to an energy convergence of 0.1 kJ/mol Å by use of the Polak–Ribière conjugate gradient method. After this search the found conformations were again minimized to an energy convergence of 0.01 kJ/mol Å. In both strategies, duplicate structures and those greater than 50 kJ/mol above the global minimum were discarded. The generated structures were clustered in families with Xcluster 1.7. An RMSD value of 0.2 Å was used.

4.3. X-ray crystallography

Crystal of **11** suitable for single crystal X-ray diffraction was fixed at glass fiber using epoxy resin. Diffraction data were collected at rt on Nonius BV MACH3 diffractometer with graphite monochromated Cu Kα radiation (λ=1.54178 Å). The structure was solved by direct methods using SHELXS97[59] and refined on F^2 by using full-matrix least-squares methods with program SHELXL97.[60] Software used to prepare material for publication was WinGX.[61] Anisotropic thermal parameters were refined for non-H atoms. Hydrogen atoms were localized from $\Delta\rho$ maps and refined with isotropic thermal parameters. CCDC-705574 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at 'http://www.ccdc.cam.ac.uk/conts/retrieving.html' [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; E-mail: deposit@ccdc.cam.sc.uk].

Crystal data for ***11***: *a*=15.3280(3), *b*=26.3269(9), *c*=7.7231(12) Å; *V*=3116.6(5)Å^3; *F*(000)=1296; (Cu Kα)=0.740; orthorhombic space group *Pccn*; 3077 reflections collected, 1706 unique (R_{int}=0.0614); *R*1=0.0594, and for all data *wR*2=0.1928.

4.4. Opioid receptor binding assay

Opioid radioreceptor binding assays have been performed on brain homogenates of male Wistar rats (250–300 g body weight) as reported in detail elsewhere.[65] Binding affinities of the compounds to the μ- and δ-opioid receptors were measured against tritiated compounds [^3H]DAMGO and [^3H][Ile^{5,6}]deltorphin 2, respectively.

4.5. Phthaloyl-*erythro*-(2*S**,3*S**)-β-methyl phenylalanine 19 and *threo*-(2*S**,3*R**)-β-methyl phenylalanine 20

To a solution of β-Me-Phe·HCl and Na_2CO_3 (6.0 equiv *erythro*; 4.0 equiv *threo*) in H_2O/CH_3CN (3:5), methyl-2-((succinimidooxy) carbonyl)benzoate (MSB, 1.0 equiv) was added and the reaction mixture was stirred until HPLC analysis indicated the disappearance of the starting material (typically 24 h). The mixture was acidified with a 2 N HCl solution to pH 1–2, diluted with EtOAc, and extracted with a 1 N HCl solution (2×) and H_2O (1×). The organic layer was dried ($MgSO_4$), filtered, and evaporated. The residue was crystallized from hot EtOH to yield the phthaloyl-protected compounds for the *erythro* derivative. The *threo* derivative was purified by flash column chromatography (EtOAc/petroleum ether 3:7+1% AcOH).

Compound ***19***. Yield: 3.7 g (65%). Mp 135.8–142.2 °C. HPLC t_R=17.0 min. R_f 0.61 ($CHCl_3$/EtOH 3:1). MS 310 ($M+H^+$), 332 ($M+Na^+$), 348 ($M+K^+$), 641 ($2M+Na^+$). ^{1}H NMR (DMSO, 250 MHz) 1.50 (3H, d, β-CH_3, ^3J=6.8 Hz), 3.78 (1H, m, β-H), 4.89 (1H, d, α-H, ^3J=10.8 Hz), 6.95–7.10 (5H, M, H-arom), 7.67–7.79 (4H, M, H-Phth) ppm. ^{13}C NMR (DMSO, 63 MHz) 21.33 (β-CH_3), 39.44 (β-CH), 56.52 (α-CH), 123.52, 126.91, 127.62 (CH-arom), 128.42, 135.12 (CH-Phth), 130.83 (C_q-Phth), 143.31 (C_q-arom), 167.02 (C=O-Phth), 170.22 (C=O COOH) ppm.

Compound ***20***. Yield: 125 mg (72%). Mp 114.7–117.4 °C. HPLC t_R=16.2 min. R_f 0.59 ($CHCl_3$/EtOH 3:1). MS 310 ($M+H^+$), 163 (M−Phth), 264 (M−COOH), 292 (M−OH), 332 ($M+Na^+$), 348 ($M+K^+$). ^{1}H NMR (DMSO, 500 MHz) 1.13 (3H, d, β-CH_3, ^3J=7.2 Hz), 3.80 (1H, m, β-H), 4.95 (1H, d, α-H, ^3J=8.9 Hz), 7.09–7.30 (5H, M, H-arom), 7.89–7.95 (4H, M, H-Phth), 12.96 (1H, br s, COOH) ppm. ^{13}C NMR (DMSO, 63 MHz) 18.79 (β-CH_3), 38.64 (β-CH), 56.44 (α-CH), 123.54, 126.36, 127.36 (CH-arom), 128.30, 135.00 (CH-Phth), 130.84 (C_q-Phth), 144.05 (C_q-arom), 167.32 (C=O-Phth), 169.10 (C=O COOH) ppm.

4.6. Phthaloyl-*erythro*-(2*S**,3*S**)-β-methyl phenylalaninyl-glycine methyl ester 21 and phthaloyl-*threo*-(2*S**,3*R**)-β-methyl phenylalaninylglycine ethyl ester 22

In an oven dried flask, the phthaloyl-protected compound was dissolved in dry CH_2Cl_2. The solution was basified by addition of Et_3N (3.0 equiv), after which the coupling reagent TBTU (1.1 equiv) and the hydrochloric salt of Gly-OMe or Gly-OEt (1.1 equiv) were added. The mixture was kept at pH 8 by means of Et_3N and stirred for 1 h at rt. Subsequently, the solution was washed with a 1 N HCl solution (3×), a saturated $NaHCO_3$ solution (3×) and brine (3×). The organic layer was dried ($MgSO_4$), filtered, and evaporated. The residue was crystallized from a minimum amount of hot EtOH.

Compound ***21***. Yield: 2.8 g (64%). Mp 129.3–131.2 °C. HPLC t_R=17.0 min. R_f 0.51 ($CHCl_3$/EtOAc 1:1). MS 381 ($M+H^+$), 264 (M−$CONHCH_2COOCH_3$), 292 (M−$NHCH_2COOCH_3$), 403 ($M+Na^+$), 419 ($M+K^+$). ^{1}H NMR (DMSO, 250 MHz) 1.43 (3H, d, β-CH_3, ^3J=6.9 Hz), 3.61 (3H, s, OCH_3), 3.75–4.00 (3H, M, β-H+α-CH_2-Gly), 4.90 (1H, d, α-H, ^3J=11.2 Hz), 6.95–7.11 (5H, M, H-arom), 7.66–7.78 (4H, M, H-Phth), 8.63 (1H, t, NH, ^3J=5.8 Hz) ppm. ^{13}C NMR (DMSO, 63 MHz) 21.00 (β-CH_3), 38.69 (β-CH), 41.23 (α-CH_2-Gly), 52.05 (OCH_3), 58.36 (α-CH), 123.32, 126.90, 127.62 (CH-arom), 128.45, 134.86 (CH-Phth), 131.13 (C_q-Phth), 143.38 (C_q-arom), 167.34 (C=O-Phth), 168.66 ($COOCH_3$), 170.39 (CO-NH) ppm.

Compound ***22***. Yield: 5.1 g (30%). Mp 120.1–123.2 °C. HPLC t_R=16.9 min. R_f 0.57 ($CHCl_3$/EtOAc 1:1). MS 395 ($M+H^+$), 264 (M−$CONHCH_2COOCH_2CH_3$), 292 (M−$NHCH_2COOCH_2CH_3$), 321 (M−$COOCH_2CH_3$), 349 (M−OCH_2CH_3). ^{1}H NMR (DMSO, 250 MHz) 1.05–1.10 (6H, M, CH_3-Et+β-CH_3), 3.59 (1H, dd, Hα-Gly, ^3J=5.6 Hz, ^2J=17.3 Hz), 3.73 (1H, dd, Hα′-Gly, ^3J=5.8 Hz, ^2J=17.2 Hz), 3.82–4.03 (3H, M, CH_2-Et+β-H), 4.88 (1H, d, α-H, ^3J=9.1 Hz), 7.08–7.35 (5H, M, H-arom), 7.86–7.94 (4H, M, H-Phth), 8.35 (1H, t, NH, ^3J=5.6 Hz) ppm. ^{13}C NMR (DMSO, 63 MHz) 13.86 (CH_3 Et), 18.45 (β-CH_3), 38.11 (β-CH), 40.85 (α-CH_2-Gly), 57.27 (α-CH), 60.27 (CH_2 Et), 123.31, 126.29, 127.42 (CH-arom), 128.27, 134.68 (CH-Phth), 131.26 (C_q-Phth), 144.11 (C_q-arom), 167.27 (C=O-Phth), 167.38 (C=O ester), 169.39 (CO-NH) ppm.

4.7. Phthaloyl-*erythro*-(2*S**,3*S**)-β-methyl phenyl-alaninylglycine 23 and *threo*-(2*S**,3*R**)-β-methyl phenylalaninylglycine 24

Phthaloyl-β-Me-Phe-Gly-OMe/OEt **21** or **22** was dissolved in acetone. To this solution a 1 N HCl solution was added slowly. The reaction mixture was refluxed in an oil bath at 90 °C, until HPLC analysis indicated the absence of the starting material (typically 16–20 h). The mixture was cooled down to rt and evaporated. The obtained white solid was used in the next step without further purification.

Compound ***23***. Yield: 2.6 g (100%). Mp 187.4–196.2 °C. HPLC t_R=15.5 min. R_f 0.40 ($CHCl_3/CH_3OH$ 1:1). MS 367 ($M+H^+$), 264 (M−$CONHCH_2COOH$), 292 (M−$NHCH_2COOH$), 349 (M−OH), 733 (2M+1). ^{1}H NMR (DMSO, 250 MHz) 1.43 (3H, d, β-CH_3, ^3J=6.9 Hz), 3.68 (1H, dd, Hα-Gly, ^3J=5.6 Hz, ^2J=17.4 Hz), 3.88 (1H, dd, Hα′-Gly, ^3J=6.0 Hz, ^2J=17.6 Hz), 3.97 (1H, m, β-H), 4.90 (1H, d, α-H, ^3J=11.2 Hz), 6.95–7.11 (5H, M, H-arom), 7.64–7.74 (4H, M, H-Phth), 8.53 (1H, t, NH, ^3J=5.8 Hz) ppm. ^{13}C NMR (DMSO, 63 MHz) 21.05 (β-CH_3), 38.70 (β-CH), 41.32 (α-CH_2-Gly), 58.52 (α-CH), 123.31, 126.89, 127.61 (CH-arom), 128.45, 134.85 (CH-Phth), 131.14 (C_q-Phth), 143.46 (C_q-arom), 167.39 (C=O-Phth), 168.48 (COOH), 171.30 (CO-NH) ppm.

Compound ***24***. Yield: 3.7 g (100%). Mp 55.8–58.3 °C. HPLC t_R=15.9 min. R_f 0.52 ($CHCl_3/CH_3OH$ 1:1). MS 367 ($M+H^+$), 264 (M−$CONHCH_2COOH$), 292 (M−$NHCH_2COOH$), 389 ($M+Na^+$), 405 ($M+K^+$). ^{1}H NMR (DMSO, 250 MHz) 1.07 (3H, d, β-CH_3, ^3J=7.2 Hz), 3.48 (1H, dd, Hα-Gly, ^3J=5.4 Hz, ^2J=17.4 Hz), 3.70 (1H, dd, Hα′-Gly, ^3J=6.1 Hz, ^2J=17.4 Hz), 3.88 (1H, m, β-H), 4.87 (1H, d, α-H, ^3J=9.0 Hz), 7.14–7.27 (5H, M, H-arom), 7.85–7.93 (4H, M, H-Phth), 8.23 (1H, t, NH, ^3J=5.8 Hz) ppm. ^{13}C NMR (DMSO, 63 MHz) 18.41 (β-CH_3), 38.23 (β-CH), 40.76 (α-CH_2-Gly), 57.36 (α-CH), 123.29, 126.28, 127.43 (CH-arom), 128.27, 134.63 (CH-Phth), 131.29 (C_q-Phth), 144.14 (C_q-arom), 167.18 (C=O-Phth), 167.46 (COOH), 170.87 (CO-NH) ppm.

4.8. Phthaloyl-*erythro*-(2*S**,3*S**)-β-methyl phenylalaninyl-glycine oxazolidinone 25 and *threo*-(2*S**,3*R**)-β-methyl phenylalaninylglycine oxazolidinone 26

In an oven dried one necked flask, the hydrolyzed compound was suspended in dry benzene (45 ml/g **23** or **24**), followed by the addition of $(CH_2O)_n$ (15 equiv) and a catalytic amount of *p*-TosOH (0.2 equiv). A Dean–Stark apparatus was mounted on the flask and the mixture was brought to reflux in an oil bath at 90 °C for 5 h (**23**) or 12 h (**24**). After every 1.5 h, $(CH_2O)_n$ (5 equiv) was added. The reaction mixture was cooled down to rt and evaporated. The residue was dissolved in $CHCl_3$ and extracted with a 6% $NaHCO_3$ solution (2×) and H_2O (1×). The $CHCl_3$ layer was dried ($MgSO_4$),

filtered, and evaporated to yield the corresponding oxazolidinones. No further purification was required.

Compound ***25***. Yield: 2.3 g (89%). Mp 205.7–208.7 °C. HPLC t_R=16.1 min. R_f 0.40 (EtOAc/petroleum ether 3:2). MS 379 ($M+H^+$), 264 ($M-CONCH_2OCOCH_2$), 292 ($M-NCH_2OCOCH_2$). ^{1}H NMR (DMSO, 250 MHz) 1.34 (3H, d, β-CH_3, ^3J=6.8 Hz), 3.99 (2H, m, α-CH_2-Gly), 4.41 (1H, m, β-H), 5.02 (1H, d, α-H, ^3J=10.9 Hz), 5.27 (1H, d, H1 N-CH_2-O, ^2J=4.9 Hz), 5.59 (1H, d, H1′ N-CH_2-O, ^2J=4.7 Hz), 6.95–7.13 (5H, M, H-arom), 7.68–7.75 (4H, M, H-Phth) ppm. ^{13}C NMR (DMSO, 63 MHz) 20.09 (β-CH_3), 38.40 (β-CH), 44.87 (α-CH_2-Gly), 56.05 (α-CH), 79.31 (N-CH_2-O), 123.44, 127.04, 127.99 (CH-arom), 128.39, 134.94 (CH-Phth), 130.88 (C_q-Phth), 142.32 (C_q-arom), 165.03 (C=O-Phth), 167.14 (C=O oxazolidinone), 170.98 (CO-NR_2) ppm.

Compound ***26***. Yield: 3.3 g (89%). Mp 168.7–173.1 °C. HPLC t_R=17.7 min. R_f 0.37 (EtOAc/petroleum ether 3:2). MS (ES^+) 379 ($M+H^+$), 264 ($M-CONCH_2OCOCH_2$), 292 ($M-NCH_2OCOCH_2$), 401 ($M+Na^+$), 417 ($M+K^+$). ^{1}H NMR (DMSO, 250 MHz) 0.87 (3H, d, β-CH_3, ^3J=6.7 Hz), 3.65–4.00 (3H, M, α-CH_2-Gly+β-H), 4.84 (1H, d, α-H, ^3J=10.4 Hz), 4.93 (1H, d, H1 N-CH_2-O, ^2J=5.4 Hz), 5.07 (1H, d, H1′ N-CH_2-O, ^2J=4.9 Hz), 6.91–7.20 (5H, M, H-arom), 7.68–7.80 (4H, M, H-Phth) ppm. ^{13}C NMR (DMSO, 63 MHz) 18.62 (β-CH_3), 37.49 (β-CH), 44.14 (α-CH_2-Gly), 55.92 (α-CH), 78.54 (N-CH_2-O), 123.60, 126.57, 127.69 (CH-arom), 128.33, 134.95 (CH-Phth), 130.80 (C_q-Phth), 143.22 (C_q-arom), 164.33 (C=O-Phth), 167.30 (C=O oxazolidinone), 170.23 (CO-NR_2) ppm.

4.9. *erythro*-2-(4*S**,5*S**)-(4-Phthaloyl-5-methyl-3-oxo-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)acetic acid [Phth-*erythro*-(4*S**,5*S**)-5-Me-Aba-Gly-OH] 27 and *threo*-2-(4*S**,5*R**)-4-phthaloyl-5-methyl-3-oxo-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)acetic acid [Phth-*threo*-(4*S**,5*R**)-5-Me-Aba-Gly-OH] 28

In an oven dried one necked flask the oxazolidinone was dissolved in dry CH_2Cl_2 and kept under Ar atmosphere. Then TFMSA (10 equiv) was added dropwise, which turned the solution dark brown. The flask was sealed with a septum and stirring was continued overnight under Ar atmosphere and at rt. The reaction mixture was cooled down to 0 °C by use of an ice bath and quenched by careful addition of a cold mixture of CH_2Cl_2 and water. The phases were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were washed with H_2O (1×) and diluted with acetone to obtain complete dissolution of all product. This phase was dried ($MgSO_4$), filtered, and evaporated to obtain the benzazepinones.

Compound ***27***. Yield: 1.5 g (100%). Mp 190.7–193.5 °C. HPLC t_R=15.6 min. R_f 0.63 (petroleum ether/CH_3OH 3:2+1% CH_3COOH). MS 379 ($M+H^+$), 333 (M−COOH), 361 (M−OH), 401 ($M+Na^+$). ^{1}H NMR (DMSO, 250 MHz) 1.33 (3H, d, β-CH_3, ^3J=6.7 Hz), 3.97 (1H, m, β-H), 4.04 (1H, d, Hα-Gly, ^2J=17.3 Hz), 4.38 (1H, d, Hα′-Gly, ^2J=17.3 Hz), 4.40 (1H, br s, ε′-H), 5.15 (1H, d, ε-H, ^2J=15.7 Hz), 5.32 (1H, d, α-H, ^3J=3.8 Hz), 7.10–7.34 (4H, M, H-Aba), 7.83 (4H, br s, H-Phth) ppm. ^{13}C NMR (DMSO, 63 MHz) 18.16 (β-CH_3), 36.22 (β-CH), 51.26 (α-CH_2-Gly), 52.63 (ε-CH_2), 57.55 (α-CH), 123.55, 135.08 (CH-Phth), 126.75, 127.05, 128.26, 128.75 (CH-Aba), 131.39 (C_q-Phth), 135.67, 142.18 (C_q-Aba), 167.74 (C=O-Phth), 168.09 (C=O-Aba), 170.85 (C=O Gly) ppm.

Compound ***28***. Yield: 3.18 g (100%). Mp 85.3–87.6 °C. HPLC t_R=16.3 min. R_f 0.65 (petroleum ether/CH_3OH 3:2+1% CH_3COOH). MS 379 ($M+H^+$), 333 (M−COOH), 361 (M−OH), 401 ($M+Na^+$). ^{1}H NMR (DMSO, 250 MHz) 1.32 (3H, d, β-CH_3, ^3J=6.8 Hz), 3.86 (1H, d, Hα-Gly, ^2J=17.3 Hz), 4.13 (1H, m, β-H), 4.20 (1H, d, Hα′-Gly, ^2J=17.3 Hz), 4.45 (1H, d, ε′-H, ^2J=15.7 Hz), 4.95 (1H, d, ε-H, ^2J=15.6 Hz), 5.14 (1H, d, α-H, ^3J=10.7 Hz), 7.17–7.36 (4H, M, H-Aba), 7.87–7.96 (4H, M, H-Phth) ppm. ^{13}C NMR (DMSO, 63 MHz) 21.72 (β-CH_3), 35.46 (β-CH), 49.35 (α-CH_2-Gly), 50.97 (ε-CH_2), 58.23 (α-CH), 123.36, 134.83 (CH-Phth), 126.30, 128.36, 128.75, 128.98 (CH-Aba), 131.17 (C_q-Phth), 135.61, 141.15 (C_q-Aba), 166.93 (C=O-Phth), 167.79 (C=O-Aba), 170.10 (C=O Gly) ppm.

4.10. *erythro*-(4*S**,5*S**)-2-(4-Acetamido-5-methyl-3-oxo-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)-*N*-methylacetamide [Ac-*erythro*-(4*S**,5*S**)-5-Me-Aba-Gly-NH-Me] 12 and *threo*-(4*S**,5*R**)-2-4-(acetamido-5-methyl-3-oxo-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)-*N*-methylacetamide [Ac-*threo*-(4*R**,5*S**)-5-Me-Aba-Gly-NH-Me] 13

To a solution of the benzazepinone product in EtOH, hydrazine hydrate (6.0 equiv) was added. The solution was refluxed for 1.5 h, at an oil bath temperature of 95 °C. After cooling the reaction mixture to rt, the solvent was evaporated. The residue was re-dissolved in H_2O and the pH was adjusted to 5 by means of careful addition (pH-meter) of AcOH. This suspension was stirred at rt for 1 h, after which the formed precipitate was filtered and the filtrate was evaporated. The residue was dissolved in H_2O. The pH was adjusted to 6 with Et_3N and Ac_2O (7.0 equiv) was added in three portions. Meanwhile the pH was kept at 6 with Et_3N. After stirring 2 h at rt the reaction mixture was evaporated. The crude product was not purified at this stage and directly used in the next step. To a solution of Ac-*erythro*- or *threo*-β-Me-Aba-Gly-OH in CH_3CN/H_2O (3:1), pyridine was added until a pH of 8 was reached. The reaction mixture was cooled down to 0 °C, followed by the addition of *N*,*N*-dicyclohexylcarbodiimide (1.4 equiv). After 10 min, $CH_3NH_2\cdot HCl$ (4.0 equiv) and pyridine (5.0 equiv) were added. The coupling was continued for 2.5 h. HPLC analysis did indicate that starting material was still present. So 30–60 extra equivalents of $CH_3NH_2\cdot HCl$ and 9–13 extra equivalents of *N*,*N*-dicyclohexylcarbodiimide were added and the pH was kept at 8. The solution was evaporated and the residue was re-dissolved in CH_2Cl_2 (10 mM solution). The precipitated DCU was filtered and the filtrate was evaporated. The residue was dissolved again in CH_2Cl_2 (130 mM solution), and after filtration of DCU and evaporation of the filtrate, a sample of the product was purified by prep HPLC. After lyophilization a white solid was obtained.

Compound ***12***. Mp 145.6–149.8 °C. t_R=11.5 min. R_f 0.50 (CH_3CH_2OH). MS 304 ($M+H^+$), 273 ($M-NHCH_3$), 326 ($M+Na^+$), 342 ($M+K^+$). ^{1}H NMR (DMSO, 500 MHz) 1.05 (3H, d, β-CH_3, ^3J=7.2 Hz), 1.95 (3H, s, CH_3 Ac), 2.60 (3H, d, CH_3 NHMe, ^3J=4.5 Hz), 3.10 (1H, m, β-H), 3.75 (1H, d, Hα-Gly, ^2J=16.3 Hz), 4.12 (1H, d, ε′-H, ^2J=17.3 Hz), 4.24 (1H, d, Hα′-Gly, ^2J=16.3 Hz), 5.19 (1H, d, ε-H, ^2J=17.2 Hz), 5.45 (1H, m, α-H, $^3J_{\alpha,\beta}\approx$2.6 Hz), 7.14–7.25 (4H, M, H-Aba), 7.84 (1H, br s, NH-CH_3), 8.04 (1H, d, NH-Ac, ^3J=6.7 Hz) ppm. ^{13}C NMR (DMSO, 126 MHz) 19.03 (β-CH_3), 22.50 (CH_3-Ac), 25.39 (NH-CH_3), 39.84 (β-CH), 50.45 (α-CH_2-Gly), 51.04 (α-CH), 52.54 (ε-CH_2), 125.91, 127.38, 128.51, 130.50 (CH-Aba), 132.98, 141.87 (C_q-Aba), 168.11 (C=O-NHMe), 168.60 (C=O-Ac), 170.51 (C=O Aba) ppm.

Compound ***13***. Mp 257.1–263.9 °C. HPLC t_R=10.3 min. R_f 0.55 (CH_3CH_2OH). MS 304 ($M+H^+$), 245 ($M-CONHCH_3$), 273 ($M-NHCH_3$), 326 ($M+Na^+$), 342 ($M+K^+$). ^{1}H NMR (DMSO, 500 MHz) 1.28 (3H, d, β-CH_3, ^3J=6.8 Hz), 1.92 (3H, s, CH_3 Ac), 2.57 (3H, d, CH_3 NHMe, ^3J=4.4 Hz), 2.91 (1H, m, β-H), 3.35 (1H, d, Hα-Gly, ^2J=16.2 Hz), 3.96 (1H, d, ε′-H, ^2J=16.1 Hz), 4.18 (1H, d, Hα′-Gly, ^2J=16.2 Hz), 5.08 (1H, d, ε-H, ^2J=15.9 Hz), 5.17 (1H, m, α-H, $^3J_{\alpha,\beta'}\approx$11.0 Hz), 7.09–7.25 (4H, M, H-Aba), 7.75 (1H, br s, NH-CH_3), 8.19 (1H, d, NH-Ac, ^3J=8.5 Hz) ppm. ^{1}H NMR ($CDCl_3$, 500 MHz) 1.38 (3H, d, β-CH_3, ^3J=6.8 Hz), 2.06 (3H, s, CH_3 Ac), 2.60 (3H, d, CH_3 NHMe, ^3J=4.5 Hz), 2.90 (1H, m, β-H), 3.73 (1H, d, Hα-Gly, ^2J=16.1 Hz), 3.88 (1H, d, ε′-H, ^2J=16.1 Hz), 4.16 (1H, d, Hα′-Gly, ^2J=16.0 Hz), 5.08 (1H, d, ε-H, ^2J=15.9 Hz), 5.24 (1H, m, α-H), 5.95 (1H, br s, NH-CH_3), 6.40 (1H, d, NH-Ac, ^3J=7.8 Hz), 7.04–7.23 (4H, M, H-Aba) ppm. ^{13}C NMR (DMSO, 126 MHz) 22.58 (CH_3-Ac), 23.67

(β-CH_3), 25.45 (NH-CH_3), 39.53 (β-CH), 48.62 (α-CH_2-Gly), 51.03 (ε-CH_2), 54.34 (α-CH), 125.75, 128.08, 128.94, 130.68 (CH-Aba), 134.51, 141.69 (C_q-Aba), 168.15 (C=O-NHMe), 169.22 (C=O-Ac), 170.47 (C=O Aba) ppm.

4.11. Ethyl (*R,S*)-2-(benzylideneamino)propanoate 29

(*R,S*)-Ala-OEt·HCl (8.85 g, 58.0 mmol, 1.0 equiv) was dissolved in dry CH_2Cl_2 (120 mL) to which benzaldehyde (6.15 g, 58.0 mmol, 1.0 equiv), Et_3N (16 mL, 116 mol, 2.0 equiv), and 4.8 g $MgSO_4$ were added. After 24 h stirring at rt, $MgSO_4$ was filtered and the filtrate was evaporated. The residue was dissolved in Et_2O and washed with brine, after which the organic layer was dried ($MgSO_4$), filtered, and evaporated. A light yellow oil was obtained and was used without further purification.

Yield: 11.1 g (93%). R_f 0.7 (EtOAc/cyclohexane 2:1). MS 206 ($M+H^+$), 132 (M−COOEt), 118 (M−Ph−CH=N), 91 (tropylium ion). ^{1}H NMR ($CDCl_3$, 250 MHz) 1.27 (3H, t, CH_3 ethyl, ^3J=7.1 Hz), 1.53 (3H, d, α-CH_3, ^3J=6.8 Hz), 4.10–4.29 (3H, M, OCH_2+α-H), 7.38–7.80 (5H, M, H-arom), 8.32 (1H, s, imine H) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz) 14.19 (CH_3 ethyl), 19.39 (α-CH_3), 61.01 (OCH_2), 67.99 (α-CH), 128.50, 128.60, 131.00 (CH-arom), 135.80 (C_q-arom), 162.80 (CH=N), 172.50 (COO−) ppm.

4.12. Ethyl (*R,S*)-2-(benzylideneamino)-3-(2-cyanophenyl)-2-methylpropanoate 30

To a solution of ethyl (*R,S*)-2-(benzylideneamino)propanoate **29** (10.0 g, 48.7 mmol, 1.0 equiv) in CH_2Cl_2 (300 mL), *o*-cyanobenzyl bromide (11.5 g, 57.4 mmol, 1.2 equiv), KOH (4.1 g, 73.1 mmol, 1.5 equiv), K_2CO_3 (20.2 g, 146.0 mmol, 3.0 equiv), and $(Et)_3$BnNCl (1.11 g, 4.87 mmol, 0.1 equiv) were added. After overnight stirring, the mixture was filtered and the precipitate was washed with CH_2Cl_2 (2×15 mL). The filtrate was washed with water until neutral. The organic phase was dried ($MgSO_4$), filtered, and evaporated to yield a pale yellow oil.

Yield: 15.3 g (98%). MS 321 ($M+H^+$), 233 (hydrolyzed imine). ^{1}H NMR ($CDCl_3$, 250 MHz) 1.27 (3H, t, CH_3 ethyl, ^3J=7.1 Hz), 1.52 (3H, s, α-CH_3), 3.48 (1H, d, β-H, ^2J=13.7 Hz), 3.62 (1H, d, β-H′, ^2J=13.7 Hz), 4.23 (2H, q, OCH_2, ^3J=7.1 Hz), 7.26–7.78 (9H, M, H-arom), 8.16 (1H, s, imine H) ppm. ^{13}C NMR ($CDCl_3$, 63 MHz) 14.56 (CH_3 ethyl), 22.97 (α-CH_3), 43.88 (β-CH_2), 61.83 (OCH_2), 69.33 (α-C_q), 115.00 (arom C_q-CN), 119.10 (CN), 127.50–135.40 (CH-arom), 136.60 (arom C_q-CH=N), 160.60 (CH=N), 173.50 (COO−) ppm.

4.13. Ethyl (*R,S*)-2-amino-3-(2-cyanophenyl)-2-methylpropanoate hydrochloride 32

Ethyl (*R,S*)-2-(benzylideneamino)-3-(2-cyanophenyl)-2-methylpropanoate **30** (17.5 g, 54.6 mmol, 1.0 equiv) was suspended in an Et_2O/1.5 N HCl (200 mL/140 mL) mixture. After 3 h stirring, the layers were separated and the aqueous phase was evaporated. The residue was dissolved in a minimum amount of EtOH and dropwise addition of EtOAc/Et_2O (1:1) led to the formation of white crystals.

Yield: 14.7 g (63%) (four steps). Mp 103–108 °C. HPLC t_R=13.2 min. R_f 0.50 ($CH_3CN/CH_3OH/H_2O$ 4:1:1). MS 233 ($M+H^+$), 159 (M−COOEt). ^{1}H NMR (DMSO, 250 MHz) 1.14 (3H, t, CH_3 ethyl, ^3J=7.1 Hz), 1.53 (3H, s, α-CH_3), 3.37 (1H, d, β-H, ^2J=13.9 Hz), 3.44 (1H, d, β-H′, ^2J=13.9 Hz), 4.13 (2H, m, OCH_2), 7.49–7.86 (4H, M, H-arom), 9.07 (3H, br s, NH_3^+) ppm. ^{13}C NMR (DMSO, 63 MHz) 13.90 (CH_3 ethyl), 21.00 (α-CH_3), 40.64 (β-CH_2), 59.60 (α-C_q), 62.80 (OCH_2), 113.50 (arom C_q-CN), 118.10 (CN), 128.90, 132.40, 133.60, 133.70 (CH-arom), 137.60 (C_q-arom), 170.10 (COO−) ppm.

4.14. (*R,S*)-2-Amino-3-(2-cyanophenyl)-2-methylpropanoic acid hydrochloride 33

A solution of ethyl (*R,S*)-2-amino-3-(2-cyanophenyl)-2-methylpropanoate hydrochloride **32** (5.0 g, 18.7 mmol, 1.0 equiv) in 6 N HCl (70 mL) was stirred for 50 h at 65 °C and was subsequently cooled down to rt, which induced crystallization. The white crystals were filtered and rinsed with a minimum amount of cold 6 N HCl.

Yield: 4.5 g (81%). Mp 139–144 °C. HPLC t_R=9.6 min. R_f 0.40 ($CH_3CN/CH_3OH/H_2O$ 4:1:1). MS 205 ($M+H^+$), 159 (M−COOH). ^{1}H NMR (DMSO, 250 MHz) 1.47 (3H, s, α-CH_3), 3.34 (1H, d, β-H, ^2J=14.2 Hz), 3.41 (1H, d, β-H′, ^2J=14.2 Hz), 7.47–7.84 (4H, M, H-arom), 8.87 (3H, br s, NH_3^+) ppm. ^{13}C NMR (DMSO, 63 MHz) 21.50 (α-CH_3), 40.40 (β-CH_2), 59.52 (α-C_q), 113.70 (arom C_q-CN), 118.30 (CN), 128.80, 132.20, 133.50, 133.50 (CH-arom), 137.80 (C_q-arom), 171.80 (COO−) ppm.

4.15. (*R,S*)-3-(2-Cyanophenyl)-2-(methoxycarbonylamino)-2-methylpropanoic acid 34

To a solution of (*R,S*)-2-amino-3-(2-cyanophenyl)-2-methylpropanoic acid hydrochloride **33** (2.0 g, 8.43 mmol, 1.0 equiv) in a 1 N NaOH solution (60 mL), methyl chloroformate (2.6 mL, 33.7 mmol, 4.0 equiv) was added. After 45 min stirring, the reaction mixture was cooled down by use of an ice bath and acidified to pH 2–3 with a 6 N HCl solution. The aqueous phase was extracted with cold CH_2Cl_2 (4×100 mL). The combined organic layers were washed with water (pH 2, 1×100 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated. A white solid was obtained and used in the next step without further purification.

Yield: 1.5 g (69%). Mp 145.0–147.5 °C. HPLC t_R=15.3 min. R_f 0.70 ($CH_3CN/CH_3OH/H_2O$ 4:1:1). MS 263 ($M+H^+$), 203 (M−CH_3OCO), 525 (2M+1). ^{1}H NMR (DMSO, 250 MHz) 1.14 (3H, s, α-CH_3), 3.25 (1H, d, β-H, ^2J=13.7 Hz), 3.50 (1H, d, β-H′, ^2J=13.7 Hz), 3.57 (3H, s, MocCH_3), 7.26–7.81 (4H, M, H-arom), 12.70 (1H, br s, COOH) ppm. ^{13}C NMR (DMSO, 63 MHz) 22.30 (α-CH_3), 38.51 (β-CH_2), 51.37 (CH_3-Moc), 58.67 (α-C_q), 113.60 (arom C_q-CN), 118.40 (CN), 127.60, 132.00, 132.70, 132.90 (CH-arom), 140.50 (C_q-arom), 155.60 (C=O-Moc), 174.90 (COO−) ppm.

4.16. (*R,S*)-3-(2-(Aminomethyl)phenyl)-2-(methoxycarbonylamino)-2-methylpropanoic acid 35

To a suspension of (*R,S*)-3-(2-cyanophenyl)-2-(methoxy carbonylamino)-2-methylpropanoic acid **34** (1.86 g, 7.1 mmol, 1.0 equiv) in EtOH/H_2O (3:1, 60 mL), 10% Pd/C (40w%, 0.744 g) and a 10% aqueous AcOH solution (5.5 mL, 10.6 mmol, 1.5 equiv) were added. The suspension was hydrogenated in a Parr apparatus (50 psi, rt, 2 days). The mixture was filtered over dicalite and rinsed with water. After evaporation of the solvent, the residue was crystallized from a minimum amount of hot EtOH.

Yield: 1.4 g (74%). Mp 218.4–219.2 °C. HPLC t_R=9.8 min. R_f 0.50 ($CH_3CN/CH_3OH/H_2O$ 4:1:1). MS 267 ($M+H^+$), 533 (2M+1). ^{1}H NMR (DMSO, 250 MHz) 1.41 (3H, s, α-CH_3), 3.12 (1H, d, β-H, ^2J=14.0 Hz), 3.25 (1H, d, β-H′, ^2J=14.0 Hz), 3.58 (3H, s, MocCH_3), 3.87 (1H, d, CH-NH_2, ^2J=13.4 Hz), 4.03 (1H, d, CH′-NH_2, ^2J=13.4 Hz) 7.09–7.39 (4H, M, H-arom) ppm. ^{13}C NMR (DMSO, 63 MHz) 24.52 (α-CH_3), 36.92 (β-CH_2), 40.06 (CH_2-NH_2), 51.56 (CH_3-Moc), 60.38 (α-C_q), 126.80, 128.40, 131.10, 131.40 (CH-arom), 133.40, 137.80 (C_q-arom), 155.20 (C=O-Moc), 175.50 (COO−) ppm.

4.17. (*R,S*)-4-Methyloxycarbonylamino-4-methyl-1,2,4,5-tetrahydro-2-benzazepin-3-one [Moc-(*R,S*)-4-Me-Aba] 36

A solution of (*R,S*)-3-(2-(aminomethyl)phenyl)-2-(methoxy carbonylamino)-2-methylpropanoic acid **35** (0.5 g, 1.88 mmol,

1.0 equiv) and pyridine (303 μL, 3.76 mmol, 2.0 equiv) in CH_3CN/H_2O (3:1, 170 mL) was cooled in an ice bath for 10 min, followed by the addition of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (0.469 g, 2.4 mmol, 1.3 equiv, EDC). After 30 min the ice bath was removed and the reaction mixture was stirred overnight. After evaporation of the solvent, a white solid was obtained. This residue was re-dissolved in water (100 mL) and extracted with CH_2Cl_2 (4×100 mL). The organic layer was washed with H_2O (2×80 mL), dried ($MgSO_4$), filtered, and evaporated (yield: 60%). The combined aqueous phases were evaporated again, re-dissolved in H_2O (100 mL), and extracted with CH_2Cl_2 (3×100 mL). The organic layer was washed with H_2O (50 mL), dried ($MgSO_4$), filtered, and evaporated to yield a white solid.

Yield: 0.45 g (97%). Mp 231.4–233.2 °C. HPLC t_R=14.8 min. R_f 0.40 (Et_2O/hexane 1:1). MS 249 ($M+H^+$), 217 ($M-OCH_3$), 174 ($M-CH_3OCO-NH$), 497 (2M+1). ^1H NMR (DMSO, 250 MHz) 1.00 (3H, s, α-CH_3), 2.51 (1H, d, β-H, ^2J=13.7 Hz), 3.53 (3H, s, MocCH_3), 3.79 (1H, m, ε-H), 3.98 (1H, d, β-H′, ^2J=13.7 Hz), 4.57 (1H, d, ε-H′, ^2J=14.3 Hz), 7.20–7.29 (4H, M, H-Aba), 7.64 (1H, s, NHMoc), 7.80 (1H, d, NH-Aba, ^3J=6.2 Hz) ppm. ^{13}C NMR (DMSO, 63 MHz) 25.84 (α-CH_3), 41.25 (ε-CH_2), 44.30 (β-CH_2), 51.50 (CH_3Moc), 58.61 (α-C_q), 127.10, 127.50, 128.10, 130.50 (CH-Aba), 137.90, 139.00 (C_q-Aba), 156.20 (C=O-Moc), 174.70 (C=O-Aba) ppm.

4.18. (*R*,*S*)-*tert*-Butyl-2-(4-(methoxycarbonylamino)-4-methyl-3-oxo-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)-acetate [Moc-(*R*,*S*)-4-Me-Aba-Gly-O^{t}Bu] 37

Pentane was added four times to 20 g 60% NaH in oil and decanted into EtOH. The residual pentane was each time evaporated by use of a dry rotavap. A white powder was obtained (11.4 g, 95%). To a cooled solution of (*R*,*S*)-4-methyloxycarbonylamino-4-methyl-1,2,4,5-tetrahydro-2-benzazepin-3-one **36** (79.5 mg, 0.320 mmol, 1.0 equiv) in *N*,*N*-dimethylformamide (6 mL), NaH (11.1 mg, 0.480 mmol, 1.5 equiv) was added, followed by the addition after 20 min of *tert*-butyl-bromoacetate (103 μL, 0.640 mmol, 2.0 equiv). After 20 min the ice bath was removed and the mixture was stirred for 1 h. The mixture was diluted with EtOAc (20 mL) and the organic phase was subsequently extracted with a saturated $NaHCO_3$ solution (3×15 mL) and H_2O (2×20 mL), dried ($MgSO_4$), filtered, and evaporated. The product was purified by flash column chromatography (EtOAc/hexane 12:20) to yield a white solid.

Yield: 91.6 mg (79%). Mp 103.1–104.6 °C. HPLC t_R=22.4 min. R_f 0.40 (EtOAc). MS 363 ($M+H^+$), 385 ($M+Na^+$), 289 ($M-O^tBu$). ^1H NMR (DMSO, 250 MHz) 1.04 (3H, s, α-CH_3), 1.26 (9H, s, H-^{t}Bu), 2.56 (1H, d, β-H, ^2J=14.1 Hz), 3.53 (3H, s, MocCH_3), 3.90 (1H, d, Hα-Gly, ^2J=16.6 Hz), 3.97–4.15 (2H, M, β-H′+ε-H), 4.25 (1H, d, Hα′-Gly, ^2J=16.6 Hz), 5.03 (1H, d, ε-H′, ^2J=14.8 Hz), 7.21–7.30 (4H, M, H-Aba), 7.73 (1H, s, NHMoc) ppm. ^{13}C NMR (DMSO, 63 MHz) 26.54 (α-CH_3), 27.86 (CH_3 ^{t}Bu), 41.90 (β-CH_2), 51.50 (CH_3Moc), 52.28 (α-CH_2-Gly), 53.67 (ε-CH_2), 59.30 (α-C_q), 80.62 (C_q-^{t}Bu), 127.00, 128.20, 130.20 (H-Aba), 137.70, 138.00 (C_q-Aba), 156.20 (C=O-Moc), 168.70 (COO^{t}Bu), 173.70 (C=O-Aba) ppm.

4.19. (*R*,*S*)-2-(4-Amino-4-methyl-3-oxo-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)acetic acid hydrobromide ((*R*,*S*)-4-Me-Aba-Gly-OH) [HBr·(*R*,*S*)-4-Me-Aba-Gly-OH] 38

(*R*,*S*)-Moc-4-Me-Aba-Gly-O^{t}Bu **37** (453 mg, 1.25 mmol, 1.0 equiv) was dissolved in 33% HBr/AcOH (24 mL) and stirred at 55 °C for 1.5 h. The reaction mixture was evaporated and repeatedly re-dissolved in AcOH and evaporated to yield an orange solid.

Yield: >100%. HPLC t_R=10.8 min. R_f 0.20 (CH_3CN/CH_3OH/H_2O). MS 249 ($M+H^+$), 232 ($M-NH_2$), 497 (2M+1). ^1H NMR (DMSO, 250 MHz) 1.25 (3H, s, α-CH_3), 3.00–4.72 (6H, M, β-CH_2+α-CH_2-Gly+ε-CH_2), 7.29–7.41 (4H, M, H-Aba), 8.45 (3H, s, NH_3^+) ppm. ^{13}C NMR (DMSO, 63 MHz) 21.39 (α-CH_3), 39.02 (β-CH_2), 52.17–52.32 (α-CH_2-Gly+ε-CH_2), 59.57 (α-C_q), 127.90, 128.90, 130.10 (CH-Aba), 135.60, 137.30 (C_q-Aba), 170.40+171.20 (C=O-Aba+C=O Gly) ppm.

4.20. (*R*,*S*)-2-(4-Acetamido-4-methyl-3-oxo-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)-*N*-methylacetamide [(*R*,*S*)-Ac-4-Me-Aba-Gly-NHMe] 11

HBr·(*R*,*S*)-4-Me-Aba-Gly-OH **38** (185 mg, 0.745 mmol, 1.0 equiv) was dissolved in H_2O (9 mL). The pH was adjusted to 6 with Et_3N and Ac_2O (490 μL, 5.22 mmol, 7.0 equiv) was added in five portions over 5 min. Meanwhile the pH was kept at 6 with Et_3N. After 30 min stirring at rt the reaction mixture was evaporated. The residue was dissolved in a minimum amount 2 N HCl and extracted with CH_2Cl_2 (4×40 mL). The organic layer was again extracted with 2 N HCl (5 mL), dried ($MgSO_4$), filtered, and evaporated. A transparent oil was obtained and used without further purification. To a solution of (*R*,*S*)-Ac-4-Me-Aba-Gly-OH (177 mg, 0.584 mmol, 1.0 equiv) in CH_3CN/H_2O (3:1, 30 mL), pyridine (96.2 μL, 1.17 mmol, 2.0 equiv) was added. The reaction mixture was cooled down to 0 °C, followed by the addition of *N*,*N*-dicyclohexylcarbodiimide (176 mg, 0.817 mmol, 1.4 equiv). After 10 min CH_3NH_2·HCl (164 mg, 2.33 mmol, 4.0 equiv) and pyridine (481 μL, 2.92 mmol, 5.0 equiv) were added. The coupling was continued for 2.5 h. The reaction mixture was evaporated and the product was purified by prep HPLC. After lyophilization a white solid was obtained.

Yield: 106 mg (60%). HPLC t_R=13.4 min. MS 304 ($M+H^+$), 273 ($M-CH_3NH$), 245 ($M-CH_3CONH$ of $-CH_3NHCO$). ^1H NMR (DMSO, 250 MHz) 1.11 (3H, s, α-CH_3), 1.90 (3H, s, CH_3Ac), 2.55 (1H, d, β-H, ^2J=14.0 Hz), 2.63 (3H, s, CH_3 $NHCH_3$), 3.63 (1H, d, Hα-Gly, ^2J=16.6 Hz), 3.91 (1H, d, β-H′, ^2J=14.0 Hz), 4.10 (1H, d, ε-H, ^2J=14.6 Hz), 4.40 (1H, d, H′α-Gly, ^2J=16.6 Hz), 5.05 (1H, d, ε-H′, ^2J=14.5 Hz), 7.21–7.39 (4H, M, H-Aba), 7.68 (1H, s, NH-CH_3), 8.77 (1H, s, NH-Ac) ppm. ^{13}C NMR (DMSO, 63 MHz) 22.36 (CH_3-Ac), 25.47 (α-CH_3), 25.60 (NH-CH_3), 41.37 (β-CH_2), 50.95 (ε-CH_2), 53.89 (α-CH_2-Gly) 58.91 (α-C_q), 126.90, 127.80, 128.10, 129.80 (CH-Aba), 136.90, 137.70 (C_q-Aba), 168.50 (C=O-NHMe), 170.20 (C=O-Ac), 172.60 (C=O-Aba) ppm.

4.21. (1*R*,4*S*)-2-(4-Acetamido-3-oxo-1-phenyl-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)-*N*-methylacetamide [(1*R*,4*S*)-Ac-1-phenyl-Aba-Gly-NHMe] 14 and (1*S*,4*S*)-2-(4-acetamido-3-oxo-1-phenyl-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)-*N*-methylacetamide [(1*S*,4*S*)-Ac-1-phenyl-Aba-Gly-NHMe] 15

(1*S*,4*S*)-Phth-1-phenyl-Aba-Gly-OEt and (1*R*,4*S*)-Phth-1-phenyl-Aba-Gly-OEt were prepared as previously described by us.[37] They were transformed into (1*R*,4*S*)-Ac-1-phenyl-Aba-Gly-NHMe **14** and (1*S*,4*S*)-Ac-1-phenyl-Aba-Gly-NHMe **15** by the reaction sequence described above for the 5-methyl analogs. Final purification was performed by prep HPLC.

4.22. (1*R*,4*S*)-2-(4-Acetamido-3-oxo-1-phenyl-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)-*N*-methylacetamide [(1*R*,4*S*)-Ac-1-phenyl-Aba-Gly-NHMe] 14

Yield: 25.4 mg (49%). HPLC t_R=13.5 min. R_f 0.75 (EtOAc/BuOH/AcOH/H_2O 1:1:1:1). MS 366 ($M+H^+$). ^1H NMR ($CDCl_3$, 250 MHz): 2.08 (3H, s, CH_3Ac), 2.77 (3H, br s, CH_3 NHMe), 2.86 (1H, dd, β-H, ^2J=16.0 Hz, ^3J=4.0 Hz), 2.98 (1H, pseudo t, β′-H, $^2J \approx {}^3J$=8.5 Hz), 3.63 (1H, Hα-Gly, ^2J=17 Hz), 4.45 (1H, m, α-H), 4.99 (1H, Hα′-Gly, ^2J=17 Hz), 5.80 (1H, s, ε-H), 6.28 (1H, br s, NH-CH_3), 6.60 (1H, br s, NH-Ac) 7.07 (2H, m, H-arom), 7.22–7.46 (7H, M, H-arom) ppm. ^{13}C NMR (DMSO-d_6, 63 MHz) 22.0 (NH-CH_3) (HMBC), 25.3 (CH_3-Ac) (HMBC), 35.3 (β-CH_2), 53.4 (α-CH_2-Gly), 54.0 (C_α Aba), 68.7 (ε-CH), 126.7 (CH-arom), 127.3 (CH-arom), 128.6 (CH-arom), 129.3

(CH-arom), 130.0 (CH-arom), 130.7 (CH-arom), 137.7 (C_q-arom), 139.9 (C_q-arom), 140.6 (C_q-arom), 168.2 (C=O-NHMe), 168.3 (C=O-Ac), 171.4 (C=O-Aba) ppm.

4.23. (1*S*,4*S*)-2-(4-Acetamido-3-oxo-1-phenyl-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)-*N*-methylacetamide [(1*S*,4*S*)-Ac-1-phenyl-Aba-Gly-NHMe] 15

Yield: 22.6 mg (44%). HPLC t_R=13.0 min. R_f 0.75 (EtOAc/BuOH/AcOH/H_2O 1:1:1:1). MS 366 ($M+H^+$). ^1H NMR ($CDCl_3$, 250 MHz) 1.99 (3H, s, CH_3Ac), 2.76 (3H, br s, CH_3 NHMe), 2.91 (1H, dd, β-H, ^2J=14.0 Hz, ^3J=10 Hz), 3.40 (1H, dd, β′-H, ^2J=14 Hz, ^3J=4.5 Hz), 4.35 (1H, Hα-Gly, ^2J=17 Hz), 4.55 (1H, Hα′-Gly, ^2J=17 Hz), 4.87 (1H, m, α-H), 5.78 (1H, s, ε-H), 6.38 (1H, br s, NH-CH_3), 6.87 (1H, br s, NH-Ac) 7.00 (2H, m, H-arom), 7.05–7.34 (7H, M, H-arom) ppm. ^{13}C NMR (DMSO-d_6, 63 MHz) 22.1 (NH-CH_3) (HMBC), 26.0 (CH_3-Ac) (HMBC), 36.0 (β-CH_2), 48.3 (C_α Aba), 53.5 (α-CH_2-Gly), 67.3 (ε-CH), 125.3 (CH-arom), 126.0 (CH-arom), 127.3 (CH-arom), 128.0 (CH-arom), 128.7 (CH-arom), 131.8 (CH-arom), 132.0 (CH-arom), 134.7 (C_q-arom), 135.3 (C_q-arom), 142.7 (C_q-arom), 168.1 (C=O-Ac), 168.4 (C=O-NHMe), 171.9 (C=*O*-Aba) ppm.

4.24. Protocol for peptide synthesis

Compounds **42** to **44** were prepared on a 0.4 mmol scale by manual solid phase synthesis using MBHA resin (loading 0.95 mmol/g) as a solid support and following standard Boc amino protection procedures. The Boc-deprotection was performed in a mixture of TFA/CH_2Cl_2/2% anisole (5 min+20 min). After filtration of the TFA mixture and neutralization with 20% DIPEA/CH_2Cl_2, the couplings were performed by using a threefold excess of the amino acids and activating agent (TBTU), and a ninefold excess of *N*-methylmorpholine. The completeness of the couplings was checked with the Kaiser or NF 31 color tests.[62,63] Cleavage of the peptide from the resin and side chain deprotection was accomplished by treatment with HF_{liq} for 1 h at 0 °C. The crude peptide was purified by preparative HPLC and their structure was confirmed by mass spectrometry. (*S*)-Aba-Gly[56] and (*S*)-spiroAba-Gly[55] were obtained following the literature procedures, whereas (4*S*)-Me-Aba-Gly-OH was prepared as described for racemate **38**, starting from (*S*)-**33**.[64] *H-(S)-Dmt-(R)-Ala-(S)-Aba-Gly-NH*$_2$ ***42***. HPLC t_R=11.5 min. R_f 0.66 (EtOAc/BuOH/AcOH/H_2O 1:1:1:1). MS 496 ($M+H^+$). *H-(S)-Dmt-(R)-Ala-(4S)-Me-(S)-Aba-Gly-NH*$_2$ ***43***. HPLC t_R=10.3 min. R_f 0.71 (EtOAc/BuOH/AcOH/H_2O 1:1:1:1). MS 510 ($M+H^+$). *H-(S)-Dmt-(R)-Ala-(S)-spiroAba-Gly-NH*$_2$ ***44***. HPLC t_R=10.8 min. R_f 0.69 (EtOAc/BuOH/AcOH/H_2O 1:1:1:1). MS 536 ($M+H^+$).

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