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Exploring new dipeptides based on phenylglycine and C$^{\alpha}$-methyl phenylglycine as hosts in inclusion resolutions

Simona Müller,[a] Gerry J. A. Ariaans,[a] Bernard Kaptein,[b,*] Quirinus B. Broxterman,[b] Fernando Formaggio,[c,*] Elisa Battan,[c] Marco Crisma,[c] Claudio Toniolo[c] and Alle Bruggink[a,b]

[a]*Department of Organic Chemistry, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands*
[b]*DSM Pharma Chemicals, Advanced Synthesis and Catalysis, PO Box 18, 6160 MD Geleen, The Netherlands*
[c]*Institute of Biomolecular Chemistry, CNR, Department of Chemistry, University of Padova, via Marzolo 1, 35131 Padova, Italy*

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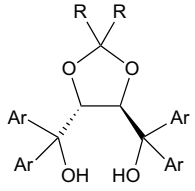
Abstract—Twelve homo-dipeptides derived from phenylglycine, Phg, and C$^{\alpha}$-methyl phenylglycine, (αMe)Phg, were synthesized and tested as resolving agents in resolutions through selective crystallization of inclusion compounds. The 3D-structure of a hydrated (αMe)Phg dipeptide host was also solved by single crystal X-ray diffraction. These dipeptides were examined in the co-crystallization with 15 different racemic guests, mainly alcohols and sulfoxides. Next to confirming the literature results for the resolution of methylphenylsulfoxide, a rather limited scope was found for new resolutions. Only racemic solketal could be resolved with H-(*S*)-(αMe)Phg-(*S*)-(αMe)Phg-OH in modest efficiency using various experimental techniques. This resolution was complicated by the formation of polymorphic host–guest crystals. Whereas a wide array of similar dipeptides could be explored as resolving agents, it is expected to be difficult to rationally design potentially successful molecular structures. Compared to resolution by diastereomeric salt formation, inclusion complexes are less readily formed and therefore of a more limited scope and preparative applicability.

1. Introduction

Access to pure enantiomers through selective crystallization of diastereomeric inclusion compounds would extend the scope of traditional racemate resolution beyond salt forming molecules. The pioneering work by Toda and co-workers[1,2] showed a promising potential of this approach. Using a limited number of diols (Fig. 1) derived from tartaric acid, almost 200 successful inclusions were reported and about 100 racemates were resolved. In more than half of the examples ee's of 80% or higher were obtained, combined, in about 20 cases, to yields of 40% or higher (based on the racemate).

Ogura and co-workers[3–8] reported that some phenylglycine (Phg) dipeptides, such as **1** (Fig. 2), are highly efficient hosts for the inclusion resolution of racemic alkyl phenyl sulfoxides,[3–5] 1-arylethylamines,[6] α-hydroxy esters,[7] and ethers.[8] With the aim at investigating the scope and limitations of inclusion resolutions we extended the selective crystallization of diastereomeric inclusion compounds to new racemic guests. In addition, we tested new host dipeptides, based on the conformationally restricted C$^{\alpha}$-methyl phenylglycine [(αMe)Phg] α-amino acid residue.[9–11] The easy access to a wide range of simple peptides would greatly expand the potential applications of the inclusion resolution methods.



R,R	Ar
methyl	phenyl
	o-tolyl
	p-tolyl
$(CH_2)_4$	phenyl
$(CH_2)_5$	phenyl

Figure 1. Successful taddols (2,2-dialkyl-α,α,α′,α′-tetraaryldioxolane-4,5-dimethanols) for inclusion resolutions.

* Corresponding authors. Tel.: +31-46-4767416; fax: +31-46-4767604 (B.K.); tel.: +39-049-8275277; fax: +39-049-8275239 (F.F.); e-mail addresses: bernard.kaptein@dsm.com; fernando.formaggio@unipd.it

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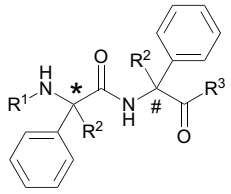


(*,#)	R^1	R^2	R^3	Entry
(*R*,*R*)	H	H	OH	**1**
(*S*,*S*)	H	H	OH	**2**
(*R*,*S*)	H	H	OH	**3**
(*S*,*R*)	H	H	OH	**4**
(*R*,*R*)	Z	H	OBn	**5**
(*S*,*S*)	Z	H	OBn	**6**
(*R*,*S*)	Z	H	OBn	**7**
(*S*,*R*)	Z	H	OBn	**8**
(*S*,*S*)	H	CH_3	OH	**9**
(*S*,*R*)	H	CH_3	OH	**10**
(*R*,*R*)	H	CH_3	NH_2	**11**
(*S*,*R*)	H	CH_3	NH_2	**12**

Figure 2. Dipeptide hosts used in this study.

2. Results and discussion

2.1. Selection of hosts and guests

We decided to prepare 12 dipeptides (Fig. 2) to be investigated for their inclusion capabilities. Known synthetic procedures were followed for the preparation of the Phg-containing peptides **1**–**8**: the carboxyl group was protected as benzyl ester (–COOBn), while the amino group was masked by the benzyloxycarbonyl (Z) moiety. The N-protected residue was coupled to the C-protected residue by means of the DCC/HOBt (DCC, 1,3-dicyclohexylcarbodiimide; HOBt, 1-hydroxy-1,2,3-benzotriazole) method.[12] Simultaneous N- and C-deprotection was achieved through catalytic hydrogenation.[5] Purification of intermediates and desired products was found to be laborious but necessary to obtain pure compounds for inclusion experiments. The tendency of the Phg-peptides to include solvents[13–15] appeared a promising feature in view of the inclusion experiments.

The four C^{α}-methyl substituted dipeptides **9**–**12** were prepared starting from H-(*S*)-(αMe)Phg-OH and H-(*R*)-(αMe)Phg-NH_2. Both starting materials were synthesized by a chemo-enzymatic process and available at DSM Pharma Chemicals on a multi-ton scale.[16,17] For the synthesis of dipeptides **9** and **10** two orthogonal N- and C-protecting groups, Z and *tert*-butoxy (–O*t*-Bu), respectively, were selected. This choice, implying a double step for the protecting group removal, allowed us to obtain peptides of higher purity. As (αMe)Phg is a slow-reacting residue in peptide bond formation,[18] to prepare dipeptides **9**–**12** we looked for a more effective activation procedure, as compared to the DCC/HOBt method employed for peptides **1**–**8**. Initially, the dipeptides were prepared by the EDC/HOAt [EDC, N-ethyl,N′-(3-dimethylamino)propylcarbodiimide; HOAt, 7-aza-1-hydroxy-1,2,3-benzotriazole] coupling method.[19] However, on large scale the symmetrical anhydride method proved to be more convenient and cost effective. The symmetrical anhydride of Z-protected (*S*)- or (*R*)-(αMe)Phg was prepared in good yield (about 80%) by mixing in anhydrous CH_3CN an equimolar amount of (*S*)- or (*R*)-Z-(αMe)Phg-OH and its 5-(4*H*)oxazolone, this latter obtained, in turn, by treating (*S*)- or (*R*)-Z-(αMe)Phg-OH with EDC in anhydrous CH_2Cl_2.

We were able to solve the crystal structure of H-[(*S*)-(αMe)Phg]$_2$-OH dihydrate by X-ray diffraction. The molecular structure is illustrated in Figure 3. As expected, the molecule of the free homo-dipeptide is zwitterionic. Indeed, the C2–O2 and C2–OT bond lengths, 1.239(5) and 1.251(5) Å, respectively, differing by less than 3σ, strongly point to the carboxylate form of the C-terminal group. In addition, three H-atoms bound to the N1 atom could be located on a difference Fourier map. The peptide backbone is fully extended, with $\psi_1 = -168.5(3)^\circ$ and $\phi_2 = -174.8(4)^\circ$.[20,21] As a consequence, two consecutive, intra-residue H-bonded C_5-ring structures are observed, one between the protonated N1 amino group and the peptide carbonyl oxygen O1 atom, and the other between the peptide N2–H group and the C-terminal (carboxylate) O2 atom, with N···O separations of 2.645(4) and 2.560(4) Å, respectively. In each molecule the phenyl rings of the two (αMe)Phg residues are nearly parallel to each other (the angle between the normals to their average planes is 20°), and roughly perpendicular to the peptide backbone.

In the packing mode of this peptide dihydrate (Fig. 4) there is only one direct peptide···peptide intermolecular H-bond, between the protonated N1 amino group and the (carboxylate) OT oxygen atom of a (1/2−*x*, −*y*, 1/2+*z*) symmetry related molecule, with an N···O separation of 2.772(5) Å, which links molecules head-to-tail in a zig-zag motif parallel to the *c* direction. All other intermolecular H-bonds involve the co-crystallized water molecules, as bridges between peptide molecules related through one of the crystallographic twofold screw axes. More specifically, the O1W water molecule acts as H-bond donor to the (carboxylate) O2 atom within the same asymmetric unit with an O1W···O2 separation of 2.731(5) Å, and to the (peptide) O1 atom of a (1/2−*x*, −*y*, −1/2+*z*) symmetry related molecule with an O1W···O1 separation of 2.886(4) Å, thus bridging peptide molecules along the *c* direction. In addition, O1W is the acceptor of a H-bond from the N1 amino

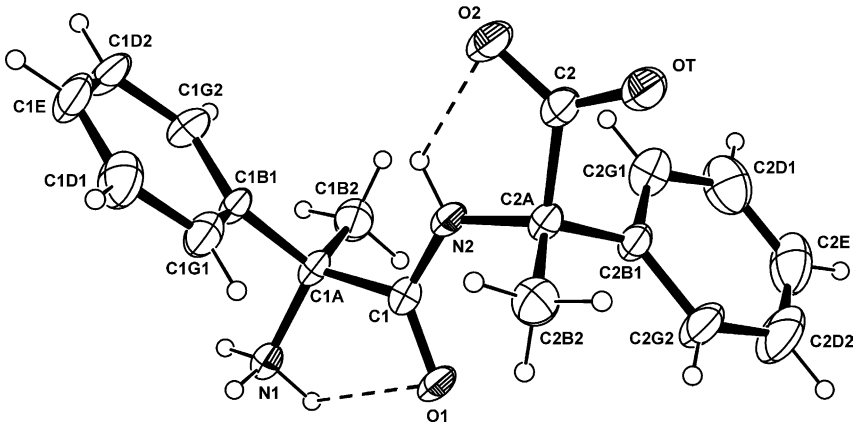


Figure 3. ORTEP view of the X-ray diffraction structure of H-[(*S*)-(αMe)Phg]$_2$-OH dihydrate with atom numbering. Anisotropic displacement ellipsoids are drawn at the 30% probability level. The two intramolecular H-bonds are indicated by dashed lines.

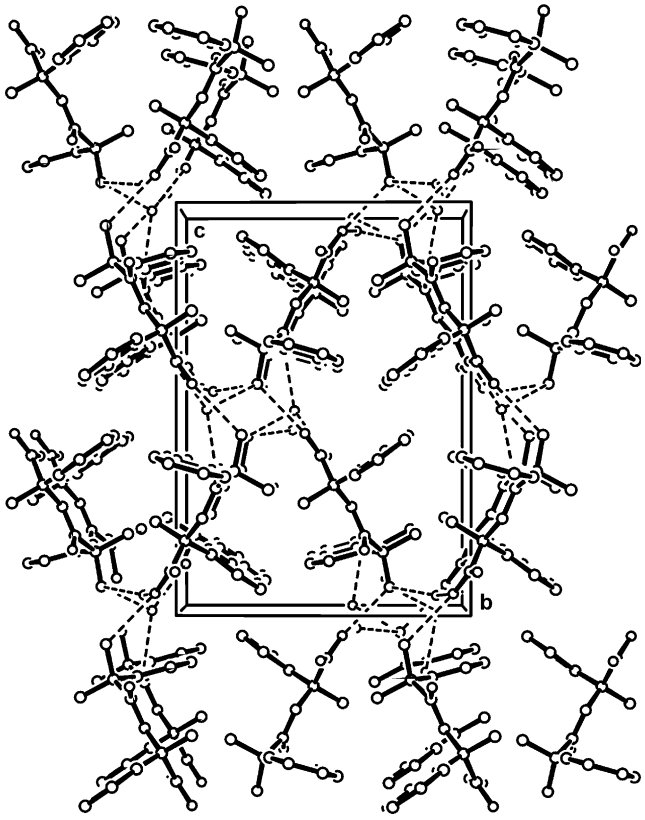


Figure 4. Packing mode of the H-[(*S*)-(αMe)Phg]$_2$-OH dihydrate molecules in the crystal state as viewed down the *a* axis. Intermolecular H-bonds are represented by dashed lines.

group of a (1−*x*, −1/2+*y*, 1/2−*z*) symmetry related peptide molecule [N1···O1W distance 2.785(5) Å]. This latter interaction provides water-mediated connection of peptide molecules along the *b* direction. The second water molecule, O2W, is the acceptor of a H-bond from the protonated N1 amino group within the same asymmetric unit, the N1···O2W distance being 2.675(5) Å, and the H-bond donor to the (carboxylate) OT atom of a (−*x*, 1/2+*y*, 1/2−*z*) symmetry related peptide molecule with an O2W···OT separation of 2.700(5) Å. As a consequence, O2W acts as a bridge between symmetry related peptide molecules along the *b* direction.

The remaining H atom of the O2W molecule is involved in a O–H···π interaction with the phenyl ring of the N-terminal residue of a (−1/2+*x*, 1/2−*y*, 1−*z*) symmetry related peptide molecule. The distances between the O2W oxygen atom and the ring carbon atoms range from 3.284(5) Å (C1G1) to 3.518(5) Å (C1D2), while the related H···C distances vary from 2.57(5) to 3.21(5) Å. This latter interaction occurs along the *a* direction. Conversely, all of the water-mediated and the single direct peptide–peptide H-bonds connect molecules in the *bc* plane. This layered packing motif is similar to the 'water-buried sheet' mode reported by Ogura and co-workers[14] for (1-naphthyl)glycyl-phenylglycine hydrated inclusion complexes. Interestingly, the latter complexes and the structure described in this work, although differing in significant details, share the same symmetry as they belong to the same space group ($P2_12_12_1$).

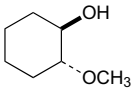
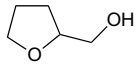
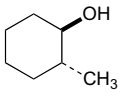
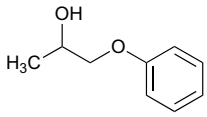
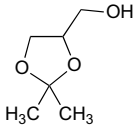
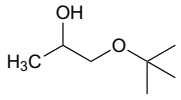
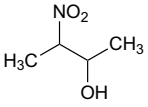
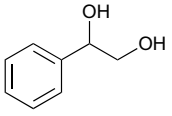
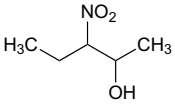
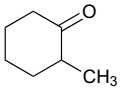
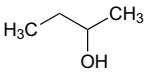
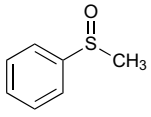
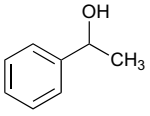
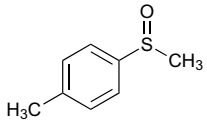
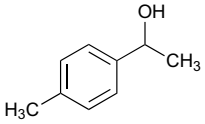
A selection of 15 racemates, depicted in Table 1, as potential guests was used to test the resolving capability of dipeptides **1**–**12** through inclusion compound formation. Priority was given to chiral alcohols, as many amines were already successfully resolved by Ogura and co-workers[6] using dipeptide **1**. Chiral separation of alcohols by inclusion resolution is of special interest because this class of compounds cannot be resolved directly via diastereomeric salt formation.

In order to investigate the possible influence of the oxidation state of the sulfur atom in the inclusion phenomenon, we extended our investigation to the reduced (methylphenylsulfide, also called thioanisole) and oxidized (methylphenylsulfone) achiral forms of methylphenylsulfoxide tested by Ogura and co-workers.[4]

2.2. Inclusion experiments

Given our experience with the sometimes poor reproducibility of crystallizations, in particular in resolution

Table 1. Selected examples of racemic guests tested in the inclusion resolution with hosts **1**–**12**

trans-2-Methoxycyclohexanol		Tetrahydrofurfurylalcohol	
trans-2-Methylcyclohexanol		1-Phenoxy-2-propanol	
2,2-Dimethyl-1,3-dioxolane-4-methanol (solketal)		1-*tert*-Butoxy-2-propanol	
3-Nitro-2-butanol		1-Phenyl-1,2-ethanediol	
3-Nitro-2-pentanol		2-Methylcyclohexanone	
2-Butanol		Methylphenylsulfoxide	
1-Phenylethylalcohol		Methyl-*p*-tolylsulfoxide	
1-*p*-Tolylethylalcohol			

studies, we initially re-investigated a literature experiment. Ogura and co-workers[3–5] found a high (*R*)-enantioselectivity (92–93% ee) for methylphenylsulfoxide when forming an inclusion complex with dipeptide host **1**. Accordingly, in a reproduction of this experiment we obtained an ee of 90%. Apparently, the sulfoxide group is of crucial importance for complex formation, as inclusions with the corresponding sulfone and sulfide as guests did not occur. Ogura and co-workers[3–5] showed that in the crystal state the sulfoxide molecules are accommodated in a cavity between adjacent layers of dipeptide **1**, linked by the intermolecular salt bridges formed between the $-NH_3^+$ and $-COO^-$ groups. In this way host and guest can experience three different types of interactions: hydrogen bonding between a $-NH_3^+$ and the sulfoxide group, phenyl–phenyl edge-to-face interactions and C–H$\cdots\pi$ interactions between phenyl and alkyl groups.[3] Small structural changes can easily upset these subtle interactions, thus hampering a successful resolution. This observation may probably account for the lack of inclusion crystal formation when the methylphenylsulfide and methylphenylsulfone (and also the racemic methyl-*p*-tolylsulfoxide) were used as guests. Surprisingly, also all other racemic guests depicted in Table 1 failed to form inclusion crystals, not only when employing **1** as host, but with all other dipeptide hosts as well. All these inclusion experiments were performed according to the 'sorption' method,[4] that is by stirring at room temperature in water for one or more days a suspension of host (usually sparingly soluble in water) and 1 or 2 equiv of guest. As shown above, host **9** tends to include water and therefore in all experiments only hydrated crystals of **9** were found. Various other practical procedures were tested, that is crystallization from solution, employing increased pressure, or grinding the substrates in a mortar, again without success. Another known alternative approach is to prepare the inclusion compound through a slurry of the crystalline dipeptide and racemic guest compounds in heptane at room temperature. After one or two days the solid is filtered off. Treatment with water and methylene chloride separates the unreacted host peptide from the racemic guest or inclusion product. Testing this 'heptane sorption' method with all other hosts and guests allowed us to identify two new inclusion complexes: dipeptide **9** with (i) methylphenylsulf-

oxide [36% ee with a (*R*)-enantiomer preference] and (ii) solketal [48% ee, also with a preference for the (*R*)-enantiomer]. As the latter inclusion complex appeared to be novel and the most promising one, it has been analyzed in more detail.

2.3. Inclusion resolution of solketal

The resolution of solketal with dipeptide host **9** through the heptane sorption method could be repeated using various slurrying agents such as hexane, methanol, and also water in host–guest ratios from 1:2 to 1:10. The ee values for the (*R*)-enantiomer range from 0% to 2% in water (employing 10 equiv of solketal; with 2 equiv no crystals were obtained) to 30–33% in methanol and 45–48% in hexane. Starting from a solution with excess of solketal no resolution was obtained: from methanol (not dried; 2–10 equiv of solketal) only crystals of host hydrates were formed, whereas with neat solketal incl
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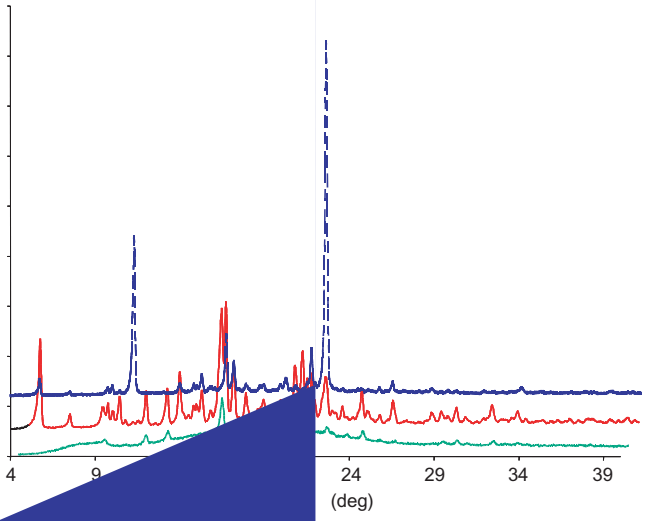

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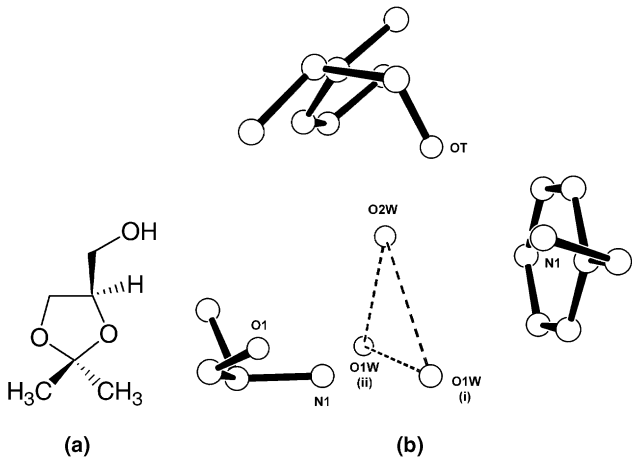

Figure 7. (a) Model of (*R*)-solketal. (b) Portion of the packing mode of H-[(*S*)-(αMe)Phg]$_2$-OH dihydrate. Only atoms within 4.6 Å from O2W are shown. The (1/2−*x*, −*y*, 1/2+*z*) and (1−*x*, 1/2+*y*, 1/2−*z*) symmetry equivalents of O1W are indicated as (i) and (ii), respectively.

easily accommodate the solketal guest, particularly its hydrophobic *gem*-dimethyl group. The geometrical considerations reported above are based on the spatial relationships among the three oxygen atoms of (*R*)-solketal, which define a plane. The same considerations also hold for its mirror image, (*S*)-solketal. Therefore, at the present stage our analysis is unable to provide any significant clue on the observed enantioselectivity in the formation of the inclusion compounds of **9** with solketal.

3. Conclusions

The limited scope of resolution through selective crystallization of inclusion compounds, found in all our studies thus far,[22] has been confirmed in this work. Compared to selective crystallization of diastereomeric salts, in which ionic interactions are determining the energy minima of the crystals, the substrates in inclusion resolutions have far more possibilities to reach stable crystal forms, that is single host crystals and dimers, trimers, etc., or solvation forms thereof. Inclusions of a racemic or chiral guest are other options, but not necessarily adding to crystal stability. In fact, a rational approach to the design of inclusion resolutions is even more complicated than in diastereomeric salts.[23,24]

We have found a new resolving homo-dipeptide, (**9**), characterized by an aromatic C$^{\alpha}$-tetrasubstituted α-amino acid, although with limited efficiency and some practical limitations. Whereas a wide array of similar dipeptides could be explored as resolving agents, it will be rather difficult to design a rational approach toward potentially successful molecular structures. We confirmed Ogura's three-points interaction hypothesis required for an effective inclusion[3–8,13–15] by extending the analysis of a sulfoxide to its reduced and oxidized forms.

4. Experimental section

4.1. Peptide synthesis and characterization

Well-known procedures were used for the preparation of peptides **1**–**8**.[6,12] Repeated recrystallizations are required after each reaction step to allow for efficient subsequent manipulations. Experimental details for dipeptides **9**–**12** are given below.

Melting points were determined using a Leitz model Laborlux 12 apparatus and are not corrected. Optical rotations were measured using a Perkin–Elmer model 241 polarimeter equipped with a Haake model D thermostat. Thin-layer chromatography was performed on Merck Kieselgel 60/F_{254} precoated plates. The solvent systems used are: (1) chloroform/ethanol (9:1); (2) 1butanol/acetic acid/water (3:1:1); (3) toluene/ethanol (7:1); (4) ethyl acetate/petroleum ether (1:1). The chromatograms were developed by quenching of UV fluorescence, chlorine–starch–potassium iodide or ninhydrin chromatic reaction, as appropriate. The IR absorption spectra were recorded on a Perkin–Elmer 1720X FTIR spectrophotometer using the KBr disk technique. The ^{1}H NMR spectra were obtained with a Bruker AC 250 spectrometer. Measurements were carried out in deuterochloroform (99.96% *d*, Merck) or in deuterated dimethylsulfoxide (DMSO, 99.96% d_6 Acros Organics) with tetramethylsilane as the internal standard. High-resolution mass spectra were obtained by electrospray ionization (ESI) on a Perseptive Biosystem Mariner API-TOF spectrometer. A 1 nM solution of neurotensin, angiotensin I, and bradykinin in an acetonitrile/water 1:1 mixture, containing 1% formic acid, was used for calibration.

4.1.1. Z-(*S*)-(αMe)Phg-OH. H-(*S*)-(αMe)Phg-OH (5.00 g, 30.0 mmol) was suspended in dioxane (30 mL) and cooled to 0 °C. Then, a solution of 2.0 M NaOH (16.0 mL, 32 mmol) and $NaHCO_3$ (2.68 g, 32 mmol) was added. Then, Z–OSu (5.00 g, 20 mmol) was added to the solution, and a suspension formed. The reaction was stirred at rt for 4 d. Then, again Z–OSu (5.00 g, 20 mmol) was added and the reaction was stirred for additional 12 d. The solvent mixture was evaporated under reduced pressure. The oily product was dissolved in 5% $NaHCO_3$ (100 mL) and the unreacted Z–OSu was extracted with Et_2O. The aqueous layer was acidified to pH 3 with $KHSO_4$ and the product was extracted with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. Oil; yield 95%; R_{f1} 0.50, R_{f2} 0.90, R_{f3} 0.30; $[\alpha]_D^{20} = +24.9$ (*c* 0.5, MeOH); IR (film) ν_{max} 3300, 1717, 1654 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.45 (m, 5H, Z phenyl CH), 7.32 [m, 5H, (αMe)Phg phenyl CH], 6.35 (s, 1H, NH), 5.06 (s, 2H, Z CH_2), 2.03 (s, 3H, β-CH_3). MS (ESI-TOF) *m*/*z* calcd for $C_{17}H_{18}NO_4$: 300.1235; found: 300.1301 $[M+H]^+$.

4.1.2. Z-(*R*)-(αMe)Phg-OH. This compound was prepared from H-(*R*)-(αMe)Phg-OH and Z–OSu as described above for its (*S*)-enantiomer. Oil; yield 93%; R_{f1}

0.50, R_{f2} 0.90, R_{f3} 0.30; $[\alpha]_D^{20} = -24.8$ (*c* 0.5, MeOH); IR (film) ν_{max} 3303, 1718, 1652 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.45 (m, 5H, Z phenyl CH), 7.32 [m, 5H, (αMe)Phg phenyl CH], 6.38 (s, 1H, NH), 5.06 (s, 2H, Z CH_2), 2.03 (s, 3H, β-CH_3). MS (ESI-TOF) *m/z* calcd for $C_{17}H_{18}NO_4$: 300.1235; found: 300.1286 $[M+H]^+$.

4.1.3. 5-(4*H*)Oxazolone from Z-(*S*)-(αMe)Phg-OH. Z-(*S*)-(αMe)Phg-OH (40.7 g, 136 mmol) was dissolved in anhydrous CH_2Cl_2 and EDC·HCl (26.3 g, 137 mmol) was added. The reaction was stirred at rt for 2 h. Then, the solvent was removed in vacuo and the residue dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated to dryness. Oil; yield 96%; R_{f4} 0.95; $[\alpha]_D^{20} = +49.0$ (*c* 0.5, MeOH); IR (film) ν_{max} 1833, 1688 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.55–7.25 [m, 10H, Z and (αMe)Phg phenyl CH], 5.49 (s, 2H, Z CH_2), 1.80 (s, 3H, β-CH_3).

4.1.4. 5-(4*H*)Oxazolone from Z-(*R*)-(αMe)Phg-OH. This compound was prepared as described above for its (*S*)-enantiomer starting from Z-(*R*)-(αMe)Phg-OH. Oil; yield 94%; R_{f4} 0.95; $[\alpha]_D^{20} = -45.5$ (*c* 0.5, MeOH); IR (film) ν_{max} 1835, 1688 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.55–7.25 [m, 10H, Z and (αMe)Phg phenyl CH], 5.49 (s, 2H, Z CH_2), 1.80 (s, 3H, β-CH_3).

4.1.5. [Z-(*S*)-(αMe)Phg]$_2$O. Z-(*S*)-(αMe)Phg-OH (40.7 g, 136 mmol) was added to a solution of the 5-(4*H*)oxazolone from Z-(*S*)-(αMe)Phg-OH (38.2 g, 136 mmol) in anhydrous CH_3CN. After stirring the reaction mixture overnight at rt the solvent was removed in vacuo and the residue dissolved in EtOAc. The solution was washed with 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated to dryness. Oil; yield 82%; R_{f4} 0.90; $[\alpha]_D^{20} = +28.5$ (*c* 0.5, MeOH); IR (film) ν_{max} 3404, 3330, 1822, 1721 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.55–7.08 [m, 20H, Z and (αMe)Phg phenyl CH], 5.85 (2s, 2H, NH), 5.15–4.90 (m, 4H, Z CH_2), 1.85 (s, 6H, β-CH_3).

4.1.6. [Z-(*R*)-(αMe)Phg]$_2$O. This compound was prepared as described above for its (*S*)-enantiomer starting from Z-(*R*)-(αMe)Phg-OH and the corresponding 5-(4*H*)oxazolone. Oil; yield 81%; R_{f4} 0.90; $[\alpha]_D^{20} = -33.5$ (*c* 0.5, MeOH); IR (film) ν_{max} 3407, 3328, 1822, 1721 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.55–7.10 [m, 20H, Z and (αMe)Phg phenyl CH], 5.85 (2s, 2H, NH), 5.15–4.90 (m, 4H, Z CH_2), 1.85 (s, 6H, β-CH_3).

4.1.7. Z-(*S*)-(αMe)Phg-O*t*-Bu. Isobutylene (20 mL) was slowly bubbled into a solution of Z-(*S*)-(αMe)Phg-OH (4.00 g, 13.4 mmol) in anhydrous CH_2Cl_2 (50 mL) and cooled to −60 °C. Concentrated H_2SO_4 (0.1 mL) was added and the pressure resistant reaction flask was hermetically closed. After keeping the reaction vessel at rt for 7 d, the content was poured into a 5% aqueous solution of $NaHCO_3$ (50 mL). CH_2Cl_2 was removed under reduced pressure and the aqueous phase was extracted with EtOAc. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4, filtered, and evaporated to dryness. Yield 81%; mp 61–63 °C (from EtOAc); R_{f1} 0.90, R_{f2} 0.85, R_{f3} 0.90; $[\alpha]_D^{20} = +9.5$ (*c* 0.5, MeOH); IR (KBr) ν_{max} 3345, 1714 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.41 (m, 5H, Z phenyl CH), 7.35 [m, 5H, (αMe)Phg phenyl CH], 6.27 (s, 1H, NH), 5.03 (dd, 2H, Z CH_2), 2.00 (s, 3H, β-CH_3), 1.33 (s, 9H, O*t*-Bu CH_3). MS (ESI-TOF) *m/z* calcd for $C_{21}H_{26}NO_4$: 356.1860; found: 356.1811 $[M+H]^+$.

4.1.8. Z-(*R*)-(αMe)Phg-O*t*-Bu. This compound was prepared from Z-(*R*)-(αMe)Phg-OH and isobutylene as described above for its (*S*)-enantiomer. Yield 83%; mp 59–61 °C (from EtOAc); R_{f1} 0.90, R_{f2} 0.85, R_{f3} 0.90; $[\alpha]_D^{20} = -9.3$ (*c* 0.5, MeOH); IR (KBr) ν_{max} 3347, 1715 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.41 (m, 5H, Z phenyl CH), 7.35 [m, 5H, (Me)Phg phenyl CH], 6.31 (s, 1H, NH), 5.03 (dd, 2H, Z CH_2), 2.00 (s, 3H, β-CH_3), 1.33 (s, 9H, O*t*-Bu CH_3). MS (ESI-TOF) *m/z* calcd for $C_{21}H_{26}NO_4$: 356.1860; found: 356.1912 $[M+H]^+$.

4.1.9. Z-(*S*)-(αMe)Phg-(*S*)-(αMe)Phg-O*t*-Bu. To a solution of [Z-(*S*)-(αMe)Phg]$_2$O (71.4 g, 123 mmol) in anhydrous CH_2Cl_2, H-(*S*)-(αMe)Phg-O*t*-Bu [obtained by catalytic hydrogenation of the corresponding Z-derivative (44 g, 123 mmol) in anhydrous CH_2Cl_2] and 0.5 equiv of NMM were added. The reaction mixture was stirred at rt for 5 d. Then, the solvent was removed in vacuo and the residue dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated to dryness. The product was crystallized from EtOAc/PE. Yield 54%; mp 128–130 °C (EtOAc/PE); R_{f1} 0.95, R_{f2} 0.95, R_{f3} 0.90; $[\alpha]_D^{20} = +7.5$ (*c* 0.5, MeOH); IR (KBr) ν_{max} 3386, 1735, 1722, 1681 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.55–7.15 [m, 15H, Z and (αMe)Phg phenyl CH], 6.88 (s, 1H, NH), 6.65 (s, 1H, NH), 5.00 (m, 2H, Z CH_2), 2.00 and 1.89 (2s, 6H, β-CH_3), 1.25 (s, 9H, O*t*-Bu CH_3). MS (ESI-TOF) *m/z* calcd for $C_{30}H_{35}N_2O_5$: 503.2540; found: 503.2514 $[M+H]^+$.

4.1.10. Z-(*S*)-(αMe)Phg-(*R*)-(αMe)Phg-O*t*-Bu. This compound was prepared as described above for Z-(*S*)-(αMe)Phg-(*S*)-(αMe)Phg-O*t*-Bu starting from [Z-(*S*)-(αMe)Phg]$_2$O and H-(*R*)-(αMe)Phg-O*t*-Bu. The product was isolated by flash chromatography (eluant EtOAc/PE 1:1). Oil; yield 53%; R_{f1} 0.95, R_{f2} 0.95, R_{f3} 0.90; $[\alpha]_D^{20} = -5.6$ (*c* 0.25, MeOH); IR (KBr) ν_{max} 3385, 1732, 1684 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.55–6.90 [m, 16H, Z and (αMe)Phg phenyl CH, and 1NH], 6.68 (s, 1H, NH), 5.00 (m, 2H, Z CH_2), 2.05 and 1.95 (2s, 6H, β-CH_3), 1.27 (s, 9H, O*t*-Bu CH_3). MS (ESI-TOF) *m/z* calcd for $C_{30}H_{35}N_2O_5$: 503.2540; found: 503.2614 $[M+H]^+$.

4.1.11. Z-(*S*)-(αMe)Phg-(*S*)-(αMe)Phg-OH. Z-(*S*)-(αMe)Phg-(*S*)-(αMe)Phg-O*t*-Bu (14.7 g, 29.2 mmol) was

dissolved in a 1:1 CH_2Cl_2/TFA mixture. After stirring at rt for 1 h, the solvent was removed in vacuo, Et_2O was added, and the compound collected by filtration. Yield 90%; mp 191–193 °C (from MeOH/Et_2O); R_{f1} 0.65, R_{f2} 0.95, R_{f3} 0.20; $[\alpha]_D^{20} = +22.5$ (*c* 0.5, MeOH); IR (KBr) ν_{max} 3373, 3351, 1738, 1675 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.55–7.15 [m, 16H, Z and (αMe)Phg phenyl CH, and 1NH], 6.32 (s, 1H, NH), 5.05 (m, 2H, Z CH_2), 1.92 and 1.82 (2s, 6H, β-CH_3). MS (ESI-TOF) *m/z* calcd for C_{26} $H_{27}N_2O_5$: 447.1914; found: 447.2001 $[M+H]^+$.

4.1.12. Z-(*S*)-(αMe)Phg-(*R*)-(αMe)Phg-OH. This compound was prepared as described above for Z-(*S*)-(αMe)Phg-(*S*)-(αMe)Phg-OH starting from the corresponding dipeptide *tert*-butyl ester. Yield 89%; mp 66–68 °C (from MeOH/Et_2O); R_{f1} 0.50, R_{f2} 0.95, R_{f3} 0.15; $[\alpha]_D^{20} = -9.0$ (*c* 0.5, MeOH); IR (KBr) ν_{max} 3381, 1730, 1682 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.55–6.95 [m, 16H, Z and (αMe)Phg phenyl CH, and 1NH], 6.40 (s, 1H, NH), 5.00 (m, 2H, Z CH_2), 1.98 and 1.90 (2s, 6H, β-CH_3). MS (ESI-TOF) *m/z* calcd for C_{26} $H_{27}N_2O_5$: 447.1914; found: 447.1982 $[M+H]^+$.

4.1.13. H-(*S*)-(αMe)Phg-(*S*)-(αMe)Phg-OH (9). Z-(*S*)-(αMe)Phg-(*S*)-(αMe)Phg-OH (11.7 g, 26.3 mmol) was dissolved in MeOH containing Pd/C. Under stirring the mixture was flushed with N_2 and then H_2 was bubbled for 30 min. The catalyst was filtered off and the solvent was removed in vacuo. Yield 92%; mp 310 °C (phase transition around 145 °C, formation of crystals at about 160 °C); R_{f1} 0.05, R_{f2} 0.80, R_{f3} 0.05; $[\alpha]_D^{20} = +82.9$ (*c* 0.5, MeOH); IR (KBr) ν_{max} 3415, 3357, 1683 cm^1 ; ^1H NMR (250 MHz, DMSO, d_6) δ 7.55–7.05 [m, 13H, (αMe)Phg phenyl CH and NH], 1.78 (s, 6H, β-CH_3). MS (ESI-TOF) *m/z* calcd for $C_{18}H_{21}N_2O_3$: 313.1547; found: 313.1609 $[M+H]^+$.

4.1.14. H-(*S*)-(αMe)Phg-(*R*)-(αMe)Phg-OH (10). This compound was prepared as described above for **9** starting from Z-(*S*)-(αMe)Phg-(*R*)-(αMe)Phg-OH. Yield 96%; mp 158–160 °C; R_{f1} 0.05, R_{f2} 0.75, R_{f3} 0.05; $[\alpha]_D^{20} = +58.9$ (*c* 0.5, MeOH); IR (KBr) ν_{max} 3416, 1680 cm^{-1}; ^1H NMR (250 MHz, DMSO, d_6) δ 7.65–7.05 [m, 13H, (αMe)Phg phenyl CH and NH], 1.82 and 1.78 (2s, 6H, CH_3). MS (ESI-TOF) *m/z* calcd for $C_{18}H_{21}N_2O_3$: 313.1547; found: 313.1603 $[M+H]^+$.

4.1.15. Z-(*R*)-(αMe)Phg-(*R*)-(αMe)Phg-NH_2. To a solution of [Z-(*R*)-(αMe)Phg]$_2$O (64.9 g, 112 mmol) in CH_2Cl_2 H-(*R*)-(αMe)Phg-NH_2 (18.3 g, 112 mmol), obtained by the partial Strecker synthesis of the α-amino acid,[16,17] and 0.5 equiv of NMM were added. The reaction was stirred at rt for 6 d. Then, the solvent was removed in vacuo and the residue dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated to dryness. The product was crystallized from EtOAc/PE. Yield 52%; mp 189–190 °C (EtOAc/PE); R_{f1} 0.85, R_{f2} 0.95, R_{f3} 0.30; $[\alpha]_D^{20} = -19.0$ (*c* 0.2, MeOH); IR (KBr) ν_{max} 3386, 1735, 1722, 1681 cm^{-1}; ^1H NMR (250 MHz, DMSO, d_6) δ 8.14 (s, 1H, NH), 7.87 (s, 1H, NH), 7.45–7.15 [m, 17H, Z and (αMe)Phg phenyl CH, and 2 NH], 5.07 (m, 2H, Z CH_2), 1.81 and 1.67 (2s, 6H, β-CH_3). MS (ESI-TOF) *m/z* calcd for $C_{26}H_{28}N_3O_4$: 446.2074; found: 446.2058 $[M+H]^+$.

4.1.16. Z-(*S*)-(αMe)Phg-(*R*)-(αMe)Phg-NH_2. This compound was prepared as described above for Z-(*S*)-(αMe)Phg-(*S*)-(αMe)Phg-NH_2 starting from [Z-(*S*)-(αMe)Phg]$_2$O and H-(*R*)-(αMe)Phg-NH_2. The product was isolated by flash chromatography (eluant EtOAc/PE 1:1) and crystallized from EtOAc/PE. Yield 62%; mp 75–76 °C (EtOAc/PE); R_{f1} 0.85, R_{f2} 0.95, R_{f3} 0.30; $[\alpha]_D^{20} = -9.0$ (*c* 0.2, MeOH); IR (KBr) ν_{max} 3360, 1726, 1673 cm^{-1}; ^1H NMR (250 MHz, DMSO, d_6) δ 8.19 (s, 1H, NH), 7.74 (s, 1H, NH), 7.40–7.10 [m, 17H, Z and (αMe)Phg phenyl CH, and 2NH], 5.04 (m, 2H, Z CH_2), 1.83 and 1.72 (2s, 6H, β-CH_3). MS (ESI-TOF) *m/z* calcd for C_{26} $H_{28}N_3O_4$: 446.2074; found: 446.2138 $[M+H]^+$.

4.1.17. H-(*R*)-(αMe)Phg-(*R*)-(αMe)Phg-NH_2 (11). Z-(*R*)-(αMe)Phg-(*R*)-(αMe)Phg-NH_2 (20.8 g, 46.7 mmol) was dissolved in MeOH containing Pd/C. Under stirring, the mixture was flushed with N_2 and then H_2 was bubbled for 30 min. The catalyst was filtered off and the solvent was removed in vacuo. Yield 98%; mp 69–70 °C; R_{f1} 0.70, R_{f2} 0.70, R_{f3} 0.20; $[\alpha]_D^{20} = -27.6$ (*c* 0.5, MeOH); IR (KBr) ν_{max} 3327, 1662 cm^{-1}; ^1H NMR (250 MHz, DMSO, d_6) δ 8.69 (s, 1H, NH), 7.48–7.25 [m, 10H, (αMe)Phg phenyl CH], 5.92 (s, 1H, NH), 5.43 (s, 1H, NH), 2.24 (br s, 2H, 2NH), 1.90 and 1.76 (2s, 6H, β-CH_3). MS (ESI-TOF) *m/z* calcd for $C_{18}H_{22}N_3O_2$: 312.1707; found: 312.1801 $[M+H]^+$.

4.1.18. H-(*S*)-(αMe)Phg-(*R*)-(αMe)Phg-NH_2 (12). Z-(*S*)-(αMe)Phg-(*R*)-(αMe)Phg-NH_2 (46 g, 103 mmol) was treated as described above for **11**. Yield 88%; mp 86–88 °C; R_{f1} 0.80, R_{f2} 0.75, R_{f3} 0.25; $[\alpha]_D^{20} = -72.1$ (*c* 0.5, MeOH); IR (KBr) ν_{max} 3449, 3314, 1660 cm^{-1}; ^1H NMR (250 MHz, DMSO, d_6) δ 9.25 (s, 1H, NH), 7.65–7.05 [m, 12H, (αMe)Phg phenyl CH and 2NH], 3.12 (br s, 2H, 2NH), 1.83 and 1.55 (2s, 6H, β-CH_3). MS (ESI-TOF) *m/z* calcd for $C_{18}H_{22}N_3O_2$: 312.1707; found: 312.1764 $[M+H]^+$.

4.2. X-ray diffraction

Single crystals of H-[(*S*)-(αMe)Phg]$_2$-OH dihydrate were grown from an ethyl acetate–methanol solution by diffusion of petroleum ether vapour. $C_{18}H_{20}N_2O_3 \times 2H_2O$. Orthorhombic, space group ($P2_12_12_1$). Unit cell parameters $a = 9.559(2)$, $b = 11.680(2)$, $c = 16.417(3)$ Å. $V = 1832.9(6)$ Å^3; $Z = 4$; $D_{calcd} = 1.262$ Mg m^{-3}. Data collection was performed on a Philips PW1100 diffractometer, using graphite monochromated Cu Kα radiation ($\lambda = 1.54178$ Å) in the θ-2θ scan mode up to $\theta = 60°$. Limiting indices: $0 \leqslant h \leqslant 10$; $0 \leqslant k \leqslant 13$; $0 \leqslant l \leqslant 18$. A total of 1593 reflections were collected, 1575 of which

independent. Three standard reflections, periodically monitored, showed a linear decay that reached 20% at the end of data collection. Data were rescaled accordingly. The structure was solved by direct methods of the SHELXS 97 program,[25] and refined by full-matrix block least-squares on F_2, using all data, by application of the SHELXL 97 program,[26] with all non-H atoms anisotropic, and allowing the positional parameters and the anisotropic displacement parameters of the non-H atoms to refine at alternate cycles. The positions of the H-atoms of the N-terminal NH_3^+ group and the two co-crystallized water molecules were recovered from a difference Fourier map. All other H-atoms were calculated at idealized positions. H-atoms of the peptide molecule were refined as riding, while the positional parameters of H-atoms bound to the water molecules were refined with a common bond distance restraint of 0.82 Å. Data/restraints/parameters: 1575/4/214. Refinement converged to $R_1 = 0.0672$ [on $F \geqslant 4(\sigma)F$] and $wR_2 = 0.1771$ (on F^2 all data). Goodness-of-fit on F^2 1.047. $\Delta\rho$ max. and min. +0.330 and −0.352 e Å^{-3}.

CCDC-232008 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336-033; e-mail: deposit@ccd.cam.ac.uk].

4.3. Resolution experiments

Several experimental techniques were used to reach successful crystallization in resolutions, including all common methods to induce nucleation and crystal formation. Most techniques employed for inclusion resolutions have already been described.[6] A representative description is given below.

4.3.1. General procedure for crystallization experiments using slurry systems. The dipeptide was stirred together with the respective guest compound in water or in an organic solvent. Most experiments were performed in 1:1, 1:2 and 1:10 host/guest ratios at room temperature. In those cases in which crystals were obtained, they were filtered off, washed with heptane, and analyzed for the presence of the respective guest compound by ^{1}H NMR. If an inclusion complex was obtained, the ee of the included guest was determined using HPLC (Chiralcel OB) or GC (β-CD). In other cases a H_2/CH_2Cl_2 work-up was used to separate non-included guest from host compounds.

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