Design, Synthesis, NMR-Solution and X-Ray Crystal Structure of *N*-Acyl-γ-dipeptide Amides That Form a βII′-Type Turn

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Conformational analysis of γ-amino acids with substituents in the 2-position reveals that an *N*-acyl-γ-dipeptide amide built of two enantiomeric residues of *unlike* configuration will form a 14-membered H-bonded ring, *i.e.*, a γ-peptidic turn (*Figs. 1–3*). The diastereoselective preparation of the required building blocks was achieved by alkylation of the doubly lithiated *N*-Boc-protected 4-aminoalkanoates, which, in turn, are readily available from the corresponding (*R*)- or (*S*)-α-amino acids (*Scheme 1*). Coupling two such γ-amino acid derivatives gave *N*-acetyl and *N*-[(*tert*-butoxy)carbonyl] (Boc) dipeptide methyl amides (**1** and **10**, resp.; *Fig. 2*, *Scheme 2*); both formed crystals suitable for X-ray analysis, which confirmed the turn structures in the solid state (*Fig. 4* and *Table 4*). NMR Analysis of the acetyl derivative **1** in CD_3OH, with full chemical-shift and coupling assignments, and, including a 300-ms ROESY measurement, revealed that the predicted turn structure is also present in solution (*Fig. 5* and *Tables 1–3*). The results described here are yet another piece of evidence for the fact that more stable secondary structures are formed with a decreasing number of residues, and with increasing degree of predictability, as we go from α- to β- to γ-peptides. Implications of the superimposable geometries of the actual turn segments (with amide bonds flanked by two *quasi*-equatorial substituents) in α-, β-, and γ-peptidic turns are discussed.

1. Introduction. – Oligomers consisting of γ-amino acids (γ-peptides) with appropriate substitution patterns form stable *2.6*$_{14}$ helices in solution [1–5] and in the solid state [4]. Most interestingly, this structure has been observed for γ-peptides consisting of as few as four residues. Particularly stable helices are formed by oligomers consisting of 2,4-disubstituted γ-amino acid residues with relative configurations *like*[2]) [2–4]. 2,4-Disubstituted γ-amino acid residues do also show strong conformational preferences in nonpeptidic molecules such as the natural products calyculin C [6] and bleomycin A_2 [7]. It has been shown that changes of configuration and of the substitution pattern of the γ-amino acid residues in bleomycin A_2 alter its DNA-cleaving activity [8]. This observation was explained by different conformational preferences of the incorporated γ-amino acid residues. *Hoffmann et al.* have synthesized a number of

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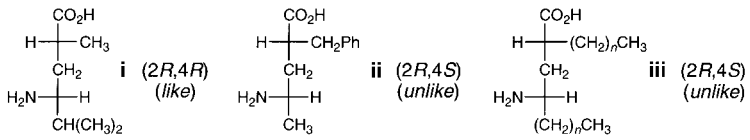
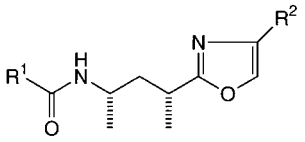
[1]) Part of the Ph.D. thesis of *M.B.*, ETH-Zürich, 2001.

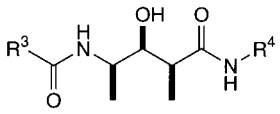
[2]) The CIP-configurational designations of homochiral γ-amino acid residues may reverse, depending on the priorities of the substituents (*cf.* **i**/**ii**). In the general discussion of this paper (*Figs. 1* and *3*) we assign *CIP* nonspecified substituents R as if they were nonbranched alkyl groups (**iii**).



2,4-disubstituted γ-amino acid derivatives and investigated their conformations in solution by NMR spectroscopy [9].



γ-Amino acid residue in calyculin C



γ-Amino acid residue in bleomycin A_2

The conformational preferences of 2,4-disubstituted γ-amino acids can – at least partially – be explained by the fact, that, out of the nine conformers obtained by rotation around the $C(sp^3)-C(sp^3)$ backbone bonds only two do not have unfavorable *syn*-pentane interactions[3]) (*Fig. 1*). This intrinsic preference of 2,4-disubstituted γ-amino acid residues for certain conformations ought to be useful for the design of new γ-peptides with defined secondary structures. The residue **A** shown in *Fig. 1* is known to stabilize *2.6*$_{14}$-helical structures of γ-peptides (*vide supra*), but it would also fit into a pleated-sheet structure. On the other hand, residue **B** should be suitable for the construction of a turn structure[4]) (*vide infra*).

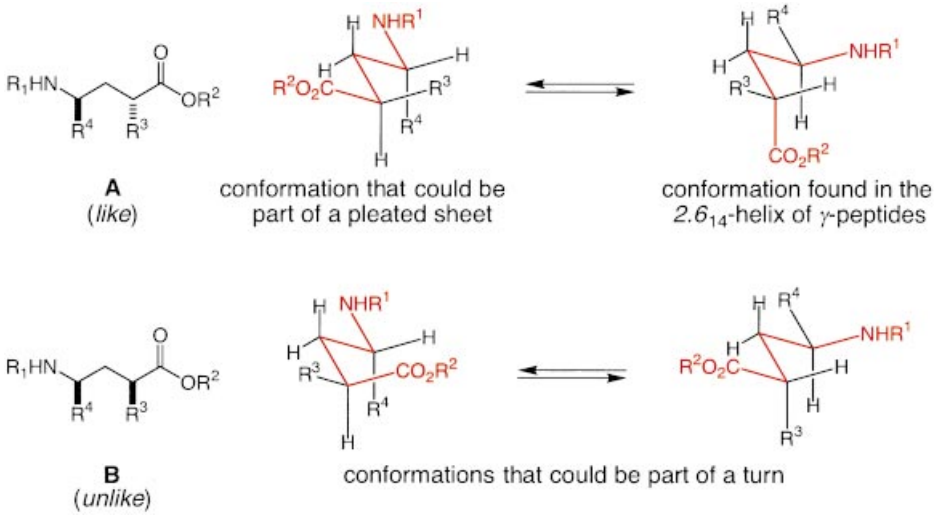


Fig. 1. *Conformations of 2,4-disubstituted γ-amino acid residues without unfavorable* syn*-pentane interactions.* Assumed *CIP* priorities[2]): $N > CH_2 > R^4$ and $CO > CH_2 > R^3$.

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[3]) The *syn*-pentane interaction in an open-chain structure is equivalent to the 1,3-diaxial interactions in a 1,3-disubstituted cyclohexane (for a review on the influence of *syn*-pentane interactions on the conformation of molecules, see [10]).

[4]) *Hanessian et al.* have described a reverse turn structure of a γ-tetrapeptide consisting of homochiral type-**B** residues [3].

Considering the conformations of residues of type **B**, we conclude that a dipeptide consisting of two such residues of opposite sense of chirality should fold to a stable turn structure. Thus, we decided to synthesize the γ-dipeptide derivative **1** and to determine its secondary structure (*Fig. 2*). There is a remarkable similarity between **1** and the 11-aminoundec-6-enoic acid derivative **C**, which has been described by *Hoffmann* and co-workers [11] to form a nonpeptidic, turn-like structure in CCl_4 and $CDCl_3$ solution.

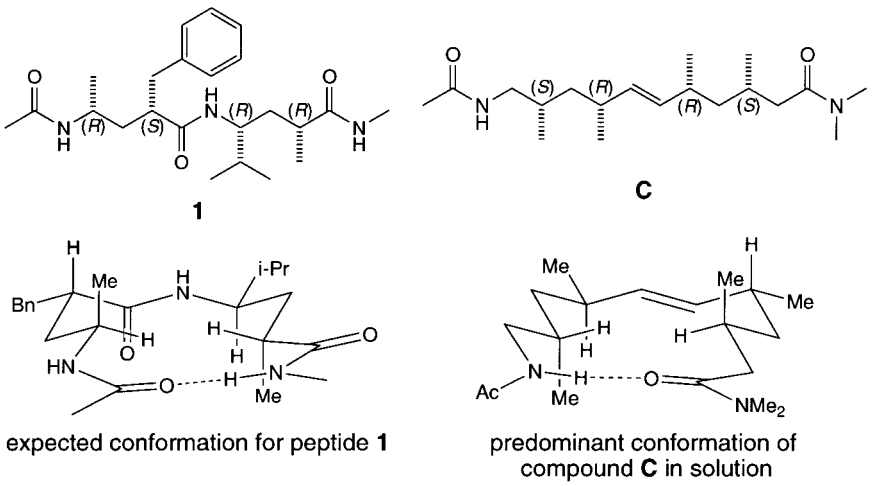

Fig. 2. *Presentation of* **1** *and its expected most stable conformation, and presentation of* **C** *and its proposed turn-like structure according to* Hoffmann *and co-workers* [11].

The turn structure predicted for compound **1** exhibits a strong resemblance to the type-II′ 'β-turn'[5]) of α-oligopeptides containing a D-amino acid residue[6]) in position 2 and to the turn motif of β-peptides [14–16] (*Fig. 3*). Each of these three structures have the same arrangement of the central (R)CH–NH–CO–CH(R) moiety (for another type of reverse-turn motif of β-peptides involving a cyclic β-amino acid residue, see [17]). Thus, β- and γ-peptides are candidates for pharmacological mimics of 'β-turn'-forming α-peptides [15]. Since the β- and γ-peptides are resistant to degradation by peptidases [18], these compounds should have better bioavailability than corresponding α-peptides [18c].

2. Preparation of γ-Dipeptide 1. – The required 2,4-disubstituted γ-amino acid derivatives were obtained as outlined in *Scheme 1*. The γ^4-amino acid ester **2** was prepared, as described previously, by double homologation of commercially available L-Boc-valine [1]. Alkylation of **2** with MeI furnished the 4-amino-2-methylalkanoate **3** in 87% yield. The remarkably high diastereoselectivities for alkylations of γ^4-amino

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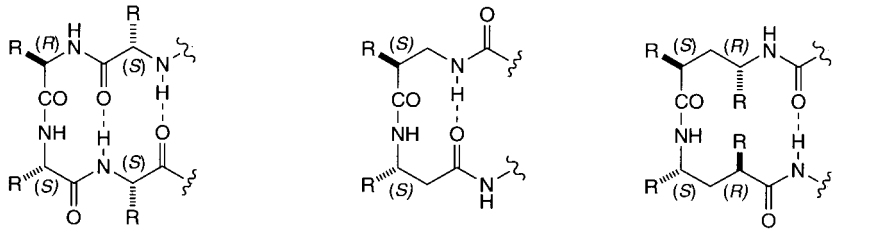
5) We write 'β-turn' in quotation marks to reduce the risk of confusion in papers on β- and γ-amino acids and -peptides. We also refer to normal, proteinogenic amino acids and peptides as α-amino acids and α-peptides, respectively (*cf.* the terms 'α-helix' and 'β-sheet').

6) The incorporation of a D-amino acid residue is strongly 'β-turn'-stabilizing [12], other amino acid residues often found in 'β-turns' are Pro and Gly (for work on 'β-hairpin'-forming α-tetrapeptides, see [13]).



β-turn of a peptide consisting of L-α-amino acids with a type II' β-turn-stabilizing D-amino acid residue in position 2

turn of a β-peptide with an (*S*)-β^2–(*S*)-β^3-residue motif

expected turn of a γ-peptide with a (2*S*,4*R*)-γ^{2,4}–(2*R*,4*S*)-γ^{2,4}-residue motif

Fig. 3. *Comparison of the 'β-turn' motif of an α-peptide with the turn structure found in β-peptides* [14–16] *and the expected turn structure of γ-peptide* **1**. For (*R*)/(*S*) assignments, see *Footnote 2*.

acid derivatives was previously reported by *Hanessian* and *Schaum* [19][7]). Finally, the methyl ester **3** was hydrolyzed, and the resulting acid was converted to the *N*-methyl amide **4** in 81% yield over the two steps.

In a similar manner as described above, the γ-amino acid derivative **5** was alkylated with BnBr. In this case, a 4:1 mixture of the (2*S*,4*R*)-methyl ester **6** and of the (3*R*,5*R*)-pyrrolidinone **7** was obtained[8]). Performing the deprotonation and the alkylation at lower temperature (−100° instead of −78°) did not lead to improvement of the product ratio. Since we did not succeed in separating the two products by column chromatography, the mixture was directly hydrolyzed by treatment with LiOH and crystallized upon salt formation with dicyclohexylamine. The salt **9** (dr 93:7) was obtained in 49% overall yield from the ester **5**.

The amide **4** was Boc-deprotected and coupled with the acid **8** (*Scheme 2*). The γ-dipeptide derivative **10** was isolated in 70% yield as a single diastereoisomer. Removal of the Boc group, followed by treatment with Ac_2O/DMAP, finally furnished target compound **1** in 68% yield.

3. Investigation of the γ-Dipeptides by X-Ray Crystal Structure Analysis and NMR-Spectroscopy. – The γ-dipeptide derivatives **1** and **10** turned out to be highly crystalline compounds. Thus, we were able to obtain crystals suitable for X-ray single-crystal structure analysis. Both compounds show the expected turn structure (*Fig. 4*)[9]). The structures are characterized by a 14-membered ring containing a H-bond between the NH group of the C-terminal amide functionality and the CO group of the N-terminal

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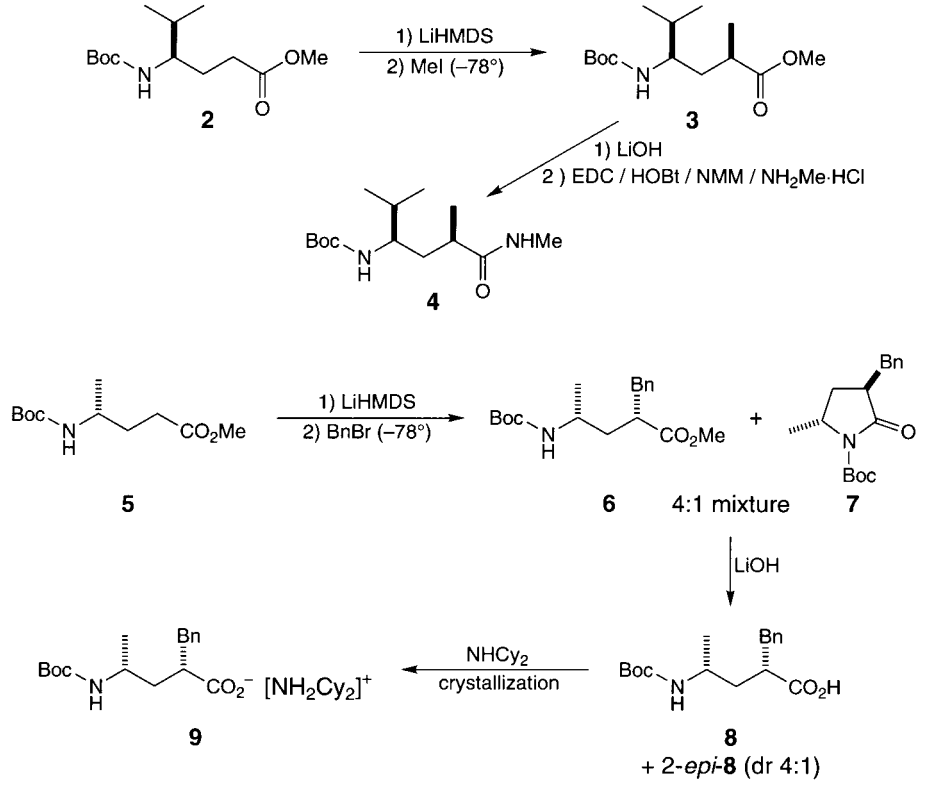
[7]) Highly diastereoselective alkylations of doubly lithiated 5-hydroxyalkanoates were described by *Narasaka et al.* [20a–c] (see also work of *Wittenberger et al.* [20d]).

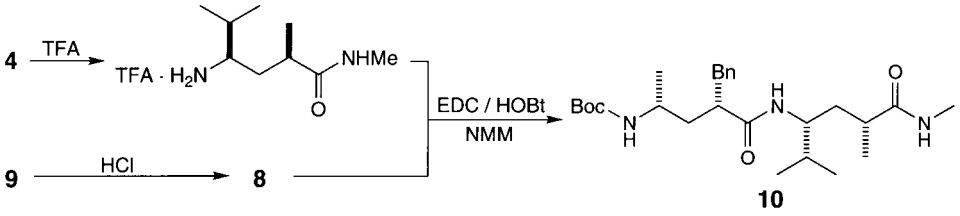
[8]) The *trans*-arrangement of the substituents in **7** was confirmed by NOE measurements.

[9]) Crystallographic data (excluding structure factors; see also *Table 4* in the *Exper. Part*) for the structures reported in this paper have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-164715 (**1**) and No. CCDC-164714 (**10**). Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

Scheme 1. *Preparation of γ-Amino Acid Derivatives* **4** *and* **9**



Scheme 2. *Preparation of γ-Dipeptide Derivative* **10**



Ac and Boc, respectively. The γ-Me group of residue 1 and the α-Me group of residue 2 are arranged in *quasi*-axial positions on the ring, while the Bn and the i-Pr groups are in *quasi*-equatorial positions. In the crystal lattice, the molecules form stacks with intermolecular H-bonds extending in two dimensions between the CO and NH groups not involved in intramolecular H-bonding.

To investigate the solution structure of peptide **1**, we determined the vicinal ^{1}H-NMR coupling constants. As solvent, we chose CD_3OH to break down any aggregates and because this medium is more similar to the natural solvent H_2O than other common solvents for NMR spectroscopy. This is important with regard to a

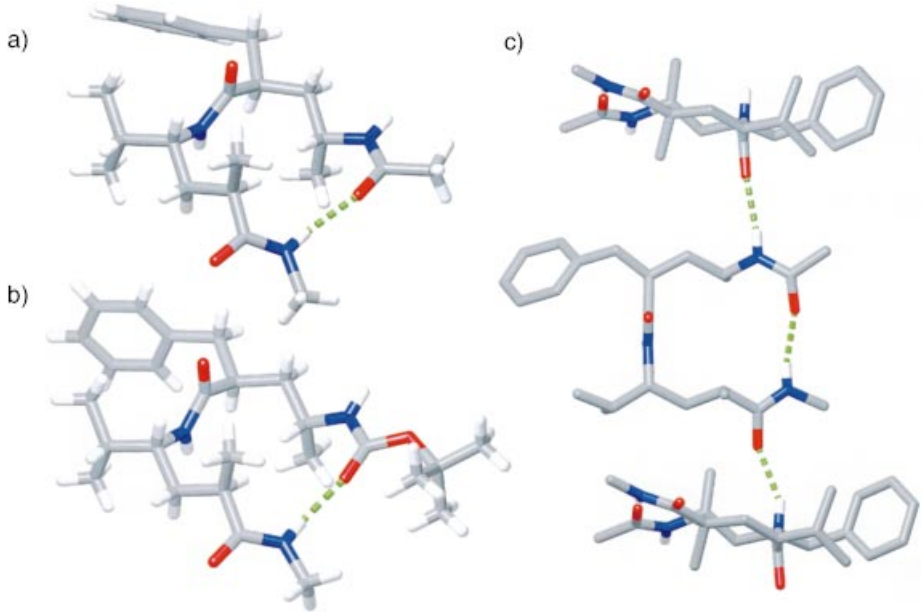


Fig. 4. *X-Ray crystal structures of the γ-dipeptides* **1** *and* **10**. C-Atoms in grey, N-atoms in blue, O-atoms in red, and H-atoms in white. The structures were determined by Dr. *B. Schweizer*. *a*) Molecular structure of **1**. The unit cell contains two independent molecules with similar structures (only one is shown). *b*) Molecular structure of **10**. *c*) Part of the crystal packing of **1**. The type of stacking of molecules, as shown here for one dimension, is found in two approximately perpendicular directions of the crystal lattice.

possible pharmacological application of this kind of turn-forming γ-peptides. The ^{1}H-NMR resonances were assigned by a DQF-COSY experiment. To obtain accurate values for the coupling constants, we used the program *gNMR* to fit a calculated spectrum to the experimental spectrum by varying the values for the chemical shifts, coupling constants, and peak widths. The important signals of the experimental spectrum together with the resonances calculated with the optimized values are shown in *Fig. 5*.

The values determined for the chemical shifts and coupling constants are listed in *Tables 1* and *2*. For the assignment of the $H^{Si}-C(\beta)$ and $H^{Re}-C(\beta)$ resonances, it was assumed that a conformation similar to the crystal structure is predominantly populated[10]). In residue 2, the coupling constants of 3.7 and 10.1 Hz for $C(\alpha)H/C(\beta)H_2$ and of 2.7 and 11.2 Hz for $C(\gamma)H/C(\beta)H_2$ indicate a strong preference of this residue for a conformation in agreement with the conformation found in the solid state. In residue 1, the vicinal coupling constants are closer to an averaged value, indicating that more than one conformation is populated[11]).

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[10]) This assumption is verified by NOE measurements (*vide infra*).

[11]) *Hoffmann et al.* have found for γ-amino acid derivatives of type **B** (*Fig. 1*) a preference for a *gauche* conformation of the amido substituent with respect to the main chain [9]. In the turn structure of **1**, such a conformation was observed in residue 2 but not in residue 1.

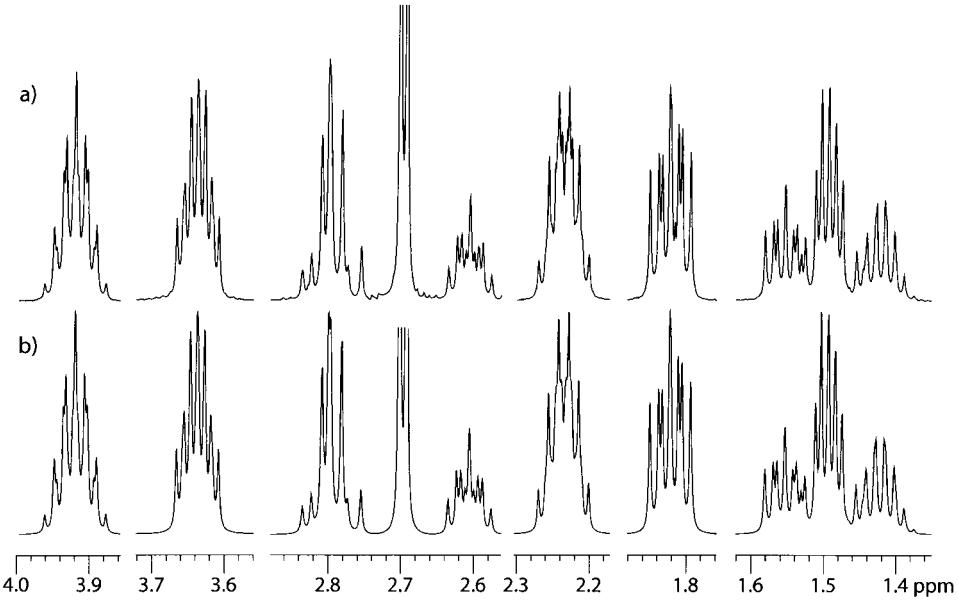
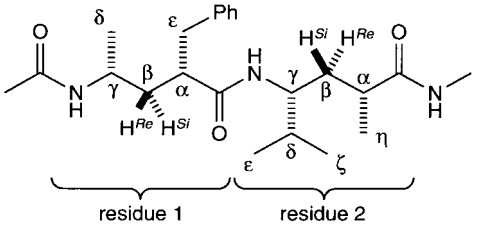


Fig. 5. *Parts of the 500-MHz ^{1}H-NMR Spectrum of* **1** *in CD_3OH*. The scales for the intensities of the shown signals/signal sets are different. *a*) Measured spectra. *b*) Calculated spectra with optimized values for chemical shifts and coupling constants. The program *gNMR* V3.6 (by *Peter H. M. Budzelaar*, published by *Cherwell Scientific Publishing*) was used for the optimization. The obtained values are given in *Tables 1* and *2*.

Table 1. *^{1}H-NMR Chemical Shifts of the Dipeptide* **1** *in CD_3OH*

Residue	NH	α	β^{Re}	β^{Si}	γ	δ	ε	ε^1	ε^2	ζ	η
1	7.92	2.61	1.82	1.55	3.92	1.15		2.81	2.78		
2	7.49	2.24	1.51	1.48	3.64	1.42	0.56			0.57	1.11



To obtain more information about the solution structure of peptide **1**, we measured a ROESY spectrum with a mixing time of 300 ms. The NOEs extracted from this spectrum are listed in *Table 3*. All of the observed NOEs are in agreement with the proposed turn structure. Particularly indicative for a high population of this conformation are the NOEs observed between NH of the terminal methylamide group and H–C(γ) of residue 1, as well as between H–C(γ) of residue 1 and H–C(α) of residue 2.

Table 2. *^{1}H-NMR Coupling Constants of the Dipeptide* **1** *in CD_3OH.* For specifications, see *formula* in *Table 1.*

Residue 1			Residue 2		
H-Atom(s)	H-Atom(s)	J [Hz]	H-Atom(s)	H-Atom(s)	J [Hz]
α	β^{Si}	5.7	α	β^{Si}	3.7
α	β^{Re}	8.6	α	β^{Re}	10.1
β^{Si}	γ	8.4	β^{Si}	γ	11.2
β^{Re}	γ	6.0	β^{Re}	γ	2.7
β^{Si}	β^{Re}	13.5	β^{Si}	β^{Re}	13.9
γ	NH	8.3	γ	NH	9.4
γ	δ	6.5	γ	δ	5.5
α	ε^1	5.7	δ	ε	6.9
α	ε^2	9.5	δ	ζ	6.8
ε^1	ε^2	13.3	α	η	6.9

Table 3. *NOEs Observed in the ROESY (*300 ms*) NMR Spectrum of Dipeptide* **1** *in CD_3OH.* The NOEs are classified according to their intensities in three categories: strong (s), medium (m), and weak (w). NOEs indicating a turn structure are highlighted. For specification of the H-atoms, see *formula* in *Table 1.*

Residue	H-Atom(s)	Residue	H-Atom(s)	NOE	Residue	H-Atom(s)	Residue	H-Atom(s)	NOE
1	NH	1	γ	m	1	γ	1	β^{Si}	m
1	NH		$CH_3C(O)$	s	1	γ	1	δ	s
1	NH	1	β^{Re}	m	1	γ	2	η	w
1	NH	1	β^{Si}	m	2	γ	2	α	m
1	NH	1	δ	m	2	γ	2	β	m
	NHMe	1	γ	m	2	γ	2	δ	m
	NHMe		NHMe	s	2	γ	2	η	s
	NHMe	2	α	m	2	γ	2	ε/ζ	s
	NHMe	2	β	w	1	ε	1	β^{Re}	m
	NHMe	1	δ	w	1	ε	1	β^{Si}	m
	NHMe	2	η	m	1	ε	1	δ	m
2	NH	1	γ	w		NHMe	2	β	w
2	NH	2	γ	m		NHMe	1	δ	w
2	NH	1	ε	m		NHMe	2	η	w
2	NH	1	α	s	1	α	1	β^{Re}	m
2	NH	2	α	m	1	α	1	β^{Si}	m
2	NH	2	β	m	1	α	1	δ	m
2	NH	2	δ	w	1	α	2	ε/ζ	w
2	NH	1	δ	w	2	α	2	β	m
2	NH	2	ε/ζ	m	2	α	1	δ	w
	Ph	1	ε	s	2	α	2	η	m
	Ph	1	α	m	2	α	2	ε/ζ	w
	Ph	1	δ	w	1	β^{Re}	1	β^{Si}	s
	Ph	2	ε/ζ	w	1	β^{Re}	1	δ	w
1	γ	1	ε	m	1	β^{Si}	1	δ	m
1	γ	1	α	m	2	β	2	η	m
1	γ	2	α	m	2	β	2	ε/ζ	s
1	γ		$CH_3C(O)$	m	2	δ	2	ε/ζ	s
1	γ	1	β^{Re}	m					

4. Conclusion. – We have shown that the tendency of 2,4-disubstituted γ-amino acid residues to populate certain conformations can be used for the design of a γ-dipeptide that forms a turn structure in the solid state and in solution. There is a remarkable structural similarity between this γ-dipeptide and the type II 'β-turn' motif of naturally occurring oligopeptides.

We thank Dr. *B. Schweizer* of our Crystallography Service Division for determining the X-ray crystal structures and Mrs. *B. Brandenberger* for recording NMR spectra. We gratefully acknowledge the *Novartis Stipendienfonds* for a scholarship granted to *M. B.* and *Novartis Pharma AG*, Basel, for ongoing financial support.

Experimental Part

1. *General.* Abbreviations: Boc_2O: di(*tert*-butyl) dicarbonate, DMAP: 4-(dimethylamino)pyridine, dr: diastereoisomer ratio (determined by ^{1}H-NMR). EDC: 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, FC: flash chromatography, HOBt: 1-hydroxy-1*H*-benzotriazole, h.v.: high vacuum (0.01–0.1 mbar), LiHMDS: lithium bis(trimethylsilyl)amide, NMM: 4-methylmorpholine. Solvents for chromatography and workup procedures were distilled from *Sikkon* (anh. $CaSO_4$; *Fluka*) and from KOH (Et_2O), resp. THF was freshly distilled over Na under Ar before use. All other solvents and reagents were used as received from *Fluka* or *J. T. Baker*. TLC: *Merck* silica gel *60 F$_{254}$* plates; detection with UV, $KMnO_4$ soln. (12 g of NaOH, 1.5 g of $KMnO_4$, 300 ml of H_2O), or anisaldehyde soln. (9.2 ml of anisaldehyde, 3.75 ml of AcOH, 12.5 ml of conc. H_2SO_4, 340 ml of EtOH). FC: *Fluka* silica gel *60* (40–63 μm). M.p.: *Büchi 510* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241* polarimeter (10 cm, 1-ml cell) at r.t. IR Spectra: *Perkin-Elmer 782* spectrophotometer. NMR Spectra: *Bruker AMX-II-500* (^{1}H: 500 MHz, ^{13}C: 125 MHz), *AMX-400* (^{1}H: 400 MHz, ^{13}C: 100 MHz); chemical shifts (δ) in ppm downfield from internal TMS ($\delta = 0.0$ ppm); *J* values in Hz. ESI-MS: *Finnigan-MAT TSQ-7000*; in *m/z* (% of basis peak). Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

2. *Alkylation of γ-Amino Acid Methyl Esters. General Procedure 1* (*GP 1*). Similar to a reported procedure [19], the appropriate *N*-Boc-γ^4-amino acid methyl ester was dissolved in THF (0.25M), and a LiHMDS soln. (1M in THF, 5 equiv.) was added at $-78°$. After stirring for 45 min at $-78°$, the electrophile (neat, 5 equiv.) was added. The resulting soln. was stirred for 1.5 h at $-78°$, and the reaction was quenched with 1M HCl. The mixture was diluted with Et_2O, and the two layers were separated. The org. layer was washed with 1M HCl (2×), sat. $NaHCO_3$ (2×) and NaCl (1×) solns., dried ($MgSO_4$), and concentrated under reduced pressure. FC afforded the pure product.

3. *Hydrolysis of γ-Amino Acid Methyl Esters and* N*-Boc-Pyrrolidin-2-ones. General Procedure 2* (*GP 2*). To a soln. of the appropriate γ-amino acid methyl ester or *N*-Boc-pyrrolidin-2-one in THF (0.25M), 1N LiOH (4 equiv.) was added. The resulting emulsion was stirred at r.t. for 12 h. The mixture was cooled to 0°, acidified with a 10% $KHSO_4$ soln. (to *ca.* pH 2), and extracted with AcOEt (2×). The org. layers were combined, washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was used for the next step without further purification.

4. *Boc Deprotection. General Procedure 3* (*GP 3*). The *N*-Boc-protected compound was dissolved in CH_2Cl_2 (0.3M), and the mixture was cooled to 0°. An equal volume of TFA was added, and the mixture was stirred for 1 h at 0°. Concentration under reduced pressure and drying (h.v.) yielded the crude TFA salt, which was used without further purification.

*Methyl (2*R*,4*R*)-4-{[(*tert*-Butoxy)carbonyl]amino}-3,5-dimethylhexanoate* (**3**). The γ-amino acid derivative **2** (1.56 g, 6.0 mmol) was treated with MeI (1.9 ml, 30 mmol) according to *GP 1*. FC (pentane/Et_2O 4:1 → 3:1) yielded **3** (1.43 g, 87%). Colorless oil. R_f 0.30 (pentane/Et_2O 3:1). $[\alpha]_D^{rt} = -19.1$ ($c = 1.12$, $CHCl_3$). IR ($CHCl_3$): 3440*m*, 2975*m*, 1708*s*, 1504*s*, 1462*m*, 1437*m*, 1392*m*, 1367*m*, 1090*w*, 988*w*, 861*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, major rotamer): 0.87 (*d*, $J = 6.9$, Me); 0.90 (*d*, $J = 6.8$, Me); 1.19 (*d*, $J = 7.1$, Me); 1.43 (*s*, *t*-Bu); 1.46–1.53 (*m*, 1 H, CH_2); 1.65–1.76 (*m*, 1 H of CH_2, Me_2CH); 2.45–2.53 (*m*, CHCO); 3.49–3.56 (*m*, CHN); 3.68 (*s*, MeO); 4.26 (*d*, $J = 10.0$, NH). ^{13}C-NMR (100 MHz, $CDCl_3$, major rotamer): 17.2, 17.8, 18.8, 28.4 (Me); 33.1 (CH); 35.9 (CH_2); 37.0 (CH); 51.7 (Me); 53.9 (CH); 78.9, 155.9, 177.8 (C). MS: 569.4 (8, $[2M + Na]^+$), 312.4 (10, $[M + K]^+$), 296.4 (100, $[M + Na]^+$), 274.4 (50, $[M + H]^+$). Anal. calc. for $C_{14}H_{27}NO_4$ (273.37): C 61.51, H 9.95, N 5.12; found: C 61.59, H 9.86, N 5.13.

*(2*R*,4*R*)-4-[[(*tert*-Butoxy)carbonyl]amino]-2,5,*N*-trimethylhexanamide* (**4**). Hydrolysis of **3** (1.36 g, 5.0 mmol) according to *GP 2* yielded the corresponding acid (1.28 g, 98%) as a colorless oil. A part of the acid obtained (727 mg, 2.80 mmol) was dissolved in CH_2Cl_2 (14 ml), and the soln. was cooled to 0°. To this soln., HOBt (88% purity, 516 mg, 3.36 mmol), $MeNH_2 \cdot HCl$ (567 mg, 8.40 mmol), NMM (1.23 ml, 11.3 mmol), and EDC (590 mg, 3.08 mmol) were added successively. The mixture was stirred at 0° for 2 h and allowed to warm to r.t. After stirring for 10 h at r.t., the mixture was diluted with AcOEt. The org. layer was separated, washed with 1N HCl (3×), sat. $NaHCO_3$ (3×) and NaCl (1×) solns., dried ($MgSO_4$), and evaporated. FC (Et_2O) yielded **4** (624 mg, 82%). White solid. M.p. 142–143°. R_f 0.39 (Et_2O). $[\alpha]_D^{r.t.} = -17.9$ ($c = 0.77$, $CHCl_3$). IR ($CHCl_3$): 3458*m*, 2971*m*, 1701*s*, 1664*s*, 1506*s*, 1455*w*, 1414*w*, 1392*w*, 1367*m*, 1094*w*, 861*w*. ^{1}H-NMR (400 MHz, $CDCl_3$, major rotamer): 0.79 (*d*, $J = 6.9$, Me); 0.81 (*d*, $J = 6.9$, Me); 1.08 (*d*, $J = 6.9$, Me); 1.35 (*s*, *t*-Bu); 1.35–1.40 (*m*, 1 H, CH_2); 1.60 (*sept. d*, $J = 6.8$, 5.1, Me_2CH); 1.72 (*ddd*, $J = 14.1$, 11.6, 7.9, 1 H, CH_2); 2.10–2.18 (*m*, CHCO); 2.72 (*d*, $J = 4.8$, MeN); 3.35–3.43 (*m*, CHN); 4.34 (*d*, $J = 9.7$, NH); 5.63 (br. *m*, MeN*H*). ^{13}C-NMR (100 MHz, $CDCl_3$, major rotamer): 17.8, 18.8, 26.5, 28.4 (Me); 33.2 (CH); 36.0 (CH_2); 39.6, 55.0 (CH); 78.8, 156.2, 177.7 (C). MS: 567.4 (48, $[2M + Na]^+$), 295.3 (100, $[M + Na]^+$), 273.3 (52, $[M + H]^+$). Anal. calc. for $C_{14}H_{28}N_2O_3$ (272.39): C 61.73, H 10.36, N 10.28; found: C 61.80, H 10.16, N 10.15.

*Dicyclohexylammonium (2*S*,4*R*)-2-Benzyl-4-[[(*tert*-butoxy)carbonyl]amino]pentanoate* (**9**). Compound **5** (1.85 g, 8.0 mmol) was treated with BnBr (4.8 ml, 40 mmol) according to *GP 1*. FC (pentane/Et_2O 4:1 → 2:1) yielded a mixture of ester **6** and pyrrolidinone **7** (2.23 g, ratio: 4:1 mol/mol) as colorless oil. Hydrolysis of the obtained mixture according to *GP 2* yielded a mixture of **8** and *epi*-**8** (2.22 g, dr 4:1) as colorless oil. The crude product was dissolved in $(i\text{-}Pr)_2O$ (30 ml) and treated with $NHCy_2$ (1.10 ml, 5.6 mmol). The soln. was cooled to 3° in a refrigerator. After several hours, the product started to crystallize. The mixture was cooled to −20° and held at this temp. for additional 12 h. After filtration and drying (h.v.), **9** (1.90 g, dr 93:7, 49% overall yield) was obtained. A sample for anal. purposes was further purified by recrystallization from $(i\text{-}Pr)_2O$ (dr 98:2).

Ester **6**: ^{1}H-NMR (300 MHz, $CDCl_3$): 1.10 (*d*, $J = 6.2$, Me); 1.44 (*s*, *t*-Bu); 1.50–1.68 (*m*, 1 H–C(3)); 1.72–1.85 (*m*, 1 H–C(3)); 2.64–2.95 (*m*, H–C(2), PhCH_2); 3.58 (*s*, MeO); 3.64–3.82 (br. *m*, CHN); 4.20–4.35 (br. *d*, NH); 7.12–7.30 (*m*, 5 arom. H).

Pyrrolidin-2-one **7**: ^{1}H-NMR (500 MHz, $CDCl_3$): 1.25 (*d*, $J = 6.4$, Me); 1.54 (*s*, *t*-Bu); 1.70 (*ddd*, $J = 12.6$, 8.2, 1.2, 1 H–C(4)); 1.84–1.91 (*m*, 1 H–C(4)); 2.61 (*dd*, $J = 13.9$, 9.8, 1 H, PhCH_2); 2.86–2.93 (*m*, CHCO); 3.32 (*dd*, $J = 13.9$, 4.1, 1 H, PhCH_2); 4.10–4.16 (*m*, CHN); 7.17–7.31 (*m*, 5 arom. H).

Salt **9**: colorless crystals. M.p. 124–125°. $[\alpha]_D^{r.t.} = +5.0$ ($c = 1.04$, MeOH). IR ($CHCl_3$): 2939*s*, 2859*m*, 1702*s*, 1503*m*, 1453*m*, 1392*m*, 1367*m*, 1052*w*. ^{1}H-NMR (400 MHz, CD_3OD): 1.09 (*d*, $J = 6.5$, Me); 1.10–1.52 (*m*, 20 H); 1.68–1.89 (*m*, 7 H); 2.02–2.08 (*m*, 4 H); 2.49–2.56 (*m*, CHCO); 2.69–2.74 (*m*, 1 H, PhCH_2); 2.85–2.91 (*m*, 1 H, PhCH_2); 3.10–3.16 (*m*, C*H*NH_2C*H*); 3.65–3.75 (*m*, C*H*NH); 7.08–7.12 (*m*, 1 arom. H); 7.17–7.24 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, CD_3OD): 21.9 (Me); 25.6, 26.2 (CH_2); 28.9 (Me); 30.7, 39.6, 40.8 (CH_2); 45.9, 54.4 (CH); 79.6 (C); 126.7, 129.0, 130.2 (CH); 142.5, 157.8, 183.6 (C). MS: 306.3 (100, $[\mathbf{8} - H]^-$). Anal. calc. for $C_{29}H_{48}N_2O_4$ (488.71): C 71.27, H 9.90, N 5.73; found: C 71.41, H 9.90, N 5.70.

γ-Dipeptide Derivative **10**. A soln. of the salt **9** (1.11 g, 2.27 mmol) in Et_2O (150 ml) was washed with 1N HCl (4×), dried ($MgSO_4$), and evaporated. The acid **8** obtained was dried (h.v.). In a second flask, **4** (620 mg, 2.28 mmol) was Boc-deprotected according to *GP 3*. The obtained TFA salt together with **8** was dissolved in CH_2Cl_2 (10 ml), and the soln. was cooled to 0°. To this soln., HOBt (88% purity, 421 mg, 2.74 mmol), NMM (1.26 ml, 11.4 mmol), and EDC (481 mg, 2.51 mmol) were added successively. The mixture was stirred at 0° for 2 h and allowed to warm to r.t. After stirring for 10 h at r.t., the mixture was diluted with AcOEt. The org. layer was washed with 1N HCl (3×), sat. $NaHCO_3$ (3×) and NaCl (1×) solns., dried ($MgSO_4$), and evaporated. FC ($CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 100:8) yielded **10** (735 mg, 70%). White solid. M.p. 229–230°. R_f 0.23 (CH_2Cl_2/MeOH 20:1). $[\alpha]_D^{r.t.} = +9.0$ ($c = 0.78$, $CHCl_3$). IR ($CHCl_3$): 3440*w*, 3008*m*, 2966*m*, 1710*s*, 1500*m*, 1454*w*, 1365*m*, 1094*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.56 (*d*, $J = 6.8$, Me); 0.63 (br. *d*, $J = 6.5$, Me); 1.13 (*d*, $J = 6.4$, Me); 1.17 (*d*, $J = 6.9$, Me); 1.44 (*s*, *t*-Bu); 1.44–1.62 (*m*, 4 H); 1.93–2.01 (*m*, 1 H); 2.12–2.21 (*m*, 1 H); 2.25–2.35 (*m*, 1 H); 2.69–2.73 (*m*, 1 H, PhCH_2); 2.75 (*d*, $J = 4.7$, MeN); 2.89 (*dd*, $J = 13.4$, 9.4, 1 H, PhCH_2); 3.51–3.62 (*m*, CHN); 3.70–3.80 (*m*, CHN); 4.71 (br. *d*, NH); 5.25 (*d*, $J = 9.3$, NH); 6.89 (br. *q*, MeN*H*); 7.15–7.53 (5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.9, 18.3, 18.6, 21.4, 26.2, 28.4 (Me); 32.3 (CH); 35.3 (CH_2); 38.1 (CH); 39.6, 39.7 (CH_2); 45.6, 48.0, 52.5 (CH); 79.5 (C); 126.4, 128.5, 129.0 (CH); 139.2, 155.7, 173.9, 177.9 (C). MS: 945.8 (1, $[2M + Na]^+$), 500.2 (12, $[M + K]^+$), 484.3 (100, $[M + Na]^+$), 462.4 (20, $[M + H]^+$). Anal. calc. for $C_{26}H_{43}N_3O_4$ (461.64): C 67.65, H 9.39, N 9.10; found: C 67.65, H 9.34, N 9.09.

γ-Dipeptide Derivative **1**. Compound **10** (462 mg, 1.00 mmol) was Boc-deprotected according to *GP 3*. The crude TFA salt obtained was dissolved in CH_2Cl_2 (10 ml) and cooled to 0°. To this soln., DMAP (611 mg, 5.00 mmol) and Ac_2O (0.28 ml, 3.0 mmol) were added. The mixture was stirred for 30 min at 0° and allowed to

warm to r.t. After stirring for 2.5 h at r.t., AcOEt was added. The org. layer was washed with 1N HCl (3×), sat. $NaHCO_3$ (3×) and NaCl (1×) solns., dried ($MgSO_4$), and evaporated. FC ($CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 20:1) yielded **1** (274 mg, 68%). White solid. M.p. 234–235°. R_f 0.33 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D^{r.t.} = -41.1$ ($c = 0.84$, $CHCl_3$). IR ($CHCl_3$): 3338*w*, 3007*m*, 2968*m*, 1654*s*, 1533*m*, 1454*w*, 1372*w*. ^{1}H-NMR (500 MHz, CD_3OH): 0.56 (*d*, $J = 6.9$, Me); 0.57 (*d*, $J = 6.8$, Me); 1.11 (*d*, $J = 6.9$, Me); 1.15 (*d*, $J = 6.5$, Me); 1.39–1.45 (*m*, Me_2CH); 1.47–1.51 (*m*, CH_2CN); 1.53–1.58 (*m*, 1 H, CH_2CN); 1.79–1.91 (*m*, 1 H, CH_2CN); 1.95 (*s*, MeCO); 2.20–2.27 (*m*, CHCO); 2.58–2.63 (*m*, CHCO); 2.70 (*d*, $J = 4.7$, MeN); 2.75–2.84 (*m*, $PhCH_2$); 3.61–3.67 (*m*, CHN); 3.89–3.95 (*m*, CHN); 7.12–7.24 (*m*, 5 arom. H); 7.49 (*d*, $J = 9.4$, NH); 7.78 (br. *q*, MeN*H*); 7.92 (*d*, $J = 8.1$, NH); ^{13}C-NMR (125 MHz, CD_3OH): 17.8, 18.6, 19.2, 20.9, 22.9, 26.6 (Me); 33.6 (CH); 36.4 (CH_2); 39.1 (CH); 40.0, 40.9 (CH_2); 44.8, 47.7, 53.2, 127.4, 129.4, 130.3 (CH); 140.8, 172.4, 176.9, 180.3 (C). MS: 442.3 (24, $[M+K]^+$), 426.4 (100, $[M+Na]^+$), 404.4 (16, $[M+H]^+$). Anal. calc. for $C_{23}H_{37}N_3O_3$ (403.56): C 68.45, H 9.24, N 10.41; found: C 68.55, H 9.29, N 10.55.

X-Ray Crystal-Structure Determination of **1** *and* **10** (see *Table 4* and *Fig. 4*). The reflections were measured on an *Enraf Nonius CAD-4* diffractometer with CuK_α radiation (graphite monochromator, $\lambda = 1.54184$ Å). Part of the structure of **1** was solved by direct methods with SIR97 [21], and the remaining non-H-atoms were found from a difference *Fourier* map. The structure of **10** was solved by direct methods with SIR97. The non-H-atoms were refined anisotropically with SHELXL-97 [22]. The H-atoms were calculated at idealized positions and included in the structure-factor calculation with fixed isotropic displacement parameters. Two symmetrically independent molecules were found in the unit cell of **1**.

Table 4. *Crystallographic Data for* **1** *and* **10**

	1	**10**
Empirical formula	$C_{46}H_{74}N_6O_6$	$C_{26}H_{43}N_3O_4$
Formula weight	807.11	461.64
Crystallized from	CH_2ClCH_2Cl/MeOH	CH_2ClCH_2Cl/MeOH
Crystal temp. [K]	293(2)	293(2)
Crystal dimensions [mm]	0.30 × 0.15 × 0.05	0.40 × 0.40 × 0.05
Crystal system	triclinic	orthorhombic
Lattice parameters		
2θ range [°]	$3.71 < 2\theta < 61.81$	$3.09 < 2\theta < 62.66$
a [Å]	9.001(4)	8.993(2)
b [Å]	11.260(5)	11.245(3)
c [Å]	12.269(4)	28.646(7)
α [°]	88.12(3)	90
β [°]	76.51(3)	90
γ [°]	89.71(4)	90
V [Å^3]	1208.5(9)	2896.9(12)
Space group	$P1$	$P2_12_12_1$
Z	1	4
D_x [g·cm^{-3}]	1.109	1.058
μ [mm^{-1}]	0.583	0.566
Total reflections measured	3732	2436
Independent reflections	3732	2436
Reflections observed	1811	1264
Criterion	$I > 3\sigma(I)$	$I > 3\sigma(I)$
Restraints	3	0
Variables	535	306
Final R	0.0506	0.0729
wR_2	0.1255	0.1640
Goodness-of-fit	1.047	1.342
$\Delta\rho$ (max, min) [e·Å^{-3}]	0.142, −0.178	0.222, −0.288

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