Stabilizing unusual conformations in small peptides and glucopeptides using a hydroxylated cyclobutane amino acid†

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The synthesis and the conformational study in the solid state and in aqueous solution of a peptide and a glucopeptide containing the non-natural (1*S*,2*S*)-1-amino-2-hydroxycyclobutanecarboxylic acid (c_4Ser) residue are reported. This is the first example of a glycopeptide containing a carbohydrate moiety linked to an underlying non-natural amino acid residue. The conformational analysis in solution combines NOEs and coupling constants data with Molecular Dynamics (MD) simulations with time-averaged restraints. The study reveals that the c_4Ser-Ala-Ala diamide peptide shows a conformation of two consecutive β-turn type III structures (the basic structural element of a 3_{10} helix). However, none of the turns observed in the peptide are present in the derived glucopeptide. The influence of the carbohydrate moiety on the peptide backbone can be explained by means of the existence of two simultaneous hydrogen bonds, between the endocyclic oxygen of the glucose and two amidic protons of the peptide. In addition, the non-natural residue favors the existence of an unusual high energy conformation for the glycosidic linkage, the so-called anti-ϕ conformation.

Introduction

Small natural peptides are in general conformationally flexible and are therefore not suitable to study or control secondary peptide structures. Taking into account that one attractive approach to restrict peptide conformation involves the incorporation of quaternary α-amino acids in the peptidic chain,[1] the synthesis of α,α-disubstituted α-amino acids has been the concern of many synthetic organic chemists.[2] Therefore, with the aim of increasing the possibilities in terms of producing modified peptides with new properties or functions, the synthesis and conformational study of peptides containing non-natural amino acids is still undergoing continual advancement and optimization. It is well-known, for instance, that the Cα tetrasubstitution in small homopeptides favours the stabilization of 3_{10} or α-helixes.[3] Additionally, in short peptides, α,α-disubstituted α-amino acids stabilize turn structures,[4] which have received particular attention because they play an important role in proteins from both structural and functional points of view.[5] On this basis, and in order to modify the active conformation of a peptide, several artificial turns have been reported in the past.[6]

To go one step further, and taking into account the important role that glycopeptides play in fundamental biological processes,[7] we are working on the design of novel glycopeptides that incorporate non-natural amino acids in their structures. Our preliminary results, carried out on the simplest non-natural glycosylated amino acids,[8] show that these molecules can stabilize conformations present in naturally occurring molecules or exhibit some atypical conformations. This feature could be a powerful tool to design and modulate the conformational space of the different synthesized glycopeptides.

With the intention of expanding our novel small systems, we herein report the synthesis and the conformational analysis in aqueous solution of peptide **1** and its corresponding β-*O*-glucopeptide **2** (Fig. 1). In addition, the structures found in solution are compared to that obtained in the solid state for peptide **1**. It is important to emphasize that, to the best of our knowledge, this is the first synthesized glycopeptide reported to date that incorporates a non-natural amino acid at the underlying residue.

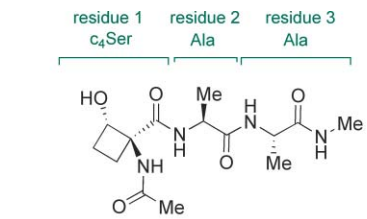

Fig. 1 Compounds studied in this work.

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† Electronic supplementary information (ESI) available: NMR spectra for all new compounds, full assignment of protons and $^nJ_{H,H}$ couplings for compound **2**, sections of the 800 ms 2D NOESY spectra (400 MHz) in H_2O/D_2O (9:1) for compound **2** and X-ray diffraction structure for compound **9**. CCDC reference number 716958. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b907091p

Results and discussion

Synthesis

The synthesis of peptide **1** was started from the commercially available *N*-Boc-Ala derivative **3**. This compound was treated with $MeNH_2$·HCl and *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU) as a coupling agent in the presence of diisopropylethylamine (DIEA) as a base to give the amide **4**. Removal of the Boc group and the subsequent treatment of the resulting amine **5** with an additional equivalent of compound **3** gave the dipeptide **6**. This compound was firstly treated with trifluoroacetic acid (TFA) and then with the racemic mixture of cyclobutane derivative **8**, which was previously synthesized by our group,[2] to give a mixture of protected peptides **9** and **10**. The purification of compound **9** by column chromatography on silica gel and hydrogenolysis of the benzyl group with hydrogen in the presence of Pd/C as a catalyst gave the desired peptide **1** (Scheme 1). Single crystals suitable for X-ray analysis were obtained for compound **1**, which allowed us to determine the relative stereochemistry. Knowing the absolute *S* configuration of two of the stereogenic centers allowed the configuration of stereogenic centers in the cyclobutane ring also to be deduced as *S*.

The corresponding model glycopeptide **2** was obtained following the modified conditions of the Koenigs–Knorr glycosylation.[9] The treatment of peptide **1** with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide in the presence of AgTfO gave derivative **11**. The hydroxyl groups of this compound were deprotected using MeONa/MeOH to give the glycopeptide **2** with a moderate overall yield (Scheme 2).

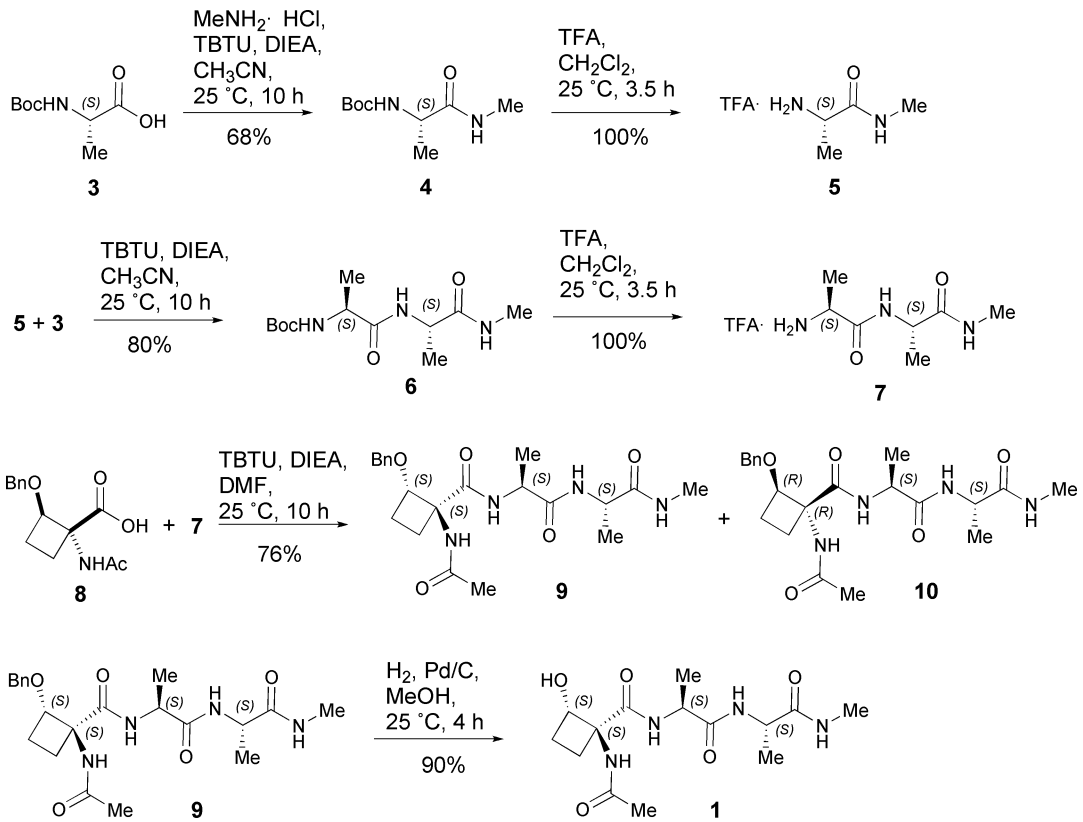
Solid state structure of peptide 1

The atom numeration and the labels of the different torsional angles of compounds **1** and **2** are shown in Fig. 2.

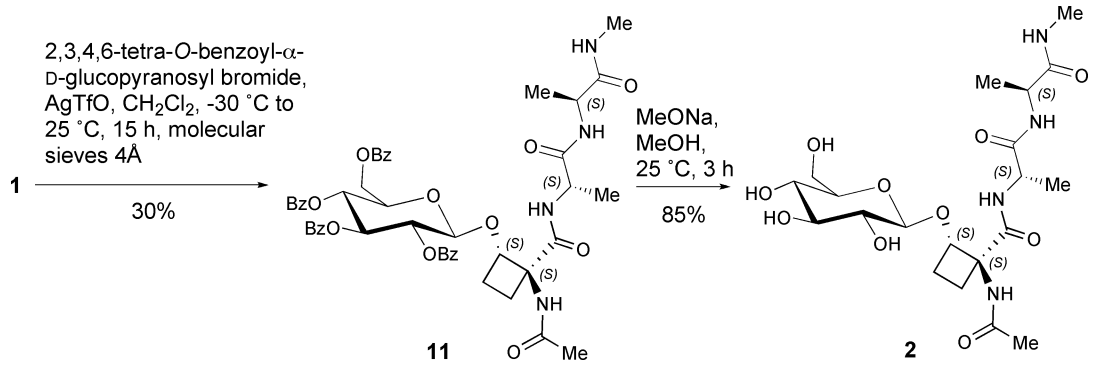
Some important conclusions can be drawn with respect to the solid state (Fig. 3 and Supporting Information†). Firstly, a conformation consisting of two consecutive β-turns type III, slightly deviating from an ideal 3_{10} helix structure, was obtained in the solid state for compound **1**. The torsion angles (ϕ,ψ) shown in Fig. 3 resemble the typical values for a β-turn type III (–60°, –30° and –60°, –30°). The structure is stabilized by two intramolecular hydrogen bonds (with N–H···O distances of 2.02 and 2.06 Å, respectively and N–H···O angles of 166° and 161°, respectively). A similar structure has been recently found for a tripeptide containing a tetrahydrofuran Cα-tetrasubstituted amino acid.[4] Secondly, the cyclobutane ring adopts only one conformation, with a pucker angle θ equal to –28.3°. In addition, the substituent at carbon Cβ occupies an equatorial position. This result is in accordance with the crystal structure of different cyclobutane derivatives previously studied in the solid state.[10]

Conformational preferences of peptide 1 in aqueous solution

The conformational preferences of the tripeptide **1** were examined with experimentally restrained MD simulations using a protocol[11]



Scheme 1 Synthesis of peptide diamide **1**.



Scheme 2 Synthesis of glucopeptide diamide **2**.

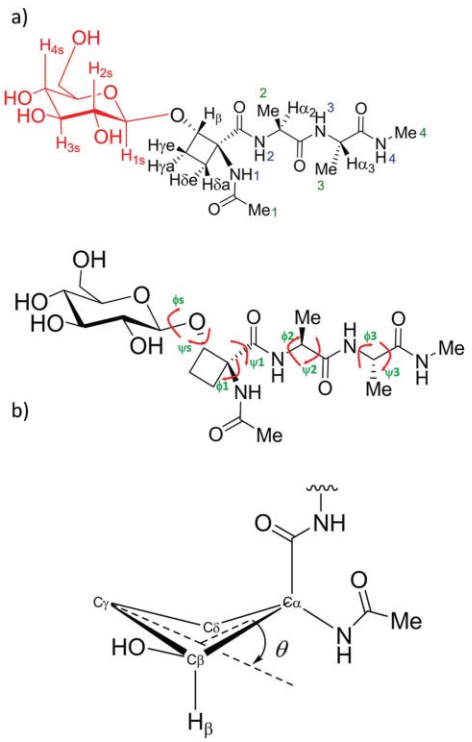


Fig. 2 Molecular structure of glucopeptide **2**, showing the atom numeration and the most relevant torsional angles. Letter 's' makes reference to the carbohydrate moiety. The blue and green numbers make reference to the numeration of the NH and methyl groups, respectively. The same numbering and labels were used for peptide **1** (a). The pucker angle θ of the cyclobutane ring is defined as the acute angle between the planes Cα-Cβ-Cδ and Cβ-Cγ-Cδ (b).

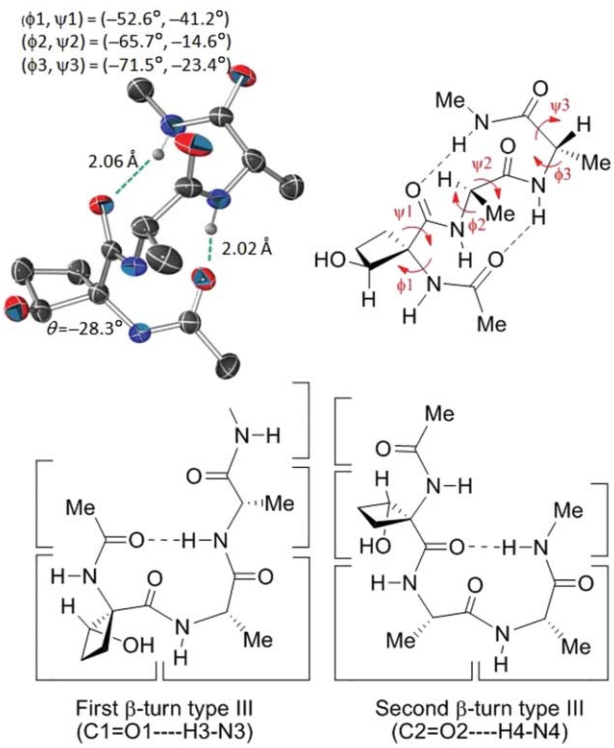


Fig. 3 Some geometrical features of the crystal structure of peptide **1**. Heteroatoms are shown in color; blue for the nitrogen atom and red for oxygen atom. Thermal ellipsoids drawn at the 50% probability level.

similar to that recently applied by our group on different model glycopeptides.

NMR data. In a first step, full assignment of the protons of compounds **1** and **2** was carried out using COSY and HSQC experiments and it is given in Table 1 and in the Supporting Information (Table S1†). Selective 1D-NOESY experiments in D_2O (25 °C, pH = 5.2) and 2D-NOESY experiments in H_2O/D_2O (9/1) (25 °C, pH = 4.84) were then carried out (see Experimental Section).

The medium-strong Hα2–NH3, Hα3–NH4 (typical of extended conformations), Hα2–NH2, and Hα3–NH3, as well as NH2–NH3 and NH3–NH4 NOEs (characteristic of folded ones), shown in Fig. 4, suggests the coexistence of both conformations in aqueous solution for the two alanine residues of peptide **1**. On the other hand, the medium NOE observed between NH1 and NH2 suggests an important population of a helix-like conformation for the cyclobutane residue.[12]

The next step was to experimentally determine some proton-proton distances relevant to the conformational analysis. The distances involving NH protons were semi-quantitatively determined by integrating the volume of the corresponding cross-peaks in the 2D NOESY spectrum. Additionally, some proton-proton distances were obtained from the corresponding NOE build-up curves[13] (see Supporting Information†). On the other hand, $^3J_{NH,H\alpha}$ coupling constants were measured from the splitting of the resonance signals in the 1D spectrum. All these experimentally

Table 1 Full assignment of protons and $^{n}J_{H,H}$ couplings for compound **1**[a]

δ (in ppm)	proton	splitting[b] (# protons)	$^{n}J_{H,H}$ (in Hz)
1.10–1.27	Me2,Me3	m (6H)	—
1.39	Hδa	m (1H)	—
1.63–1.78	Hγa	m (1H)	—
1.83	Me1	s (3H)	—
2.11	Hγe	m (1H)	—
2.47	Hδe	m (1H)	—
2.56	Me4	s (3H)	—
4.05	Hα3	q (1H)	$^{3}J_{H\alpha3,Me3} = 7.1$
4.18	Hα2	q (1H)	$^{3}J_{H\alpha2,Me2} = 7.1$
4.26	Hβ	't' (1H)	$^{3}J_{H\beta,H\gamma a} = {}^{3}J_{H\beta,H\gamma e} = 7.1$
7.49–7.57[c]	NH4[c]	m (1H)[c]	—
7.67[c]	NH3[c]	d (1H)[c]	$^{3}J_{NH3,H\alpha3} = 6.2$[c]
8.08[c]	NH2[c]	d (1H)[c]	$^{3}J_{NH2,H\alpha2} = 5.8$[c]
8.69[c]	NH1[c]	s (1H)[c]	—

[a] Data extracted from 1D ^{1}H NMR experiment carried out in D_2O (25 °C, pH = 5.2) at 400 MHz. [b] s = singlet, d = doublet, q = quartet, m = multiplet. [c] Data extracted from 1D ^{1}H NMR experiment carried out in H_2O/D_2O (9/1) (25 °C, pH = 4.84) at 400 MHz.

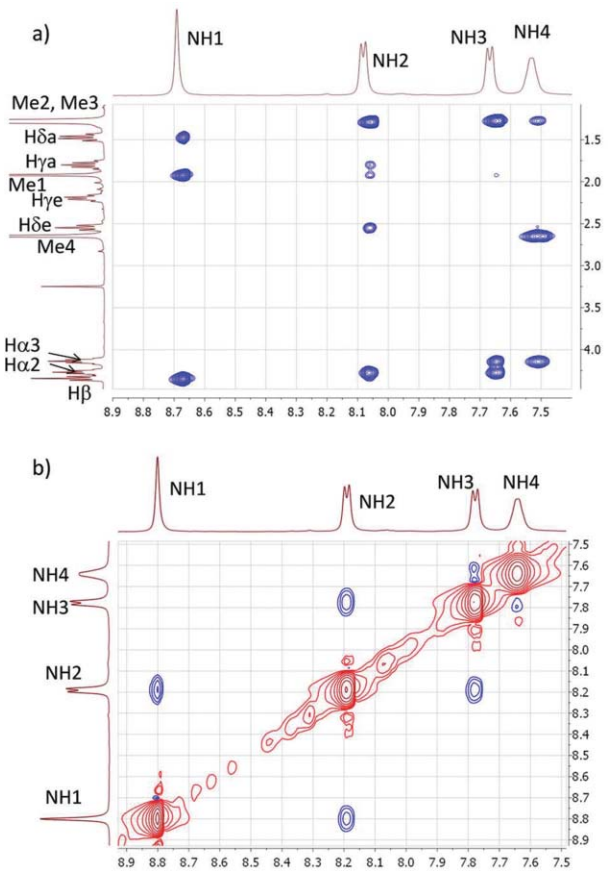


Fig. 4 Sections of the 800 ms 2D NOESY spectra (400 MHz) in H_2O/D_2O (9:1) at 25 °C and pH = 4.84 of compound **1**.

determined distances, as well as the measured $^{3}J_{NH,H\alpha}$, are shown in Table 2. All these data were used as restraints in MD-tar (MD with time-averaged restraints) simulations[14] with the aim of obtaining a distribution of conformers in aqueous solution able to quantitatively reproduce the NMR data.

Table 2 Comparison of the experimental and MD-tar simulations derived distances, θ angle and ^{3}J coupling constants for compounds **1** and **2**[a]

	Compound **1**		Compound **2**	
	Exptl	MD-tar	Exptl	MD-tar
$d_{NH4,H\alpha3}$	2.4	2.4	2.4	2.3
$d_{NH3,H\alpha3}$	2.5	2.8	2.5	2.8
$d_{NH3\text{-}H\alpha2}$	2.4	2.6	2.6	2.3
$d_{NH2,H\alpha2}$	2.4	2.7	2.6	2.8
$d_{NH1,H\delta a}$	2.7	2.7	3.1	3.0
$d_{H\beta,NH1}$	2.2	2.1	2.3	2.5
$d_{NH4,NH3}$	3.0	2.8	3.0	2.9
$d_{NH3,NH2}$	2.5	2.3	2.8	2.6
$d_{NH2,NH1}$	2.6	2.8	2.7	2.6
$d_{H\beta,H\delta a}$	2.7	2.9	2.7	2.8
$d_{H\beta,H\gamma e}$	2.4	2.3	2.4	2.5
$d_{H\beta,H1s}$	—	—	2.4	2.4
$d_{H\beta,H2s}$	—	—	2.9	2.8
$<\theta>$	—	−18.8	—	−23.4
$^{3}J_{NH2,H\alpha2}$	5.8	6.0	5.6	6.0
$^{3}J_{NH3,H\alpha3}$	6.2	6.4	6.5	6.7

[a] Distances are given in Å, ^{3}J in Hz, and θ in degrees.

The ϕ_i/ψ_i (i = 1, 2 or 3) distribution obtained from the MD-tar simulations for peptide **1** is shown in Fig. 5a. As can be seen, the cyclobutane residue presents ϕ_1/ψ_1 typical values for helix-like conformations, such as the right and left handed α-helix (α_D, 85%; α_L, 13%), with a clear preference for the α_D. It is important to mention that these two major conformers lie at the local minima previously found for a model peptide previously studied by our group.[8] Concerning residue Ala2, it exhibited dispersed values of ϕ_2/ψ_2, being the most populated helix-like conformation (about 53% of the total trajectory time). On the contrary, the extended conformations (β, 23% and PPII, 50%) were the most populated ones for Ala3. From these data, it can be stated that the peptide **1** is rather flexible in aqueous solution. Indeed, only the β-turn exhibited in the crystalline structure between the carbonyl of the *N*-acetyl group and the NH3 was observed in aqueous solution. According to our MD-tar simulations, this β-turn is present about 30% of the total trajectory time.

The second β-turn (between C2=O2 and NH4) was only present about 7% of the total time. Fig. 6a shows a superimposition of 10 conformers of peptide **1**, randomly collected from the MD-tar simulations, together with the solid state conformation. Interestingly, the new peptide **1** presents a completely different conformational behavior when it is compared with, *i.e.* the natural derived tripeptide Z-Thr-Ala-Ala-CO_2Me.[15] The studies carried out on the naturally derived molecule state that this molecule takes mainly extended conformations in DMSO.

Finally, as in the solid state, the cyclobutane ring adopts mainly (ca. 95% of the total trajectory time) one conformation in aqueous solution, with $<\theta> = -18.8°$. This result is in good agreement with the short distance (2.7 Å) shown between the atoms Hβ and Hδa.

Conformational preferences of glycopeptide 2 in aqueous solution

The protocol above described for peptide **1** was also used to quantitatively determine the conformers present in aqueous solution for glucopeptide **2** (see Supporting Information and

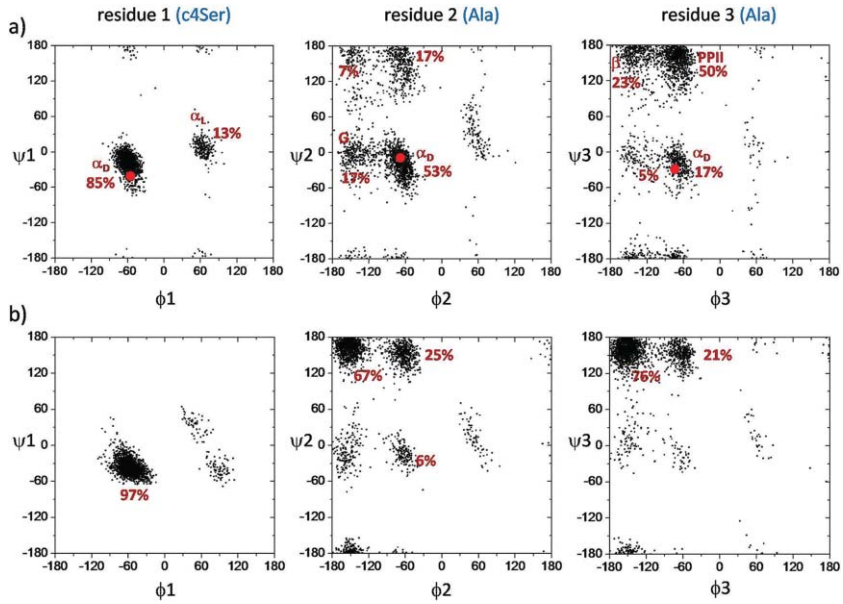


Fig. 5 ϕ_i/ψ_i distributions obtained from the MD-tar simulations for compounds **1** (a) and **2** (b). The red dots correspond to the values of ϕ and ψ dihedrals observed in the solid state.

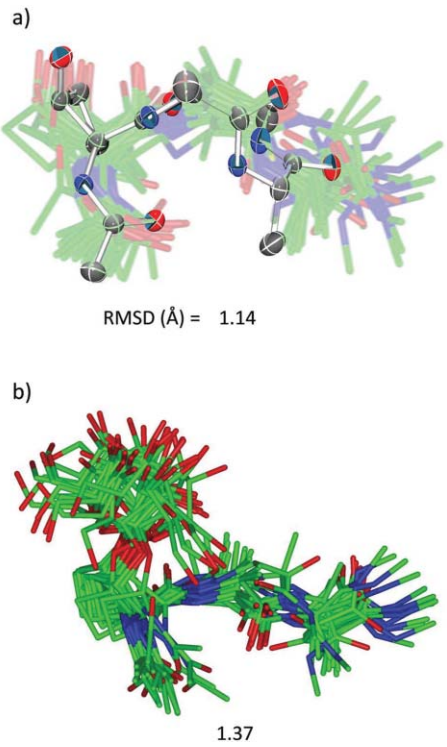


Fig. 6 Calculated ensembles obtained from the MD-tar simulations for compounds **1** (a) and **2** (b), showing the RMSDs for heavy atoms superimposition. In the case of compound **1**, the crystal structure is also shown.

Table 2). As can be seen in Fig. 5b, the cyclobutane residue adopts almost exclusively the helix-like conformation (97% of the total trajectory time). This result is similar to that observed for its parent peptide. The major difference shown between compounds **1** and **2** was concerning Ala of residue **2**. While in the former, this residue showed a clear preference for a helix-like conformation, in the glucopeptide the extended conformers were the most populated ones. As a consequence, the β-turns observed in the crystal structure of compound **1** were present less than a 4% of the total trajectory time in water solution for the glycopeptide **2**. As can be seen in Fig. 6b, the glucopeptide adopts an extended conformation when compared with its parent peptide (Fig. 6a). Therefore, it can be concluded that glycosylation of the peptide **1** results in a distinct conformational change of the glycopeptide. This conformational influence of glycosylation on the underlying peptide has been frequently observed in natural glycopeptides.[16]

Concerning the glycosidic linkage, the values of the ϕ_s dihedral angle are mainly determined by the exo-anomeric effect.[17] Consequently, the glycosidic linkage adopts mostly the *syn* conformation in aqueous solution [ϕ_s(O5s-C1s-O1s-Cβ)= −60° for β-*O*-linkages], which corresponds to the minimum energy. In the case of glucopeptide **2**, we observe not only a strong-medium NOE between Hβ-H1s, which is representative of a *syn* conformation of ϕ_s dihedral, but also a medium one between Hβ-H2s (Fig. 7a). These NOEs can never be explained without assuming the existence of the *anti*-ϕ population (the Hβ-H2s distance for a *syn* conformation is longer than 4.5 Å).[8] The MD-tar simulations suggest a population of *ca.* 20% for this high energy conformation (Fig. 7). This feature is important since although the existence of this higher energy conformation has been experimentally confirmed for some oligosaccharides,[18] to the best of our knowledge this is the first time that this exceptionally large percentage of *anti*-ϕ conformation is experimentally (NOE) observed in a glycopeptide.

In order to gain insights into the structural changes induced by the β-*O*-glycosylation on both the peptide backbone of **2** and the ϕ_s dihedral angle, we decided to study the possible hydrogen bonds in the glycopeptide. As a consequence, the relatively large proportion of the *anti*-ϕ conformers in aqueous solution could be stabilized by the formation of intramolecular hydrogen bonds (Fig. 8). In fact,

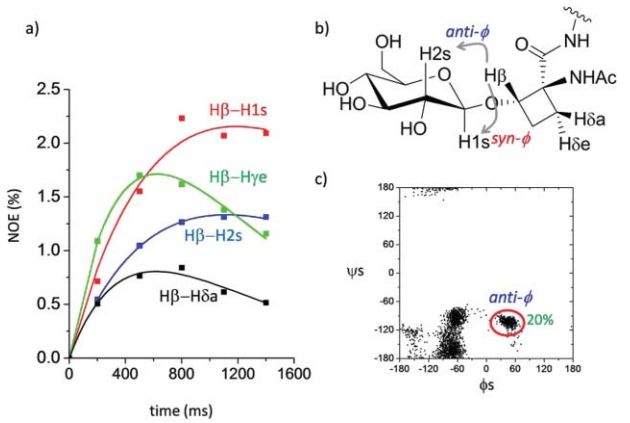


Fig. 7 NOE build-up curve corresponding to Hβ of compound **2** (a). Schematic representation of the characteristic NOEs of *syn*- and *anti*-ϕ conformations in **2** (b). (c) ϕ_s/ψ_s distribution obtained from the MD-tar simulations for glucopeptide **2e** (c).

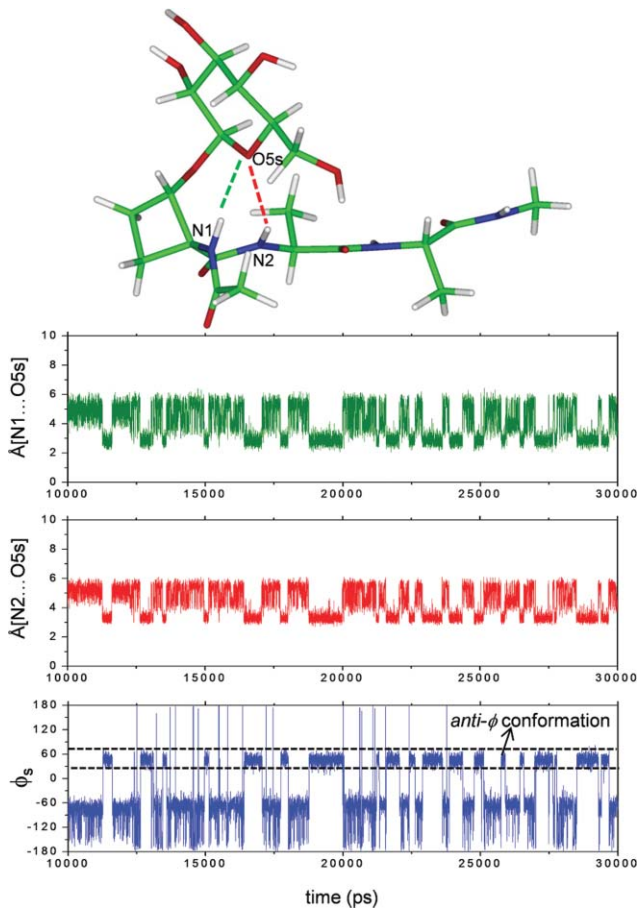


Fig. 8 Time series monitoring the ϕs dihedral angle and distances N1 ··· O5s and N2 ··· O5s in the MD-tar simulation for compound **2**.

there is a strong correlation between the *anti*-ϕ conformation and the existence of two simultaneous hydrogen bonds, one between the N1 and O5s and another one between N2 and O5s.

These hydrogen bonds are also responsible for the absence, in glycopeptide **2**, of the first β-turn in the peptide backbone observed in peptide **1**, which explains the effect of the carbohydrate moiety on the peptide conformation. Therefore, and taking into account that glycosylation of peptides, especially those containing β-*O*-glycosidic linkages, results generally in induction of turn conformations,[16] the example shown in the present work is an interesting contrast that emphasizes the use of non-natural amino acids as a useful tool to obtain new compounds with novel conformational behaviour.

Conclusions

Herein we report the first example of a glycopeptide containing a non-natural amino acid residue. Our study reveals that the c_4Ser-Ala-Ala diamide peptide shows a conformation of two consecutive β-turn type III structures in the solid state, the basic structural element of a 3_{10} helix. Remarkably, only the first β-turn is partially present in solution. However, the β-*O*-glucosylation notably modifies the conformation of the peptide backbone. Indeed, none of the turns observed in the peptide remain in the glucopeptide derivative. In this case, although the c_4Ser residue retains the helix-like conformations, the two alanine residues prefer extended conformations. This result differs from previous studies with glycopeptides containing natural amino acids. The influence of the carbohydrate moiety on the peptide backbone can be explained by means of the existence of two simultaneous hydrogen bonds between endocyclic oxygen of the glucose and the amidic protons H-N1 and H-N2 of the peptide. Moreover, the non-natural residue favors the existence of high energy conformations for the glycosidic linkage, such as the anti-ϕ conformation.

Experimental

General synthetic procedures

Melting points are uncorrected. All manipulations with air-sensitive reagents were carried out under a dry argon atmosphere using standard Schlenk techniques. Solvents were purified according to standard procedures. The chemical reagents were purchased from Aldrich Chemical Co. Analytical TLC was performed using Polychrom SI F_{254} plates. Column chromatography was performed using Kieselgel 60 (230–400 mesh). Organic solutions were dried over anhydrous Na_2SO_4 and, when necessary, concentrated under reduced pressure using a rotary evaporator. NMR spectra were recorded at 300 or 400 MHz (^{1}H) and at 75 or 100 MHz (^{13}C) and signals are reported in ppm downfield from TMS. Microanalyses were carried out on a CE Instruments EA-1110 analyser and are in good agreement with the calculated values.

Synthesis of compound 4. A solution of **3** (1.00 g, 5.29 mmol) in acetonitrile (40 mL) was treated with DIEA (4.4 mL, 26.4 mmol), methylamine hydrochloride (714 mg, 10.60 mmol) and TBTU (2.21 g, 6.88 mmol). The reaction mixture was stirred at 25 °C for 10 h, the solvent was then removed and the mixture was dissolved in ethyl acetate (40 mL). The organic layer was washed with aqueous 1M HCl (20 mL) and aqueous 5% $NaHCO_3$ (20 mL), dried over Na_2SO_4, filtered, and evaporated to give a residue that was purified by silica gel column chromatography, eluting with dichloromethane/MeOH (15:1) to give **4** (730 mg), as a white solid, in 68% yield. Mp 106–108 °C. $[\alpha]_D^{25}$: –26.1 (*c* 1.18, $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 1.36 (d, 3H, $J = 7.0$ Hz), 1.44 (s, 9H), 2.81 (d, 3H, $J = 4.7$ Hz), 4.08–4.26 (m, 1H), 5.20 (d, 1H, $J = 7.2$ Hz), 6.36–6.57 (m, 1H). δ_C (100 MHz, $CDCl_3$) 18.6, 26.2, 28.3, 50.0,

80.0, 155.5, 173.4. Anal. calcd for $C_9H_{18}N_2O_3$: C, 53.45; H, 8.97; N, 13.85. Found: C, 53.61; H, 8.99; N, 13.80%.

Synthesis of compound 6. TFA (5 mL) was added to a solution of **4** (226 mg, 1.12 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The reaction was maintained at 0 °C for 30 min, at 25 °C for 3 h, and then concentrated to give derivative **5** (1.09 mmol). This compound was then dissolved at 25 °C in CH_3CN (15 mL) and treated with **3** (247 mg, 1.30 mmol), DIEA (0.9 mL, 5.44 mmol) and TBTU (454 mg, 1.41 mmol), following the same protocol described for the synthesis of derivative **4**, to give compound **6** (238 mg), as a white solid, in 80% yield. Mp 140–142 °C. $[\alpha]_D^{25}$: −39.5 (*c* 1.07, CH_3OH). δ_H (400 MHz, $CDCl_3$): δ 1.37 (d, 3H, $J = 4.3$ Hz), 1.40 (d, 3H, $J =$ 4.3 Hz), 1.46 (s, 9H), 2.80 (d, 3H, $J = 4.8$ Hz), 4.02–4.18 (m, 1H), 4.38–4.54 (m, 1H), 4.89–4.99 (m, 1H), 6.48 (br s, 1H), 6.58 (d, 1H, $J = 7.3$ Hz). δ_C (100 MHz, $CDCl_3$): δ 18.0, 26.3, 28.3, 48.8, 50.8, 80.7, 155.8, 172.5. Anal. calcd for $C_{12}H_{23}N_3O_4$: C, 52.73; H, 8.48; N, 15.37. Found: C, 52.75; H, 8.39; N, 15.40%.

Synthesis of compound 9. TFA (1 mL) was added to a solution of **6** (52 mg, 0.19 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The reaction was maintained at 0 °C for 30 min, at 25 °C for 3 h, and then concentrated to give derivative **7** (0.18 mmol). This compound was then dissolved at 25 °C in DMF (3 mL) and treated with racemic cyclobutane derivative **8** (48 mg, 0.18 mmol), DIEA (0.2 mL, 0.90 mmol) and TBTU (75 mg, 0.23 mmol), following a similar protocol to described for the synthesis of derivative **4**, to give a mixture of compounds **9** and **10**. The mixture was purified by silica gel column chromatography, eluting with dichloromethane/MeOH (15:1) to give pure compounds **9** (30 mg, 40%) and **10** (27 mg, 36%) as white solids. *Compound **9**:* Mp 173–175 °C. $[\alpha]_D^{25}$: +21.2 (*c* 1.22, CH_3OH). δ_H (400 MHz, $CDCl_3$): δ 1.27 (d, 3H, $J = 7.4$ Hz), 1.43 (d, 3H, $J = 7.3$ Hz), 1.69 ('q', 1H, $J = 11.0$ Hz), 1.94 (s, 3H), 1.97–2.13 (m, 1H), 2.14–2.32 (m, 1H), 2.49 ('t', 1H, $J = 10.6$ Hz), 2.74 (d, 3H, $J = 4.6$ Hz), 4.13–4.30 (m, 2H), 4.39 ('t', 1H, $J = 7.7$ Hz), 4.52 (d, 1H, $J = 11.0$ Hz), 4.63 (d, 1H, $J = 11.0$ Hz), 7.03–7.15 (m, 1H), 7.31 (br s, 5H), 7.71 (d, 1H, $J = 8.2$ Hz), 8.01 (s, 1H), 8.56 (d, 1H, $J = 5.2$ Hz). δ_C (100 MHz, $CDCl_3$): δ 16.8, 17.2, 22.5, 24.5, 24.7, 26.2, 49.7, 51.0, 63.7, 71.6, 79.6, 127.8, 128.1, 128.4, 137.0, 171.4, 173.0, 173.2, 173.8. Anal. calcd for $C_{21}H_{30}N_4O_5$: C, 60.27; H, 7.23; N, 13.39. Found: C, 59.98; H, 7.21; N, 13.37%. *Compound **10**:* δ_H (400 MHz, $CDCl_3$): δ 1.06 (d, 3H, $J = 7.3$ Hz), 1.44 (d, 3H, $J = 7.3$ Hz), 1.45–1.51 (m, 1H), 1.72–1.82 (m, 1H), 2.05 (s, 3H), 2.17–2.25 (m, 1H), 2.70–2.75 (m, 1H), 2.76 (d, 3H, $J = 4.6$ Hz), 4.08–4.23 (m, 2H), 4.29–4.39 (m, 1H), 4.67 (d, 1H, $J = 11.9$ Hz), 4.74 (d, 1H, $J = 11.9$ Hz), 7.02–7.10 (m, 1H), 7.22 (d, 1H, $J = 8.2$ Hz), 7.33 (br s, 5H), 8.31 (d, 1H, $J = 4.8$ Hz). δ_C (100 MHz, $CDCl_3$): δ 17.0, 17.1, 22.3, 23.2, 25.8, 26.3, 48.9, 51.4, 66.1, 73.0, 80.1, 128.2, 128.3, 128.6, 137.0, 171.3, 171.4, 172.3, 173.1. Anal. calcd for $C_{21}H_{30}N_4O_5$: C, 60.27; H, 7.23; N, 13.39. Found: C, 60.08; H, 7.29; N, 13.35%.

Synthesis of compound 1. A solution of compound **9** (50 mg, 0.12 mmol) in MeOH (3 mL) was hydrogenolyzed, using 10 mg of 10% Pd/C as a catalyst, at 25 °C for 4 h. The catalyst and solvent were removed and further purification of the residue with C_{18} reverse-phase sep-pak cartridge gave **1** (35 mg), as a white solid in 90% yield. Mp 129–131 °C. $[\alpha]_D^{25}$: +17.4 (*c* 1.03, H_2O). δ_H (400 MHz, D_2O): δ 1.10–1.27 (m, 6H), 1.31–1.47 (m, 1H), 1.63–1.78 (m, 1H), 1.83 (s, 3H), 2.05–2.18 (m, 1H), 2.41–2.52 (m, 1H), 2.56 (s, 3H), 4.05 (q, $J = 7.1$ Hz, 1H), 4.18 (q, $J = 7.1$ Hz, 1H), 4.26 ('t', $J = 7.1$ Hz, 1H). δ_H (400 MHz, H_2O/D_2O): δ 7.49–7.57 (m, 1H, NH4), 7.67 (d, $J = 6.2$ Hz, 1H, NH3), 8.08 (d, $J = 5.8$ Hz, 1H, NH2), 8.69 (s, 1H, NH1). δ_C (100 MHz, D_2O): 16.1, 16.4, 21.8, 23.7, 25.6, 25.9, 49.8, 50.2, 65.8, 70.8, 172.9, 174.5, 175.1, 175.3. Anal. calcd for $C_{14}H_{24}N_4O_5$: C, 51.21; H, 7.37; N, 17.06. Found: C, 51.01; H, 7.36; N, 17.02%.

Synthesis of compound 11. Silver triflate (60 mg, 0.25 mmol) was added to a suspension of **1** (50 mg, 0.15 mmol) and powdered molecular sieves (4 Å, 20 mg) in CH_2Cl_2 (4 mL), under an inert atmosphere. The mixture was stirred at −30 °C and 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (570 mg, 0.86 mmol) in CH_2Cl_2 (4 mL) was then added. The mixture was stirred at this temperature for 1 h and was then warmed to 25 °C and stirred for additional 14 h. The crude was filtered, concentrated, and purified by silica gel column chromatography, eluting with CH_2Cl_2/MeOH (95:5), to give **11** (39 mg, 30% yield) as a colourless oil. $[\alpha]_D^{25}$: +11.4 (*c* 0.98, CH_3OH). δ_H (400 MHz, $CDCl_3$): δ 1.22 (d, $J = 7.3$ Hz, 3H), 1.35 (d, $J = 7.4$ Hz, 3H), 1.51–1.62 (m, 1H), 1.83 (s, 3H), 2.10–2.19 (m, 1H), 2.22–2.33 (m, 1H), 2.42–2.54 (m, 1H), 2.70 (d, $J = 4.6$ Hz, 3H), 4.17–4.44 (m, 4H), 4.56 (dd, $J_1 = 12.3$ Hz, $J_2 = 5.3$, 1H), 4.70 (dd, $J_1 = 12.4$ Hz, $J_2 = 2.6$, 1H), 5.07 (d, $J =$ 7.8 Hz, 1H), 5.50–5.58 (m, 1H), 5.68 (t, $J = 9.7$ Hz, 1H), 5.93 (t, $J = 9.7$ Hz, 1H), 6.73 (s, 1H), 6.81 (d, $J = 4.7$ Hz, 1H), 7.17–7.82 (m, 14H), 7.88–8.07 (m, 8H). δ_C (100 MHz, $CDCl_3$): 16.5, 16.9, 22.6, 25.2, 26.0, 26.3, 49.2, 51.1, 62.9, 64.4, 69.3, 71.3, 72.4, 73.0, 77.9, 100.1, 128.4, 128.5, 128.5, 128.6, 128.7, 129.4, 129.6, 129.7, 129.8, 129.9, 133.4, 133.6, 133.7, 133.8, 165.2, 165.4, 165.7, 166.3, 171.1, 171.9, 172.3, 173.4. Anal. calcd for $C_{48}H_{50}N_4O_{14}$: C, 63.57; H, 5.56; N, 6.18. Found: C, 63.70; H, 5.65; N, 6.10%.

Synthesis of compound 2. A solution of **11** (30 mg, 0.04 mmol) in MeOH (5 mL) was treated with MeONa/MeOH (0.5M) to pH = 9. After stirring for 3 h at 25 °C, the mixture was neutralized with Dowex 50-X8, filtered, and concentrated. Purification of the residue with C18 reverse-phase sep-pak cartridge gave 15 mg of **2**, as a colourless oil in 85% yield. $[\alpha]_D^{25}$: −12.5 (*c* 1.05, H_2O). δ_H (400 MHz, D_2O): δ 1.28–1.35 (m, 6H), 1.57 (dd, $J_1 = 20.8$ Hz, $J_2 = 11.2$ Hz, 1H), 1.95 (s, 3H), 1.95–2.06 (m, 1H), 2.25 (dd, $J_1 =$ 19.0, Hz, $J_2 = 9.8$ Hz, 1H), 2.54–2.63 (m, 1H), 2.67 (s, 3H), 3.20–3.32 (m, 2H), 3.35–3.44 (m, 2H), 3.59–3.67 (m, 1H), 3.84–3.90 (m, 1H), 4.17 (q, $J = 7.3$ Hz, 1H), 4.24 (q, $J = 7.2$ Hz, 1H), 4.53 (d, $J = 7.9$ Hz, 1H), 4.61 (t, $J = 8.8$ Hz, 1H). ^{1}H NMR (400 MHz, H_2O/D_2O): δ 7.52–7.59 (m, 1H, NH4), 7.72 (d, $J = 6.7$ Hz, 1H, NH3), 8.37 (d, $J = 5.6$ Hz, 1H, NH2), 8.87 (s, 1H, NH1). δ_C (100 MHz, D_2O): δ 15.8, 16.4, 21.8, 24.3, 24.6, 25.9, 49.8, 50.6, 60.9, 65.1, 69.7, 72.3, 75.0, 75.7, 76.3, 100.5, 172.8, 174.2, 175.3, 175.3. Anal. calcd for $C_{20}H_{34}N_4O_{10}$: C, 48.97; H, 6.99; N, 11.42. Found: C, 48.88; H, 6.93; N, 11.38%.

2D NMR experiments

NMR experiments were recorded on a Bruker Avance 400 spectrometer at 298 K. Magnitude-mode ge-2D COSY spectra were recorded with gradients and using the cosygpqf pulse program with 90 degree pulse width. Phase-sensitive ge-2D HSQC spectra were recorded using z-filter and selection before t1 removing the decoupling during acquisition by use of invigpndph pulse program

with CNST2 (JHC) = 145. 2D NOESY experiments were made using phase-sensitive ge-2D NOESY for $CDCl_3$ spectra and phase-sensitive ge-2D NOESY with WATERGATE for H_2O/D_2O (9:1) spectra. Selective ge-1D NOESY experiments were carried out using the 1D-DPFGE NOE pulse sequence. NOEs intensities were normalized with respect to the diagonal peak at zero mixing time. Experimental NOEs were fitted to a double exponential function, $f(t) = p_0(e^{-p1t})(1 - e^{-p2t})$ with p_0, p_1 and p_2 being adjustable parameters.[13] The initial slope was determined from the first derivative at time t = 0, $f'(0) = p_0p_2$. From the initial slopes, interproton distances were obtained by employing the isolated spin pair approximation.

MD simulations: MD-tar simulations

MD-tar simulations were performed with AMBER[19] 6.0 and AMBER94 force field,[20] which was implemented with GLYCAM 04[21] and the General Amber Force Field (GAFF)[22] parameters to accurately simulate the conformational behavior of the carbohydrate moiety and the cyclobutane ring, respectively. NOE-derived distances were included as time-averaged distance constraints, and scalar coupling constants *J* as time-averaged coupling constraints. A $<r^{-6}>^{-1/6}$ average was used for the distances and a linear average was used for the coupling constants. Final trajectories were run using an exponential decay constant of 8000 ps and a simulation length of 80 ns with a dielectric constant $\varepsilon = 80$.

X-Ray crystallography

The diffraction data for the crystalline compounds were collected at −173 °C on a Nonius kappa diffractometer with a CCD detector using Mo Kα radiation ($\lambda = 0.71073$ Å). The structures were solved by direct methods and refined by a full-matrix least-squares procedure with the SHELXL suite of programs.[23] Hydrogen atoms were located from mixed methods (electron-density maps and theoretical positions). Further details on the crystal structures of the compound **1** are available on the CIF data of the Supporting Information.‡

Notes and references

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‡ Crystal data for compound **1**: $C_{14}H_{28}N_4O_7$, $M_w = 364.40$, colorless prism of 0.32 × 0.25 × 0.20 mm, $T = 293(2)$ K, orthorhombic, space group $P2_12_12_1$, $Z = 4$, $a = 7.8140(5)$ Å, $b = 14.5374(8)$ Å, $c = 16.0994(10)$ Å, $V = 1828.72(19)$ Å^3, $d_{calc} = 1.309$ g cm^{-3}, $F(000) = 768$, $\lambda = 0.71073$ Å (Mo, Kα), $\mu = 0.105$ mm^{-1}, Nonius kappa CCD diffractometer, θ range 3.63–28.25°, 3068 collected reflections, 4471 unique ($R_{int} = 0.000$), full-matrix least-squares (SHELXL97),[23] $R_1 = 0.0652$, $wR_2 = 0.1490$ ($R_1 = 0.1125$, $wR_2 = 0.1761$ all data), goodness of fit = 1.019, residual electron density between 0.238 and –0.336 *e* Å^{-3}. Hydrogen atoms were located from mixed methods (electron-density maps and theoretical positions). CCDC 716958.

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