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Structure of Thyroxine: Role of Thyroxine Hydroxyl in Protein Binding

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Abstract

The crystal and molecular structures of thyroxine (3,5,3′,5′-tetraiodo-L-thyronine, T_4) have been accurately determined from an X-ray diffraction study on crystals of a T_4–*N*-diethanolamine (NDEA) salt $[C_{15}H_{10}I_4NO_4]^-.[C_4H_{12}NO_2]^+$. There are two independent thyroxine conformations in the crystal: *cisoid* and *transoid*. This is the first observation of both conformers in the same crystal. The geometry of the thyroxine 4′-oxygen is consistent with phenoxide-ion formation: short C(4′)—O(4′1) bond length, contraction of the C(3′)–C(4′)–C(5′) angle and expansion of the C(3′) and C(5′) angles. The amino-acid function is a zwitterion. The conformations of the amino-acid side chain with respect to the inner phenyl ring differ significantly between the two T_4 molecules; one is nearly perpendicular, normal for aromatic amino acids, while the other is nearly coplanar, an unusual observation among thyroid-hormone structures. The crystal structure shows a directional specificity of the hydrogen bonds and illustrates specific hydrogen-bonding patterns between the thyroid hormones, thyroxine and triiodothyronine. This structure determination reveals geometrical and conformational differences consistent with the degree of iodination and ionization state of the 4′-OH which may explain protein-binding differences among thyroactive compounds. The crystals are triclinic, space group $P\bar{1}$, $Z = 2$, with $a = 7{\cdot}842\ (1)$, $b = 14{\cdot}108\ (2)$, $c = 12{\cdot}194\ (2)$ Å, $\alpha = 95{\cdot}67\ (1)$, $\beta = 108{\cdot}47\ (1)$, $\gamma = 77{\cdot}45\ (1)°$, $V = 1248{\cdot}4$ Å^3, $M_r = 881{\cdot}97$, $D_c = 2{\cdot}35$ Mg m^{-3}, λ(Mo $K\alpha$) = 0·7107 Å, $\mu = 5{\cdot}08$ mm^{-1}. Final $R = 0{\cdot}039$ for 4573 observed reflections.

Introduction

Thyroid-hormone–protein binding is central to the transport, distribution in tissue, and metabolic rates of the various thyroid hormones, analogues and precursors. Specifically, the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) bind to three classes of proteins: plasma proteins which transport the hormones through the general circulation, membrane-bound proteins which interact with the hormones at the cell surface, and nuclear proteins through which hormonal activity is expressed (Jorgensen, 1978). Since thyroid-hormone binding is postulated to involve hydrogen-bond formation through the 4′-OH, the nature of these intermolecular interactions is of importance. Theoretical energy calculations of the hydrogen-bond strengths of *ortho*-substituted phenols and phenoxides were computed (Dietrich, Jorgensen, Kollman & Rothenberg, 1976; Andrea, Dietrich, Murray, Kollman, Jorgensen & Rothenberg, 1979) in order to predict likely orientations of the thyroid hormones at their binding sites. These studies predict an average O···O distance of 2·63 Å and a C—O···O angle of 125°, irrespective of whether the 4′-OH is acting as a hydrogen-bond donor or acceptor.

The acidity of the 4′-OH increases with *ortho* iodine substitution and has values of 6·73 and 8·45 for T_4 and T_3 respectively. At physiological pH the phenolic hydroxyl of T_4 is about 80% ionized whereas in T_3 it is only about 10% ionized (Korcek & Tabachnick, 1976). In previous crystal-structure determinations of T_3, the 4′-oxygen has been consistently observed as a hydroxyl group (Cody, 1979*a*). However, in previous studies of thyroxine (Camerman & Camerman, 1974*a*), the

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ionization state of the 4′-oxygen has been ambiguous because of poor data. The structure determination of a well-formed crystal of the thyroxine–*N*-diethanolamine salt, reported here, permits an accurate comparison of the geometries of T_3 and T_4 and an examination of their hydrogen-bonding patterns. The structural results are contrasted with other physical data and models of hormone–protein-binding interactions.

Experimental

Single crystals of the 1:1 salt of thyroxine (Sigma Chemical Co.) and *N*-diethanolamine (Sigma Chemical Co.) were grown from methanol at room temperature. The cell parameters were determined from the least-squares refinement of 71 reflections with a θ range of 29·9 to 33·8° using Mo $K\alpha$ radiation. The intensities of 5668 independent reflections were measured at 291 K on an automated diffractometer with a θ–2θ sweep of $1{\cdot}0° + 0{\cdot}1° \tan(\theta)$. Zr-filtered Mo $K\alpha$ radiation was used during data collection. The crystal, of dimensions 0·4 × 0·3 × 0·12 mm, was air-stable and showed no significant changes in the intensities of the standard reflections over the period of data collection. Intensities were corrected for Lorentz and polarization factors but not for extinction or absorption. On the basis of a $2\sigma(I)$ test, 4573 data were considered observed. The variance of each F was calculated according to the method of Stout & Jensen (1968).

The structure was solved by heavy-atom procedures and refined by full-matrix least squares using anisotropic thermal parameters for the non-H atoms. The H positions were located in difference electron density Fourier maps and included in the structure-factor calculations, but were not refined. The final residual $R = \sum||F_o| - |F_c||/\sum|F_o|$ was 0·039 for the 4573 observed reflections.

The least-squares programs are part of the Enraf–Nonius crystallographic package for the PDP 11/45 computer. All scattering factors were taken from *International Tables for X-ray Crystallography* (1974). The final fractional coordinates and equivalent B_{iso} values for the T_4.NDEA salt are listed in Table 1.*

* Lists of structure factors, anisotropic thermal parameters and H-atom coordinates have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36073 (32 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 1. *Fractional coordinates* (×10⁴) *and equivalent* B_{iso} (×10²) *for the thyroxine–N-diethanolamine salt*

$B_{iso} = \frac{4}{3}\sum_i\sum_j B_{ij}(\mathbf{a}_i.\mathbf{a}_j)$ taken from Hamilton (1959). The starred atoms refer to molecule T_4-2.

	x	y	z	B_{iso} (Å²)		x	y	z	B_{iso} (Å²)
C(1)	8741 (10)	8903 (5)	2762 (6)	208 (16)	C(1′*)	8269 (11)	7299 (6)	8568 (6)	205 (16)
C(2)	7025 (12)	8694 (6)	2108 (7)	255 (19)	C(2′*)	8358 (11)	7235 (6)	7464 (6)	219 (18)
C(3)	6943 (11)	7744 (6)	1715 (7)	255 (18)	C(3′*)	8937 (11)	7964 (6)	7075 (6)	219 (16)
C(4)	8440 (11)	6998 (5)	1962 (6)	215 (16)	C(4′*)	9400 (10)	8805 (6)	7736 (7)	208 (16)
C(5)	10164 (11)	7223 (6)	2618 (7)	234 (17)	C(5′*)	9298 (11)	8802 (6)	8899 (7)	222 (18)
C(6)	10263 (11)	8172 (7)	2971 (7)	250 (19)	C(6′*)	8748 (12)	8089 (6)	9301 (7)	249 (19)
O(41)	8385 (8)	6064 (4)	1491 (5)	272 (13)	O(4′1*)	9817 (8)	9531 (4)	7336 (5)	273 (14)
C(1′)	7662 (10)	5435 (6)	1955 (6)	198 (16)	C(7*)	7788 (12)	2589 (6)	8083 (7)	250 (18)
C(2′)	7764 (11)	5500 (6)	3132 (7)	247 (18)	C(8*)	8016 (10)	2159 (6)	6926 (7)	211 (17)
C(3′)	7137 (10)	4783 (6)	3536 (6)	209 (17)	N(8*)	7329 (9)	1215 (5)	6663 (6)	250 (16)
C(4′)	6498 (11)	4004 (6)	2855 (7)	230 (18)	C(9*)	9973 (11)	1938 (6)	6841 (8)	254 (18)
C(5′)	6433 (10)	4001 (6)	1678 (7)	200 (16)	O(9*)	10246 (8)	1478 (5)	5979 (5)	319 (15)
C(6′)	7051 (10)	4701 (6)	1246 (7)	214 (17)	O(10*)	11188 (10)	2233 (7)	7665 (8)	514 (23)
O(4′1)	5996 (8)	3306 (4)	3253 (5)	268 (13)	I(3*)	11793 (1)	5471 (1)	9890 (1)	402 (2)
C(7)	9034 (12)	9885 (7)	3349 (8)	279 (21)	I(3′*)	9071 (1)	7836 (1)	5367 (1)	327 (1)
C(8)	7606 (11)	10789 (6)	2915 (7)	236 (18)	I(5*)	3648 (1)	6297 (1)	7282 (1)	391 (2)
N(8)	8428 (9)	11635 (5)	3498 (6)	236 (15)	I(5′*)	10000	10000	10000	384 (2)
C(9)	5771 (11)	10864 (6)	3201 (8)	246 (18)	O(13)	4570 (11)	1205 (6)	8069 (8)	453 (22)
O(9)	5771 (9)	11121 (6)	4206 (6)	384 (18)	C(23)	4399 (18)	438 (11)	8704 (12)	500 (35)
O(10)	4429 (10)	10690 (7)	2391 (8)	434 (20)	C(33)	4441 (16)	−493 (10)	7962 (1	450 (31)
I(3)	4368 (1)	7468 (1)	684 (1)	390 (2)	N(43)	2911 (11)	−384 (6)	6868 (8)	363 (20)
I(5)	12545 (1)	6164 (1)	3187 (1)	407 (2)	C(53)	3295 (18)	−1052 (9)	5917 (11)	437 (31)
I(3′)	7293 (1)	4853 (1)	5290 (1)	308 (1)	C(63)	4392 (16)	−667 (9)	5330 (12)	442 (30)
I(5′)	5553 (1)	2849 (1)	559 (1)	327 (1)	O(73)	3373 (11)	278 (7)	4927 (7)	473 (20)
C(1*)	7796 (11)	3673 (6)	8239 (7)	238 (17)	O(14)	1170 (8)	1683 (6)	2392 (6)	382 (17)
C(2*)	9376 (11)	4021 (6)	8784 (7)	267 (19)	C(24)	1248 (14)	2515 (8)	1848 (9)	377 (25)
C(3*)	9317 (11)	5025 (6)	8987 (7)	224 (17)	C(34)	1323 (14)	3363 (7)	2683 (10)	358 (25)
C(4*)	7704 (11)	5694 (6)	8657 (7)	250 (18)	N(44)	3025 (10)	3179 (6)	3709 (7)	298 (18)
C(5*)	6107 (11)	5338 (7)	8017 (7)	246 (19)	C(54)	2862 (15)	3776 (8)	4776 (9)	386 (25)
C(6*)	6200 (11)	4346 (7)	7846 (7)	255 (19)	C(64)	1999 (14)	3270 (9)	5443 (9)	403 (25)
O(41*)	7556 (8)	6650 (4)	9007 (5)	279 (13)	O(74)	3180 (11)	2343 (6)	5724 (7)	514 (21)

Results and discussion

The molecular conformations of the two independent thyroxine–*N*-diethanolamine salts, illustrated in Fig. 1, show that the two T_4 molecules differ significantly from one another in the amino-acid side chain and thus in their overall conformation. Molecule T_4-1 has the side-chain group nearly coplanar with the inner ring [χ^2 = C(6)–C(1)–C(7)–C(8) = 162·8 (8)°], an unusual observation for aromatic amino-acid structures, and has an overall *transoid* conformation [the outer ring and C(8) on the opposite sides of the inner ring] (Cody, 1975). Molecule T_4-2, on the other hand, shows the usual amino-acid conformation of χ^2 = 87·5 (10)° and has an overall *cisoid* conformation. This is the first observation of both conformers in the same crystal lattice. Both molecules have the amine function extended [χ^1 = C(1)–C(7)–C(8)–N(8) = 169·6 (7), −160·0 (7)°, respectively]. The only other thyroactive structures which do not have the side chain perpendicular to the inner ring plane are two thyropropionic acid derivatives, a 3,5,3′-triiodothyropropionic methyl ester analogue (χ^2 = −16°) (Cody, Hazel & Osawa, 1978) and an ethyl ester analogue (χ^2 = 46°) (Camerman & Camerman, 1974*b*).

The molecular conformation of thyroxine, reported as the HCl salt (Camerman & Camerman, 1974*a*), is significantly different from the two conformers in this structure (Fig. 1). The overall conformation of T_4.HCl is transoid with the diphenyl ether conformation (φ/φ' = 105/−34°) similar to that in T_4-1. The side-chain conformation (χ^2 = 98°) is near that found in T_4-2. The amino-acid group is rotated (χ^1 = 67°) such that both functional groups are directed toward the inner ring.

A comparison of the bond lengths and angles between the two T_4 molecules shows that there are no significant differences among these parameters (Fig. 2). The same is true of the NDEA molecules. The observed C(4′)–O(4′1) bond lengths [1·31 (1), 1·32 (1) Å] are indicative of a phenoxide bond. This phenoxide-ion geometry causes a contraction of the C(3′)–C(4′)–C(5′) angle [115·0 (6), 112·4 (6)°], a slight increase in the bonds adjacent to the C(4′) atom, and an expansion of the C angles at both the C(3′) and C(5′) positions when compared to the normal phenolic geometry found in T_3 structures (angles all near 120°). No changes are observed in the C–I bond lengths. Other compounds

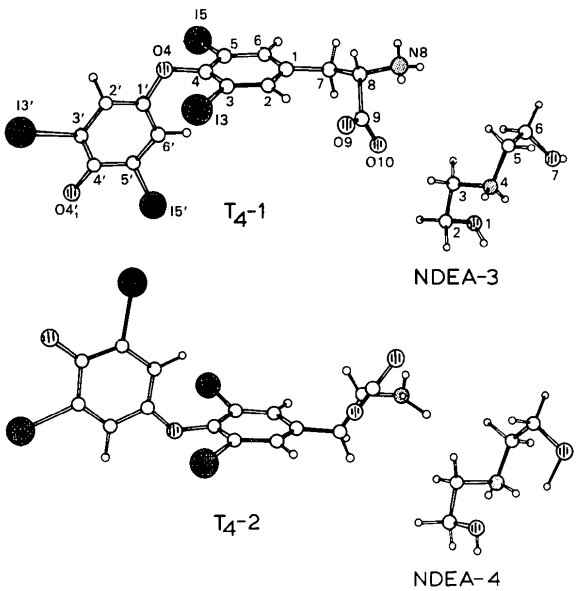

Fig. 1. Molecular conformation and numbering scheme of the two independent thyroxine.NDEA salts observed in this structure determination. The torsion angles for T_4-1 and T_4-2 respectively are: φ C(5)–C(4)–O(4′1)–C(1′): 108·1 (8), −112·7 (9)°; φ' C(4)–O(4′1)–C(1′)–C(6′): −30·0 (11), 32·5 (12)°; χ^2 C(2)–C(1)–C(7)–C(8): 162·8 (8), 87·5 (10)°; χ^1 C(1)–C(7)–C(8)–N(8): −169·6 (7), −160·0 (7)°; ψ N(8)–C(8)–C(9)–O(9): −40·8 (11), 48·7 (10)°.

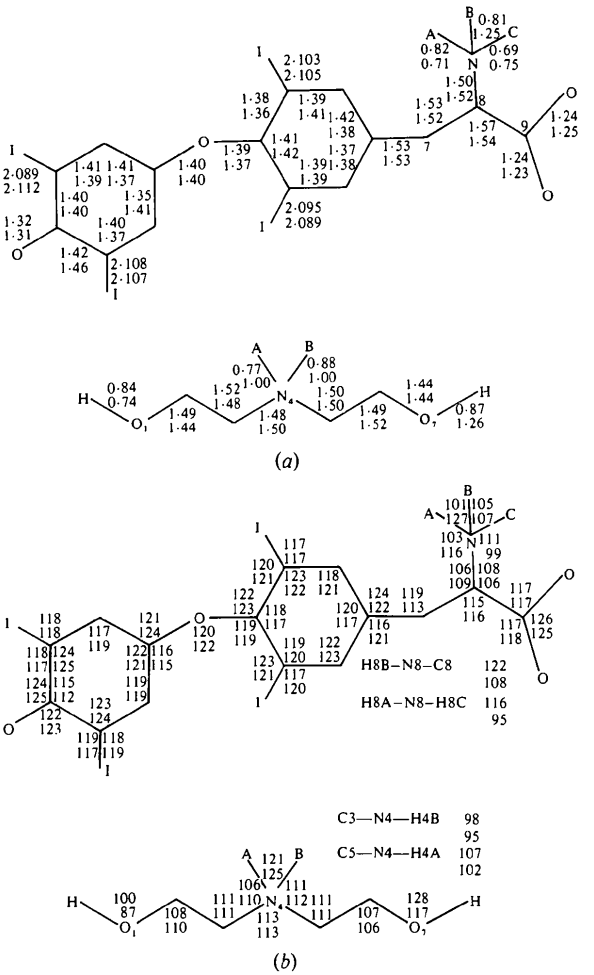

Fig. 2. (*a*) Bond lengths (Å) in molecules T_4-1 and T_4-2 (upper and lower numbers respectively) and NDEA-3 and NDEA-4. (*b*) Bond angles (°) in the T_4.NDEA salts. The e.s.d.'s for bonds and angles are 0·01 Å and 0·6° respectively.

which exhibit similar ring geometry are 3,5-dinitrotyrosine and picric acids (1,3,5-trinitrophenols), which resemble the outer ring of thyroxine (Cody, Langs & Hazel, 1979). The apparent effect of di-*ortho* substitution by electron-withdrawing groups is to enhance these geometrical changes.

As shown in Table 2, the 4′-phenolic ion in both T_4 molecules participates in strong O···N hydrogen bonds with the NDEA and N function of adjacent T_4 molecules. As illustrated in Fig. 3, the two N donor atoms approach the phenoxide O symmetrically from above and below the plane of the phenoxide ring.

The geometry and conformation of the two NDEA molecules (Fig. 1) are similar to those observed in other NDEA structures (Cody, Hazel & Langs, 1977), where the molecule has a claw-shaped bidentate conformation.

A comparison of the hydrogen bonding observed in this structure with that found in other thyroid-hormone structures (Cody, 1979*b*) reveals that there is a high degree of specificity in the location of hydrogen-bond donors and acceptors (Fig. 4). An analysis of the 4′-O···*X* hydrogen bonds in these structures shows that the average O···O distance is 2·75 Å and the O···N distance is 2·76 Å. When the 4′-OH is considered separately as a hydrogen-bond donor or an acceptor, the O···O values become 2·66 and 2·81 Å respectively. The hydrogen-bond donor values are in agreement with those predicted from theoretical energy calculations (Andrea *et al.*, 1979). In this structure the 4′-oxygen, as a phenoxide ion, can only act as a hydrogen-bond acceptor and has two donor atoms which approach the ring symmetrically from directions above and below the ring. The average N···O distance (Table 2) is 2·87 Å and the O···O is 2·75 Å.

Table 2. *Hydrogen-bond geometry for the* T_4.NDEA *salt*

E.s.d.'s for distances are ±0·01 Å.

D–H···*A*	*D*···*A*	*D*–H	H···*A*	*D*–H···*A*
N(8)–H(*A*)···O(14)	2·92 Å	0·82 Å	2·10 Å	174°
–H(*B*)···O(4′1*)	2·65	0·81	1·88	159
–H(*C*)···O(10*)	2·89	0·69	2·23	165
N(8*)–H(*A**)···O(13)	3·18	0·71	2·62	138
–H(*B**)···O(4′1)	2·72	1·25	1·52	160
–H(*C**)···O(9)	2·84	0·75	2·11	163
N(43)–H(*A*)···O(10*)	2·99	0·88	2·31	135
–H(*B*)···O(4′1)	2·70	0·77	1·97	158
N(44)–H(*A*)···O(9)	3·18	0·99	2·41	133
–H(*B*)···O(4′1*)	2·60	1·01	1·70	146
O(13)–H···O(9*)	2·62	0·83	1·80	173
O(14)–H···O(10)	2·61	0·74	2·00	140
O(73)–H···O(9)	2·83	0·87	2·04	149
O(74)–H···O(10*)	2·95			

* Refers to molecule T_4-2.

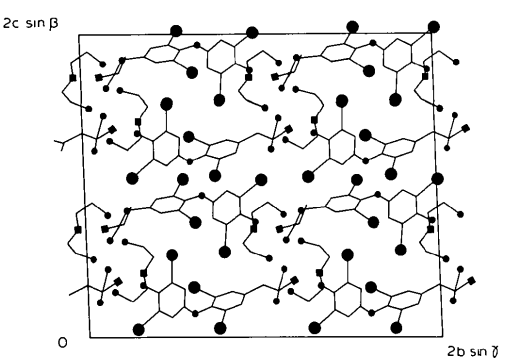

Fig. 3. Packing diagram of the T_4.NDEA salt projected down **a**. The large circles represent I, squares N and small circles O.

These data permit an experimental description of the hydrogen-bond directionality of the 4′-OH in contrast to the results of the theoretical energy calculations (Andrea *et al.*, 1979) of hydrogen-bond strengths in model *ortho*-substituted phenols and phenoxides. These theoretical studies show general agreement with the crystallographic results where the 4′-OH is acting as a hydrogen-bond donor. The observation that in T_3 structures the hydrogen-bonding atoms approach the 4′-OH from a direction opposite the 3′-iodine is additional verification of the quantitative structure–activity data (Dietrich, Bolger, Kollman & Jorgensen, 1977) which suggest that *in vitro* binding of T_3 probably involves hydrogen-bond donation to the 5′ side of the nuclear receptor. These data also indicate that the degree of 4′-OH ionization contributes to the degree of analogue binding to the serum proteins and that binding is probably through a 4′-phenoxide ion. The acidity difference between T_4 and T_3 is proposed as one factor governing the differences between the relative affinity and activity of the two hormones.

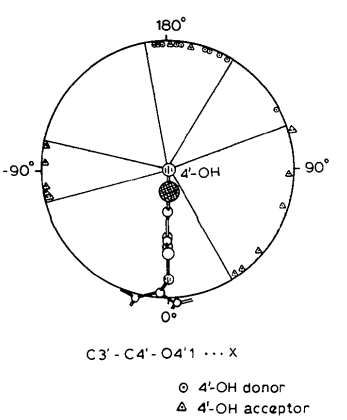

Fig. 4. Distribution of hydrogen-bond directionality with 4′-OH as acceptor and donor. Plot is of hydrogen-bond torsion angle C(3′)–C(4′)–O(4′1)···*X*. The outer ring is viewed edge on with O(4′1) as the center.

The observation of both the *cisoid* and *transoid* conformers of T_4 in the same crystal lattice suggests that they are both stable low-energy conformers. Further studies are required to establish the relevance of these conformations to binding.

In conclusion, this crystallographic determination of thyroxine has provided structural evidence which shows: (1) a 4′-phenoxide geometry, (2) possible hydrogen-bonding geometry to binding sites of a receptor protein, and (3) structural confirmation of theoretical structure–activity models. Since the outer ring of thyroxine, as a phenoxide ion, is of major importance in binding to serum proteins, these structural observations may shed light on the mechanisms of thyroxine binding and help to explain binding-affinity differences among the thyroactive compounds.

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The Structure of 1,2-*O*-Ethylene-β-D-glucopyranose at 120 K

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Abstract

$C_8H_{14}O_6$, tetragonal, $I4$, $a = b = 15{\cdot}545\ (6)$, $c = 7{\cdot}703\ (2)$ Å, $V = 1861{\cdot}4$ Å^3 at 120 K, $Z = 8$, $D_{calc} = 1{\cdot}46$ Mg m^{-3}. The 182 structural parameters were refined *versus* the 1219 (84%) most significant unique reflections ($d > 0{\cdot}71$ Å) collected at 120 K, to a linear R of 0·033. Owing to the 1,2-*O*-ethylene substitution the conformational preference ascribed to the *exo*-anomeric effect is cancelled. The molecule can thus be considered as a 'pseudo-disaccharide' with the glycosidic-link conformation *trans–trans* as described by the conventional torsion angles θ and φ. Both rings of the molecule adopt chair conformations. The pyranose ring is slightly distorted owing to an intermolecular hydrogen bond. Bond distances and angles around the anomeric carbon resemble those found in glycosides with the conventional *gauche–gauche* and

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