Rotational Barriers and the Number of Stereoisomers of Iodixanol, an X-Ray Contrast Agent

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The diastereomeric composition of iodixanol resulting from chiral centres and hindered rotation has been elucidated by two approaches. Rotational barriers for the nine bond types in iodixanol have been calculated by molecular mechanics and semiempirical quantum mechanical methods (MOPAC) as an aid in determining which bonds contribute to the diastereomeric composition by virtue of high rotational barriers. The results of the calculations suggest that rotation around the phenyl–N bond is sufficiently high (118–140 kJ mol⁻¹; 28–33 kcal mol⁻¹) to give rise to non-interconvertible rotamers at room temperature. In this case the diastereomeric composition of iodixanol will consist of six racemates and four meso forms, as opposed to three racemates and three meso forms if phenyl–N rotation is allowed.

X-ray contrast agents generally contain heavy atoms such as iodine as an X-ray absorbent and hydrophilic parts like carboxylic acid, carbamide or hydroxy groups to achieve good water solubility. The type of hydrophilic group classifies a tri-iodinated aromatic compound as an ionic (1st generation) or non-ionic (2nd generation) contrast agent. Third generation X-ray contrast agents like iodixanol are dimers of non-ionic tri-iodinated aromatic compounds in a pharmaceutical formulation which are isotonic and isosmotic with blood.

Isomerism in iodixanol has influence on the ¹H/¹³C NMR spectra, HPLC retention time, dipole moment and the crystallization behaviour. Different isomers of iodixanol have identical constitutional formulas, but different three-dimensional shapes, thus making crystallization from solution difficult.

Iodixanol is a complex mixture of stereoisomers and rotational isomers due to several stereocentres and chiral axes arising from hindered rotation. Knowledge of the exact composition of iodixanol is important for several reasons, e.g., compliance with the requirements of drug regulatory authorities that apply to documentation, and analytical aspects such as knowledge of the maximum number of separable entities on chiral or achiral chromatography. However, the assessment of the number of stereoisomers of iodixanol is an intricate problem due to elements of symmetry that render the classical formulae of computation inapplicable. These formulae are limited to molecules having n asymmetric atoms, [R-(CXY)ₙ-R' ], and none, or one centre of symmetry only [R = R'].

Calculations of rotational barriers to determine rotamers of high stability at room temperature and the enumeration of the stereoisomers of iodixanol constitute the subject of the present paper.

Molecular structure and symmetry elements. Iodixanol accommodates five stereocentres carrying four different substituents and six chiral axes arising from hindered rotation caused by steric interactions between the bulky iodine atoms and the side chains attached to the aromatic moieties. However, at room temperature only the two chiral axes connecting the iodinated benzene rings with the tertiary nitrogen atoms are likely to contribute to the number of stable stereoisomers.

For an analysis of the symmetry elements of iodixanol the molecule is stretched out into the form of highest symmetry. The phenyl–N nitrogen atom is treated as a planar sp² hybridized atom with its substituents in a plane orthogonal to the phenyl ring plane. The molecular structure and symmetry elements are shown in Fig. 1.

Whether the central carbon atom in the bridge is chiral or not is decided by the symmetry behaviour of all other parts of the molecule. Therefore the chirality of this carbon atom is treated last. The –CHOH– group in the bridge is temporarily replaced by the prochiral –CH₂– group only for the symmetry evaluation of all other parts of the iodixanol molecule. Only then has the molecule a
C₂ axis through the central carbon atom. Here it is important to emphasize that iodixanol itself does not have a C₂ axis. Symmetry operations are used to determine whether two isomers are identical, enantiomer or meso forms. In order to discuss the properties and contributions of all stereogenic units it is useful to define parts of the iodixanol molecule as shown in Fig. 2.

Calculations of rotational barriers

Restricted rotation is the cause of axial chirality and some other forms of isomerism in iodixanol. Therefore a qualitative and quantitative description of rotational barriers is not only helpful but necessary for further analysis of isomerism types.

Methods. The rotational barriers for rotation around the nine different single bonds in iodixanol have been calculated using both the molecular mechanics method and semiempirical quantum mechanical methods (MOPAC 5.0). The programs were used on a Silicon Graphics 4D/35 workstation, running under the IRIS 3.2.2 operating system. To reduce the size of MOPAC geometry calculations we have calculated the rotational barriers for the three smaller model molecules shown in Fig. 3. The model molecules contain all relevant partial structures of iodixanol.

The rotational barrier of a single bond A–B is predominantly determined by the nature of the bond A–B and the groups attached to A and B. Therefore, the errors introduced by using these model molecules are judged to be small. Furthermore, by using two methods of calculation that are based on different theories, one can better assess the validity of the results.

Molecular mechanics.

The three molecules were built using the Builder option in INSIGHT 2.3.0. The molecules were energy minimized with DISCOVER 2.9.5 using the CFF91 force-field with supplied atomic charges. The VA09A minimization procedure was used until the gradient norm was less than 0.041 kJ Å⁻² (0.001 kcal mol⁻²). Rotational barriers were calculated using a dihedral driver to rotate the specific bonds in steps of 10⁶. The structures were fully geometry optimized at each point, constraining only the specific torsion angle to the specific step value. All calculations were performed using a distance dependent dielectric function (ε = ε₀).

MOPAC. The rotational barriers for bonds 1, 2, 5 and 6 were also determined by use of MOPAC 5.0, incorporating the SADDLE option by the following procedure. 1. The MM geometry optimized structures were re-optimized using MOPAC to obtain the starting geometry. 2. The respective bonds were rotated 180° and the structure’s geometry optimized with MOPAC to obtain the final geometry. 3. The initial and final coordinates were used for SADDLE calculations. The keywords MMOK
and XYZ were used, with default convergence criteria.  
4. In the case of bond 1 the MM calculations strongly 
suggest that phenyl–N rotation is accompanied by amide 
rotation, i.e., an exo/endo transition. Therefore the amide 
bond was also rotated 180° under step 2. In the case of 
bond 5, two SADDLE calculations were performed, one 
with the amide bond rotated 180° in the final geometry 
(cis) and one keeping the amide bond trans. 
The calculations 1–4 were performed using both the 
AM1 and the PM3 methods. The rotational barriers for 
the single bonds 3, 4, 7, 8 and 9 were calculated using 
the procedure described below. Only the AM1 method was 
used here. 1. The staggered forms of the molecules were 
geometry optimized. 2. A SADDLE calculation was then 
performed using all combinations of starting points. In 
the case of three starting points a, b and c this results in 
the SADDLE calculations ab, ac and bc. 3. The energy 
barrier was taken as the difference in energy between 
the highest energy transition-state structure and the lowest-
energy staggered structure.  

Results  
The calculated energy barriers for the nine different bonds 
are given in Table 1, together with results from NMR and 
HPLC.  
Although the three calculational methods are not always 
consistent with respect to the barrier heights, they 
do collectively give an ordering of the barriers that is 
consistent: Ph–N > PhN–COCH3 > PhCO–NH > Ph–CO 
> CH2OH–CH2OH ≥ PhNCH2–CH2OH ≥ NHCH2– 
CH2OH ≥ NH–CH2 ≥ PhN–CH2. Furthermore, the 
calculated barriers and the experimental results support 
the conclusion that only Ph–N rotation will be disallowed 
at room temperature.  

Discussion  
The rotational barriers for the bonds 3,4 and 7–9 are all 
calculated to be below 44.7 kJ mol⁻¹ (10.7 kcal mol⁻¹).  

<table>
<thead>
<tr>
<th>Bond number and type</th>
<th>Molecular modeling</th>
<th>Related experimental data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average MM AM1 PM3</td>
<td>NMR HPLC</td>
</tr>
<tr>
<td>1 Ph–N</td>
<td>118.0 (28.2)</td>
<td>109.3 (28.1) 104.7 (25.0)</td>
</tr>
<tr>
<td>2 PhN–COCH3</td>
<td>69.4 (16.6)</td>
<td>59.9 (14.3)  44.4 (10.6)</td>
</tr>
<tr>
<td>3 PhN–CH2</td>
<td>23.8 (5.7)</td>
<td>29.3 (7.0)</td>
</tr>
<tr>
<td>4 PhNCH2–CHOH</td>
<td>36.0 (8.6)</td>
<td>32.7 (7.8)</td>
</tr>
<tr>
<td>5 Ph–CO</td>
<td>76.4 (18.2)</td>
<td>69.1 (16.5)  91.8 (21.9)</td>
</tr>
<tr>
<td>6 PhCO–NH</td>
<td>84.2 (20.1)</td>
<td>92.0 (22.0)  91.3 (21.8)</td>
</tr>
<tr>
<td>7 NH–CH2</td>
<td>27.4 (6.5)</td>
<td>30.2 (7.2)</td>
</tr>
<tr>
<td>8 NHCH2–CHOH</td>
<td>32.8 (7.8)</td>
<td>39.0 (9.3)</td>
</tr>
<tr>
<td>9 CH2OH–CH2OH</td>
<td>39.7 (9.5)</td>
<td>34.8 (8.3)</td>
</tr>
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</table>

* Kinetic data by Hagen and Tanseth.  
16  

** Two different transition-state (TS) structures are arrived at when using the PM3 method. The lowest energy TS structure is arrived at from the cis final geometry. The trans TS-structure is 8.4 kJ mol⁻¹ (2.0 kcal mol⁻¹) higher in energy. Both structures possess a pyramidal nitrogen. They differ mainly in the C–N–C–O torsion angle that is 34.4° in the cis TS-structure and −4.9° in the trans TS structure.  
† Determined for serinol derivatives.  
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© Kinetic data by Berg and Fagervoll.  
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i.e., rotation around the bonds would be expected to occur readily at room temperature. The results from the MM and MOPAC methods are in only fair agreement, the average difference for the five bonds being 8.8 kJ mol\(^{-1}\) (2.1 kcal mol\(^{-1}\)). A closer examination of the relative barrier heights and comparisons to relevant experimental data for these bonds is outside the scope of this paper. For comparison, the rotational barrier around the central bond in butane is 25–29 kJ mol\(^{-1}\) (6–7 kcal mol\(^{-1}\)) and 260–264 kJ mol\(^{-1}\) (62–63 kcal mol\(^{-1}\)) in 2-butene.\(^{11}\)

**Bond 1 (Ph–N).** Bond 1 is calculated to possess the highest barrier by all three methods. However the molecular mechanics method predicts a significantly higher barrier than MOPAC AM1 and PM3. Since it is known that the AM1 and PM3 methods usually underestimate barrier heights,\(^{12}\) we believe the MM value 140.1 kJ mol\(^{-1}\) (33.5 kcal mol\(^{-1}\)) to be closest to the true value. This means that rotation around this bond should not occur readily even at elevated temperatures. All three methods agree that phenyl–N rotation is accompanied by amide bond rotation, i.e., an *exo-endo* transition. The concerted amide rotation avoids the severe I⋯Me contacts that would result from a planar transition-state (TS) structure. However, inspection of the calculated structures show that, in spite of amide bond rotation, there are large out-of-plane distortions of the amine group. Since all other bonds can rotate more easily than the phenyl–N bond it is difficult to detect the phenyl–N bond rotation relative to an intramolecular reference position.

**Bond 2 (PhN–CO).** Bond 2 has a barrier\(^{13,14}\) of 104.1 kJ mol\(^{-1}\) (24.9 kcal mol\(^{-1}\)) according to the MM method. The AM1 and PM3 barriers are not in keeping with this result since they are only half this magnitude. Using HPLC kinetic data for the *exo-endo* equilibrium in iodoxanol\(^{15}\) and iopentol \(^{16}\) = 5-[N-(2-hydroxy-3-methoxypropyl)acetamido]-N,N′-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamidc and the Arrhenius equation we obtain activation energies for the *exo-endo* transition of 105.8 kJ mol\(^{-1}\) (25.3 kcal mol\(^{-1}\)) for iodoxanol and 102.4 kJ mol\(^{-1}\) (24.5 kcal mol\(^{-1}\)) for iopentol. Thus appears that the molecular mechanics results are most reliable also for this bond. The barriers for bond 1 and 2 are in agreement with the finding that the barrier for bond 2 is superimposed on the barrier for bond 1. We know from experience that rotation around the PhN–COCH\(_3\) bond in iodoxanol occurs in aqueous solution even at room temperature.

**Bond 3 (Ph–CO).** The calculated barriers for bond 3 are fairly consistent, and also in keeping with experimental NMR results. All three methods agree that Ph–C rotation is not accompanied by amide bond rotation, i.e., there is no coupling between rotation around bonds 5 and 6. The fact that the AM1 method arrives at the same TS structure from both starting points as opposed to PM3, gives more credit to this method. It is also in better keeping with the MM result and the NMR result\(^{17}\) 73.3 kJ mol\(^{-1}\) (17.5 kcal mol\(^{-1}\)) found for serinol (2-aminopropane-1,3-diol) derivatives such as iopamidol [ = N,N′-bis(2-hydroxy-1-hydroxymethylethyl)-5-(2-hydroxypropionlamido)-2,4,6-triiodoisophthalamide].

**Bond 6 (PhCO–NH).** The AM1 and PM3 methods both give a barrier of 92.0 kJ mol\(^{-1}\) (22.0 kcal mol\(^{-1}\)), which is also in good keeping with NMR results for serinol derivatives containing the PhCO–NHR fragment 87.8 kJ mol\(^{-1}\) (21.0 kcal mol\(^{-1}\)).\(^{13,17,18}\) The MM method slightly underestimates this barrier 69.4 kJ mol\(^{-1}\) (16.6 kcal mol\(^{-1}\)).

**Types of stereoisomerism in iodoxanol**

In iodoxanol three types of stereoisomer are possible: (A) diastereomers and enantiomers from, e.g., chiral carbon atoms in the side chains, the pseudoasymmetric carbon atom in the bridge and chiral axes; (B) torsion diastereomers (rotamers), e.g., *exo-endo* isomerism, *cis*–*trans* isomerism, *syn*/*anti* isomerism and chiral axes; (C) conformers.

The convergence approach focuses on part A. Every amide side chain (groups 1, 2, 6 and 7) contains one asymmetric carbon atom. The number of isomers can be calculated from the formula \(2^n\), where \(n\) is the number of chiral elements but here the actual number is less because of *meso* forms. Torsion diastereomers (rotamers) are isomers resulting from restricted rotation. Here the expression rotamer is used for stereoisomers with a rotational barrier that is sufficiently high to prevent rapid equilibration at ordinary temperatures (bonds 1, 2, 5 and 6 in Fig. 4). Conformers are formed by rotation about all other single bonds excluding terminal single bonds and those that have been mentioned previously. However, there is no sharp borderline between rotamers and conformers.

**Chiral axis through 1-phenyl–N.**\(^8\) This exists only under two conditions: groups A and B are different (*condition 1*) and the phenyl–N bond rotation is highly restricted (*condition 2*). A high rotational barrier of the phenyl–N bond

![Fig. 4. Bond number assignment of single bond rotations in iodoxanol.](image-url)
prevents the interchange of the side chain groups A and B (Fig. 5).

Any rotation around the phenyl–N bond will lead to racemization of the axial chirality. The priority of groups involved is A > B and COCH$_3$ > CH$_2$–C. The groups A and B in iodoxan correspond to the previously defined side chain groups 1 and 2 or 6 and 7 (Fig. 2). In iodoxan a difference in group A and B may result from different chiral carbon atoms in the side chains, syn/anti isomerism or cis/trans isomerism of two side chain carbonyl groups. Since axial chirality depends on two conditions, it is not treated as an independent type of isomerism by the symmetry species approach. But for every type of isomerism it is distinguished that condition 1 is true when group 1 + 2 or 6 + 7 are not equal; condition 1 is false when group 1 + 2 or 6 + 7 are equal (Fig. 2). The diastereomeric composition (optical isomers) of iodoxan is calculated separately for the two cases of condition 2: condition 2 is true when phenyl–N bond rotation is forbidden; condition 2 is false when phenyl–N bond rotation is allowed. Today there is not enough experimental evidence to answer the question, does the phenyl–N bond in iodoxan rotate or not? Therefore we distinguish and describe two borderline cases: the allowed and the forbidden phenyl–N bond rotation. The real diastereomeric composition of iodoxan must necessarily be between these two borderline cases.

**Chiral carbon atoms in the side chains.** These have either R or S configuration.

**syn/anti Isomerism.** Restricted rotation about the amide PhCO–NH bond 6 (Fig. 4) may lead to two different forms as shown in Fig. 6. The primary amide functions have either syn or anti configuration.

The bond order of the CO–N bond is increased due to partial double bond character. Higher carboxylic amides contain only about 3% syn isomer. cis/trans Isomerism. Restricted rotation about the Ph–CO bond 5 (Fig. 4) results in cis/trans isomerism of the carboxamido groups as shown in Fig. 7. The carbonyl oxygens of the carboxamido functions may be located on either the upper side of the phenyl ring as the N–acetyl carbonyl oxygen atom or on the lower side. Here the U U or L L locations of two carbonyl oxygens on the same phenyl ring both represent a cis configuration. Severe restricted rotation about the phenyl–N bond 1 combined with cis/trans isomerism results in several rotamers.

**exo/endo Isomerism.** Hindered rotation about the amide CO–N bond 2 (Fig. 4) results in two thermodynamically preferred orientations of the acetyl groups. As shown in Fig. 8 the carbonyl group may be directed away from the phenyl ring (exo) or towards the ring (endo).

**The pseudoasymmetric carbon atom.** The central carbon atom in the bridge (group 4) is stereochemically similar to the central carbon atom in 2,3,4-pentanetriol (Fig. 9). The middle carbon atom is asymmetric when the 2- and 4-carbons have different configurational assignments. Such a
ior under symmetry operations correlates each isomer to a symmetry species. Symmetry operations are used to identify identical isomers and to simplify the mathematical combination of different types of isomerism. Potential stereoisomerism from restricted bond rotation and from chiral carbon atoms are discussed separately. Characteristics of the method of convergence: step 1: tabulation of the configurational permutations for a compound having \( n = 7 \) stereocenters; step 2: assignment of achiral stereocenters in iodixanol; step 3: find equivalent stereocenters in iodixanol.

The symmetry species approach. The symmetry elements of the iodixanol molecular structure were analyzed. Symmetry operations are used to recognize whether two isomers are identical, enantiomer or meso forms. Some molecular properties change their sign under a certain symmetry operation, e.g., \( R \) configuration becomes \( S \) under a \( \sigma \) operation. In the point groups \( C_{1}, C_{2}, C_{s}, \) or \( C_{v} \) properties can only show symmetrical \((+1)\) or asymmetrical \((-1)\) behaviour (also called character). A combination of characters +1 and −1 relative to the elements of the point group is called symmetry species e.g., \( A_{1} \) or \( B_{1} \). The symmetry species is an irreducible representation of the symmetrical behaviour of any stereoisomer. Low symmetry combined with higher symmetry forms low symmetry products. All possible types of stereoisomerism of iodixanol form partial structures that are members of the point groups \( C_{1}, C_{2}, C_{s}, \) or \( C_{2v} \). The \( C_{2} \) axis is identical with the \((z)\)-coordinate axis and goes through the central carbon atom. The hydrogen and hydroxy group attached to the pseudoasymmetric carbon atom are not symmetrical under \( C_{2} \) or \( \sigma_{v}(zx) \) symmetry operations. The diastereomeric composition of iodixanol can be described as a combination of all types of isomerism mentioned above. The combination starts with the chiral carbon atoms in the side chains as the outer shell, then the inner shells and finally the pseudoasymmetric carbon atom. Each type of isomerism is treated separately. Its contribution to the total number of stereoisomers (\( \Sigma \)) is evaluated according to the total geometry of the molecule. Identical forms are ruled out at an early stage to simplify the mathematical combination of different types of isomerism. The following rules were found.

Chiral carbon atoms in the side chains. For forbidden phenyl–N bond rotation: \( \Sigma_{R/S} = 2A_{1} + 2A_{2} + B_{1} + B_{2} + 4A = 10 \) isomers. For allowed phenyl–N bond rotation: \( \Sigma_{R/S} = A_{1} + 2A_{2} + 2B_{1} + 2B_{2} + 2A = 6 \) isomers. Since \( meso \) forms of different species \( A_{1} \) and \( B_{1} \) equilibrate by phenyl–N bond rotation their contribution is represented by the species with highest symmetry \( A_{1} + 0B_{1} \). Since the chiral carbon atoms in the side chains are the first type of isomerism evaluated as the outer shell, there are no combination partners and no combination rules yet.

syn/anti Isomerism. Rules for forbidden phenyl–N bond rotation: in combination with \( A_{1}, A_{2}, \Sigma_{syn/anti} = 2A_{1} \).
\[ + 2A_2 + B_1 + B_2 + 4A = 10 \text{ isomers. In combination with} \\
B_1, B_2 \text{ or } A, \Sigma_{\text{symm/sym}} = 2A_1 + 2A_2 + 2B_1 + 2B_2 + 8A = 16 \\
isomers. Rules for allowed phenyl-\(N\) bond rotation: in combination with \\
A_1, A_2, \Sigma_{\text{trans/trans}} = 2A_1 + 2A_2 + B_1 + B_2 + 4A = 10 \text{ isomers. In combination with } \\
A_1, A_2, \Sigma_{\text{trans/trans}} = 2A_1 + 0A_2 + B_1 + B_2 + 4A = 8 \text{ isomers. In combination with } B_1, B_2 \\
or \text{ then } \Sigma_{\text{trans/trans}} = 0A_1 + 2A_2 + 2B_1 + 2B_2 + 4A = 9 \text{ isomers. Since }
forms of different species } A_1 \text{ and } B_1 \text{ equilibrate by phenyl-\(N\) bond rotation their contribution is represented by the species of highest symmetry } 0A_1 + B_2.
\]

**cis/trans Isomerism.** Rules for forbidden phenyl-\(N\) bond rotation: in combination with \\
A_1, A_2, \Sigma_{\text{cis/trans}} = 2A_1 + 2A_2 + B_1 + B_2 + 4A = 10 \text{ isomers. In combination with } \\
B_1, B_2 \text{ or } A \text{ then } \Sigma_{\text{cis/trans}} = 0A_1 + 2A_2 + 2B_1 + 2B_2 + 8A = 16 \text{ isomers. Rules for allowed phenyl-\(N\) bond rotation: in combination with } \\
A_1, A_2, \text{ then } \Sigma_{\text{cis/trans}} = 1A_1 + 2A_2 + 0B_1 + B_2 + 2A_2 + 2B_1 + 2A_2 + 0B_1 + B_2 + 2A_2 + 2B_1 + 2A_2 + 0B_1 + B_2 = 6 \text{ isomers. In combination with } \\
B_1, B_2 \text{ or } A, \Sigma_{\text{cis/trans}} = 2A_1 + 2A_2 + B_1 + B_2 + 4A = 9 \text{ isomers. Since forms of different species } A_1 \text{ and } B_1 \text{ equilibrate by phenyl-\(N\) bond rotation their contribution is represented by the species of highest symmetry } 1A_1 + 0B_1.
\]

**exo/endo Isomerism.** Rules for forbidden phenyl-\(N\) bond rotation: in combination with \\
A_1, A_2, \Sigma_{\text{exo/endo}} = 2A_1 + B_1 + B_2 = 3 \text{ isomers. In combination with } B_1, B_2 \text{ or } A, \Sigma_{\text{exo/endo}} = 2A_1 + 2A_2 + B_1 + B_2 = 4 \text{ isomers. Rules for allowed phenyl-\(N\) bond rotation: in combination with } A_1, A_2, \Sigma_{\text{exo/endo}} = 1A_1 + B_1 + B_2 = 1 \text{ stereoisomer. In combination with } \\
B_1, B_2 \text{ or } A, \Sigma_{\text{exo/endo}} = 1A_1 + 0B_1 + B_2 = 1 \text{ stereoisomer. Since forms of different species } A_1 \text{ and } B_1 \text{ equilibrate by phenyl-\(N\) bond rotation their contribution is represented by the species of highest symmetry } 1A_1 + 0B_1.
\]

**The pseudoasymmetric carbon atom.** In combination with \\
A or B, the carbon atom is chiral and \(\Sigma_{\text{PC}} = 2A + 2 \text{ isomers. In combination with } B_1, B_2 \text{ or } A, \Sigma_{\text{PC}} = 2B_1 + 2B_2 = 2 \text{ isomers. In combination with } A_1 \text{ or } A_2 \text{ the carbon atom is achiral and } \Sigma_{\text{PC}} = B_1 = 1 \text{ stereoisomer.}
\]

**Theoretical considerations.** The theoretical considerations are based on the following hypothesis. All rotational dia-
sterereomers are stable and do not interchnage and the phenyl-\(N\) bond rotation is forbidden. If you combine the total 
contribution \(\Sigma\) of each type of isomerism straightforward by and with no differentiation, then the maximum number of combinations \(N(\text{max})\) is calculated from eqn. (1).

\[
N(\text{max}) = \Sigma_{\text{cis/trans}}(\text{max}) \cdot \Sigma_{\text{symm/sym}}(\text{max}) \cdot \Sigma_{\text{cis/trans}}(\text{max}) \cdot \Sigma_{\text{exo/endo}}(\text{max}) \cdot \Sigma_{\text{PC}}(\text{max})
\]

\[
N(\text{max}) = 16 \cdot 16 \cdot 16 \cdot 4 \cdot 2 = 32768.
\]

However the real number of stereoisomers is lower be-
cause combinations with \(A_1\) or \(A_2\) form two identical products. This is due to the symmetrical behaviour of \(A_1\) 
or \(A_2\) under the \(C_4\) operation. \(\Sigma\) is then less than \(\Sigma(\text{max})\). Since all other types of isomerism determine in 
combination whether the pseudoasymmetric carbon atom 
is chiral or not, the pseudoasymmetric carbon is treated at 
last.

The maximum theoretical number of combinations \(C\) is 
calculated from eqn. (2)

\[
C = \Sigma_{\text{PC}} \cdot \left( \Sigma_{\text{exo/endo}} \cdot \left( \Sigma_{\text{symm/sym}} \cdot \Sigma_{\text{R,S}} \right) \right)
\]

by successive combination of the upper matrices giving 
eqn. (3) as a result.

\[
C = 128 B_2 + 16000 A = 16128 \text{ isomers.}
\]

Symmetry species such as \(A\) or \(A_2\) with no \(\sigma\) symmetry plane form enantiomers whereas \(B_1, B_2\) or \(A_1\) form meso 
forms. Therefore \(C\) represents 8000 pairs of enantiomers and 
128 meso forms. The maximum theoretical diaste-
reomic composition of iodoxanol is summarized in 
Table 2. Nevertheless, the premises for this result are 
unrealistic since rotational diastereomers are not stable and 
and can interchanged. It is useful to calculate the number of 
combinations separately for permanent (optical) and 
temporary (rotational) diastereomers.

**Permanent diastereomers / optical isomers.** Permanent dia-
astereomers are formed from the chiral carbon atoms in 
the side chains and the pseudoasymmetric carbon atom 
in the bridge. They are not able to equilibrate in solution. 
Axial chirality is only preserved when the phenyl-\(N\) bond 
rotation is forbidden. However, there are no exact bor-
derlines between the bond rotation attributes: 'forbidden; 
never' or 'a scarce event' or 'possible at high temperature' 
or 'allowed'. The way to distinguish between allowed 
and forbidden phenyl-\(N\) bond rotation describes two bor-
derlines. The real diastereomeric composition of iodoxan-
ol must necessarily be between these two boundaries.

Based on forbidden phenyl-\(N\) bond rotation the 
number of maximum permanent combinations \(M\) is calculated from 
\[
M = \Sigma_{\text{PC}} \cdot \Sigma_{\text{R,S}} = 12 A + 4 B_1 \cdot M \text{ represents six pairs of enantiomers and four meso forms (Table 2).}
\]

Based on allowed phenyl-\(N\) bond rotation the number of 
permanent combinations \(P\) is calculated from 
\[
P = \Sigma_{\text{PC}} \cdot \Sigma_{\text{R,S}} = 6 A + 3 B_2 \cdot P \text{ represents three pairs of enantiomers and three meso forms (Table 2).}
\]

**Temporary diastereomers/rotational isomers.** Temporary diastereomers (rotamers) are formed from restricted ro-
tation (Fig. 4) about the amide CO–NH bond 4 (syn/ 
anti), the phenyl–CO bond 3 (cis/trans), the amide 
\(\text{CH}_3\text{CO–N}\) bond 2 (exo/endo) and the phenyl–\(N\) bond 1. 
Rotational isomers can equilibrate.

Based on forbidden phenyl-\(N\) bond rotation the number 
of maximum temporary combinations \(T\) is calculated from 
\[
T = \Sigma_{\text{exo/endo}} \cdot \left( \Sigma_{\text{cis/trans}} \cdot \Sigma_{\text{symm/sym}} \right) \text{, } T = 8 A_1 + 24 A_2 + 24 B_1 + 12 B_2 + 436 A = 504 \text{ isomers. } T \text{ represents 230}
\]

pairs of enantiomers and 44 meso forms (Table 2).

Based on allowed phenyl-\(N\) bond rotation the number of 
temporary combinations \(L\) is calculated from \(L = \)
Table 2. The diastereomeric compositions of iodoxanol. Abbreviations: R/S=configuration of chiral carbon atoms.

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<thead>
<tr>
<th>Diastereomeric composition name</th>
<th>phenyl-N bond rotation</th>
<th>evaluated types of isomerism</th>
<th>Number of isomers and isomer type</th>
<th>Point group</th>
<th>Symmetry species</th>
</tr>
</thead>
<tbody>
<tr>
<td>maximum theoretical</td>
<td>forbidden</td>
<td>all</td>
<td>8000 pairs of enantiomers</td>
<td>$C_1$</td>
<td>$A$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>128 meso forms</td>
<td>$C_s$</td>
<td>$B_2$</td>
</tr>
<tr>
<td>maximum permanent</td>
<td>forbidden</td>
<td>only R/S</td>
<td>6 pairs of enantiomers</td>
<td>$C_i$</td>
<td>$A$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 meso forms</td>
<td>$C_s$</td>
<td>$B_2$</td>
</tr>
<tr>
<td>permanent</td>
<td>allowed</td>
<td>only R/S</td>
<td>3 pairs of enantiomers</td>
<td>$C_i$</td>
<td>$A$</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>3 meso forms</td>
<td>$C_s$</td>
<td>$B_2$</td>
</tr>
<tr>
<td>maximum temporary</td>
<td>forbidden</td>
<td>all except R/S</td>
<td>8 meso forms</td>
<td>$C_{2v}$</td>
<td>$A_1$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 pairs of enantiomers</td>
<td>$C_2$</td>
<td>$A_2$</td>
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<tr>
<td></td>
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<td>24 meso forms</td>
<td>$C_2$</td>
<td>$B_1$</td>
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<tr>
<td></td>
<td></td>
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<td>12 meso forms</td>
<td>$C_s$</td>
<td>$B_2$</td>
</tr>
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<td>$A$</td>
<td>$C_1$</td>
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<td>total:</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td>230 pairs of enantiomers</td>
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<td>44 meso forms</td>
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<td>$A_1$</td>
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<tr>
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<td></td>
<td></td>
<td>4 pairs of enantiomers</td>
<td>$C_2$</td>
<td>$A_2$</td>
</tr>
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<td></td>
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<td>1 meso form</td>
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<td>$B_1$</td>
</tr>
<tr>
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<td></td>
<td>4 meso forms</td>
<td>$C_s$</td>
<td>$B_2$</td>
</tr>
<tr>
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<td></td>
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</tr>
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<td>22 pairs of enantiomers</td>
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<td>7 meso forms</td>
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</tbody>
</table>

Table 3. Chiral carbon atom configuration and isomer types of the maximum permanent and permanent diastereomeric composition (Table 2); abbreviations: E=pair of enantiomers, M=meso form, R/S/a=configuration, a=achiral, —=identical form.

<table>
<thead>
<tr>
<th>Configuration in group</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>No. 5</th>
<th>No. 6</th>
<th>No. 7</th>
<th>Point group and symmetry species</th>
<th>Isomer type; forbidden Ph-N bond rotation</th>
<th>Isomer type; allowed Ph-N bond rotation</th>
</tr>
</thead>
<tbody>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>C_1</td>
<td>A</td>
<td></td>
<td></td>
<td>E1</td>
<td>E1</td>
<td>E1</td>
</tr>
<tr>
<td>S S</td>
<td>a</td>
<td>S</td>
<td>S</td>
<td>C_1</td>
<td>A</td>
<td></td>
<td></td>
<td>E1</td>
<td>E1</td>
<td>E1</td>
</tr>
<tr>
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<td>R</td>
<td>R</td>
<td>S</td>
<td>C_1</td>
<td>A</td>
<td></td>
<td></td>
<td>E2</td>
<td>E2</td>
<td>E2</td>
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<tr>
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<td>R</td>
<td>R</td>
<td>S</td>
<td>C_1</td>
<td>A</td>
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<td></td>
<td>E2</td>
<td>E2</td>
<td>E2</td>
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<td>S</td>
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<td>C_1</td>
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<td>E3</td>
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<td>S</td>
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<td>R</td>
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<td>E4</td>
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<td>S</td>
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<td></td>
<td>E5</td>
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<td>E5</td>
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<td>S</td>
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<td>A</td>
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<td></td>
<td>E6</td>
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<td>A</td>
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<td>M4</td>
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</tr>
</tbody>
</table>
Experimental

In the synthesis of iodoxanol\textsuperscript{24} only racemic forms of epichlorohydrin and 3-amino-1,2-propanediol were used. No optical activity was found in iodoxanol drug substance. The population of \textit{exo} and \textit{endo} rotamers of iodoxanol in D\textsubscript{2}O was measured by means of \textsuperscript{1}H NMR spectroscopy (300 MHz) and found to be 77.8\% \textit{exo} and 22.2\% \textit{endo} after reaching a state of equilibrium between isomers. The equilibrium was reached after a period of about 30 h at 25 °C. The establishment of an isomeric \textit{exo/endo} equilibrium in both DMSO-\textit{d}_{6}, DMF-\textit{d}_{7}, and CD\textsubscript{3}OD was accompanied by an increase of the \textit{exo} isomer and a corresponding decrease of the \textit{endo} isomer. Neither pH nor concentration affected the \textit{exo/endo} population ratio of iodoxanol in D\textsubscript{2}O. Distinct \textit{exo} and \textit{endo} isomers were preserved in the temperature range 25–130 °C.

\textit{exo/endo}- and [\textit{exo/endo} and \textit{endo/endo}]-isomers were separated and isolated by preparative HPLC on RP-18. The fractions were immediately frozen during collection and freeze dried. However, the high isomeric purity achieved through separation was not maintained during lyophilization.

The isomerization of isolated \textit{exo/exo} iodoxanol to an equilibrium of \textit{exo/exo}, \textit{exo/endo} and \textit{endo/endo} isomers was measured by analytical HPLC at 10, 25 and 40 °C. The results clearly show that the isomerization rate is temperature dependent with the \textit{exo/exo} isomer as the most stable and the \textit{endo/endo} isomer as the least stable. The reaction rate constants, the equilibrium constants as well as the time to reach equilibrium have been determined. The time to reach equilibrium was found to be 250 h at 10°C, 27 h at 25°C, 4 h at 40°C.

The X-ray crystal structure\textsuperscript{25} of iodoxanol is reported separately.

References

5. Conclusions in this article supersede a previous report of Gulbrandsen, T. Kjeml 6 (1990) 6.
9. Quantum Chemistry Program Exchange (QCPE), program No. 455, Indiana University, Bloomington, Indiana 47405, USA.
10. by Biosym Technologies Inc., San Diego, CA 92121.
15. Results published with agreement from Hagen, E. and Tønseth, C. P. (Dept. of Analytical Chemistry), Thomassen, T. (Dept. of Chemical Synthesis), Skjeld, W. (Dept. of Development Chemistry) at Nycomed Imaging, Oslo.
22. An exhaustive mathematical treatment of the symmetry species approach including character tables, multiplication table, isomeric composition tables is available on request from H. Priebe.
23. An exhaustive description of the convergence approach including permutation tables and operation sequences is available on request from A. J. Aasen.

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