

## Synthesis, Analysis and Toxicity of Three Compounds Formed during the Synthesis of Iodixanol

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Priebe, H., Dugstad, H., Heglund, I. F., Sande, R. and Tønseth, C. P., 1995. Synthesis, Analysis and Toxicity of Three Compounds Formed during the Synthesis of Iodixanol. - Acta Chem. Scand. 49: 737-743 © Acta Chemica Scandinavica 1995.

The origin of 4-acetyl-2-[*N*-acetyl-3,5-bis(2,3-dihydroxypropylcarbamoyl)-2,4,6-triiodoanilinomethyl]-5,7-diiodo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6,8-dicarboxylic acid bis(2,3-dihydroxypropylamide) = *N*-acetyl cyclized iodixanol, 2-[*N*-acetyl-3,5-bis-(2,3-dihydroxypropylcarbamoyl)-2,4,6-triiodoanilinomethyl]-5,7-diiodo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6,8-dicarboxylic acid bis(2,3-dihydroxypropylamide) = cyclized iodixanol and 5,5'-(*N*-acetyl-2-hydroxypropane-1,3-diyl)diamino)bis[*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide] = deacetyl iodixanol in the manufacturing process of the X-ray contrast agent 3,3',5,5'-tetrakis(2,3-dihydroxypropylcarbamoyl)-2,2',4,4',6,6'-hexaiodo-*N,N'*-(2-hydroxypropane-1,3-diyl)diacetanilide = iodixanol is discussed and their synthesis and purification are described. Their physical and toxicological properties, and analytical and spectroscopic data are summarized.

X-Ray contrast agents generally contain heavy atoms such as iodine as an X-ray absorbent and hydrophilic parts like carboxylic acid, carboxamide 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 such as iodixanol<sup>1</sup> are dimers of non-ionic tri-iodinated aromatic compounds in a pharmaceutical formulation<sup>2</sup> which is isotonic<sup>3</sup> and isoosmotic<sup>4</sup> with blood.

In order to avoid the presence of toxic components in the iodixanol drug substance all impurities or degradation products above a certain concentration level are characterized by their origin, synthesis and analytical, spectroscopic and toxicological properties. *N*-Acetyl cyclized iodixanol **1**, cyclized iodixanol **2** and deacetyl iodixanol **3** are three representatives of possible impurities in the iodixanol process. They may be formed during the synthesis<sup>5</sup> or by degradation of iodixanol. Their molecular structures are summarized in Fig. 1.

In this text molecule numbers and trivial names of their abbreviations are used instead of the corresponding IUPAC names.<sup>6</sup> Their intercorrelation is given below.

**1** = *N*-acetyl cyclized iodixanol = 4-acetyl-2-[*N*-acetyl-3,5-bis(2,3-dihydroxypropylcarbamoyl)-2,4,6-triiodoanili-

nomethyl]-5,7-diiodo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6,8-dicarboxylic acid bis(2,3-dihydroxypropylamide).

**2** = Cyclized iodixanol = 2-[*N*-acetyl-3,5-bis(2,3-dihydroxypropylcarbamoyl)-2,4,6-triiodoanilinomethyl]-5,7-diiodo-3,4-dihydro-2*H*-benzo[1,4]oxazine-6,8-dicarboxylic acid bis(2,3-dihydroxypropylamide).

**3** = Deacetyl iodixanol = 5,5'-(*N*-acetyl-2-hydroxypropane-1,3-diyl)diamino)bis[*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide].

**4** = Compound 5400 = 5-amino-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-isophthalamide.

**5** = TFA-allyl-5400 = 5-(*N*-allyl-2,2,2-trifluoroacetamido)-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide.

**6** = Compound 5410 = 5-acetamido-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-isophthalamide.

**7** = TFA-5400-bromohydrin = 5-[*N*-(3-bromo-2-hydroxypropyl)-2,2,2-trifluoroacetamido]-*N,N'*-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide.

**8** = Iodixanol = 3,3',5,5'-tetrakis(2,3-dihydroxypropylcarbamoyl)-2,2',4,4',6,6'-hexaiodo-*N,N'*-(2-hydroxypropane-1,3-diyl)diacetanilide.

**9** = Peracetyl cyclized iodixanol = acetic acid 2-acetoxy-3-[3-{acetyl-[4-acetyl-6,8-bis-(2,3-diacetoxypropylcarbamoyl)-5,7-diiodo-3,4-dihydro-2*H*-benzo[1,4]oxazin-2-ylmethyl]amino}-5-(2,3-diacetoxypropylcarbamoyl)-2,4,6-triiodobenzoylamino]propyl ester.

**10** = TFA-5400 = *N,N'*-Bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-(2,2,2-trifluoroacetamido)isophthalamide.

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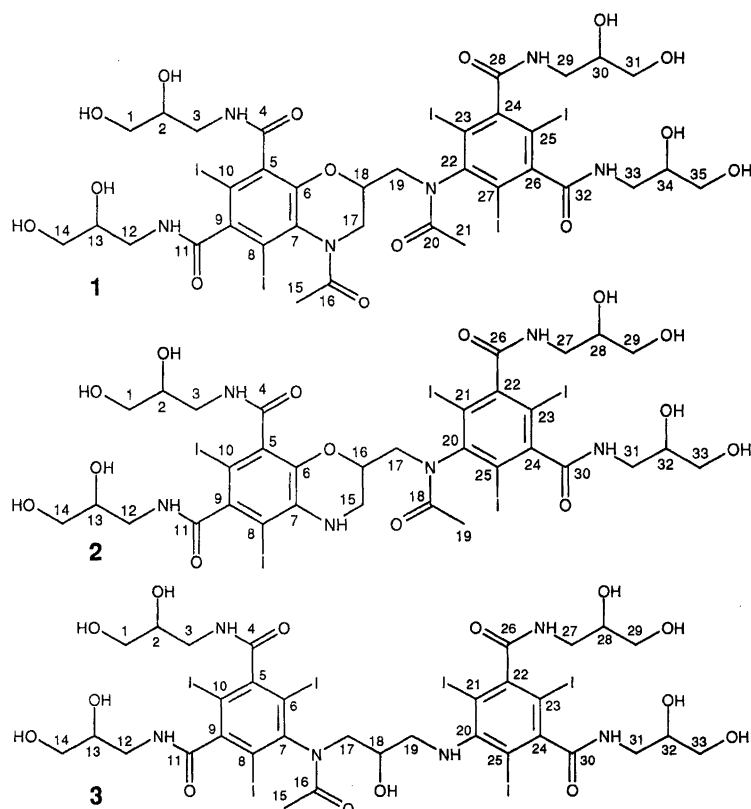


Fig. 1. Molecular structures and numbering system of  $^{13}\text{C}$  NMR carbon atoms of *N*-acetyl cyclized iodixanol **1**, cyclized iodixanol **2** and deacetyl iodixanol **3**.

### Origin and alternative synthesis

Partial loss of acetic acid from iodixanol can generally occur under hydrolysis or solvolysis conditions to form deacetyl iodixanol **3**. The synthesis of **3** is based on the ability of trifluoroacetamides to undergo solvolytic cleavage more easily than the corresponding acetamide. The key step to **3** was the alkylation of **6** with TFA-5400-bromohydrin **7** in basic aqueous medium. The bromohydrin **7** was prepared from **5** and bromine in aqueous solution. **5** was prepared from compound **4** by acetylation with trifluoroacetic anhydride (TFAA) – 4-dimethylaminopyridine (DMAP) followed by allylation with allyl bromide (Fig. 2).

*N*-Acetyl cyclized iodixanol **1** is formed when iodixanol is exposed to basic conditions<sup>7</sup> at elevated temperatures or at room temperature, e.g., when a solution of iodixanol is left in contact too long with a strong basic ion exchange resin. Under the conditions of its formation **1** is mainly deacetylated and forms cyclized iodixanol **2**. This second reaction strongly diminishes the remaining concentration of **1** and makes a different synthetic approach necessary.

**2** was synthesized by the sequence shown in Fig. 3. The synthesis of **1** was based on the ability of acetates to undergo solvolytic cleavage more easily than the corresponding acetamides. **1** was synthesized from **2** by peracetylation with acetic acid anhydride and *p*-toluenesulfonic acid. Peracetyl cyclized iodixanol **9** was formed

as an intermediate and was deacetylated under basic conditions in aqueous methanol to **1**.

### Acute intravenous toxicity

Deacetyl iodixanol **3**, *N*-acetyl cyclized iodixanol **1** and cyclized iodixanol **2** were tested for acute toxicity in mice. The administration of **2** and **3** caused no effects on clinical signs and body weight development. Mortality and morbidity was seen in only three out of five mice to which **1** had been given. The mortality incidence of 3/5 at the dose  $14.7 \text{ gI kg}^{-1}$  of **1**, is within the lower range of the  $\text{ALD}_{50}$  (approximated median lethal dose) reported for iodixanol of  $15\text{--}18 \text{ gI kg}^{-1}$  in NMRI mice.<sup>8</sup> The lethal toxicity of **1** is probably similar or marginally higher than iodixanol. The doses of **3**, **2** and **1** injected correspond to about 500, 5000 and 15000 times the range of the specified amounts in a high clinical dose of  $500 \text{ mgI kg}^{-1}$  iodixanol. The presence of **1**, **2** or **3** was therefore considered to be of no significance for the toxicity of the clinical formulation<sup>9</sup> of iodixanol.

### Experimental, material and methods

UV spectra were run on a Philips PU 8750 UV/VIS spectrometer. IR spectra were measured on a Bruker IFS 66.

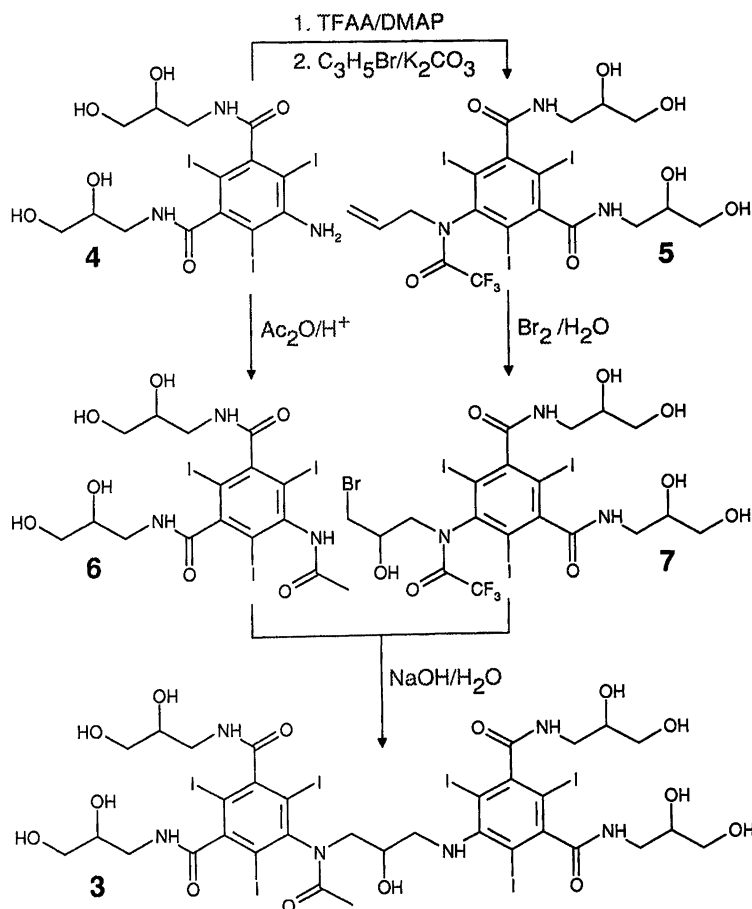


Fig. 2. Preparation of deacetyl iodixanol **3**.

FAB-MS spectra were obtained with a V6 TRIO-2. All NMR spectra were recorded at 25°C on a Varian VXR-300 S spectrometer with DMSO- $d_6$  as the solvent and 1% v/v TMS as an internal reference. Chemical shifts are reported as ranges when single resonances are split due to the presence of numerous rotational isomers<sup>10</sup> in solution at 25°C. The chemical shift assignments to C-atom numbers are valid only for the simulated <sup>13</sup>C NMR spectra<sup>11</sup> of the corresponding compounds. Elemental analyses were performed by Ilse Beetz, Mikroanalytisches Laboratorium, Postfach 1164, D-96301 Kronach, Germany. The preparative HPLC work was carried out on a Jobin Yvon Prepamatic LC – II by using a self-packed axial compression column with 80 mm ID, in reversed-phase mode. The column was packed from a slurry containing isopropyl alcohol. The separation was monitored by refractive index coupled in series with a UV detector. The analytical HPLC was performed by using two methods, an RP-18 column system and an amino-column system. Substances were detected with UV spectrophotometric detection (254 nm). The RP-18 column system utilised a Brownlee Labs column (cat. No. OD-5A, 5  $\mu$ m, 0.25 m long and 4.6 mm in internal diameter). The chromatographic parameters of the RP-18 HPLC method were a linear gradient from 3–10% acetonitrile in water

for 30 min and then a concave gradient from 10 to 50% acetonitrile in water for 40 min (flow 1 ml min<sup>-1</sup>). To improve the detection limits and the quantification, the RP-18 method uses a 'high-low' procedure<sup>12</sup> which combines data obtained with sample preparations with different concentrations (2.5 mg ml<sup>-1</sup> and 25 mg ml<sup>-1</sup>). The amino column system utilised a Supelcosil LC-NH2, Supelco column (cat. No. 5-8338, 5  $\mu$ m, 0.25 m long and 4.6 mm in internal diameter). The chromatographic parameters were a linear gradient from 85–66% acetonitrile in water for 25 min (flow 2.5 ml min<sup>-1</sup>).

*Acute intravenous toxicity.* Male NMRI mice, weighing 20  $\pm$  2 g were injected with a single dose of **3**, **1** or **2** intravenously in the tail at the rate of 1.2 ml min<sup>-1</sup>. All animals were fed a standard mouse diet (Ewos R3 pellets) and water *ad libitum*. 4–10 mice per group were dosed according to Table 1. Clinical signs, body weight and mortality were registered over a period of 7 days. Because of the low aqueous solubility **3** was formulated in DMSO (9.24%) to a final iodine concentration of 17 mg ml<sup>-1</sup>. The amount of DMSO given in the vehicle group was equivalent to the volumes given to the mice dosed with **3**. **1** was formulated to a final concentration of 300 mg ml<sup>-1</sup> of which 87% of the iodine content was

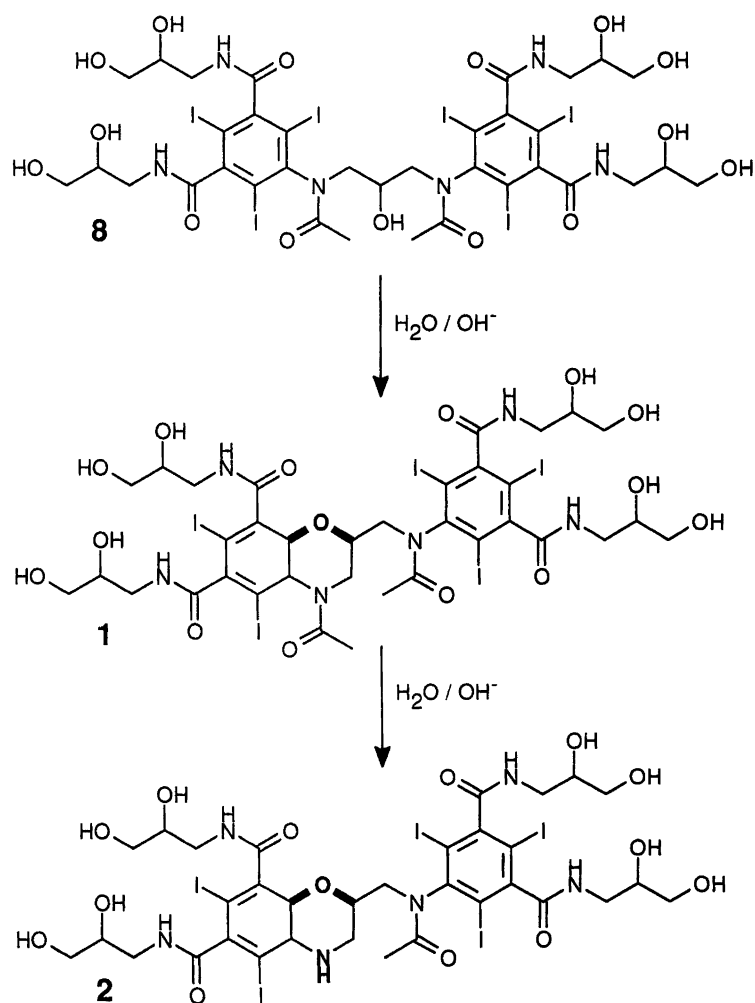


Fig. 3. Decomposition of iodixanol **8** at high pH forming *N*-acetyl cyclized iodixanol **1** and cyclized iodixanol **2**.

Table 1. Dosages and mortality of mice after a single intravenous injection of **1**, **2** or **3**; mortality is expressed as incidence.

| Test substance                                     | Dose (gI kg <sup>-1</sup> ) | Mortality at day 7 |
|--|-----------------------------|--------------------|
| Deacetyl iodixanol <b>3</b>                        | 0.4                         | 0/4                |
|  | 0.6                         | 0/4                |
|  | 0.8                         | 0/4                |
|  | 1.0                         | 0/4                |
| <i>N</i> -acetyl cyclized iodixanol <b>1</b> (87%) | 14.7                        | 3/5                |
| Cyclized iodixanol <b>2</b>                        | 5.4                         | 0/10               |
| DMSO   | 0.50 ml                     | 0/4                |
|  | 0.75 ml                     | 1/4                |
|  | 1.00 ml                     | 0/4                |
|  | 1.20 ml                     | 0/4                |

**1**, **2** was formulated to an iodine concentration of 50 mgI ml<sup>-1</sup>. Because of the low solubility of **3** and **2**, and the volume limits of 1.2 ml per 20 g mouse, 5.4 gI kg<sup>-1</sup> and 1.0 gI kg<sup>-1</sup>, respectively was the maximum dose.

#### Preparation of deacetyl iodixanol **3**.

**Trifluoroacetylation of 4.** Trifluoroacetic anhydride (TFAA, 845 mmol, 177.5 g, 117.5 ml) and 4-*N,N'*-dimethylaminopyridine (DMAP, 1.78 mmol, 200 mg) were added to a suspension of **4** (141.8 mmol, 100 g) in toluene (500 ml) at 20–25°C. The reaction mixture was stirred at 40°C. After 30 min all solid material was dissolved. Stirring at 40°C was continued for 1 day. The mixture was evaporated to dryness and redissolved in methanol–water (400 ml/40 ml). Activated carbon (5 g) was added and the mixture was stirred at 60–70°C for 30 min. The solution was filtered cold and the filtrate was evaporated to dryness. Yield: 106.7 g, 133.2 mmol, 94% TFA-5400 **10**.

**Allylation of 10.** **10** (124.8 mmol, 100 g) was dissolved in *N,N'*-dimethylacetamide (DMA, 400 ml) and potassium carbonate (149.8 mmol, 20.7 g, 1.2 equiv.) was added. Allyl bromide (149.8 mmol, 18.1 g, 12.6 ml, 1.2 equiv.) was added slowly to the reaction mixture. Stirring was continued at room temperature for 1 day. Additional allyl

bromide (12.4 mmol, 1.5 g, 1.05 ml, 0.1 equiv.) was added and stirring was continued at room temperature for 1 day. Acetic acid (8.6 ml) was added and the mixture was evaporated to dryness under high vacuum. The residue was dried at 50°C *in vacuo* and suspended in water (300 ml) with intensive stirring for 4–6 h. The white crystalline substance formed was filtered off cold. The filter cake was washed with cold water and dried (50°C/50 mbar/1 day). Yield: 88.1 g, 104.7 mmol, 84% TFA-allyl-5400 **5**, purity 83%.

**Hydroxybromination of 5.** **5** (11.9 mmol, 10.0 g) was suspended in water (1.1 l) at 50°C. The solution was cooled to 0°C. A solution of bromine (13.1 mmol, 2.1 g, 1.1 equiv.) in water (200 ml) was added slowly over 2 h during which the suspension almost dissolved. Amberlite IRA-68 ion exchange resin (20 ml) was added and stirred at 0°C for 30 min. The ion exchange resin was filtered off and the filtrate was freeze dried. Yield: 10.5 g, 11.2 mmol, 94% TFA-5400-bromohydrin **7**.

**Alkylation of 6 with 7.** **6** (51.14 mmol, 38.2 g, 1.2 equiv.) was dissolved in water (200 ml) at 50°C pH 12.0. **7** (42.64 mmol, 40.0 g) was added slowly over 2 h. The pH was kept constant at 12.0 for 2 days/room temperature. Some product precipitated. The reaction mixture was neutralized, evaporated to dryness, resuspended in 400 ml 80% methanol, diluted with water to 50% methanol and filtered. The filter cake was washed with 500 ml water and dried (16 g, 88 area % purity). The filtrate was treated with IRA-68 and AMB-200 ion exchange resin and the ion exchanger was filtered off. The filtrate **3** was evaporated to dryness (60 g, 61% purity) and purified by preparative HPLC.

**Purification by preparative HPLC.** **3** was ion exchanged by using a mixed bed ion exchanger. The yield after ion exchange was approx. 85%. 99 grams were dissolved in 510 ml 20% methanol in RO water and filtered through a 0.45 µm filter before chromatography. Six repetitive cycles were run by injecting 16.5 g/85 ml on each cycle. Six fractions were taken and each worked up by vacuum evaporation. Fractions 3 and 4 (36.43 g) were 99.2% pure **3** (stationary phase: Merck Lichroprep RP-18, 60 Å, 25–40 µm, 700 g,  $V_d = 1$  l; mobile phase: solvent A = 20% methanol in water; solvent B = 80% methanol in water; flow rate: approx. 85 ml min<sup>-1</sup>; elution pressure: 7.6–7.8 bar; cycle time: 80 min).

**Final treatment for biological purpose.** **3** (28.4 g, 18.8 mmol) was dissolved in water (2 l) at room temperature. Traces remained undissolved. Ion exchange resin IRA-68 (ca. 0.8 g) and IR-200 (ca. 0.8 g) were added until the pH remained stable at 6.5. Activated carbon (ca. 0.5 g) was added to the solution and stirred at room temperature for 1 h. The suspension was filtered using a Millipore filter. Neutral aluminium oxide (grain size 50–200 µm, ca. 0.5 g) was added to the filtrate. The

suspension was allowed to stand at room temperature without stirring for 1 h and was filtered using a Millipore filter. The filtrate was evaporated to dryness (50°C/20 mbar) and the residue was dried *in vacuo* (50°C/1 day). Recovery: 24.5 g **3** (86.2%).

#### Characterization and analysis of 3.

**Isomerism.** There is only one *N*-acetyl group that can form two *exo/endo* isomers<sup>10</sup> from restricted rotation about the CO–N bond. One carbon atom in every side chain and the central carbon atom in the bridge are chiral and form 32 *R/S* isomers.

**HPLC.** Achiral column material will not separate optical isomers resulting from chiral carbon atoms. The resolution is usually to separate other types of rotamers except *exo/endo* isomers. The analytical HPLC (RP-18 column and amino column) shows two peaks due to *exo/endo* isomerism with  $t_R = 35.2 + 37.1$  min and  $t_R = 10.8 + 11.5$  min, respectively. The purity was found to be 99.1 area % (RP-18 column) and 97.1 area % (amino column).

Elemental anal: Found: C 25.85; H 2.83; N 5.83; I 50.77. Calc. for C<sub>33</sub>H<sub>42</sub>I<sub>6</sub>N<sub>6</sub>O<sub>14</sub>: C 26.28; H 2.81; N 5.57; I 50.49. **TLC:**  $R_f$ -values are very similar to iodixanol on silica and RP-18. On silica: methyl acetate–methanol–acetic acid = 6:3:1;  $R_f = 0.5$ ; ethyl acetate–methanol–acetic acid = 6:3:1;  $R_f = 0.35$ ; on RP-18 W/UV254 'Macherey-Nagel' (cat. No. 811071): methanol–water = 2:8;  $R_f = 0.6$ . M.p. 295–296°C. <sup>1</sup>H NMR, <sup>13</sup>C NMR. The observed <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts of **3** are summarized in Table 2 and Table 3, respectively. The results of the <sup>13</sup>C NMR spectrum simulation of **3** are given in Table 3. **UV:**  $C = 0.949 \times 10^{-5}$  mol l<sup>-1</sup> (H<sub>2</sub>O);  $\lambda_{max} = 243$  nm;  $E = 0.447$ ;  $\log \epsilon = 4.652$ . IR<sup>13</sup> (KBr): 3400, 3250 (s, N–H, O–H), 3100 (m, N–H), 2940, 2880 (m, C–H), 1640 (vs, C=O primary amide sidechains), 1550 (s, C=O primary amide sidechains), 1400, 1280 (m,  $\delta$  C–H), 1115, 1040 (m). Solubility in water: 10 g l<sup>-1</sup>; in aqueous iodixanol (350 mg l/ml): 30 g l<sup>-1</sup>. FAB-MS:  $m/z$  (% rel. int.) = 1509 (100, *M* + H); 1383 (65, *M* + 2H – I); 1257 (25, *M* + 3H – 2I); 1129 (15, *M* + 2H – 3I).

**Preparation of *N*-acetyl cyclized iodixanol 1.** A suspension of **2** (3.0 g, MW 1379.77, 2.17 mmol) and *p*-toluenesulfonic acid (0.06 g, MW 172.22, 0.34 mmol) in acetic

Table 2. <sup>1</sup>H NMR chemical shifts and assignments of **1**, **2** and **3** in DMSO-*d*<sub>6</sub>.

| Chemical group                     | Chemical shift of <b>1</b> (ppm) | Chemical shift of <b>2</b> (ppm) | Chemical shift of <b>3</b> (ppm) |
|------------------------------------|----------------------------------|----------------------------------|----------------------------------|
| CO–NH                              | 8.8–8.0                          | 8.8–7.9                          | 8.7–8.0                          |
| Ar–NH                              |                                  | 5.4–5.2                          | 5.1                              |
| OH                                 | 4.9–4.4                          | 4.9–4.4                          | 4.9–4.4                          |
| CH, CH <sub>2</sub>                | 4.3–2.7                          | 4.3–2.9                          | 4.4–2.8                          |
| CO–CH <sub>3</sub> ( <i>endo</i> ) | 2.34–2.02                        | 2.28–2.24                        | 2.25                             |
| CO–CH <sub>3</sub> ( <i>ring</i> ) | 2.34–2.02                        |                                  |                                  |
| CO–CH <sub>3</sub> ( <i>exo</i> )  | 1.88–1.72                        | 1.85–1.77                        | 1.78                             |

Table 3.  $^{13}\text{C}$  NMR chemical shifts and assignments of deacetyl iodixanol **3** in  $\text{DMSO}-d_6$ .

| Chemical shift of <b>3</b> (ppm) | Simulation <sup>11</sup> (ppm) | Assignment to C-atom No. in Fig. 1 |
|----------------------------------|--------------------------------|------------------------------------|
| 170.2–170.1                      | 171.4±2.9                      | 16                                 |
| 169.8–169.4                      | 170.7±1.7                      | 4 11 26 30                         |
| 149.8                            | 149.9±                         | 20                                 |
| 151.4–150.5                      | 150.0±3.1                      | 5 9 22 24                          |
| 147.8–147.4                      | 147.6±1.8                      | 7                                  |
| 101.3–99.5                       | 98.6±4.4                       | 6 8                                |
| 92.3–91.5                        | 98.6±4.4                       | 21 25                              |
| 83.3–82.9                        | 89.9±2.9                       | 10 23                              |
| 70.0–69.7                        | 70.4±0.7                       | 2 13 28 32                         |
| 68.4–68.0                        | 69.7±                          | 18                                 |
| 63.8–63.7                        | 64.3±0.6                       | 1 14 29 33                         |
| 54.9–54.2                        | 53.4±                          | 17                                 |
| 53.6–53.3                        | 50.0±                          | 19                                 |
| 42.4–42.2                        | 43.0±0.6                       | 3 12 27 31                         |
| 22.7–22.1                        | 22.9±0.7                       | 15                                 |

acid anhydride (15 ml) was heated to 70°C. Within 3 h the reaction mixture became a homogeneous solution of **9**. The solution was evaporated to dryness and redissolved in methanol–water (80:20, 15 ml). In order to deacetylate only *O*-acetyl groups the pH of the solution was adjusted to 11.5. After 3 h of stirring at room temperature the solution was neutralized and evaporated to dryness. The crude **1** (3.5 g) was purified by preparative HPLC.

**Purification by preparative HPLC.** **1** (101.25 g) was dissolved in 380 ml 18% methanol in RO-water and filtered through a 0.45  $\mu\text{m}$  filter before chromatography. Four repetitive cycles were run by injecting 25.3 g/95 ml each cycle. Six fractions were taken and each was worked up by vacuum evaporation. Based on total area % of an analytical HPLC chromatogram on RP-18, the fractions 3, 4 and 5 (22 g) were >98.5% pure (stationary phase: Merck Lichroprep RP-18, 60 Å, 25–40  $\mu\text{m}$ ; mobile phase: solvent A = 21% methanol in water; solvent B = 80% methanol in water; flow rate: approx. 85 ml  $\text{min}^{-1}$ ; elution pressure: 8.9–9.1 bar; cycle time: 80 min).

#### Characterization and analysis of **1**.

**Isomerism.** The rotation of the *N*-acetyl group connected to the oxazine ring is fixed. However, the other *N*-acetyl group can form 2 *exo/endo* isomers<sup>10</sup> from restricted rotation about the CO–N bond. One carbon atom in every side chain and position 2 of the benzoxazine ring are chiral and the structure forms 32 *R/S* isomers.

**HPLC.** The resolution is usually insufficient to separate other types of rotamer except *exo/endo* isomers. The analytical HPLC (RP-18 column and amino column) shows two peaks due to *exo/endo* isomerism with  $t_{\text{R}} = 35.5 + 43.1$  min and  $t_{\text{R}} = 8.7 + 11.0$  min, respectively. The purity was found to be 99.4 area % (RP-18 column) and 99.1 area % (amino column). Elemental anal: Found: C 29.27; H 3.08; N 6.08; I 44.68. Calc. for

Table 4.  $^{13}\text{C}$  NMR chemical shifts and assignments of *N*-acetyl cyclized iodixanol **1** in  $\text{DMSO}-d_6$ .

| Chemical shift of <b>1</b> (ppm) | Simulation <sup>11</sup> (ppm) | Assignment to C-atom No. in Fig. 1 |
|----------------------------------|--------------------------------|------------------------------------|
| 170.7–168.7                      | 171.4±2.9                      | 20                                 |
| 170.7–168.7                      | 170.7±1.7                      | 11 32 28                           |
| 170.7–168.7                      | 169.6±0.2                      | 16                                 |
| 166.2–165.7                      | 166.1±0.5                      | 4                                  |
| 151.3–150.7                      | 150.0±3.1                      | 9 26 24                            |
| 147.5–146.6                      |                                |                                    |
| 144.7                            | 147.6±1.8                      | 22                                 |
| 142.0–141.4                      | 142.4±1.7                      | 6                                  |
| 133.1                            | 132.5±3.4                      | 5                                  |
| 130.5–130.0                      | 130.2±0.5                      | 7                                  |
| 100.5–99.0                       | 98.6±4.4                       | 23 27                              |
| 96.5–95.6                        |                                |                                    |
| 92.2–91.9                        | 89.9±2.9                       | 25                                 |
| 90.7–90.4                        | 89.0±6.8                       | 8                                  |
| 89.4                             | 82.3±7.6                       | 10                                 |
| 77.7–76.7                        | 77.4±0.2                       | 18                                 |
| 70.7–69.6                        | 70.4±0.7                       | 2 13 30 34                         |
| 63.8–63.4                        | 64.3±0.6                       | 1 14 31 35                         |
| 52.2–51.4                        | 51.9±0.0                       | 19                                 |
| 44.5–43.9                        | 45.4±0.0                       | 17                                 |
| 42.5–41.9                        | 43.0±0.6                       | 3 12 29 33                         |
| 23.7; 22.4                       | 22.9±0.7                       | 15 21                              |

$\text{C}_{35}\text{H}_{43}\text{I}_5\text{N}_6\text{O}_{15}$ : C 29.56; H 3.05; N 5.91; I 44.61. TLC: TLC system on silica: chloroform–methanol–acetic acid = 50:50:2;  $R_f$ : 0.52. M.p. 234–236°C.  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR: the observed  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts of **1** are summarized in Table 2 and Table 4, respectively. The results of the  $^{13}\text{C}$  NMR spectrum simulation of **1** are given in Table 4. UV:  $C = 1.077 \times 10^{-5}$  mol  $\text{l}^{-1}$  ( $\text{H}_2\text{O}$ );  $\lambda_{\text{max}} = 240$  nm;  $E = 0.610$ ;  $\log \epsilon = 4.753$ . IR (KBr): 3360, 3250 (s, N–H, O–H), 3080 (m, N–H); 2930, 2880 (m, C–H), 1745, 1700 (s, C=O *N*-acetyl), 1640 (vs, C=O primary amide sidechains), 1570 (s, C=O primary amide sidechains); 1370, 1390 (s,  $\delta$  C–H acetyl); 1030, 1050 (m). FAB-MS:  $m/z$  (% rel. int.) = 1423 (100,  $M+H$ ); 1297 (70,  $M+2H-I$ ); 1171 (38,  $M+3H-2I$ ); 1042 (20,  $M+H-3I$ ).

**Preparation of cyclized iodixanol 2.** Iodixanol **8** (110 g, 71 mmol) was dissolved in a solution of sodium hydroxide (35.6 g, 0.89 mol) in water (3.2 l). The solution was heated to 75°C for 20 h. The mixture was neutralized with conc. HCl and the solvent was removed under reduced pressure. The residue was dissolved in methanol (1.3 l) at 50°C. The solution was cooled to 0°C and precipitated salts were removed by filtration. The filtrate was treated with active carbon at 40–50°C for 1.5 h before further filtration. The filtrate was evaporated to dryness and gave crude **2** (96.7 g, 88.3% purity, 87% yield).

**Purification by preparative HPLC.** **2** (127 g) was dissolved in 500 ml 21% methanol in water and filtered through a 0.45  $\mu\text{m}$  filter before chromatography. Five repetitive cycles were run by injecting approx. 25.4 g/100 ml each

cycle. Eight fractions were taken and fractions 2–8 were worked up by vacuum evaporation. Based on total area % of an analytical HPLC chromatogram on RP-18 the fractions 3–6 (20.3 g) were >99.1% pure (stationary phase: Merck Lichroprep RP-18, 60 Å, 25–40 µm, 700 gram, V.d. = 1 l; mobile phase: solvent A = 21% methanol in water, solvent B = 80% methanol in water; flow rate: approx. 86 ml min<sup>-1</sup>; elution pressure: approx. 11 bar; detection: RI sens. 8, UV out of function; cycle time: 75 min).

#### Characterization and analysis of 2.

**Isomerism.** The *N*-acetyl group can form 2 *exo/endo* isomers<sup>10</sup> from restricted rotation about the CO–N bond. One carbon atom in every side chain and position 2 of the benzoxazine ring are chiral and 32 *R/S* isomers are formed.

**HPLC.** Since the resolution is insufficient to separate *syn/anti* and *cis/trans* isomers we expect only two isomers to be separated. The analytical HPLC (RP-18 column and amino column) shows three peaks most probably due to *exo/endo* isomerism with  $t_R = 27.9 + 34.4 + 35.0$  min and  $t_R = 9.6 + 12.7 + 14.9$  min, respectively. Isomerisation studies showed that the three peaks all belong to the product. The purity was found to be 99.5 area % (RP-18 column) and 99.5 area % (amino column). Elemental anal: Found: C 28.81; H 3.14; N 5.74; I 45.92. Calc. for C<sub>33</sub>H<sub>41</sub>I<sub>3</sub>N<sub>6</sub>O<sub>14</sub>: C 28.72; H 2.99; N 6.09; I 45.97. TLC: TLC system on silica: chloroform–methanol–acetic acid = 50:50:5; two spots with  $R_f$ : 0.52 and 0.29. Solubility: in water: ~2 mg ml<sup>-1</sup> measured by TLC. Attempts to make a solution of 2 mgI ml<sup>-1</sup> (4.3 mg ml<sup>-1</sup>) in water failed. Attempts to make a solution of 20 mgI ml<sup>-1</sup> (43 mg ml<sup>-1</sup>) in methanol failed. M.p. 225–230°C. <sup>1</sup>H NMR, <sup>13</sup>C NMR. The observed <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts of 2 are summarized in Table 2 and

Table 5, respectively. The results of the <sup>13</sup>C NMR spectrum simulation of 2 are given in Table 5. UV:  $C = 1.811 \times 10^{-5}$  mol l<sup>-1</sup> (H<sub>2</sub>O);  $\lambda_{max} = 234$  nm;  $E = 1.036$ ;  $\log \epsilon = 4.769$ . IR diffuse reflectance measurement of 1.99% 2 in KBr: 3360, 3230 (m, N–H, O–H), 3070 (m, N–H), 2935, 2880 (w, C–H), 1653, 1630 (vs, C=O primary amide sidechains), 1570 (s, C=O primary amide sidechains); 1435, 1390 (s,  $\delta$  C–H acetyl), 1036 (m). FAB-MS:  $m/z$  (% rel. int.) = 1403 (50,  $M + Na$ ), 1381 (100,  $M + H$ ), 1277 (25,  $M + H + Na - I$ ); 1255 (49,  $M + 2H - I$ ); 1129 (10,  $M + 3H - 2I$ ).

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Table 5. <sup>13</sup>C NMR chemical shifts and assignments of cyclized iodixanol 2 in DMSO-*d*<sub>6</sub>.

| Chemical shift of 2 (ppm) | Simulation <sup>11</sup> (ppm) | Assignment to C-atom No. in Fig. 1 |
|---------------------------|--------------------------------|------------------------------------|
| 170.2–169.3               | 171.4 ± 2.9                    | 18                                 |
| 170.2–169.3               | 170.7 ± 1.7                    | 11 26 30                           |
| 166.7–166.5               | 166.1 ± 0.5                    | 7                                  |
| 151.4–150.4               | 150.0 ± 3.1                    | 9 22 24                            |
| 147.4–146.7               |                                |                                    |
| 141.0                     | 147.6 ± 1.8                    | 20                                 |
| 138.0–137.6               | 142.4 ± 1.7                    | 6                                  |
| 134.6                     | 132.5 ± 3.4                    | 5                                  |
| 131.8–131.5               | 131.8 ± 0.1                    | 7                                  |
| 100.9–99.5                | 98.6 ± 4.4                     | 21 25                              |
| 92.1–91.6                 | 89.9 ± 2.9                     | 23                                 |
| 90.6                      | 89.0 ± 6.8                     | 8                                  |
| 83.7–82.1                 | 82.3 ± 7.6                     | 10                                 |
| 75.6–75.0                 | 72.5 ±                         | 16                                 |
| 72.3–71.6                 |                                |                                    |
| 70.0–69.7                 | 70.4 ± 0.7                     | 2 13 28 32                         |
| 63.8–63.5                 | 64.3 ± 0.6                     | 1 14 29 33                         |
| 50.9–50.2                 | 51.0 ±                         | 15                                 |
| 43.9–43.6                 | 44.0 ±                         | 17                                 |
| 42.5–42.3                 | 43.0 ± 0.6                     | 3 12 27 31                         |
| 22.5; 22.3; 21.7          | 22.9 ± 0.7                     | 19                                 |

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