Smiles Rearrangement in the Synthesis of Di- and Triiodinated 5-(Acylamino)-1,3-Benzenedicarboxamides

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Abstract: Both the number and the position of iodine atoms on the aromatic ring of polyiodinated *N,N'*-bis(2,3-dihydroxypropyl)-5-[2-methylamino-2-oxoethoxy]-1,3-benzenedicarboxamides are crucial to define the structure of the product obtained by treatment with aqueous NaOH. The 2,6-diiodo and the 2,4,6-triiodo derivatives give the corresponding Smiles rearrangement products in 77 and 96% yields, respectively. Conversely, under the same conditions, the 2,4-diiodo isomer predominantly undergoes hydrolysis of the phenoxyacetamide moiety.

INTRODUCTION

The Smiles rearrangement is industrially applied to the preparation of Iomeprol (**1**, *N,N'*-bis(2,3-dihydroxypropyl)-5-[(hydroxyacetyl)methylamino]-2,4,6-triiodo-1,3-benzenedicarboxamide), a widely used X-ray contrast agent, according to a process [1a-d] which is more environmental friendly than the one previously used [2] as most of the steps are run in water, thus avoiding potentially dangerous solvents. The final Smiles rearrangement of **2** in the presence of aqueous NaOH (Scheme **1**) is possible because of: *i*) its stability towards hydrolysis [1a-d,3]; *ii*) the presence of the electron withdrawing iodine atoms in *ortho* and *para* with respect to the position where the rearrangement occurs.
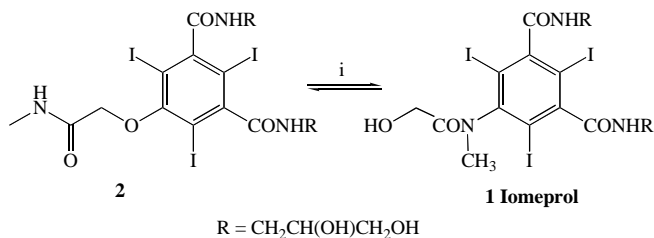
Scheme 1. Reagents and conditions: (i) aq. NaOH, 5 h; 95 °C; 85%.

The second issue has been discussed in several papers [4], although Smiles rearrangements on aromatic substrates deactivated towards nucleophilic substitutions have also been reported [5]. However, it is important to underline that, in cases involving the migration of an aromatic ring from oxygen to amide nitrogen, strictly anhydrous conditions were always used. In this context, excluding the examples we already reported [3], no Smiles rearrangement was ever successfully performed in either water or aqueous media. Our experience on the Smiles rearrangement of *N*-methyl phenoxyacetamides performed with aqueous bases had shown a

completely different behaviour for **2** and the corresponding substrate without the iodine atoms (*i.e. N,N'*-bis(2,3-dihydroxypropyl)-5-[2-methylamino-2-oxoethoxy]-1,3-benzenedicarboxamide) [3]. While the former rearranged almost quantitatively, the latter was completely hydrolysed to the corresponding phenoxyacetic acid. The aims of this work are: *i*) to investigate whether treatment with aqueous NaOH of di-iodinated isomers **3** and **4** (Scheme **2**) leads to the corresponding Smiles rearrangement products (*i.e.* **5** and **6**, which are possible side-products in the synthesis of **1**); *ii*) to elucidate, on the basis of computational studies, the role played by the iodine atoms on the different behaviour shown by the two isomers **3** and **4**.
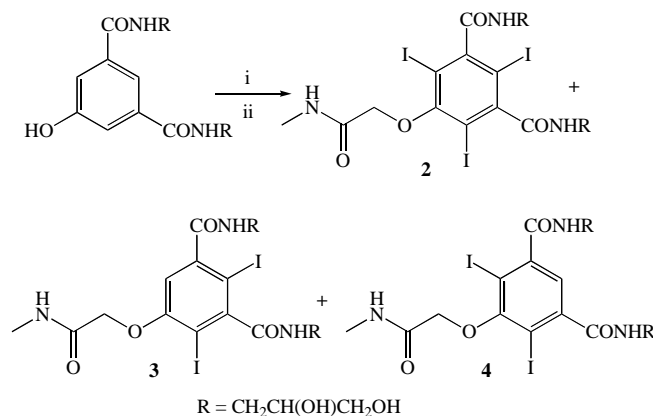
Scheme 2. Reagents and conditions: (i) H₂O, ICl/HCl (1.4 mol equiv.), aq. NaOH to maintain pH 1.5, 3 h, 5-7 °C; (ii) H₂O, aq. NaOH to pH 7, ClCH₂CONHCH₃, 7 h, 95 °C, preparative HPLC separation, 1.1% (**3**), 1.9% (**4**).

RESULTS AND DISCUSSION

The synthesis of the starting materials for the study was performed according to Scheme **2**. Iodination of *N,N'*-bis(2,3-dihydroxypropyl)-5-hydroxy-1,3-benzenedicarboxamide with 1.4 mol equiv. of ICl, instead of the theoretical 3 mol equiv required to afford **1**, gave a mixture which was sub-

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jected to alkylation reaction with *N*-methyl chloroacetamide [1d]. After concentration and filtration of precipitated **2**, a preparative HPLC separation performed on the mother liquor afforded the diiodinated regioisomers **3** and **4**.

The synthesis of **5** and **6**, *i.e.* the reference compounds to test the rearrangement of **3** and **4**, was initially approached by controlled monodeiodination of **1** (Scheme 3). Under the reaction conditions (NaBH$_4$, H$_2$O), the reaction gave a mixture of products, from which the major product **5** could be obtained pure by preparative HPLC [6]. A second approach was then pursued to prepare both diiodinated regioisomers by selective diiodination of **7**.


R = CH$_2$CH(OH)CH$_2$OH
R^1 = CH$_2$CH(OCOCH$_2$OAc)CH$_2$OCOCH$_2$OAc
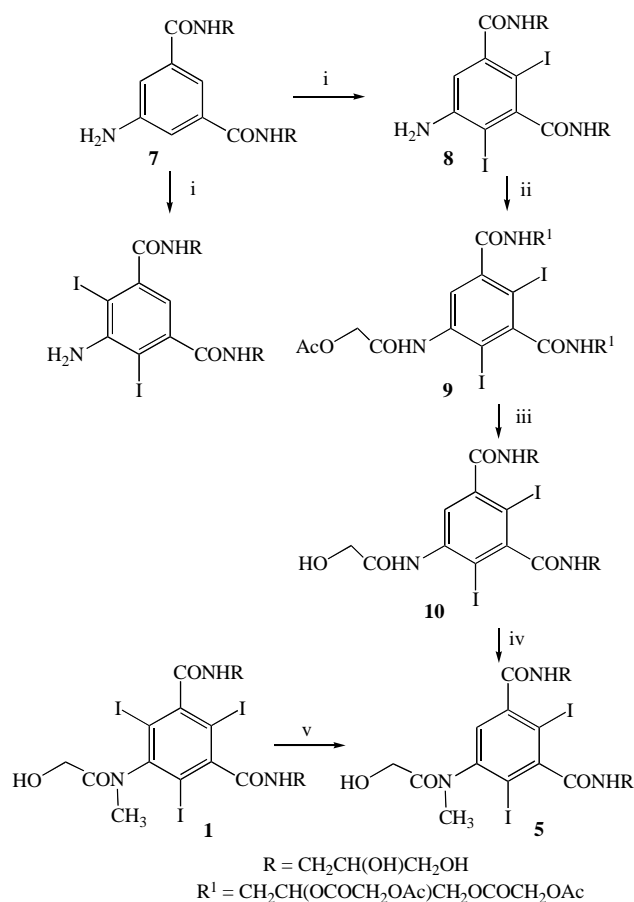
Scheme 3. Reagents and conditions: (i) H$_2$O pH 1, ICl/HCl, 2 h, 1-4 °C, 43%; (ii) DMAc, CH$_3$COOCH$_2$COCl, 11 h, 35 °C; (iii) H$_2$O/MeOH, NaOH to maintain pH 9.5, 12 h, 35 °C, 82% (from **8**); (iv) DMF, MeONa, CH$_3$I, 24 h, 20 °C, 52%; (v) NaBH$_4$ in aq. NaOH, HCl, 1.5 h, 25-50 °C, preparative HPLC separation, 20%.

To our delight, the reaction was highly regioselective and the formation of the 4,6-diiodo regioisomer was not observed and only the 2,4-diiodo isomer **8** was obtained. The latter was peracylated with acetoxyacetyl chloride, deprotected and, finally, *N*-methylated to afford **5**. None of the above routes proved successful to afford compound **6**.

The industrial conditions used [7] to transform **2** into **1** *via* Smiles rearrangement with substoichiometric amount of NaOH give **1** in almost quantitative yield. Indeed, at the end of the reaction the content of **1** is about 96% while **2**, which is in equilibrium, is reduced to 1.5-2%. Very small amounts of the phenoxyacetic acid produced by hydrolysis of **2** and

the benzoxazinone derivative through cyclisation of the hydroxy group of the glycolic moiety of **1** with one of the ortho iodine atoms are also present, but their formation can be kept under control [3]. When we reacted **3** under similar conditions (Scheme 4), it was clear that a Smiles rearrangement was not occurring. Indeed, a substoichiometric amount of NaOH was not sufficient, as it should be for a secondary phenoxyacetamide, and the conversion of **3** could only be completed after addition of 1 mol equiv. of NaOH. However, the main product was **11** [8].

R = CH$_2$CH(OH)CH$_2$OH
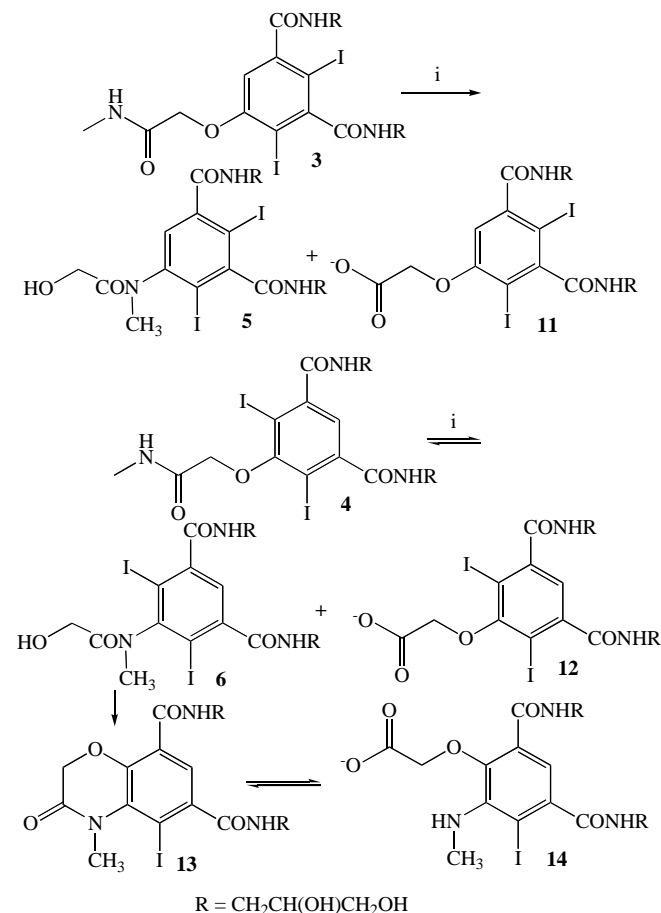
Scheme 4. Reagents and conditions: (i) H$_2$O, NaOH (0.1-1.0 mol equiv.), 3.5-6.0 h, 85-95 °C, 96% (**1**), 77% (**6**), 3% (**5**).

Conversely, Smiles rearrangement of **4** afforded a complex mixture as it was partly hydrolysed to **12**, while the expected rearranged derivative **6** also underwent the subsequent cyclisation to benzoxazinone **13**, in its turn in equilibrium with the amino acid **14**. Few trials [9] aimed at increasing the content of **6** in the reaction mixture met only with moderate success as its yield never exceeded 68%. Although prolonging the reaction time could improve the conversion of **4**, the amount of **6** did not raise because of the transformation into **13** and **14**. However, a striking difference with the reaction outcome of **3** was observed, as **4** underwent rearrangement to a great extent and only a small amount of the starting material was hydrolysed [10]. The results were quite unexpected and we wondered if they could be related to electronic/steric features, in either the starting compounds and/or in the Jackson-Meisenheimer intermediates [4,11]. Computational studies were undertaken and molecules **II**, **III** and **IV**,

deriving from **2**, **3** and **4** by substitution of the carboxamide moieties with hydrogen atoms (Fig. **1**) were selected as model compounds on the basis of following considerations.
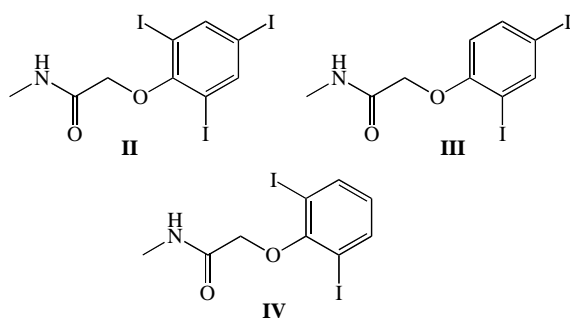

Fig. (1). Model compounds used for computational studies.

It is known that secondary aromatic carboxamides, bearing in *ortho* position one isopropyl substituent, which is as bulky as an iodine atom, adopt a conformation in which the aromatic ring and the plane of the carboxamide lie more or less in a orthogonal array. This prevents the electron delocalization originated from the π conjugated system of the carboxamide residues [12]. Accordingly, since the carboxamides do not contribute to the electron density on the phenyl ring, it was reasonable to replace them with hydrogen atoms. This simplification also saved substantial computational time.

Accordingly, we performed *ab initio* calculations in the gas phase, at HF/6-31G* and at HF/6-31+G** levels of theory, to obtain the relative values of both potentials (ΔE) and Gibbs free energies (ΔG) of: *i*) the anions deriving from the abstraction of the NH proton of the *N*-methylamide moiety (**An**); *ii*) the transition states (**TS**); *iii*) the Jackson-Meisenheimer intermediates (**JM**). We also monitored: *i*) the interatomic distance between the C_5 carbon atom and the nitrogen atom of the methylamide moiety, *i.e.* the electrophilic and the nucleophilic sites, respectively, involved in the intramolecular cyclisation; *ii*) the C_5 atomic charge, derived from electrostatic potential. The results, at both levels of theory, are reported in Table **1**.

A few points deserve some comments: *i*) the atomic charge at C_5 depends on both the number and the position of

the iodine atoms on the benzene ring. Moving from the triiodinated compound **II** to the diiodinated **IV**, the charge diminishes as the inductive effect of the *para* iodine atom is lacking. The trend is amplified in the diiodinated compound **III**, because the lack of the *ortho* iodine atom is not compensated by the presence of the *para* one, which has a minor effect on the atomic charge; *ii*) the initial C_5-N distance, especially in **An** species, is higher in **III** than in **II** and **IV**; *iii*) both the potential (ΔE) and the free energy (ΔG) hypersurfaces predict significant barriers exist for all the reactions from the reactants (**An**) to the transition states (**TS**). However, the barrier for compound **III** is higher and, furthermore, only the reactions of **II** and **IV** are thermodynamically favourable. The afore mentioned differences obviously depend on the presence of the bulky iodine atom in *ortho* position, which is crucial from a sterical point of view too. Indeed, in compound **III**, bearing an hydrogen atom in the *ortho* position, the side chain can occupy a conformational space otherwise sterically precluded in **II** and **IV** (Fig. **2**).

Fig. (**2**) gives a picture of the reaction coordinate referred to compounds **II** and **III**, while those of compound **IV**, closely resembling that of **II**, is not shown. It is worth noting that the geometry of **An II** (and **An IV**) is more "product like" than the one of **An III**. Indeed, the bulky iodine atoms in positions 4 and 6 favour the perpendicular arrangement with respect to the benzene ring which is necessary for the formation of the five member ring in the Jackson-Meisenheimer intermediate. Despite the adopted approximations, *i.e.* use of simplified molecules and gas phase reactions for the calculations, the results allow the interpretation of the behaviour of compounds **2-4** as follows: *i*) turning from **2** to **4** the lack of the *para* iodine makes the rearrangement slower and in the meantime some hydrolysis takes place; *ii*) the formation of the Jackson-Meisenheimer intermediate is more difficult for **3** than for **2** and **4**, as the lack of the *ortho* iodine atom allows an arrangement of the side chain that increases the distance between the reactive centres. Moreover, the charge of C_5 is lower and the energetic barrier becomes higher: the rearrangement becomes negligible and hydrolysis is the preferred reaction.

CONCLUSIONS

In diiodinated *N*-methylphenoxyacetamides, both the number and the position of the iodine atoms in the benzene

Table 1. C_5 Atomic Charges, C_5-N Distances, Relative Electronic and Gibbs Free Energies as Obtained from *ab initio* Calculations

Species	C5-N Distance (Å)[a,b]	C5 Atomic Charge[a,b] (e.u.)	ΔE[a,b] (kcal. mol⁻¹)	ΔG[a] (kcal. mol⁻¹)
An II	2.71 (2.76)	1.41 (1.55)	0.0 (0.0)	0.0
TS II	2.03 (2.00)	1.63 (1.73)	7.6 (10.0)	10.4
JM II	1.47 (1.47)		-12.9 (-8.6)	-7.7
An III	3.28 (3.21)	0.93 (0.95)	0.0 (0.0)	0.0
TS III	1.97 (1.95)	1.08 (1.21)	18.2 (20.0)	18.1
JM III	1.48 (1.49)		4.0 (5.4)	4.0
An IV	2.75 (2.78)	1.20 (1.34)	0.0 (0.0)	0.0
TS IV	2.00 (1.98)	1.48 (1.71)	10.3 (11.6)	12.7
JM IV	1.47 (1.47)		-8.2 (-5.0)	-2.6

[a]Calculated at HF/6-31G* level. [b](In parentheses) calculated at HF/6-31+G** level.
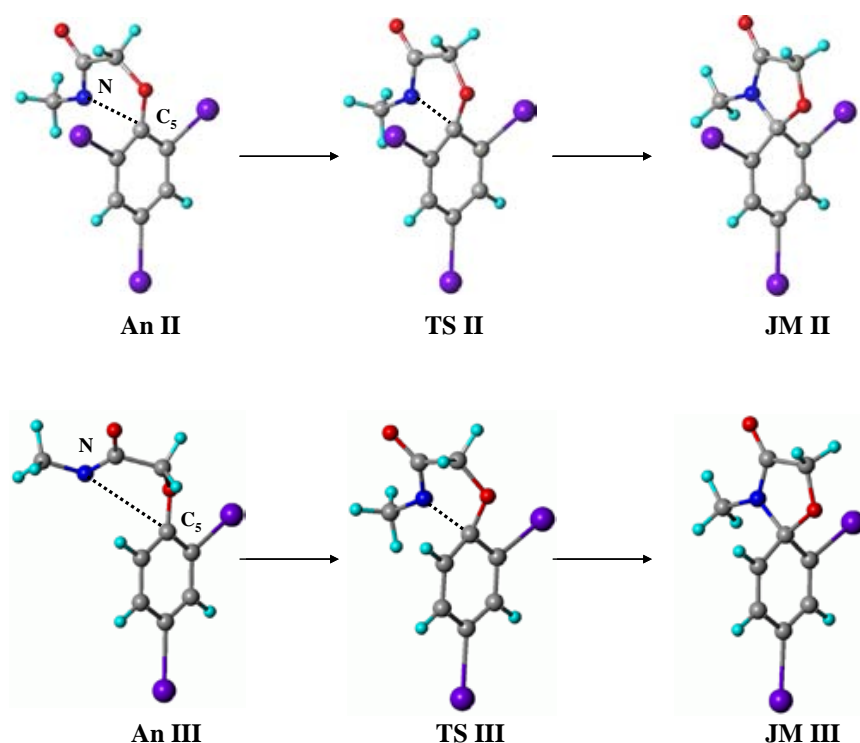
Fig. (2). HF/6-31+G** optimized structures for the cyclisation reactions of II and III.

ring are crucial to address the reaction in aqueous NaOH to either Smiles rearrangement or hydrolysis of the *N*-methylamide moiety. The 2,4-diiodo isomer, **3,** affords only the hydrolysis product whereas for the 2,6-diiodo isomer, **4,** the rearrangement occurs to a large extent. The different behaviour of the two isomers can be explained on the basis of steric and electronic differences. Although the present study is limited to substrates **3** and **4** we think that it is indicative of a general behaviour. Accordingly, in order to synthesise iodinated acetanilides through Smiles rearrangement of the related phenoxyacetamides in water, it is mandatory that the latter have three iodine atoms *ortho* and *para* to the ipso aromatic carbon. Two iodine atoms are still sufficient, provided that they are in the *ortho* positions. The lack of one iodine atom in ortho position preferentially lead to the hydrolysis of the phenoxyacetamide.

EXPERIMENTAL SECTION

General Information

All reagents and solvents, obtained from commercial sources, were used without further purification. Melting points (°C, uncorrected) were measured with a Büchi 510 instrument. TLC were carried out on 60 F_{254} silica gel plates using 6:3:1 $CHCl_3/CH_3OH/25\%$ NH_4OH as the eluent, UV light (254 nm) and alkaline $KMnO_4$ for the visualization. Preparative HPLC separations were performed with a Merck KGaA Prepbar 100 instrument using a Prepbar-Lichroprep RP18 column (250 x 50 mm, 10 μm) and methods A or B. Method A: injection: 20 mL of a 90 mg/mL solution in H_2O; mobile phase: a) H_2O, b) 1:1 H_2O/CH_3CN; timetable as follows: the percentage of b) is held 5 for 15 min, then increased linearly to reach 35 at 40 min, maintained for 15 min, increased linearly to reach 50 at 60 min and maintained

for 10 min; flow rate: 30 mL/min; UV detection: 240 nm. Method B: injection: 200 mL of a 12 mg/mL solution in H_2O; mobile phase: a) H_2O, b) 99.5:0.5 H_2O/CH_3CN; timetable as follows: the percentage of b) is held 0 for 40 min, then switched to 100 and maintained for 20 min; flow rate: 30 mL/min for 10 min, then 70 mL/min for 30 min and, again 30 mL/min for 20 min; UV detection: 254 nm. Analytical HPLC analyses were performed with a Hewlett-Packard HP 1100 instrument using methods A, B, C and D. Method A: Zorbax SB-C18 column (150 x 4.6 mm, 3.5 μm); oven temperature: 60 °C; injection: 20 μL of a 1 mg/mL solution in H_2O; mobile phase: a) 0.01 M KH_2PO_4, b) 1:1 0.01 M KH_2PO_4/CH_3OH; timetable as follows: the percentage of b) is held 5 for 10 min, then increased linearly to reach 35 at 25 min and maintained for 10; flow rate: 1 mL/min; UV detection: 240 nm. Method B: Superspher RP-18 column (250 x 4.6 mm, 4 μm); oven temperature: 58 °C; injection: 10 μL of a 1 mg/mL solution in H_2O; mobile phase: a) 0.0125 M KH_2PO_4, b) 55:40:3 $H_2O/CH_3CN/2$-BuOH; timetable as follows: the percentage of b) is held 3 for 5 min, then increased linearly to reach 25 at 15 min and 75 at 30 min; flow rate 1.2 mL/min; UV detection at 245 nm. Method C: Lichrospher 100 RP-18 column (250 x 4 mm, 5 μm); oven temperature: 30 °C; injection: 10 μL of a 1 mg/mL solution in H_2O; mobile phase: a) 0.017 M H_3PO_4, b) 1:1 H_2O/CH_3CN; timetable as follows: the percentage of b) is held 5 for 5 min, then increased linearly to reach 10 at 10 min, held at 10 for 5 min then increased linearly to reach 50 at 20 min and held for 5 min; flow rate: 1 mL/min; UV detection: 245 nm. Method D: Kromasil C4 column (250 x 4.6 mm, 5 μm); oven temperature: 40 °C; injection: 15 μL of a 1 mg/mL solution in H_2O; mobile phase: a) 0.017 M H_3PO_4, b) CH_3CN; timetable as follows: the percentage of b) is held 0 for 8 min, then increased linearly to reach 50 at 40 min; flow rate: 1.5

mL/min; UV detection: 254 nm. Mass spectra were acquired on a TSQ700 ThermoFinnigan Spectrometer using CH_3OH as the solvent. ^{13}C-NMR spectra were recorded at 298 K and 100.61 MHz with a Bruker DRX 400 spectrometer. The chemical shifts are given in δ units (ppm) relative to TMS (δ=0). ^{1}H-NMR spectra are not reported since they are not useful for the assignment of the structures due to the broadness and extreme overlapping of the signals. Numbering of the basic skeleton of the molecule and mention of the neighbouring groups have been adopted to unambiguously identify the carbon atoms. Elemental analyses were carried out at the Redox Laboratories (Monza, Milano, Italy); the results are referred to the dry compound while the water percentage (K.F.) is reported in parentheses.

N,N'-Bis(2,3-dihydroxypropyl)-2,4-diiodo-5-[2-(methylamino)-2-oxoethoxy]-1,3-benzenedicarboxamide (3) and N,N'-bis(2,3-dihydroxypropyl)-4,6-diiodo-5-[2-(methylamino)-2-oxoethoxy]-1,3-benzenedicarboxamide (4)

A solution of *N,N'*-bis(2,3-dihydroxypropyl)-5-hydroxy-1,3-benzenedicarboxamide [1d] (145 g; 0.44 mol) in water (3 L) was iodinated at 5-7 °C with a 56.3% solution of ICl in 15% aq HCl, maintaining the pH at 1.5 by addition of 2 M NaOH. The selectivity was poor as during the addition of the iodinating agent we mainly observed the presence of the starting material and *N,N'*-bis(2,3-dihydroxypropyl)-5-hydroxy-2,4,6-triiodo-1,3-benzenedicarboxamide. Only small amounts of diiodinated products (LC/MS), *i.e.* the precursors of **3** and **4**, were detected. The reaction was stopped after 3 h and the addition of 1.4 mol equiv. of ICl in HCl, when their sum was around 10%. The reaction mixture was neutralised with 30% aq NaOH, concentrated to about 0.7 L and precipitated *N,N'*-bis(2,3-dihydroxypropyl)-5-hydroxy-2,4,6-triiodo-1,3-benzenedicarboxamide was filtered. The mother liquor was subjected to alkylation with *N*-methylchloroacetamide, according to the literature.[1d] After filtration of precipitated **2**, the mother liquor was concentrated to about 0.2 L, again filtered to get rid of some more **2** and subjected to preparative HPLC (method A). The crop (5.7 g) obtained by evaporation of the fractions containing almost pure **3** was dissolved in H_2O (75 mL) and the solution eluted through ion exchange resins (Amberlite Amberjet® 1200H, 9 mL and Relite ® MG1, 6 mL). The neutral eluate was evaporated to dryness and the waxy residue (5.1 g) was crystallised with EtOH (45 mL) to afford **3** (3.2 g; 4.9 mmol). Yield 1.1%. Mp 219-222 °C; TLC Rf 0.44; HPLC 99.4% (r.t. 14.0 min with method A or 11.5 min with method B); ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.4 (CH$_3$), 43.6 (NHCH$_2$), 64.7 (CH$_2$OH), 69.4 (CH$_2$O), 71.0 (CHOH), 83.1 and 88.4 (C2 and C4), 112.5 (C6), 145.7 (C3), 151.0 (C1), 157.5 (C5), 168.0, 169.4 and 170.5 (CH$_2$CO and ArCO); MS *m/z* (ESI) 674 (M + Na)$^+$, 1325 (2M + Na)$^+$; Anal. Calcd for $C_{17}H_{23}I_2N_3O_8$: C, 31.36; H, 3.56; I, 38.98; N, 6.45. Found: C, 31.41; H, 3.67; I, 38.90; N, 6.33; (H$_2$O, 2.18). The crop (6.6 g) obtained by evaporation of the fractions from preparative HPLC containing almost pure **4** was dissolved in H_2O (75 mL) and the solution eluted through ion exchange resins (Amberlite Amberjet® 1200H, 9 mL and Relite ® MG1, 6 mL). The neutral eluate was evaporated to dryness and the waxy residue (10.6 g) was crystallised with EtOH (66 mL) to afford **4** (5.6 g; 8.6 mmol). Yield 1.9%. Mp 253-254 °C; TLC Rf 0.41; HPLC 99.0% (r.t. 20.8 min with method A or 14.5 min with method B); ^{13}C NMR (100 MHz, DMSO-d_6) δ

26.5 (CH$_3$), 43.6 (NHCH$_2$), 64.7 (CH$_2$OH and CH$_2$O), 71.1 (CHOH), 92.5 (C4 and C6), 123.8 (C2), 148.2 (C1 and C3), 157.4 (C5), 167.6 and 168.8 (CH$_2$CO and ArCO); MS *m/z* (ESI) 674 (M + Na)$^+$, 1325 (2M + Na)$^+$; Anal. Calcd for $C_{17}H_{23}I_2N_3O_8$: C, 31.36; H, 3.56; I, 38.98; N, 6.45. Found: C, 31.48; H, 3.75; I, 38.86; N, 6.26; (H$_2$O, 3.22).

N,N'-Bis(2,3-dihydroxypropyl)-5-[(hydroxyacetyl)methylamino]-2,4-diiodo-1,3-benzenedicarboxamide (5) (from 1)

NaBH$_4$ (0.76 g; 20 mmol) was dissolved in a solution of *N,N'*-bis(2,3-dihydroxypropyl)-5-[(hydroxyacetyl)methylamino]-2,4,6-triiodo-1,3-benzenedicarboxamide (**2**) (7 g; 9 mmol) in 2 M NaOH (100 mL), then 37% HCl (19 mL) was dropped in within 3 h. A brisk hydrogen evolution was observed and the temperature raised from 25 to 50 °C. After further 1.5 h the yellow solution was concentrated to half volume and directly used for a preparative HPLC (method A) separation to afford **5** (1.2 g; 1.84 mmol). Yield 20.4%. Mp 160-165 °C; HPLC 99.2% (r.t. 6.8 min with method B); ^{13}C NMR (100 MHz, DMSO-d_6); δ 35.0 (CH$_3$), 42.7 (NHCH$_2$), 60.9 (CH$_2$CO), 63.8 (CH$_2$OH), 70.2 (CHOH), 92.6 (C2), 100.9 (C4), 127.1 (C6), 144.0, 145.3 and 151.3 (C1, C3 and C5), 168.0, 169.7 and 170.7 (ArCO and CH$_2$CO); MS *m/z* (ESI) 674 (M + Na)$^+$, 690 (M + K)$^+$; Anal. Calcd for $C_{17}H_{23}I_2N_3O_8$: C, 31.36; H, 3.56; I, 38.98; N, 6.45. Found: C, 31.60; H, 3.71; I, 36.63; N, 6.31; (H$_2$O 2.87).

5-Amino-N,N'-bis(2,3-dihydroxypropyl)-2,4-diiodo-1,3-benzenedicarboxamide (8)

A 56.3% solution of ICl in 15% aq HCl (20.5 g; 71 mmol) was dropped over 2 h into a solution of **7·HCl** (13 g; 35.7 mmol) in H_2O (300 mL) and 1 M HCl (40 mL; 40 mmol) stirred at 1-4 °C. At the end of the addition, the reaction mixture at pH 1 was evaporated under vacuum and the residue was purified by preparative HPLC (method B) to afford **8** (8.8 g; 15.3 mmol). Yield 43%. Mp 170 °C; TLC: Rf 0.30; HPLC 99.0% (method C); ^{13}C NMR (100 MHz, D$_2$O); 44.9 (NHCH$_2$), 66.4 (CH$_2$OH), 72.7 (CHOH), 76.9 (C2), 86.3 (C4), 116.7 (C6), 145.5 (C5), 150.4 and 151.2 (C1 and C3), 174.5 and 175.4 (ArCO); MS *m/z* (ESI) 580 (M + H)$^+$, 602 (M + Na)$^+$; Anal. Calcd for $C_{14}H_{19}I_2N_3O_6$: C, 29.03; H, 3.31; I, 43.83; N, 7.26. Found: C, 29.31; H, 3.42; I, 42.93; N, 7.47; (H$_2$O 6.36).

N,N'-Bis(2,3-dihydroxypropyl)-5-(hydroxyacetyl)amino-2,4-diiodo-1,3-benzenedicarboxamide (10)

Acetoxyacetyl chloride (11.0 g; 80.6 mmol) was dropped over 20 min into a solution of **8** (4.8 g; 8.29 mmol) in DMA (50 mL) stirred at rt. After 11 h at 35 °C, the reaction mixture was evaporated under vacuum, the residue dissolved in CHCl$_3$ (100 mL) and the solution extracted with H_2O (3x100 mL). The separated organic phase was washed with 8% NaHCO$_3$ (3x75 mL), dried over Na$_2$SO$_4$ and evaporated. The residue containing crude **9** was dissolved with 1.5/1 MeOH/H_2O (100 mL) then 1 M NaOH (25 mL; 25 mmol) was dropped into the solution stirred at 35 °C, at such a rate to maintain pH 9.5 [13]. After 12 h at 35 °C the solution was loaded onto ion exchange resin columns (Duolite® C20MB, 20 mL and Duolite® A30B, 22 mL) which were eluted with water. The eluate was concentrated to about 30 mL, then EtOH (100 mL) was added. After 15 h at 10 °C the precipitate was filtered, washed with EtOH (2x10 mL) and dried to afford **10** (4.35 g; 6.83 mmol). Yield 82%. Mp 235 °C; TLC:

Rf 0.23; HPLC 98.2% (method D); ^{13}C NMR (100 MHz, DMSO-d$_6$); δ 42.6 (NHCH$_2$), 61.7 (*C*H$_2$CO), 63.9 (CH$_2$OH), 70.2 (CHOH), 85.7 (C2), 90.3 (C4), 118.5 (C6), 139.3 (C5), 144.6 (C3), 149.9 (C1), 168.7, 169.9 and 171.6 (ArCO and CH$_2$*C*O); MS *m/z* (ESI) 638 (M + H)$^+$, 660 (M + Na)$^+$; Anal. Calcd for C$_{16}$H$_{21}$I$_2$N$_3$O$_8$: C, 30.16; H, 3.32; I, 39.83; N, 6.59. Found: C, 30.42; H, 3.34; I, 39.88; N, 6.68; (H$_2$O 1.2).

N,N'-Bis(2,3-dihydroxypropyl)-5-[(hydroxyacetyl)methyl-amino]-2,4-diiodo-1,3-benzenedicarboxamide (5) (from 10)

A solution of **10** (3.8 g; 5.96 mmol) in DMF (35 mL) was added with 1.1 M MeONa in MeOH (5.4 mL; 5.96 mmol) then kept under vacuum to get rid of MeOH. A solution of iodomethane (0.93 g; 6.56 mmol) in DMF (5 mL) was dropped in 20 min and, after 24 h at rt, the solvent was evaporated to obtain a residue which was dissolved in H$_2$O (100 mL). After extraction with CH$_2$Cl$_2$ (6x100 mL), the aqueous phase was loaded onto ion exchange resin columns (Duolite$^®$ C20MB, 15 mL and Amberlite$^®$ IRA 400, 15 mL) which were eluted with water. The crude obtained from evaporation to dryness of the eluate was purified by silica gel flash-chromatography (6:4:1 CHCl$_3$/CH$_3$OH/25% NH$_4$OH) and the recovered sample was further crystallised with EtOH (30 mL). After 24 h at rt, the precipitate was filtered, washed with EtOH (10 mL) and dried to afford **5** as a white product containing 2 mol of EtOH of crystallisation (2.3 g; 3.09 mmol). Yield 52%. Mp 260 °C (dec.); HPLC 99.4% (r.t. 6.8 min with method B); ^{13}C NMR (100 MHz, D$_2$O); δ 19.6 (*C*H$_3$CH$_2$OH), 39.3 (CH$_3$), 44.9 (NHCH$_2$), 60.1 (CH$_3$*C*H$_2$OH), 63.5 (*C*H$_2$CO), 66.2 (CH$_2$OH), 72.6 (CHOH), 93.8 (C2), 102.6 (C4), 129.7 (C6), 147.2, 149.2 and 152.9 (C1, C3 and C5), 173.2, 174.9 and 176.1 (ArCO and CH$_2$*C*O); MS *m/z* (ESI) 674 (M + Na)$^+$, 690 (M + K)$^+$; Anal. Calcd for C$_{17}$H$_{23}$I$_2$N$_3$O$_8$ 2C$_2$H$_6$O: C, 33.91; H, 4.74; I, 34.12; N, 5.65. Found: C, 33.80; H, 4.57; I, 34.34; N, 5.70; (H$_2$O 0.8).

N,N'-Bis(2,3-dihydroxypropyl)-5-[(hydroxyacetyl)methyl-amino]-4,6-diiodo-1,3-benzenedicarboxamide (6)

To a solution of **4** (4.17 g; 6.42 mmol) in H$_2$O (42 mL) heated at 85 °C 1 M NaOH (963 + 642 µL; 1.605 mmol total) was added at 0 and 1.5 h. After further 2 h at the same temperature, the solution was neutralised (1 M HCl; 0.3 mL) and loaded onto an Amberlite$^®$ 16.00 XAD column (240 mL) which was eluted at first with H$_2$O (2.1 L) then with 95/5 H$_2$O/CH$_3$CN (20 x 150 mL). The fractions containing the desired product were evaporated to obtain a crop (3.0 g) which was crystallised with 2-BuOH (24 mL) to afford **6** (1.87 g; 2.87 mmol). Yield 44.8%. Mp 111-114 °C; HPLC 95% (r.t. 11.8 min with method B); ^{13}C NMR (100 MHz, D$_2$O) δ 34.8 (CH$_3$), 42.7 (NHCH$_2$), 61.6 (*C*H$_2$CO), 63.8 (CH$_2$OH), 70.4 (CHOH), 99.3 (C4 and C6), 125.9 (C2), 145.3 (C1 and C3), 146.6 (C5), 171.2 and 173.4 (ArCO and CH$_2$*C*O); MS *m/z* (ESI) 674 (M + Na)$^+$, 690 (M + K)$^+$; Anal. Calcd for C$_{17}$H$_{23}$I$_2$N$_3$O$_8$: C, 31.36; H, 3.56; I, 38.98; N, 6.45. Found: C, 31.51; H, 3.92; I, 38.59; N, 6.38; (H$_2$O, 4.12).

N,N'-Bis(2,3-dihydroxypropyl)-3,4-dihydro-5-iodo-4-methyl-3-oxo-2H-1,4-benzoxazine-6,8-dicarboxamide (13)

The fractions discharged from the chromatography to obtain **6** were concentrated to about 30 mL and subjected to preparative HPLC to afford **13** (0.12 g). Mp 96-98 °C; HPLC

100% (r.t. 16.5 min with method B); ^{13}C NMR (100 MHz, D$_2$O) δ 37.2 (CH$_3$), 42.2 (NHCH$_2$), 63.4 (CH$_2$OH), 68.9 (C2), 69.9 (CHOH), 87.1 (C5), 122.1 (C8), 124.0 (C7), 135.8 and 139.0 (C6 and C4a), 148.1 (C8a), 166.1, 169.3 and 172.2 (ArCO and C3); MS *m/z* (ESI) 546 (M + Na)$^+$, 1070 (2M + Na)$^+$; Anal. Calcd for C$_{17}$H$_{22}$IN$_3$O$_8$: C, 39.02; H, 4.24; I, 24.25; N, 8.03. Found: C, 38.96; H, 4.21; I, 24.03; N, 7.99; (H$_2$O, 5.51).

N,N'-Bis(2,3-dihydroxypropyl)-6-[2-(carboxy)ethoxy]-4-iodo-5-(methylamino)-1,3-benzenedicarboxamide (14)

Although we did not isolate the compound because of the very low amount of **13** at disposal, we are confident that **14** has the proposed structure because of the following experiment. A solution of **13** (10 mg; 0.019 mmol) in H$_2$O (0.5 mL) was diluted with 2 M NaOH (95.5 µL; 0.19 mmol) and heated to 90 °C for 1 h. The starting material (HPLC r.t. 16.5 min with method B) was almost quantitatively (> 97%) transformed into a single product, **14** (r.t. 7.6 min), which we believe originated by hydrolysis of the lactam bond. Indeed, after cooling to rt and acidification to pH 1 with 6 M HCl, the lactam moiety was quickly reformed to quantitatively give **13**.

Computational Methods

RHF-SCF gas-phase calculations were carried out with either GAUSSIAN 94 [14] or GAUSSIAN 98 [15] programs. Full geometry optimizations were performed using both 6-31G* and, as the inclusion of diffuse functions has been shown important for anionic species [16], 6-31+G** basis sets; the basis set for the iodine atoms was that associated with the pseudopotential [17], with a standard double-ζ LANL2DZ contraction, as internally-stored in the programs. The reliability of the stationary points and the thermodynamics properties (calculated at 298 K and 1 atm) were evaluated by means of the vibrational frequencies at the HF/6-31G* and, limiting to the structures of the transition states (**TS**) due to the very time-consuming calculation, at HF/6-31+G** level of theory. The atomic charges were derived from electrostatic potential using the Merz-Kollman-Singh scheme [18,19].

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[6] Subsequently, when we had at disposal an authentic sample of **6** obtained by rearrangement and reviewed the HPLC chromatograms of the deiodination, we found that a compound with the same retention time was present in the mixture, although in extremely small amount.

[7] The reaction is performed at 95 °C in H$_2$O with NaOH (0.1-1.0 mol equiv. with respect to **2**) [1b].

[8]   In the few mg scale reactions performed on **3**, varying temperature (75-100 °C) and quantity of NaOH (0.1-1.0 mol equiv.) in order to eventually find more proper conditions, evolution of methylamine was observed in any case and at least 1 mol equiv. of base was necessary for the completion. The content of **5**, which was identified by LC/MS, in the reaction mixtures was never higher than 3% and the almost unique reaction was hydrolysis to **11**. Accordingly, the recovery of **5** from the reaction mixture was not pursued and **5** was prepared by an independent synthesis (see Experimental).

[9]   Some trials on a few mg scale of **4** were performed varying the reaction conditions. At 100 °C with 0.25 mol equiv. of NaOH the maximum conversion was obtained at 30 min when the HPLC composition of the mixture, %, was: 66.2 (**6**), 9.5 (**4**), 10.5 (**13**), 2.9 (**14**) and 5.1 (**12**). At 85 °C; with 0.2 mol equiv. of NaOH the best result was achieved at 2 h with the following HPLC composition, %: 65.2 (**6**), 12.9 (**4**), 6.7 (**13**), 2.8 (**14**) and 5.6 (**12**). Finally, at 65 °C in MeOH with KOH/MeOH (0.25 mol equiv.) no appreciable result was observed because the starting material was suspended. Even after DMF addition in order to have a solution, the reaction was sluggish and the same by-products were observed in large quantities. In the preparative scale reaction (see Experimental) the base was added in two portions to avoid a too high pH in the mixture. This led to a mixture with the following HPLC composition, %: 68.1 (**6**), 12.3 (**4**), 5.6 (**13**), 2.1 (**14**) and 6.8 (**12**). Accordingly, it is likely that the reaction could be better performed in a buffer around pH 10.

[10]   The sum of the HPLC content (%) of compounds **6**, **13** and **14** (76.7 mean) [9] gives a rough indication of the extent of rearrangement of **4**, as the three products derive from such reaction.

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