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Formation of *N,O*-Acetals in the Production of X-ray Contrast Agents

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Supporting Information

ABSTRACT: Acetylation of **1** with acetic anhydride, an important step in the preparation of iohexol, gave the corresponding acetanilide and minor amounts of a few byproducts, whose structures have been elucidated. Among the byproducts were some 1,3-oxazolidines which survived when the reaction was quenched by adding methanol and water under strong acidic conditions.

1. INTRODUCTION

Process development has always been in focus in the chemical industry, but with increasing production costs in recent years due to the price of chemicals, the improvement of chemical processes has become more important than ever. Many aspects are important to consider in such an endeavor, but two issues are particularly important to address, chemical yield and byproduct formation. Overall the best way to improve a chemical process is by reducing the latter in every step. Lower levels of impurities may not only pave the way for more efficient purification; in the best cases it may even be possible to skip one such step and significantly reduce production costs without compromising product quality.

To reduce the level of impurities formed in a synthesis, it is important to know the structures of the byproducts and understand how they are formed and react. This approach has successfully been applied to improve many industrial syntheses. One such example is the acetylation of the aniline derivative 5-amino-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide (**1**) to make the corresponding acetamide, 5-acetamido-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide (**2**) (Scheme 1), which is a key intermediate in the production of iohexol, the active ingredient in the X-ray contrast agent Omnipaque. Over the years thorough studies of byproduct formation and reactivity have made it possible to manufacture pure **2** both simpler and more efficiently.

Although process development increased the yield and purity of **2**, there were still unknown impurities present that would be beneficial to study with the aim, ultimately, to prevent their formation completely. The presence of such impurities is illustrated in Figure 1, which shows a typical HPLC chromatogram of an unpurified sample of **2**. The structures of all compounds with a retention time shorter than 12 min have been known for some time, whereas several of those giving rise to the peaks with retention times between 15 and 22 min were unknown. In order to improve the production of **2** further, it was therefore decided to study the latter group of impurities more thoroughly, and our findings are reported here.

2. RESULTS AND DISCUSSION

In order to get an indication of the structural differences between the impurities under investigation, here a mixture containing these compounds was analyzed by LC-MS. Somewhat to our surprise the compounds denoted **a**–**f** in Figure 1 gave rise to two different mass spectra only; the spectra of compounds **a**–**d** were in essence identical and so were those of compounds **e** and **f**. This observation clearly indicates that the six compounds constitute two groups of isomers, and we decided to focus our attention on the isolation and structure elucidation of these impurities.

2.1. Byproducts e and f. In order to obtain impurities **e** and **f** in reasonable amounts for structure elucidation, a reaction mixture from the manufacturing process was used to isolate the two impurities. Unfortunately, neither compound could be obtained pure in reasonable quantities even after multiple preparative HPLC separations, but by investigating three mixtures of the two compounds (approximate ratios of 1:9, 1:1, and 9:1) by two-dimensional NMR techniques, it could be concluded that **e** and **f** are stereoisomers of 3-acetamido-*N*-(2,3-dihydroxypropyl)-5-(5-hydroxymethyl-2,2-dimethyloxazolidine-3-carbonyl)-2,4,6-triiodobenz-amide (**3**) (Figure 2).

The formation of **3** can be explained by a two-step transformation from **1**, i.e., acetylation of the amino group and *N*,*O*-ketalization of one of the benzamide moieties. The latter reaction requires the presence of acetone to occur, which is a significant observation because acetone was not added to the reaction mixture. Therefore, acetone must have been generated in situ or been present as an impurity in one or several of the reagents (vide infra). It is also noteworthy that an oxazolidine and not a 1,3-dioxolane is obtained, because the latter group of compounds should be formed much more easily than the former, by reaction with the vicinal diol in the side chain.[1]

2.2. Byproducts a–d. To elucidate the structure(s) of byproducts **a**–**d**, another reaction mixture from the manufacturing process was used for the isolation, which was performed in three steps by preparative HPLC. It was necessary to use an acidified eluent in the isolation to achieve stable retention times for compounds **a**–**d**, and this indicates that the byproducts contain a carboxylic acid moiety. Compounds **a** and **b** were obtained with purities of 93 % and 97 %, respectively. Unfortunately, compounds **b** and **c** could not be obtained pure

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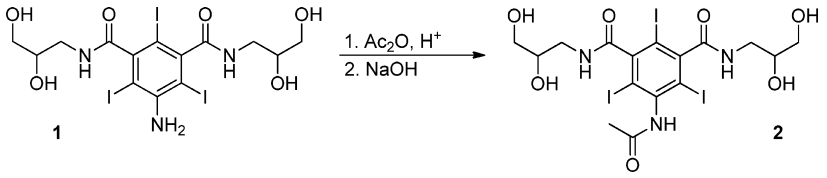
Scheme 1. Acetylation step in the manufacturing of iohexol



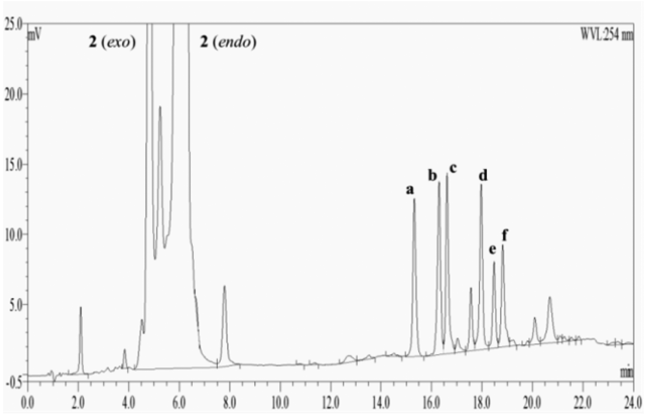


Figure 1. A typical impurity profile prior to purification of **2**. In this study the focus has been on impurities **a**–**f**.

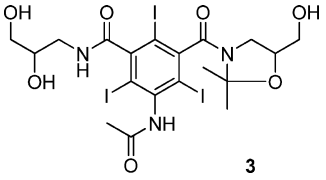


Figure 2. 1,3-Oxazolidine **3**, which appeared to be formed as a stereoisomeric mixture when **1** was acetylated under standard conditions.

due to very similar retention under a variety of conditions, but two mixtures with a **b**:**c** ratio of approximately 3:2 and 1:9 were obtained.

Notably, most of the IR and NMR spectra of **a**, **d**, and the two mixtures of **b** and **c** were so similar to parts of the corresponding spectra of the two isomers of oxazolidine **3** that the structures of the four impurities were believed to be almost identical to that of **3**. The only important differences were the presence of a broad IR absorption in the 3300–2800 cm^{-1} region, indicative of a COOH group,[2] and an additional carbonyl signal in the 170–171 ppm region in the ^{13}C NMR spectrum, which also indicates the presence of a COOH moiety.[3] This, combined with the absence of one methyl signal, means it could be concluded that **a**–**d** are stereoisomers of 2-(3-(3-acetamido-5-(2,3-dihydroxypropylcarbamoyl)-2,4,6-triiodobenzoyl)-5-hydroxymethyl-2-methyloxazolidin-2-yl)acetic acid (**4**) (Figure 3).

Just like for compound **3** the formation of **4** can be explained by a two-step transformation from **1**, i.e., acetylation of the amino group and *N*,*O*-ketalization at one of the benzamide moieties to give a five-membered ring. The latter step requires the presence of acetoacetic acid or a corresponding ester of this acid, and this is a significant observation since this acid was not added to the reaction mixture (vide infra). Again it is noteworthy that **4** is an oxazolidine and not a 1,3-dioxolane,

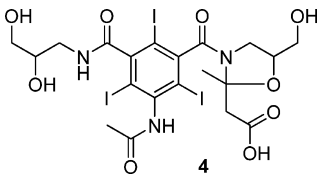


Figure 3. 1,3-Oxazolidine **4**, which appeared to be formed as a stereoisomeric mixture when **1** was acetylated under standard conditions.

because the latter group of compounds is in general formed much more easily than the former.[1]

2.3. Impurities in Reagents. The formation of impurities **3** and **4** in the synthesis of **2** in the production of iohexol is intriguing since acetone and acetoacetic acid are not used in the process at all. One explanation for the formation of these impurities may be that one or several of the reagents applied contain a low level of acetone and acetoacetic acid. All of the reagents were therefore analysed thoroughly with respect to acetone, but not even traces were detected. Another possibility is that the two ketones are formed during the reaction. If this is the case, it is reasonable to believe that chemicals recovered in the manufacturing process should contain at least acetone. Samples collected from fractions of chemicals recovered during the manufacturing of **2** were therefore analysed by GC-MS, and significant amounts of acetone were indeed detected in recovered acetic anhydride. Thus, the source of this ketone is at least one acetic-anhydride impurity which is able to form acetone under the reaction conditions prevailing during the acetylation of **1**.

The potential acetone precursors are of course limited by the processes applied to manufacture acetic anhydride on an industrial scale. Today this anhydride is produced predominantly by two processes. The most recent method involves carbonylation of methyl acetate in the presence of a rhodium-iodide catalyst, and in this process ethylidene diacetate, acetone, carbon dioxide and methane are known byproducts.[4] The other process involves addition of acetic acid to ketene, and acetic anhydride manufactured in this way is known to contain ketene-related byproducts including diketene.[5,6] This is significant because diketene reacts with nucleophiles, and when exposed to water under acidic conditions acetoacetic acid is indeed formed.[6] This acid can undergo a number of reactions, including oxazolidine formation to give **4** and decarboxylation to give acetone which is required to form impurity **3**.

2.4. *N*,*O*-Acetal versus *O*,*O*-Acetal Formation. Formation of 1,3-dioxolanes from vicinal diols and aldehydes and ketones is a well-known transformation in organic chemistry,[7] but conversion of 2-aminoethanols to 1,3-oxazolidines by reaction with aldehydes and ketones has also been reported.[8−11] In order to get a good yield of both heterocycles, an acid has to be used as catalyst, and the water generated in both reactions has to be removed, for instance by using

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molecular sieves, performing azeotropic distillation with benzene or toluene, or by adding a chemical that traps water by a chemical reaction.

Although syntheses of oxazolidines are reported in the literature, it was surprising to find that oxazolidines are formed in considerable amounts directly from amides under acidic conditions since amides are significantly less nucleophilic than amines. Therefore, it was suspected that acetic anhydride plays the role as a water-removing reagent and changes the equilibrium position in the oxazolidine direction. In order to check if this might be the case a model compound was mixed with acetone and PTSA and heated to reflux (ca. 60 °C) (vide infra), and indeed, in the absence of acetic anhydride oxazolidines were not formed.

On this basis, it is interesting to observe that 1,3-dioxolane impurities were not detected at all in the reaction mixture from the industrial production of **2**. One reason could of course be that such compounds are not formed at all, but a more likely explanation is that 1,3-dioxolanes are indeed generated but decompose during the reaction or during the workup. In order to explore the latter possibility, the starting material (**1**) was added to a mixture of acetone, acetic anhydride, and acetic acid in the presence of PTSA and stirred under reflux. A sample was collected after 15 min and after modified workup (hydrolysis by aqueous base treatment followed by adjustment of pH to 4−5), it was analysed by HPLC. The analysis revealed as expected the presence of acetanilide **2** and oxazolidine **3** (as a stereoisomeric mixture), but in addition that two other major products, denoted **5** and **6**, had been formed (Figure 4a). The sample was then acidified with sulfuric acid until the pH was approximately 0, and subsequent analysis showed that only the main product **2** and oxazolidine **3** were present; not even traces of **5** and **6** could be detected (Figure 4b).

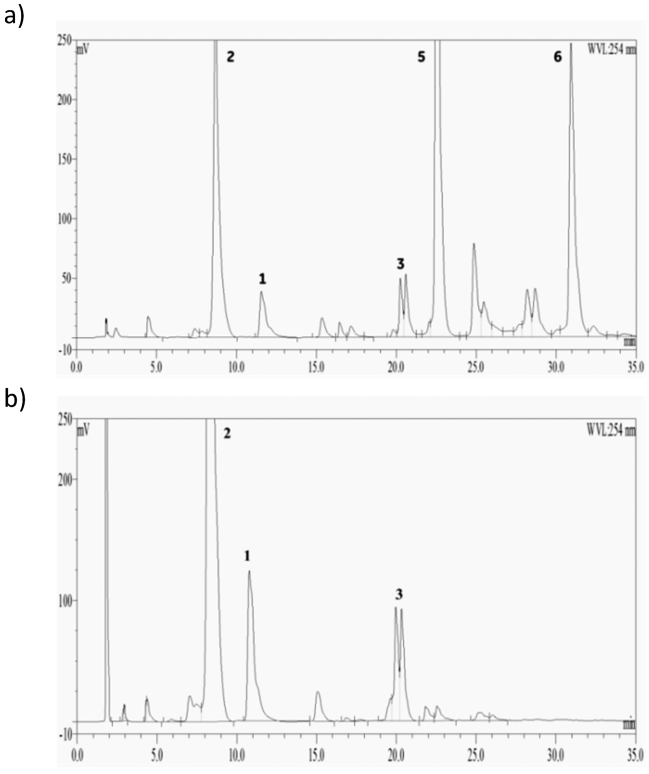

Figure 4. Chromatograms from HPLC analyses of a sample collected from the reaction between aniline **1**, acetone, acetic anhydride, acetic acid, and PTSA. (a) Sample collected after reflux for 15 min; worked up by hydrolysis with addition of NaOH and neutralization to pH 4−5; (b) sample in a after subsequent treatment with concentrated sulfuric acid until pH approximately 0.

To determine the structures of **5** and **6**, the reaction above was repeated with a short reaction time (15 min) and subsequently worked up by applying the modified procedure described above (hydrolysis by addition of NaOH and neutralization to pH 4−5). Due to the significant difference in the retention of the two compounds, the separation by preparative HPLC was straightforward, and samples of **5** and **6** were obtained in adequate quantity and purity for spectroscopic and spectrometric investigations (see Figure 5). The data obtained proved that the two products were 3-(3-acetamido-5-(2,2-dimethyl-1,3-dioxolan-4-yl)methylcarbamoyl-2,4,6-triiodobenzamido)propane-1,2-diol (**5**) and 5-acetamido-*N*,*N*′-bis(2,2-dimethyl-1,3-dioxolan-4-yl)methyl-2,4,6-triiodoisophthalamide (**6**).

With the structures of **5** and **6** elucidation of the impurity profile of **2**, prepared by acetylation of **1** in the manufacturing process, can be understood. All compounds **3**−**6** are indeed formed initially, but 1,3-dioxolanes **5** and **6**, which are formed the fastest and in the highest amounts, are much more easily cleaved than oxazolidines **3** and **4** and revert to the corresponding tetraol (**2**) under strongly acidic aqueous conditions.

2.5. Studies with Model Compounds. The fact that oxazolidines **3** and **4** are formed as mixtures of stereoisomers is not really surprising since the starting material contains two chiral centres and the three iodine atoms are so sterically demanding that rotation around the C−C and N−C bonds to the benzene ring is prevented.[12] The steric hindrance caused by the iodine atoms is illustrated by the ChemDraw-generated drawing in Figure 6, but to substantiate the effect experimentally we decided to study the model compound *N*,*N*′-bis(2,3-dihydroxypropyl)-5-nitro-isophthalamide (**7**), a common, iodine-free starting material used to produce X-ray contrast agents.

When **7** was exposed to a mixture of acetic anhydride and acetone under strongly acidic conditions, HPLC analysis of the crude product showed that several new compounds had been formed (Figure 7). Four compounds were by means of preparative HPLC obtained in adequate quantity and purity for subsequent spectroscopic and spectrometric investigations, and the data obtained proved that the four products were 5-nitro-*N*-(2,3-dihydroxypropyl)-*N*′-(2,2-dimethyl-1,3-dioxolan-4-yl)-methylisophthalamide (**8**), 5-nitro-*N*,*N*′-bis(2,2-dimethyl-1,3-dioxolan-4-yl)-methylisophthalamide (**9**), *N*-(2,3-dihydroxypropyl)-3-(5-hydroxymethyl-2,2-dimethyloxazoli-dine-3-carbonyl)-5-nitrobenzamide (**10**), and *N*-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl-3-(5-hydroxymethyl-2,2-dimethyloxazolidine-3-carbonyl)-5-nitrobenzamide (**11**) (Scheme 2). The structures of the compounds giving rise to the peaks with retention times 21.5 and 25.1 min are still unknown, but their mass spectra prove that they are not isomers of **10** and **8**, respectively. Thus, unlike oxazolidines **3** and **4**, each of the acetals **8**−**11** gives rise to one chromatographic peak only, and this clearly indicates that the stereoisomerism exhibited by **3** is due to hindered rotation caused by the iodine atoms, whereas that of **4** is a combination of hindered rotation and *cis*/*trans* isomerism in the oxazolidine moiety.

Another relevant point to consider is the potential formation of tetrahydro-1,3-oxazines during the acetylation of **1**. Such

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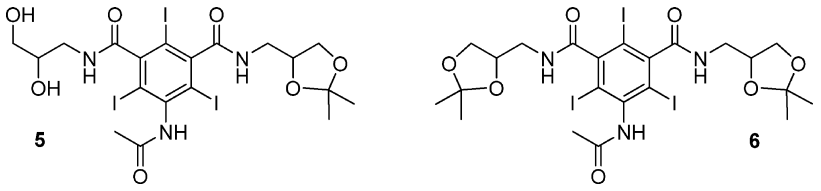

Figure 5. 1,3-Dioxolane derivatives **5** and **6** formed during the acetylation of aniline **1**. The compounds were isolated when the reaction time was short, and the workup was carried out under slightly acidic conditions.

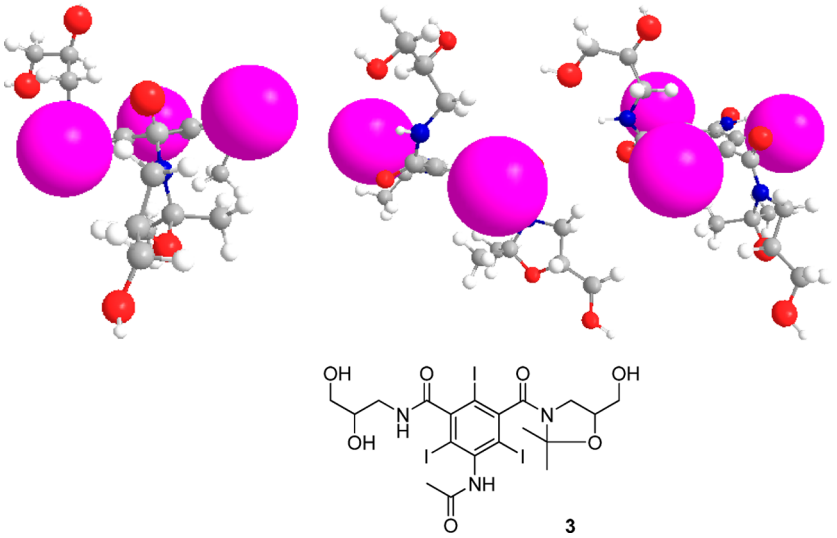

Figure 6. A ChemDrawn-generated drawing of oxazolidine **3**. The following colour code has been applied: Hydrogen atoms are white, carbon grey, nitrogen blue, oxygen red, and iodine violet.

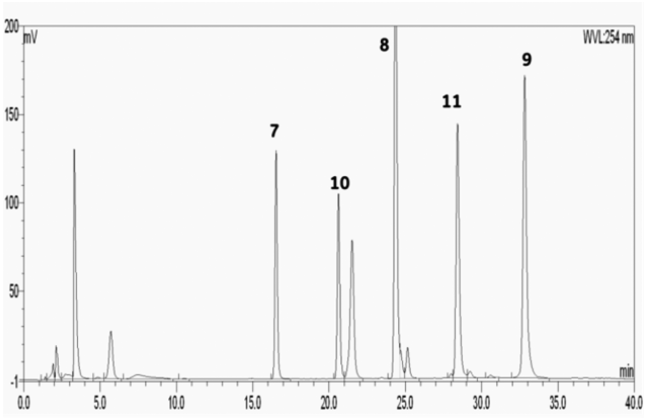

Figure 7. A HPLC chromatogram of the crude product after acetylation of **7** and workup using a modified procedure that makes it possible for 1,3-dioxolanes to survive.

compounds are known in the literature,[13] but no such product was obtained from **1**. Several reasons for this outcome are conceivable, but the most likely one is that tetrahydro-1,3-oxazines are formed more reluctantly than 1,3-oxazolidines and 1,3-dioxolanes under the reaction conditions prevailing under the acetylation. In order to see if this is the case *N*,*N*′-disubstituted isophthalamides **12a**−**12d** were acetylated by acetic anhydride in acetone in the presence of PTSA. The workup was carried out under mild conditions to make sure that any *N*,*O*-acetals formed did not decompose (Scheme 3). Thorough LC-MS analyses of the crude product mixtures were performed before they were subjected to preparative HPLC for product isolation and full product characterization, and the results were conclusive: *N*,*O*-acetal formation did only take place with the bis(2-hydroxyethyl)amides (**12a** and **12b**), not with bis(3-hydroxypropyl)-amides (**12c** and **12d**). On the basis of these observations the absence of 1,3-oxazine impurities in **2** is in accordance with expectations.

3. EXPERIMENTAL SECTION

General. All commodity chemicals were purchased from commercial suppliers and used without further purification. Also, unless otherwise stated, all reagents were commercially available and used as received. All melting points reported are uncorrected. IR absorptions are given in wave numbers (cm^{-1}), and intensities are characterized as (s) for strong, (m) for medium, (w) for weak, and (br) for broad. ^{1}H NMR spectra were recorded at ambient temperature at 400 or 600 MHz with DMSO-d_6 (δ_H = 2.50 ppm) as solvent and internal reference. Chemical shifts are reported in ppm. Multiplicity is given as (s) for singlet, (d) for doublet, (t) for triplet, (q) for quartet, (qui) for quintet, (dd) for doublet of doublet, (m) for multiplet, and (bs) for broad singlet. ^{13}C NMR spectra were recorded at ambient temperature at 100 or 150 MHz with the central peak of the DMSO-d_6 septet (δ_C = 39.50 ppm) as internal reference. Mass spectra were obtained on a Waters qTOF-micro or a JEOL AccuTOF T100LC, which both were operated in the ESI mode.

Analysis and Isolation by HPLC. Analytical HPLC was performed on an Agilent 1100 or a TSP (Thermo Separation products) instrument with UV detection (254 nm). Four different RP-18 columns were used, two from Supelco (75 × 4.6 mm, 3 μ; 250 × 4.6 mm, 5 μ), one from LUNA (250 × 4.6

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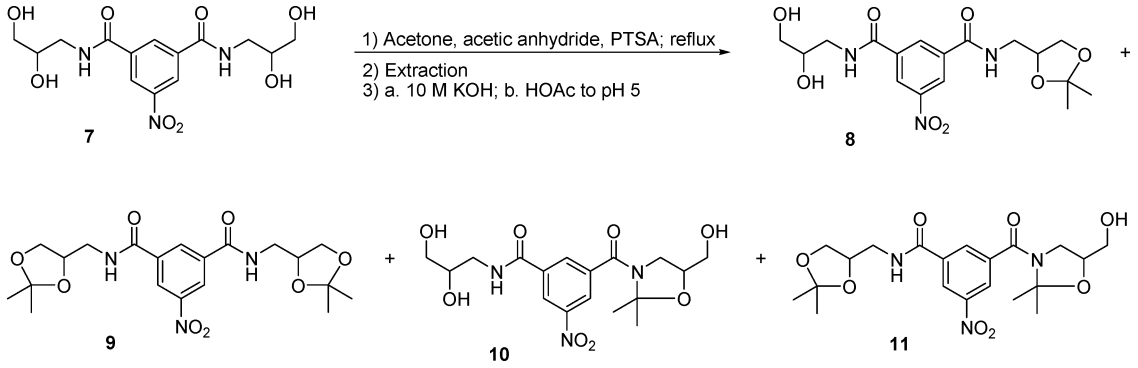
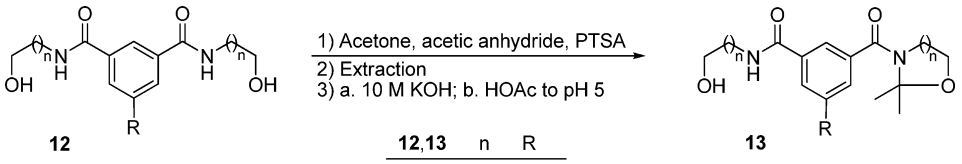
Scheme 2. *N,O*- and *O,O*-Acetals from treatment of model compound 7 with acetone in acetic anhydride containing *p*-toluenesulfonic acid (PTSA)



Scheme 3. *N,O*-Acetals from treatment of model compounds 12a−12d with acetone in acetic anhydride containing *p*-toluenesulfonic acid (PTSA)



12,13	n	R
a	1	H
b	1	NO_2
c	2	H
d	2	NO_2

mm, 10 μ), and one from Brownlee (250 × 4.6 mm, 5 μ). Five different gradient programs with mixtures of water (A) and acetonitrile (B) as eluent were applied. All gradients are linear.

Program 1: 0.4−2.2% B (0−10 min), 2.2−14.3% B (10−20 min), 14.3% B (20−24 min).

Program 2: 2.0−5.0% B (0−10 min), 5.0−25.0% B (10−20 min), 25.0% B (20−25 min).

Program 3: 2.5−5.0% B (0−10 min), 5.0−30.0% B (10−30 min), 30.0% B (30−50 min).

Program 4: 5.0−10.0% B (0−10 min), 10.0−50.0% B (10−30 min), 50.0% B (30−35 min).

Program 5: 2.5−5.0% B (0−10 min), 5.0−30.0% B (10−30 min), 30.0% B (30−60 min).

The flow rate was 1.0 mL/min (Program 1) or 1.5 mL/min (Programs 2−5). No acid was added into the eluents unless otherwise stated. The amount of each component is given in % on the basis of its relative area.

Preparative HPLC was carried out by using a Shimadzu LC-8A or a Varian 800 mL SDS instrument, both with UV detection (254 nm). A LUNA, RP-18, 250 × 50 mm, 10 μ column or a LUNA, RP-18, 260 × 101 mm, 10 μ column was used. The flow rate was 75 mL/min (Shimadzu) or 250 mL/min (Varian). No acid was added into the eluents (water and acetonitrile) unless otherwise stated

*Isolation of 3-Acetamido-N-(2,3-dihydroxypropyl)-5-(5-hydroxymethyl-2,2-dimethyl-oxazolidine-3-carbonyl)-2,4,6-triiodobenzamide (**3**).* Aniline **1** was acetylated on a production scale by acetic anhydride in the presence of a strong acid catalyst. When the reaction was complete, the mixture was concentrated, and remaining acetic anhydride was quenched by addition of methanol and water under strong acidic conditions. Then deacetylation was performed by addition of sodium hydroxide, and a small portion of the reaction mixture obtained was used for the isolation, which was performed by means of preparative HPLC. The injection solution was prepared by diluting 100 mL of the reaction mixture with H_2O until 1 L and then adjusting the pH to 7−9 by adding HCOOH. Fifteen runs were performed on a LUNA 260 × 101 mm column, and the two compounds were collected in the same fraction and then concentrated under vacuum on a rotary evaporator. The resulting concentrate was purified further on a LUNA 250 × 50 mm column in two steps. In the first the two compounds were again collected in the same fraction, which was concentrated on a rotary evaporator and freeze-dried to give a white solid (530 mg). The solid was dissolved in water for the second purification step. Six runs were performed, and three fractions were collected, concentrated on a rotary evaporator, and freeze-dried. The weights of the obtained white solids were 83 mg (fraction 1), 130 mg (fraction 2), and 65 mg (fraction 3). The HPLC analyses (Program 1, Supelco 75 × 4.6 mm) of the solids gave the following results (stereoisomers of **3** are denoted ***α*** and ***β***, in Figure 1 they are given as e and f): Solid 1: Purity 97%, **3*α***: **3*β*** (90:10). Solid 2: Purity 96%, **3*α***: **3*β*** (54:46). Solid 3: Purity 89%, **3*α***: **3*β*** (12:88). Data for **3*α***: Mp 265 °C (dec); IR (ATR) 3251 (br), 2983 (w), 2937 (w), 2885 (w), 1635 (s), 1540 (m), 1362 (m), 1248 (m), 1044 (m) cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 9.86 (m, 1H, Ar−NH), 8.60−8.35 (m, 1H, HN−CH_2), 4.80 (m, 1H, N−CH_2−CH−CH_2−OH), 4.55 (m, 1H, HN−CH_2−CH−OH), 4.37 (bs, 1H, HN−CH_2−CH−CH_2−OH), 4.17 (m, 1H, N−CH_2−CH−CH_2−OH), 3.71 (m, 1H, HN−CH_2−CH), 3.53 and 3.48 (2m in a 1:1 ratio, 2H, N−CH_2−CH−CH_2−OH), 3.48 and 3.42 (2m in a 1:1 ratio, 2H, HN−CH_2−CH−CH_2−OH), 3.31 and 3.17 (2m in a 1:1 ratio, 2H, HN−CH_2), 3.19 and 3.01 (2m in a 1:1 ratio, 2H, N−CH_2), 2.03 (s, 3H, HN−C(O)−CH_3), 1.69 (s, 3H, C−CH_3), 1.63 (s, 3H, C−CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.2 (Ar-C(O)−NH), 167.5 (Ar−NH−C(O)), 165.1 (Ar−

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C(O)−N), 150.5 (ArC−C(O)−NH), 148.4 (ArC−C(O)−N), 143.7 (ArC−NH), 99.1 (ArC−I), 97.8 (ArC−I), 94.5 (CH_3−C−CH_3), 89.2 (ArC−I), 75.1 (N−CH_2−CH), 69.8 (HN−CH_2−CH), 63.8 (HN−CH_2−CH−CH_2−OH), 61.3 (N−CH_2−CH−CH_2−OH), 49.0 (N−CH_2), 42.5 (HN−CH_2), 25.0 (C−CH_3), 23.3 (C−CH_3), 22.8 (C(O)−CH_3); HRMS Calcd for $C_{19}H_{24}I_3N_3O_7Na^+$ [M + Na^+] 809.8646, found 809.8645. Data for **3*β***: Mp 265 °C (dec); IR (ATR) 3231 (br), 2988 (w), 2936 (w), 2880 (w), 1631 (s), 1523 (m), 1361 (m), 1249 (m), 1108 (m), 1043 (m) cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 9.88 (m, 1H, Ar−NH), 8.60−8.35 (m, 1H, HN−CH_2), 4.80 (bs, 1H, N−CH_2−CH−CH_2−OH), 4.55 (bs, 1H, HN−CH_2−CH−OH), 4.36 (bs, 1H, HN−CH_2−CH−CH_2−OH), 4.17 (m, 1H, N−CH_2−CH), 3.72 (m, 1H, HN−CH_2−CH), 3.55 and 3.49 (2m in a 1:1 ratio, 2H, N−CH_2−CH−CH_2−OH), 3.49 and 3.42 (2m in a 1:1 ratio, 2H, HN−CH_2−CH−CH_2−OH), 3.32 and 3.17 (2m in a 1:1 ratio, 2H, HN−CH_2), 3.16 and 3.09 (2m in a 1:1 ratio, 2H, N−CH_2), 2.04 (s, 3H, HN−C(O)-CH_3), 1.70 (s, 3H, C−CH_3), 1.63 (s, 3H, C−CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.2 (Ar−C(O)−NH), 167.5 (Ar−NH−C(O)), 165.1 (Ar−C(O)−N), 150.5 (ArC−C(O)−NH), 148.4 (ArC−C(O)−N), 143.6 (ArC−NH), 99.2 (ArC−I), 97.9 (ArC−I), 94.5 (CH_3−C−CH_3), 88.9 (ArC−I), 75.1 (N−CH_2−CH), 69.8 (HN−CH_2−CH), 63.8 (HN−CH_2−CH-CH_2−OH), 61.4 (N−CH_2−CH−CH_2−OH), 49.1 (N−CH_2), 42.5 (HN−CH_2), 25.0 (C−CH_3), 23.2 (C−CH_3), 22.8 (C(O)−CH_3); HRMS Calcd for $C_{19}H_{24}I_3N_3O_7Na^+$ [M + Na^+] 809.8646, found 809.8646.

*Isolation of 2-(3-(3-Acetamido-5-(2,3-dihydroxypropylcarbamoyl)-2,4,6-triiodobenzo-yl)-5-hydroxymethyl-2-methyl-oxazolidin-2-yl)acetic Acid (**4**).* The reaction mixture used for the isolation by preparative HPLC was obtained as described for **3**. Thirteen runs were performed on a LUNA 260 × 101 mm column, and HCOOH (0.2%) was added to the eluents. The injection solution was prepared by diluting 50 mL of the solution from the experiment described above with water until 1 L. Then the pH was adjusted to approximately 4 by adding HCOOH. One fraction was collected and then concentrated on a rotary evaporator. The concentrate was purified further on a LUNA 250 × 50 mm column in two steps. For the first step, six runs were performed. Three fractions were collected, and they were then concentrated on a rotary evaporator and freeze-dried. The weight of the white solids obtained from the fractions was 0.6 g, 1.9 g, and 0.7 g, respectively. The solid from fraction 3 (0.7 g) was dissolved in water (60 mL) for the second purification step on the LUNA 250 × 50 mm column. Three runs were performed, and one fraction was collected followed by concentration on a rotary evaporator and freeze-drying. (Stereoisomers of **4** are denoted **4*α*−4*δ***; in Figure 1 they are given as **a**−**d**, respectively). The purity (HPLC, LUNA 250 × 4.6 mm, Program 2, 0.1% HCOOH) was 97% (**4*δ***). The solid from fraction 1 (0.6 g) was dissolved in water (40 mL) for the second purification step on the LUNA 250 × 50 mm column. Two runs were performed, and one fraction was collected followed by concentration on a rotary evaporator and freeze-drying. The purity (HPLC, LUNA 250 × 4.6 mm, Program 2, 0.1% HCOOH) was 93% (**4*α***). The solid from fraction 2 (1.9 g) was dissolved in water (60 mL) for the second purification step on the LUNA 250 × 50 mm column. Three runs were performed, and two fractions were collected, concentrated on a rotary evaporator and then purified further in additional purification steps. Finally, after freeze-drying, two white solids were obtained. The HPLC analyses (Method 2, LUNA 250 × 4.6 mm, 0.1% HCOOH) of the solids gave the following results: Solid 1:93%, **4*β*:4*γ*** (59:41). Solid 2:99%, **4*β*:4*γ*** (12:88). Data for **4*α***: Mp 230 °C (dec); IR (ATR) 3231 (br), 2930 (w), 1712 (m), 1626 (s), 1525 (m), 1356 (m), 1237 (m), 1108 (m), 1032 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.00 (m, 1H, Ar−NH), 8.75−8.50 (m, 1H, HN−CH_2), 5.30 (bs, 3H, OH), 4.23 (m, 1H, N−CH_2−CH), 3.68 (m, 1H, HN−CH_2−CH), 3.54 and 3.50 (2m in a 1:1 ratio, 2H, N−CH_2−CH−CH_2−OH), 3.47 and 3.39 (2m in a 1:1 ratio, 2H, HN−CH_2−CH−CH_2−OH), 3.30 and 3.16 (2m in a 1:1 ratio, 2H, N−CH_2), 3.30 and 3.15 (2m in a 1:1 ratio, 2H, HN−CH_2), 3.24 and 2.85 (2m in a 1:1 ratio, 2H, CH_2−COOH), 2.03 (s, 3H, HN−C(O)−CH_3), 1.83 (s, 3H, C−CH_3), carboxylic acid proton not observed; ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.3 (COOH), 169.5 (Ar−C(O)−NH), 167.9 (Ar−NH−C(O)), 165.6 (Ar−C(O)−N), 150.7 (ArC−C(O)−NH), 148.1 (ArC−C(O)−N), 143.8 (ArC−NH), 100.1 (ArC−I), 98.5 (ArC−I), 94.3 (CH_3−C−CH_3), 89.5 (ArC−I), 75.9 (N−CH_2−CH), 70.0 (HN−CH_2−CH), 64.0 (HN−CH_2−CH−CH_2−OH), 61.3 (N−CH_2−CH−CH_2−OH), 49.0 (N−CH_2), 42.6 (HN−CH_2), 41.0 (CH_2−COOH), 23.3 (C−CH_3), 23.0 (C(O)−CH_3); HRMS Calcd for $C_{20}H_{24}I_3N_3O_9Na^+$ [M + Na^+] 853.8544, found 853.8547. Data for the mixtures of **4*β*** and **4*γ***: IR (ATR) 3247 (br), 2935 (w), 1714 (m), 1631 (s), 1522 (m), 1357 (m), 1242 (m), 1112 (m), 1042 (m) cm^{-1}; HRMS Calcd for $C_{20}H_{24}N_3I_3O_9Na^+$ [M + Na^+] 853.8544, found 853.8547. Data for **4*β***: ^1H NMR (400 MHz, DMSO-d_6) δ 10.00 (m, 1H, Ar−NH), 8.75−8.50 (m, 1H, HN−CH_2), 4.21 (bs, 3H, OH), 4.21 (m, 1H, N−CH_2−CH), 3.68 (m, 1H, HN−CH_2−CH), 3.54 and 3.50 (2m in a 1:1 ratio, 2H, N−CH_2−CH−CH_2−OH), 3.47 and 3.39 (2m, 2H, HN−CH_2−CH−CH_2−OH), 3.37 and 2.75 (2m in a 1:1 ratio, 2H, CH_2−COOH), 3.30 and 3.16 (2m in a 1:1 ratio, 2H, N−CH_2), 3.30 and 3.15 (2m in a 1:1 ratio, 2H, HN−CH_2), 2.03 (s, 3H, HN−C(O)−CH_3), 1.79 (s, 3H, C−CH_3), carboxylic acid proton not observed; ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4 (COOH), 169.5 (Ar−C(O)−NH), 167.9 (Ar−NH−C(O)), 165.6 (Ar−C(O)−N), 150.7 (ArC−C(O)−NH), 148.1 (ArC−C(O)−N), 143.8 (ArC−NH), 100.0 (ArC−I), 98.3 (ArC−I), 94.3 (CH_3−C−CH_3), 89.5 (ArC−I), 76.0 (N−CH_2−CH), 70.0 (HN−CH_2−CH), 64.0 (HN−CH_2−CH−CH_2−OH), 61.1 (N−CH_2−CH−CH_2−OH), 48.9 (N−CH_2), 42.6 (HN−CH_2), 42.6 (CH_2−COOH), 23.1 (C(O)−CH_3), 21.4 (C−CH_3). Data for **4*γ***: ^1H NMR (400 MHz, DMSO-d_6) δ 9.99 (m, 1H, Ar−NH), 8.75−8.50 (m, 1H, HN−CH_2), 4.99 (m, 1H, N−CH_2−CH−CH_2−OH), 4.75 (bs, 1H, HN−CH_2−CH−OH), 4.53 (bs, 1H, HN−CH_2−CH−CH_2−OH), 4.21 (m, 1H, N−CH_2−CH), 3.68 (m, 1H, HN−CH_2−CH), 3.54 and 3.50 (2m in a 1:1 ratio, 2H, N−CH_2−CH−CH_2−OH), 3.47 and 3.39 (2m in a 1:1 ratio, 2H, HN−CH_2−CH−CH_2−OH), 3.41 and 3.22 (2m in a 1:1 ratio, 2H, N−CH_2), 3.30 and 3.15 (2m in a 1:1 ratio, 2H, HN−CH_2), 3.24 and 2.85 (2m in a 1:1 ratio, 2H, CH_2−COOH), 2.03 (s, 3H, HN−C(O)−CH_3), 1.83 (s, 3H, C−CH_3), carboxylic acid proton not observed; ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5 (COOH), 169.4 (Ar−C(O)−NH), 167.8 (Ar−NH−C(O)), 165.6 (Ar−C(O)−N), 150.7 (ArC−C(O)−NH), 148.1 (ArC−C(O)−N), 143.9 (ArC−NH), 100.0 (ArC−I), 98.3 (ArC−I), 94.3 (CH_3−C−CH_3), 90.0 (ArC−I), 75.9 (N−CH_2−CH), 70.0 (HN−CH_2−CH), 63.9 (HN−CH_2−CH−CH_2−OH), 61.0 (N−CH_2−CH−CH_2−OH), 48.8 (N−CH_2), 42.5 (HN−CH_2), 41.0 (CH_2−COOH), 23.1 (C−CH_3), 23.0 (C(O)−CH_3). Data for **4*δ***: Mp 270 °C (dec); IR (ATR) 3230 (br), 2929 (w), 1718 (m), 1627 (s), 1526 (m),

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1359 (m), 1205 (m), 1107 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.00 (m, 1H, Ar−NH), 8.75−8.52 (m, 1H, HN−CH_2), 4.95 (bs, 1H, N−CH_2−CH−CH_2−OH), 4.73 (m, 1H, HN−CH_2−CH−OH), 4.51 (bs, 1H, HN−CH_2−CH−CH_2−OH), 4.19 (m, 1H, N−CH_2−CH), 3.68 (m, 1H, HN−CH_2−CH), 3.53 and 3.50 (2m in a 1:1 ratio, 2H, N−CH_2−CH−CH_2−OH), 3.47 and 3.39 (2m in a 1:1 ratio, 2H, HN−CH_2−CH−CH_2−OH), 3.37 and 2.76 (2m in a 1:1 ratio, 2H, CH_2−COOH), 3.30 and 3.15 (2m in a 1:1 ratio, 2H, HN−CH_2), 3.17 and 3.11 (2m in a 1:1 ratio, 2H, N−CH_2), 2.03 (s, 3H, HN−C(O)−CH_3), 1.79 (s, 3H, C−CH_3), carboxylic acid proton not observed; ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4 (COOH), 169.4 (Ar−C(O)−NH), 167.9 (Ar−NH−C(O)), 165.6 (Ar−C(O)−N), 150.6 (ArC−C(O)−NH), 148.1 (ArC−C(O)−N), 143.8 (ArC−NH), 100.1 (ArC−I), 98.5 (ArC−I), 94.4 (CH_3−C−CH_3), 89.4 (ArC−I), 75.9 (N−CH_2−CH), 69.9 (HN−CH_2−CH), 64.0 (HN−CH_2−CH−CH_2−OH), 61.3 (N−CH_2−CH−CH_2−OH), 49.3 (N−CH_2), 42.6 (HN−CH_2), 42.6 (CH_2−COOH), 23.0 (C(O)−CH_3), 21.3 (C−CH_3); HRMS Calcd for $C_{20}H_{24}N_3I_3O_9Na^+$ [M + Na^+] 853.8544, found 853.8547.

*Synthesis of 3-(3-Acetamido-5-((2,2-dimethyl-1,3-dioxolan-4-yl)methylcarbamoyl)-2,4,6-triiodobenzamido)-propane-1,2-diyl Diacetate (**5**) and 5-Acetamido-N,N′-bis-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl-2,4,6-triiodoisophthaldiamide (**6**).* Acetone (14 mL, 11 g, 0.19 mol), acetic anhydride (7.6 mL, 8.2 g, 0.081 mol), and acetic acid (22 mL) were mixed at rt, and PTSA (0.4 g) and **1** (7.0 g, 0.0099 mol) were then added. The reaction mixture was heated to reflux (90−95 °C), and after 15 min the solution was cooled to rt. Precipitation started during the cooling, and diethyl ether (40 mL) was added at rt to precipitate more solid. The precipitate was filtered, washed with diethyl ether, and finally dried in a vacuum oven at 50 °C. A HPLC sample was treated with a drop of NaOH (50%, aq), and the pH was approximately 14 (pH paper). After neutralizing with a little bit of acetic acid (pH 4−5), the HPLC sample was analyzed (Supelco 250 × 4.6 mm; Program 3). The main compounds were **2** (38%), **5** (39%), and **6** (17%).

Preparative HPLC was used for isolation which was carried out in one step. Three runs were performed on the LUNA 250 × 50 mm column. For each run, solid (200−1100 mg) from the experiment was diluted with methanol (5 mL) and then water (45 mL). Some drops of an aqueous KOH solution (10 M) were added to hydrolyse the ester groups. When the hydrolysis was complete, the pH was adjusted to 4−5 by adding some drops of acetic acid. After the pH adjustment, the solution was injected into the column, and two fractions were collected. The fractions were then concentrated on a rotary evaporator in the presence of small amounts of ammonia to keep a weak alkaline solution during the concentration. Precipitation occurred in the second fraction, and the precipitate was filtered and air-dried. The concentrate from the first fraction was freeze-dried, and the weight of the solid obtained was 525 mg. For the second fraction the weight was 160 mg (white solid). The purity of **5** (HPLC; LUNA 250 × 4.6 mm; Program 4) was >99% and of **6** 98%. Data for **5**: Mp 285 °C (dec); IR (ATR) 3244 (br), 2984 (w), 2935 (w), 2880 (w), 1633 (s), 1553 (m), 1510 (m), 1370 (m), 1268 (m), 1216 (m), 1047 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H, Ar−NH), 8.84−8.66 and 8.19−8.09 (m, 1H, HN−CH_2−CH−O), 8.62−8.44 (m, 1H, HN−CH_2−CH−OH), 4.71 (m, 1H, CH−OH), 4.50 (m, 1H, CH_2−OH), 4.24 (m, 1H, HN−CH_2−CH−O), 4.06 and 3.82 (2m in a 1:1 ratio, 2H, HN−CH_2−CH−CH_2−O), 3.69 (m, 1H, HN−CH_2−CH−OH), 3.47 and 3.40 (2m in a 1:1 ratio, 2H, HN−CH_2−CH−CH_2−OH), 3.38 and 3.20 (2m in a 1:1 ratio, 2H, NH−CH_2−CH−O), 3.30 and 3.15 (2m in a 1:1 ratio, 2H, NH−CH_2−CH−OH), 2.02 (s, 3H, HN−C(O)−CH_3), 1.35 (s, 3H, O−C−CH_3), 1.27 (s, 3H, O−C−CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7 (Ar−C(O), 2C), 167.7 (Ar−NH−C(O)), 150.1 (ArC−C(O)), 149.7 (ArC−C(O)), 143.4 (ArC−NH), 108.5 (CH_3−C−CH_3), 99.4 (ArC−I, 2C), 90.2 (ArC−I), 73.4 (HN−CH_2−CH−O), 70.0 (HN−CH_2−CH−OH), 67.7 (HN−CH_2−CH−CH_2−O), 64.0 (HN−CH_2−CH−CH_2−OH), 42.6 (NH−CH_2−CH−OH), 41.9 (HN−CH_2−CH−O), 27.0 (O−C−CH_3), 25.4 (O−C−CH_3), 23.0 (HN−C(O)−CH_3); LC-HRMS Calcd for $C_{19}H_{25}I_3N_3O_7^+$ [M + H^+] 787.8821, found 787.8851. Data for **6**: Mp 306 °C (dec); IR (ATR) 3246 (m), 2984 (w), 2935 (w), 2876 (w), 1633 (s), 1553 (m), 1509 (m), 1369 (m), 1267 (m), 1212 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H, Ar−NH), 8.86−8.43 (m, 2H, HN−CH_2), 4.25 (m, 2H, HN−CH_2−CH), 4.06 and 3.81 (2m in a 1:1 ratio, 4H, HN−CH_2−CH−CH_2), 3.39 and 3.19 (2m in a 1:1 ratio, 4H, HN−CH_2), 2.02 (s, 3H, HN−C(O)−CH_3), 1.36 (s, 6H, (O)C(O)−CH_3), 1.27 (s, 6H, (O)C(O)−CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.6 (Ar−C(O), 2C), 167.7 (Ar−NH−C(O)), 149.8 (ArC−C(O), 2C), 143.4 (ArC−NH), 108.5 (CH_3−(O)C(O)−CH_3, 2C), 99.5 (ArC−I, 2C), 90.2 (ArC−I), 73.3 (HN−CH_2−CH, 2C), 67.7 (HN−CH_2−CH−CH_2, 2C), 41.9 (HN−CH_2, 2C), 27.0 ((O)C(O)−CH_3, 2C), 25.4 ((O)C(O)−CH_3, 2C), 23.0 (HN−C(O)−CH_3); LC-HRMS Calcd for $C_{22}H_{29}I_3N_3O_7^+$ [M + H^+] 827.9134, found 827.9086.

Decomposition of 5 and 6 under Aqueous Conditions. Acetone (7.0 mL, 5.5 g, 0.095 mol), acetic anhydride (3.8 mL, 4.1 g, 0.040 mol), and acetic acid (11 mL) were mixed at room temperature, and PTSA (0.2 g) and **1** (3.5 g, 0.0050 mol) were then added. The resulting mixture was heated to reflux, and after 15 min a sample was collected for HPLC analysis (Supelco 250 × 4.6 mm; Program 3). Prior to analysis the sample was diluted with water, treated with a drop of aqueous NaOH (50%), and neutralized with acetic acid (pH 4−5). The chromatogram revealed that the product mixture contained **2** (19%), **3** (5%), **5** (32%), and **6** (17%) (see Figure 4a). The sample was further treated with concentrated H_2SO_4 until the pH was approximately 0 (pH paper), and after storage for 5−10 min the sample was analyzed again under the conditions mentioned above. The chromatogram revealed that the product mixture now contained **2** (70%) and **3** (10%), but not even traces of **5** and **6** (see Figure 4b).

*Synthesis of N,N′-Bis(2,3-dihydroxypropyl)-5-nitroisophthaldiamide (**7**).* Dimethyl 5-nitroisophthalate (40.0 g, 0.167 mol) and 3-amino-1,2-propandiol (36.4 g, 0.400 mol) were mixed in methanol (140 mL). The mixture was heated to reflux (70−75 °C), stirred for 70 h, and then cooled to rt. During the cooling crystallization occurred. The slurry was filtered, the solid was washed with methanol, and the crystals were finally dried under vacuum at 50 °C to give **7** (54.4 g, 91%) as a white solid. Mp 131−134 °C (literature 128−132 °C).[14] The purity (HPLC; Brownlee 250 × 4.6 mm; Program 5) was 99%. NMR data was in accordance to the literature.[15]

*N-(2,3-Dihydroxypropyl)-N′-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl-5-nitroisophthaldi-amide (**8**) and N,N′-bis((2,2-dimethyl-1,3-dioxolan-4-yl)methyl-5-nitroisophthaldiamide (**9**).* Acetone (50 mL, 40 g, 0.68 mol) and acetic anhydride (8.0 mL, 8.6 g, 0.085 mol) were mixed at rt, and PTSA (0.4 g) and 7

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(3.5 g, 0.0098 mol) were then added. After stirring the mixture for 3 h at rt, the solution was diluted with CH_2Cl_2 (150 mL), and the organic phase was washed with 2 M aqueous NaOH (2 × 50 mL), H_2O (50 mL), and brine (30 mL). The organic phase was then dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator. A HPLC sample of the residue was treated with NaOH and then neutralized with acetic acid before being analysed by HPLC (Supelco 250 × 4.6 mm; Program 3) which revealed the presence of **7** (2%), **8** (20%), and **9** (56%). Preparative HPLC was used for isolation which was carried out in two steps. For the first step, two runs were performed on the LUNA 250 × 50 mm column. For each run, the solution injected was made from some of the residue from the experiment (10−15 mL) which was diluted with methanol (10−20 mL) followed by water (60 mL). One fraction only was collected during the two runs. The fraction was concentrated under vacuum on a rotary evaporator in the presence of ammonia to keep it alkaline. The resulting concentrate was then treated with KOH and stirred for 5 min followed by acetic acid until pH was approximately 5 (pH strip). The second purification step was carried out on the same column as the first step, and two fractions were collected, followed by freeze-drying. The weight of the pale yellow, highly viscous liquid obtained from the first fraction was 270 mg. The purity of **8** (HPLC; LUNA 250 × 4.6 mm; Program 4) was >99%. The weight of the off-white glazed solid from the second fraction was 1030 mg. The purity of **9** (HPLC; LUNA 250 × 4.6 mm; Program 4) was >99%. Data for **8**: IR (ATR) 3305 (br), 3085 (w), 2986 (w), 2936 (w), 2882 (w), 1643 (s), 1529 (s), 1211 (m), 1045 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.11 (t, 1H, HN−CH_2−CH−O), 8.92 (t, 1H, HN−CH_2−CH−OH), 8.82 (t, 1H, ArH), 8.80 (t, 1H, ArH), 8.76 (t, 1H, ArH), 4.88 (d, 1H, CH−OH), 4.61 (t, 1H, CH_2−OH), 4.24 (qui, 1H, HN−CH_2−CH), 4.01 and 3.71 (2dd in a 1:1 ratio, 2H, HN−CH_2−CH−CH_2), 3.69 (m, 1H, HN−CH_2−CH−OH), 3.45 and 3.23 (2m in a 1:1 ratio, 2H, NH−CH_2−CH−OH), 3.43 (m, 2H, NH−CH_2−CH−O), 3.37 (m, 2H, HN−CH_2−CH−CH_2−OH), 1.35 (s, 3H, CH_3), 1.26 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.1 (Ar−C(O)), 164.0 (Ar−C(O)), 147.8 (ArC−NO_2), 136.3 (ArC−C(O)), 135.8 (ArC−C(O)), 132.5 (ArC), 124.4 (ArC), 124.2 (ArC), 108.5 (CH_3−C−CH_3), 74.1 (HN−CH_2−CH−O), 70.2 (HN−CH_2−CH−OH), 66.8 (HN−CH_2−CH−CH_2−O), 64.0 (HN−CH_2−CH−CH_2−OH), 43.3 (HN−CH_2−CH−OH), 42.4 (HN−CH_2−CH−O), 26.9 (CH_3), 25.4 (CH_3); LC-HRMS Calcd for $C_{17}H_{24}N_3O_8^+$ [M + H^+] 398.1558, found 398.1554. Data for **9**: Mp 83−86 °C; IR (ATR) 3310 (w), 3082 (w), 2985 (w), 2936 (w), 2882 (w), 1644 (s), 1529 (s), 1349 (m), 1210 (m), 1154 (m), 1080 (m), 1051 (m), 834 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.11 (t, 2H, NH), 8.81 (s, 2H, ArH), 8.76 (s, 1H, ArH), 4.24 (qui, 2H, HN−CH_2−CH), 4.01 and 3.71 (2dd in a 1:1 ratio, 4H, HN−CH_2−CH−CH_2), 3.43 (m, 4H, HN−CH_2), 1.35 (s, 6H, CH_3), 1.26 (s, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0 (Ar−C(O), 2C), 147.8 (ArC−NO_2), 135.9 (ArC−C(O), 2C), 132.5 (ArC), 124.4 (ArC, 2C), 108.5 (CH_3−C−CH_3, 2C), 74.1 (HN−CH_2−CH, 2C), 66.8 (HN−CH_2−CH−CH_2, 2C), 42.4 (HN−CH_2, 2C), 26.9 (CH_3, 2C), 25.4 (CH_3, 2C); LC-HRMS Calcd for $C_{20}H_{28}N_3O_8^+$ [M + H^+] 438.1871, found 438.1829.

*N-(2,3-Dihydroxypropyl)-3-(5-hydroxymethyl-2,2-dimethyloxazolidine-3-carbonyl)-5-nitrobenzamide (**10**) and N-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-3-(5-hydroxymethyl-2,2-dimethyloxazolidine-3-carbonyl)-5-nitrobenzamide (**11**).* Acetone (25 mL, 20 g, 0.34 mol) and acetic anhydride (4.0 mL, 4.3 g, 0.042 mol) were mixed at room temperature, and PTSA (0.2 g) and **7** (1.8 g, 5.0 mmol) were then added. The reaction mixture was stirred at rt for 72 h. The mixture was then diluted with ethyl acetate (200 mL), and the organic phase was washed with 2 M aqueous NaOH (3 × 50 mL), water (50 mL), and brine (30 mL). Then the organic phase was dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator. A HPLC sample of the residue was treated with NaOH and then neutralized with acetic acid before being analysed by HPLC (Supelco 250 × 4.6 mm; Program 3) which revealed the presence of **7** (9%), **8** (23%), **9** (19%), **10** (8%), and **11** (14%).

Preparative HPLC was used for isolation which was carried out in two steps. For the first step, two runs were performed on the LUNA 250 × 50 mm column. For each run, residue (10−15 mL) from the experiment was diluted with methanol (30−40 mL) and then water (60 mL). One fraction was collected followed by concentration on a rotary evaporator in the presence of small amounts of ammonia to keep a weak alkaline solution during the concentration. The concentrate was then freeze-dried. Prior to the second purification step the solid (250 mg) was dissolved in methanol (20 mL) and then diluted with water (30 mL). Aqueous 10 M KOH was then added, and after stirring for 5 min at rt acetic acid was added until pH was approximately 5 (pH strip). The second purification step was carried out on the same column as the first step. Several fractions were collected during the two runs. Each fraction was analysed by HPLC, and based on the HPLC-results some of them were combined to give two fractions. The weight of the off-white solid (freeze-dried) obtained from the first fraction was 19 mg. The purity of **10** (HPLC, Program 4, LUNA 250 × 4.6 mm) was 88%. The weight of the off-white solid (freeze-dried) from the second fraction was 99 mg. The purity of **11** (HPLC) was 89%. Data for **10**: Mp 240 °C (dec); IR (ATR) 3307 (m), 3080 (w), 2983 (w), 2934 (w), 2875 (w), 2728 (w), 1628 (s), 1533 (s), 1348 (m), 1040 (s) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 8.95 (t, 1H, NH), 8.75 (t, 1H, ArH), 8.44 (t, 1H, ArH), 8.39 (t, 1H, ArH), 4.90 (m, 1H, CH−OH), 4.90 (m, 1H, N−CH_2−CH−CH_2−OH), 4.62 (t, 1H, HN−CH_2−CH−CH_2−OH), 4.12 (m, 1H, N−CH_2−CH), 3.67 (m, 1H, HN−CH_2−CH), 3.49 (t, 2H, N−CH_2−CH−CH_2), 3.47 and 3.40 (2m in a 1:1 ratio, 2H, N−CH_2), 3.45 and 3.21 (2m in a 1:1 ratio, 2H, HN−CH_2), 3.36 (m, 2H, HN−CH_2−CH−CH_2), 1.67 (s, 3H, CH_3), 1.63 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9 (Ar−C(O)), 163.7 (Ar−C(O)), 147.8 (ArC−NO_2), 139.3 (ArC−C(O)−N), 136.2 (ArC−C(O)−NH), 131.5 (ArC), 123.6 (ArC), 123.0 (ArC), 94.8 (CH_3−C−CH_3), 75.4 (N−CH_2−CH), 70.1 (HN−CH_2−CH), 64.0 (HN−CH_2−CH−CH_2−OH), 61.2 (N−CH_2−CH−CH_2−OH), 50.2 (N−CH_2), 43.4 (HN−CH_2), 25.6 (CH_3), 23.7 (CH_3); LC-HRMS Calcd for $C_{17}H_{24}N_3O_8^+$ [M + H^+] 398.1558, found 398.1555. Data for **11**: Mp 115−120 °C; IR (ATR) 3326 (br), 3082 (w), 2984 (w), 2936 (w), 2882 (w), 1630 (s), 1534 (s), 1348 (m), 1247 (m), 1209 (m), 1048 (s), 841 (m), 716 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.13 (t, 1H, NH), 8.75 (s, 1H, ArH), 8.46 (s, 1H, ArH), 8.39 (s, 1H, ArH), 4.90 (t, 1H, OH), 4.24 (qui, 1H, HN−CH_2−CH), 4.13 (m, 1H, N−CH_2−CH), 4.01 (dd, 1H, HN−CH_2−CH−CH_2), 3.71 (m, 1H, HN−CH_2−CH−CH_2), 3.49 (m, 2H, N−CH_2−CH−CH_2−OH), 3.48 and 3.41 (2m in a 1:1 ratio, 2H, N−CH_2), 3.43 (m, 2H, NH−CH_2), 1.68 (s, 3H, N−C−CH_3), 1.63 (s, 3H, N−C−CH_3), 1.35 (s, 3H, O−C−CH_3), 1.26 (s, 3H, O−C−CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8

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(Ar−C(O)), 163.7 (Ar−C(O)), 147.8 (ArC−NO_2), 139.4 (ArC−C(O)−N), 135.8 (ArC−C(O)−NH), 131.1 (ArC), 123.8 (ArC), 123.0 (ArC), 108.5 (CH_3−(O)C(O)−CH_3), 94.8 (CH_3−(O)C(N)−CH_3), 75.4 (N−CH_2−CH), 74.1 (HN−CH_2−CH), 66.8 (HN−CH_2−CH−CH_2), 61.2 (N−CH_2−CH−CH_2), 50.2 (N−CH_2), 42.4 (HN−CH_2), 26.9 ((O)C(O)−CH_3), 25.6 ((N)C(O)−CH_3), 25.4 ((O)C(O)−CH_3), 23.7 ((N)C(O)-CH_3); LC-HRMS Calcd for $C_{20}H_{28}N_3O_8^+$ [M + H^+] 438.1871, found 438.1848.

Synthesis of Model Compounds 12. *N,N′-Bis(2-hydroxyethyl)isophthalamide (**12a**).* Dimethyl isophthalate (70.0 g, 0.360 mol) was added to 2-methoxyethanol (140 mL), and then ethanol amine (56.0 g, 0.918 mol) was added to the mixture. The solution was immediately heated to reflux (125−130 °C), and the mixture was refluxed in 20 h. Complete conversion was confirmed by HPLC. The solution was cooled to room temperature, and then diethyl ether (250 mL) was added for crystallization. The slurry was filtered, and the solid was washed with a mixture of diethyl ether and 2-methoxyethanol. The white solid was finally dried in a vacuum oven to obtain **12a** (51.0 g, 56%). The purity (HPLC, Program 5, Brownlee 250 × 4.6 mm) was >99%. Data for **12a**: Mp 145−146 °C; IR (ATR) 3384 (m), 3254 (br), 3075 (w), 2970 (w), 2925 (w), 2870 (w), 1635 (s), 1542 (s), 1301 (m), 1056 (s), 875 (m), 687 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.54 (t, 2H, NH), 8.33 (s, 1H, ArH), 7.97 (d, 2H, ArH), 7.54 (t, 1H, ArH), 4.77 (t, 2H, OH), 3.53 (m, 4H, HN−CH_2−CH_2), 3.36 (m, 4H, HN−CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0 (Ar−C(O), 2C), 134.7 (ArC−C(O), 2C), 129.7 (ArC, 2C), 128.3 (ArC), 126.2 (ArC), 59.8 (HN−CH_2−CH_2, 2C), 42.3 (HN−CH_2, 2C); HRMS Calcd for $C_{12}H_{16}N_2O_4Na^+$ [M + Na^+] 275.1008, found 275.1007.

*N,N′-Bis(2-hydroxyethyl)-5-nitroisophthaldiamide (**12b**).* Dimethyl 5-nitroisophthalate (40.0 g, 0.167 mol) was added to methanol (140 mL), and then ethanol amine (24.9 g, 0.408 mol) was added to the mixture. The solution was immediately heated to reflux (70−75 °C), and after 70 h it was cooled to room temperature. During the cooling crystallization occurred. The slurry was filtered, and the solid was washed with methanol. The white solid was finally dried in a vacuum oven to give **12b** (46.8 g, 94%). The purity (HPLC; Brownlee 250 × 4.6 mm; Program 5) was >99%. Data for **12b**: Mp 150−151 °C; IR (ATR) 3281 (br), 3076 (w), 2921 (w), 1647 (s), 1549 (m), 1529 (s), 1346 (m), 1327 (m), 1300 (m), 1083 (m), 1049 (m), 918 (m), 674 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.95 (t, 2H, NH), 8.80 (s, 2H, ArH), 8.76 (s, 1H, ArH), 4.81 (t, 2H, OH), 3.55 (m, 4H, HN−CH_2−CH_2), 3.38 (m, 4H, HN−CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.9 (Ar−C(O), 2C), 147.8 (ArC−NO_2), 136.2 (ArC−C(O), 2C), 132.3 (ArC), 126.2 (ArC, 2C), 59.5 (HN−CH_2−CH_2, 2C), 42.5 (HN−CH_2, 2C); HRMS Calcd for $C_{12}H_{15}N_3O_6Na^+$ [M + Na^+] 320.0859, found 320.0858.

*N,N′-Bis(3-hydroxypropyl)isophthalamide (**12c**).* Dimethyl isophthalate (100 g, 0.515 mol) was added to 3-amino-1-propanol (117 g, 1.56 mol). The solution was immediately heated to 120−140 °C, and after 2.5 h methanol (330 mL) was added for crystallization. Then the solution was carefully cooled to 5 °C. During the cooling crystallization occurred. The slurry was stirred overnight at 5 °C, and then the solid was filtered and washed with methanol. The white solid was finally dried in a vacuum oven to obtain **12c** (130 g, 90%). The purity (HPLC; Brownlee 250 × 4.6 mm; Program 5) was 96%. The crude was dissolved in methanol (400 mL) at 60 °C for recrystallization. The solution was carefully cooled to 5 °C, and during the cooling diethyl ether (650 mL) was added. Next day the slurry was filtered and washed with a mixture of methanol and diethyl ether. The recrystallized white solid was finally dried in a vacuum oven to obtain **12c** (106 g, 74%). The purity (HPLC) was 98%. Data for **12c**: Mp 128−129 °C; IR (ATR) 3283 (br), 3094 (w), 2952 (w), 2868 (w), 1628 (s), 1567 (m), 1546 (s), 1336 (m), 1314 (m), 1281 (m), 1052 (s), 1000 (m), 675 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.57 (t, 2H, NH), 8.28 (s, 1H, ArH), 7.95 (d, 2H, ArH), 7.54 (t, 1H, ArH), 4.51 (bs, 2H, OH), 3.47 (t, 4H, HN−CH_2−CH_2−CH_2), 3.33 (q, 4H, HN−CH_2), 1.69 (qui, 4H, HN−CH_2−CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9 (Ar−C(O), 2C), 134.8 (ArC−C(O), 2C), 129.6 (ArC, 2C), 128.3 (ArC), 126.1 (ArC), 58.6 (HN−CH_2−CH_2−CH_2, 2C), 36.7 (HN−H_2, 2C), 32.4 (HN−CH_2−CH_2, 2C); HRMS Calcd for $C_{14}H_{20}N_2O_4Na^+$ [M + Na^+] 303.1321, found 303.1320.

*N,N′-Bis(3-hydroxypropyl)-5-nitroisophthalamide (**12d**).* Dimethyl 5-nitroisophthalate (40.0 g, 0.167 mol) was added to methanol (140 mL), and then 3-amino-1-propanol (30.2 g, 0.402 mol) was added to the mixture. The solution was immediately heated to reflux (70−75 °C). After 70 h the solution was cooled to room temperature, and during the cooling crystallization occurred. The slurry was filtered, and the white solid was washed with methanol before drying in a vacuum oven to obtain **12d** (50.0 g, 92%). The purity (HPLC; Brownlee 250 × 4.6 mm; Program 5) was >99%. Data for **12d**: Mp 129−130 °C; IR (ATR) 3279 (br), 3084 (w), 2943 (w), 2878 (w), 1638 (s), 1555 (m), 1534 (s), 1330 (s), 1303 (m), 1050 (s), 1033 (s), 1000 (s), 696 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.94 (t, 2H, NH), 8.77 (s, 2H, ArH), 8.73 (s, 1H, ArH), 4.51 (t, 2H, OH), 3.48 (q, 4H, HN−CH_2−CH_2−CH_2), 3.36 (m, 4H, HN−CH_2), 1.71 (qui, 4H, HN−CH_2−CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.7 (Ar−C(O), 2C), 147.9 (ArC−NO_2), 136.3 (ArC−C(O), 2C), 132.2 (ArC), 124.1 (ArC, 2C), 58.6 (HN−CH_2−CH_2−CH_2, 2C), 37.0 (HN−CH_2, 2C), 32.2 (HN−CH_2−CH_2, 2C); HRMS Calcd for $C_{14}H_{19}N_3O_6Na^+$ [M + Na^+] 348.1172, found 348.1171.

*3-(2,2-Dimethyloxazolidine-3-carbonyl)-N-(2-hydroxyethyl)benzamide (**13a**).* Acetone (75 mL, 59 g, 1.0 mol) and acetic anhydride (6.0 mL, 6.5 g, 0.064 mol) were mixed at room temperature. PTSA (0.6 g) and **12a** (3.8 g, 0.015 mol) were then added. After 3 h the solution was diluted in dichloromethane (200 mL), and the organic phase was then washed with aqueous NaOH (2 M, 2 × 50 mL), water (50 mL) and brine (30 mL). The organic phase was finally dried over $MgSO_4$, and solvents were removed on a rotary evaporator. The white solid obtained was dried further under vacuum at 50 °C and weighed 2.7 g. A HPLC sample of the solid was treated with NaOH, neutralized with acetic acid, and analysed by HPLC (Supelco 250 × 4.6 mm; Program 3). The amount of **13a** was 5%. Preparative HPLC was used for isolation. Two runs were performed on the LUNA 250 × 50 mm column. Solid (1150−1300 mg) was dissolved in methanol (20 mL) and then diluted with water (80 mL). Some aqueous KOH (10 M) was added, and when the pH was stable at 13−14 (pH paper), it was adjusted to approximately 5 by adding a little acetic acid. One fraction was collected and subsequently concentrated on a rotary evaporator in the presence of small amounts of ammonia to keep the solution slightly alkaline. The concentrate was finally freeze-dried, and the weight of the pale yellow, highly viscous liquid obtained was 41 mg. The purity of **13a** (HPLC; LUNA 250 × 4.6 mm; Program 4) was 83%. IR (ATR) 3310

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(br), 3077 (w), 2983 (w), 2937 (w), 2883 (m), 1619 (s), 1535 (s), 1433 (m), 1240 (s), 1145 (m), 1054 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.58 (t, 1H, NH), 7.97 (s, 1H, ArH), 7.94 (d, 1H, ArH), 7.64 (d, 1H, ArH), 7.52 (t, 1H, ArH), 4.76 (bs, 1H, OH), 3.90 (t, 2H, N−CH_2−CH_2), 3.50 (m, 2H, N−CH_2), 3.50 (m, 2H, HN−CH_2−CH_2), 3.34 (m, 2H, HN−CH_2), 1.61 (s, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1 (Ar−C(O)−N), 165.5 (Ar−C(O)−NH), 137.8 (ArC−C(O)−NH), 134.5 (ArC−C(O)−N), 128.9 (ArC), 128.5 (ArC), 128.4 (ArC), 125.1 (ArC), 94.9 (CH_3−C−CH_3), 63.1 (N−CH_2−CH_2), 59.7 (HN−CH_2−CH_2), 48.2 (N−CH_2), 42.2 (HN−CH_2), 24.2 (CH_3, 2C); LC-HRMS Calcd for $C_{15}H_{21}N_2O_4^+$ [M + H^+] 293.1496, found 293.1460.

*3-(2,2-Dimethyloxazolidine-3-carbonyl)-N-(2-hydroxyethyl)-5-nitrobenzamide (**13b**).* Acetone (100 mL, 79.1 g, 1.36 mol) and acetic anhydride (8.0 mL, 8.7 g, 0.085 mol) were mixed at rt. PTSA (0.8 g) and **12b** (5.8 g, 0.015 mol) were then added. After 5 h the solution was diluted with ethyl acetate (300 mL), and the organic phase was washed with NaOH (2 M, 2 × 50 mL), water (50 mL) and brine (30 mL). The organic phase was finally dried over $MgSO_4$, and solvents were removed on a rotary evaporator. A HPLC sample of the residue was treated with NaOH, neutralized with acetic acid, and analysed by HPLC (Supelco 250 × 4.6 mm; Program 3). The amount of **13b** was 8%. Preparative HPLC was used for isolation. Three runs were performed on the LUNA 250 × 50 mm column. The residue (5−10 mL) was diluted in methanol (10 mL) and then diluted further with water (80 mL). Some aqueous KOH (10 M) was added, and when the pH was stable at 13−14 (pH paper), it was adjusted to approximately 5 by adding a little acetic acid. One fraction was collected and subsequently concentrated on a rotary evaporator in the presence of small amounts of ammonia to keep the solution slightly alkaline. The concentrate was finally freeze-dried, and the weight of the white solid obtained was 205 mg. The purity of **13b** (HPLC; LUNA 250 × 4.6 mm; Program 4) was 97%. Mp 173−174 °C; IR (ATR) 3424 (br), 3292 (m), 3088 (w), 2991 (w), 2935 (w), 2880 (w), 1622 (s), 1535 (m), 1328 (m), 1251 (m), 1057 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.99 (t, 1H, NH), 8.74 (t, 1H, ArH), 8.45 (t, 1H, ArH), 8.41 (t, 1H, ArH), 4.80 (bs, 1H, OH), 3.92 (t, 2H, N−CH_2−CH_2), 3.54 (t, 4H, N−CH_2 and HN−CH_2−CH_2), 3.37 (q, 2H, HN−CH_2), 1.63 (s, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8 (Ar−C(O)−N), 163.5 (Ar−C(O)−NH), 147.8 (ArC−NO_2), 139.3 (ArC−C(O)−N), 136.2 (ArC−C(O)−NH), 131.1 (ArC), 123.6 (ArC), 123.1 (ArC), 94.3 (CH_3−C−CH_3), 63.3 (N−CH_2−CH_2), 59.5 (HN−CH_2−CH_2), 48.1 (N−CH_2), 42.6 (HN−CH_2), 24.1 (CH_3, 2C); LC-HRMS Calcd for $C_{15}H_{20}N_3O_6^+$ [M + H^+] 338.1347, found 338.1320.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H NMR and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): T. Hland is employed by GE Healthcare which is a co-funder of the scholarship that paid for his PhD studies.

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