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Process Development and Scale-up of T3-Sulfate, A New Prodrug Alternative to the Conventional Hormone Therapy of Hypothyroidism

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ABSTRACT: An efficient and scalable preparation of the thyroid hormone analogue *O*-[3-iodo-4-(sulfooxy)phenyl]-3,5-diiodo-L-tyrosine sodium salt (T3-sulfate, **1**) is reported. The synthesis involved monoiodination of *O*-(4-hydroxyphenyl)-3,5-diiodo-L-tyrosine to give liothyronine (**2**) which was sulfated with chlorosulfonic acid in *N*,*N*-dimethylacetamide. Crude T3-sulfate was initially purified by chromatography on polystyrene resin Amberlite XAD 1600 and then crystallized with ethanol. This strategy was scaled-up to give a process suitable for the production of kilogram quantities of API, needed to support preclinical and clinical studies.

INTRODUCTION

Hypothyroidism is a common and well-known endocrine disease, occurring in about 4−5% of the adult population in Western countries.[1] Hypothyroidism is related to an insufficient blood concentration of thyroid hormones,[2] precisely liothyronine[3] (**2**) and thyroxine[4] (**3**) (Figure 1), due to several causes

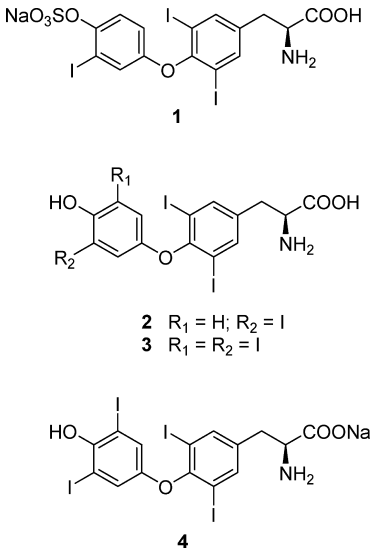

Figure 1. Structures of thyroid hormones and analogues: T3-sulfate **1**, liothyronine **2**, thyroxine **3**, and sodium levothyroxine **4**.

such as autoimmune thyroiditis, thyroidectomy, radioiodine therapy, or to iodine deficiency in the diet, especially in poor countries. Symptoms associated with hypothyroidism are quite severe and include tiredness, depression, bradycardia, weight gain, poor concentration, muscle pain, dry skin, hair loss, and cold intolerance.

The treatment of choice for hypothyroidism is the life-long oral administration of synthetic sodium levothyroxine (**4**), which is the sodium salt of thyroxine (Figure 1). Sodium levothyroxine is absorbed in the small intestine,[4c] it has an in vivo half-life of a week[1b] and undergoes enzymatic deiodination in peripheral tissues to liothyronine,[1,2] which is the most active biological hormone. Sodium levothyroxine is usually taken daily in a single dose (e.g., 125 μg per day in a 70 kg adult)[1c] reducing the symptoms of hypothyroidism.

The administration of both thyroxine and liothyronine has been also studied since the addition of the more active liothyronine could help in reaching the optimal plasma level of both hormones, consequently attaining their physiological concentrations in target tissues. A controversial debate is still ongoing since the results of several studies performed following this approach did not always show a real improvement in patients' quality of life. Moreover, the much shorter half-life of liothyronine (1 day) does not allow a single daily dosage, which would make therapeutical treatment more awkward for patients.[5]

A possible pharmacological alternative to liothyronine could be its derivative T3-sulfate **1** (Figure 1).[6] In fact, one of the ways the organism disposes of lyothyronine is by an in vivo sulfation, mainly performed in the liver.[7] On the other hand, it has been found that in some tissues there is a desulfation process capable of restoring liothyronine from T3-sulfate.[8] Preclinical studies demonstrated that T3-sulfate could be considered as a pro-drug of liothyronine, and its in vivo enzymatic conversion is able to ensure a stable level of liothyronine for more than 48 h.[9] Therefore, the thyroxine/T3-sulfate combination therapy could represent a reliable alternative to the conventional sodium levothyroxine treatment. Clinical studies are currently ongoing in this respect.[10]

This manuscript describes the development of a scalable process for the preparation of T3-sulfate on a kilogram scale and the synthesis of the related impurities.

RESULTS AND DISCUSSION

Process Development. The syntheses of T3-sulfate (**1**) reported in literature were performed only with ^{125}I or ^{131}I-

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labelled liothyronine in a microgram scale or less, employing sulfuric acid, chlorosulfonic acid, and trimethylamine or pyridine complexes of sulfur trioxide as sulfating agents.[6] Among these and other sulfating agents we have chosen chlorosulfonic acid[11] because it is widely available from different commercial sources, economical and more effective in terms of yields.[6a] The following parameters were studied for the process optimization: (i) solvent, (ii) stoichiometric excess of chlorosulfonic acid, (iii) time and temperature reaction, (iv) quenching conditions, (v) purification.

Solvent. Our preliminary experiments were carried out on gram scale using chlorosulfonic acid and *N,N*-dimethylformamide (DMF) as solvent, as reported.[6a] The results were disappointing since the yield of T3-sulfate was lower than expected, and several byproducts were formed, in particular compounds **5** and **6** (Figure 2), whose structures were assigned

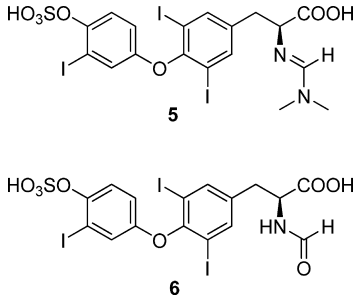


Figure 2. Byproducts formed when chlorosulfonic acid is used in DMF.

on the basis of HPLC-MS analysis. The explanation could be the formation of a chlorosulfonic acid−DMF adduct[12] such as **7** (Figure 3). Liothyronine can react with **7** following two

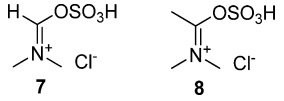


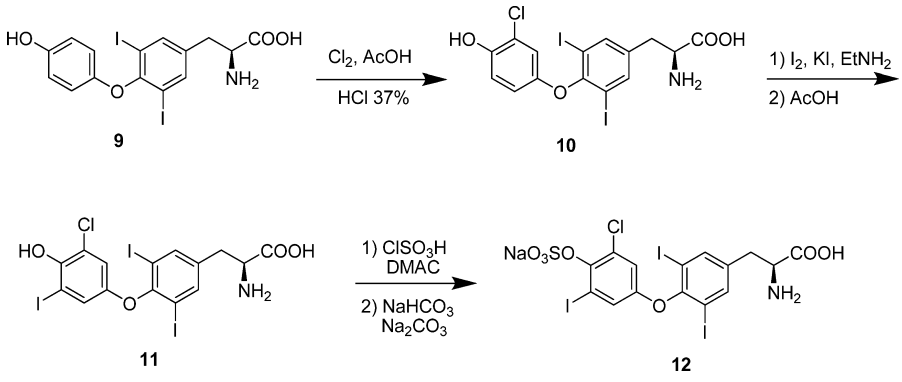
Figure 3. Possible adducts chlorosulfonic acid−DMF **7** and chlorosulfonic acid−DMAC **8**.

different pathways: (a) nucleophilic attack of phenol group at the sulfur atom giving the desired product **1**, and (b) nucleophilic attack of amino group at the carbon atom generating byproduct **5**. After screening several different solvents, the best results were obtained with *N,N*-dimethylacetamide (DMAC). In this case the reaction between chlorosulfonic acid and DMAC to give the adduct **8** (Figure 3) cannot be excluded, but the formation of a byproduct such as **5** was not observed. This is owing to the fact that probably path b is suppressed due to the greater steric hindrance of the iminium carbon atom in **8**.

Chlorosulfonic Acid. The excess of chlorosulfonic acid employed in the reaction was found to be a critical issue. According to relevant literature,[6a] a huge ratio of 100 mol of chlorosulfonic acid per mol of liothyronine should be used, but these conditions are unlikely for economic reasons and temperature control, since the addition of chlorosulfonic acid to DMAC is exothermic. Moreover, we found that the amount of chlorosulfonic acid is directly related to the formation of the byproduct **12** (Scheme 1). This byproduct cannot be eliminated during the purification step, and because of this it must be kept as low as possible in the crude. In order to ensure good conversion of starting material and at the same time a lower amount of byproduct **12**, an optimized ratio of 8 mol of chlorosulfonic acid per mol of liothyronine was found to be the best compromise. It must be pointed out that small traces of sulfuryl chloride (SO_2Cl_2) and/or pyrosulfuryl chloride ($ClSO_2OSO_2Cl$) in commercial chlorosulfonic acid might contribute to the formation of **12**. Indeed, an increased amount of **12** was found when chlorosulfonic acid doped with sulfuryl chloride (up to 8%) was employed. This byproduct was synthesized following the strategy shown in Scheme 1 in order to confirm the structure and to get a sufficient amount as a reference standard. The synthesis involved chlorination of *O*-(4-hydroxyphenyl)-3,5-diiodo-L-tyrosine (**9**)[13] with a solution of chlorine in acetic acid then iodination with iodine, potassium iodide, and ethylamine, to give compound **11**. Then the final reaction with chlorosulfonic acid in DMAC gave **12** with an overall yield of 40%. This strategy was preferred over the direct chlorination of liothyronine (**2**) which resulted in incomplete conversion and formation of a dichlorinated byproduct which was difficult to separate.

Time and Temperature Reaction. A calorimetric evaluation showed that the reaction between chlorosulfonic acid and liothyronine is exothermic with an adiabatic increasing of temperature of 27 °C and a heat release of 176 kcal mol^{-1}, based on liothyronine. Moreover, it was found that the heat evolution rate is dependent on chlorosulfonic acid addition rate, and no accumulation occurred at the end of the addition. In all the initial laboratory trials the temperature of the reaction mixture was set at 0 °C. The optimization work revealed that a temperature range of 5−15 °C was easier to control during

Scheme 1. Synthesis of chlorinated byproduct 12



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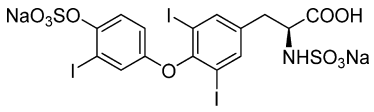
scale-up without increasing the amount of byproducts. With all the chlorosulfonic acid added the reaction mixture should be left for completion at 20 °C for at least 2 h. Longer reaction times should be avoided as they displayed an increase in byproducts. This is especially true for compound **13** (Figure 4) deriving from sulfation of both phenol and amino groups of liothyronine. Also **13** was independently synthesized to confirm the structure.



13

Figure 4. Structure of disulfated byproduct **13**.

Quenching Conditions. Stability tests showed that aqueous solutions of T3-sulfate are not stable in acidic conditions, regenerating liothyronine by hydrolysis of the sulfate moiety. For this reason, the reaction mixture cannot be simply quenched by addition of water but needs to be slowly poured into an aqueous solution of sodium bicarbonate or sodium carbonate in excess, maintaining the temperature at 30 °C. Similarly, when the reaction is ongoing, the same quenching step must be always carried out on each sample for HPLC analysis, in order to obtain realistic information on liothyronine conversion.

Purification. The aqueous solution obtained from the quenching step is a complex mixture containing T3-sulfate, several byproducts, DMAC, and a large quantity of inorganic salts. We found an easy and convenient purification was the elution of this mixture through a column of Amberlite XAD 1600, a polystyrene, nonionic, macroreticular resin. Elution with water allowed to dispose of all inorganic salts and the majority of DMAC, while T3-sulfate and the byproducts remained fixed on the resin. All of the last traces of DMAC and hydrophilic byproducts, such as **13** and **14**, were removed carrying on the elution with water/acetone 95:5 (v/v). Compound **14**, as well as **15** (Scheme 2), derive from the sulfation of *O*-(4-hydroxyphenyl)-3,5-diiodo-L-tyrosine (**9**) and thyroxine (**3**). These two impurities are always present in small amounts in liothyronine starting material. Byproducts **14** and **15** were also synthesized as reported in Scheme 2. T3-sulfate was recovered from the column simply by increasing the acetone amount in the eluent. For the synthesis of laboratory lots, the fractions with a content of product greater than 99% (HPLC area) were pooled, concentrated, and treated with acetone to give, after filtration and drying, T3-sulfate as a white powder. The column was finally washed with pure acetone to elute all of the more lipophilic compounds such as unreacted liothyronine (**2**) and byproduct **15**. At this point the column, after being reconditioned with water, was ready to be employed for another trial of purification.

Scale-up. The scale-up of T3-sulfate required the availability of significant amounts of the costly starting material liothyronine (**2**). We decided to synthesize this key intermediate in house. For its preparation, among the several strategies reported in literature,[14] we chose the monoiodination of *O*-(4-hydroxyphenyl)-3,5-diiodo-L-tyrosine (**9**) (Scheme 3).[14d] The choice of this strategy was also based on the expertise that we had previously acquired in the manufacturing plant synthesis of sodium levothyroxine (**4**) on a multikilogram scale.[15] The intermediate **9** was synthesized in kilogram quantities starting from L-tyrosine and following the procedure reported by Chalmers and co-workers.[13]

Monoiodination of **9** was achieved with iodine and sodium iodide in an aqueous solution of ethylamine.[16] This method was preferred over the classical route employing aqueous ammonia, since it avoids the risk of formation of explosive nitrogen iodides. Moreover, **9** is much more soluble in aqueous solution of primary or secondary amines than aqueous ammonia. Amines can also increase the reactivity of iodine by the formation of a charge transfer complex, which has also been documented in the case of morpholine.[17]

Liothyronine (**2**), obtained as sodium salt, was converted into the zwitterionic form by treatment with acetic acid then reacted with chlorosulfonic acid in DMAC (Scheme 3). The reaction mixture was quenched with sodium carbonate and purified by elution through a column of Amberlite XAD 1600. The fractions with suitable purity were concentrated, and T3-sulfate was crystallized by addition of ethanol and cooling to 0 °C. After filtration and drying, T3-sulfate was isolated 68% yield and 99.0% purity (HPLC area %). The scale-up of this process enabled us to obtain 0.98 kg of pure T3-sulfate needed for preclinical and clinical evaluation as API.

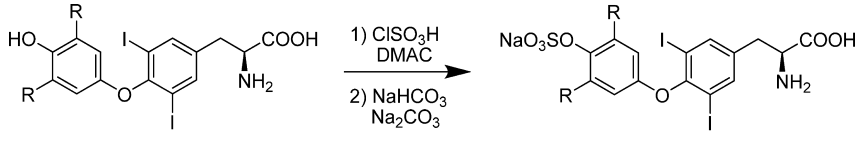
CONCLUSION

In conclusion, we have developed a practical and scalable process for the synthesis of T3-sulfate, a thyroid hormone analogue. The synthesis has been optimized and scaled-up to a kilogram scale, with an overall yield of 68% starting from *O*-(4-hydroxyphenyl)-3,5-diiodo-L-tyrosine (**9**). In addition, the syntheses of the main impurities have been reported.

EXPERIMENTAL SECTION

Instrumentation and Materials. ^{1}H- NMR and ^{13}C NMR spectra were recorded on Bruker DRX400 NMR spectrometer. The mass spectra were recorded on a ThermoFinnigan mass spectrometer TSQ700, electrospray ionization. FT-IR spectra were determined in KBr matrix on a PerkinElmer SPECTRUM2000 spectrophotometer. HPLC analyses were collected on Agilent 1100 or 1200 liquid chromatographs using a diode array detector, and the results are reported in area percentage (area %). Elemental analyses and calorimetry (RC1 Mettler

Scheme 2. Synthesis of sulfated byproducts 14 and 15



9 R = H
3 R = I

14 R = H
15 R = I

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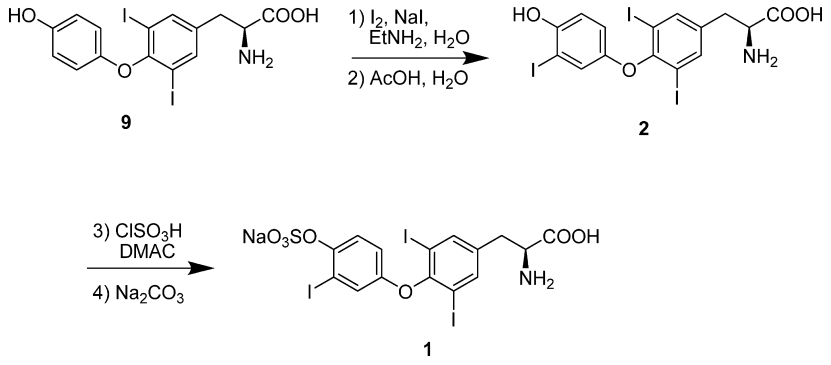
Scheme 3. Synthesis of T3-sulfate



calorimeter) were performed by Redox laboratories, Monza, Italy. *O*-(4-Hydroxyphenyl)-3,5-diiodo-L-tyrosine (**9**)[13] and *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine (thyroxine, **3**)[15] were synthesized following the procedures reported in literature. Chlorosulfonic acid was purchased from CABB, Merck, Sigma-Aldrich, or Acros. Amberlite XAD 1600 and XE 750 were purchased from Rohm&Haas. All other materials were purchased from commercial suppliers and used without any further purification. **Caution must be taken in handling chlorine, chlorosulfonic acid, and all the compounds here reported, which should be considered as highly potent substances**.

HPLC conditions. Stationary phase: Gemini C18, 5 μm, 150 mm × 4.6 mm i.d. (Phenomenex); mobile phase: eluent A = 0.05 M KH_2PO_4, eluent B = MeCN, gradient elution: t = 0 min (10% B), t = 45 min (70% B), flow rate: 1.5 mL min^{-1}; column temperature: 50 °C; UV detector at 225 nm.

***O*-[3-Iodo-4-(sulfooxy)phenyl]-3,5-diiodo-L-tyrosine Sodium Salt (1).** A solution of iodine (0.48 kg, 1.90 mol) and sodium iodide (0.65 kg, 4.34 mol) in water (3.5 L) was added to a solution of *O*-(4-hydroxyphenyl)-3,5-diiodo-L-tyrosine (**9**) (1 kg, 1.90 mol), sodium iodide (0.32 kg, 2.13 mol), and 70% aq ethylamine (2.8 kg, 43.5 mol) in water (3 L). The reaction mixture was stirred for 6 h at room temperature then cooled to 0 °C, stirred for 4 h, and filtered. The solid was washed with water (2.4 L), then suspended in water (15 L), and acetic acid (0.62 L) was added. The suspension was stirred for 2 h and the solid filtered and washed with water (3.5 L). The solid was resuspended in water (15 L), stirred, filtered, and washed with water (3.5 L) to give crude liothyronine (**2**). The solid was suspended in DMAC (13 L) and the remaining water was eliminated by distillation under vacuum. The suspension was cooled to 5 °C, and under nitrogen atmosphere, chlorosulfonic acid (1.54 kg, 13.21 mol) was slowly added, keeping the temperature below 10 °C. The temperature was raised to 20 °C, and the mixture stirred for 1 h then added to a solution of sodium carbonate (2.27 kg, 21.45 mol) in water (30 L). The solution was eluted through an Amberlite XAD 1600 (12.5 L) resin column. The resin was initially eluted with water (87.5 L) then with water/acetone solutions starting with 95:5 (v/v) and up to 70:30 (v/v). Fractions with the suitable purity were pooled and concentrated by distillation under vacuum. The suspension was cooled to 40 °C, and ethanol (6.6 L) was added obtaining a clear solution which was cooled to 0 °C causing precipitation. The solid was filtered, washed with 96% ethanol (4 L), and dried at 40 °C under vacuum to give **1** (0.98 kg; 68%) as a white powder. HPLC purity 99.0 area %; ^{1}H NMR (DMSO-d_6) δ 2.87 (1H, m), 3.17 (1H, m), 3.40 (2H, bm), 3.55 (1H, m), 6.74 (1H, d, J = 9 Hz), 7.09 (1H, s), 7.44 (1H, d, J = 9 Hz), 7.86 (2H, s); ^{13}C NMR (DMSO-d_6) δ 35.9, 55.9, 91.3, 92.8, 116.4, 121.8, 125.1, 140.0, 141.7, 149.5, 152.6, 170.5; MS m/z: calcd. for $[C_{15}H_{11}I_3NNaO_7S-Na]^-$ 729.7, found 729.8; IR (υ_{max}, cm^{-1}) 3595, 3300, 1629, 1246, 1182, 1054, 1027; Elem. Anal.: Calcd for $C_{15}H_{11}I_3NNaO_7S$: C 23.93, H 1.47, N 1.86, Na 3.05, I 50.56, S 4.26. Found: C 23.62, H 1.88, N 1.80, Na 2.91, I 51.30, S 4.28.

***O*-[3-Chloro-4-hydroxyphenyl]-3,5-diiodo-L-tyrosine (10).**[18] To a vigorously stirred mixture of *O*-(4-hydroxyphenyl)-3,5-diiodo-L-tyrosine (**9**) (10.0 g; 19 mmol) in AcOH (500 mL), 37% HCl (4.7 g; 48 mmol) was slowly added obtaining a clear solution. A solution of chlorine (**CAUTION**) in AcOH (1.5% w/w; 90 g; 19 mmol) was added in one portion, at room temperature. After 30 min a second amount of chlorine in acetic acid (1.5% w/w; 18 g; 4 mmol) was added, and after further 30 min nitrogen was bubbled into the solution to remove any trace of chlorine. The solution was evaporated, and the residue was crystallized from 2 M HCl (160 mL) and ethanol (30 mL). The solid obtained was filtered then dissolved in EtOH (50 mL) and water (80 mL), and a 30% (w/w) aqueous solution of sodium acetate was added until pH 5. The resulting suspension was filtered and the solid washed with water (10 mL) then dried at 40 °C for 20 h under vacuum to give **10** (10.5 g; 99%) as white powder. HPLC purity area 95.1%; ^{1}H NMR (DMSO-d_6) δ 2.92 (1H, m), 3.16 (1H, m), 3.45 (2H, bm), 3.62 (1H, m), 6.61 (1H, d, J = 6 Hz), 6.71 (1H, s), 7.01 (1H, d, J = 6 Hz), 7.86 (2H, s); ^{13}C NMR (DMSO-d_6) δ 35.2, 55.2, 92.6, 115.4, 116.6, 117.7, 120.3, 139.1, 141.4, 149.0, 149.2, 152.4, 170.1; MS m/z: calcd. for $[C_{15}H_{12}ClI_2NO_4 + H]^+$ 559.9, found 559.8.

***O*-[3-Chloro-5-iodo-4-hydroxyphenyl]-3,5-diiodo-L-tyrosine (11).** A 70% aqueous solution of ethylamine (69 g; 1070 mmol) was slowly added in 30 min to a suspension of **10** (10 g; 17.9 mmol) in water (85 mL) keeping the temperature below 20 °C. A solution of KI (9.38 g; 57 mmol) and iodine (5.62 g; 22.2 mmol) in water (27 mL) was added in 2 h, keeping the temperature below 20 °C. The solution was stirred for 20 h; then a 5% (w/w) solution of $Na_2S_2O_5$ was added until complete reaction of excess iodine. The solution was heated to 50 °C; acetic acid (75.2 g; 1250 mmol) was added until final pH 5. The resulting suspension was heated to 60 °C and filtered. The solid was washed with hot water (485 g. 65 °C) then dried at 40 °C for 20 h under vacuum to give **11** (10.9 g; 89%) as a white powder. HPLC purity area 88.8%; ^{1}H NMR (DMSO-d_6) δ 2.85 (1H, m), 3.17 (1H, m), 3.40 (2H, bm), 3.57 (1H, m), 6.81 (1H, s), 7.10 (1H, s), 7.86 (2H, s); ^{13}C NMR (DMSO-d_6) δ 35.5, 55.4, 89.8, 92.4, 116.9, 121.4, 124.4, 139.6,

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141.4, 148.5, 150.0, 151.9, 169.9; MS *m/z*: calcd. for $[C_{15}H_{11}ClI_3NO_4-H]^-$ 683.7, found 683.9.

***O*-[3-Chloro-5-iodo-4-(sulfooxy)phenyl]-3,5-diiodo-L-tyrosine Sodium Salt (12).** In a nitrogen atmosphere chlorosulfonic acid (63.4 g; 544 mmol) was slowly added over 1.5 h to a vigorously stirred suspension of **11** (15.55 g; 22.7 mmol) in DMAC (300 mL), maintaining the temperature at 0 °C. The reaction mixture was allowed to warm to room temperature and reacted for 4 h. The mixture was slowly poured into a stirred 8% (w/w) aq solution of $NaHCO_3$ (684 g; 651 mmol); then a 15% (w/w) aq solution of Na_2CO_3 was added until pH 7. The resulting solution was loaded onto an Amberlite XE 750 (650 mL) resin column. The resin was initially eluted with water then with an acetone/water gradient up to acetone/water 1:1 (v/v). The fractions containing the product were concentrated, and 1 M HCl was added until pH 6.2. The solution was evaporated, and the solid was dried at 40 °C for 20 h under vacuum to give **12** (8.0 g; 45%) as a white powder. HPLC purity area 99%; ^{1}H NMR (DMSO-d_6) δ 2.86 (1H, m), 3.18 (1H, m), 3.39 (2H, bm), 3.57 (1H, m), 6.81 (1H, d, J = 1.5 Hz), 7.12 (1H, d, J = 1.5 Hz), 7.86 (2H, s); ^{13}C NMR (DMSO-d_6) δ 35.8, 55.5, 92.7, 96.0, 117.5, 125.0, 129.2, 139.9, 141.8, 146.6, 152.2, 153.0, 170.5; MS *m/z*: calcd. for $[C_{15}H_{10}ClI_3NNaO_7S-Na]^-$ 763.7, found 763.8; Elem. Anal.: Calcd for $C_{15}H_{10}ClI_3NNaO_7S$: C 22.88, H 1.28, N 1.78, Cl 4.50, Na 2.92, I 48.35, S 4.07. Found: C 22.68, H 0.89, N 1.76, Cl 4.61, Na 3.48, I 48.69, S 3.76.

***O*-[3-Iodo-4-(sulfooxy)phenyl]-3,5-diiodo-*N*-sulfo-L-tyrosine (13).** In nitrogen atmosphere chlorosulfonic acid (6.15 g; 53 mmol) was slowly added over 15 min to a vigorously stirred suspension of *O*-(4-hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosine (**2**) (5 g; 7.7 mmol) in pyridine (100 mL), maintaining the temperature at 15−20 °C. The reaction mixture was stirred for 1 h then slowly poured into a stirred solution of $NaHCO_3$ (2.77 g; 34 mmol) in water (150 mL), and 1 M NaOH was added until pH 8.2. The resulting solution was concentrated to about 50 mL then loaded onto an Amberlite XAD 1600 (130 mL) resin column. The resin was initially eluted with water then with an acetone/water gradient. The fractions containing the product were evaporated, and the glassy residue obtained was treated twice with acetone (20 mL). The suspension was filtered and the solid dried at 40 °C for 48 h under vacuum to give **13** (1.73 g; 27%) as a white powder. HPLC purity 99.4 area %; ^{1}H NMR (D_2O) δ 2.91 (2H, d, J = 6 Hz), 3.86 (1H, t, J = 6 Hz), 6.90 (1H, dd, J_1 = 3 Hz, J_2 = 9 Hz), 7.28 (1H, d, J = 3 Hz), 7.35 (1H, d, J = 9 Hz), 7.81 (1H, s); ^{13}C NMR (D_2O) δ 37.7, 59.7, 90.2, 91.3, 117.2, 122.8, 126.2, 140.0, 141.8, 147.1, 151.8, 154.2, 178.8; MS *m/z*: calcd. for $[C_{15}H_{10}I_3NNa_2O_{10}S_2-H-Na]^{-2}$ 415.3, found 415.7; Elem. Anal.: Calcd for: $C_{15}H_{10}I_3NNa_2O_{10}S_2$: C 21.07, H 1.18, N 1.64, Na 5.38, I 44.52, S 7.50. Found: C 20.34, H 1.31, N 1.62, Na 7.86, I 39.42, S 7.94.

***O*-[4-(Sulfooxy)phenyl]-3,5-diiodo-L-tyrosine Sodium Salt (14).** In nitrogen atmosphere chlorosulfonic acid (8.39 g; 72 mmol) was slowly added in 45 min to a vigorously stirred suspension of *O*-(4-hydroxyphenyl)-3,5-diiodo-L-tyrosine (**9**) (5.04 g; 9.6 mmol) in DMAC (100 mL), maintaining the temperature at 0 °C. The reaction mixture was allowed to warm to room temperature and reacted for 4 h. The mixture was slowly poured into a stirred solution of $NaHCO_3$ (19.6 g; 233 mmol) in water (225 mL). The resulting solution was eluted through an Amberlite XAD 1600 (125 mL) resin column. The resin was initially eluted with water then with an acetone/water gradient. The fractions containing the product were evaporated under vacuum and the solid residue stirred for 2 h with CH_2Cl_2 (15 mL). The solid was filtered and treated again with CH_2Cl_2 (15 mL), as described above, in order to eliminate the last traces of DMAC. The solid was filtered, dried, and dissolved in water, and 1 M HCl was added until pH 6.2. The suspension was evaporated, and the solid was dried at 40 °C for 20 h under vacuum to give **14** (3.28 g; 54.5%) as a white powder. HPLC purity 98.8 area %; ^{1}H NMR (DMSO-d_6) δ 2.87 (1H, m), 3.19 (1H, m), 3.46 (2H, bm), 3.55 (1H, m), 6.67 (2H, d, J = 9 Hz), 7.10 (2H, d, J = 9 Hz), 7.86 (2H, s); ^{13}C NMR (DMSO-d_6) δ 35.8, 55.9, 93.0, 116.0, 122.8, 139.7, 141.7, 149.0, 152.8, 153.0, 170.9; MS *m/z*: calcd for $[C_{15}H_{12}I_2NNaO_7S-Na]^-$ 603.8, found 604.0; Elem. Anal.: Calcd for $C_{15}H_{12}I_2NNaO_7S$: C 28.73, H 1.93, N 2.23, Na 3.67, I 40.47, S 5.11. Found: C 28.69, H 1.36, N 2.23, Na 4.28, I 39.97, S 4.91.

***O*-[3,5-Diiodo-4-(sulfooxy)phenyl]-3,5-diiodo-L-tyrosine Sodium Salt (15).** In nitrogen atmosphere chlorosulfonic acid (14.0 g; 120 mmol) was slowly added over 1 h to a vigorously stirred suspension of *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosine (**3**) (10 g; 13 mmol) in DMAC (200 mL), maintaining the temperature at 0 °C. The reaction mixture was then allowed to warm to room temperature and reacted for 4 h. The mixture was slowly poured in 1.5 h into a stirred solution of $NaHCO_3$ (32.8 g; 390 mmol) in water (450 mL). The gelatinous solid precipitated was completely dissolved by addition of Na_2CO_3 (4.65 g; 44 mmol). The resulting solution was eluted through an Amberlite XAD 1600 (250 mL) resin column. The resin was initially eluted with water to eliminate salts and DMAC; then an acetone/water gradient was applied. The fractions containing the product were collected and evaporated, and the residue was treated with acetone (8 mL). The suspension was filtered and the solid dried at 40 °C for 48 h under vacuum to give **15** (2.06 g; 18%) as a white powder. HPLC purity 97.0 area %; ^{1}H NMR (DMSO-d_6) δ 2.80 (1H, m), 3.14 (1H, m), 3.51 (1H, m), 7.14 (2H, s), 7.87 (2H, s); ^{13}C NMR (DMSO-d_6) δ 37.4, 56.5, 92.5, 93.5, 126.5, 141.5, 141.7, 150.1, 151.9, 153.0, 173.6; MS *m/z*: calcd. for $[C_{15}H_{10}I_4NNaO_7S-Na]^-$ 855.6, found 855.7; Elem. Anal.: Calcd for: $C_{15}H_{10}I_4NNaO_7S$: C 20.50, H 1.15, N 1.59, Na 2.62, I 57.76, S 3.65. Found: C 20.70, H 0.91, N 1.63, Na 4.26, I 57.04, S 3.44.

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The authors declare no competing financial interest.

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