Synthesis of Human Ultraviolet Filter Compounds: *O*-β-D-Glucopyranosides of 3-Hydroxykynurenine and 2-Amino-3-hydroxy-γ-oxobenzenebutanoic Acid

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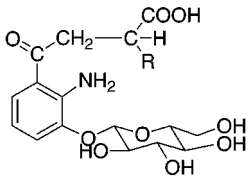
Received November 24, 1998

The role of endogenous tryptophan-derived UV filters in aging lenses and in human cataract is becoming increasingly important. The two major UV filters found in the lenses of primates, the *O*-β-D-glucopyranosides of 3-hydroxykynurenine and 2-amino-3-hydroxy-γ-oxobenzenebutanoic acid, **1** and **2**, were synthesized from the common benzoyl acrylate precursor 2-amino-3-hydroxybenzoylacrylic acid **10**. Synthesis of compound **3**, the α-*N*-acetyl derivative of **1**, was achieved using coupling of 2-nitro-3-benzyloxyacetophenone **4** with the sodium salt of diethyl acetamidomalonate as a key step. This is the first reported synthesis of the lenticular glucopyranoside **2** and the *N*-acetyl compound **3**.

The lenses of many vertebrates contain low molecular weight fluorescent compounds, known as UV filters. These play an important role in protecting the retina, and possibly the lens, from UV-induced damage by absorbing the near-UV radiation that is transmitted by the cornea.[1] Kynurenine, 3-hydroxykynurenine, and the *O*-β-D-glucopyranosides of 3-hydroxykynurenine and 2-amino-3-hydroxy-γ-oxobenzenebutanoic acid, **1** and **2**, fulfill such a role in human lenses.[2,3]

A number of as yet unidentified UV filters are contained in the human lens. As enzymes capable of acetylating amino groups of lens proteins (such as α- and β-crystallins) and small peptides are known to be active in the lens,[4] the *N*-acetyl derivative of the most abundant UV filter **1** may also be present. An acetylated derivative, *N*-acetyl-3-hydroxykynurenine, is the major low molecular weight pigment found in the lens of the gray squirrel.[5] Synthesis of the authentic *N*-acetyl compound **3** would serve as a reference compound for lenticular studies to test this hypothesis.



1 R = NH_2
2 R = H
3 R = NHAc

Compound **1** has previously been synthesized in a low, but unspecified, yield by the direct coupling of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (ABG) to 3-hydroxykynurenine, followed by hydrolysis of the crude reaction mixture.[6] Compounds **2** and **3** have not previously been synthesized. Consequently, this paper outlines the synthesis of the lenticular glucopyranosides **1** and **2** and the *N*-acetyl compound **3**.

The synthesis of the β-glucopyranoside of (±) *N*-acetyl-3-hydroxykynurenine **3** is depicted in Scheme 1. 3-Hydroxyacetophenone was nitrated and benzylated according to the method of Butenandt[7] to afford 2-nitro-3-benzyloxyacetophenone **4**, which served as the starting material for the synthesis. Selective bromination of the methyl ketone moiety in **4** to afford the α-bromoketone **5** was accomplished in 82% yield employing dioxane dibromide. Coupling of **5** with the sodium salt of diethyl acetamidomalonate[8] in ethanol resulted in a low yield (21%) of the coupled adduct **6**. This is presumably a consequence of competing Favorski-type rearrangement reactions of **5** under the basic reaction conditions.[9] Removal of the benzyl protecting group in **6** was conveniently achieved employing neat trifluoroacetic acid to yield the *o*-nitrophenol **7**. This compound was glycosylated via the Helferich method[10] using ABG and mercuric cyanide as a catalyst to afford the *O*-β-glucopyranoside **8** in 61% yield. The β configuration was confirmed by the characteristic $J_{1,2}$ coupling constant of 7.3 Hz for the anomeric proton.[11] Catalytic hydrogenation of **8** employing Adam's catalyst was straightforward and afforded the amino glucoside **9** in excellent yield (92%). Deacetylation of **9** via Zemplen's method resulted in virtually quantitative O-deacetylation as evidenced by the presence of a solitary *N*-acetyl resonance in the ^{1}H NMR spectrum of the crude reaction product. De-esterification of this product was followed by ^{1}H NMR in D_2O/CH_3OD employ-

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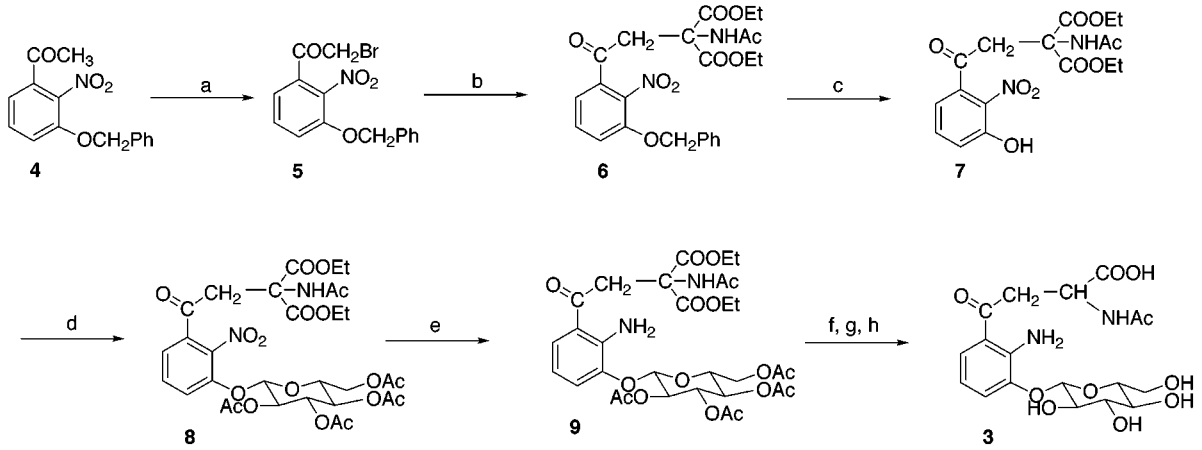
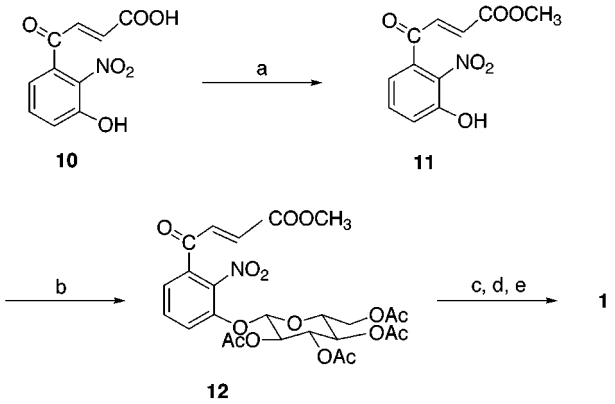
10.1021/jo982321n CCC: $18.00
Published on Web 05/04/1999

Scheme 1[a]



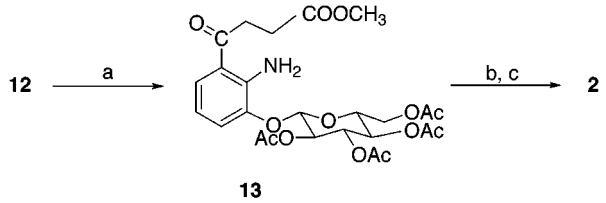
[a] Key: (a) dioxane·Br_2; (b) $CH(COOEt)_2NHAc/NaOEt$; (c) TFA; (d) $ABG/Hg(CN)_2/K_2CO_3$; (e) $H_2/Pd(C)$ (f) NaOEt; (g) NaOH; (h) H^+.

Scheme 2[a]



[a] Key: (a) MeOH/DCC; (b) $ABG/Hg(CN)_2/K_2CO_3$; (c) NH_4OH; (d) $H_2/Pd(C)$; (e) (i) NaOH, (ii) H^+.

Scheme 3[a]



[a] Key: (a) H_2/PtO_2; (b) NaOEt/EtOH; (c) (i) NaOH, (ii) H^+.

ing 4 equiv of NaOD. This indicated an initial rapid (30 min) mono de-esterification followed by slow (12 h) hydrolysis of the remaining ethyl ester. Removal of solvent and incubation of the residue in acetic acid at 50 °C for 3 h resulted in smooth decarboxylation to yield **3** as a mixture of diastereoisomers in 44% yield after purification by preparative reversed-phase HPLC. When human lenses were extracted with 80% ethanol and the UV filters separated by HPLC[3] it was found that none of the acetylated material **3** was detected.

It was expected that enzymic N-deacetylation of D,L-**3** would be affected enantiospecifically to yield L-**1** and unreacted D-**3**. Compound **3** was, however, found to be refractory to a number of enzymes commonly employed to affect the conversion of N-acetylated α-amino acids to α-amino acids.[12] Conversely alkaline hydrolysis of **3** under conditions to affect N-deacetylation[13] resulted in extensive decomposition. Consequently, an alternative synthetic method for the preparation of (±)-**1** was employed (Scheme 2).

The acrylic acid derivative **10**[7] was methylated employing DCC and a large excess of methanol to afford a 45% yield of the methyl ester **11**. Preparation of the tetraacetyl *β*-glucopyranoside **12** was accomplished, in a 61% yield, by coupling of ABG to the methyl ester **11** under the conditions employed for the preparation of the *O*-*β*-glucopyranoside **8**. Incubation of **12** in a 1:1 solution of methanol/ammonia (33%) overnight resulted in smooth incorporation of ammonia and exhaustive O-deacetylation to afford an amino acid, as indicated by the presence of a solitary new compound on TLC that stained with ninhydrin. This product was not purified but subjected directly to catalytic hydrogenation ($H_2/Pd(C)$) followed by alkaline hydrolysis. Purification by preparative reversed-phase HPLC afforded the *O*-*β*-D-glucopyranoside of (±)-3-hydroxykynurenine, **1**, in an overall yield of 48% from **12**. Two unresolved peaks of equal intensity were observed for the diastereoisomers of **1**. Use of different solvent conditions and flow rates did not allow their complete separation. The first eluting peak increased in size upon spiking with an authentic sample of the *O*-*β*-D-glucopyranoside of L-3-hydroxykynurenine isolated from human lenses.[3]

The protected glucoside **12** was also utilized for the synthesis of the *O*-*β*-glucopyranoside **2** (Scheme 3). Catalytic hydrogenation of **12** employing PtO_2 resulted in reduction of the nitro and olefinic functionalities to afford **13**. Complete O-deacetylation of **13** via Zemplen's procedure followed by alkaline hydrolysis yielded 60% of **2** after purification by semipreparative HPLC. An authentic sample of the *O*-*β*-D-glucopyranoside of 2-amino-3-hydroxy-*γ*-oxobenzenebutanoic acid obtained from human lenses[3] coeluted with the synthesized sample of **2** in the HPLC.

To confirm the structural integrity of the *β*-glucopyranosides, compounds **1**−**3** were incubated with *β*-glucosidase (pH 5.6, 37 °C) and the reactions monitored by analytical reversed-phase HPLC. After 12 h incubation, **1**−**3** had reacted completely, indicating that each com-

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pound was a glucopyranoside exclusively in the β configuration. In the case of **1**, it was also confirmed that the aglycon component was 3-hydroxykynurenine, by comparison (by HPLC) with commercial 3-hydroxykynurenine.

The ^{1}H NMR spectra of the β-glucopyranosides **1**−**3** exhibited similar aromatic resonances, each with a doublet of doublets at δ ~6.7 ppm, doublet at δ ~7.3, and doublet at δ ~7.6, with ortho J values of ~8 Hz. Such a pattern is typical of a 3-hydroxykynurenine derivative.[14] The ^{1}H NMR spectrum of the *N*-acetyl derivative **3**, while similar to that of **1**, also exhibited a characteristic acetyl resonance (δ 1.93) and a pronounced downfield shift of 0.48 ppm for the Hα proton in accordance with substitution of an α-NH_2 for an α-NHAc group. Compound **2**, which lacks an α-amino substituent, showed the presence of an isolated CH_2CH_2 group with two triplets at δ 2.60 and 3.25 and was in excellent agreement with the values found for **2** isolated from human lenses.[3]

Conclusions

The two major UV filter compounds found in the lenses of primates, the *O*-β-D-glucopyranosides of 3-hydroxykynurenine and 2-amino-3-hydroxy-γ-oxobenzenebutanoic acid, **1** and **2**, were successfully synthesized from the common benzoyl acrylate precursor 2-amino-3-hydroxybenzoylacrylic acid **10**. Synthesis of compound **3**, the α-*N*-acetyl derivative of **1**, was achieved using coupling of 2-nitro-3-benzyloxyacetophenone **4** with the sodium salt of diethyl acetamidomalonate as a key step. Compound **3** was not detected in human lenses.

Experimental Section

All experiments were carried out using instrumentation and manipulations as described previously.[15] The NMR spectra were measured in $CDCl_3$ unless otherwise stated. Microanalyses (C, H, N) were performed by the Australian National University Analytical Services, Canberra. Analytical HPLC was carried out on Millipore Waters HPLC equipment comprising a 6000A series solvent delivery system with an automated gradient controller and model 746 data module. Separations were performed using a 100 × 8 mm Nova Pak C_{18} reversed-phase radial pack cartridge. Elution was carried out using linear gradients from 0 to 80% CH_3CN over 10 min at a flow rate of 2 mL/min. Semipreparative HPLC was performed using a Varian 2010 HPLC pump with a 250 mm × 10 mm Alltech C18 10 μm column, eluting with 0.1% HOAc (pH 3) for 30 min, followed by 10% CH_3CN for 30 min, at a flow rate of 3 mL/min. Preparative HPLC was performed using a Millipore Waters Prep 500 HPLC with a Prep PAK 500-C_{18} and 55−105 μm particle size column eluting with a linear gradient of 0−50% CH_3CN over 30 min at a flow rate of 20 mL/min.

Reactions were followed by TLC on Merck F254 silica gel plates. Merck silica gel (70−230 mesh) was used for column chromatography. All solvents and reagents were obtained from commercial sources and purified before use as required.

2-Bromo-1-(2-nitro-3-phenylmethoxyphenyl)ethanone (5). Dioxane dibromide[16] (1.0 g, 4.06 mmol) was added to a solution of 2-nitro-3-benzyloxyacetophenone **4**[7] (1.0 g, 3.69 mmol) in CH_2Cl_2 (20 mL) and stirred at room temperature for 1.5 h. The light yellow solution was washed with water (20 mL), followed by $NaHCO_3$ (5% solution, 20 mL). The organic phase was dried (Na_2SO_4) and solvent removed to give an orange oil. This was dissolved in hot ether, which afforded white crystals of **5** (1.06 g, 82%) on cooling: mp 116−118 °C dec; ^{1}H NMR δ 4.34 (2H, s), 5.23 (2H, s), 7.28−7.40 (7H, m), 7.50 (1H, t, J = 8.0 Hz); ^{13}C NMR δ 31.3, 71.3, 119.0, 120.9, 127.0, 128.4, 128.7, 128.7, 129.0, 131.4, 134.8, 150.7, 189.4; IR (Nujol mull) ν_{max} 1710, 1541, 1291 cm^{-1}. Anal. Calcd for $C_{15}H_{12}BrNO_4$: C, 51.43; H, 3.43; N, 4.00. Found: C, 51.63; H, 3.37; N, 3.91.

Diethyl(Acetylamino)[2-(2-nitro-3-phenylmethoxyphenyl)-2-oxoethyl]propanedioate (6). To a solution of diethyl acetamidomalonate (573 mg, 2.64 mmol) and NaOEt (180 mg, 2.64 mmol) in EtOH (15 mL) was added in one portion the bromoketone **5** (0.88 g, 2.51 mmol) and the mixture stirred at room temperature for 2 h. Solvent was removed under vacuum and the residue partitioned between EtOAc (120 mL) and 0.01 M NaOH (50 mL). The organic phase was washed sequentially with water (50 mL) and 0.001 M HCl (50 mL). After drying ($MgSO_4$) and removal of solvent under vacuum, the residue was chromatographed (EtOAc/hexane 2:3−3:2) to afford **6** as a light yellow oil. Crystallization from Et_2O afforded pure **6** (260 mg, 21%) as a white solid: mp 107 °C; ^{1}H NMR δ 1.25 (6H, t, J = 7.1 Hz), 2.00 (3H, s), 4.22 (2H, s), 4.26 (4H, q, J = 7.1 Hz), 5.21 (2H, s), 7.07 (1H, br s), 7.25−7.39 (7H, m), 7.46 (1H, t, J = 8.1 Hz); ^{13}C NMR δ 13.60, 22.59, 43.40, 62.86, 63.48, 71.03, 118.63, 120.42, 126.81, 128.18, 128.50, 130.49, 131.06, 134.74, 138.97, 150.09, 166.45, 169.56, 195.14; IR (Nujol mull) ν_{max} 3236, 1750 (br), 1699, 1640 cm^{-1}. Anal. Calcd for $C_{24}H_{26}N_2O_9$: C, 59.26; H, 5.35; N, 5.76. Found: C, 59.14; H, 5.40; N, 5.57.

Diethyl (Acetylamino)[2-(2-nitro-3-hydroxyphenyl)-2-oxoethyl]propanedioate (7). Trifluoroacetic acid (3 mL) was added to **6** (150 mg, 0.31 mmol) and the mixture stirred at room temperature for 24 h. Solvent was removed under vacuum and the oily residue dissolved in EtOAc (2 mL). Hexane was added slowly until the mixture became turbid. After refrigeration for 2 h rosettes of yellow crystals formed. These were collected by filtration to afford **7** (78 mg, 64%): mp 132−133 °C (EtOAc/hexane); ^{1}H NMR δ 1.29 (6H, t, J = 7.2 Hz), 2.11 (3H, s), 4.07 (2H, s), 4.29 (4H, q, J = 7.2 Hz), 6.77 (1H, dd, J = 7.3, 1.1 Hz), 7.15 (1H, br s), 7.22 (1H, dd, J = 8.5, 1.1 Hz), 7.58 (1H, dd, J = 8.5, 7.3 Hz), 10.44 (1H, br s); ^{13}C NMR δ 13.80, 22.80, 45.53, 63.03, 63.46, 118.34, 121.55, 131.16, 136.19, 137.91, 154.62, 166.64, 169.99, 199.26; IR (Nujol mull) ν_{max} 3295, 1746, 1695 cm^{-1}; UV λ_{max} (EtOH) = 216 nm (ϵ 16 980), 248 (ϵ 6310), 316 (ϵ 3090). Anal. Calcd for $C_{17}H_{20}N_2O_9$: C, 51.52; H, 5.05; N, 7.07. Found: C, 51.36; H, 5.41; N, 6.84.

Diethyl (Acetylamino)[2-[2-nitro-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyphenyl)]-2-oxoethyl]propanedioate (8). A mixture of **7** (40 mg, 0.10 mmol), mercuric cyanide (26 mg, 0.10 mmol), potassium carbonate (17 mg, 0.12 mmol), and ABG[17] (50 mg, 0.12 mmol) in CH_3CN (3 mL) was stirred at room temperature for 2 d. Solvent was removed under vacuum and the residue partitioned between EtOAc (20 mL) and water (15 mL). The organic phase was dried ($MgSO_4$), solvent removed under vacuum, and the residue chromatographed (EtOAc/hexane, 2:1−4:1) to afford **8** (41 mg, 61%): mp 94−96 °C (EtOH); ^{1}H NMR δ 1.20 (6H, t, J = 6.8 Hz), 1.96 (3H, s), 1.99 (3H, s), 2.01 (3H, s), 2.06 (3H, s), 2.08 (3H, s), 3.84 (1H, dt, J = 3.4, 9.9 Hz), 4.15−4.30 (8H, m), 4.97−5.29 (4H, m), 7.07 (1H, br s), 7.53−7.61 (3H, m); ^{13}C NMR δ 13.76, 20.36, 20.50 (2), 20.62, 22.82, 43.44, 61.56, 63.10, 63.20, 63.62, 67.90, 70.20, 72.02, 72.33, 100.37, 123.85, 124.00, 130.47, 131.19, 140.56, 148.20, 166.50, 166.56, 169.24, 169.78, 170.07, 170.39, 194.74; IR (Nujol mull) ν_{max} 3384, 1751, 1661 cm^{-1}. Anal. Calcd for $C_{31}H_{38}N_2O_{18}$: C, 51.24; H, 5.23; N, 3.86. Found: C, 51.26; H, 5.05; N, 3.97.

Diethyl (Acetylamino)[2-[2-amino-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyphenyl)]-2-oxoethyl]pro-

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panedioate (9). To a solution of **8** (1 g, 1.38 mmol) in EtOAc/EtOH (21 mL, 2:1) was added Adam's catalyst (300 mg) and the mixture placed under a hydrogen atmosphere (1 atm) for 24 h. The mixture was filtered and the filtrate taken to dryness under vacuum. The light yellow residue was recrystallized from CCl_4/hexane to afford **9** (920 mg, 92%) as an off-white solid: mp 108−110 °C; ^{1}H NMR δ 1.24 (6H, t, J = 7.3 Hz), 1.98 (3H, s), 2.05 (6H, s), 2.07 (3H, s), 2.09 (3H, s), 3.87 (1H, ddd, J = 10.2, 4.9, 2.0 Hz), 4.22 (2H, s), 4.26−4.35 (6H, m), 4.97 (1H, d, J = 7.8 Hz), 5.13−5.33 (3H, m), 6.49 (2H, br s), 6.55 (1H, t, J = 7.8 Hz), 7.03 (1H, d, J = 7.8 Hz), 7.11 (1H, s), 7.53 (1H, d, J = 7.8 Hz); ^{13}C NMR δ 13.85, 20.55 (br), 22.94, 42.94, 61.76, 62.77, 63.98, 68.21, 71.14, 72.80 (m), 100.06, 114.08, 117.43, 119.21, 125.90, 142.31, 144.40, 167.42, 167.57, 169.35, 169.83, 170.05, 170.46, 198.34; IR (Nujol mull) ν_{max} 3381, 3362, 1747, 1670 cm^{-1}; UV λ_{max} (EtOH) = 232 nm (ϵ 17 313), 264 (ϵ 4500), 370 (ϵ 4370). Anal. Calcd for $C_{31}H_{40}N_2O_{16}$: C, 53.45; H, 5.75; N, 4.02. Found: C, 53.66; H, 5.65; N, 3.87.

α-Acetylamino-2-amino-3-(β-D-glucopyranosyloxy)-γ-oxobenzenebutanoic Acid (3). To a suspension of **9** (500 mg, 0.72 mmol) in EtOH (10 mL) was added a solution of NaOEt (14 mg, 0.20 mmol) and the mixture stirred for 1 h. Solvent was removed under vacuum and the residue dissolved in H_2O (10 mL). NaOH (115 mg, 2.88 mmol) was added and the mixture stirred at rt for 12 h. The solution was lyophilized, following which HOAc (5 mL) was added and stirring continued at 50 °C for 3 h. Solvent was removed under vacuum and the residue purified by preparative HPLC eluting with H_2O to afford **3** (136 mg, 44%) as a yellow solid after lyophilization: mp 157−160 °C (H_2O); ^{1}H NMR (D_2O) δ 1.93 (3H, s), 3.48−3.95 (8H, m), 4.63 (1H, t, J = 6.1 Hz), 5.00 (1H, d, J = 6.7 Hz), 6.73 (1H, dd, J = 7.9, 7.9 Hz), 7.28 (1H, d, J = 7.9 Hz), 7.61 (1H, d, J = 7.9 Hz); ^{13}C NMR (D_2O) δ 21.84, 40.83, 49.03, 60.66, 69.52, 72.97, 75.63, 76.22, 101.54, 116.01, 118.23, 120.64, 125.78, 141.11, 144.93, 173.84, 175.25, 199.98. Anal. Calcd for $C_{18}H_{24}N_2O_{10}$: C, 50.47; H, 5.61; N, 6.54. Found: C, 50.66; H, 5.65; N, 6.21.

Methyl (*E*)-4-Oxo-4-(3-hydroxy-2-nitrophenyl)-2-butenoate (11). To a solution of 2-amino-3-hydroxybenzoylacrylic acid **10** (3 g, 12.7 mmol)[7] in MeOH (20 mL) at 0 °C was added a solution of DCC (2.61 g, 12.7 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred at room temperature overnight and the solvent removed under vacuum. The residue was suspended in CH_2Cl_2 (100 mL) and filtered and the filtrate taken to dryness under vacuum. The residue was chromatographed (CH_2Cl_2/hexane, from 1:1 to 5:3 to 3:1) to afford **11** (1.42 g, 45%) as a bright yellow solid: mp 111−112 °C; ^{1}H NMR δ 3.80 (3H, s), 6.39 (1H, d, J = 16.1 Hz), 6.84 (1H, d, J = 7.1 Hz), 7.27 (1H, d, J = 16.1 Hz), 7.31 (1H, d, J = 8.6 Hz), 7.66 (1H, dd, J = 8.6, 7.1 Hz). ^{13}C NMR 52.30, 119.89, 122.09, 132.08, 131.34 (q), 136.94, 139.45, 140.94 (q), 155.33, 165.25, 191.06; IR (Nujol mull) ν_{max} 1728, 1671, 1603 cm^{-1}; UV λ_{max} (EtOH) = 220 nm (ϵ 20 890), 272 (ϵ 5130 inflex), 334 (ϵ 2820). Anal. Calcd for $C_{11}H_9NO_6$: C, 52.59; H, 3.59; N, 5.58. Found: C, 52.72; H, 3.66; N, 5.53.

Methyl (*E*)-4-Oxo-4-[2-nitro-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyphenyl]-2-butenoate (12). To a solution of **11** (200 mg, 0.80 mmol) in CH_3CN (5 mL) was added mercuric cyanide (20 mg) followed by ABG (327 mg, 0.80 mmol) and potassium carbonate (55 mg, 0.40 mmol). The mixture was stirred at room temperature for 4 d. Solvent was removed under vacuum and the residue dissolved in EtOAc (20 mL) and washed with 0.01 M HCl (20 mL). The organic phase was dried ($MgSO_4$), solvent removed under vacuum, and the residue chromatographed (CH_2Cl_2/hexane 5:1) to afford **12** (284 mg, 61%): mp 110−112 °C (EtOH); ^{1}H NMR δ 2.01 (3H, s), 2.03 (3H, s), 2.08 (3H, s), 2.11 (3H, s), 3.82 (3H, s), 3.82−3.95 (1H, m), 4.24 (2H, d, J = 3.9 Hz), 5.05−5.30 (4H, m), 6.73 (1H, d, J = 15.6 Hz), 7.52 (1H, d, J = 15.6 Hz), 7.45−7.56 (3H, m); ^{13}C NMR δ 20.41 (4), 52.48, 61.58, 67.92, 70.24, 72.04, 72.33, 100.22, 123.47, 124.02, 131.31, 131.54, 134.08, 136.33, 140.96, 148.46, 165.11, 169.12, 169.17, 169.97, 170.30, 187.50; IR (Nujol mull) ν_{max} 1749, 1680, 1541 cm^{-1}. Anal. Calcd for $C_{25}H_{27}NO_{15}$: C, 51.64; H, 4.65; N, 2.41. Found: C, 51.55; H, 4.81; N, 2.09.

α,2-Diamino-3-(β-D-glucopyranosyloxy)-γ-oxobenzenebutanoic Acid (1). To a suspension of **12** (100 mg, 0.17 mmol) in MeOH (1 mL) was added 1 mL of a 33% NH_3 solution and the mixture stirred at room temperature for 12 h. Solvent was removed under vacuum, the residue dissolved in H_2O (2 mL) and adjusted to pH 6.0 with HOAc, Pd(C) (20 mg of 10%) added, and the mixture placed under an atmosphere of H_2 for 12 h. The mixture was filtered, NaOH (28 mg, 0.68 mmol) added to the filtrate, and the mixture stirred for 2 h. The pH was adjusted to 6.5 with HOAc and the mixture purified by preparative reversed-phase HPLC eluting with H_2O to afford **1** (32 mg, 48%) as a yellow solid after lyophilization: ^{1}H NMR (D_2O) δ 3.60−4.00 (8H, m), 4.15 (1H, t, J = 5.5 Hz), 5.04 (1H, d, J = 5.5 Hz), 6.75 (1H, dd, J = 8.5, 7.9 Hz), 7.32 (1H, d, J = 7.9 Hz), 7.61 (1H, d, J = 8.5 Hz); m/z (ES$^+$) 387 (MH$^+$, 100). Anal. Calcd for $C_{16}H_{22}N_2O_9$: C, 49.74; H, 5.70; N, 7.25. Found: C, 49.66; H, 5.72; N, 7.09.

Methyl 4-Oxo-4-[2-amino-3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxyphenyl]-2-butanoate (13). To a solution of **12** (200 mg, 0.34 mmol) in EtOAc/EtOH (10 mL, 4:1) was added Adam's catalyst (20 mg) and the mixture placed under a hydrogen atmosphere (1 atm) for 12 h. The mixture was filtered and the filtrate taken to dryness under vacuum. The light yellow residue was chromatographed (CH_2Cl_2/EtOAc, 95:5) to afford **13** (121 mg, 63%) as a golden oil: ^{1}H NMR δ 2.05−2.09 (12H, m), 2.71 (2H, t, J = 6.6 Hz), 3.30 (2H, t, J = 6.6 Hz), 3.71 (3H, s), 3.82−3.92 (1H, m), 4.15−4.22 (2H, m), 4.96−5.36 (4H, m), 6.50 (2H, br s), 6.55 (1H, dd, J = 8.3, 7.8 Hz), 7.04 (1H, d, J = 7.8 Hz), 7.53 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 20.50, 28.12, 33.99, 51.72, 61.74, 68.21, 71.09, 72.03, 72.20, 100.18, 113.84, 117.77, 118.91, 125.46, 142.14, 144.57, 169.35, 169.82, 170.04, 170.46, 173.51, 199.62. Anal. Calcd for $C_{25}H_{31}NO_{13}$: C, 54.25; H, 5.61; N, 2.53. Found: C, 53.99; H, 5.45; N, 2.68.

2-Amino-3-(β-D-glucopyranosyloxy)-γ-oxobenzenebutanoic Acid (2). To a solution of **13** (500 mg, 0.90 mmol) in EtOH (4 mL) was added a solution of NaOEt in EtOH (14 mg, 0.20 mmol). The mixture was stirred at rt for 2 h, solvent was then removed, and the residue dissolved in H_2O (5 mL) to which was added NaOH (144 mg, 3.6 mmol). The mixture was stirred at rt for 4 h, adjusted to pH 3.0 with 1 M HCl, and lyophilized. The solid was purified via semipreparative reversed-phase HPLC, eluting with 0.1% HOAc, followed by 10% CH_3CN, to afford **3** (200 mg, 60%) as a yellow solid after lyophilization. ^{1}H NMR spectral data were in agreement with the partial spectral data published:[3] ^{1}H NMR (D_2O) δ 2.60 (2H, t, J = 6.3 Hz), 3.25 (2H, t, J = 6.3 Hz), 3.40−3.60 (5H, m), 3.76 (1H, dd, J = 12.6, 5.1 Hz), 3.83 (1H, dd, J = 12.6, 2.0 Hz), 6.66 (1H, dd, J = 8.1, 8.1 Hz), 7.21 (1H, dd, J = 8.1, ∼1 Hz), 7.59 (1H, dd, J = 8.1, ∼1 Hz); HRMS calcd for $C_{16}H_{22}NO_9$ (MH$^+$) 372.129 457, found 372.130 025.

JO982321N