

Synthesis of 3,3',5-Trihydroxybiphenyl-2-carboxylic Acid, a Component of the Bitterest Natural Product Amarogentin and Its Coenzyme A and *N*-Acetyl Cysteamine Thiol Esters

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3,3',5-Trihydroxybiphenyl-2-carboxylic acid (**6**), an ester component of the bitter-tasting natural products amarogentin and amaroswerin, was synthesized in six steps in 13.6% overall yield. Its *N*-acetyl cysteamine thiol ester (**9**) and its coenzyme A thiol ester (**8**), a likely biosynthetic precursor of the amarums, were also prepared.

3,3',5-Trihydroxybiphenyl-2-carboxylic acid (**6**) is an ester component of amarogentin, which is the bitterest natural product known.¹ It has been shown that this acid could be formed in the plant by aldol condensation of 7-(3'-hydroxyphenyl)-3,5,7-triketeto acid.^{2,3} This compound could, in turn, be formed from biologically activated CoA esters of 3-hydroxybenzoic acid and three units of malonate.^{2,3} To study the biosynthesis of amarogentin at the enzyme level, we required the coenzyme A thiol ester of the biphenylcarboxylic acid. Herein we report the first total chemical synthesis of this biphenylcarboxylic acid (**6**) as well as its coenzyme A and *N*-acetyl cysteamine thiol esters (**8** and **9**).

Results and Discussion

Cyclization of 1,3,5,7-tetracarbonyl compounds,⁴ conversion of pyranopyrone,⁵ and condensation of diketene with 1,3-dicarbonyl compounds^{6,7} should lead to the formation of β -resorcylic acids or esters. In this report, the desired biphenylcarboxylic acid ethyl ester was synthesized by reaction of diketene with ethyl-3-phenyl-3-oxopropionate.⁷

Ethyl 3-(3'-methoxyphenyl)-3-oxopropionate (**1**) was first generated by reaction of diethyl carbonate with 3-methoxyacetophenone in the presence of sodium hydride. When **1** was allowed to react with diketene in the presence of sodium hydride in THF, 3'-methoxy-3,5-dihydroxybiphenyl-2-carboxylic acid ethyl ester (**2**) was obtained directly in 31% yield. Demethylation of **2** was achieved through treatment with 5 equivalents of BBr₃ in dry CH₂Cl₂ to give 3,3',5-trihydroxy-biphenyl-2-carboxylic acid ethyl ester (**3**) (Scheme 1).

Conversion of **3** to its acid (**6**) was first necessary. Direct cleavage of the ester group through hydrolysis of **3** with NaOH or potassium-*tert*-butoxide⁸ resulted in decarboxylation. The use of hog liver esterase in Na₂HPO₄ buffer (pH 8.0) to hydrolyze **3** also was unsuccessful. It was assumed that, on the one hand, the carbonyl ester is sterically hindered and difficult to hydrolyze at room temperature,

and on the other hand, the carboxylic acid would, however, undergo decarboxylation by treatment with acid and base at elevated temperatures because of the presence of hydroxyl groups at the 2- and 4-positions. For this reason the hydroxyl groups must be protected with a group stable toward both acidic and basic conditions, but easily removable under mild and neutral conditions.

Due to the ease of cleavage of **3**, we selected a benzyl protecting group. Therefore, **3** was converted to **4** by treatment with benzyl chloride and anhydrous potassium carbonate. Hydrolysis of **4** with 10% NaOH in EtOH afforded 3,3',5-tribenzyloxybiphenyl-2-carboxylic acid (**5**) in 87% yield. Catalytic hydrogenation of **5** with 10% Pd/C in absolute EtOH gave 3,3',5-trihydroxybiphenyl-2-carboxylic acid (**6**) in almost quantitative yield (Scheme 1).

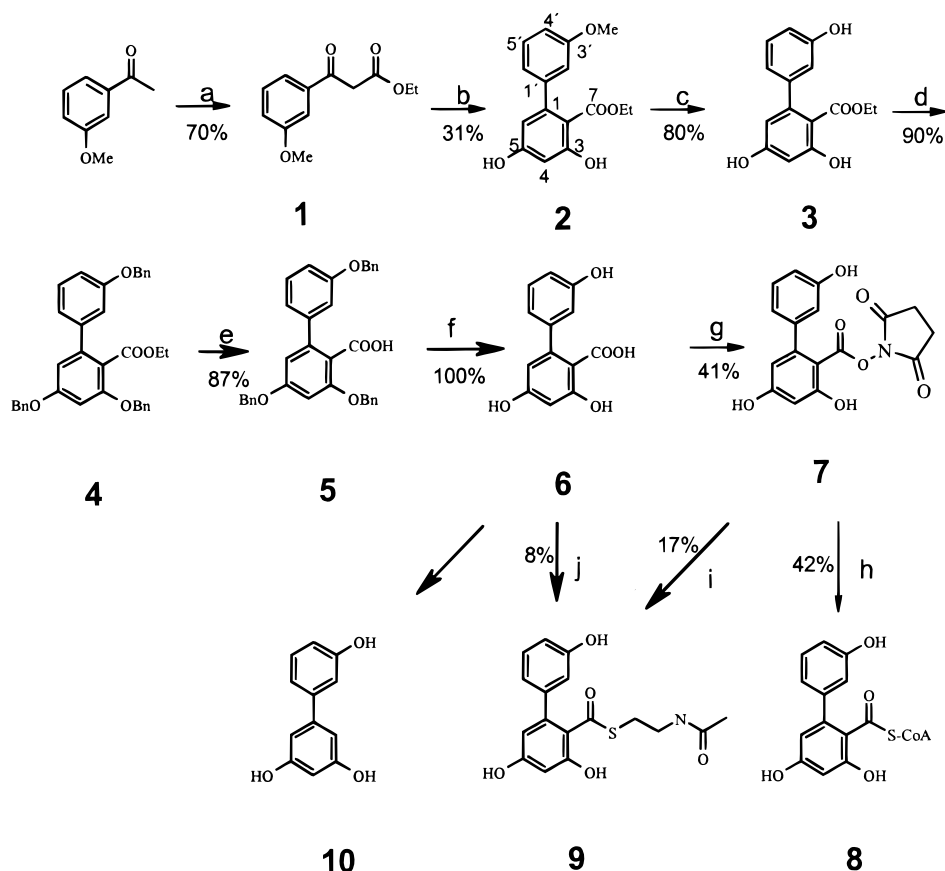
To investigate the enzymatic formation of amarogentin from sweroside and 3,3',5-trihydroxybiphenyl-2-carboxylic acid (**6**), both the *N*-acetyl cysteamine and coenzyme A (CoA) thiol esters of this acid (**9** and **8**) were also synthesized. For preparation of the CoA ester, the desired biphenylcarboxylic acid was activated via a succinimide derivative.⁹ Compound **6** reacted with *N*-hydroxysuccinimide in the presence of *N,N*-dicyclohexylcarbodiimide (DCC) in dry ethyl acetate to produce biphenylcarboxylic acid *N*-hydroxysuccinimide ester (**7**). The biphenylcarbonyl-CoA thiol ester (**8**) was prepared by transesterification of **7** with coenzyme A (CoA-SH) in 50 mM aqueous NaHCO₃. The CoA ester was identified by a specific color reaction with nitroprusside spray reagent as described in the Experimental Section. The "delayed" nitroprusside reaction as well as the chromatographic properties and the difference spectrum upon base-catalyzed hydrolysis showed the characteristics of the CoA thiol ester.¹⁰ The maximal UV absorption of the thiol ester was registered at 316 nm. The biphenylcarboxylic acid *N*-acetyl cysteamine ester (**9**) was also synthesized as a model compound through *N*-hydroxysuccinimide ester by transesterification with *N*-acetyl cysteamine (NAC-SH) under the same conditions as the CoA ester synthesis. The general method for the synthesis of thiol esters directly from free acids in the presence of DCC and 4-(dimethylamino)pyridine (DMAP) in anhydrous CH₂Cl₂¹¹ could not be used in the CoA ester synthesis. However, it worked for the synthesis of biphenylcarboxylic acid *N*-acetyl cysteamine ester. With both methods, the main side reaction was decarboxylation of the biphenylcarboxylic acid to form the biphenyl **10**.

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Scheme 1. Synthesis of 3,3',5-Trihydroxybiphenyl-2-carboxylic Acid (**6**) and Its CoA (**8**) and *N*-Acetyl Cysteamine (**9**) Thiol Esters^a

^a (a) NaH, diethyl carbonate, benzene, reflux, 1.5 h; (b) diketene, NaH, THF, 0 °C to room temperature, 1 h; (c) BBr₃, CH₂Cl₂, -10 °C to room temperature, overnight; (d) BnCl, K₂CO₃, KI, EtOH, reflux, 48 h; (e) 10% NaOH/EtOH, reflux, 3 h; (f) H₂, Pd/C, EtOH, room temperature, 48 h; (g) *N*-hydroxysuccinimide, DCC, EtOAc, room temperature, 72 h; (h) CoA-SH, 50mM aqueous NaHCO₃, 0 °C, overnight; (i) NAC-SH, 50 mM aqueous NaHCO₃, 0 °C, 24 h; (j) NAC-SH, DCC, DMAP, CH₂Cl₂, 0 °C to room temperature, 3 h.

The UV spectra of 3,3',5-trihydroxybiphenyl-2-carboxylic acid (**6**) and its *N*-acetyl cysteamine thiol ester (**9**) taken in 0.1 M of phosphate buffer (pH 7.0) are given in Figure 1. The difference spectrum between **9** and **6** shows a maximum at 314 nm, which is in accord with similar compounds reported in the literature.¹⁰ With the synthesis of the biphenylcarboxyl-CoA thiol ester in hand and the availability of sweroside, it should now be possible to detect and characterize the acyl CoA transferase necessary for completing the chemo-enzymatic synthesis of amarogentin from sweroside and the biphenylcarboxyl-CoA thiol ester.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker AM 360 spectrometer operating at 360 MHz for ¹H and 90 MHz for ¹³C. CIMS and HREIMS were measured on a Finnigan SSQ 700 and MAT 90 mass spectrometers, respectively. IR spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrometer and UV spectra on a UVIKON 930 spectrophotometer. HPLC was performed on a Merck Hitachi system with L-6200 Intelligent Pump and L-4200 UV-vis detector. Fluka Si gel 60 (220–440 mesh) was employed for column chromatography, and thin-layer chromatography (TLC) was performed on Merck Si gel 60 F₂₅₄ plates.

Ethyl 3-(3'-Methoxyphenyl)-3-oxopropionate (1).^{7,12} Sodium hydride (55% dispersion, 2.4 g, 50 mmol) was added with stirring to a solution of diethyl carbonate (6 mL) in dry benzene (20 mL). A solution of 3-methoxyphenylacetone (3.0 g, 20 mmol) in diethyl carbonate (2.6 mL) was added dropwise, and the mixture was refluxed for 1.5 h and then cooled. A small amount of EtOH was added to the reaction mixture to destroy excess NaH. The resulting mixture was poured into a mixture

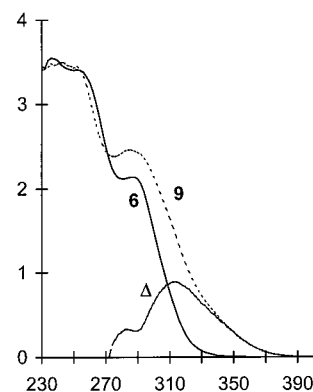


Figure 1. The UV spectra of 3,3',5-trihydroxybiphenyl-2-carboxylic acid (**6**) and its *N*-acetyl cysteamine (**9**) thiol ester and the difference spectrum of **9** and **6** (Δ) taken in 0.1 M of phosphate buffer (pH 7.0)

of 10% aqueous HCl (20 mL) and ice (20 g) and extracted with ether (2 × 50 mL). The ether layer was washed with H₂O and dried over Na₂SO₄. Ether was removed in vacuo, and the residue was purified by distillation at 11 mbar with collection at bp 174–182 °C to afford **1** (3.11 g, 14 mmol, 70%). Spectral data, see literature.¹³

3'-Methoxy-3,5-dihydroxybiphenyl-2-carboxylic Acid Ethyl Ester (2). NaH (55% dispersion, 2.8 g, 20 mmol) was added to a solution of **1** (12.46 g, 56 mmol) in THF (100 mL) with stirring and cooling in an ice-water bath. The mixture was stirred for 10 min, followed by the dropwise addition of a solution of diketene (5 mL) in absolute THF (20 mL). The whole mixture was stirred for 1 h at room temperature and then poured into a mixture of concentrated HCl (10 mL) and ice (120 g). The resulting mixture was extracted with ether (2

× 100 mL). The ether layer was dried over Na₂SO₄ and then evaporated. The residue was chromatographed by column chromatography (CC) over Si gel eluting with hexane/ether (5:1) to afford **2** (5.0 g, 17 mmol, 31%): UV (CHCl₃) λ_{max} (log ε) 241 (4.27), 264 (4.17), 305 (3.95) nm; IR (KBr) ν_{max} 3320, 1642, 1570, 1453, 1241 cm⁻¹; ¹H NMR (CDCl₃, 360 MHz) δ 7.22 (1H, dd, *J* = 8.0, 8.0 Hz, H-5'), 6.87 (1H, br d, *J* = 8.0 Hz, H-6'), 6.76 (1H, br d, *J* = 1.8, 8.0 Hz, H-4'), 6.75 (1H, br s, H-2'), 6.41 (1H, d, *J* = 1.8 Hz, H-6), 6.28 (1H, d, *J* = 1.8 Hz, H-4), 3.92 (2H, q, -OCH₂CH₃), 3.80 (3H, s, -OMe), 1.27 (3H, t, -OCH₂CH₃); ¹³C NMR (CDCl₃, 90 MHz) δ 170.7 (C-7), 161.9 (C-3'), 159.0 (C-5), 156.6 (C-3), 147.2 (C-1), 144.2 (C-1'), 128.5 (C-5'), 120.7 (C-6'), 113.6 (C-2'), 112.5 (C-4'), 111.0 (C-6), 107.6 (C-2), 102.4 (C-4), 60.7 (-OCH₂CH₃), 55.3 (-OCH₃), 13.0 (-OCH₂CH₃); CIMS *m/z* 289 [M + 1]⁺ (100), 243 (9); HREIMS *m/z* 288.1004 (calcd for C₁₆H₁₆O₅, 288.0998).

3,3',5-Trihydroxybiphenyl-2-carboxylic Acid Ethyl Ester (3). Compound **2** (2.0 g, 7 mmol) was dissolved in dry CH₂Cl₂ (150 mL) and chilled to -10 °C. Then, 1.0 M BBr₃ in CH₂Cl₂ (35 mL) was added dropwise. The mixture was stirred overnight, beginning at 0 °C and gradually increasing to room temperature. Small pieces of ice were added and the mixture extracted with EtOAc. The EtOAc was evaporated under reduced pressure and the residue purified on CC over Si gel eluting with CHCl₃/MeOH (20:1) to afford **3** (1.53 g, 80%): UV (MeOH) λ_{max} (log ε) 216 (4.42), 264 (3.97), 299 (3.77) nm; IR (KBr) ν_{max} 3277, 2984, 1642, 1577, 1453, 1404, 1366, 1318, 1274, 1225, 1171, 1106 cm⁻¹; ¹H NMR (CD₃OD, 360 MHz) δ 7.01 (1H, dd, *J* = 7.9, 7.9 Hz, H-5'), 6.60 (1H, dd, *J* = 2.4, 7.9 Hz, H-6'), 6.53 (1H, br d, *J* = 7.9 Hz, H-4'), 6.50 (1H, br s, H-2'), 6.17 (1H, d, *J* = 1.9 Hz, H-6), 6.08 (1H, d, *J* = 1.9 Hz, H-4), 3.81 (2H, q, *J* = 7.2 Hz, -OCH₂CH₃), 0.69 (3H, t, *J* = 7.2 Hz, -OCH₂CH₃); ¹³C NMR (CD₃OD, 90 MHz) δ 172.0 (C-7), 164.0 (C-5), 162.9 (C-3), 157.9 (C-3'), 148.0 (C-1), 145.7 (C-1'), 129.7 (C-5'), 120.5 (C-6'), 116.2 (C-2'), 114.7 (C-4'), 111.6 (C-6), 106.7 (C-2), 102.6 (C-4), 61.5 (-OCH₂CH₃), 13.6 (-OCH₂CH₃); CIMS *m/z* 275 [M + 1]⁺ (100), 229 [M - OC₂H₅]⁺ (2); HREIMS *m/z* 274.0846 (calcd for C₁₅H₁₄O₅, 274.0841).

3,3',5-Tribenzyloxybiphenyl-2-carboxylic Acid Ethyl Ester (4). Compound **3** (274 mg, 1.0 mmol) and anhydrous potassium carbonate (828 mg, 6.0 mmol) in absolute ethanol (20 mL) were refluxed for 10 min. Benzyl chloride (0.6 mL, 5.2 mmol) and a small amount of potassium iodide were added, and the mixture was refluxed for 48 h. After cooling, ether (20 mL) was added, and the precipitate was removed by filtration and washed with ether. The combined filtrate was evaporated, H₂O (20 mL) was added to the residue, and the solution was extracted with ether (3 × 30 mL). The ether layer was dried, evaporated, and chromatographed by column over Si gel eluting with hexane/EtOAc (15:1) to afford **4** (490 mg, 0.9 mmol, 90%): UV (CHCl₃) λ_{max} (log ε) 240 (4.20), 282 (3.65) nm; IR (KBr) ν_{max} 3431, 2956, 2925, 2854, 1729, 1600, 1462, 1378, 1263, 1167, 1043, 740, 698 cm⁻¹; ¹H NMR (CDCl₃, 360 MHz) δ 7.70 (1H, dd, *J* = 8.0, 8.0 Hz, H-5'), 7.53 (1H, d, *J* = 2.0 Hz, H-2'), 7.01 (1H, dd, *J* = 8.0, 2.0 Hz, H-4'), 6.96 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.59 (1H, d, *J* = 2.0 Hz, H-6), 6.57 (1H, d, *J* = 2.0 Hz, H-4), 5.11, 5.07, 5.05 (each 3H, s, 3 × -CH₂- of benzyl groups), 4.07 (2H, q, *J* = 7.1 Hz, -OCH₂CH₃), 0.99 (3H, t, *J* = 7.1 Hz, -OCH₂CH₃); ¹³C NMR (CDCl₃, 90 MHz) δ 167.7 (C-7), 160.2 (C-5), 158.6 (C-3), 157.1 (C-3'), 144.8 (C-1), 142.5 (C-1'), 130.9 (C-5'), 129.3-127.0 (aromatic carbons of benzyl groups), 121.0 (C-6'), 114.6 (C-2'), 114.5 (C-4'), 107.5 (C-6), 100.0 (C-2, 4), 70.5, 70.3, 70.0 (3 × -CH₂- of benzyl groups), 61.0 (-OCH₂CH₃), 14.0 (-OCH₂CH₃); CIMS *m/z* 545 [M + 1]⁺ (100), 455 (19), 365 (16), 275 (7). HREIMS *m/z* 544.2276 (calcd for C₃₆H₃₂O₅, 544.2250).

3,3',5-Tribenzyloxybiphenyl-2-carboxylic Acid (5). A solution of **4** (490 mg, 0.9 mmol) in 10% NaOH/EtOH (10 mL) was refluxed for 3 h and monitored by TLC to determine the reaction progress. The mixture was adjusted to pH 3-4 with concentrated HCl with stirring in an ice-water bath. The white salt was washed with ether, the ether layer was evaporated, and the residue was separated by CC eluting with hexane/EtOAc (5:1) to afford pure **5** (404 mg, 0.78 mmol, 87%): UV (MeOH) λ_{max} (log ε) 210 (4.87), 258 (sh) (4.01), 286

(3.80) nm; IR (KBr) ν_{max} 3424, 3063, 3030, 2925, 2867, 1697, 1599, 1574, 1496, 1454, 1377, 1340, 1285, 1247, 1167, 1026, 736, 696 cm⁻¹; ¹H NMR (CD₃OD, 360 MHz) δ 7.25-7.10 (15H, m, aromatic H of benzyl groups), 7.06 (1H, dd, *J* = 8.0, 8.0 Hz, H-5'), 6.88 (1H, d, *J* = 2.0 Hz, H-2'), 6.82 (1H, d, *J* = 8.0 Hz, H-4'), 6.76 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.49 (1H, d, *J* = 2.0 Hz, H-6), 6.35 (1H, d, *J* = 2.0 Hz, H-4), 4.92, 4.83, 4.82 (each 2H, s, -CH₂- of benzyl groups); ¹³C NMR (CD₃OD, 90 MHz) δ 172.1 (C-7), 161.5 (C-5), 160.1 (C-3), 158.0 (C-3'), 142.9 (C-1), 138.2 (C-1'), 130.4 (C-5'), 129.5-128.0 (aromatic carbons of benzyl groups), 122.1 (C-6'), 115.9 (C-2'), 115.5 (C-4'), 109.0 (C-6), 100.8 (C-2, 4), 71.5, 71.2, 71.0 (3 × -CH₂- of benzyl groups); CIMS *m/z* 517 [M + 1]⁺ (73), 426 (22), 383 (18), 293 (9), 203 (7), 112 (100); HREIMS *m/z* 516.1945 (calcd for C₃₄H₂₈O₅, 516.1937).

3,3',5-Trihydroxybiphenyl-2-carboxylic Acid (6). To a solution of **5** (720 mg, 1.39 mmol) in absolute EtOH (20 mL) was added 10% Pd/C (240 mg) suspended in absolute EtOH (10 mL). The whole mixture was stirred under a H₂ atmosphere at room temperature for 48 h. The catalyst was filtered off and washed with EtOH (10 mL). The combined filtrate was evaporated in vacuo to afford **6** (345 mg, 1.39 mmol, 100%): UV (0.1 M phosphate buffer, pH 7.0) λ_{max} (log ε) 252 (3.93), 287 (3.73) nm; IR (KBr) ν_{max} 3362, 1637, 1604, 1583, 1458, 1288, 1242, 1172, 848, 807 cm⁻¹; ¹H NMR (CD₃OD, 360 MHz) δ 6.82 (1H, dd, *J* = 8.0, 8.0 Hz, H-5'), 6.54-6.57 (3H, H-2', -4', and -6'), 6.15 (1H, d, *J* = 1.9 Hz, H-6), 6.04 (1H, d, *J* = 1.9 Hz, H-4); ¹³C NMR (CD₃COCD₃, 90 MHz) δ 172.8 (C-7), 161.7 (C-5), 160.3 (C-3), 158.9 (C-3'), 148.2 (C-1), 146.1 (C-1'), 129.1 (C-5'), 120.6 (C-6'), 116.4 (C-2'), 115.1 (C-4'), 111.3 (C-6), 102.5 (C-2, -4); CIMS *m/z* 247 [M + 1]⁺ (89), 203 (100); HREIMS *m/z* 246.0527 (calcd for C₁₃H₁₀O₅, 246.0528).

3,3',5-Trihydroxybiphenyl-2-carboxylic Acid Succinimide Ester (7). Compound **6** (140 mg, 0.57 mmol) was dissolved in anhydrous EtOAc (20 mL), and *N*-hydroxysuccinimide (66 mg, 0.57 mmol) and *N,N*-dicyclohexylcarbodiimide (124 mg, 0.60 mmol) were added. Then the whole mixture was stirred at room temperature for 72 h. The dicyclohexylurea was filtered off and washed with EtOAc (10 mL). The combined filtrate and washing were evaporated in vacuo. The residue was separated on CC eluting with CHCl₃/acetone (5:1) to afford pure succinimide ester **7** (80 mg, 41%) as white amorphous powder: UV (MeOH) λ_{max} (log ε) 202 (4.25), 215 (sh) (4.18), 230 (sh) (4.06), 274 (3.72), 287 (3.46) nm; IR (KBr) ν_{max} 3421, 2925, 1729, 1615, 1583, 1437, 1361, 1209, 1063 cm⁻¹; ¹H NMR (CD₃COCD₃, 360 MHz) δ 7.12 (1H, dd, *J* = 8.8, 8.0 Hz, H-5'), 6.84 (2H, H-2' and -4'), 6.75 (1H, dd, *J* = 2.8, 8.0 Hz, H-6'), 6.44 (1H, d, *J* = 2.0 Hz, H-6), 6.39 (1H, d, *J* = 2.0 Hz, H-4), 2.81 (4H, s, H of succinimide moiety); ¹³C NMR (CD₃COCD₃, 90 MHz) δ 170.4 (2 × -CO- of succinimide moiety), 165.8 (C-7), 163.6 (C-5), 163.0 (C-3), 157.7 (C-3'), 147.7 (C-1), 142.8 (C-1'), 129.9 (C-5'), 120.7 (C-6'), 115.9 (C-2'), 115.3 (C-4'), 112.2 (C-6), 103.0 (C-2), 102.7 (C-4), 26.0 (2 × -CH₂- of succinimide moiety); CIMS *m/z* 344 [M + 1]⁺ (62), 229 [M - succinimide]⁺ (100); HREIMS *m/z* 343.0680 (calcd for C₁₇H₁₃NO₇, 343.0692).

3,3',5-Trihydroxybiphenyl-2-carboxylic Acid CoA Ester (8). CoA (10 mg, 0.013 mmol) and **7** (20 mg, 0.058 mmol) were suspended in a microcentrifuge tube containing 0.5 mL of 50 mM aqueous NaHCO₃ and was chilled to 0 °C. Two drops of acetone were added to dissolve **7** completely. The mixture was maintained at 0 °C overnight and separated by PC on Whatman no. 3 paper developed in *n*-butanol-acetic acid-H₂O (5:2:3). The biphenylcarbonyl-CoA thiol ester was detected by fluorescence at 366 nm, eluted with H₂O, and lyophilized to give **8** (5.4 mg, 42%). After ascending chromatography on Whatman no. 3 paper, the biphenylcarbonyl-CoA thiol ester (*R_f* 0.44) turned a characteristic pink on treatment with nitroprusside spray reagent under alkaline conditions. Free phenolic groups were detected by spraying with diazotized sulfanilic acid in 10% KOH and turned yellow: UV (0.1 M phosphate buffer, pH 7.0) λ_{max} (log ε) 254 (3.80), 330 (2.91) nm; IR (KBr) ν_{max} 3416, 1754, 1649, 1573, 1419, 1385, 1261, 1100, 1057, 1021, 736, 668 cm⁻¹; ¹H NMR (D₂O, 360 MHz) δ 8.32 (1H, s, H-5 of CoA), 8.13 (1H, s, H-2 of CoA), 7.10 (1H, dd, *J* = 8.0, 8.0 Hz, H-5'), 6.72 (1H, br s, H-2'), 6.70 (1H, d, *J* = 8.0

Hz, H-4'), 6.63 (1H, br d, $J = 8.0$ Hz, H-6'), 6.27 (1H, br s, H-6), 6.20 (1H, br s, H-4), 6.00 (1H, br s, H-6 of CoA), 4.47 (1H, d, H-9 of CoA), 4.14 (2H, t, H-10 of CoA), 4.00 (1H, m, H-7 of CoA), 3.90 (1H, m, H-15 of CoA), 3.51 (1H, m, H-8 of CoA), 3.40–3.05 (m, H-17, -18, -11 of CoA), 2.93–2.20 (m, H-20, -21 of CoA), 1.21, 1.19 (each 3H, s, H-13, -14 of CoA).

3,3',5-Trihydroxybiphenyl-2-carboxylic Acid *N*-Acetyl Cysteamine Ester (9). Method 1: **7** (22 mg, 0.06 mmol) and *N*-acetyl cysteamine¹⁴ (HS-NAC, 25 μ L) were suspended in 50 mM of aqueous NaHCO₃ (0.7 mL). Acetone was added until the mixture formed a single phase. The solution was kept at 0 °C for 24 h and crude **9** was isolated by preparative TLC eluting with CHCl₃/MeOH (5:1) and further purified by HPLC (UV: 254 nm; column: RP₁₈, 5 μ m, 4.6 \times 250 mm; flow rate: 3 mL/min) eluted with MeOH/H₂O (6:4) to afford **9** (4.0 mg, 17%). Method 2: To 3,3',5-trihydroxybiphenyl-2-carboxylic acid (**6**) (0.1 mmol) in dry CH₂Cl₂ (2 mL), DMAP (2.0 mg, 0.016 mmol) and HS-NAC (20 mg, 0.17 mmol) were added. At 0 °C, DCC (0.11 mmol, 23 mg) was added and the mixture kept in an ice-water bath for 5 min followed by 3 h in 20 °C. The precipitate was removed by filtration, and water (3 mL) was added to the filtrate. The CH₂Cl₂ layer was separated and the water phase extracted with EtOAc. The combined organic layers were dried and concentrated, and the residue was separated by preparative TLC. Further purification by HPLC gave **9** in 8% yield: UV (0.1 M phosphate buffer, pH 7.0) λ_{\max} (log ϵ) 252 (3.94), 286 (3.79) nm; IR (KBr) ν_{\max} 3405, 2923, 1618, 1567, 1444, 1356, 1281, 1212, 1170, 1114, 909 cm⁻¹; ¹H NMR (CD₃COCD₃, 360 MHz) δ 7.21 (1H, dd, $J = 7.8, 7.8$ Hz, H-5'), 7.07 (1H, br s, -NH), 6.79–6.86 (3H, H-2', -4', -6'), 6.41 (1H, d, $J = 2.5$ Hz, H-6), 6.34 (1H, d, $J = 2.5$ Hz, H-4), 3.22 (2H, s, -N-CH₂), 2.93 (2H, m, -S-CH₂), 1.83 (3H, s, -COCH₃); ¹³C NMR (CD₃COCD₃, 90 MHz) δ 196.2 (CO-S), 170.4 (-CO-CH₃), 161.6 (C-5), 159.5 (C-3), 158.1 (C-3'), 145.2 (C-1), 142.8 (C-1'), 130.1 (C-5'), 121.4 (C-6'), 117.3 (C-4'), 115.8 (C-2'), 110.8

(C-6), 102.8 (C-2, -4), 39.4 (-N-CH₂), 30.4 (-S-CH₂), 22.8 (-CO-CH₃); CIMS m/z 348 [M + 1]⁺ (15), 257 (15), 229 (65), 203 (100), 158 (66); HREIMS m/z 347.0826 (calcd for C₁₇H₁₇-NO₅S, 347.0828).

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