SYNTHESIS OF FORPHENICINOL AND FORPHENICINE

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(Received for publication June 28, 1982)

Forphenicinol, L-2-(3-hydroxy-4-hydroxymethylphenyl)glycine, was synthesized by two methods starting from dimethyl hydroxyterephthalate or DL-2-(3-hydroxyphenyl)glycine. Forphenicine, L-2-(4-formyl-3-hydroxyphenyl)glycine, was prepared from L-forphenicinol by oxidation, thus establishing configuration of forphenicine to be L. D-Forphenicinol was determined to be biologically inactive.

Forphenicine was a microbial fermentation product isolated by UMEZAWA *et al.*[1] as a novel inhibitor of alkaline phosphatase (prepared from chick intestine). The structure was elucidated to be 2-(4-formyl-3-hydroxyphenyl)glycine[2], excluding configuration of the asymmetric carbon which was not determined. As reported in this paper, the configuration of forphenicine was determined to be the L-form by synthesis.

Intraperitoneal injection but not oral administration of forphenicine enhanced delayed-type hypersensitivity and increased the number of antibody-forming cells[3]. Among synthetic derivatives of forphenicine reported in earlier papers, L-2-(3-hydroxy-4-hydroxymethylphenyl)glycine, forphenicinol, was found to enhance immune responses and exhibit inhibition against mouse tumors by oral administration[4,5].

In the present paper, we report the synthesis of forphenicinol, D-forphenicinol and forphenicine. Our approach was to synthesize forphenicinol and oxidize it to forphenicine. Based on easily obtainable starting materials, two strategies were elaborated. One was the introduction of C-1 unit to the benzene ring of 2-(3-hydroxyphenyl)glycine, and the other was the formation of the glycine portion from one of the carboxyl group of a hydroxyterephthalic acid and followed by a reduction of the other carboxyl group. The latter approach which utilized a Strecker reaction was tried first (Scheme 1).

ASAKAWA *et al.* have reported that ester derivatives of the carboxylic acid of salicylic acid are reduced to the alcohol with sodium borohydride in tetrahydrofuran, dioxane, diglyme, dimethylsulfoxide or ethyl acetate in good yield[6]. We applied these reaction conditions to the reduction of dimethyl hydroxyterephthalate[7] (**1**). With sodium borohydride in tetrahydrofuran this afforded **2** in 91% yield. Reduction of **1** with sodium dihydrobis-(2-methoxyethoxy)aluminate in refluxing benzene for 2.5 hours gave **3** in a low yield. Salicylalcohol moiety of **2** was protected by a isopropylidene ketal in order to prevent the formation of sparingly soluble metal chelate product during the next reduction process. Treatment of **2** with 2,2-dimethoxypropane in benzene containing a catalytic amount of *p*-toluenesulfonic acid afforded compound **4** in quantitative yield. Reduction of **4** with sodium dihydrobis-(2-methoxyethoxy)aluminate in benzene at room temperature afforded **5** in good yield. Compound **5** was converted to **6** by oxidation with chromium trioxide in the presence of 3,5-dimethylpyrazole[8].

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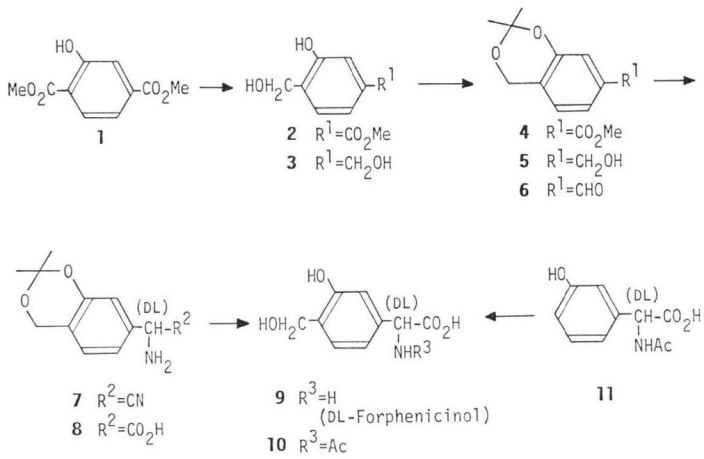
Scheme 1.

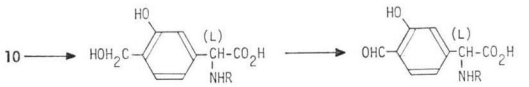


Addition of sodium cyanide in aqueous ammonia to **6** in methanol containing ammonium chloride afforded compound **7** in 85% yield. Hydrolysis of **7** with barium hydroxide followed by the removal of isopropylidene ketal by mild acid treatment afforded DL-forphenicinol (**9**) in 50% yield. For the purpose of the optical resolution by asymmetric hydrolysis with an acylase, DL-forphenicinol (**9**) was converted to the *N*-acetyl derivative (**10**) with acetic anhydride in water.

According to the other approach, *N*-acetyl-DL-forphenicinol (**10**) was also obtained by hydroxymethylation of *N*-acetyl-DL-2-(3-hydroxyphenyl)glycine (**11**) with formaldehyde (Scheme 1). DL-2-(3-Hydroxyphenyl)glycine prepared from 3-hydroxybenzaldehyde by a Strecker reaction was acetylated with acetic anhydride in water to afford *N*-acetyl-DL-2-(3-hydroxyphenyl)glycine (**11**). Hydroxymethylation of **11** with formaldehyde in 1 M sodium hydroxide gave **10** in 47% yield. The acetyl group of **10** was removed stereospecifically with a mold aminoacylase, yielding forphenicinol (**12**) in 44% yield. *N*-Acetyl-D-forphenicinol was obtained in 50% yield (Scheme 2).

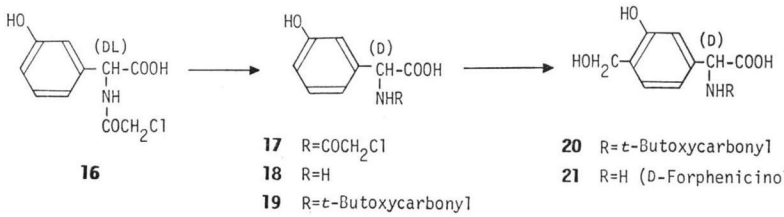
D-Forphenicinol was prepared by a similar hydroxymethylation process described above from *N*-*t*-butoxycarbonyl-D-2-(3-hydroxyphenyl)glycine (**19**) (Scheme 3). D-Forphenicinol was found not to enhance immune responses.

Scheme 2.



Forphenicine was synthesized from forphenicinol by oxidation. The amino group of forphenicinol could be conveniently protected with the *t*-butoxycarbonyl group, since forphenicine had been confirmed to be stable in acid treatment needed in the deprotection process. Oxidation of *N*-*t*-butoxy-

Scheme 3.



carbonylforphenicinol (**13**) with activated manganese dioxide followed by the removal of the protecting group with trifluoroacetic acid afforded forphenicine (**15**) in 44% yield. Synthetic forphenicine was identical with the natural product in all physicochemical properties and in its activity to inhibit alkaline phosphatase. Thus, the structure of forphenicine was elucidated to be L-2-(4-formyl-3-hydroxyphenyl)-glycine synthetically.

Experimental

Melting points were determined on a hot-stage microscope and are uncorrected. Nuclear magnetic resonance spectra were recorded by a Varian A-60 spectrometer. Chemical shifts in D_2O were recorded in ppm using tetramethylsilane as an external reference. All chemical shifts in other solvents were recorded in ppm downfield from internal tetramethylsilane. The abbreviations s, d, t and dd indicate singlet, doublet, triplet and double doublet, respectively. Optical rotations were determined by Japan Spectroscopic Co., Ltd. DIP-140 polarimeter. Merck silica gel (Kieselgel 60, Art. 7734) was used for column chromatography. Thin-layer chromatography (TLC) was used routinely for monitoring the reactions; precoated plates (Merck, Art. 5715) were used and the substances were detected with ultraviolet absorption, or visualized with 2,4-dinitrophenylhydrazine or ninhydrin reagents.

Methyl 3-Hydroxy-4-hydroxymethylbenzoate (**2**)

To a solution of dimethyl hydroxyterephthalate (**1**) (2.1 g, 10 mmole) in tetrahydrofuran (50 ml) was added sodium borohydride (756 mg, 20 mmole) and the resulting solution was refluxed for 1.5 hours. After the removal of the solvent by evaporation, water was added to the residue and the solution was brought to pH 1.0 with 1 M hydrochloric acid. The solution was allowed to stand at 0°C to give colorless crystals (1.7 g, 93%). Mp 104.5~105°C; NMR (CD_3OD) 3.88 (3H, s, OMe), 4.72 (2H, s, Ar–CH_2OH), 7.3~7.7 (3H, Ar).

Isopropylidene Derivative (**4**)

Methyl 3-hydroxy-4-hydroxymethylbenzoate (**2**) (1,274 mg, 7 mmole) was suspended in dry benzene (30 ml), and 2,2-dimethoxypropane (5 ml) and *p*-toluensulfonic acid monohydrate (50 mg) were added under stirring at room temperature. After 1 hour the reaction mixture was clear. After 16 hours the solution was passed through the column of silica gel (100 ml) equilibrated with benzene and developed with benzene. Evaporation of eluate (250 ml) afforded colorless crystals of **4** (1,462 mg, 94%). Mp 47.5~48°C; NMR ($CDCl_3$) 1.53 (6H, s, isopropylidene), 3.89 (3H, s, OMe), 4.88 (2H, s, Ar–CH_2O–), 7.04 (1H, d, J=8 Hz, Ar), 7.52 (1H, d, J=1.8 Hz, Ar), 7.60 (1H, dd, Ar).

Reduction of **4**

To a solution of **4** (1,462 mg, 6.6 mmole) in dry benzene (20 ml) was added 70% benzene solution of sodium dihydrobis-(2-methoxyethoxy)aluminate (2.8 ml, 9.7 mmole) dropwise with stirring at 0°C and subsequently the solution was allowed to come to room temperature. After 2 hours the solution was cooled in an ice bath with stirring during which 9 ml of 1 M hydrochloric acid was added slowly. The solution was diluted with benzene and water. After the removal of the white precipitate by filtration, the benzene layer was separated and aqueous layer was extracted twice with benzene. The benzene layers were combined and concentrated to dryness yielding colorless crystals of **5** (1,213 mg). Mp 62~74°C. Recrystallization from benzene - cyclohexane afforded colorless crystals (928 mg, 73%). Mp 73.5~75.5°C. NMR ($CDCl_3$) 1.53 (6H, s, isopropylidene), 1.82 (1H, t, OH), 4.62 (2H, d, J= 6.0 Hz, Ar–CH_2–OH), 4.85 (2H, s, Ar–CH_2O–), 6.80~7.0 (3H, Ar).

Oxidation of **5**

To a suspension of chromium trioxide (250 mg, 2.5 mmole) in dry dichloromethane (10 ml) was added 3,5-dimethylpyrazole (240 mg, 2.5 mmole) with stirring at room temperature. After 2 hours the solution turned dark brown and became almost clear. To this was added **5** (194 mg, 1 mmole) dissolved in dry dichloromethane (2 ml). After 2 hours the residual 3,5-dimethoxypyrazole - chromium trioxide complex was precipitated by the addition of ethyl ether (100 ml) and the supernatant was evaporated to dryness. The brown residue was chromatographed on a column of silica gel developed with benzene -

ethyl acetate (50: 1). Fractions containing **6** were combined and concentrated to dryness yielding colorless syrup of **6** (164 mg, 85%). NMR ($CDCl_3$) 1.55 (6H, s, isopropylidene), 4.91 (2H, s, Ar–CH_2O–), 7.15 (1H, d, J=7.8 Hz, Ar), 7.34 (1H, d, J=1.5 Hz, Ar), 7.47 (1H, dd, Ar), 9.91 (1H, s, aldehyde).

α-Amino Nitrile (**7**)

To a solution of sodium cyanide (216 mg, 4.4 mmole) in 28% aqueous ammonia (5 ml) were added ammonium chloride (236 mg, 2.2 mmole) and aldehyde compound (**6**) (206 mg, 1.1 mmole) in methanol. After stirring at room temperature for 4.5 hours, ammonia and methanol were removed by evaporation. The solution was diluted with water and extracted 3 times with *n*-butanol (10 ml). The organic phase was concentrated to dryness and the residue was purified by column chromatography on silica gel developed with benzene - ethyl acetate (4: 1). The fractions containing **7** were combined and evaporated to dryness to give a pale yellow solid **7** (198 mg, 85%). NMR ($CDCl_3$) 1.53 (6H, s, isopropylidene), 3.27 (2H, broad s, amino), 4.83 (2H, s, Ar–CH_2O–), 4.88 (1H, broad s, α-methine), 6.8~7.2 (3H, Ar).

DL-2-(3-Hydroxy-4-hydroxymethylphenyl)glycine (DL-Forphenicinol) (**9**)

To a solution of **7** (198 mg, 0.9 mmole) in ethanol (5 ml) were added barium hydroxide (2 g) and water (3 ml). After refluxing for 5 hours, the reaction mixture was diluted with water, adjusted to pH 2.0 with 1 M sulfonic acid, and then refluxed for 30 minutes to remove isopropylidene ketal. After the resulting barium sulfonate was removed by centrifugation (3000 r.p.m., 30 minutes), the supernatant was concentrated and chromatographed on a column of SP-Sephadex C-25 (H^+ form, 10 ml) developed with water. The fractions containing DL-forphenicinol were combined and concentrated to dryness yielding pale yellow powder of DL-forphenicinol (**9**) (89 mg, 50%). Crystallization from water afforded colorless crystals (62 mg, 35%). Mp >200°C (dec.), *Anal.* Calcd. for $C_9H_{11}NO_4$: C 54.82, H 5.62, N 7.10. Found: C 54.96, H 5.55, N 7.04. NMR (D_2O) 5.12 (2H, s, Ar–CH_2OH), 5.18 (1H, s, α-methine), 7.35~7.55 (2H, Ar), 7.82 (1H, d, J=8.0 Hz, Ar).

DL-2-(3-Hydroxyphenyl)glycine Hydrochloride Monohydrate

To a solution of sodium cyanide (22 g, 0.45 mole) and ammonium chloride (22 g, 0.41 mole) in 28% aqueous ammonia (130 ml) was added 3-hydroxybenzaldehyde (24.4 g, 0.2 mole) over 30 minutes at 5~10°C. After stirring at 15~20°C for 4 hours, the solution was cooled in an ice bath to afford crystals of DL-[α-amino-α-(3-hydroxyphenyl)]acetonitrile (24 g, 0.16 mole, 81%). The crystals were dissolved in 6 M hydrochloric acid (240 ml) and refluxed for 2 hours. The hot solution, decolorized with charcoal, was cooled in an ice bath to give crystals of DL-2-(3-hydroxyphenyl)glycine hydrochloride monohydrate (27 g, 61%). Recrystallization from water - acetone afforded colorless needles. Mp 154°C (dec.). *Anal.* Calcd. for $C_8H_9NO_3 \cdot HCl \cdot H_2O$: C 43.35, H 5.46, N 6.32. Found: C 43.09, H 5.59, N 6.16.

N-Acetyl-DL-2-(3-hydroxyphenyl)glycine (**11**)

To a stirred mixture of DL-2-(3-hydroxyphenyl)glycine hydrochloride monohydrate (25 g, 0.11 mole) and sodium hydroxide (15 g, 0.375 mole) in water (37 ml) was added acetic anhydride (12.5 g, 0.12 mole) over 1 hour at 0~10°C. Additional acetic anhydride (12.5 g, 0.12 mole) and sodium hydroxide (5 g, 0.125 mole) were slowly added for 1 hour. After 30 minutes 5 M sodium hydroxide was added and stirred for 10 minutes. The solution was acidified with concentrated hydrochloric acid (75 ml) and cooled to 0°C to afford crystals (21.5 g, 89.5%). Recrystallization from water gave colorless crystals of *N*-acetyl-DL-2-(3-hydroxyphenyl)glycine (**11**). Mp 168~170°C. *Anal.* Calcd. for $C_{10}H_{11}NO_4$: C 57.41, H 5.30, N 6.70. Found: C 57.26, H 5.21, N 6.49.

N-Acetyl-DL-2-(3-hydroxy-4-hydroxymethylphenyl)glycine (*N*-Acetyl-DL-forphenicinol) (**10**)

Hydroxymethylation of *N*-Acetyl-DL-(3-hydroxyphenyl)glycine (**11**): To a solution of **11** (10.5 g, 50 mmole) in 1 M sodium hydroxide (100 ml) was added 37% aqueous formaldehyde (5.25 ml, 75 mmole) and stirred at 50°C for 6 hours. The solution was cooled in an ice bath and acidified with concentrated hydrochloric acid (10 ml). Storage at 0°C overnight yielded crystals of **10** (3.99 g). Recrystallization from water afforded pure crystals of **10** (3.36 g, 28%). Mp 185°C (dec.). *Anal.* Calcd. for $C_{11}H_{13}NO_5$: C 55.23, H 5.48, N 5.85. Found: C 54.94, H 5.53, N 5.79. NMR (CD_3SOCD_3) 1.88 (3H, s, Ac), 4.44

(2H, s, Ar–CH_2OH), 5.16 (1H, d, J=7.2 Hz, α-methine), 6.64~6.87 (2H, Ar), 7.23 (1H, d. J=8.0 Hz, Ar), 8.42 (1H, d, J=7.2 Hz, amide), 9.10~9.50 (1H, broad s, CO_2H). The filtrates after the crystallization were combined, concentrated to about 40 ml, and subjected to the column chromatography of Diaion HP-20 (Mitsubishi Chemical Industries Ltd., Tokyo, 500 ml); *N*-acetyl-DL-forphenicinol (**10**) (2.26 g, 19%) and the starting material (**11**) (1.24 g) were recovered.

N-Acetylation of DL-Forphenicinol (**9**): To a solution of DL-forphenicinol (354 mg, 1.8 mmole) in water (25 ml) was added acetic anhydride (3 ml) and stirred at room temperature for 7 hours. Cold water (10 ml) was added to the solution and chromatographed on a column of DEAE-Sephadex A-25 ($HCOO^-$) developed with a linear gradient of water and 0.5 M ammonium formate, and desalted by a column of SP-Sephadex C-25 (H^+) to give colorless powder of *N*-acetyl-DL-forphenicinol (**10**) (280 mg, 65%).

L-2-(3-Hydroxy-4-hydroxymethylphenyl)glycine (Forphenicinol) (**12**)

N-Acetyl-DL-forphenicinol (**10**) (3.0 g, 12.6 mmole) was dissolved in 0.5 M sodium hydroxide and adjusted pH 7.5 (total volume 60 ml). To the solution were added cobalt chloride hexahydrate (8 mg) and mold aminoacylase (100 mg, 1500 units, Acylase Amano 15000, Amano Pharmaceutical Co., Ltd., Nagoya), and hydrolyzed at 37°C for 48 hours. The reaction mixture was decolorized with charcoal and passed through the column of Dowex 50 X4 (H^+, 30 ml). The column was washed with water, and forphenicinol (L-forphenicinol) was eluted with 1 M aqueous ammonia (250 ml). The eluate was concentrated to dryness yielding forphenicinol (**12**) (1.09 g, 44%). Crystallization from water gave colorless crystals. Mp 223°C (dec.), $[\alpha]_D^{20}$ +131° (c 1.0, 1 M HCl), *Anal.* Calcd. for $C_9H_{11}NO_4$: C 54.82, H 5.62, N 7.10. Found: C 54.69, H 5.69, N 7.06. The effluent of the column of Dowex 50 was concentrated to 50 ml and chromatographed on a column of Diaion HP-20 developed with water to afford *N*-acetyl-D-2-(3-hydroxy-4-hydroxymethylphenyl)glycine (1.5 g, 50%). The analytical sample was recrystallized from water. Mp 172°C (dec.), $[\alpha]_D^{20}$ −206° (c 1.0, EtOH).

N-*t*-Butoxycarbonyl-L-2-(3-hydroxy-4-hydroxymethylphenyl)glycine (*N*-Boc-L-Forphenicinol) (**13**)

To a solution of L-forphenicinol (379 mg, 1.9 mmole) (**12**) in water (5 ml) were added triethylamine (0.42 ml, 3 mmole) and *t*-butyl *S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate (528 mg, 2.2 mmole) dissolved in dioxane (5 ml). After 23 hours the solution was diluted with water (30 ml) and the unreacted carbonate was extracted twice with ethyl acetate (10 ml). The aqueous layer was adjusted pH 2 at 0°C by addition of cold 1 M hydrochloric acid and extracted 3 times with ethyl acetate (10 ml). The organic solvent phase was washed with water, dried with anhydrous magnesium sulfate, and concentrated to dryness affording the crude product. The pale yellow solid was washed with ethylenechloride to give colorless powder (436 mg, 76%). Mp 143~145°C, $[\alpha]_D^{20}$ +132° (c 1.0, MeOH), *Anal.* Calcd. for $C_{14}H_{19}NO_6$: C 56.56, H 6.44, N 4.71. Found: C 56.79, H 6.31, N 4.69. NMR (CD_3OD) 1.43 (9H, s, Boc), 4.67 (2H, s, Ar–CH_2OH), 5.10 (1H, s, α-methine), 6.88 (1H, d, J=1.5 Hz, Ar), 6.89 (1H, dd, Ar), 7.29 (1H, d, J=8.0 Hz).

L-2-(4-Formyl-3-hydroxyphenyl)glycine (Forphenicine) (**15**)

To a solution of *N*-Boc-L-forphenicinol (119 mg, 0.4 mmole) (**13**) in ethyl acetate (18 ml) was added active manganese dioxide (1.5 g) and stirred at room temperature for 4 hours. Manganese dioxide was filtered and washed with 50% aqueous methanol (30 ml) and methanol (30 ml). The filtrate was concentrated to dryness and the residual solid was dissolved in trifluoroacetic acid (1 ml). After standing at room temperature for 30 minutes, trifluoroacetic acid was removed by evaporation. The residue was dissolved in water (10 ml) and chromatographed on a column of SP-Sephadex C-25 (H^+) developed with water. The fractions containing forphenicine were combined and concentrated to afford pale yellow crystals of forphenicine (34 mg, 44%). $[\alpha]_D^{20}$ +141° (c 1.0, 1 M HCl) [lit.[2]), $[\alpha]_D^{20}$ +140° (c 1.0, 1 M HCl)]. *Anal.* Calcd. for $C_9H_9NO_4$: C 55.38, H 4.65, N 7.18. Found: C 55.18, H 4.63, N 7.12. 2-(4-Carboxy-3-hydroxyphenyl)glycine (5 mg, 6%) was obtained as a by-product and L-forphenicinol (11 mg, 14%) was recovered.

N-Chloroacetyl-DL-2-(3-hydroxyphenyl)glycine (**16**)

To a solution of DL-2-(3-hydroxyphenyl)glycine hydrochloride monohydrate (15.3 g, 74.5 mmole) and sodium hydroxide (7 g, 175 mmole) in water (50 ml) were added chloroacetyl chloride (17.5 g, 155

mmole) in toluene (15 ml) over 40 minutes at 5~10°C keeping pH 11.5~13.5 by addition of 5 M sodium hydroxide (55 ml). After additional 2 hours the solution was cooled in an ice bath and adjusted pH 1 with concentrated hydrochloric acid. After 1.5 hours an insoluble material was removed by filtration and the filtrate was extracted with ethyl acetate. The extract was concentrated and the residue was crystallized from water (50 ml) to give crystals of *N*-chloroacetyl-DL-2-(3-hydroxyphenyl)glycine (**16**) (9.4 g, 55%). Mp 165~167°C. Concentration of the mother liquor gave a second crop (4.4 g, 26%), mp 165~167°C.

N-Chloroacetyl-D-2-(3-hydroxyphenyl)glycine (**17**)

To a solution of *N*-chloroacetyl-DL-2-(3-hydroxyphenyl)glycine (**16**) (10 g, 41 mmole) in 0.5 M sodium hydroxide (adjusted pH 7.5, total volume 200 ml) were added cobalt chloride hexahydrate (20 mg) and mold aminoacylase (250 mg, 3,750 units), and hydrolyzed at 37°C for 28 hours. The reaction mixture was adjusted pH 1.5 with hydrochloric acid, decolorized with charcoal, and extracted with ethyl acetate. Concentration of the extract followed by addition of benzene (50 ml) afforded crystals of *N*-chloroacetyl-D-2-(3-hydroxyphenyl)glycine (**17**) (4.7 g, 47%). Mp 182°C (dec.), $[\alpha]_D^{26}$ −173° (*c* 1.0, EtOH).

D-2-(3-Hydroxyphenyl)glycine (**18**)

The solution of *N*-chloroacetyl-D-2-(3-hydroxyphenyl)glycine (**17**) (10 g, 41 mmole) in 3 M hydrochloric acid (20 ml) was refluxed for 3.5 hours. The solution, diluted with water, was passed through the column of Dowex 50X4 (H^+, 50 ml). The column was washed with water and the eluate with 300 ml of 1 M aqueous ammonia was collected. The eluate was concentrated to dryness and the residual solid was suspended in ethanol. Collection of the solid by filtration gave D-2-(3-hydroxyphenyl)glycine (**18**) (6.5 g, 95%). $[\alpha]_D^{26}$ −144° (*c* 1.0, 1 M HCl).

N-*t*-Butoxycarbonyl-D-2-(3-hydroxyphenyl)glycine (**19**)

Using the method described for the preparation of **13**, **19** was prepared from D-2-(3-hydroxyphenyl)-glycine (**18**) (5 g, 29.9 mmole) and *t*-butyl *S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate (8.26 g, 34.4 mmole). Crystallization from ethyl acetate - chloroform afforded 6.69 g (84%) of *N*-*t*-butoxycarbonyl-D-2-(3-hydroxyphenyl)glycine (**19**). Mp 119~120°C (dec.), $[\alpha]_D^{23}$ −130° (*c* 1.0, MeOH).

N-*t*-Butoxycarbonyl-D-2-(3-hydroxy-4-hydroxymethylphenyl)glycine (*N*-Boc-D-Forphenicinol) (**20**)

To a solution of *N*-*t*-butoxycarbonyl-D-2-(3-hydroxyphenyl)glycine (**19**) (2.67 g, 10 mmole) in water (18 ml) were added sodium hydroxide (0.8 g, 20 mmole) and 35% aqueous formaldehyde (1.5 ml, 20 mmole) at 80°C. The solution was heated at 90°C for 10 minutes, and then cooled in an ice bath and adjusted pH 2.0. The product was extracted with ethyl acetate. The extract was concentrated and the residue was chromatographed on a column of silica gel developed with benzene - ethyl acetate (2: 1). The eluate was concentrated to dryness yielding *N*-Boc-D-forphenicinol (**20**) (1.24 g, 42%). $[\alpha]_D^{20}$ −140° (*c* 1.0, MeOH).

D-2-(3-Hydroxy-4-hydroxymethylphenyl)glycine (D-Forphenicinol) (**21**)

N-Boc-D-Forphenicinol (**20**) (1.1 g, 3.7 mmole) was dissolved in trifluoroacetic acid (9 ml) and stirred in an ice bath for 15 minutes. The reaction mixture was diluted with methanol and evaporated in reduced pressure. The residue was dissolved in methanol (60 ml) and adjusted pH 6.5 with triethylamine. Concentration of the solution afforded crystals of D-forphenicinol (**21**) (510 mg, 70%). An analytical sample was recrystallized from water. $[\alpha]_D^{20}$ −126° (*c* 1.0, 1 M HCl).

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