Stereoselective Synthesis of Desmethoxy-(+)-verruculogen TR-2

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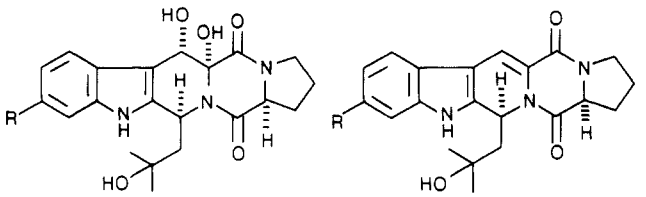
An efficient five-step stereoselective synthesis of (+)-desmethoxyverruculogen TR-2 from L-tryptophan is presented.

Verruculogen TR-2 (1),[1] a mycotoxin isolated from *Penicillum simplissimum*, is a member of a family of compounds that induce sustained tremors in animals.[2] A number of these compounds have been found in mold-contaminated foods and grains,[2d] and some of them have been implicated in neurological disorders of farm animals.[3,4] The mode of action of these compounds is not well understood[2d,3] but is perceived to involve inhibition of the presynaptic release of γ-aminobutyric acid (GABA) in the central nervous system.[5]

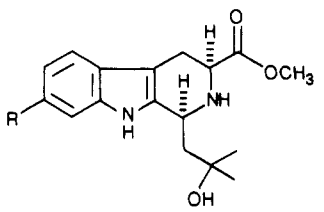
Verruculogen TR-2, like the other members of the fumitremorgen–verruculogen group of tremorgenic mycotoxins, possesses a 6-methoxytryptophan–proline–diketopiperazine unit, together with one (or more for other members) isoprenyl unit. The biosynthesis of this class of mycotoxins has been studied, indicating that these compounds indeed derive from tryptophan, proline, and mevalonate.[6]

Retrosynthetically, we envisioned the *cis*-diol functionality could be obtained stereoselectively by an osmium tetroxide hydroxylation of the key intermediate **3**.[7] We describe here an efficient stereoselective synthesis of the desmethoxy analogue of verruculogen TR-2 (**2**), which incorporates this proposed transformation.



1, R = OCH_3
2, R = H

3, R = OCH_3
4, R = H



5, R = OCH_3
6, R = H

Results and Discussion

The first problem to be addressed was the construction of the 1,3-disubstituted 1,2,3,4-tetrahydro-2-carboline framework of TR-2 with establishment of the C-1 stereocenter. While stereoselective methodologies for the synthesis of either cis- or trans-1,3-disubstituted tetrahydro-2-carbolines have been reported, none of these were suitable for the preparation of the tetrahydrocarboline hydroxy ester **5**.[9-11] The Pictet–Spengler condensation[8] of L-tryptophan methyl ester hydrochloride (**7**) with 3-hydroxy-3-methylbutanol (**8**)[12] in water at 20 °C provided a 71% yield of an inseparable mixture of diastereomeric tetrahydro-2-carbolines **6** and **9** in a 1.9:1 ratio (based on ^1H NMR). It was not possible to unambiguously determine which isomer was which by the spectra.[13] Variations in temperature (−10 to +60 °C) or pH (3–8) of the reaction had little effect on the ratio of the two isomers. With the hope that we could separate these two diastereomers at a later

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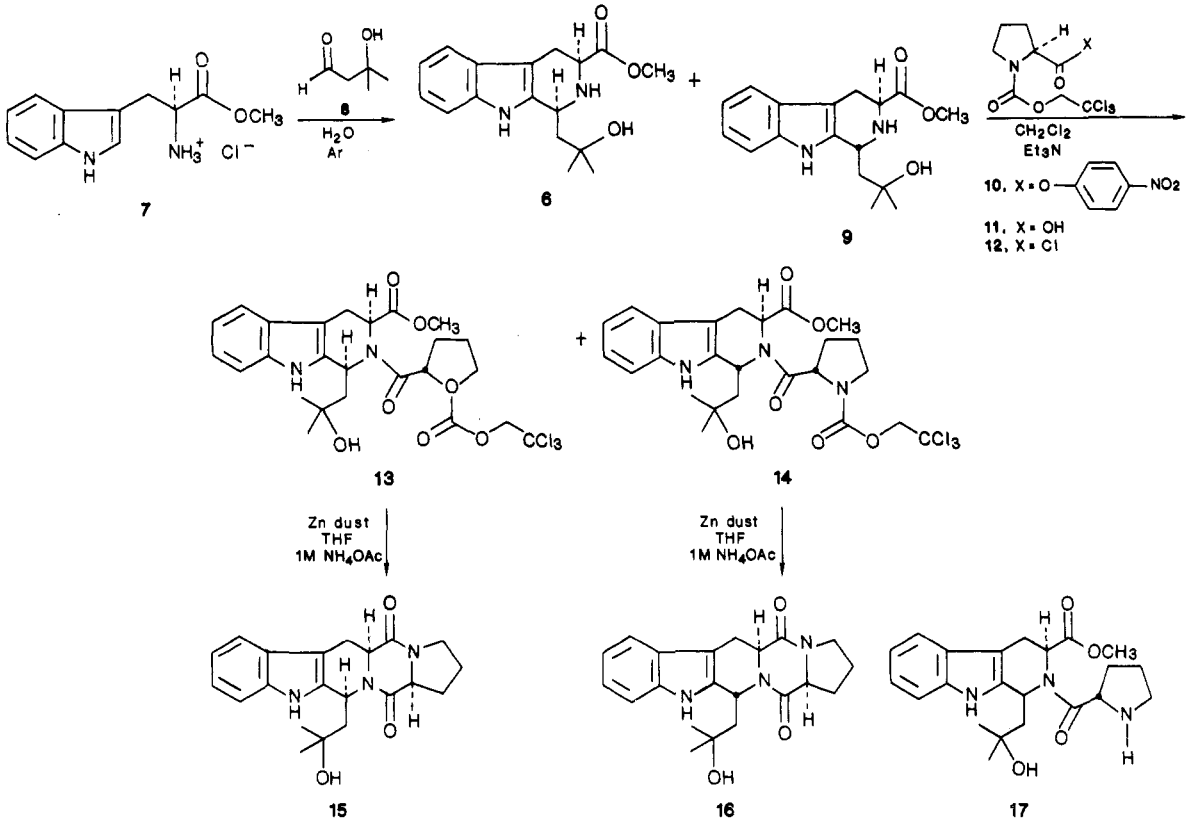
Scheme I



stage, we proceeded to the acylation of this mixture with the L-proline residue. With the *p*-nitrophenyl ester[14] of *N*-[(2,2,2-trichloroethoxy)carbonyl]-L-proline (TrOC-L-proline)[15] (**10**) in refluxing chloroform or benzene, peptide formation was not observed. Dicyclohexylcarbodiimide-catalyzed coupling of TrOC-L-proline with the mixture of **5** and **6** also failed, indicating the high degree of steric hinderance of the tetrahydro-2-carboline nitrogen. However, this difficulty was circumvented by utilizing the acid chloride of TrOC-L-proline (**12**) in dichloromethane at 0 °C, with warming to room temperature for 18 h, which produced the mixture of dipeptides **13** and **14** in 69% purified yield. With use of freshly prepared acid chloride **12**, no epimerized dipeptides were detected by TLC or ^{1}H NMR. Diastereomers **13** and **14** were separable by chromatography[16] at this stage.

To serve as our simpler model compounds, the diketopiperazines **15** and **16** were produced directly from the prolinamides **13** and **14**. In each case, reductive removal of the (2,2,2-trichloroethoxy)carbonyl group under neutral conditions (zinc dust in tetrahydrofuran–1.0 M aqueous ammonium acetate)[17] for 24 h afforded compounds that had suffered loss of the methyl ester, as shown by ^{1}H NMR and IR (broad band at 1660 cm^{-1}). Additionally, the ^{1}H NMR spectrum did not exhibit multiple peaks due to the amide rotomers, a complicating feature of the spectra of **13** and **14**. This clearly indicated that the initially formed amino ester **17** had cyclized to the diketopiperazine under the reaction conditions.

The minor diastereomeric diketopiperazine was a highly crystalline compound, and the opportunity to determine its relative stereochemistry presented itself. Thus, a single-crystal X-ray diffraction study showed that this minor diastereomer corresponded to the trans isomer **16**.[18] An ORTEP drawing of this is presented in Figure 1. Therefore, the initial Pictet–Spengler condensation provided a 1.9:1 excess of the desired *cis*-tetrahydro-2-carboline **6**.

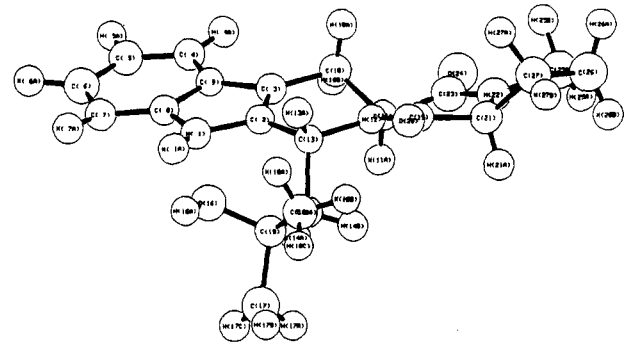


Figure 1. ORTEP drawing of **16**.

Conversion of the ester **13** into the unsaturated ester **18** was achieved by deprotonation using 2.2 equiv of potassium hydride at −10 °C,[22] followed by reaction with benzeneseleninic anhydride[19] in tetrahydrofuran–dimethyl

(14) For a review of peptide synthesis, see: Bodanszky, M.; Klausner, Y. S.; Ondetti, M. A. *Peptide Synthesis*; Wiley-Interscience: New York, 1976.
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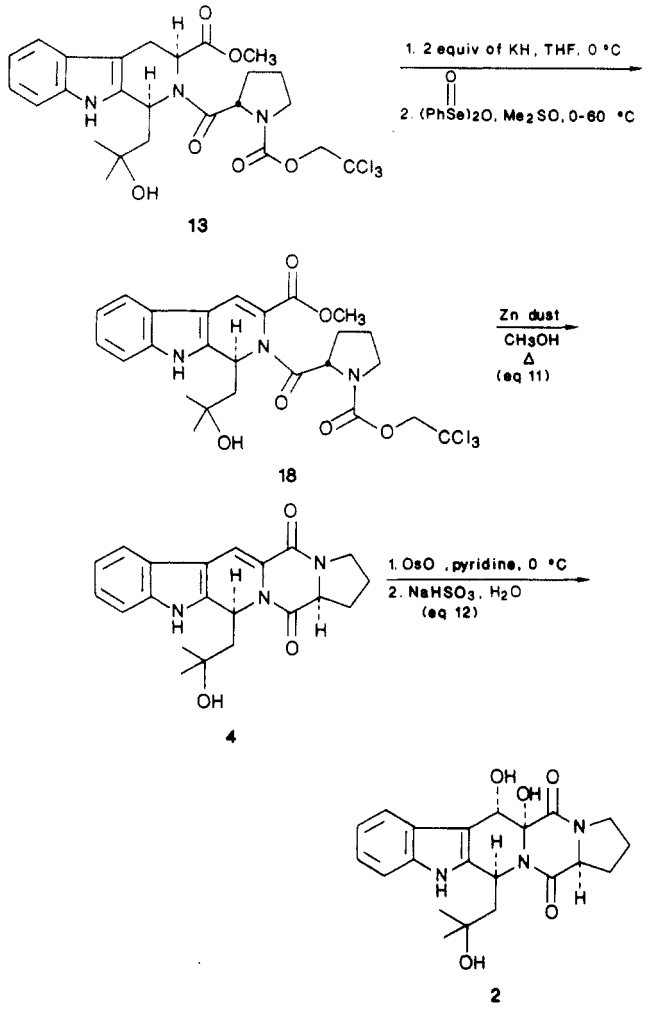
(18) Crystallography data for **16**: space group $P2_12_12_1$; a = 10.372 (4) Å, b = 15.471 (5) Å, c = 11.768 (9) Å; α = 90.00 (0)°, β = 90.00 (0)°, γ = 90.00 (0)°; V = 1888 Å^3; $C_{21}H_{25}N_3O_3$, M_r 367.19; Z = 4, ρ_{calcd} = 1.29 g/cm^3. Diffraction data were collected on a Syntex four-circle diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.7107 Å) at 300 K, in the θ–2θ mode, 2θ < 50°; data analysis was performed with the UCLA crystallographic computing package (the structure was solved with MULTAN78). A total of 1945 independent reflections were collected; 880 reflections were unobserved weak ($I < 3\sigma$). Hydrogen atoms were located in the Fourier difference map. All non-hydrogen atoms were refined anisotropically and H atoms refined isotropically, to a final R = 0.074 (R_w = 0.083). The final Fourier difference map contained no significant features.
(19) Ayrey, G.; Barnard, D.; Woodbridge, D. T. *J. Chem. Soc.* **1962**, 2555.

Scheme II



sulfoxide at −35 °C to room temperature for 18 h.[20] The ^{1}H NMR spectrum exhibited the presence of a new olefinic proton (δ 7.90 and 7.93, pair of singlets due to amide rotamers) and the loss of the C-10 methylene and C-20 methine proton signals. Upon removal of the (trichloroethyl)urethane protecting group with zinc dust in refluxing methanol,[23] spontaneous cyclization occurred and the stable, crystalline dehydrodiketopiperazine **4** was isolated in 94% yield.

The final transformation of the key intermediate **4** was accomplished with a slight excess of osmium tetroxide in pyridine followed by reductive workup with aqueous sodium bisulfite.[7d] The only product observed by TLC or ^{1}H NMR (75% isolated yield) possessed spectral characteristics nearly identical with those of verruculogen TR-2.[2] The olefinic resonance (δ 7.46, s) of **4** was replaced by a doublet (δ 5.2, J = 8.6 Hz) due to splitting by the hydroxyl proton and a new singlet (δ 4.90) for the tertiary hydroxyl proton. The stereochemistry was assigned as that for **2**, based on the expected steric influence of the hydroxyisobutyl side chain and on the close analogy to the spectra of the natural product. Application of these methods to (+)-verruculogen TR-2 (**1**) requires only that 6-methoxy-L-tryptophan be employed as starting material; this will be the subject of a future report.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker WP-200 instrument. IR spectra were recorded in $CHCl_3$ on a Beckman 4250 or Perkin-Elmer 521 instrument. Melting points were obtained on a Thomas-Hoover apparatus and are uncorrected. Mass spectra were recorded on a double-focusing AEI, Model MS-902, which was interfaced to a computer. Optical rotations were obtained on a Perkin-Elmer polarimeter.

Thin-layer chromatography was performed using E. Merck silica gel 60 F-254 0.25-mm plates. Visualization was accomplished with ultraviolet light and iodine. Flash chromatography was carried out on E. Merck 320–400-mesh silica gel.[16] Eluents are reported as either v/v mixtures of volume percent mixtures.

Tetrahydrofuran was distilled from the sodium ketyl of benzophenone. Dichloromethane was distilled from phosphorus pentoxide. Hexamethyldisilazane, diisopropylamine, triethylamine, and hexamethylphosphoramide were distilled from calcium hydride. Dimethyl sulfoxide was fractionally distilled under reduced pressure and dried over activated 4-Å molecular sieves. Thionyl chloride was distilled from linseed oil. All solvents were distilled under dry nitrogen.

L-Tryptophan and L-proline were gifts from Ajinomoto. Butyllithium in hexane was obtained from Aldrich and standardized according to the method of Duhamel and Planquevant.[24] All other commercial reagents were used without further purification. The following compounds were prepared according to literature procedures: L-tryptophan methyl ester hydrochloride;[25] 3-hydroxy-3-methylbutanal;[12] benzeneselenic anhydride.[19]

1-(2-Hydroxy-2-methylpropyl)-3-carbomethoxy-1,2,3,4-tetrahydro-2-carboline (6, 9). L-Tryptophan methyl ester hydrochloride (4.12 g, 16.2 mmol) and 3-hydroxy-3-methylbutanal [8; 2.23 g, 21.8 mmol; freshly distilled; bp 78–80 °C (27 torr] were dissolved in 25 mL of water, and the solution was degassed with of argon for 5 min. The solution was allowed to react at 19 °C for 14 days. The brownish solution was adjusted to pH 10 with saturated aqueous $NaHCO_3$ and extracted with 4 × 50 mL of CH_2Cl_2. The combined organic extracts were washed with 50 mL of brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield 5.79 g of brown solid. The crude product was purified by flash chromatography (5% CH_3OH–CH_2Cl_2), affording 3.48 g (71%) of the mixture of diastereomeric tetrahydro-2-carbolines **6** and **9**, as a yellow waxy solid: R_f 0.38 (5% CH_3OH–$CHCl_3$); ^{1}H NMR δ 1.24 (s), 1.34 (s), 13.7 (s) and 1.39 (s, 6 H total), 1.6–2.2 (m, 3 H), 2.85 (m, 1 H), 3.1 (m, 1 H), 2.6–3.3 (br s, 1 H), 3.73 (s), 3.79 (s) and 3.6–4.0 (m, 4 H total), 4.4 (br m, 1 H), 7.1 (m, 2 H), 7.3 (m, 1 H), 7.44 (m, 1 H), 8.25 (br s) and 9.13 (br s, 1 H total); ^{13}C NMR δ 25.4, 26.0, 28.7, 29.6, 31.1, 31.2, 45.8, 47.1, 48.4, 50.7, 51.2, 52.2, 56.8, 70.7, 107.1, 110.9, 111.1, 117.6, 118.0, 119.3, 119.5, 121.7, 122.0, 126.9, 127.1, 135.0, 135.7, 135.9, 173.6; IR 3493, 3345, 3010, 2980, 1744, 1470, 1445, 1280, 1180 cm^{-1}; high-resolution mass spectral analysis for $C_{17}H_{22}N_2O_3$, calcd 302.1632, found 302.1637.

[[(2,2,2-Trichloroethyl)oxy]carbonyl]-L-proline (11). L-Proline (5.0 g, 43.4 mmol) was placed in a 100-mL three-neck flask equipped with magnetic stirrer and thermometer, dissolved in 21.7 mL of 2.0 N aqueous NaOH, and cooled to −9 °C. 2,2,2-Trichloroethyl chloroformate (6.4 mL, 46.5 mmol) and 30 mL of 2.0 N NaOH were then added dropwise simultaneously to the stirred solution, maintaining the temperature below 0 °C. After addition was complete (1 h), the mixture was stirred an additional 2 h, warming slowly to 15 °C. The mixture was extracted with ether (3 × 40 mL) and the aqueous phase acidified to pH 2 with 10% HCl. The product was extracted with 4 × 50 mL of EtOAc; the combined EtOAc extracts were washed with 30 mL of brine,

(20) Attempts to convert either of the diastereomeric diketopiperazines **15** or **16** into the dehydrodiketopiperazines **4** (or its diastereomer) with either DDQ,[21a] phenylselenyl chloride,[21b] or benzeneseleninic anhydride[19] as oxidant were unsuccessful. Either *N*-hydroxyindole, a complex mixture, or recovered starting material, respectively, was isolated as product. Also, other attempts at effecting the conversion of **13** into **18** through phenylselenation (lithium diisopropylamide or hexamethyl disilazide followed by phenylselenyl chloride or diphenyl diselenide) all failed in a similar manner.

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(24) Duhamel, L.; Planquevant, J. *J. Org. Chem.* **1979**, *44*, 3404.

(25) Peter, J.; Brugger, M.; Schrieber, J.; Eschenmoser, A. *Helv. Chim. Acta* **1963**, *46*, 1577.

dried ($MgSO_4$), and concentrated in vacuo to yield 12.16 g (96%) of a clear oil, which became a low-melting amorphous solid upon storage in the freezer: R_f 0.56 (EtOAc); $[\alpha]_D$ −53.7° (*c* 1.705, $CHCl_3$); ^1H NMR δ 1.8–2.24 (m, 4 H), 3.6 (m, 2 H), 4.44 (m, 1 H), 4.78 (m, 2 H), 9.1 (br s, 1 H); IR 3500–2800, 1724, 1422, 1354, 1135 cm^{-1}.

[[(2,2,2-Trichloroethyl)oxy]carbonyl]-L-prolinyl Chloride (12). Acid **11** (2.79 g, 9.58 mmol) was dissolved in 5 mL of thionyl chloride and the resultant solution warmed to reflux for 3 h. Excess thionyl chloride was removed by distillation and the yellow residue purified by Kugelrohr distillation [160 °C (0.014 torr)], yielding 2.79 g (94%) of clear oil: $[\alpha]_D$ −43.1° (*c* 1.915, $CHCl_3$); ^1H NMR δ 2.05 (m, 2 H), 2.36 (m, 2 H), 3.67 (m, 2 H), 4.71 (m), 4.74 (m) and 4.78 (s, 3 H total); IR 3060, 2990, 2960, 2895, 1795, 1730, 1415, 1350, 1138, 1070, 975, 965, 765, 720 cm^{-1}.

Hydroxy Prolinamides 13 and 14. Tetrahydro-2-carbolines **6** and **9** (1.98 g, 6.53 mmol) were dissolved in 7 mL of dry CH_2Cl_2 and 2 mL of dry Et_3N, and the solution was cooled (0 °C) under dry nitrogen. A solution of acid chloride **12** (2.23 g, 7.22 mmol) in 5 mL of dichloromethane was added slowly dropwise over 30 min. The resulting mixture was allowed to slowly warm to room temperature and stir an additional 21 h. The mixture was diluted with 20 mL of CH_2Cl_2 and extracted sequentially with 20 mL of water, 2 × 20 mL of 0.1 N HCl, 2 × 20 mL of saturated $NaHCO_3$, 20 mL of water, and 20 mL of brine; then, the organic layer was dried ($MgSO_4$), filtered, and concentrated to give 3.47 g of the crude amide as a yellow foamy solid. Flash chromatography (EtOAc–hexanes, 3:2) afforded 1.42 g of the less polar isomer (**13**) and 1.17 g of the more polar isomer (**14**) (69% total).

13: mp 197–197.5 °C; R_f 0.41 (ethyl acetate–hexanes, 3:2); $[\alpha]_D$ +10.4° (*c* 1.59, $CHCl_3$); ^1H NMR δ 1.35 (s), 1.60 (s), 1.62 (s) and 1.65 (s, 6 H total), 1.9–2.5 (several m, 7 H), 3.05 (dd, 1 H), 3.5–3.9 (m), 3.59 (s) and 3.66 (s, 6 H total), 4.65–5.0 (m, 4 H), 5.68 (overlapping dd, 1 H), 7.1 (m, 2 H), 7.3 (m, 1 H), 7.5 (m, 1 H), 10.0 (s) and 10.06 (s, 1 H total); IR 3610, 3350, 3010, 2980, 1720, 1660, 1630, 1600, 1415, 1340, 1180, 1130 cm^{-1}. Anal. Calcd for $C_{25}H_{30}N_3O_6Cl_3$: C, 52.23; H, 5.26; N, 7.31. Found: C, 52.15; H, 5.25; N, 6.97.

14: mp 146–148 °C; R_f 0.30 (EtOAc–hexanes, 3:2); $[\alpha]_D$ −47.2° (*c* 1.11, $CHCl_3$); ^1H NMR δ 1.40 (s), 1.42 (s), 1.56 (s) and 1.65 (s, 6 H total), 1.7–2.5 (br m, 7 H), 3.0–3.85 (br m), 3.65 (s), 3.66 (s), 3.72 (s) and 3.75 (s, 7 H total), 4.4–5.8 (several m, 5 H), 7.15 (m, 2 H), 7.35 (m, 1 H), 7.50 (m, 1 H), 8.6 (s), 9.95 (s) and 10.0 (s, 1 H total); IR 3475, 3410, 3005, 2970, 1740, 1724, 1670, 1420, 1355, 1340, 1137 cm^{-1}. Anal. Calcd for $C_{25}H_{30}N_3O_6Cl_3$: C, 52.23; H, 5.26; N, 7.31. Found: C, 52.40; H, 5.54; N, 6.98.

***cis*-Diketopiperazine 15.** Prolinamide **13** (230 mg, 0.40 mmol) was dissolved in 20 mL of THF and 4 mL of 1.0 M aqueous NH_4OAc, then zinc dust (1.0 g) was adjusted, and the slurry was stirred (under nitrogen atmosphere) at room temperature for 24 h. The resulting mixture was filtered, washing the white solid with 4 × 5 mL of THF. The combined filtrate and washings were concentrated under reduced pressure, and the residue was partitioned between 40 mL of EtOAc and 10 mL of 0.1 N aqueous HCl. The organic layer was washed sequentially with 10 mL of 0.1 N HCl, 10 mL of water, 2 × 10 mL of saturated aqueous $NaHCO_3$, 10 mL of water, and 10 mL of brine, dried ($MgSO_4$), and filtered; the filtrate was concentrated under reduced pressure and the residue placed under high vacuum to yield 140 mg of the crude diketopiperazine. Recrystallization from ether–hexanes yielded 101 mg (69%) of pure **15** as white needles: mp 213–214 °C; R_f 0.29 (5% CH_3OH–$CHCl_3$); $[\alpha]_D$ −81.6° (*c* 0.29, $CHCl_3$); ^1H NMR δ 1.12 (s, 3 H), 1.30 (s, 3 H), 1.75–2.1 (m, 4 H), 2.25 (m, 2 H), 3.07 (dd, 1 H), 3.4–3.65 (m, 3 H), 3.85–4.0 (m, 2 H), 4.08 (s, 1 H), 5.65 (overlapping dd, 1 H), 7.05 (m, 2 H), 7.3 (m, 1 H), 7.55 (m, 1 H), 9.69 (s, 1 H); ^{13}C NMR δ 21.3, 23.3, 28.2, 29.7, 31.3, 45.4, 49.7, 50.5, 57.4, 59.3, 70.3, 106.3, 111.5, 118.1, 119.8, 121.9, 126.2, 135.3, 135.9, 165.9, 170.2; IR 3440, 3000, 2960, 2875, 1660, 1620, 1450, 1404, 1330, 1295, 1160, 1140, 1000 cm^{-1}; high-resolution mass spectral analysis for $C_{21}H_{25}N_3O_3$, calcd 367.1898, found 367.1888.

***trans*-Diketopiperazine 16.** Prolinamide **14** (203 mg, 0.353 mmol) was dissolved in 15 mL of THF and 3 mL of 1.0 M NH_4OAc and zinc dust (1.84 g) added. The resulting mixture was stirred under a nitrogen atmosphere for 3 days at room temperature. The zinc was removed by filtration, washing the solid with 4 × 5 mL of THF. The combined filtrate and washings were concentrated under reduced pressure to an aqueous residue, and the product was extracted with 2 × 20 mL of EtOAc. The organic extracts were combined, washed sequentially with 2 × 10 mL of 0.1 N HCl, 2 × 10 mL of saturated $NaHCO_3$, 10 mL of water, and 10 mL of brine, and dried ($MgSO_4$); the filtrate was concentrated to afford 156 mg of crude diketopiperazine. Recrystallization from ether–hexanes yielded 85 mg (66%) of pure **16** as colorless rods: mp 219–222 °C dec; R_f 0.24 (5% CH_3OH–$CHCl_3$); $[\alpha]_D$ −230.0° (*c* 0.39, $CHCl_3$); ^1H NMR δ 1.34 (s, 3 H), 1.51 (s, 3 H), 1.8–2.1 (m, 4 H), 2.17 (dd, 1 H), 2.5 (m, 1 H), 2.85 (dd, 1 H), 3.44 (m, 1 H), 3.55 (dd, 1 H), 3.9 (m, 1 H), 4.1 (m, 1 H), 4.4 (dd, 1 H), 6.0 (dd, 1 H), 7.1 (m, 2 H), 7.3 (m, 1 H), 7.5 (m, 1 H), 9.36 (s, 1 H); ^{13}C NMR δ 21.5, 28.6, 29.1, 30.1, 31.9, 45.0, 47.2, 48.4, 54.8, 59.5, 405.8, 111.1, 118.2, 119.5, 121.9, 126.4, 133.1, 136.1, 164.4, 164.8; IR 3380, 3010, 2890, 1660, 1457, 1340, 1310, 1170 cm^{-1}. Anal. Calcd for $C_{21}H_{25}N_3O_3$: C, 68.64; H, 6.86; N, 11.44. Found: C, 68.35; H, 7.18; N, 11.39.

Unsaturated Ester 18. Potassium hydride (78 mg, 1.89 mmol, dry weight after washing oil dispersion with 5 × 3 mL of dry THF and removing volatiles under vacuum) was suspended in 3 mL of dry THF and cooled to −3 °C in an ice–salt bath (under dry argon atmosphere); then, a solution of the indole ester **13** [428 mg, 0.744 mmol; dried at 76 °C (0.008 torr) over P_2O_5 for 3 days] in 3 mL of THF was added, causing vigorous gas evolution. Stirring was continued at 0 °C for 1 h, and then a solution of benzeneseleninic anhydride [285 mg, 0.787 mmol, dried at 76 °C (0.005 torr) over P_2O_5 for 24 h] in 1.5 mL of Me_2SO was added, producing a yellow suspension. This mixture was allowed to warm slowly to room temperature and stir overnight. To ensure complete reaction, the mixture was warmed to 60 °C for 4 h, and then the reaction was quenched with 5 mL of water. The mixture was concentrated under reduced pressure to an aqueous suspension, which was extracted with 30 mL of EtOAc. The organic phase was washed sequentially with 2 × 20 mL of 0.1 N HCl, 3 × 20 mL of saturated $NaHCO_3$, 20 mL of water, and 20 mL of brine and dried ($MgSO_4$); the filtrate was concentrated to yield 464 mg of crude product. Flash chromatography (EtOAc–hexanes, 1:1) afforded 151 mg (35%) of pure **18** as a yellowish foamy solid: mp 134–137 °C; $[\alpha]_D$ −17.9° (*c* 0.355, $CHCl_3$); ^1H NMR δ 1.25 (m), 1.5 (m) and 1.55–2.5 (br m, 13 H total), 3.6 (m, 2 H), 3.91 (s) and 3.94 (s, 3 H total), 4.65 (m, 2 H), 4.9 (m, 1 H), 6.45 (m, 1 H), 7.25 (m, 2 H), 7.45 (m, 1 H), 7.65 (m, 1 H), 7.91 (s) and 7.93 (s, 1 H total), 10.45 (br s) and 10.53 (br s, 1 H total); ^{13}C NMR δ 23.0, 23.7, 28.9, 29.1, 29.8, 31.2, 31.5, 43.5, 43.8, 46.8, 47.3, 49.0, 49.2, 52.3, 59.9, 60.3, 70.6, 70.7, 74.8, 75.0, 95.7, 107.5, 112.4, 117.3, 117.5, 118.2, 121.5, 123.0, 124.2, 126.6, 136.5, 136.9, 142.6, 143.0, 152.7, 153.2, 164.6, 164.9, 173.9, 174.7; IR 3430, 3280, 3020, 2970, 1717, 1660, 1600, 1567, 1526, 1488, 1450, 1431, 1410, 1352, 1245, 1163, 1125, 1086, 1043, 970, 820, 700 cm^{-1}; high-resolution spectral analysis for $C_{25}H_{28}N_3O_6Cl_3$, calcd 571.1046, found 571.1007.

Dehydrodiketopiperazine 4. Unsaturated ester **27** (85 mg, 0.149 mmol) was dissolved in 10 mL of methanol, and zinc dust (35 mg) was added. The mixture was stirred at reflux (under nitrogen atmosphere) for 24 h. The reaction mixture was filtered, washing the solids with 20 mL of methanol, and the combined filtrate and washings were concentrated under reduced pressure to a yellow solid. The crude diketopiperazine was purified by flash chromatography (3% CH_3OH–$CHCl_3$) to yield 51 mg (94%) of pure **4** as a yellow–green solid, which could be recrystallized from CH_2Cl_2–ether to afford chartreause needles: mp 223 °C dec; R_f 0.36 (5% CH_3OH–$CHCl_3$); $[\alpha]_D$ +150° (*c* 0.705, $CHCl_3$); ^1H NMR δ 1.17 (s, 3 H), 1.39 (s, 3 H), 1.9–2.2 (br m, 6 H), 2.4 (br m, 1 H), 2.95 (br s, 1 H), 3.7 (m, 2 H), 4.14 (m, 1 H), 6.25 (overlapping dd, 1 H), 7.2 (m, 2 H), 7.4 (m, 1 H), 7.46 (s, 1 H), 7.7 (m, 1 H), 9.85 (br s, 1 H); ^{13}C NMR δ 22.1, 28.8, 29.5, 29.7, 31.7, 45.0, 48.2, 58.9, 70.2, 106.5, 111.8, 112.3, 118.4, 121.2, 122.1, 122.7, 128.3, 136.5, 159.9, 166.8 (two low-field carbons coincident); IR 3390, 3010 2960, 2870, 1650, 1605, 1567, 1540, 1430, 1395, 1375, 1235, 1156, 1130, 1070, 1005, 929, 800 cm^{-1}; high-resolution mass spectral analysis for $C_{21}H_{23}N_3O_3$, calcd 365.1741, found 365.1756.

Desmethoxy-TR-2 (2). Dehydrodiketopiperazine **4** (5.5 mg, 0.015 mmol) was dissolved in 0.5 mL of dry pyridine, cooled to 0 °C (under an argon atmosphere), and then treated with a solution of osmium tetroxide (50 μL, 0.39 M in pyridine, 0.019 mmol). The orange solution was stirred at 0 °C for 2 h and then was treated with 0.5 mL of saturated aqueous $NaHSO_3$, and the

mixture was allowed to react at 25 °C for 30 min, at which time an orange aqueous layer separated. The mixture was extracted with 2 × 5 mL of $CHCl_3$, the combined organic extracts were washed with 5 mL of water and 10 mL of brine, dried ($MgSO_4$), and filtered, the filtrate was concentrated in vacuo to yield 4.5 mg (75%) of essentially pure **2**. The crude material could be recrystallized from CH_2Cl_2–cyclohexane to afford white feathery crystals: mp 180–182 °C; R_f 0.29 (5% CH_3OH–$CHCl_3$); $[\alpha]_D$ +116° (*c* 0.73, $CHCl_3$); ^1H NMR δ 1.34 (s, 3 H), 1.52 (s, 3 H), 1.7–2.25 (br m, 5 H), 2.3–2.6 (m, 2 H), 3.09 (d, J = 8.6 Hz, 1 H), 3.6 (m, 1 H), 3.8 (m, 1 H), 4.2 (overlapping dd, 1 H), 4.92 (s, 3 H), 5.2 (d, J = 8.6 Hz, 1 H), 5.95 (overlapping dd, 1 H), 7.1 (m, 2 H), 7.3 (m, 1 H), 7.8 (m, 1 H), 9.4 (br s, 1 H); ^{13}C NMR δ 22.2, 28.7, 29.8, 32.5, 46.2, 47.7, 49.0, 60.0, 69.4, 71.5, 83.5, 106.1, 111.3, 119.9 (2C), 122.0, 126.3, 135.2, 136.0, 165.4, 165.8; IR 3380, 3010, 2965, 2935, 1660, 1450, 1380, 1226, 1150 cm^{-1}; high-resolution mass spectral analysis for $C_{21}H_{25}N_3O_5$, calcd 399.1796, found 399.1808.

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Synthesis of Nucleotide 5′-Diphosphates from 5′-*O*-Tosyl Nucleosides

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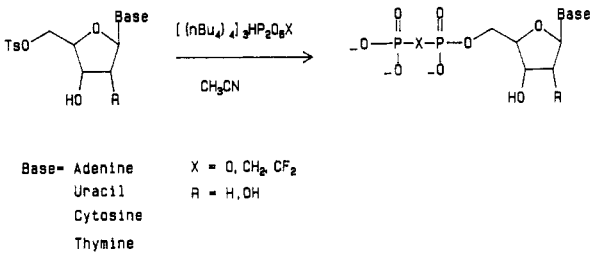
Procedures are described for the synthesis of nucleoside 5′-diphosphates, methanediphosphonates, and difluoromethanediphosphonates. The general strategy involves protection of the nucleosides as amidine, 2′,3′-methoxymethylidene, and 3′-(*tert*-butyldimethylsilyl) derivatives prior to tosylation with tosyl chloride and (*N*,*N*-dimethylamino)pyridine. Deprotection, followed by displacement of the tosyl moiety with the tris(tetra-*n*-butylammonium) pyrophosphate, methanediphosphonate, or difluoromethanediphosphonate salts gave the desired products. The ammonium salts of the nucleotides were purified by flash chromatography on cellulose or medium pressure ion-exchange chromatography on DEAE Fractogel. Syntheses are reported for UDP (**18**), CDP (**19**), TDP (**20**), GDP (**21**), ADP (**23**), 2′,3′-isopropylidene-ADP (**22**), adenosine 5′-methanediphosphonate (**24**), adenosine 5′-difluoromethanediphosphonate (**25**), and deoxyadenosine 5′-difluoromethanediphosphonate (**27**). In addition, ATP (**26**) was prepared by treatment of 5′-*O*-tosyladenosine with tetrakis(tetra-*n*-butylammonium) triphosphate. Yields for the displacement reactions ranged from 43% to 93%.

Nucleoside 5′-diphosphates are central compounds in numerous biochemical and pharmacological studies. As a result, they and their various analogues have been the targets of numerous synthetic efforts over the past 3 decades. This work has produced a large repertoire of synthetic organic and biochemical methods for the phosphorylation of nucleosides.[1] In general, nucleoside phosphorylation is achieved by nucleophilic addition of the 5′-ribosyl hydroxyl to an activated phosphate derivative. The P–O–P linkage is then generated by a variety of activation and displacement sequences at phosphorus. Although phosphorylation with electrophilic phosphorus derivatives serves in many applications, a transformation that introduces phosphorus as a nucleophile in a single step is an important alternative with numerous applications. There are few phosphorylation strategies that rely upon nucleophilic displacement at carbon for establishment of the C–O–P linkage. Reported cases include the synthesis of methanediphosphonate nucleotides and their analogues by displacements on 5′-halogen or sulfonate ester derivatives[2-5] and polymerization of O^2,5′-cyclonucleosides.[6]

Scheme I



We recently developed a single-step diphosphorylation procedure for the synthesis of various isoprenoid natural products and their analogues.[7-9] An adaptation of this approach to the phosphorylation of nucleosides is now presented.[10] The procedure utilizes a nucleophilic displacement of 5′-*O*-tosyl nucleosides by the tris(tetra-*n*-butylammonium) form of pyrophosphoric acid at room temperature as outlined in Scheme I. The resulting diphosphates are purified by a simple absorption chroma-

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