Supporting Information

Synthesis of $p$-Aminophenyl Aryl H-Phosphinic Acids and Esters via Cross-Coupling Reactions: Elaboration to Phosphinic Acid Pseudopeptide Analogs of Pteroyl Glutamic Acid and Related Antifolates

Yonghong Yang and James K. Coward*

Departments of Medicinal Chemistry and Chemistry, University of Michigan, Ann Arbor, Michigan, 48109-1055

jkcoward@umich.edu

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**General Procedures.** All reactions involving moisture-sensitive reagents were conducted in oven-dried glassware under an inert atmosphere of nitrogen or argon. All solvents used in moisture-sensitive reactions were treated as follows: Tetrahydrofuran was freshly distilled from benzophenone ketyl. Methylene chloride, acetonitrile, and pyridine were freshly distilled from calcium hydride (CaH₂). Triethylamine was distilled from sodium. Acetic acid was distilled from acetic anhydride and CrO₃. Thin-layer chromatography was performed with aluminum-backed silica gel 60-F254 plates. Column chromatography was carried out using silica gel 60 (230–400 mesh) as the stationary phase. All reagents were purchased from chemical suppliers and used without further purification except 4-iodoaniline, which was purified by a silica gel chromatography using a mixture of 20% ethyl acetate in hexanes as an eluant. Using a CEM Discovery reactor, microwave heating was carried out in a closed reaction vessel with magnetic stirring. Temperature control was by IR detection. Melting points were taken on a Thomas-Hoover Mel-Temp apparatus and are uncorrected. ¹H NMR, ¹³C NMR and ³¹P NMR spectra were recorded on a Bruker AVANCE DPX300 300-MHz or DRX 500 500-MHz spectrometers. CDCl₃ was used as the solvent unless otherwise indicated. ¹H NMR spectra were recorded at 300 MHz or 500 MHz, and are reported as follows: chemical shifts in ppm downfield from internal tetramethylsilane reference (δ 0.00) with integrated intensity, multiplicity, coupling constant in Hz (¹H NMR spectra of compounds (1, 10) purified by preparative HPLC contain signals due to the NH₄OAc buffer used as a component of the mobile phase. These peaks are noted by an asterisk (*) on the corresponding attached spectra). ¹³C NMR spectra were obtained at 75 MHz or 126 MHz and referenced to CDCl₃ (δ 77.0). The ¹³C NMR spectra of 4eEt and 10c
include one peak marked by ‘x’ due to an impurity. $^{31}$P spectra were recorded at 121 MHz with 1% aqueous H$_3$PO$_4$ as an external reference. All $^{13}$C and $^{31}$P NMR spectra were proton-decoupled. Mass spectra were recorded on a VG Analytical system, Model 70-250S. UV spectra were recorded on a Beckman DU® 640B spectrophotometer.

Analytical HPLC method: flow rate, 0.7 mL/min; eluant A, ammonium acetate buffer (20 mM, pH 6.2); eluant B, acetonitrile; gradient, 0 min, 2% B, 40 min, 80% B, 50 min, 80% B; column, Chrompack Kromasil 100 C18 (250 × 4.6 mm). Detection was by UV/vis, $\lambda$ = 254 nm. Preparative HPLC method: flow rate, 15.0 mL/min; eluant A, ammonium acetate buffer (20 mM, pH 6.2); eluant B, acetonitrile; gradient, 0 min, 2% B, 40 min, 80% B, 50 min, 80% B; column, Varian Dynamax 60 C18 (250 × 21.4 mm). Detection was by UV/vis, $\lambda$ = 254 nm. Experimental details on the synthesis of Benzyl 4-iodophenylcarbamate (2b) under base-free condition are given below, since such details are not available in the literature. tert-Butyl 4-iodophenylcarbamate (2c) was prepared according to the literature$^1$ with minor modification. Anilinium hypophosphite (3) was prepared according to the literature.$^2$ 2-Methyleneglutarate esters 5a and 5b were prepared as described previously$^3$ with minor modifications to give much better yields.$^4$

The heterocyclic alkylating agents, 2-amino-6-(bromomethyl)-4(1H)-pteridinone hydrobromide (8a)$^5$ and 6-(bromomethyl)-2,4-pteridinediamine hydrobromide (8b)$^6-8$, as well as the heterocyclic aldehydes, 2-pivaloyl-6-formylpterin (9a)$^9-12$, 2-pivaloyl-5-deaza-6-formylpterin (9b)$^{13-15}$ and 2-actyl-5-deaza-6-formylpterin (9c)$^{16}$ were obtained by following literature procedures.
**Caution:** Tetramethyl orthosilicate (Si(OCH₃)₄ is very toxic and could cause blindness. Therefore, the less volatile tetraethyl orthosilicate (Si(OCH₂CH₃)₄ was used to optimize reaction conditions in this research (Eq. 1, Scheme 4).

**Benzyl 4-bromophenyl(methyl)carbamate (2a).** To a round-bottom flask containing 4-bromoaniline (2.53 g, 14.7 mmol) and K₂CO₃ (2.24 g, 16.2 mmol), were added CHCl₃ (45 mL) and H₂O (0.25 mL), and then the flask was allowed to cool to 0 °C. CbzCl (2.30 mL, 16.1 mmol) was added dropwise to the cold solution with vigorous stirring over a period of 10 min. The reaction mixture thickened to slurry. After an additional 5 h, the reaction was checked with TLC (20% ethyl acetate in hexanes), and TLC indicated the 4-bromoaniline was completely consumed. The reaction was quenched by the addition of ice-water (50 mL), followed by a saturated solution of NaHCO₃ (20 mL). The layers were separated, and the aqueous layer was back-extracted with ethyl acetate with 2% of methanol (4 × 100 mL). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The product was purified by crystallization from CHCl₃/Hexanes to afford benzyl 4-bromophenylcarbamate (3.27 g, 10.7 mmol, 73%) as white crystals: mp 118–120 °C; ¹H NMR δ 7.40–7.11 (9 H, m), 6.76 (1 H, s), 5.18 (2 H, s), which was used for the next step methylation reaction. To an oven-dried round-bottom flask was added NaH (0.246 g, 8.2 mmol) as a 80% dispersion in mineral oil, followed by THF (10 mL) via syringe, and then the flask was allowed to cool to 0 °C under a N₂ atmosphere. A solution of benzyl 4-bromophenylcarbamate (1.80 g, 5.88 mmol) in THF (40 mL) was added into the flask dropwise over a period of 15 min, followed by iodomethane (0.51 mL, 8.2 mmol) over an additional period of 10 min. The reaction mixture was allowed to warm to room temperature. After an additional 5 h, the
starting material disappeared as indicated by TLC (20% ethyl acetate in hexanes). The reaction was quenched by adding water (40 mL), followed by a saturated solution of NaHCO₃ (50 mL). The reaction mixture was extracted from ethyl acetate (3 × 100 mL). The combined organic layers were dried over Na₂SO₄ and filtered. The solvent was removed in vacuo to give the crude product. The crude product was purified by flash column chromatography (20% ethyl acetate in hexanes) to afford 2a as a light yellow oil (1.73 g, 5.40 mmol, 92%): ¹H NMR δ 7.44–7.41 (2 H, m), 7.36–7.24 (5 H, m), 7.14–7.08 (2 H, m), 5.19 (2 H, s), 3.27 (3 H, s); ¹³C NMR δ 155.0, 142.1, 136.2, 131.7, 128.3, 127.9, 127.7, 127.1, 119.1, 67.3, 37.4.

**Benzyl 4-iodophenylcarbamate (2b).** To an oven-dried flask containing 4-iodoaniline (1.23 g, 5.62 mmol) in THF (20 mL), was added CbzCl (0.96 mL, 6.74 mmol) dropwise over a period of 10 min at 0 °C. After an additional 20 h at room temperature, the reaction was worked up by adding a saturated solution of NaHCO₃ (50 mL), followed by ethyl acetate (100 mL). The organic layer was separated. The aqueous layer was back-extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude product as a white solid was washed hexanes (3 × 5 mL) and collected by filtration to afford 2b (1.96 g, 5.55 mmol, 99%) as a white solid: mp 132–134 °C; ¹H NMR δ 7.61–7.16 (9 H, m), 6.63 (1 H, s), 5.20 (2 H, s); ¹³C NMR δ 153.1, 137.9, 137.6, 135.7, 128.6, 128.4, 128.3, 120.5, 86.4, 67.2.

**(4-(N-Benzylloxycarbonyl-amino)-phenyl)phosphinic Acid (4b).** The same procedure was repeated as described for 4a except that a mixture of 2b (776 mg, 2.20 mmol), anilinium hypophosphite (3) (815 mg, 5.47 mmol), and Pd(PPh₃)₄ (80 mg, 70 µmol),
Et₃N (1 mL) in CH₃CN (12 mL) was heated under reflux for 5 h and the reaction mixture was extracted with CH₂Cl₂ to afford 4b as a brown oil (576 mg, 90% (³¹P NMR yield)):

¹H NMR δ 7.87–7.65 (2 H, m), 7.48 (1 H, d, Jₚₜ = 616 Hz), 7.40–7.02 (7 H, m), 6.88 (1 H, br s), 5.22 (2 H, s); ³¹P NMR δ 21.8 (t, J = 111.5 Hz); MS (ESI) m/z 290.0 ([M – H]⁻, 100).

(4-(N-tert-Butoxycarbonyl-amino)-phenyl)phosphinic Acid (4c). The same procedure was repeated as described for 4a except that a mixture of 2c (591 mg, 1.85 mmol), anilinium hypophosphite (3) (605 mg, 4.08 mmol), Pd(PPh₃)₄ (67 mg, 58 µmol), and Et₃N (0.8 mL) in CH₃CN (11 mL) was heated under reflux for 24 h to afford 4c as a brown oil (452 mg, 95% (³¹P NMR yield)): ¹H NMR δ 10.91 (1 H, br s), 7.86–7.24 (4 H, m), 7.55 (1 H, d, Jₚₜ = 613 Hz), 6.98 (1 H, br s), 1.50 (9 H, s); ³¹P NMR δ 21.0 (t, J = 157.9 Hz); MS (ESI) m/z 256.0 ([M – H]⁻, 100).

(4-(N-Benzylxocarbonyl-amino)-phenyl)phosphinic ethyl ester (4dEt, Table S1, entry 1 precursor). The same procedure was repeated as described for 4d except that a mixture of 2b (603 mg, 1.71 mmol), anilinium hypophosphite (3) (838 mg, 5.27 mmol), DABCO (589 mg, 5.25 mmol), tetraethyl orthosilicate (1.14 mL, 5.14 mmol), dppp (60 mg, 0.145 mmol), and Pd(OAc)₂ (30 mg, 0.134 mmol) in CH₃CN (20 mL) was heated under reflux for 3 h to afford 4dEt (400 mg, 1.25 mmol, 73%) as a white solid: mp 138–140 °C; ¹H NMR δ 7.72–7.67 (2 H, m), 7.61–7.58 (3 H, m), 7.53 (1 H, d, Jₚₜ = 564 Hz), 7.39–7.32 (5 H, m), 5.20 (2 H, s), 4.16–4.04 (2 H, m), 1.34 (3 H, t, J = 7.0 Hz); ¹³C NMR δ 153.0, 142.7 (d, ⁴JCP = 3.0 Hz), 135.7, 132.2 (d, ²JCP = 12.8 Hz), 128.6, 128.4, 128.3, 123.5 (d, ¹JCP = 137.1 Hz), 118.1 (d, ³JCP = 14.3 Hz), 67.3, 61.9 (d, ²JPOC = 6.1 Hz), 16.3 (d, ³JPOC ...
= 6.3 Hz); 31P NMR δ 25.6; MS (ESI) m/z 320.1 ([M + H]+, 100); ESI-HRMS (m/z) calcd for C16H19NO4P [M + H]+ 320.1052, found 320.1045.

If Pd(PPh3)4 was used as catalyst, the diarylphosphinic acid, 11a, was isolated as a white solid: 1H NMR δ 7.78–7.63 (4 H, m), 7.48–7.46 (4 H, m), 7.41–7.26 (10 H, m), 7.18 (2 H, br s), 5.18 (4 H, s), 4.03 (2 H, p, J = 7.2 Hz), 1.31 (3 H, t, J = 7.0 Hz); 13C NMR δ 153.0, 141.5, 135.7, 132.8 (d, JCP = 11.2 Hz), 128.6, 128.4, 128.3, 125.8 (d, JCp = 143.0 Hz), 118.0 (d, JCp = 13.3 Hz), 67.2, 61.0 (d, JFOC = 5.7 Hz), 16.4 (d, JCp = 6.6 Hz); 31P NMR δ 32.6; MS (ESI) m/z 545.2 ([M + H]+, 100).

(4-(N-tert-Butoxycarbonyl-amino)-phenyl)phosphinic methyl ester (4e). The same procedure was repeated as described for 4d except that a mixture of 2c (201 mg, 0.630 mmol), anilinium hypophosphite (3) (306 mg, 1.92 mmol), DABCO (215 mg, 1.92 mmol), tetramethyl orthosilicate (0.28 mL, 1.88 mmol), dppp (20 mg, 48 µmol), and Pd(OAc)2 (7 mg, 31 µmol) in CH3CN (10 mL) was heated under reflux for 3 h to afford 4e (130 mg, 0.479 mmol, 76%) as a white solid: mp 138–140 °C; 1H NMR δ 7.70–7.58 (5 H, m), 7.52 (1 H, d, JHP = 566.6 Hz), 3.75 (3 H, d, J = 12.0 Hz), 1.51 (9 H, s); 13C NMR δ 152.3, 143.4, 132.0 (d, JCP = 12.7 Hz), 121.8 (d, JCp = 137.8 Hz), 117.8 (d, JCp = 14.3 Hz), 80.8, 51.7 (d, JFOC = 6.5 Hz), 28.0; 31P NMR δ 27.2; MS (ESI) m/z 272.1 ([M + H]+, 100); ESI-HRMS (m/z) calcd for C12H19NO4P [M + H]+ 272.1052, found 272.1049.

(4-(N-tert-Butoxycarbonyl-amino)-phenyl)phosphinic ethyl ester (4eEt, Table S1, entries 2 and 3 precursor). The same procedure was repeated as described for 4d except that a mixture of 2c (205 mg, 0.642 mmol), anilinium hypophosphite (3) (307 mg, 1.93 mmol), DABCO (216 mg, 1.93 mmol), tetraethyl orthosilicate (0.43 mL, 1.94 mmol),
dppp (30 mg, 73 µmol), and Pd(OAc)$_2$ (10 mg, 45 µmol) in CH$_3$CN (10 mL) was heated under reflux for 3 h to afford 4e$_{Et}$ (150 mg, 0.53 mmol, 82%) as a white solid: mp 114–116 °C; $^1$H NMR δ 7.73–7.70 (2 H, m), 7.54–7.52 (2 H, m), 7.56 (1 H, d, $J_{HP}$ = 563 Hz), 6.79 (1 H, br s), 4.16–4.10 (2 H, m), 1.53 (9 H, s), 1.37 (3 H, t, $J$ = 7.0 Hz); $^{13}$C NMR δ 152.3, 143.1, 132.2 (d, $^2J_{CP}$ = 12.7 Hz), 123.0 (d, $^1J_{CP}$ = 137.4 Hz), 117.9 (d, $^3J_{CP}$ = 14.2 Hz), 81.2, 61.7 (d, $^2J_{POC}$ = 6.3 Hz), 28.2, 16.3 (d, $^3J_{POC}$ = 6.5 Hz); $^{31}$P NMR δ 24.5; MS (ESI) m/z 286.1 ([M + H]$^+$, 100); ESI-HRMS (m/z) calcd for C$_{13}$H$_{21}$NO$_4$P [M + H]$^+$ 286.1208, found 286.1210. Note: The sample for the NMR spectra contains contaminant of Ph$_3$P=0, which can’t be remove completely.

If Pd(PPh$_3$)$_4$ was used as catalyst, the diarylphosphinic acid, 11b, was isolated as a white solid: $^1$H NMR δ 7.69 (4 H, t, $J$ = 10.0 Hz), 7.46 (4 H, d, $J$ = 6.9 Hz), 7.02 (2 H, s), 4.05 (2 H, p, $J$ = 7.1 Hz), 1.50 (18 H, s), 1.33 (3 H, t, $J$ = 7.0 Hz); $^{13}$C NMR δ 152.3, 141.9, 132.8 (d, $^2J_{CP}$ = 11.1 Hz), 125.4 (d, $^1J_{CP}$ = 143.0 Hz), 117.8 (d, $^3J_{CP}$ = 13.5 Hz), 81.1, 60.9 (d, $^2J_{POC}$ = 5.7 Hz), 28.3, 16.5 (d, $^3J_{POC}$ = 6.6 Hz); $^{31}$P NMR δ 32.8; MS (ESI) m/z 477.2 ([M + H]$^+$, 100).

2-[[((Phenyl)(methoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid dimethyl ester (Table 1, entry 1). The same procedures were repeated as described for 6a except that a mixture of phenylphosphinic acid (69 mg, 0.486 mmol), dimethyl 2-methylene glutarate 5a (174 mg, 1.01 mmol), and BSA (0.60 mL, 2.42 mmol) in anhydrous CH$_2$Cl$_2$ (1 mL) was stirred at room temperature for 24 h to afford of crude product (226 mg) as a light brown oil: $^{31}$P NMR δ 44.3. It was used for next step reaction without further purification. The crude product (226 mg) in MeOH (5 mL) was treated with a large excess of a 2 M solution of TMS-diazomethane in diethyl ether to afford the title
compound (123 mg, 0.375 mmol, 77% (2 steps)) as a colorless oil: $^1$H NMR δ 7.77 (2 H, m), 7.58 (1 H, m), 7.51 (2 H, m), 3.78–3.49 (9 H, s), 2.87–2.82 (1 H, m), 2.48–2.31 (3 H, m), 2.10–1.96 (3 H, m); $^{13}$C NMR δ 174.1 (dd, $^3J_{CP} = 20.5$, 7.3 Hz), 172.6 (d, $^5J_{CP} = 5.8$ Hz), 132.4, 131.5 (dd, $^2J_{CP} = 15.2$, 10.1 Hz), 129.4 (dd, $^1J_{CP} = 125.0$, 43.5 Hz), 128.5 (dd, $^3J_{CP} = 11.1$, 10.3 Hz), 51.7 (d, $^2J_{POC} = 16.6$ Hz), 51.4, 51.0 (d, $^5J_{POC} = 5.9$ Hz), 38.2, 31.4 (dd, $^1J_{CP} = 101.1$, 33.3 Hz), 31.0, 28.4 (d, $^2J_{CP} = 11.3$ Hz); $^{31}$P NMR δ 44.7, 44.3; MS (ESI) m/z 351.1 ([M + Na]$^+$, 100).

2-[(Phenyl)(methoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid di-tert-butyl ester (Table 1, entry 2). The same procedures were repeated as described for 6a except that a mixture of phenylphosphinic acid (38 mg, 0.267 mmol), di-tert-butyl 2-methyleneglutarate 5b (125 mg, 0.488 mmol), and BSA (0.40 mL, 1.61 mmol) in anhydrous CH$_2$Cl$_2$ (4 mL) was heated under reflux for 3 d to afford of crude product (130 mg) as a colorless oil: $^{31}$P NMR δ 44.3. It was used for next step reaction without further purification. The crude product (130 mg) in MeOH (5 mL) was treated with a large excess of a 2 M solution of TMS-diazomethane in diethyl ether to afford the title compound (64 mg, 0.155 mmol, 58% (2 steps)) as a colorless oil: $^1$H NMR δ 7.80–7.77 (2 H, m), 7.58–7.55 (1 H, m), 7.51–7.48 (2 H, m), 3.63–3.59 (3 H, m), 2.77–2.57 (1 H, m), 2.48–2.32 (1 H, m), 2.25–2.13 (2 H, m); 1.99–1.80 (3 H, m), 1.45–1.37 (18 H, m); $^{13}$C NMR δ 173.2 (dd, $^3J_{CP} = 19.6$, 6.8 Hz), 171.9 (d, $^5J_{CP} = 8.0$ Hz), 132.5, 131.8 (dd, $^2J_{CP}$ = 11.6, 10.2 Hz), 130.0 (dd, $^1J_{CP} = 124.6$, 43.2 Hz), 128.7 (dd, $^3J_{CP} = 12.5$, 7.1 Hz), 81.1, 80.4 (d, $^5J_{CP} = 1.6$ Hz), 51.1 (d, $^2J_{POC} = 6.4$ Hz), 39.3 (dd, $^4J_{CP} = 24.3$, 3.1 Hz), 32.7, 31.6 (dd, $^1J_{CP} = 101.2$, 40.7 Hz), 29.3 (t, $^2J_{CP} = 11.4$ Hz), 28.03, 27.99, 27.90; $^{31}$P NMR δ 44.2, 43.8; MS (ESI) m/z 413.0 ([M + H]$^+$, 100).
2-[(4-(N-Benzoxycarbonyl-amino)-phenyl)(ethoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid dimethyl ester (6b<sub>Et</sub>, Table 1, entry 6, footnote ‘e’). The same procedures were repeated as described for 6b (Method B) except that a mixture of 4d<sub>Et</sub> (62 mg, 0.194 mmol), dimethyl 2-methylene glutarate 5a (72 mg, 0.418 mmol), and BSA (0.50 mL, 2.02 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (8 mL) was heated under reflux for 7 d to afford 6b<sub>Et</sub> (50 mg, 0.102 mmol, 53%) as a colorless oil: ¹H NMR δ 7.91 (1 H, br s), 7.70–7.65 (2 H, m), 7.60–7.59 (2 H, m), 7.36–7.31 (5 H, m), 5.19 (2 H, s), 4.04–3.97 (1 H, m), 3.80–3.74 (1 H, m), 3.67–3.48 (6 H, m), 2.88–2.73 (1 H, m), 2.46–2.25 (3 H, m), 2.03–1.84 (3 H, m), 1.23 (3 H, td, J = 7.0, 4.8 Hz); ¹³C NMR δ 174.4 (dd, ³JCₚ = 23.8, 7.0 Hz), 172.8 (d, ⁵JCₚ = 6.3 Hz), 153.1, 142.3, 135.7, 132.9 (dd, ³JCₚ = 13.6, 11.2 Hz), 128.6, 128.4, 128.3, 123.8 (dd, ¹JCₚ = 130.2, 43.8 Hz), 118.1 (dd, ³JCₚ = 12.7, 8.2 Hz), 67.1, 60.6 (dd, ²JCₐₜ = 5.8, 3.4 Hz), 51.9 (d, ⁵JCₐₜ = 9.6 Hz), 51.6, 38.5 (dd, ⁴JCₚ = 9.5, 2.7 Hz), 31.9 (dd, ¹JCₚ = 101.9, 31.2 Hz), 31.2, 28.6 (dd, ²JCₚ = 15.4, 11.7 Hz), 16.3 (dd, ³JCₐₜ = 6.4, 3.6 Hz); ³¹P NMR δ 41.85, 41.77, 41.5, 41.4; MS (ESI) m/z 514.1 ([M + Na⁺], 100); ESI-HRMS (m/z) calcd for C<sub>24</sub>H<sub>30</sub>NO<sub>8</sub>PNa [M + Na⁺]⁺ 514.1607, found 514.1611.

2-[(4-(N-tert-Butyloxycarbonyl-amino)-phenyl)(methoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid dimethyl ester (6c, Table 1, entry 8). The same procedure was repeated as described for 6b (Method B) except that a mixture of 4d (22 mg, 81 µmol), dimethyl 2-methylene glutarate 5a (70 mg, 0.407 mmol), and BSA (0.30 mL, 1.21 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (4 mL) was heated under reflux for 7 d to afford 6c (19 mg, 43 µmol, 53%) as a colorless oil: ¹H NMR δ 7.70–7.65 (2 H, m), 7.57–7.48 (2 H, m), 6.83 (1 H, s), 3.66–3.52 (9 H, m), 2.89–2.74 (1 H, m), 2.45–2.28 (3 H, m), 2.03–1.89 (3 H, m), 1.52 (9 H, S); ¹³C NMR δ 174.4 (dd, ³JCₚ = 22.4, 7.2 Hz), 172.9 (d, ⁵JCₚ = 6.2
Hz), 152.2, 142.5 (t, $^4J_{CP} = 3.1$ Hz), 133.1 (dd, $^2J_{CP} = 14.6, 10.9$ Hz), 122.9 (dd, $^1J_{CP} = 130.2, 44.5$ Hz), 117.9 (dd, $^3J_{CP} = 12.9, 10.2$ Hz), 81.3, 51.9 (d, $^2J_{POC} = 11.2$ Hz), 51.7 (d, $^7J_{POC} = 1.5$ Hz), 51.1 (d, $^5J_{POC} = 6.4$ Hz), 38.5 (d, $^4J_{CP} = 5.4$ Hz), 31.8 (dd, $^1J_{CP} = 102.2, 37.9$ Hz), 31.3, 28.6 (d, $^2J_{CP} = 11.4$ Hz), 28.2; $^{31}$P NMR δ 43.4, 43.0; MS (ESI) m/z 466.0 ([M + Na]$^+$, 100); ESI-HRMS (m/z) calcd for C$_{20}$H$_{30}$NO$_8$PNa [M + Na]$^+$ 466.1607, found 466.1611.

2-[[((4-(N-tert-Butoxycarbonyl-amino)-phenyl)(ethoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid dimethyl ester (6cEt, Table 1, entry 8, footnote ‘e’). The same procedure was repeated as described for 6b (Method B) except that a mixture of 4eEt (46 mg, 0.161 mmol), dimethyl 2-methylene glutarate 5a (56 mg, 0.325 mmol), and BSA (0.60 mL, 2.42 mmol) in anhydrous CH$_2$Cl$_2$ (5 mL) was heated under reflux for 6 d to afford 6cEt (35 mg, 77 µmol, 47%) as a colorless oil: $^1$H NMR δ 7.71–7.66 (2 H, m), 7.51–7.49 (2 H, m), 6.81 (1 H, br s), 4.07–4.01 (1 H, m), 3.83–3.77 (1 H, m), 3.65 (3 H, d, $J = 11.3$ Hz), 3.58 (3 H, d, $J = 66.0$ Hz), 2.89–2.75 (1 H, m), 2.48–2.30 (3 H, m), 2.06–1.87 (3 H, m), 1.53 (9 H, s), 1.26 (3 H, td, $J = 7.0, 4.8$ Hz); $^{13}$C NMR δ 174.5 (dd, $^3J_{CP} = 26.8, 7.1$ Hz), 172.9 (d, $^5J_{CP} = 6.7$ Hz), 152.2, 142.4 (t, $^4J_{CP} = 14.7, 11.0$ Hz), 133.0 (dd, $^2J_{CP} = 14.7, 11.0$ Hz), 123.8 (dd, $^1J_{CP} = 130.0, 46.1$ Hz), 117.9 (dd, $^3J_{CP} = 12.8, 9.7$ Hz), 81.3, 60.6 (dd, $^2J_{POC} = 6.2, 3.6$ Hz), 51.9 (d, $^5J_{POC} = 10.9$ Hz), 51.7, 38.6 (d, $^4J_{CP} = 6.8$ Hz), 32.0 (dd, $^1J_{CP} = 101.9, 35.8$ Hz), 31.3, 28.6 (dd, $^2J_{CP} = 17.3, 11.6$ Hz), 28.2, 16.3 (dd, $^3J_{POC} = 6.4, 3.6$ Hz); $^{31}$P NMR δ 41.5, 41.2; MS (ESI) m/z 480.1 ([M + Na]$^+$, 100); ESI-HRMS (m/z) calcd for C$_{21}$H$_{32}$NO$_8$PNa [M + Na]$^+$ 480.1763, found 480.1761.

2-[[((4-(N-Benzoyloxycarbonyl-amino)-phenyl)(methoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid di-tert-butyl ester (6d, Table 1, entry 9). The same
The same procedure was repeated as described for 6b (Method B) except that a mixture of 4d (72 mg, 0.236 mmol), di-tert-butyl 2-methylene glutarate 5b (121 mg, 0.472 mmol), and BSA (0.60 mL, 2.42 mmol) in THF (5 mL) was heated under reflux for 36 h to afford 6d (33 mg, 59 µmol, 25%) as a colorless oil: 1H NMR δ 7.76–7.74 (1 H, d, J = 8.4), 7.71–7.68 (2 H, m), 7.60–7.58 (2 H, m), 7.40–7.33 (5 H, m), 5.21 (2 H, s), 3.56–3.53 (3 H, m), 2.76–2.68 (0.5 H, m), 2.64–2.55 (0.5 H, m), 2.45–2.31 (1 H, m), 2.26–2.14 (2 H, m), 1.95–1.78 (3 H, m), 1.45–1.38 (18 H, m); 13C NMR δ 173.3 (dd, 3JCP = 18.7, 7.0 Hz), 171.9 (d, 3JCP = 7.0 Hz), 153.2, 142.3, 135.8, 133.0 (t, 2JCP = 10.5 Hz), 128.6, 128.44, 128.36, 123.5 (dd, 1JCP = 129.6, 41.4 Hz), 118.2 (dd, 3JCP = 12.7, 8.7 Hz), 81.1, 80.4, 67.2, 51.0 (d, 2JPOC = 6.3 Hz), 39.4 (dd, 1JCP = 29.1, 2.3 Hz), 32.7, 31.7 (dd, 1JCP = 101.9, 36.3 Hz), 29.2 (dd, 2JCP = 14.6, 11.9 Hz), 28.05, 28.01, 27.9; 31P NMR δ 44.29, 44.25, 43.85, 43.81; MS (ESI) m/z 584.2 ([M + Na]+, 100); ESI-HRMS (m/z) calcd for C29H40NO8PNa [M + Na]+ 584.2389, found 584.2385.

2-([(4-(N-Benzylxycarbonyl-amino)-phenyl)(methoxy)-phosphinoyl]-methyl]pentane-1,5-dioic acid di-tert-butyl ester (6d, Table 1, entry 11). The same procedure was repeated as described for 6b (Method C) except that a suspension of 6b (250 mg, 0.524 mmol) and 5% Pd/C (167 mg) in THF (10 mL) was
stirred under H₂ atmosphere for 24 h to afford 7b (173 mg, 0.504 mmol, 96%) as a colorless oil: ¹H NMR δ 7.53–7.48 (2 H, m), 6.72–6.70 (2 H, m), 4.20 (2 H, br s), 3.69–3.51 (9 H, m), 2.88–2.72 (1 H, m), 2.44–2.26 (3 H, m), 2.04–1.86 (3 H, m); ¹³C NMR δ 174.5 (dd, 3 J_CP = 22.7, 7.3 Hz), 172.9 (d, 5 J_CP = 4.9 Hz), 150.6 (t, 4 J_CP = 2.7 Hz), 133.5 (dd, 2 J_CP = 18.6, 11.4 Hz), 116.6 (dd, 1 J_CP = 135.1, 52.0 Hz), 114.2 (dd, 3 J_CP = 13.7, 8.9 Hz), 51.8 (d, 7 J_POC = 10.7 Hz), 51.6 (d, 5 J_POC = 6.3 Hz), 50.8 (d, 5 J_POC = 6.3 Hz), 38.6 (dd, 4 J_CP = 5.9, 2.9 Hz), 31.9 (dd, 1 J_CP = 102.3, 33.7 Hz), 31.2, 28.5 (dd, 2 J_CP = 11.4, 3.1 Hz); ³¹P NMR δ 45.65, 45.61, 44.29, 44.25; MS (ESI) m/z 366.1 ([M + Na]⁺, 100); ESI-HRMS (m/z) calcd for C₁₅H₂₂NO₆PNa [M + Na]⁺ 366.1082, found 366.1073.

2-[[((4-Amino-phenyl)(methoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid di-tert-butyl ester (7c). The same procedure was repeated as described for 7a except that a suspension of 6d (93 mg, 0.166 mmol) and 5 % Pd/C (90 mg) in THF (10 mL) was stirred under H₂ atmosphere for 24 h to afford 7c (63 mg, 0.147 mmol, 89%) as a colorless oil: ¹H NMR δ 7.57–7.53 (2 H, m), 6.73–6.71 (2 H, m), 4.11 (2 H, br s), 3.63–3.52 (3 H, m), 2.77–2.57 (1 H, m), 2.44–2.16 (3 H, m), 1.94–1.78 (3 H, m), 1.46–1.37 (18 H, m); ¹³C NMR δ 173.4 (dd, 3 J_CP = 23.2, 7.0 Hz), 172.0 (d, 5 J_CP = 6.8 Hz), 150.3 (t, 4 J_CP = 2.5 Hz), 133.6 (dd, 2 J_CP = 14.9, 11.2 Hz), 117.3 (dd, 1 J_CP = 134.3, 48.7 Hz), 114.4 (dd, 3 J_CP = 13.6, 4.6 Hz), 80.8, 80.3 (d, 5 J_POC = 1.7 Hz), 50.7 (dd, 2 J_POC = 6.3, 3.1 Hz), 39.5 (dd, 4 J_CP = 35.0, 2.3 Hz), 32.8, 31.9 (dd, 1 J_CP = 102.1, 36.5 Hz), 29.2 (t, 2 J_CP = 11.3 Hz), 28.02, 27.98, 27.89; ³¹P NMR δ 45.2, 44.7; MS (ESI) m/z 450.4 ([M + Na]⁺, 100); ESI-HRMS (m/z) calcd for C₂₁H₃₄NO₆PNa [M + Na]⁺ 450.2021, found 450.2023.

[(4-((2-Pivaloylamino-4-hydroxy)pteroyl-amino)-phenyl)(methoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid di-tert-butyl ester (10b2). The same procedure was
repeated as described for 10b1 except that the solution of phosphinic tert-butyl ester 7c (97 mg, 0.227 mmol) and 9a (57 mg, 0.207 mmol) in AcOH (3 mL) was stirred at room temperature for 15 min, followed by treatment with a solution of BH$_3$•NHMe$_2$ (17 mg, 0.286 mmol) in AcOH (0.5 mL) to afford 10b2 (100 mg, 0.146 mmol, 71%) as a yellow solid: mp 103–105 °C; $^1$H NMR δ 12.49 (1 H, br s), 8.88 (1 H, s), 8.83 (1 H, br s), 7.56 (2 H, dd, $J = 10.9$, 8.6 Hz), 6.72 (2 H, dd, $J = 8.5$, 2.2 Hz), 5.46–5.43 (1 H, m), 4.71 (2 H, d, $J = 5.5$ Hz), 3.56 (3 H, dd, $J = 11.1$, 6.3 Hz), 2.76–2.56 (1 H, m), 2.42–2.14 (3 H, m), 1.95–1.79 (3 H, m), 1.44–1.33 (27 H, m); $^{13}$C NMR δ 180.8, 173.4 (dd, $^3$J$_{CP} = 20.8$, 6.8 Hz), 171.9 (d, $^5$J$_{CP} = 5.7$ Hz), 159.5, 154.5, 152.0, 150.4 (d, $^4$J$_{CP} = 2.2$ Hz), 149.4, 149.2, 133.6 (dd, $^2$J$_{CP} = 15.7$, 11.3 Hz), 130.7, 117.1 (dd, $^1$J$_{CP} = 134.7$, 47.5 Hz), 112.6 (dd, $^3$J$_{CP} = 13.4$, 5.2 Hz), 80.9, 80.3, 50.8 (dd, $^2$J$_{POC} = 6.4$, 2.6 Hz), 46.6, 40.5, 39.4 (dd, $^4$J$_{CP} = 31.1$, 3.1 Hz), 32.7, 31.9 (dd, $^1$J$_{CP} = 102.1$, 42.4 Hz), 29.2 (dd, $^2$J$_{CP} = 14.4$, 11.9 Hz), 28.00, 27.96, 27.88, 26.8; $^{31}$P NMR δ 43.3, 42.8; MS (ESI) m/z 709.1 ([M + Na]$^+$, 100); UV $\lambda_{max}$: (0.1 N NaOH) 257, 349, 428 nm; (0.1 N HCl) 272, 335 nm; analytical HPLC $t_R$ = 32.1, 32.4 min.

[[(4-(N-(4-Deoxy-2,4-diamino)pteroyl-N-methyl-amino)-phenyl)(methoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid dimethyl ester (10c)]. The same procedure was repeated as described for 10a except that a mixture of 7a (61 mg, 0.171 mmol) and 8b (86 mg, 0.256 mmol) in DMA (2 mL) was stirred at room temperature for 12 d to afford 10c (POMe) (40 mg, 75 µmol, 44%) as a light yellow feather-looking solid and 10c' (POH) (36 mg, 70 µmol, 41%) as a yellow solid. 10c: mp 95–97 °C; $^1$H NMR δ 8.66 (1 H, s), 7.57–7.56 (2 H, m), 6.82–6.80 (2 H, m), 4.77 (2 H, s), 3.64–3.49 (9 H, m), 3.21 (3H, s), 2.88–2.73 (1 H, m), 2.45–2.25 (3 H, m), 2.04–1.85 (3 H, m); $^{13}$C NMR
(CD$_3$OD) $\delta$ 174.5, 173.2 (d, $^3J_{CP} = 3.7$ Hz), 163.3, 162.8, 154.3 (d, $^4J_{CP} = 11.2$ Hz), 152.4, 149.6, 148.1, 147.5, 133.8 (d, $^2J_{CP} = 160.6$ Hz), 113.2 (d, $^1J_{CP} = 136.7$ Hz), 112.5 (t, $^3J_{CP} = 171.5$ Hz), 56.0, 54.9, 53.8, 52.8, 52.5, 52.1, 51.72, 51.65, 51.4, 50.91, 50.86, 50.6, 50.5, 50.2, 49.7, 49.3, 49.0, 39.1, 38.9, 38.1, 37.8, 31.6, 31.3, 30.5, 29.5, 28.5, 27.5; $^{31}$P NMR $\delta$ 44.5 44.2; MS (ESI) $m/z$ 554.1 ([M + Na]$^+$, 100); ESI-HRMS (m/z) calcd for C$_{23}$H$_{30}$N$_7$O$_6$PNa [M + Na]$^+$ 554.1893, found 554.1896. UV $\lambda_{max}$: (0.1 N NaOH) 260, 278, 375 nm; (0.1 N HCl) 243, 278, 335 nm. Analytical HPLC $t_R = 21.8$ min. 10c$: mp 173–175 °C; $^1$H NMR (CD$_3$OD) $\delta$ 8.54 (1 H, s), 7.57 (2 H, t, $J = 9.4$ Hz), 6.78 (2 H, d, $J = 8.3$ Hz), 4.81 (2 H, s), 3.55 (3 H, s), 3.37 (3 H, s), 3.19 (3 H, s), 2.67–2.58 (1 H, m), 2.19 (2 H, t, $J = 7.6$ Hz), 2.14–2.06 (1 H, m), 1.89–1.67 (3 H, m); $^{31}$P NMR (DMSO) $\delta$ 24.8 (br); MS (ESI) $m/z$ 540.1 ([M + Na]$^+$, 100); ESI-HRMS (m/z) calcd for C$_{22}$H$_{28}$N$_7$O$_6$PNa [M + Na]$^+$ 540.1736, found 540.1746. UV $\lambda_{max}$: (0.1 N NaOH) 261, 371 nm; (0.1 N HCl) 245, 274, 336 nm. Analytical HPLC $t_R = 16.7$ min.

[(4-((2-Acetylamino-4-hydroxy)-5-deazapteroyl-amino)-phenyl)(methoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid dimethyl ester (10d2). The same procedure was repeated as described for 10b1 except that a mixture of phosphinic methyl ester 7b (41 mg, 0.119 mmol), 9c (30 mg, 0.129 mmol) was stirred at rt for 27 h, followed by treatment with BH$_3$•NHMe$_2$ (11 mg, 0.187 mmol). The solvent was removed in vacuo to afford the crude product. The crude product was purified by column chromatography using a mixture of MeOH and methylene chloride (2% – 5% MeOH) as eluant to afford 10d2 (40 mg, 0.071 mmol, 67%) as a light yellow solid: mp 121–124 °C; $^1$H NMR $\delta$ 12.55 (0.6 H, br s), 11.79 (1 H, s), 8.92 (0.6 H, s), 8.84 (0.4 H, s), 8.37 (1 H, s), 7.57–7.53 (2 H, m), 6.72–6.70 (2 H, m), 5.31 (1 H, br s), 4.73 (0.4 H, s), 4.48–4.47 (2
H, m), 3.69–3.52 (9 H, m), 2.90–2.73 (1 H, m), 2.60–2.28 (6 H, m), 2.14–1.87 (3 H, m);
$^{13}$C NMR δ 174.6 (dd, $^3J_{CP} = 20.3$, 7.1 Hz), 174.3, 172.9 (d, $^5J_{CP} = 3.2$ Hz), 161.7, 157.6, 155.6, 150.8, 149.5, 135.1, 133.6 (dd, $^2J_{CP} = 15.4$, 11.5 Hz), 132.0, 116.5 (dd, $^1J_{CP} = 135.5$, 46.8 Hz), 114.4, 112.4 (dd, $^3J_{CP} = 13.3$, 8.0 Hz), 51.9 (d, $^2J_{POC} = 6.8$ Hz), 51.6 (d, $^7J_{POC} = 1.2$ Hz), 50.9 (d, $^5J_{POC} = 6.3$ Hz), 44.5, 38.6 (dd, $^4J_{CP} = 8.4$, 2.9 Hz), 31.9 (dd, $^1J_{CP} = 102.1$, 37.0 Hz), 31.3, 28.6 (dd, $^2J_{CP} = 11.4$, 2.7 Hz), 25.2; $^{31}$P NMR δ 44.8, 44.5; MS (ESI) m/z 582.1 ([M + Na]$^+$, 100); UV λ$_{max}$: (0.1 N NaOH) 245, 272, 323 nm; (0.1 N HCl) 276, 339 nm; analytical HPLC $t_R = 23.2$ min.

[(4-(N-((2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl)-N-methyl-amino)-phenyl)(methoxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid dimethyl ester (10e).

The same procedure was repeated as described for 10a except that a mixture of 7a (37 mg, 0.104 mmol) and 8a (52 mg, 0.154 mmol) in DMA (2 mL) was stirred at room temperature for 14 d to afford 10e (POME) (7 mg, 13 µmol, 13%) as a greenish yellow feather-looking solid and 10e' (POH) (19 mg, 37 µmol, 35%) as a greenish yellow solid.

10e: mp 171–173 °C; $^1$H NMR (CD$_3$OD) δ 8.50 (1 H, s), 7.54–7.50 (2 H, m), 6.94–6.91 (2 H, m), 4.85 (2 H, s), 3.61–3.40 (9 H, m), 3.28 (3H, s), 2.72–2.62 (1 H, m), 2.43–2.23 (3 H, m), 2.10–1.98 (1 H, m), 1.91–1.80 (2 H, m); $^{31}$P NMR (CD$_3$OD) δ 47.7, 47.5; MS (ESI) m/z 555.1 ([M + Na]$^+$, 100); ESI-HRMS (m/z) calcd for C$_{23}$H$_{29}$N$_6$O$_7$PNa [M + Na]$^+$ 555.1733, found 555.1727. UV λ$_{max}$: (0.1 N NaOH) 253, 280, 365 nm; (0.1 N HCl) 250, 276, 320 nm. Analytical HPLC $t_R = 20.6$ min. 10e': mp 200–202 °C (dec.); $^1$H NMR (CD$_3$OD) δ 8.48 (1 H, s), 7.56 (2 H, t, $J = 9.2$ Hz), 6.80 (2 H, d, $J = 7.5$ Hz), 4.85 (2 H, s), 3.60 (3 H, s), 3.37 (3 H, m), 3.21 (3 H, s), 2.65–2.57 (1 H, m), 2.21 (2 H, t, $J = 7.7$ Hz), 2.15–2.04 (1 H, m), 1.90–1.63 (3 H, m); $^{31}$P NMR (CD$_3$OD) δ 29.4; MS (ESI) m/z
541.1 ([M + Na]+, 100); ESI-HRMS (m/z) calcd for C_{22}H_{27}N_{6}O_{7}PNa [M + Na]+
541.1577, found 541.1578. UV λ_{max}: (0.1 N NaOH) 258, 364 nm; (0.1 N HCl) 250, 272,
320 nm. Analytical HPLC t_R = 15.8 min.

\[\text{[(4-(N-(4-Deoxy-2,4-diamino)pteroyl-amino)-phenyl}(\text{methoxy})\text{-phosphinoyl})\text{-methyl]}\text{pentane-1,5-dioic acid dimethyl ester} \ (10f) \]. The same procedure was repeated as described for 10a except that a mixture of 7b (42 mg, 0.122 mmol) and 8b (62 mg, 0.185 mmol) in DMA (2 mL) was stirred at room temperature for 11 d to afford 10f (POMe) (23 mg, 44 µmol, 37%) as a yellow feather-looking solid and 10f' (POH) (27 mg, 54 µmol, 44%) as an orange solid. 10f: mp 189–191 °C; ¹H NMR (CD_{3}OD) δ 8.75 (1 H, s), 7.53–7.47 (2 H, m), 6.88–6.85 (2 H, m), 4.63 (2 H, s), 3.65–3.56 (6 H, m), 3.43–3.37 (3H, m), 2.75–2.65 (1 H, m), 2.44–2.28 (3 H, m), 2.12–2.00 (1 H, m), 1.94–1.85 (2 H, m); ³¹P NMR (CD_{3}OD) δ 47.8, 47.7; MS (ESI) m/z 540.1 ([M + Na]+, 100); ESI-HRMS (m/z) calcd for C_{22}H_{28}N_{7}O_{6}PNa [M + Na]+ 540.1736, found 540.1741. UV λ_{max}: (0.1 N NaOH) 264, 369 nm; (0.1 N HCl) 246, 270, 333 nm. Analytical HPLC t_R = 20.7 min. 10f': mp 181–183 °C; ¹H NMR (CD_{3}OD) δ 8.72 (1 H, s), 7.50 (2 H, t, J = 9.5 Hz), 6.69 (2 H, d, J = 8.0 Hz), 4.58 (2 H, s), 3.57 (3 H, s), 3.37 (3 H, s), 2.67–2.57 (1 H, m), 2.20 (2 H, t, J = 7.6 Hz), 2.12–2.05 (1 H, m), 1.90–1.64 (3 H, m); ³¹P NMR (CD_{3}OD) δ 29.2; MS (ESI) m/z 526.1 ([M + Na]+, 100); ESI-HRMS (m/z) calcd for C_{21}H_{26}N_{7}O_{6}PNa [M + Na]+ 526.1580, found 540.1572. UV λ_{max}: (0.1 N NaOH) 260, 369 nm; (0.1 N HCl) 247, 265, 337 nm. Analytical HPLC t_R = 15.5 min.

\[\text{[(4-((2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl)-amino)-phenyl}(\text{hydroxy})\text{-phosphinoyl})\text{-methyl]}\text{pentane-1,5-dioic acid} \ (1a) \]. The same procedure was repeated as described for 1a (Method A) except that a mixture of 10b1 (49
mg, 81 µmol) and 1 N NaOH (3.0 mL) in CH₃OH (1.5 mL) was stirred at room temperature for 5 days to afford 1a (32.7 mg, 69 µmol, 84%) as a yellow solid. Spectra are identical to those obtained for 1a from 10a.

[((4-(N-(4-Deoxy-2,4-diamino)pteroyl-N-methyl-amino)-phenyl)(hydroxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid (1b). The same procedure was repeated as described for 1a (Method A) except that a mixture of 10c (14 mg, 27 µmol) and 1 N NaOH (1.5 mL) in CH₃OH (1.5 mL) was stirred at room temperature for 3 days to afford 1b (10 mg, 20 µmol, 77%) as a yellow solid: mp >300 °C (dec.); ¹H NMR (D₂O) δ 8.50 (1 H, s), 7.38 (2 H, t, J = 9.5 Hz), 6.72 (2 H, d, J = 8.8 Hz), 4.74 (2 H, s), 3.00 (3 H, s), 2.34–2.25 (1 H, m), 2.00 (2 H, t, J = 7.8 Hz), 1.96–1.87 (1 H, m), 1.65–1.49 (3 H, m); ¹³C NMR (D₂O) δ 180.8, 179.1, 163.2, 156.5, 152.0, 150.8, 149.6, 145.8, 132.5 (d, ²Jₜₚ = 10.6 Hz), 123.4 (d, ¹Jₜₚ = 134.2 Hz), 122.1, 112.4 (d, ³Jₜₚ = 12.4 Hz), 55.1, 41.0, 38.8, 34.1 (d, ¹Jₜₚ = 95.8 Hz), 32.6, 28.9 (d, ²Jₜₚ = 8.8 Hz); ³¹P NMR (D₂O) δ 32.6; MS (ESI) m/z 490.1 ([M + H]⁺, 100); ESI-HRMS (m/z) calcd for C₂₀H₂₅N₇O₆P [M + H]⁺ 490.1604, found 490.1606. UV λmax: (0.1 N NaOH) 261 nm, 371 nm; (0.1 N HCl) 245 nm, 274 nm, 336 nm. Analytical HPLC tᵣ = 10.3 min.

[((2-Amino-4-hydroxy)-5-deazapteroyl-amino)-phenyl](hydroxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid (1c). The same procedure was repeated as described for 1a (Method B) except that a mixture of 10d (23 mg, 41 µmol) and 1 N NaOH (2.0 mL) in CH₃OH (1 mL) was stirred at room temperature for 3 days to afford Phosphinic methyl ester, which was treated with TFA (0.5 mL) in CH₂Cl₂ (2.5 mL) to afford 1c (19 mg, 40 µmol, 97%) as a white solid: mp >300 °C (dec.); ¹H NMR (D₂O) δ 8.52 (1 H, s), 8.35 (1 H, s), 7.38 (2 H, t, J = 7.9 Hz), 6.71 (2 H, d, J = 7.9 Hz), 4.41 (2 H, s), 2.34–2.25 (1 H,
m), 2.01 (2 H, t, $J = 7.8$ Hz), 1.96–1.85 (1 H, m), 1.67–1.46 (3 H, m); $^{31}$P NMR (D$_2$O) $\delta$ 32.7; MS (ESI) $m/z$ 476.3 ([M + H]$^+$, 100); ESI-HRMS (m/z) calcd for C$_{20}$H$_{23}$N$_5$O$_7$P [M + H]$^+$ 476.1335, found 476.1335. UV $\lambda_{\text{max}}$: (0.1 N NaOH) 248 nm, 340 nm; (0.1 N HCl) 272 nm, 350 nm. Analytical HPLC $t_R = 6.7$ min.

$\left[(4-((2\text{-Amino}-4\text{-hydroxy})-5\text{-deazapteroyl-amino})\text{-phenyl})(\text{hydroxy})\text{-phosphinoyl})\text{-methyl}\right]\text{pentane-1,5-dioic acid (1c).}$ The same procedure was repeated as described for 1a (Method B) except that a mixture of 10d1 (23 mg, 38 $\mu$mol) and 1 N NaOH (2.0 mL) in CH$_3$OH (1.0 mL) was stirred at room temperature for 3 days to afford Phosphinic methyl ester, which was treated with TFA (0.5 mL) in CH$_2$Cl$_2$ (2.5 mL) to afford 1c (17 mg, 36 $\mu$mol, 94%) as a white solid. Spectra are identical to those obtained for 1c from 10d2.

$\left[(4-(\text{N-(2\text{-Amino}-3,4\text{-dihydro-4-oxo-6-pteridinyl})methyl})\text{-N-methyl-amino})\text{-phenyl})(\text{hydroxy})\text{-phosphinoyl})\text{-methyl}\right]\text{pentane-1,5-dioic acid (1d).}$ The same procedure was repeated as described for 1a (Method A) except that a mixture of 10e/e' (15 mg, 29 $\mu$mol) and 1 N NaOH (1.5 mL) in CH$_3$OH (1.5 mL) was stirred at room temperature for 5 d to afford 1d (6.7 mg, 14 $\mu$mol, 48%) as a yellow solid: mp $>300$ °C (dec.); $^1$H NMR (D$_2$O) $\delta$ 8.44 (1 H, s), 7.43 (2 H, t, $J = 9.6$ Hz), 6.80 (2 H, d, $J = 8.5$ Hz), 4.74 (2 H, s), 3.08 (3 H, s), 2.43–2.35 (1 H, m), 2.12 (2 H, t, $J = 7.8$ Hz), 2.03–1.95 (1 H, m), 1.71–1.55 (3 H, m); $^{31}$P NMR (D$_2$O) $\delta$ 32.5; MS (ESI) $m/z$ 491.1 ([M + H]$^+$, 100); ESI-HRMS (m/z) calcd for C$_{20}$H$_{24}$N$_6$O$_7$P [M + H]$^+$ 491.1444, found 491.1446. UV $\lambda_{\text{max}}$: (0.1 N NaOH) 261 nm, 366 nm; (0.1 N HCl) 250 nm, 272 nm, 320 nm. Analytical HPLC $t_R = 10.1$ min.
[[(4-(N-(4-Deoxy-2,4-diamino)pteroyl-amino)-phenyl)(hydroxy)-phosphinoyl)-methyl]pentane-1,5-dioic acid (1e). The same procedure was repeated as described for 1a (Method A) except that a mixture of 10f' (10 mg, 20 µmol) and 1 N NaOH (1.0 mL) in CH₃OH (1.0 mL) was stirred at room temperature for 1 day to afford 1e (9.0 mg, 19 µmol, 95%) as a yellow solid: mp >300 °C (dec.); ¹H NMR (D₂O) δ 8.59 (1 H, s), 7.38 (2 H, t, J = 9.2 Hz), 6.65 (2 H, d, J = 7.6 Hz), 4.39 (2 H, s), 2.36–2.25 (1 H, m), 2.03 (2 H, t, J = 8.1 Hz), 1.99–1.85 (1 H, m), 1.65–1.49 (3 H, m); ¹³C NMR (D₂O) δ 181.6, 180.2, 162.7, 156.9, 150.9, 149.5, 149.3, 146.6, 132.6 (d, ²J_CP = 11.2 Hz), 124.4 (d, ¹J_CP = 135.3 Hz), 121.3, 112.8 (d, ³J_CP = 12.0 Hz), 45.6, 41.7, 34.1 (dd, ¹J_CP = 99.7, 36.0 Hz), 33.4, 29.5 (d, ²J_CP = 10.0 Hz); ³¹P NMR (D₂O) δ 32.9; MS (ESI) m/z 476.1 ([M + H]⁺, 100); ESI-HRMS (m/z) calcd for C₁₉H₂₃N₇O₆P [M + H]⁺ 476.1447, found 476.1456. UV λ max: (0.1 N NaOH) 256 nm, 371 nm; (0.1 N HCl) 249 nm, 262 nm, 336 nm. Analytical HPLC t_R = 7.9 min.
Table S1. Conversion of 4 to 6 (Method B) – Optimization Experiments.

![Chemical structure](image)

Entry | Substrate | Solvent | Temp. | Time (days) | yield (%)
--- | --- | --- | --- | --- | ---
I. R<sup>3</sup> = Me, R<sup>4</sup> = Et
1. 4d | DCM | reflux | 7 | 53 |
2<sup>b</sup> | 4e | DCM | rt | 7 | 41 |
3. 4e | DCM | reflux | 6 | 47 |
II. R<sup>3</sup> = R<sup>4</sup> = Me
4. 4e | DCM | reflux | 7 | 53 |
5. 4d | DCM | reflux | 7 | 71 |
6. 4d | MeCN | reflux | 1 | 30<sup>c</sup> |
7. 4d | THF | reflux | 1.5 | 46<sup>e</sup> |
8<sup>d</sup> | 4d | DCM | 80 °C | 4 h | 50<sup>e</sup> |
III. R<sup>3</sup> = t-Bu, R<sup>4</sup> = Me
9. 4d | DCM | reflux | 9 | 0<sup>e</sup> |
10. 4d | THF | reflux | 1 | 25<sup>e</sup> |

<sup>a</sup> Isolated yield.  <sup>b</sup>Two-step process: P(III) species generated first at rt for 24 h, then 5a was added for 7 days. Otherwise, one-pot process.  <sup>c</sup>By-product detected at higher temp. Entry 6, 7 has much more by-product than entry 4.  <sup>d</sup>Microwave irradiation (250 W).  
<sup>e</sup>No desired product detected, starting material consumed.
References
