Supporting Information

Synthesis of a Diverse Series of Phosphacoumarins with Biological Activity

Xueshu Li,[†] Dongwei Zhang,[†] Hai Pang,[‡] Feng Shen,[†] Hua Fu,^{†,*} Yuyang Jiang,[±]

Yufen Zhao[†]

[†]Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology,
Ministry of Education, Department of Chemistry, Tsinghua University, Beijing
100084, P. R. China

[‡]Laboratory of Structural Biology, Tsinghua University, Beijing 100084, P. R. China

[±]Key Laboratory of Chemical Biology, Guangdong Province, Graduate School of
Shenzhen, Tsinghua University, Shenzhen 518057, P. R. China

fuhua@mail.tsinghua.edu.cn

Table of contents

Experimental Section and Characterization data for compounds 5-9	S2
PTP assay of the synthesized phosphacoumarins	S16
¹ H, ¹³ C, ³¹ P NMR spectra of the synthesized compounds 6 , 7 , and ¹ H, ¹³ C NMR spectra of the synthesized compounds 8 and 9	S18

Experimental Section

Unless otherwise noted, all reagents were purchased from commercial sources and were used without further purification. All ^1H NMR spectra are reported in δ units ppm downfield from tetramethylsilane internal standard. All ^1H NMR spectra are reported in δ units ppm relative to the central line of the triple to CDCl_3 at 77 ppm. 85% H_3PO_4 was used as the external reference of ^{31}P NMR spectra.

General procedure for the preparation of 3. 21 mmol of sodium was added to dialkyl phosphite (20 mmol) in 20 mL of dry ether at 0 $^\circ\text{C}$, the reaction lasted 1 h, and intermediate **2** was formed with a milky solution appearing. After the excess of sodium was removed by filtration, 20 mmol of *p*-substituted benzyl chloride in 5 mL of ether was added dropwise to the resulting solution in the ice-bath within 20 min, and then the reaction lasted another 1 h at room temperature. The resulting solution was washed with distilled water (10 mL), 1 M HCl (10 mL $\times$ 3), distilled water (10 mL), and the organic phase was dried over anhydrous magnesium sulfate. The solvent was removed by rotatory evaporation under reduced pressure, the residue **3** was obtained as a yellowish liquid, and it was directly used for the preparation of **4** without any purification, and total yields of the two steps from **1** to **3** are 80–85%.

General procedure for the preparation of 5. Mixture of **3** (10 mmol) and phosphoryl chloride (10.5 mmol) was heated at 60 $^\circ\text{C}$ under nitrogen atmosphere about

3 h, the low boil point byproducts and the excess of phosphoryl chloride were removed under the reduced pressure, and **4** was acquired as a viscous yellowish liquid, methyl salicylate (10 mmol) in dichloromethane (10 mL) was added dropwise to the crude product **4** in dichloromethane (5 mL) in the presence of triethylamine (2 mmol) at 0 °C, and then the temperature was slowly warmed to room temperature. The stirring lasted 2 h, 10 mL of dichloromethane was added to the resulting solution, the solution was washed with distilled water (10 mL), 1 M HCl (10 mL × 3), distilled water (10 mL). The organic phase was dried over magnesium sulfate, the solvent was removed by rotatory evaporation under reduced pressure, and the residue was purified by column chromatography on silica gel with petroleum ether/AcOEt (2:1) as eluent to give **5** as viscous yellowish liquid.

Compound 5a. Total yield of the two steps: 77%; ³¹P NMR: 26.2 ppm; HR-MS of [M+H]⁺ m/z: Cald. 321.0892, Found. 321.0889.

Compound 5b. Total yield of the two steps: 82%; ³¹P NMR: 26.7 ppm; HR-MS of [M+H]⁺ m/z: Cald. 369.0659, Found. 369.0664.

Compound 5c. Total yield of the two steps: 75%; ³¹P NMR: 26.1 ppm; HR-MS of

[M+H]⁺ m/z: Cald. 349.1205, Found. 349.1209.

Compound 5d. Total yield of the two steps: 82%; ³¹P NMR: 27.3 ppm; HR-MS of [M+H]⁺ m/z: Cald.360.1001, Found. 360.1003.

General procedures for the preparation of 6 and 7. To **5** (5 mmol) in 5 mL of dry pyridine was added KOH (10 mmol) at room temperature (KOH was crushed into powder before use). The solution was stirred at room temperature under nitrogen atmosphere for 3 h. The solvent was removed under reduced pressure, and the residue was dissolved in 10mL of CH₂Cl₂, the solution washed with 1M HCl (5 mL × 2) and distilled water (5 mL × 2). Then the organic phase was dried over anhydrous MgSO₄, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel with petroleum ether/AcOEt (2: 1) as eluent to give mixture of **6** and **7**.

Compounds 6a and 7a. White solid; mp 125–130 °C; Yield: 90%; ³¹P NMR (121.5 MHz, CDCl₃) δ 14.60, 14.71 (**7a**, ratio of two peaks 1.0:1.2), 21.08, 21.09, 21.30, 21.41 (**6a**, ratio of four peaks 1.0:1.2:1.3:1.1), the ratio of **6a** and **7a** = 6.3:1; ¹H NMR (300 MHz, CDCl₃) δ 3.74 (OCH₃ of **6a** and **7a**), 4.64 (d, ²J_{HCP} = 30 Hz, CH of **6a**), 7.01 (Ar of **6a** and **7a**), 10.95 (OH of **7a**); ¹³C NMR (75 MHz, CDCl₃) δ 53.9

(OCH₃ of **6a** and **7a**), 57.2, (d, ¹J_{CP} = 180 Hz, C-3 of **6a**), 116.1 (d, ¹J_{CP} = 165 Hz, C-3 of **7a**), 119.7–136.6 (Ar of **6a** and **7a**), 153.3 (C-8a of **6a** and **7a**), 187.5 (C-4 of **6a**); HR-MS of [M+H]⁺ m/z: Cald. 289.0630, Found. 289.0635.

Compound 6b and 7b. White solid; mp 153–159 °C; Yield: 85%; ³¹P NMR (121.5 MHz, CDCl₃) δ 13.21 (**7b**), 19.19, 19.38, 20.42, 20.69 (**6b**, ratio of four peaks 1.5:43.7:1.0:26.5), the ratio of **6b** and **7b** = 9.3 : 1; ¹H NMR (300 MHz, CDCl₃) δ 1.25 (m, OCH₂CH₃ of **6b** and **7b**), 3.72 (OCH₂ of **7b**), 4.18 (OCH₂ of **6b**), 4.60 (dd, ²J_{HCP} = 30 Hz, H-3 of **6b**), 6.87–8.02 (Ar of **6b** and **7b**), 10.97, 11.97 (OH of **7b**); ¹³C NMR (75 MHz, CDCl₃) δ 16.1 (OCH₂CH₃), 55.8 (dd, ¹J_{CP} = 118 Hz, C-3 of **6b**), 64.2 (OCH₂ of **6b** and **7b**), 119.2–136.8 (Ar of **6b** and **7b**), 153.3 (d, ²J_{COP} = 7.5 Hz, C-8a of **6b** and **7b**), 187.3 (C-4 of **6b**); HR-MS of [M+H]⁺ m/z: Cald. 337.0396, Found. 337.0389.

Compound 6c and 7c. Yellowish solid; mp 45–51 °C; Yield: 85%. ³¹P NMR (121.5 MHz, CDCl₃) δ 13.63, 14.06 (**7c**, ratio of two peaks 3.1: 1.0), 19.08, 21.36, 21.39, 21.79 (**6c**, ratio of four peaks, 1.8: 5.8: 2.9: 1.0), the ratio of **6c** and **7c** = 2.5 :

1.0; ^1H NMR (300 MHz, CDCl_3) δ 1.22 (OCH_2CH_3 of **6c** and **7c**), 2.37 (3H, PhCH_3), 4.14 (m, OCH_2 of **6c** and **7c**), 4.67 (d, $^2J_{\text{HCP}} = 30\text{Hz}$, H-3 of **6c**), 7.21–8.05 (Ar-H of **6c** and **7c**); ^{13}C NMR (75 MHz, CDCl_3) δ 16.1 (OCH_2CH_3), 20.2–21.2 (PhCH_3), 56.8 (dd, $^1J_{\text{CP}} = 113\text{ Hz}$, C-3 of **6c**), 63.9–64.2 (OCH_2 of **6c** and **7c**), 117.2 (d, $^1J_{\text{CP}} = 160\text{ Hz}$, C-3 of **7c**), 119.2–138.1 (Ar of **6c** and **7c**), 153.4 (d, $^2J_{\text{COP}} = 6.0\text{ Hz}$, C-8a of **6c** and **7c**), 188.0 (C-4 of **6c**); HR-MS of $[\text{M}+\text{H}]^+$ m/z: Cald. 317.0943, Found. 317.0945.

Compound 6d and 7d. White solid; m. p. : 173–177 °C; Yield: 88%; ^{31}P NMR (121.5 MHz, CDCl_3) δ 13.23 (**7d**), 18.19, 19.69 (**6d**, ratio of the two peaks 6.3: 3.7); the ratio of **6d** and **7d** = 7.7 : 1; ^1H NMR (300 MHz, CDCl_3) δ 1.22 (OCH_2CH_3 of **6d**), 1.85 (OCH_2CH_3 of **7d**), 3.72 (OCH_2 of **7d**), 4.17 (OCH_2 of **6d**), 4.66 (dd, $^2J_{\text{HCP}} = 30\text{ Hz}$, H-3 of **6d**), 7.26–8.02 (Ar of **6d** and **7d**); ^{13}C NMR (75 MHz, CDCl_3) δ 16.0 (OCH_2CH_3), 57.3 (d, $^1J_{\text{CP}} = 180\text{ Hz}$, C-3 of **6d**), 64.3–67.8 (OCH_2 of **6d** and **7d**), 112.3 (CN), 118.9 (d, $^1J_{\text{CP}} = 165\text{ Hz}$, C-3 of **7d**), 124.9–136.9 (Ar of **6d** and **7d**), 153.3 (C-8a of **6d** and **7d**), 186.3 (d, $^2J_{\text{CCP}} = 5.5\text{ Hz}$, C-4 of **6d**); HR-MS of $[\text{M}+\text{H}]^+$ m/z: Cald. 328.0739, Found. 328.0740.

General procedure for the preparation of 8ae–8de. Mixture of **6** (0.5 mmol) and 5mL of acetic anhydride were stirred at 25 °C, the progress of the reaction was

detected by ^{31}P NMR, and the reaction completed till the peaks at about 21 ppm disappeared. The solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel with petroleum ether/AcOEt (2:1) as eluent to give **8ae–8de**.

Compound 8ae: White foam; Yield: 92%; ^{31}P NMR (121.5 MHz, CDCl_3) δ 11.73; ^1H NMR (300 MHz, CDCl_3) δ 2.09 (s, 3H, COCH_3), 3.76 (d, $^3J_{\text{HCOP}} = 12.5$ Hz, 3H, POCH_3 , the doublet due to the coupling with phosphorus), 7.16–7.27 (m, 2H, Ar), 7.38–7.48 (m, 7H, Ar); ^{13}C NMR (75 MHz, CDCl_3) δ 20.3 (COCH_3), 54.2 (d, $^2J_{\text{COP}} = 6.5$ Hz, POCH_3), 114.6 (d, $^1J_{\text{CP}} = 169$ Hz, C-3), 118.7–131.8 (Ar), 149.6 (d, $^2J_{\text{COP}} = \sim 6$ Hz, C-8a), 150.1 (d, $^2J_{\text{CCP}} = 7.1$ Hz C-4a), 167.7 (CO); HR-MS of $[\text{M}+\text{H}]^+$ m/z: Calcd. 331.0735, Found. 331.0739.

Compound 8be: White foam; Yield: 95%; ^{31}P NMR (121.5 MHz, CDCl_3) δ 9.9 (d); ^1H NMR (300 MHz, CDCl_3) δ 1.21 (t, $^3J_{\text{HCCH}} = 7.5$ Hz, 3H, OCH_2CH_3), 2.11 (s, 3H, COCH_3), 4.13 (m, 2H, POCH_2), 7.15–7.27 (m, 2H, Ar), 7.42 (m, 6H, Ar); ^{13}C NMR

(75 MHz, CDCl₃) δ 16.0 (d, $^3J_{\text{CCOP}} = 6.2$ Hz, CH₂CH₃), 20.1 (COCH₃), 64.0 (d, $^2J_{\text{COP}} = 6.75$ Hz, OCH₂), 114.7 (d, $^1J_{\text{CP}} = 168$ Hz, C-3), 117.7–134.5 (Ar), 149.4 (d, $^2J_{\text{COP}} = 7.0$ Hz, C-8a), 150.8 (d, $^2J_{\text{CCP}} = 7.0$ Hz C-4a), 166.8 (CO); HR-MS of [M+H]⁺ m/z: Cald. 379.0502, Found. 379.0510.

Compound 8ce. White foam; Yield: 90%; ³¹P NMR (121.5 MHz, CDCl₃) δ 10.72; ¹H NMR (300 MHz, CDCl₃) δ 1.21 (t, $^3J_{\text{HCC}} = 7.1$ Hz, 3H, CH₂CH₃), 2.10 (s, 3H, COCH₃), 2.37 (s, 3H, PhCH₃), 4.13 (m, 2H, POCH₂), 7.22–7.23 (m, 4H, Ar), 7.37–7.40 (m, 4H, Ar); ¹³C NMR (75 MHz, CDCl₃) δ 16.1 (d, $^3J_{\text{CCOP}} = 5.3$ Hz, CH₂CH₃), 21.3 (COCH₃), 20.4 (PhCH₃), 64.2 (d, $^2J_{\text{COP}} = 6.75$ Hz, OCH₂), 116.0 (d, $^1J_{\text{CP}} = 168$ Hz, C-3), 118.2–138.5 (Ar), 149.58 (d, $^2J_{\text{COP}} = 6.0$ Hz, C-8a), 150.4 (d, $^2J_{\text{CCP}} = 7.2$ Hz C-4a), 167.4 (CO); HR-MS of [M+H]⁺ m/z: Cald. 359.1048, Found. 359.1045.

Compound 8de: White foam; Yield: 94%; ³¹P NMR (121.5 MHz, CDCl₃) δ 9.19; ¹H NMR (300 MHz, CDCl₃) δ 1.23 (t, $^3J_{\text{HCC}} = 7.5$ Hz, 3H, CH₂CH₃), 2.13 (s, 3H,

COCH₃), 4.17 (m, 2H, POCH₂), 7.26 (m, 2H, Ar), 7.64 (m, 2H, Ar), 7.70 (m, 4H, Ar); ¹³C NMR (75 MHz, CDCl₃) δ 16.1 (d, ³J_{CCOP} = 5.3 Hz, CH₂CH₃), 20.2 (COCH₃), 64.2 (d, ²J_{COP} = 6.7 Hz, OCH₂), 113.0 (C-CN), 115.8(CN), 117.6–135.2 (Ar), 149.6 (d, ²J_{COP} = 6.5 Hz, C-8a), 151.8 (d, ²J_{CCP} = 7.0 Hz, C-4a), 166.6 (CO); HR-MS of [M+H]⁺ m/z: Cald. 370.0844, Found. 370.0851.

General procedure for the preparation of 8af–8df. Anhydrous K₂CO₃ (1 mmol) was added to **6** (0.5 mmol) in 5 mL of anhydrous acetonitrile, the stirring lasted about 20 min, and methylsulfonyl chloride (0.6 mmol) in acetonitrile (2 mL) was added dropwise within 30 min in the ice-bath (0°C). The ice-bath was removed, and the solution was stirred at room temperature for another 30 min, the solvent was removed by rotatory evaporation, the residue was dissolved in 15 mL of dichloromethane, the solution was washed with distilled water (10 mL), 1M HCl (10 mL × 2), distilled water (10 mL) sequentially. The organic phase was dried over MgSO₄, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel with petroleum ether/AcOEt (2:1) as eluent to give pure compound **8af–8df**.

Compound 8af. White solid; mp 158–159 °C; Yield: 96%; ³¹P NMR (121.5 MHz, CDCl₃) δ 11.15; ¹H NMR (300 MHz, CDCl₃) δ 2.39 (s, 3H, SO₂CH₃), 3.77 (d, ³J_{HCO}P

= 8.0 Hz, 3H, OCH₃, the doublet due to the coupling with phosphorus), 7.26–7.31 (m, 2H, Ar), 7.46–7.49 (m, 4H, Ar), 7.60–7.63 (m, 2H, Ar), 7.82–7.90 (m, 1H, Ar); ¹³C NMR (75 MHz, CDCl₃) δ 39.6 (SO₂CH₃), 54.3 (d, ²J_{COP} = 6.1 Hz, OCH₃), 116.0 (d, ¹J_{CP} = 168.0 Hz, C-3), 118.5–118.6 (d, ¹J_{CP} = 80 Hz, C-3), 124.1–132.3 (Ar), 149.3 (d, ²J_{COP} = 7.5 Hz, C-8a), 150.7 (d, ²J_{CCP} = 7.0 Hz, C-4a); HR-MS of [M+H]⁺ m/z: Cald. 367.0405, Found. 367.0400.

Compound 8bf. White solid; mp 145–147 °C; Yield: 94%; ³¹P NMR (121.5 MHz, CDCl₃) δ 9.70; ¹H NMR (300 MHz, CDCl₃) δ 1.20–1.25 (t, ³J_{HCCH} = 7.5 Hz, 3H, CH₂CH₃), 2.54 (s, 3H, SO₂CH₃), 4.2 (m, 2H, OCH₂), 7.26–7.33 (m, 2H, Ar), 7.46–7.49 (m, 3H, Ar), 7.57–7.60 (m, 2H, Ar), 7.81–7.90 (m, 1H, Ar); ¹³C NMR (75 MHz, CDCl₃) δ 16.2 (d, ³J_{CCOP} = 5.3 Hz, CH₂CH₃), 39.81 (SO₂CH₃), 64.9 (d, ²J_{COP} = 7.5 Hz, OCH₂), 116.7 (d, ¹J_{CP} = 165.0 Hz, C-3), 118.5–135.5 (Ar), 149.3 (d, ²J_{COP} = 6.6 Hz, C-8a), 150.6 (d, ²J_{CCP} = 7.0 Hz, C-4a); HR-MS of [M+H]⁺ m/z: Cald. 415.0172, Found. 415.0179.

Compound 8cf. White solid; mp 136–138 °C; Yield: 91%; ^{31}P NMR (121.5 MHz, CDCl_3) δ 10.48 (d); ^1H NMR (300 MHz, CDCl_3) δ 1.26 (t, $^3J_{\text{HCCH}} = 7.0$ Hz, 3H, CH_2CH_3), 2.32 (s, 3H, PhCH_3), 2.43 (s, 3H, SO_2CH_3), 4.11 (m, 2H, OCH_2), 7.22–7.31 (m, 4H, Ar), 7.47–7.51 (m, 3H, Ar), 7.84–7.86 (m, 1H, Ar); ^{13}C NMR (75 MHz, CDCl_3) δ 16.21 (d, $^3J_{\text{CCOP}} = 6.0$ Hz, CH_2CH_3), 21.3 (PhCH_3), 39.6 (SO_2CH_3), 64.4 (d, $^2J_{\text{COP}} = 7.5$ Hz, OCH_2), 114.0 (d, $^1J_{\text{CP}} = 160.0$ Hz, C-3), 118.4–139.5 (Ar), 149.2 (d, $^2J_{\text{COP}} = 7.0$ Hz, C-8a), 150.1 (d, $^2J_{\text{CCP}} = 7.0$ Hz, C-4a); HR-MS of $[\text{M}+\text{H}]^+$ m/z : Cald. 395.0718, Found. 395.0725.

Compound 8df. White solid; mp 124–126 °C; Yield: 95%; ^{31}P NMR (121.5 MHz, CDCl_3) δ 8.84; ^1H NMR (300 MHz, CDCl_3) δ 1.21–1.25 (t, $^3J_{\text{HCCH}} = 7.5$ Hz, 3H, CH_2CH_3), 2.61 (s, 3H, SO_2CH_3), 4.16 (m, 2H, OCH_2), 7.24–7.31 (m, 2H, Ar), 7.51–7.60 (m, 1H, Ar), 7.84–7.87 (m, 5H, Ar); ^{13}C NMR (75 MHz, CDCl_3) δ 16.2 (d, $^3J_{\text{CCOP}} = 7.0$ Hz, CH_2CH_3), 39.8 (SO_2CH_3), 64.6 (d, $^2J_{\text{COP}} = 6.5$ Hz, OCH_2), 113.2 (C-CN), 117.3 (CN), 118.5–135.1 (Ar), 149.4 (d, $^2J_{\text{COP}} = 7.5$ Hz, C-8a), 150.4 (d, $^2J_{\text{CCP}} = 7.5$ Hz, C-4a); HR-MS of $[\text{M}+\text{H}]^+$: Cald. 406.0514, Found. 406.0510.

General procedure for the preparation of 8ag and 8bg. Anhydrous K_2CO_3 (1 mmol) was added to **6** (0.5 mmol) in 5 mL anhydrous acetonitrile, about 20 min later,

diethyl phosphorochloridate (0.6 mmol) was added dropwise in ice bath (0°C) within 30 min. After another 2 h stirring at room temperature, the solvent was removed by rotatory evaporation, and the residue was added to 15 mL of dichloromethane, washed with distilled water (10 mL), 1M HCl (10 mL × 2), distilled water (10 mL) sequentially. The organic phase was dried over MgSO₄, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel with petroleum ether/AcOEt (2:1) as eluent to give the pure compounds **8ag** and **8bg**.

Compound 8ag. Yellowish foam; Yield: 82%; ³¹P NMR (121.5 MHz, CDCl₃) δ -7.34, -7.37 (DEP-*P*), 12.97, 13.02 (P-2) (Each cluster has two peaks corresponding to the two isomers due to the chiral phosphorus). ¹H NMR (300 MHz, CDCl₃) δ 1.10 (m, 6H, 2OCH₂CH₃), 3.70 (s, 3H, OCH₃), 4.01 (m, 4H, 2OCH₂, OCH₃), 7.23–7.91 (m, 9H, Ar); ¹³C NMR (75 MHz, CDCl₃) δ 15.8(d, ³J_{CCOP} = 7.5 Hz, 2CH₂CH₃), 53.9, 54.1 (d, ²J_{COP} = 6.8 Hz, OCH₃), 64.6 (d, J_{COP} = 7.5 Hz, OCH₂), 118.5 (d, ¹J_{CP} = 165 Hz, C-3), 123.8–131.9 (Ar), 149.2 (d, ²J_{COP} = 7.5 Hz, C-8a), 150.4 (d, ²J_{CCP} = 7.5 Hz, C-4a); HR-MS of [M+H]⁺ m/z: Cald. 425.0919, Found. 425.0920.

Compound 8bg: Yellowish foam; Yield: 84%; ^{31}P NMR (121.5 MHz, CDCl_3) δ -7.28, -7.23 (DEP-P), 11.33, 11.39 ($\text{CH}_3\text{CH}_2\text{OP}(\text{O})\text{Ar}$) (Each cluster has two peaks corresponding to shows the two isomers due to the chiral phosphorus). ^1H NMR (300 MHz, CDCl_3) δ 1.16 (m, 9H, $3\text{OCH}_2\text{CH}_3$), 3.86 (m, 4H, OCH_2CH_3), 4.11 (m, 2H, OCH_2CH_3), 7.22–7.30 (m, 2H, Ar), 7.43–7.56 (m, 3H, Ar), 7.56–7.59 (m, 2H, Ar), 7.86–7.89 (m, 1H, Ar); ^{13}C NMR (75 MHz, CDCl_3) δ 15.8 (m, OCH_2CH_3), 64.8 (OCH_2), 114.4 (dd, $^1J_{\text{CP}} = 170.0$ Hz, C-3), 118.3–134.6 (Ar), 149.1 (d, $^2J_{\text{COP}} = 7.5$ Hz, C-8a), 150.2 (dd, d, $^2J_{\text{CCP}} = 8.6$ Hz, C-4a); HR-MS of $[\text{M}+\text{H}]^+$ m/z: Cald. 473.0686, Found. 473.0684.

General procedure for the preparation of 8ah and 8bh. The procedure is similar to the preparation of **8af~8df** mentioned above.

Compound 8ah. White solid; mp 138-140 °C; Yield: 97%; ^{31}P NMR (121.5 MHz, CDCl_3) δ 12.14; ^1H NMR (300 MHz, CDCl_3) δ 2.37 (s, 3H, PhCH_3), 3.67 (d, $^3J_{\text{HCO P}} = 6.1$ Hz, 3H, OCH_3), 7.03–7.91 (m, 13H, Ar); ^{13}C NMR (75 MHz, CDCl_3) δ 21.6 (ArCH_3), 54.1 (d, $^2J_{\text{COP}} = 7.5$ Hz, OCH_3), 110.1 (d, $^1J_{\text{CP}} = 165$ Hz, C-3), 118.5–132.1 (Ar), 145.2 (TsC-1), 149.2 (d, $^2J_{\text{COP}} = 6.5$ Hz, C-8a), 149.6 (d, $^2J_{\text{CCP}} = 22.0$ Hz, C-4a); HR-MS of $[\text{M}+\text{H}]^+$ m/z: Cald. 443.0718, Found. 443.0722.

Compound 8dh. White solid; mp 220-221 °C; Yield: 95%; ^{31}P NMR (243 MHz, CDCl_3) δ 9.50; ^1H NMR (600 MHz, CDCl_3) δ 1.17(t, $^3J_{\text{HCCH}} = 7.0$ Hz, 3H, CH_2CH_3), 2.45 (s, 3H, PhCH_3), 4.09 (m, 2H, OCH_2), 7.12–7.86 (m, 12H, Ar); ^{13}C NMR (150 MHz, CDCl_3) δ 16.3 (d, $^3J_{\text{CCOP}} = 5.0$ Hz, CH_2CH_3), 21.8 (PhCH_3), 64.5 (d, $^2J_{\text{COP}} = 6.0$ Hz, OCH_2), 112.2 (C-CN), 117.0 (CN), 118.3–146.1 (Ar), 149.5 (d, $^2J_{\text{COP}} = \sim 6.5$ Hz, C-8a), 151.0 (d, $^2J_{\text{CCP}} = 129.0$ Hz, C-4a); HR-MS of $[\text{M}+\text{H}]^+$ m/z: Cald. 482.0827, Found. 482.0824.

General procedure for the preparation of 9ah and 9dh. Phenylacetylene (0.45 mmol) was added to 4-tosylphosphacoumarin **8ah** or **8dh** (0.3 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (0.03 mmol), CuI (0.03 mmol), and triethylamine (0.45 mol) in dry acetonitrile (3 mL) under nitrogen atmosphere. The reaction mixture was stirred at 50 °C for 5 hours. The solution was cooled to room temperature and filtered, and the solid was washed with ethyl acetate (2×20 mL). The combined organic phase was washed with successively with distilled water (10 mL), brine (10 mL), and dried over anhydrous Na_2SO_4 . The solvent was removed under vacuum, and the residue was purified by column chromatography on silica gel with petroleum ether/AcOEt (2:1) as the eluent to give the corresponding product **9ah** or **9dh**.

9ah

Compound 9ah. Yellowish oil; Yield: 80%; ^{31}P NMR (121.5 MHz, CDCl_3) δ 9.21; ^1H NMR (300 MHz, CDCl_3) δ 3.75 (d, $^3J_{\text{HCOP}} = 5.9$ Hz, 3H, OCH_3), 7.21–8.08 (m, 14H, Ar-H); ^{13}C NMR (75 MHz, CDCl_3) δ 54.02, 54.1 (d, $^2J_{\text{COP}} = 7.5\text{Hz}$, OCH_3), 84.5 (-CC-), 100.4(C-3), 118.3–131.9 (Ar), 150.1 (d, $^2J_{\text{COP}} = 7.5$ Hz, C-8a); HR-MS of $[\text{M}+\text{H}]^+$ m/z: Cald. 373.0944, Found. 373.0941.

9dh

Compound 9dh. Yellowish oil; Yield: 77%; ^{31}P NMR (243 MHz, CDCl_3) δ 6.93; ^1H NMR (600 MHz, CDCl_3) δ 1.21–1.23 (t, $^3J_{\text{HCCH}} = 7.3$ Hz, 3H, CH_2CH_3), 4.15 (m, 2H, OCH_2), 7.30–7.50 (m, 8H, Ar), 7.70–7.76 (m, 2H, Ar), 7.89–7.91 (m, 2H, Ar), 8.1 (s, 1H, Ar); ^{13}C NMR (150 MHz, CDCl_3) δ 16.4 (d, $^3J_{\text{CCOP}} = 6.0$ Hz, CH_2CH_3), 64.2 (d, $J_{\text{COP}} = 7.5$ Hz, OCH_2), 83.9 (-CC-), 101.7 (C-3), 112.5 (CCN), 118.1–132.0 (Ar), 150.3 (d, $^2J_{\text{COP}} = 7.5$ Hz, C-8a); HR-MS of $[\text{M}+\text{H}]^+$ m/z: Cald. 412.1103, Found. 412.1100.

The preparation procedure of 9di. Phenylboronic acid (0.45 mmol) was added to 4-tosylphosphacoumarin **8dh** (0.3 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (0.03 mmol) and

triethylamine (0.60 mol) was dissolved in dry THF (3 mL) under nitrogen atmosphere. The reaction mixture was stirred at 60 °C for 5 hours, and the solution was cooled to room temperature and filtered, and the solid was washed with ethyl acetate (2 × 20 mL). The combined organic phase was washed with successively with distilled water (10 mL), brine (10 mL), and dried over anhydrous Na₂SO₄. The solvent was removed under vacuum, and the residue was purified by column chromatography on silica gel with petroleum ether-AcOEt (2:1) as the eluent to give the corresponding product **9di**.

White foam; Yield: 75%; ³¹P NMR (243 MHz, CDCl₃) δ 7.90; ¹H NMR (600 MHz, CDCl₃) δ 1.20 (t, ³J_{HCH} = 6.3 Hz, 3H, CH₂CH₃), 4.13 (m, 2H, OCH₂), 7.00–7.45 (m, 13H, Ar); ¹³C NMR (150 MHz, CDCl₃) δ 16.34 (d, ³J_{CCOP} = 6.0 Hz, OCH₂CH₃), 64.20 (d, ²J_{COP} = 6.0 Hz, OCH₂), 111.3 (CN), 116.2 (d, ¹J_{CP} = 150 Hz, C-3), 118.6–135.6 (Ar), 150.7 (C-4), 151.8 (C-8a); HR-MS of [M+H]⁺ m/z: Cald. 388.1103, Found. 388.1107.

PTP assay. The catalytic domain of SHP-1, SHP-1 (ΔSH2), was purified from a recombinant *Escherichia coli* strain as previously described.¹ All assays were carried out in 96-well microplates (Greiner) in reaction mixtures (50 μL) containing 100 mM HEPES (PH 7.0), 5 mM EDTA, 0.1 mM DTT, 0.1 mg/mL BSA, 10% (v/v) DMSO, varying concentrations of the phosphacoumarins (between 30 mM and 30 nM), and

0.08–0.8 μM (typically 0.8 μM) SHP-1. The mixture was incubated at 37 °C for 60 min, and the enzymatic reaction was then initiated by the addition of 5 μL of 100 mM pNPP. After 3 min at 37 °C, the enzyme reaction was quenched by addition of 1 M NaOH (150 μL) and the absorbance at 405 nm was measured to determine the amount of *p*-nitrophenolate released. The K_i values were obtained by fitting the data to the Michaelis-Menten equation.² All these values were usually derived from more than triple experiments using a range of phosphacoumarin concentrations.

References

- (1) Pei, D.; Neel, B. G.; Walsh, C. T. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *91*, 1092.
- (2) Fu, H.; Park, J. Pei, D. *Biochem.* **2002**, *41*, 10700.

single pulse decoupled, gated NMR