High-Yielding Staudinger Ligation of a Phosphinothioester and Azide to Form a Peptide

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General Experimental

Chemicals and solvents were purchased from Aldrich®, with the exception of Merrifield resins (Novabiochem®). Merrifield resins used were 200–400 mesh (substitution 0.63 mmol/g) and 70–90 mesh (1.26 mmol/g). Reactions were monitored by thin-layer chromatography using Whatman® TLC plates (AL SIL G/UV) with visualization by illumination with ultraviolet light or staining with I_2. NMR spectra were obtained with Bruker AC-300 and Varian UNITY-500 spectrometers. Phosphorus-31 NMR spectra were proton-decoupled and referenced against an external standard of deuterated phosphoric acid. Mass spectra were obtained with electrospray ionization (ESI) techniques at the University of Wisconsin Biotechnology Center.

Phosphine oxide 4

Chloromethylphosphonic dichloride (20 g, 120 mmol) was dissolved in freshly distilled THF (240 mL). A solution of phenylmagnesium bromide (1.0 M) in THF (240 mL, 240 mmol) was added dropwise over 1 h. The resulting mixture was stirred at reflux for 24 h. The reaction was then quenched by the addition of water (20 mL), and solvent was removed under reduced pressure. The residue was taken up in CH_2Cl_2 and washed once with water (50 mL) and once with brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$(s) and filtered, and solvent was removed under reduced pressure. The residue was purified by flash chromatography (silica gel, 3% methanol in methylene chloride). Phosphine oxide **4** was isolated as a white solid in 63% yield. **Spectral data.** ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.84–7.79 (m, 4 H), 7.62–7.58 (m, 2

H), 7.54–7.50 (m, 4 H), 4.05 (d, *J* = 7 Hz, 2 H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 132.60, 131.51 (d, *J* = 9.6 Hz), 129.64 (d, *J* = 103.9 Hz), 128.72 (d, *J* = 11.6 Hz), 37.64 (d, *J* = 71.9 Hz) ppm; ^{31}P NMR ($CDCl_3$, 202 Hz) 28.46 ppm; MS (ESI) *m/z* 250.03 (MH^+ = 251.0, M_2H^+ = 501.2 fragments at 173.0, 143.0, 91.0).

Compound 5

Phosphine oxide **4** (18.94 g, 75.6 mmol) was dissolved in THF (0.45 L). Thioacetic acid (34.3 mL, 480 mmol) was added, and the resulting solution was cooled in an ice bath. Ar(g) was bubbled through the reaction mixture for 1 h. Diisopropylethyl amine (83.6 mL, 480 mmol) was added dropwise, and the resulting mixture was heated at reflux for 24 h. Another aliquot of thioacetic acid (35.2 mL, 492 mmol) was then added, followed by triethyl amine (69.0 mL, 492 mmol). The reaction mixture was heated at reflux for another 24 h, after which solvent was removed under reduced pressure in a well-ventilated hood (stench!). The resulting black oil was dissolved in methylene chloride (0.35 L), and this solution was washed with 2 N HCl (0.15 L), saturated sodium bicarbonate solution (0.15 L), and brine (0.15 L). The organic layer was dried over anhydrous $MgSO_4$(s) and filtered. Activated charcoal was added to this solution, which was then heated at reflux for 30 min. The activated charcoal was removed by filtration, solvent was removed under reduced pressure, and the residue was purified by flash chromatography (silica gel, 70% ethyl acetate in hexanes). The pooled fractions were dissolved in methylene chloride (0.30 L), and the treatment with activated charcoal was repeated. Upon solvent removal, thioacetate **5** was isolated as an orange oil that solidified upon standing at room temperature. The yield for this reaction was 85%. **Spectral data.** ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.80–7.75 (m, 4 H), 7.56–7.52 (m, 2 H), 7.49–7.46 (m, 4 H), 3.77 (d, *J* = 8 Hz, 2 H), 2.25 (s, 3 H) ppm; ^{13}C NMR

($CDCl_3$, 125 MHz) δ 192.82, 132.11, 131.05 (d, *J* = 102 Hz), 130.86 (d, *J* = 9.75 Hz), 128.46 (d, *J* = 12.63 Hz), 29.83, 27.12 (d, *J* = 69.88 Hz) ppm; ^{31}P NMR ($CDCl_3$, 202 MHz) δ 29.14 ppm; MS (ESI) *m/z* 290.05 (MH^+ = 291.0, M_2H^+ = 581.2, fragments at 249.2, 171.0, 125.0).

Phosphine 6

Thioacetate **5** (18.65 g, 64.2 mmol) was dissolved in anhydrous chloroform (160 mL). To this solution was added trichlorosilane (97 mL, 963 mmol), and the mixture was stirred under Ar(g) for 72 h. Solvent was removed under reduced pressure (note: excess trichlorosilane in the removed solvent was quenched by slow addition of saturated sodium bicarbonate solution in a well-ventilated hood), and the residue was purified by flash chromatography (silica gel, 3% methanol in methylene chloride). Phosphine **6** was isolated as a white solid in 98% yield. **Spectral data.** ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.43–7.40 (m, 4 H), 7.33–7.30 (m, 6 H), 3.50 (d, *J* = 4 Hz, 2 H), 2.23 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.01, 136.42 (d, *J* = 13.6 Hz), 132.28 (d, *J* = 19.4 Hz), 128.69, 128.11 (d, *J* = 6.8 Hz), 29.83, 25.41 (d, *J* = 23.4 Hz) ppm; ^{31}P NMR ($CDCl_3$, 202 MHz) –15.11 ppm; MS (ESI) *m/z* 274.06 (MH^+ = 275.0, fragments at 233.0, 199.2, 121.2).

(Diphenylphosphino)methanethiol (2)

Phosphine **6** (17.27 g, 63.0 mmol) was dissolved in anhydrous methanol (0.40 L), and Ar(g) was bubbled through the solution for 1 h. Sodium hydroxide (5.04 g, 126 mmol) was then added, and the mixture was stirred under argon for 2 h. Solvent was removed under reduced pressure, and the residue was dissolved in methylene chloride (0.30 L). The resulting solution was washed with 2 N HCl (2 × 0.10 L) and brine (0.10 L). The organic layer was dried over $MgSO_4$(s) and

filtered, and solvent was removed under reduced pressure. The residue was purified by flash chromatography (alumina, 25% ethyl acetate in hexanes). (Diphenylphosphino)methanethiol **2** was isolated as a clear oil in 74% yield. **Spectral data.** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.41–7.38 (m, 4 H), 7.33-7.26 (m, 6 H), 3.02 (d, *J* = 7.8 Hz, 2 H), 1.38 (t, *J* = 7.5 Hz, 1 H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 132.54 (d, *J* = 17.1 Hz), 128.86, 128.36, 128.14, 20.60 (d, *J* = 21.7 Hz) ppm; ^{31}P NMR ($CDCl_3$, 121 MHz) δ –7.94 ppm; MS (ESI) *m/z* 232.05 (MH^+ = 233.0, fragments at 183.0, 155.0, 139.0, 91.2).

AcPheSCH$_2$PPh$_2$ (Table 1)

Method A (transthioesterification). Phosphinothiol **2** (500 mg, 2.2 mmol) was dissolved in dry THF (5 mL). The solution was deoxygenated by bubbling Ar(g) for 0.5 h. To this solution was added NaH (51.6 mg, 2.2 mmol). The mixture formed a slurry to which was added DMF (2 mL) to dissolve any precipitate. The N-methylmercapoacetamide (NMA) thioester of N-acetylphenylalanine (63 mg, 0.22 mmol) was added, and the reaction mixture was stirred for 8 h. Unreacted phophinothiol **2** was removed by adding Merrifield resin (1.5 g, 1.26 mmol/g), stirring for 6 h, and removing the resin by filtration. The residue was purified by flash chromatography (silica gel, 50% ethyl acetate in hexanes). AcPheSCH$_2$PPh$_2$ was isolated as a white solid in 92% yield.

Method B (DCC coupling). Compound **2** (500 mg, 2.15 mmol) and N-acetylphenylalanine (446 mg, 2.15 mmol) were dissolved in DMF (15 mL) under Ar(g). 1,3-Dicyclohexylcarbodiimide (DCC; 489 mg, 2.37 mmol) was then added, and the reaction mixture was stirred for 12 h at room temperature. The 1,3-dicyclohexylurea (DCU) by-product was removed by filtration, solvent was removed under reduced pressure, and the residue was

purified by chromatography as in Method A. AcPheSCH$_2$PPh$_2$ was isolated as a white solid in 84% yield.

Spectral data. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44–7.39 (m, 4 H), 7.35–7.33 (m, 6 H), 7.26–7.21 (m, 3 H), 7.11–7.09 (m, 2 H), 6.29 (d, *J* = 8.4 Hz, 1 H), 4.98–4.91 (m, 1 H), 3.57–3.44 (m, 2 H), 3.09 (dd, *J* = 14.1, 5.4 Hz, 1 H), 2.93 (dd, *J* = 14.1, 7.5 Hz, 1 H) 1.88 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 198.91, 169.86, 135.50, 132.62 (d, *J* = 19.4 Hz), 129.11 (d, *J* = 9.8 Hz) 128.79 (d, *J* = 35.9 Hz), 128.50, 128.45, 126.99, 59.56, 37.99, 25.61 (d, *J* = 24.4 Hz), 22.88 ppm; ^{31}P NMR ($CDCl_3$, 121 MHz) –44.66 ppm.

AcGlyCH$_2$PPh$_2$ (Table 1)

Method A. Phosphinothiol **2** (500 mg, 2.2 mmol) was dissolved in 5 mL of dry THF. The solution was deoxygenated by bubbling Ar(g) for 0.5 h. To this solution was added NaH (51.6 mg, 2.2 mmol). The mixture forms a slurry to which is added DMF (2 mL) to dissolve any precipitate. The NMA thioester of N-acetylglycine (44 mg, 0.22 mmol) was added, and the reaction mixture was stirred for 8 h. Unreacted phosphinothiol **2** was removed by adding Merrifield resin (1.5 g, 1.26 mmol/g), stirring for 6 h, and removing the resin by filtration. The residue was purified by flash chromatography (silica gel, 50% ethyl acetate in hexanes). AcGlyCH$_2$PPh$_2$ was isolated as a white solid in 91% yield.

Method B. Phosphinothiol **2** (100 mg, 0.43 mmol) and N-acetylglycine (55 mg, 0.47 mmol) were dissolved in DMF (3 mL) under Ar(g). DCC (98 mg, 0.47 mmol) was added, and the mixture was stirred for 12 h at room temperature. The DCU by-product was removed by filtration, solvent was removed under reduced pressure, and the residue was purified by chromatography as in Method A. AcGlyCH$_2$PPh$_2$ was isolated as a white solid in 67% yield.

Spectral data. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46–7.39 (m, 4 H), 7.38–7.36 (m, 6 H), 6.44 (bs, 1 H), 4.15 (d, *J* = 5.7 Hz, 2 H), 3.53 (d, *J* = 3.6 Hz, 2 H), 2.02 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 196.13, 170.29, 136.45 (d, *J* = 13.6 Hz), 132.62 (d, *J* = 19.1 Hz), 129.17, 128.54 (d, *J* = 6.7 Hz), 48.98, 25.29 (d, *J* = 24.2 Hz), 22.84 ppm; ^{31}P NMR ($CDCl_3$, 121 MHz) δ –15.20 ppm; MS (ESI) *m/z* 331.08 (MH^+ = 332.2, MK^+ = 370.0).

AcGlyNHBn (representative Staudinger ligation)

Thioester $AcSCH_2PPh_2$ (271 mg, 0.99 mmol) and azide N_2GlyNHBn (187 mg, 0.99 mmol) were dissolved in THF/H_2O (3:1, 9.4 mL), and the mixture was stirred at room temperature for 12 h. Solvent was removed under reduced pressure, and the residue was purified by flash chromatography (silica gel, 5% methanol in methylene chloride). AcGlyNHBn was obtained as a white solid in 91% yield. **Spectral data.** ^{1}H NMR ($CDCl_3$/CD_3OD, 1:1, 300 MHz) δ 7.32–7.24 (m, 5 H), 4.40 (s, 2 H), 3.88 (s, 2 H), 2.65 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$:CD_3OD, 1:1, 125 MHz) δ 171.76, 169.37, 137.49, 127.83, 126.77, 126.59, 42.50, 42.09, 21.32 ppm; MS (ESI) *m/z* 206.11 (MH^+ = 207.0).

Other Information

Experimental and spectral information for the other amide products and for the NMA thioesters can be found in the Supporting Information of ref 8b: Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939–1941.