Staudinger Ligation: A Peptide from a Thioester and Azide

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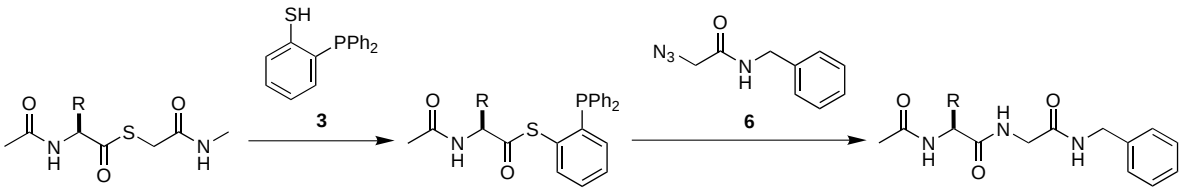
General Experimental

Chemicals and solvents were from Aldrich®, with the exception of *N*-methylmercaptoacetamide (Fluka®), bromoacetyl bromide (Acros®), and Merrifield resins (Novabiochem®). Merrifield resins (chloromethylpolystyrene-divinylbenzene) were 200–400 mesh (substitution 0.63 mmol/g) and 70–90 mesh (1.26 mmol/g). The progress of reactions was 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 using electrospray ionization (ESI) techniques at the University of Wisconsin Biotechnology Center.



Thioesters 1 and 2

An *N*-acetyl amino acid (*N*-acetyl glycine or *N*-acetyl phenylalanine) and one equivalent of *N*-methylmercaptoacetamide (NMA) were charged to a flame-dried reaction vessel under an argon atmosphere and dissolved in dry DMF to a final concentration of 0.5–0.7 M. 1,3-Dicyclohexylcarbodiimide (DCC; 1.1 equivalents) was added, and the resulting mixture was stirred at room temperature for 10–12 h. The 1,3-dicyclohexylurea (DCU) by-product was removed by filtration, and solvent was removed under reduced pressure. Products were recrystallized from CH_2Cl_2 and hexanes. Thioester **1** was obtained in a 90% yield, and thioester **2** was obtained in a 92% yield.

Thioester 1. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.62 (t, *J* = 6 Hz, 1 H), 8.05 (bs, 1 H), 4.00 (d, *J* = 6 Hz, 2 H), 3.56 (s, 2 H), 2.59 (d, *J* = 4.5 Hz, 3 H), 1.93 (s, 3 H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz) δ 197.95, 170.07, 167.13, 48.54, 31.81, 25.84, 22.26 ppm; MS (ESI) *m/z* 204.25 (M^+ = 204.9, fragments at 105.9, 100.0, 72.0).

Thioester 2. ^{1}H NMR ($CDCl_3$:CD_3OD, 1:1, 500 MHz) δ 7.30–7.27 (m, 2 H), 7.24–7.19 (m, 3 H), 4.79 (dd, *J* = 10, 5 Hz, 1 H), 3.57 (apparent q, *J* = 15 Hz, 2 H), 3.24 (ABX, *J* = 14, 5 Hz, 1 H), 2.91 (ABX, *J* = 14, 10 Hz, 1 H), 2.76 (s, 3 H), 1.95 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$:CD_3OD, 1:1, 125 MHz) δ 200.26, 172.83, 169.92, 136.93, 129.47, 128.98, 127.40, 61.24, 37.73, 32.72, 26.72, 22.40 ppm; MS (ESI) *m/z* 294.37 (MH^+ = 295.0 fragments at 190.0, 162.2, 120.2).

Phosphinothiol 3

Phosphinothiol **3** was prepared by the method of Block and coworkers, and NMR data (^{1}H and ^{31}P) correlated with their published data.[1]

Additional spectral data. ^{13}C NMR ($CDCl_3$, 125 MHz) δ 137.71 (d, *J* = 30 Hz), 135.93 (d, *J* = 8.75 Hz), 135.35 (d, *J* = 9.75 Hz), 133.98, 133.83, 130.45, 129.25, 129.00, 128.67 (d, *J* = 6.75 Hz), 125.92 ppm; MS (ESI) *m/z* 294.35 (MH^+ = 295.0).

Thioesters 4 and 5

Method A (transthioesterification). Thioester **1** or **2** (1 equivalent) and phosphinothiol **3** (10 equivalents) were charged to a flame-dried reaction vessel under an argon atmosphere and dissolved in dry DMF (0.25 M). Dry argon was bubbled through the mixture, and diisopropylethylamine (DIEA, 5 equivalents) was added. The resulting mixture was stirred for 12 h, after which additional DIEA (5 equivalents) was added. Merrifield resin (either high or low loading capacity) having a loading capacity at least equivalent to the molar amount of phosphinothiol **3** was added to the mixture to remove excess phosphinothiol **3** and NMA. The resulting slurry was stirred for an additional 12 h under argon, and the resin was removed by filtration. Solvent was removed under reduced pressure, the residue was taken up in CH_2Cl_2, and the insoluble DIEA salts were removed by filtration. Solvent was again removed, and the residue

(1) Block, E.; Ofori-Okai, G.; Zubieta, J. *J. Am. Chem. Soc.* **1989**, *111*, 2327–2329.

was used in the subsequent coupling reaction without further purification. The reaction appeared to proceed in quantitative yield, as judged by TLC.

Method B (DCC coupling). Thioester **1** or **2** (1 equivalent) and phosphinothiol **3** (1 equivalent) were added to a flame-dried reaction vessel under an argon atmosphere. DCC (1.1 equivalents) was added, and the mixture was stirred for 12 h. The DCU by-product was removed by filtration, and solvent was removed under reduced pressure. Thioesters **4** and **5** were purified by flash chromatography (silica gel, ethyl acetate:hexanes 1:1 followed by 100% ethyl acetate). Thioester **4** was obtained in 61% yield, and thioester **5** was obtained in 52% yield.

Thioester 4. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.48 (ddd, *J* = 5.5, 4, 1.5 Hz, 1 H), 7.41 (td, *J* = 7.5, 1.5 Hz, 1 H), 7.37–7.32 (m, 7 H), 7.28–7.24 (m, 4 H), 6.92 (ddd, *J* = 7.5, 3, 1.5 Hz, 1 H), 5.86 (bs, 1 H), 4.07 (d, *J* = 6 Hz, 2 H), 2.02 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.83, 170.63, 144.04, 138.00, 136.68 (d, *J* = 10.75 Hz), 134.84, 134.69, 130.95, 130.30, 129.68, 129.34 (d, *J* = 6.88 Hz), 49.78, 23.69 ppm; ^{31}P NMR ($CDCl_3$, 202 Hz) –9.91 ppm; MS (ESI) *m/z* 393.44 (MH^+ = 394.2, fragments at 295.2, 225.2).

Thioester 5. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44 (ddd, *J* = 7.5, 4, 1.5 Hz, 1 H), 7.40 (td, *J* = 7.5, 1.5 Hz, 1 H), 7.36–7.31 (m, 7 H), 7.28–7.21 (m, 7 H), 7.12–7.10 (m, 2 H), 6.89 (ddd, *J* = 8, 3, 1 Hz, 1 H), 5.63 (d, *J* = 13.5 Hz, 1 H), 4.92 (m, 1 H), 2.95 (ABX, *J* = 14.5, 5.5 Hz, 1 H), 2.64 (ABX, *J* = 14, 8 Hz, 1 H), 1.91 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 197.47, 170.43, 137.94, 136.40, 134.83, 134.75, 134.67, 134.58, 130.79, 130.30, 129.94, 129.64 (d, *J* = 5.9 Hz),

129.33, 127.77, 60.29, 38.24, 23.79 ppm; ^{31}P NMR ($CDCl_3$, 202 Hz) –10.33 ppm; MS (ESI) *m/z* 483.56 (MH^+ = 484.2, fragment at 295.2).

Azide 6

Benzyl amine (20.4 mL, 186 mmol) and methylene chloride (186 mL) were added to a flame-dried reaction vessel under an argon atmosphere and the solution was cooled to 0 °C in an ice bath. Bromoacetyl bromide (8.1 mL, 93 mmol) was added dropwise to the solution. A precipitate, presumably the HBr salt of benzyl amine, formed almost immediately. The reaction mixture was warmed to room temperature and stirred for 1 h. The precipitate was removed by filtration, and the organic phase was washed with 2 N HCl (2 × 75 mL). The organic layer was dried over anhydrous magnesium sulfate and filtered, and solvent was removed under reduced pressure. The resulting white solid was dissolved in THF (200 mL) and water (50 mL). Sodium azide (30.3 g, 466 mmol) was added, and the resulting mixture was stirred vigorously at reflux for 17 h. The organic layer was then separated from the aqueous layer, washed with a solution of saturated brine (2 × 75 mL), dried over anhydrous magnesium sulfate, and filtered. Solvent was removed under reduced pressure. Azide **6** was isolated in 98% yield, and was used without further purification.

Spectral data. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.39–7.27 (m, 5 H), 6.71 (bs, 1 H), 4.47 (d, *J* = 5.7 Hz), 4.00 (s, 2 H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.66, 137.39, 128.43, 127.45, 127.35, 52.06, 43.08 ppm; MS (ESI) *m/z* 190.20 (MH^+ = 191.0, fragment at 91.2).

Amides 7 and 8

Thioester **4** or **5** (1 equivalent) and azide **6** (1 equivalent) were dissolved in THF:H_2O (3:1) to a concentration of 0.2 M. A yellow color, presumably from liberated thiolate, formed quickly. The mixture was stirred at room temperature for 12–16 h, and then acidified with 2 N HCl until the yellow color became clear. Solvent was removed under reduced pressure, and the amide products were separated from the phosphine oxide by-products (which was characterized spectrally; data not shown) by flash chromatography (silica gel, 2.5–10% methanol in methylene chloride). Yields for amide **7** and amide **8** ranged from 15 to 35%.

Amide 7. ^{1}H NMR ($CDCl_3$:CD_3OD, 1:1, 500 MHz) δ 7.33–7.22 (m, 5 H), 4.41 (s, 2 H), 3.92 (s, 2 H), 3.86 (s, 2 H), 2.01 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$:CD_3OD, 1:1, 125 MHz) δ 173.56, 171.52, 170.67, 138.83, 129.04, 218.02, 127.78, 43.70, 43.62, 43.16, 22.45 ppm; MS (ESI) *m/z* 263.29 (MH^+ = 264.0).

Amide 8. ^{1}H NMR ($CDCl_3$:CD_3OD, 1:1, 500 MHz) δ 7.32–7.19 (m, 10 H), 4.48 (apparent t, *J* = 7.5 Hz, 1 H), 4.44 (d, *J* = 15 Hz, 1 H), 4.34 (d, *J* = 14.5 Hz, 1 H), 3.95 (d, *J* = 16.5 Hz, 1 H), 3.71 (d, *J* = 16.5 Hz, 1 H), 3.11 (dd, *J* = 13.5, 7 Hz, 1 H), 2.94 (dd, *J* = 14, 8 Hz, 1 H), 1.88 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$:CD_3OD, 1:1, 125 MHz) δ 173.42, 172.81, 170.34, 138.61, 137.18, 129.55, 129.01, 128.93, 127.86, 127.68, 127.40, 56.12, 43.53, 43.18, 37.76, 22.36 ppm; MS (ESI) *m/z* 353.42 (MNa^+ = 376.2, MH^+ = 354.2 fragments at 165.2, 120.2, 91.2).