SUPPORTING INFORMATION

Exploiting Site-Site Interactions on Solid Support to Generate Dimeric Molecules

Helen E. Blackwell, Paul A. Clemons, and Stuart L. Schreiber*

Howard Hughes Medical Institute, Department of Chemistry and Chemical Biology, Harvard Institute of Chemistry and Cell Biology (ICCB), Harvard University, Cambridge, Massachusetts 02138

e-mail: sls@slsiris.harvard.edu

General.. Reagents were obtained from Aldrich Chemical Co., Acros, Novabiochem, or J. T. Baker and used without further purification. Reaction solvents (THF, DMF, and CH_2Cl_2) were obtained from J. T. Baker (HPLC grade), and purified by passage through two solvent columns prior to use.[1] Diisopropylethylamine (DIPEA) and 2,6-lutidene were distilled from calcium hydride. Brominated polystyrene (Br-PS, 2 meq/g) was obtained from Polymer Labs, and functionalized with the silicon-based linker according to the reported protocol.[2] Ruthenium benzylidenes **3a-b** were purchased from Strem Chemical and handled in a Vacuum Atmospheres inert gas glove box.

Solid Phase Reactions. Unless otherwise noted, small-scale solid phase reactions (5-10 mg resin) were performed in 500 μL polypropylene Eppendorf tubes with mixing provided by a VWR Vortex Genie-2 vortexer fitted with a 60 microtube insert. Larger-scale solid phase reactions (20-500 mg resin) were performed in 2 mL fritted polypropylene Bio-Spin® chromatography columns (BioRad) or 10 mL fritted polypropylene PD-10 columns (Pharmacia Biotech) with 360° rotation on a Barnstead-Thermolyne Labquake™ shaker.

After solid phase reactions, resin samples were transferred to 2 mL BioSpin® columns. Resin samples in polypropylene columns were washed on a Vac-Man® laboratory vacuum manifold (Promega) fitted with nylon 3-way stopcocks (Biorad). The following standard wash procedure was used: 3 x THF, 3 x DMF. 3 x THF, 3 x CH_2Cl_2.

For cleavage, resin samples were transferred *via* spatula to 500 μL Ependorf tubes and suspended in dry, degassed THF followed by pryidine and hydrogen fluoride-pyridine (Aldrich. HF(70%)/pyridine(30%)) in a ratio of 90:5:5. Samples were then sealed with parafilm and placed on a vortexer for 90 min at room temperature. Methoxytrimethylsilane (TMSOMe, Aldrich) was added to quench the excess HF. The samples were resealed with parafilm and placed on a vortexer for an additional 30 min. The beads were filtered through a Pasteur pipette

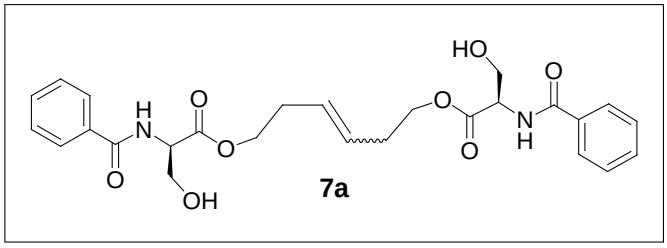
(1) The CH_2Cl_2 purification system is composed of one activated alumina (A-2) column and one supported copper redox catalyst (Q-5 reactant) column. The THF purification system is composed of two activated alumina (A-2) columns, and the DMF purification system is composed of two activated molecular sieve columns. See: Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.

(2) For a full description of our bead-linker technology platform, including standard procedures for loading substrates onto the resin, see: Tallarico, J. A.; Depew, K. M.; Pelish, H. E.; Westwood, N. J.; Lindsley, C. J.; Shair, M. D.; Schreiber, S. L.; Foley, M. A., submitted.

(plugged with glass wool) and washed 4 x with purified CH_2Cl_2. The supernatant fluid was collected in a glass vial and concentrated *in vacuo*.

Purification and Analysis. All intermediates were characterized by ^{1}H NMR, LC-MS, TLC, and/or LRMS. Flash chromatography was performed on E. Merck 60 230-400 mesh silica gel. TLC was performed on 0.25 mm E. Merck silica gel 60 F_{254} plates and visualized by UV (254 nm) and cerium ammonium molybdate. HPLC was performed on a Thermo Separation Product instrument equipped with a Vydac C18 100 Å 3 μ 4.6 mm x 6 cm column using a flow rate of 3 mL/min and a 12 min gradient of 0-99.9% CH_3CN in water/0.1% TFA, constant 0.1% MeOH with diode array UV detection. NMR spectra were recorded on a Varian Inova 400 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal solvent. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). The reported ^{1}H NMR data refer to the major olefin isomer. The reported ^{13}C NMR data include all peaks observed and no peak assignments were made. Mass spectra were obtained on JEOL AX-505H or SX-102A mass spectrometers by chemical ionization (CI) with ammonia (NH_3) or fast atom bombardment ionization (FAB) with glycerol or 3-nitrobenzyl alcohol/sodium iodide (NBA/NaI) matrices.

General Solid Phase "Intra-site" Olefin Cross-Metathesis Procedure. Solid phase-bound substrates (~1 meq/g resin) were dried prior to use for at least 24 h on a high-vacuum manifold. A resin sample was weighed out quickly in the atmosphere, placed in an oven-dried round-bottom flask, capped with a septum, and rigorously purged with Ar at room temperature. While under Ar, the resin was pre-swelled in dry CH_2Cl_2 (3/4 total final volume of CH_2Cl_2, final concentration of bound substrate = 0.03M) for at least 15 min prior to addition of ruthenium benzylidene **3a** or **3b**. Ruthenium benzylidene **3a** or **3b** (2.5 mol % relative to resin-bound substrate) was weighed out into an oven-dried round-bottom flask in a glove box, capped with a septum, and removed. Under Ar, dry CH_2Cl_2 was added to the flask containing the catalyst *via* syringe (1/8 total final volume of CH_2Cl_2, final concentration of bound substrate = 0.03M) and the flask was swirled lightly to dissolve the catalyst. The catalyst solution was immediately transferred to the flask containing the pre-swelled resin *via* cannula. The reaction flask was quickly equipped with an oven-dried reflux condenser, purged with Ar, and heated in an oil bath at 40 °C for 9 h under Ar. After 9 h, another 2.5 mol % aliquot of benzylidene **3a** or **3b** (prepared as above, 1/8 total final volume of CH_2Cl_2, final concentration of bound substrate = 0.03M) was added to the flask, and the flask was heated at 40 °C for another 9 h under Ar. (*In order to maintain a constant solvent volume for small scale reactions, it was essential to grease the round-bottom ground glass joint and seal the round-bottom/condenser connection with Teflon tape.*) The reaction mixture was then allowed to come to room temperature and filtered directly through a 2 mL BioSpin® column. The collected resin was rigorously washed in the BioSpin® column with solvents as described above. The pale brown resin was then dried on house vacuum for 16 h, and subsequently stored at room temperature in a sealed BioSpin® column or vial.



7a

Compound 7a. The 500-600 μm, linker-derivatized PS resin (1.29 g, 1.0 meq/g, 1.29 mmol bound linker) was dried prior to use for 24 h on a high vacuum manifold. FMOC-*N*-L-serine ω-butenyl ester **1a** (1.23 g, 3.23 mmol, 2.5 equiv)[3] was loaded onto the PS resin through its primary alcohol by displacement of an activated silyl triflate element on the linker, in the presence of excess 2,6-lutedine in CH_2Cl_2.[2] After rigorous washing employing the standard solvent wash procedure described above, monomer-derivatized resin **2a** was dried under house vacuum for 24 h. A portion of resin **2a** (4.7 mg, 0.99 meq/g,[4] 4.7 μmol bound substrate) was then subjected to the standard solid-phase "intra-site" cross-metathesis procedure described above. After cross-metathesis, resin **2a** (4.7 mg, 0.99 meq/g, 4.7 μmol bound substrate) was placed in a BioSpin® column and treated with 20% piperidine/DMF (500 μL) to effect FMOC deprotection. The column was capped with a plastic cap and tumbled on a Labquake™ shaker for 30 min at room temperature, after which the DMF solution was drained and the procedure was repeated. The resin was then rigorously washed employing the standard solvent wash procedure described above, and dried on house vacuum for 18 h.

The resin sample (4.7 mg, 0.99 meq/g, 4.7 μmol bound substrate) was placed in a new BioSpin® column, capped with a septum, and purged with N_2. Dry CH_2Cl_2 (50 μL) was added to the column *via* syringe, and the resin was allowed to pre-swell for 15 min at room temperature. Benzoic acid (22 mg, 0.18 mmol, 38 equiv) was weighed out into a oven-dried glass vial, capped with a septum, and purged with N_2. Dry CH_2Cl_2 (100 μL) was added to the vial *via* syringe and the vial was swirled to facilitate dissolution. Diisopropylcarbodiimide (DIC, 28 μL, 0.18 mmol, 38 equiv) was added to the vial *via* syringe. A white precipitate (urea) formed over a period of 3-4 min. This thick solution was immediately transferred to the column containing the resin *via* syringe. Freshly-distilled DIPEA (52 μL, 0.3 mmol, 64 equiv) was added to the column *via* syringe. The septum was quickly removed and DMAP (3.6 mg, 0.03 mmol, 6 equiv) was added with a spatula. The column was purged with N_2, capped with a plastic cap, sealed with parafilm, and tumbled on a Labquake™ shaker for 16 h at room temperature. The reaction mixture was then drained from the column. The pale yellow resin was washed employing the standard solvent wash procedure described above, and dried on house vacuum for 18 h.[5] A 2.0 mg aliquot of the resin was subjected to standard HF cleavage, and a light yellow oil (**7a**) was isolated.

---

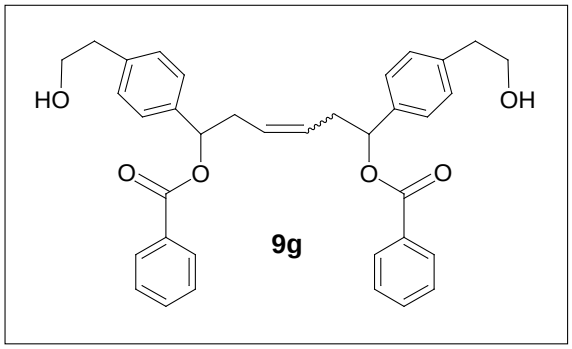
(3) Prepared *via* a PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate) coupling between FMOC-*N*-L-serine and 3-buten-1-ol in DMF. See: Coste, J.; Lenguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205-208.

(4) Resin loading determined by cleavage of the FMOC group from an aliquot of resin **2a** with 20% piperidine/DMF and quantitation of the released fulvene product by UV spectroscopy. See: Atherton, E.; Sheppard R. C. *Solid Phase Peptide Synthesis: A Practical Approach*; IRL Press: Oxford, 1989.

(5) This coupling procedure is based upon an earlier protocol developed in our laboratory for 90 μm TentaGel resin. See: Tan, D. S.; Foley, M. A.; Stockwell, B. R.; Shair, M. D.; Schreiber, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 9073-9087.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.82 (4H, m), 7.52 (2H, m), 7.47 (4H, m), 7.19 (2H, m), 5.53 (2H, m), 4.87 (2H, m), 4.31-4.21 (4H, br m), 4.11-4.07 (4H, br m), 2.47 (2H, app q, J = 7 Hz), 2.38 (2H, app q, J = 6 Hz), 2.02 (2H, br s); ^{13}C NMR (100 MHz, CDCl3): δ 178.1, 175.2, 161.6, 160.9, 128.7, 68.8, 67.7, 61.2, 59.2, 33.5, 29.9, 29.0, 28.0, 24.1, 22.4, 21.9; IR (thin film, cm^{-1}): 3465, 3196, 2860, 1772, 1460, 1420, 1349, 1319, 1175, 1114, 1070, 992, 958, 931; TLC R_f = 0.55 (67% EtOAc/Hexane); LRMS (FAB) calcd for $C_{26}H_{30}N_2O_8$ $[M+H]^+$ 499.2, found 499.0.

Representative Procedure for Library Compound Synthesis:



Compound 9g. The 500-600 μm, linker-derivatized PS resin (779 mg, 1.39 meq/g, 1.08 mmol bound linker) was dried prior to use for 24 h on a high vacuum manifold. 4-(2-hydroxyethyl)-benzaldehyde (538 mg, 3.25 mmol, 3.0 equiv) was loaded onto the PS resin through its primary alcohol by displacement of an activated silyl triflate element on the linker, in the presence of excess 2,6-lutedine in CH_2Cl_2.[2] After rigorous washing employing the standard solvent wash procedure described above, the pale yellow, aldehyde-derivatized resin was dried under house vacuum for 24 h.

A portion of the aldehyde-derivatized resin (100 mg, 1.39 meq/g, 0.14 mmol bound substrate) was placed in an oven-dried round-bottom flask, capped with a septum, and purged with N_2. Dry THF (696 μL) was added to the flask *via* syringe and the resin was allowed to pre-swell for 15 min at room temperature. A solution of allyl magnesium bromide in diethyl ether (626 μL, 1.0 M in diethyl ether, 0.63 mmol, 4.5 equiv) was added drop-wise to the flask *via* syringe over 2-3 min. The solution gradually became opaque and pale yellow in color. The reaction was allowed to proceed at room temperature for 18 h. The reaction was then quenched by the slow addition of 1.0 mL 1:1 NH_4Cl aq. (saturated)/H_2O to the reaction flask. The reaction mixture was swirled periodically over a 1 h time period, and the resin was then collected by filtration through a BioSpin® column. After rigorous washing employing the standard solvent wash procedure described above, the off-white, alcohol-derivatized resin was dried under house vacuum for 18 h.[6]

The alcohol-derivatized resin (33 mg, 1.39 meq/g, 0.05 mmol bound substrate) was placed in a new BioSpin® column, capped with a septum, and purged with N_2. Dry CH_2Cl_2 (600 μL) was added to the column *via* syringe, and the resin was allowed to pre-swell for 15 min at

(6) This solid-phase procedure is based on a literature precedent. See: Fraley, M. E.; Rubino, R. S. *Tetrahedron Lett.* **1997**, *38*, 3365-3368.

room temperature. Benzoic acid (169 mg, 1.38 mmol, 30 equiv) was weighed out into a oven-dried glass vial, capped with a septum, and purged with N_2. Dry CH_2Cl_2 (550 μL) was added to the vial *via* syringe and the vial was swirled to facilitate dissolution. DIC (216 μL, 1.38 mmol, 30 equiv) was added to the vial *via* syringe. A white precipitate (urea) formed over a period of 3-4 min. This thick solution was immediately transferred to the column containing the resin *via* syringe. Freshly-distilled DIPEA (400 μL, 2.3 mmol, 50 equiv) was added to the column *via* syringe. The septum was quickly removed and DMAP (28 mg, 0.23 mmol, 5 equiv) was added with a spatula. The column was purged with N_2, capped with a plastic cap, sealed with parafilm, and tumbled on a Labquake™ shaker for 16 h at room temperature. The reaction mixture was then drained from the column. The yellow resin was washed employing the standard solvent wash procedure described above, and dried on house vacuum for 24 h.[5]

The entire resin sample (33 mg, 1.39 meq/g, 0.05 mmol bound substrate) was subjected to the standard solid-phase "intra-site" cross-metathesis procedure described above. A 2.0 mg aliquot of the resin was then subjected to HF cleavage, and a pale brown oil (**9g**) was isolated.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.05 (4H, m), 7.53 (2H, m), 7.43 (4H, m), 7.27 (4H, masked m), 6.86 (4H, m), 5.83 (2H, m), 5.44 (2H, m), 4.05 (4H, m), 3.97 (4H, m), 2.85-2.50 (4H, br m), 2.02 (2H, masked br s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.5, 179.1, 178.8, 177.1, 173.4, 142.2, 129.9, 129.8, 128.6, 128.2, 69.6, 69.4, 61.7, 55.0, 39.5, 38.8, 38.7, 31.0, 22.5; IR (thin film, cm^{-1}): 3409, 3062, 2928, 2871, 1716, 1611, 1603, 1514, 1491, 1451, 1315, 1273, 1250, 1176, 1112, 1071, 1027, 972, 832, 713; TLC R_f = 0.38 (50% EtOAc/Hexane); LRMS (CI) calcd for $C_{36}H_{36}O_6$ $[M+Na]^+$ 587.3, found 587.0.