

Supporting Information for

Performing the Synthesis of a Complex Molecule on Sequentially-Linked Columns: Towards the Development of a “Synthesis Machine” by Stefan France, Daniel Bernstein, Anthony Weatherwax, and Thomas Lectka*

General. All reagents used were commercially available from Aldrich, Fluka, Novabiochem and Acros Chemicals. Acid chloride **8** was prepared by standard methods from the parent acid. All solvents and acid chlorides were dried and distilled by standard methods. Chlorinating agent **7** was prepared according to literature procedure.¹ L-Tert-leucine methyl amide was prepared according to literature procedures.² ¹H and ¹³C NMR spectra were acquired on a Bruker 400 MHz instrument in CDCl₃. The ¹H (400 MHz) and ¹³C (101 MHz) chemical shifts are given in parts per million (δ) with respect to internal TMS standard or residual solvent peaks. FTIR spectra were recorded on a Bruker Vector 22 spectrometer. HPLC analysis was performed with a Waters Millipore Model 510 head unit, a Regis R,R-Whelk-O1 analytical column, a Waters Millipore Lambda-Max Model 481LC spectrophotometer and a Hewlett Packard integrator. Racemate for α-chloroester **9** was prepared by using 1.2 equivalents of 4-(dimethylamino)pyridine.

General Procedure for the Assembly of Reaction Columns. The desired resin was added to a 24/40 neck, flame-dried, fritted chromatography column (13 mm x 20 cm) equipped with a septum (24/40) and flushed with nitrogen for 10 minutes. The column was prepared for use by flowing 120 mL of dry THF through the resin until the solvent level was just above the resin surface. This procedure, often referred to as “wetting” the column, was repeated for each column. In the case of columns **E** and **F**, a 1:1 mixture of THF:MeOH was used. The column tip was placed through the middle of a second septum (24/40) that allows one column to be linked directly to another.

Column A. Procedure for the Asymmetric α-Chlorination of Acid Chloride 8. A 25 mL jacketed addition funnel³ was loaded with terephthaloylquinine Wang resin **2** (2 g). The resin was wetted sequentially with 30 ml of dry THF via syringe, then 2 mL of Hunig’s base and finally 120 mL THF. The flow was stopped as the solvent reached just above the surface of the resin. 4-(1,5,5-Trimethylhydantyl)butanoyl chloride **8** (0.079 g, 0.32 mmol) and 2,2,3,4,5,6-hexachloro-2,4-dien-1-one **7** (0.096 g, 0.32 mmol) were dissolved separately in 1 mL THF and each was taken up into a 3 mL syringe equipped with a needle. The jacketed funnel was cooled to 0 °C with an ice bath. At this stage, flow was restarted at a rate of 0.1 ml/min. It was necessary to have a steady flow of nitrogen to ensure a constant flow rate. The solution of acid chloride **8** was loaded on top of the column packing. Once the level of the solution dropped to directly above the surface of the beads, the solution of **7** was added to the column. Once the level of the solution again dropped to the previous level, 9 mL of THF was added. The eluent was collected to afford 0.088 g (0.172 mmol) of **9** in 54% yield⁴ and 88% ee.⁵

2-Chloro-4-(1,5,5-trimethylhydantyl)butanoic acid pentachlorophenyl ester (**9**): (HPLC (Regis R,R-Whelk-O1: 99.9% CH₂Cl₂/0.1% i-PrOH, 1.0 mL/min) (*S*) = 8.1, (*R*) = 16.0 min. ¹H NMR 4.68 (dd, 1H), 3.86 (t, 2H), 2.93 (s, 3H), 2.61 (m, 1H), 2.45 (m, 1H), 1.40 (s, 6H) ppm; ¹³C NMR 177.0, 165.1, 143.8, 131.7, 128.8, 61.8, 54.2, 36.0, 33.7, 24.7, 22.3 ppm; IR (CHCl₃): 2950, 1773, 1710, 1384, 1362 cm⁻¹. Anal Calcd for C₁₆H₁₄N₂O₄Cl₆: C, 37.61; H, 2.76; N, 5.48; Cl, 41.63. Found C, 37.42; H, 2.71; N, 5.32; Cl, 41.82.

¹ Hedayatullah, M.; Lion, C.; Tourki, A. *Bull. Soc. Chim. Belg.* **1993**, *102*, 281-291.

² Levy, D. E.; Lapiere, F.; Liang, W.; Ye, W.; Lange, C. W. *J. Med. Chem.* **1998**, *41*, 199-223.

³ An adapter was required to connect the funnel to the subsequent column.

⁴ Yield was determined by isolation of the product from the chlorination step alone.

⁵ The only byproducts of this step are the remaining acid chloride (<10%) and the non-halogenated pentachlorophenyl ester (~45%)

Column B. Scavenging Excess Acid Chloride. Column **B** was loaded with piperazino resin **3** (0.30 g, 0.86 mmol/g) prepared according to the general procedure. Flow was started with the effective flow rate of ~0.1 mL/min. As the solvent reached just above the surface of the resin, the eluent from column **A** was dripped onto the column. Once the level of the solution dropped to just above the surface, 4 mL THF was added to flush the system.

Columns C and D. Synthesis of L-leucine-L-tert-leucine methylamide (11). Column **C** was loaded with N-cyclohexylcarbodiimide, N'-methyl polystyrene (1.0 g, 1.9 mmol/g). Flow was started at a flow rate of 2 drops/min. A solution of L-tert-leucine methyl amide (0.023 g, 0.142 mmol) and N-Fmoc-L-leucine (0.062 g, 0.150 mmol) in 2 mL of THF was added to the top of the column via syringe. When the level of solution dropped to just above the surface of the beads, 8 mL of THF was added to the top of the column. This step gave ~85% conversion⁶ to protected peptide **10**.⁷ As it eluted, the solution dripped directly onto column **D**. Column **D** was loaded with tris-(2-aminoethyl) amine polystyrene **5** (1 g, 2.2 mmol/g). The eluent from column **C** was added dropwise to the top of the column as flow from the column was started. When the level of solution dropped to just above the surface of the beads, 6 mL of THF was added to flush the column. This step affords **11** in >95% conversion (0.030 g, 0.115 mmol).⁸

Column E. Synthesis of N-[2-Chloro-4-(1,5,5-Trimethylhydantyl)butanoyl]-L-leucine-L-tert-leucine N-methylamide (12). Column **E** was loaded with Celite 545. The eluents from columns **B** and **D** were simultaneously dripped onto the column. In addition MeOH was added to the column at one-third the rate of elution from columns **B** and **D** (~0.03 mL/min). Once all of the eluents had been loaded on the column, the flow was begun at a consistent flow rate of ~0.1 mL/min. When the level of the solution dropped to just above the surface of the beads, 6 mL of 1:1 THF:MeOH solution was added to the column. A >95% conversion⁹ to **12** (0.055 g, 0.110 mmol) was obtained in this step. ¹H NMR 8.1 (d, 1H), 6.98 (d, 1H), 6.5 (bs, 1H), 4.68 (dd, 1H), 4.51-4.53 (m, 2H), 3.86 (t, 2H), 3.38 (dd, 1H), 2.93 (s, 3H), 2.77 (d, 3H), 2.61 (m, 1H), 2.45 (m, 1H), 1.75-1.83 (3H), 1.40 (s, 6H), 0.97 (s, 9H), 0.92 (d, 6H) ppm; ¹³C NMR 176.8, 171.6, 171.1, 165.1, 155.0, 63.9, 60.7, 57.9, 50.5, 41.2, 36.5, 36.2, 31.8, 31.4, 26.3, 23.7, 23.4, 22.5, 22.1 ppm; IR (CHCl₃): 3311, 3094, 2960, 2867, 1791, 1710, 1650 cm⁻¹. Anal Calcd for C₂₃H₄₀N₅O₅Cl: C, 55.02; H, 8.03; N, 13.95; Cl, 7.06. Found C, 56.01; H, 7.95; N, 13.55; Cl, 6.98.

Column F. Synthesis of BMS-275291 (1). Column **F** was loaded with Amberlite hydrogen sulfide resin **6** (0.80 g, 2.2 mmol/g) prepared by mixing Amberlite 400(Cl) and NaSH in MeOH for 15 min.¹³ The eluent from column **E** was added dropwise to the top of the column. The flow rate was ~0.1 ml/min. As the solvent reached the surface of the resin, 10 mL of THF was added to flush the column. The eluent was concentrated *in vacuo* to provide the crude product.¹⁰ Purification by column chromatography on silica, eluting with 5% MeOH/CH₂Cl₂ afforded 0.055 g (0.110 mmol) of **1** in 34% overall yield and 83% de. HPLC (Regis R,R-Whelk-O1: 1.0% i-PrOH/98% CH₂Cl₂, 1% HOAc. 1.0 mL/min) (*S,S,S*) = 19.32, (*R,S,S*) = 31.18 min. ¹H NMR 8.1 (d, 1H), 6.98 (d, 1H), 6.5 (bs, 1H), 4.51-4.53 (m, 2H), 3.86 (t, 2H), 3.61 (m, 1H), 3.38 (dd, 1H), 2.93 (s, 3H), 2.77 (d, 3H), 2.61 (m, 1H), 2.45 (m, 1H), 1.75-1.83 (3H), 1.40 (s, 6H), 0.97 (s, 9H), 0.92 (d, 6H) ppm; ¹³C NMR 176.8, 171.6, 171.1, 165.1, 155.0, 63.9, 60.7, 50.5, 41.2, 38.8, 36.5, 36.2, 31.8, 31.4, 26.3, 23.7, 23.4, 22.5, 22.1 IR (CHCl₃): 3311, 3094, 2960, 2867, 1791, 1710, 1650 cm⁻¹. Anal Calcd for C₂₃H₄₁N₅O₅S: C, 55.29; H, 8.27; N, 14.02. Found C, 56.01; H, 8.15; N, 13.87.⁷

⁶ All conversions were confirmed by TLC and ¹H NMR of an aliquot of the eluent. Each represents an average of three runs.

⁷ The mass yield was obtained from an isolated run of the pure reagents on the column.

⁸ All data are consistent with the known compound. North, J. T.; James, B. L. Patent WO 2003048119, September 4, 2003.

⁹ This conversion corresponds to the conversion of the α -chloroester to the α -chloroamide.

¹⁰ The impurities are pentachlorophenol, dibenzofulvene, and the final unsubstituted product (stemming from the non-halogenated ester resulting from column **A** reacting with peptide **11** and being carried through to the end). This is only a minor drawback as all byproducts are nonpolar, and possess a vastly different R_f and are readily separated by column chromatography. To remove them, a small column of silica can be added at the end of the column sequence, and the byproducts appear first. Since this is a trivial issue, we do not claim it as part of our column apparatus.