Supplementary Material (ESI) for Chemical Communications

A flow reactor process for the synthesis of peptides utilizing immobilized reagents, scavengers and catch and release protocols

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Materials and Methods

Dry *N*,*N*-dimethylformamide (DMF, extra dry over molecular sieves, Cat. No. 348430010) purchased from Acros organics (www.acros.com) was used throughout these experiments. The following compounds were used without further purification. From Novabiochem (www.novabiochem.com) HOBt-6-carboxamidomethyl polystyrene (PS-HOBt, Cat. No. 01-64-0425); IIDQ polystyrene (PS-IIDQ, Cat. No. 01-64-0469); Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP®, Cat. No. 01-62-0017). From Argonaut Technologies Inc. (now Biotage AB, www.biotage.com) macroporous poly(styrene-co-divinylbenzene) *p*-toluenesulfonic acid (MP-TsOH, Cat. No. 800287). From Fluka (www.sigmaaldrich.com/Brands/Fluka___Riedel_Home.html) dimethylaminopyridine on polystyrene (PS-DMAP, Cat. No. 39410); *N*-(carbobenzyloxy)-L-alanine (Cbz-Ala, Cat. No. 95850); *N*-(carbobenzyloxy)-L-phenylalanine (Cbz-Phe, Cat. No. 97000); *N*-(fluorenylmethoxycarbonyl)-L-alanine (Fmoc-Ala, Cat. No. 47616). From Aldrich (www.sigmaaldrich.com) *N*-(*tert*-butoxycarbonyl)-L-alanine (Boc-Ala, Cat. No.

134511); L-phenylalanine ethyl ester hydrochloride (Phe-OEt, Cat. No. 220701); glycine ethyl ester hydrochloride (Gly-OEt, Cat. No. G6503); L-valine methyl ester hydrochloride (Val-OMe, Cat. No. 860271). From Alfa Aesar (www.alfa.com) L-proline methyl ester hydrochloride (Pro-OMe, Cat. No. L19290). For Omnifit® columns, please see reference 9 in communication.

General Procedure A. Flow synthesis of Boc and Cbz protected dipeptides.
1.0 eq. of the HCl salt of an ester-protected amino acid (HCl.Phe-OEt, 0.10 g) was placed into a 10 mL volumetric flask and filled to line with DMF. To a separate 10 mL volumetric flask, 4.0 eq. of the Boc or Cbz protected amino acid (Cbz-Ala, 0.39 g), 4.2 eq. of PyBroP® (0.86 g), and 6.0 eq. of diisopropylethylamine (0.46 mL) was added, followed by DMF. An Omnifit column containing PS-HOBt (1.0 eq., 1.1 mmol/g, 0.40 g) was then washed/swelled with DMF at 250 µL/min. 5 mL of the 10 mL PyBroP®-activated amino acid solution was then placed in a reagent loop and passed through the PS-HOBt column at 100 µL/min for one hour. The flow rate was then increased to 250 µL/min to wash the HOBt column. Meanwhile, two separate Omnifit columns, one containing PS-DMAP (1.5 eq., 3.0 mmol/g, 0.22 g) and one containing MP-TsOH (1.5 eq., 1.42 mmol/g, 0.46 g) were washed/swelled with DMF at 250 µL/min. The columns were then placed in series and washed with DMF, connecting PS-DMAP to the activated PS-HOBt column, which is then connected to the MP-TsOH column. In the next step, 5 mL of the 10 mL HCl-amino acid solution was placed into a 5 mL reagent loop and passed through the three columns in series at 100 µL/min for two hours to ensure that all of the product had eluted from the columns, and collected into a vial. The solvent is then removed on a Vapourtec V-10®, yielding a crude product that was not in need of further purification (64.7 mg, Cbz-Ala-Phe-OEt, 74%). It should be noted that an inexpensive back pressure regulator is placed at the exit of the flow stream to help maintain a uniform flow stream and is available from Upchurch Scientific (www.upchurch.com, Cat. No. P-785). Please also note the only one-half of the contents of the volumetric flasks are pumped through the columns and the equivalents of the PS-HOBt, PS-DMAP and MP-TsOH are given in comparison to the full volumes of the flasks for ease in calculation. The true equivalents of the PS reagents are taken as 2.0 eq. PS-HOBt, 3.0

eq. PS-DMAP and 3.0 eq. MP-TsOH when compared to the actual amount of limiting reagent passed through the columns, which is 0.5 eq of the HCl salt.

General Procedure B. Flow synthesis of Fmoc protected dipeptides.

1.0 eq. of the HCl salt of an ester-protected amino acid (HCl.Gly-OEt, 61.3 mg) was placed into a 10 mL volumetric flask and filled with DMF. To a separate 10 mL volumetric flask, 8.0 eq. of the Fmoc protected amino acid (Fmoc-Ala, 1.09 g), followed by DMF. An Omnifit column was filled with PS-IIDQ (2.0 eq, 1.5 mmol/g, 0.58 g), and placed into a Vapourtec R-4 flow reactor heater, which was then connected to a separate Omnifit column containing PS-HOBt (1.0 eq., 1.1 mmol/g, 0.40 g) and both columns were then washed/swelled with DMF at 250 μL/min. The IIDQ column was heated in the Vapourtec R-4® flow reactor heater to 60ºC and 5 mL of the 10 mL Fmoc amino acid solution was then placed in a reagent loop and passed through the IIDQ-HOBt columns at 100 μL/min for 75 minutes. The flow rate was then increased to 250 μL/min to wash the IIDQ-HOBt columns free of any unreacted Fmoc-amino acid. Meanwhile, two separate Omnifit columns, one containing PS-DMAP (1.5 eq., 3.0 mmol/g, 0.22 g) and one containing MP-TsOH (1.5 eq., 1.42 mmol/g, 0.46 g) were washed/swelled with DMF at 250 μL/min. The columns were then placed in series and washed with DMF, connecting PS-DMAP to the activated PS-HOBt column, which is then connected to the MP-TsOH column. In the next step, 5 mL of the 10 mL HCl.amino acid solution was placed into a 5 mL reagent loop and passed through the three columns in series at 100 μL/min for two hours to ensure that all of the product had eluted from the column, and collected into a vial. The solvent is then removed on a Vapourtec V-10®, yielding a crude product that was not in need of further purification (61.9 mg, Fmoc-Ala-Gly-OEt, 71%). Again, as with all of our flow reactions, an inexpensive back pressure regulator is placed at the exit of the flow stream to help maintain a uniform flow stream. Please again note that only one-half of the contents of the volumetric flasks is pumped through the columns and the equivalents of the PS-IIDQ, PS-HOBt, PS-DMAP and MP-TsOH are given in comparison to the full volumes of the flasks for ease in calculation. The true equivalents of the PS reagents are taken as 4.0 eq. PS-IIDQ, 2.0 eq. PS-HOBt, 3.0 eq. PS-DMAP and 3.0 eq. MP-TsOH when compared to the actual amount of limiting reagent passed through the columns, which is 0.5 eq of the HCl salt.

General Procedure C. Flow synthesis of a tripeptide.

General procedure A was used to generate a Cbz protected dipeptide and the solvent removed using the Vapourtec V-10® solvent evaporator and re-dissolved in 4 mL of ethyl acetate:ethanol (1:1) (HCl.Gly-OEt as limiting, 61.5mg; Cbz-Ala, 0.39 g; PyBroP®, 0.86 g; DIPEA, 0.46 mL; PS-HOBt, 1.1 mmol/g, 0.40 g; PS-DMAP, 3.0 mmol/g, 0.22 g; MP-TsOH, 1.42 mmol/g, 0.46 g). General procedure A was also used to activate a second PS-$HOBt^{II}$ column using a different Cbz protected amino acid (Cbz-Phe, 0.52 g; PyBroP®, 0.86 g; DIPEA, 0.46 mL; PS-HOBt, 0.40 g). Following a standard procedure, the dipeptide from PS-$HOBt^{I}$ was placed into a 5 mL reagent loop and passed through the Thales H-cube flow hydrogenator, which was set on full H_2, 60ºC and a flow rate of 0.5 mL/min in EtOAc:EtOH (1:1). After 15 minutes, the crude product was collected and the solvent removed on the Vapourtec V-10® solvent evaporator and re-dissolved in 4 mL of DMF. The second activated PS- $HOBt^{II}$ column was then placed in series with an Omnifit® column containing 3.0 eq. of previously swelled MP-TsOH (1.42 mmol/g, 0.46 g). The free amine dipeptide was then placed in a 5 mL reagent loop and passed through the $HOBt^{II}$-TsOH columns at 100 μL/min for 90 minutes. The solution collected into a vial and the solvent removed on a Vapourtec V-10®, yielding a crude product that was not in need of further purification (58.9 mg, Cbz-Phe-Ala-Gly-OEt, 59%). It should be noted that an inexpensive back pressure regulator is placed at the exit of the flow stream to help maintain a uniform flow stream.