Supporting Information

Continuous Photochemical Cleavage of Linkers for Solid Phase Synthesis

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Contents

1. General Information

1.1 Abbreviations

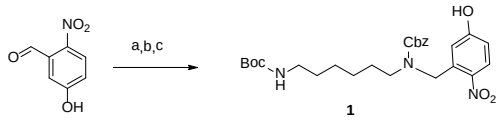
DCM, dichloromethane; PE, polyethylene; PP, polypropylene; HPLC, high performance liquid chromatography; DMF, *N*,*N*-Dimethylformamide; DIPEA, *N*,*N*-Diisopropylethylamine; FITC, Fluorescein isothiocyanate; FTU, Fluorescein thiourea; FEP, Fluorinated-Ethylene-Propylene.

1.2 General

All chemicals used were reagent grade and used as supplied except where noted. HPLC grade dichloromethane (DCM) without stabilizer was used for irradiation. Solid phase synthesis was performed in polypropylene (PP) syringe reactors equipped with a polyethylene (PE) frit.

2. Linker Preparation and Solid Support Functionalization

2.1 Synthesis of benzyl (6-((tert-butoxycarbonyl)amino)hexyl)(5-hydroxy-2-nitrobenzyl)carbamate 1.

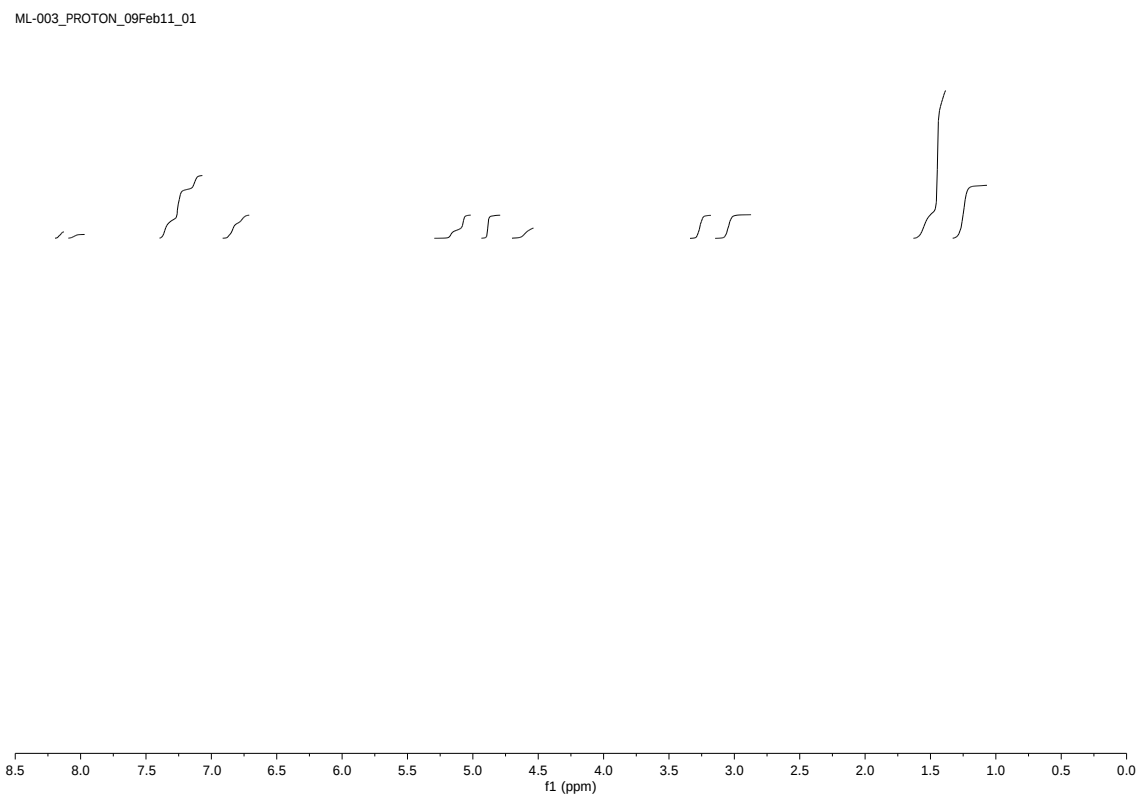


Scheme S1. Synthesis of linker **1**. Reagents and conditions: a. 1 equiv $BocHN(CH_2)_6NH_2$, toluene, 2 h, 120 ^oC; b. 1 equiv $NaBH_4$, MeOH, 30 min, 25 ^oC c. 2 equiv CbzCl, 2.2 equiv Et_3N, 3 equiv K_2CO_3, MeOH, 2 h, 25 ^oC

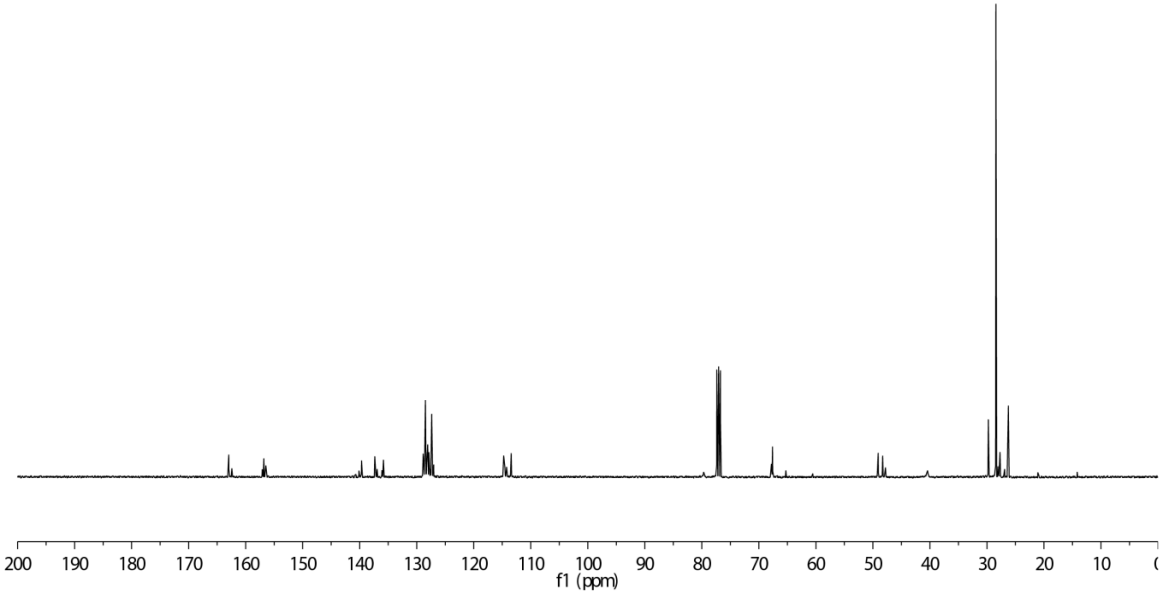
A solution of 5-hydroxy-2-nitrobenzaldehyde (2.260 g, 10.46 mmol) and *N*-Boc-1,6-hexanediamine (1.747 g, 10.46 mmol) in dry toluene (70 mL) was stirred and heated at 120 °C with a Dean-Stark apparatus until the theoretical amount of released water was reached. The solvent was then evaporated to furnish a black foam. The crude imine was dissolved in methanol (80 mL) and sodium borohydride was slowly added (0.395 g, 10.47 mmol, 1 equiv.) under bubbler control. After 30 minutes, acetone was slowly added (20 mL) and the solvent was evaporated to furnish a golden foam. To a solution of the amine in methanol (80 mL) was added Et_3N (3.2 mL, 23.03 mmol, 2.2 equiv) and benzyl chlorofromate (3.0 mL, 20.94 mmol, 2.0 equiv). After 1 h, potassium carbonate (4.33 g, 31.41 mmol, 3 equiv) was added to the mixture and stirred for an additional 1 h. The solution was filtered through celite and the solvents were

evaporated. The crude was dissolved in CH_2Cl_2 and washed successively with HCl (1M) and water. The organic layer was dried over $MgSO_4$, filtered, and the solvent was evaporated. The residue was purified by flash chromatography starting from 8:2 (cyclohexane:EtOAc) and ramped to 7:3 (cyclohexane:EtOAc) to elute compound **1**. Collected fractions were evaporated and dried under high vacuum to yield linker **1** as light yellowish foam (4.2 g, 80% over 3 steps): ^{1}H-NMR (400 MHz, $CDCl_3$): mixture of rotamers δ 8.15 (d, *J* = 8.8 Hz), 8.02 (d, *J* = 8.7 Hz), 7.35-7.11 (m, 5H), 6.87-6.72 (m, 2H), 5.15 (s, 1H), 5.06 (s, 1H), 4.88 (m, 2H), 4.69-4.67 (broad s, 1H), 3.24 (t, *J* = 6.8 Hz, 2H), 3.03 (t, *J* = 6.8 Hz, 2H), 1.57 (broad m, 2H), 1.44 (m, 11H), 1.28-1.23 (m, 4H). ^{13}C-NMR (101 MHz, $CDCl_3$): Mixture of rotamers δ 162.9, 162.4, 157.0, 156.8, 156.4, 140.1, 139.6, 137.3, 136.9, 136.1, 135.8, 128.8, 128.5, 128.5, 128.4, 128.2, 128.0, 127.8, 127.6, 127.3, 127.0, 114.7, 114.5, 114.2, 113.4, 67.7, 67.5, 65.2, 49.0, 48.2, 47.7, 40.4, 40.4, 40.3, 29.7, 28.4, 27.7, 26.2. MS ESI+-HRMS *m/z* $[M+H]^+$ calc for $C_{26}H_{36}N_3O7$ 502.2553 found 502.2561

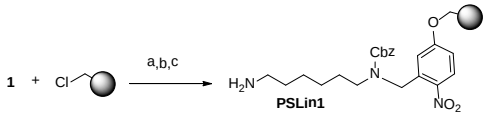
^{1}H NMR of 1 (400 MHz, $CDCl_3$)



^{13}C NMR of 1 (101 MHz, $CDCl_3$)



2.2 Solid Support Functionalization, synthesis of PSLin1.



Scheme S2. Synthesis of linker **PSLin1**. Reagents and conditions: a. **1**, Merrifield resin, 1.5 equiv TBAI, 1.5 equiv $CsCO_3$, DMF, 12 h, 60 ^{o}C; b. CsOAc, DMF, 12 h, 60 ^{o}C**;** c. 10 % TFA in DCM, 30 min, 25 ^{o}C

Linker was prepared and loaded on a Merrifield resin (0.5 mmol/g) according to a previously described procedure. [1]

To a suspension of Merrifield resin (1 g, 0.53 mmol/g, 0.53 mmol) in DMF (30 mL) was added a solution of **1** (0.319 gr, 0.636 mmol, 1.2 eq) in CH_2Cl_2 (5 mL) followed by Cs_2CO_3 (0,259 g, 0,795 mmol, 1.5 eq) and Tetrabutylammonium iodide (TBAI) (0,294 g, 0,795 mmol, 1.5 eq). The solution was allowed to rotate at 60 °C and 200 mbar on a rotavap overnight. The next morning, the resin was filtered and washed successively with DMF/Water (1/1), DMF, MeOH, CH_2Cl_2, MeOH, and CH_2Cl_2, and then allowed to swell in CH_2Cl_2 for 1 h. The swollen resin was the placed in a flask with DMF (60 mL) and CsOAc (0.203 g, 1.06 mmol, 2 eq) was added. The

[1] (a) Calin, O.; Eller, S.; Seeberger, P. H. *Angew. Chem. Int. Ed.* **2013**, *52*, 5862. (b) Eller, S.; Collot, M.; Yin, J.; Hahm, H. S.; Seeberger, P. H. *Angew. Chem. Int. Ed.* **2013**, *52*, 5858.

solution was again allowed to rotate at 60 °C and 200 mbar on a rotavap overnight. The resin was washed successively with DMF/Water (1/1), DMF, MeOH, CH_2Cl_2, MeOH, and CH_2Cl_2, the solvent was drained, and the resin was dried under vacuum.

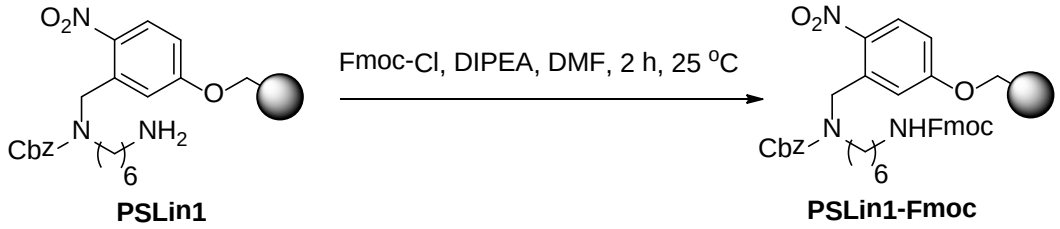
The resin was swelled in CH_2Cl_2 for 30 min and was treated twice with a solution 10% TFA/ CH_2Cl_2 for 30 min. The resin was finally washed with CH_2Cl_2 and MeOH, and dried under high vacuum overnight to give solid support **PSLin1**.

2.3 Loading Determination of solid support PSLin1

Dry resin **PSLin1** (100 mg) was placed in a syringe (5 mL) equipped with a frit. DMF (3 mL) was added to swell the resin for 1 h. The DMF was then drained and a solution of Fmoc-Cl (79 mg, 0.2 mmol) and DIPEA (0.07 mL, 0.4 mmol) in DMF (1 mL) was added to the resin. The reaction mixture was shaken for 12 h, the solution drained, and the resin washed with DMF, CH_2Cl_2, and MeOH, and dried under vacuum. Loading determination was performed using standard procedures[7] to give a typical loading between 0.12 to 0.21 mmol/g.

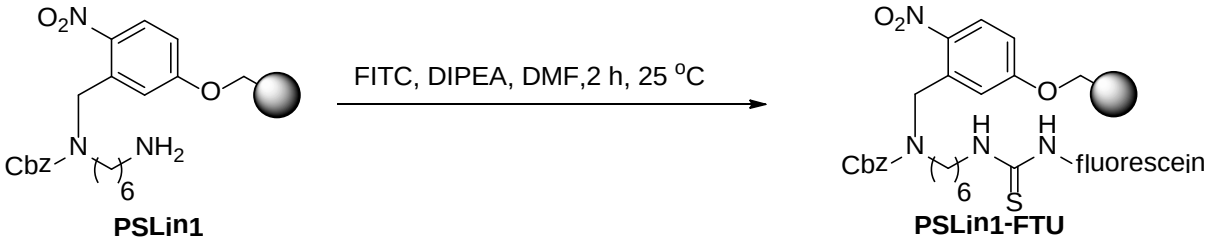
3. Solid Phase Procedures: Beads Functionalization and Fmoc Quantification

3.1 Preparation of Fmoc Labeled Solid Support PSLin1-Fmoc



Resin **PSLin1** was swollen in *N*,*N*-Dimethylformamide (DMF) for 2 h, drained and added to a mixture of Fmoc-Cl (5 equiv) and *N*,*N*-Diisopropylethylamine (DIPEA) (10 equiv) in DMF. After 2 h, the resin was drained and washed repeatedly with DMF, followed by DCM and MeOH. The obtained solid support **PSLin1-Fmoc**was dried under vacuum and kept in the dark prior to further use.

3.2 Preparation of Fluorescein Labeled Solid Support PSLin1-FTU



Resin **PSLin1** was swollen in DMF for 2 h, drained and added to a mixture of Fluorescein isothiocyanate (FITC) (1.2 equiv) and DIPEA (2 equiv) in DMF. After 2 h, the resin was drained and washed repeatedly with DMF, followed by DCM and MeOH. The obtained solid support **PSLin1-FTU** was dried under vacuum and kept in dark prior to further use.

3.4 Fmoc Quantification Test for PSLin1-Fmoc

Fmoc labeled beads (**PSLin1-Fmoc**) were irradiated in flow and in batch. 30 mg of **PSLin1-Fmoc** were swollen in DCM and irradiated in batch (as described in section 4.1) or in flow (as described in section 3.2) using 3.2 mm Fluorinated-Ethylene-Propylene (FEP) tube. The amount of Fmoc protecting group on the solid support was measured before and after irradiation following a described procedure.[2]

4. Instrumentation

4.1 Batch Photocleavage Procedure

Solid support (**PSLin1-Fmoc** or **PSLin1-FTU**, ~30 mg, loading 0.2 mmol/gr) was suspended in DCM (10 mL) and placed in a double jacketed glass reactor with a constant water circulation. A miniature ultraviolet pencil lamp (Long Wave UV Pencil Lamp Spectroline 36-380, 1,000 $\mu W/cm^2$ of 365 nm radiation at 1" (2.54cm)) was placed inside the Pyrex filter that was inserted into the reactor. The entire reactor was covered with aluminum foil and placed on an oval shaker (Vibramax 100, Heidolph). Irradiation was performed for 30 min under constant shaking of the resin to achieve maximal exposure. For comparison, the same procedure was repeated in the absence of shaking. After the irradiation light source was turned off, the aluminum foil was removed from the reaction vessel, the water circulation was stopped, and both the lamp and filter were removed. The solid support was removed using a glass pipette and filtered through a PE frit. An additional 10 mL of DCM was used to clean the reactor and remove any remains of

[2] Gude, M.; Ryf, J.; White, P. D. *Lett. Pept. Sci.* **2002**, *9*, 203.

compound and beads before the next injection. Each experiment was repeated three times and the reported results are the average of these three trials.

4.2 Continuous Flow Photocleavage Procedure

Based on a previous set-up by Hook et al.[3] a medium pressure mercury lamp (450 W) was placed inside a Pyrex filter and both were placed in double jacketed immersion well equipped with a circulating water system set to 25 ˚C. The jacket was wrapped with either a 1.5 mm (inner diameter 0.8 mm) or 3.2 mm (inner diameter 1.5 mm) FEP tubing having a total volume of 12 mL or 15 mL (small or large diameter tube, respectively). The entire setting was placed in a sealed aluminum box equipped with entry and exit holes for the FEP tubing. It was important to ensure that the water circulation was turned on and stabilized to a constant temperature of 25 ˚C prior to switching on the light source. The first injection took place only after allowing the lamp to warm up for at least 30 minutes. Solid support (**PSLin1-Fmoc** or **PSLin1-FTU**, ~30 mg), pre-swollen in DCM, was pushed through the FEP tubing using a syringe pump. In the first step, the solid support was injected using a 2 ml syringe to make sure all the resin entered the tubes. Afterwards, the small syringe was replaced by a 20 mL syringe (filled with DCM) and flow continued at a constant flow of 0.4 mL/min or 0.5 mL/min (small and large diameter, respectively). The resin was irradiated while being pushed through the tubing (residence time of 30 min) before being filtered through the PE frit. To ensure all of the desired compound was collected, about 10 mL of solvent was collected (2 mL before expected exit volume and extra 8 mL after the beads start to come out of the reactor). The collected beads were further analyzed using a Fmoc quantification test (for **PSLin1-Fmoc**) or by confocal microscopy (for **PSLin1-FTU**). The reactor was washed with an additional 10 mL of DCM before the next injection (can be done at faster flow rate to decrease the time between injections). No switching off of the light source or the water circulation is required until the end of the final injection. Each experiment was repeated three times and the reported results are the average of these three trials.

[3] Hook, B. D. A.; Dohle, W.; Hirst, P. R.; Pickworth, M.; Berry, M. B.; Booker-Milburn, K. I. *J. Org. Chem.* **2005**, *70*, 7558.

5. PSLin1-FTU Cleavage Experiments

5.1 Sample Preparation for Confocal Microscopy

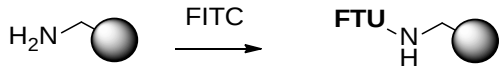
Solid support **PSLin1-FTU**was irradiated in batch and in flow using the above procedures. The solid support was washed, dried, and kept in a dark environment. The solid support was suspended in DMF and allowed to permeate into capillary micro glass slides (0.3 x 3.0 mm) before being sealed inside the slides using hot wax. The capillaries were covered in aluminum foil before use.

5.2 Visualization of Labeled Beads by Confocal Microscopy

Labeled beads were visualized using a LSM confocal microscope (Carl Zeiss AG). Fluorescein fluorescence was measured at 488 nm with 2% laser power. Fluorescence quantification was done by measuring the mean fluorescence intensity using the image J software and the data was analyzed using GraphPad Prism version 5.04 using the 1way Annova, with Bonferroni's Multiple Comparison Test to compare between samples.

6. Fluorescein Bleaching Tests

To examine if fluorescein labeled beads undergo undesired bleaching during the cleavage process, aminomethylated polystyrene beads (Novabiochem, LL) were loaded with fluorescein using FITC as described above.



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Aminomethylated polystyrene LL (100-200 mesh) (loading 0,4 mmol/gr, Novabiochem) was swollen in DMF for 2 h, drained, and added to a mixture of Fluorescein isothiocyanate (FITC) (1.2 equiv) and DIPEA (2 equiv) in DMF. After 2 h, the resin was drained and washed repeatedly with DMF, followed by DCM and MeOH. Fluorescein labeled solid support was dried under vacuum and kept in the dark before further use.

The resin was examined using confocal microscopy to evaluate the fluorescence of the beads (Figure 1A). The beads were irradiated in batch for 1, 2 and 15 h (Figure 1B, C, D respectively). No significant decrease of fluorescence was detected in any of the cases.

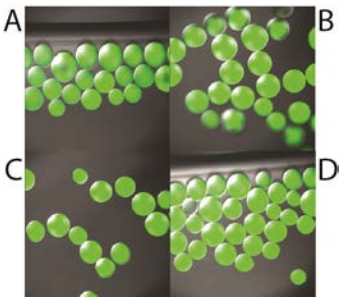
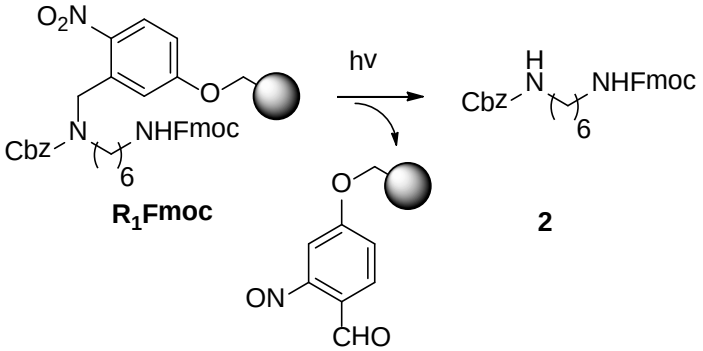


Figure 1. Fluorescein labeled polystyrene before (A) and after 1, 2 and 15 h irradiation (B, C, D respectively)

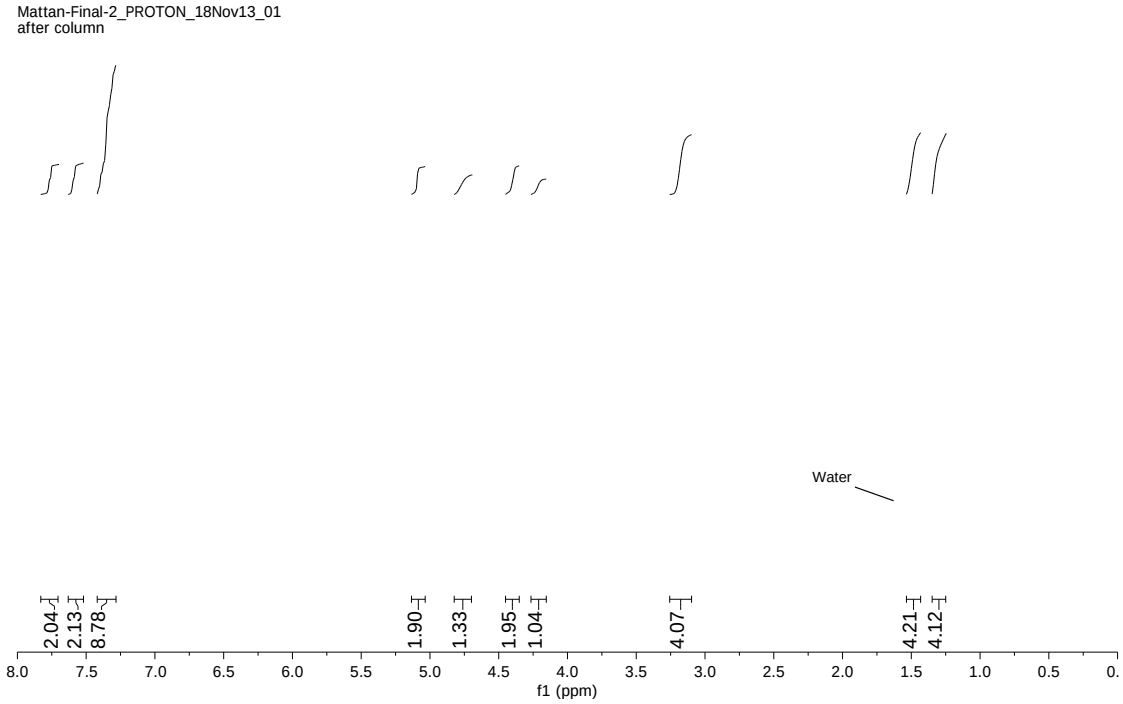
7. Cleavage of PSLin1-Fmoc and Characterization of *N*-1-Cbz-*N*-6-Fmoc-1,6-diaminohexane 2



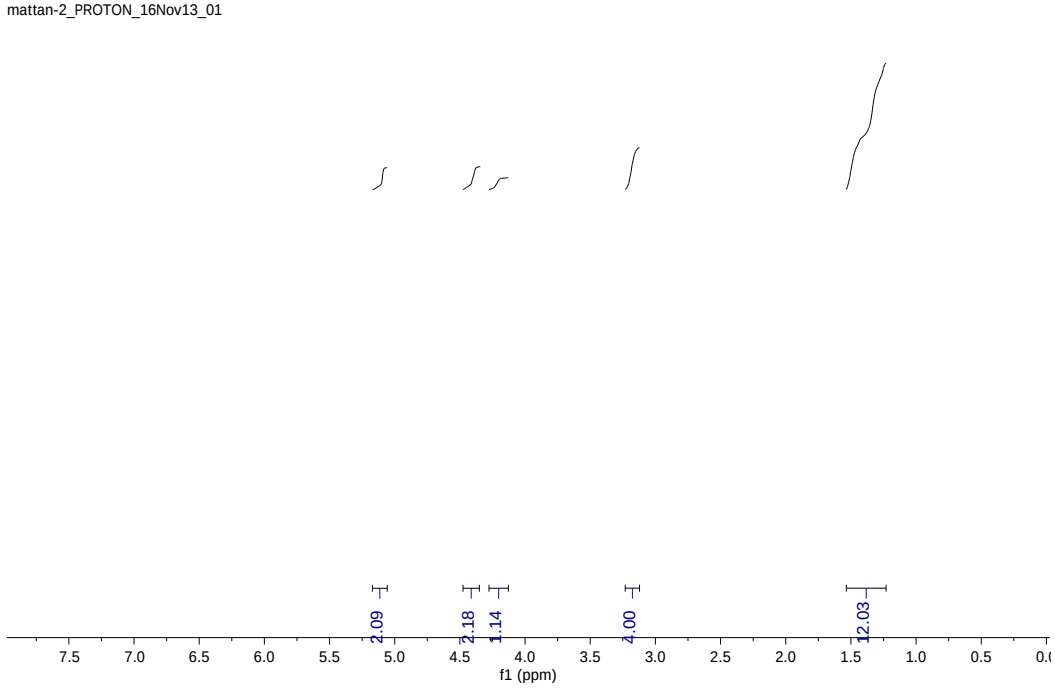
PSLin1-Fmoc (50mg, loading 0.2 mmol/gr) was cleaved under continuous flow as described above.

After cleavage, the filtrate was evaporated to dryness and the crude product was analyzed without further purification to yield a colourless oil that was purified by silica chromatography and eluted with EA:hexane 2:8 (crude 5.8 mg, 123% yield, after purification by 4 mg, 84%): A major impurity in the NMR of the crude was observed as integration indicated increased number of protons in the aliphatic region (see NMR of the crude). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.39 (t, J = 7.3 Hz, 2H), 7.27 – 7.36 (m, 9H), 5.09 (s, 2H), 4.79 (broad, 1H), 4.40 (d, J = 6.9 Hz, 2H), 4.21 (t, J = 6.9 Hz, 1H), 3.19 (m, 4H), 1.47 (broad, 4H), 1.33 (broad, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.57, 156.56, 144.12, 141.43, 136.71, 128.66, 128.25, 127.78, 127.15, 125.16, 120.10, 66.75, 66.61, 47.41, 40.95, 30.00, 26.32.: MS ESI+-HRMS *m/z* $[M+Na]^+$ calc for $C_{29}H_{32}N_2NaO_4^+$ 495.2254 found 495.2249 $[M+K]^+$ calc for $C_{29}H_{32}N_2KO_4^+$ 511.1994 found 511.1979

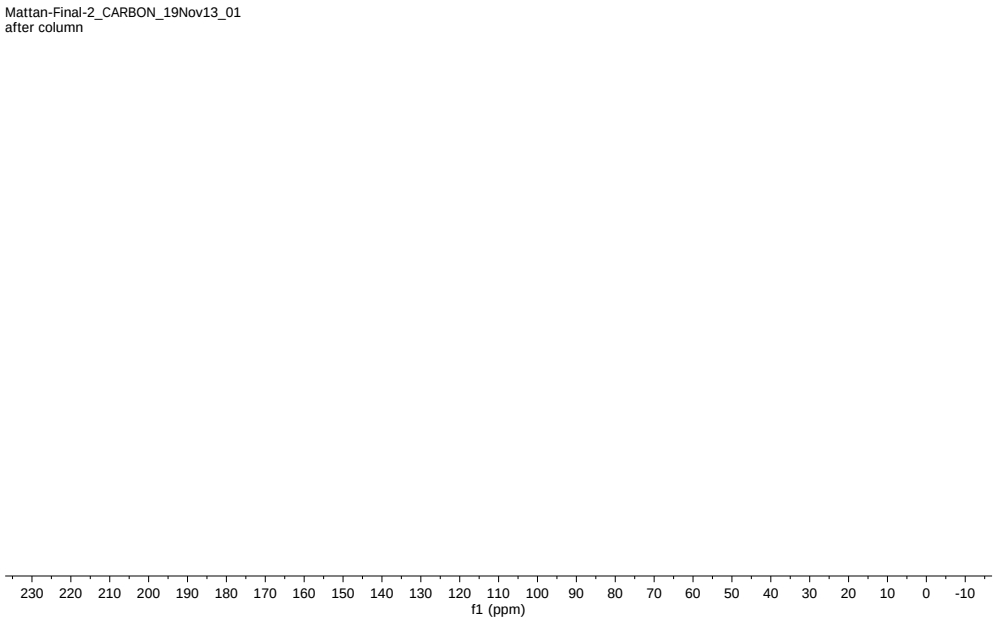
^{1}H NMR of Purified 2 (400 MHz, $CDCl_3$)



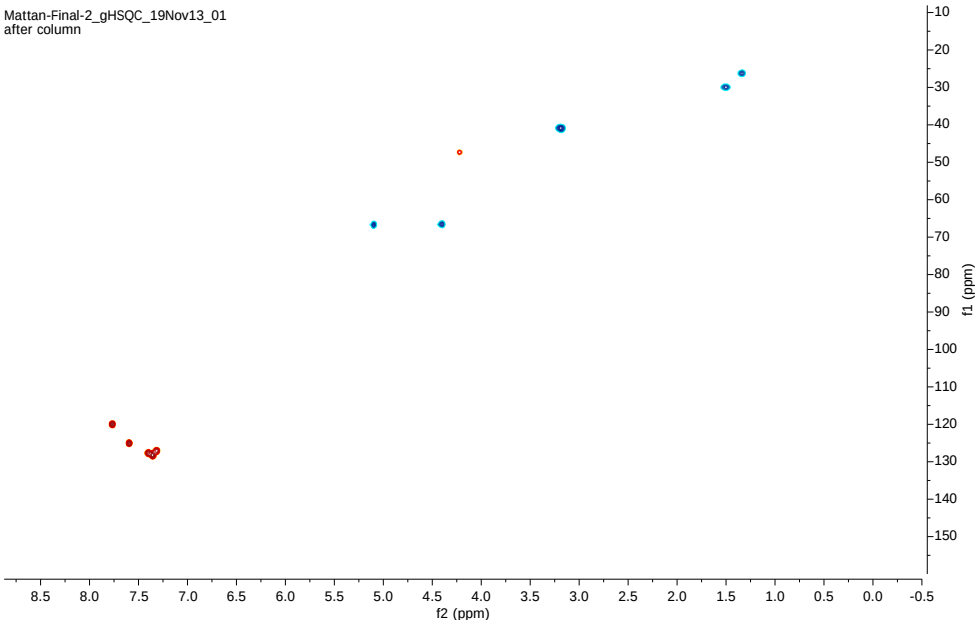
^{1}H NMR of Crude 2 (400 MHz, $CDCl_3$), Indicating the Presence of Impurities.



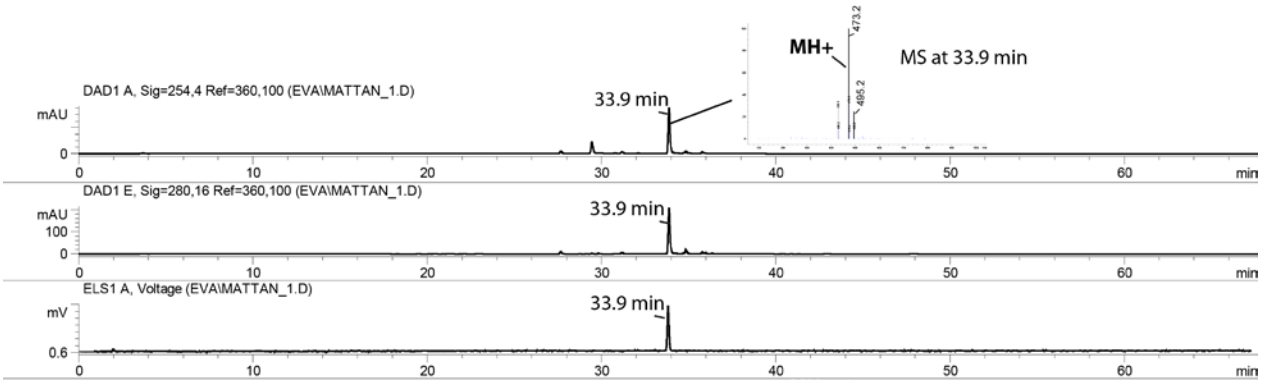
^{13}C NMR of 2 (101 MHz, $CDCl_3$)



HSQC NMR of 2 ($CDCl_3$)



RP-HPLC Trace of Crude 2



HPLC was performed using Phenomenex C5 Luna column and a linear gradient of 5% to 95% ACN in TDW over 30 min (recorded with 254 nm, 280 nm and ELSD). Compound 2 elutes at 33.9 min.

8. Pictures of flow and batch reactors

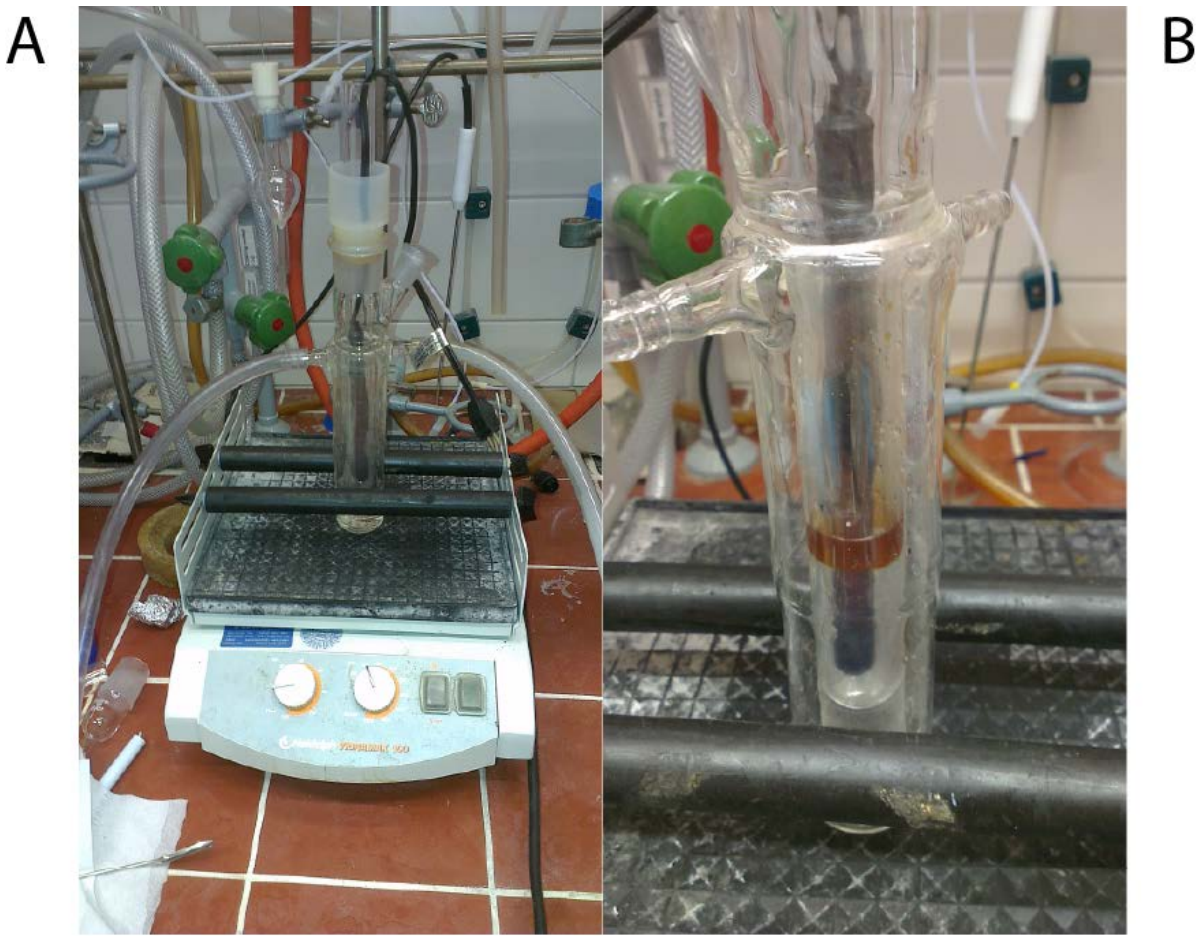


Figure 2. A. Classic batch reactor placed on an oval shaker, resin is placed inside the double jacket reactor, and pencil lamp in Pyrex filter was inserted until lamp reaches the height of the resin. B. Resin placed inside the reactor tends to form a thick rim around the lamp with very poor distribution. During cleavage, the entire reactor is covered with aluminum foil.

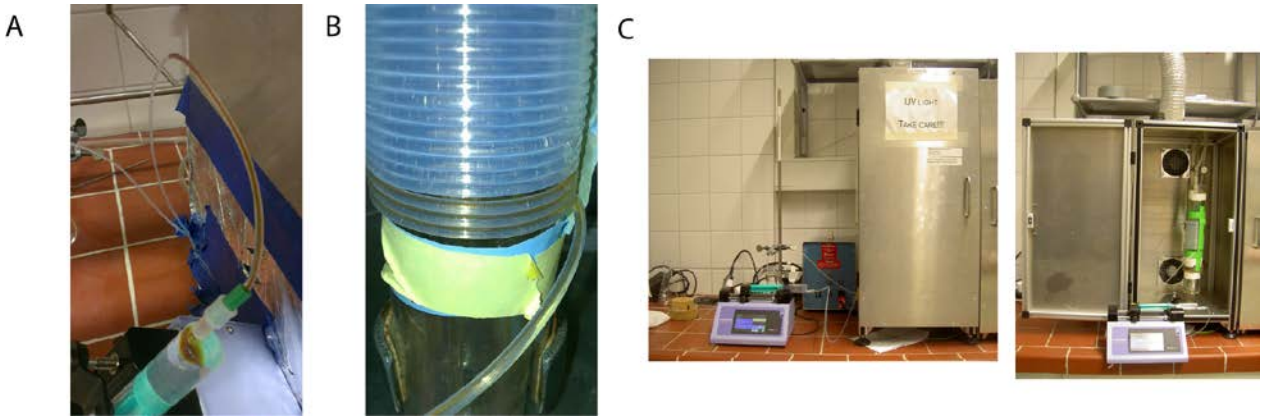


Figure 3. Cleavage in continuous flow photo-reactor. A. Injection of the solid support to the reactor is usually done vertically to assist resin suspension to enter the tubes. B. Resin is pumped through the tubes using syringe pump and is distributed evenly in the tubes of the reactor. C. The entire setting is placed inside an aluminum box fitted with exit holes for water, electricity cords and FEP tubes.

9. Movie Demonstrating Beads Movement in Large Bore Tube Flow Reactor.

Beads after photo cleavage (hence, slightly dark) were pumped through the wide tubes (1.5 mm inner diameter) to visualize the nature of beads movement inside the tubes. The attached movie demonstrates that the beads mix as they move through the tubes (movie is attached in another file).