Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


**Supporting information**

# The Breaking Beads Approach for Photocleavage from Solid Support

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# General Information

## Materials:

*N,N*-dimethylformamide (DMF), methanol (MeOH), dichloromethane (DCM), and dry toluene were purchased from BIO-LAB chemicals, Israel, Column chromatography: silica gel 60-120 mesh; thin layer chromatography (TLC): silica gel 60 F254 percolated plates; were purchased from Merck. Merrifield resin and Fmoc-Cl were purchased from CHEM-IMPEX. Pyridine, piperidine, tetrabutylammonium iodide (TBAI), sodium borohydride ($NaBH_4$), $Cs_2CO_3$, $K_2CO_3$, 1,4-Dioxane, triflic acid (TfOH) and 6-aminohexan-1-ol were purchased from Sigma-Aldrich, Israel. CsOAc and n-iodosuccinimide (NIS) purchased from ALFA-AESAR. Benzyl chlorofromate (Cbz-Cl) purchased from ACROS Organic. trimethylsilyltrifluoromethanesulfonate (TMSOTf) purchased from Apollo Scientific. Ethyl 2-O-benzoyl-3,4-di-O-benzyl-6-O-(9-fluorenylmethoxycarbonyl)-1-thio-α-D-mannopyranoside **6** was purchased from GlycoUniverse GmbH & CO KGaA, Potsdam, Germany.

## Methods:

NMR Spectra were recorded in either Brucker advance-II 500 MHz instrument. Chemical shifts are expressed in δ units. 1D and 2D-NMR were recorded in $CDCl_3$ using BBO-5mm probe unless stated otherwise. Microscope measurements were performed using ZEISS Scope.A1 equipped with AxioCamm ICc 3 camera. Mass spectrometry was recorded on Agilent 6550 iFunnel Q-TOF LC/MS system for all the photolabile linkers. The experimental molecular mass of the compounds was determined from the collected m/z ratios for all the observed multiply charged species. UV measurements were performed on Shimadzu 300pc spectrophotometer by scanning wavelengths from 400 nm to 220 nm in a quartz cuvette (114 - QS Hellma GmbH & Co. KG). The compounds were purified by InterChim puriflash XS 420 with silica flash column F0012-14g-SI-HP 5- µm, and were recorded at 254 nm wavelength at room temperature. Compound were purified using a flow rate of 15 ml/min using a mobile phase consisted of solution A: Hexane and solution B: EtOAc (for detailed MPLC gradient program see table S6).

# Experimental section:

## Photo cleavage Setup:

High power LED 365 nm lamp is connected to a benchtop low noise LED current controller (model PRZ –BLLC-04). The irradiated power of the light is 29.44 mW/Cm2 measured by PM100D-Compact Power and Energy Meter Console with 200-1100 nm silicon photodiode (model S120VC). The distance between the sample cuvette "1 cm quartz cuvette" and the LED lamp was set to 3 cm.

## Loading determination of FHP-Linker:

**FHP-Linker** (30 mg) was dried and placed in fritted Sep-Pak cartridge stoppered and covered with aluminum foil. 2 ml solution of 20% piperidine in DMF were added to the Sep-Pak and the top was corked and left to stir on an oval shaker for 2 h. In a 2 ml volumetric flask, 40 µl of this solution were placed and a flask filled with 20% of piperidine in DMF solution. The diluted solution was placed in a quartz cuvette and the absorbance was measured at 301 and 289 nm. The total Fmoc removed was calculated using a Fmoc determination equation from a previous published article with an absorbance coefficient of 6100 ,8050 [mol−1 cm−1] in piperidine/DMF solution for absorbance at 289 nm and 301 nm respectively.[10] The error is the standard deviation between three independent experiments. The absorbance measured using a Shimadzu UV-3101 PC UV-VIS-NIR Spectrophotometer. Loading studies indicated slight variations in the loading between the different batches. The typical loading was between 0.564 to 0.583 mmol/g. for each experiment in the following sections, the specific loading used will be indicated.

## General Photocleavage Procedure:

**FHP-Linker** (30 mg, 0.56 mmol/gr) of photolabile linker was suspended in DCM (2 ml) and placed in 1 cm quartz cuvette with a magnetic bar. The LED lamp was set to pass the beam directly into the solution of the cuvette. The irradiation was performed at 365 nm in a dark room for different durations and with variable stirring rates as described later for each specific experiment.

## Shaker Photocleavage Procedure*:*

**FHP-Linker** (30 mg. 0.58 mmol/gr) was suspended in DCM (2 ml) and placed in 1 cm quartz cuvette, Place the cuvette in a linear shaker (IKA® HS 260 basic) Mot 210 rpm. The LED lamp was set to pass the beam directly into the solution of the cuvette. The distance between the sample cuvette "1 cm quartz cuvette" and the LED lamp was set to 17 cm (Figure S21). The irradiation was performed at 365 nm in a dark room with and without pre-stirrer as described later for each specific experiment.

## Post irradiation treatment after each irradiation experiment:

Light source was turned off and the suspension of solid support in DCM was collected with a plastic pipette and filtered through a fritted Sep-Pak cartridge (12 ml Capacity, 20 µm porosity Frits). An additional 2 ml of DCM were used to wash the cuvette remove the remaining solid support which also filtered through the same Sep-Pak. The combined DCM filtrate containing

the cleaved compound was placed in a small 5 ml glass beaker and the DCM was allowed to evaporate. The remaining solid support was further washed with DMF, DCM, MeOH, then dried under vacuum. After each irradiation, few beads following the washing steps were taken for microscopic analysis to visualize the shape and integrity of the solid support.

### Fmoc quantification after irradiation (solid support):

The Sep-Pak containing solid support after irradiation (a mixture of **FHP-Linker** and **NA-Linker**) was stoppered and 1.5 ml of 20% piperidine in DMF was added. The Sep-Pak was sealed covered with aluminum foil and left to shake for 2 h on an oval shaker. In a 2 ml volumetric flask, 40 µl of this solution was placed and a flask filled with 20% of piperidine in DMF solution. The diluted solution was placed in a quartz cuvette and the absorbance was measured in a wavelength of (301 and 289 nm) using a Spectrophotometer.

### Preparation of N-Cbz-O-Fmoc-hexanolamine (3) calibration curve:

10 mg of **3** were placed in a 100 ml volumetric flask and the flask was filled to volume with DCM to give a 0.2 mM solution (stock solution A). 1-5 ml of the stock solution were diluted to 10 ml with DCM to provide a series of standard solutions with a concentration range between 0.02-0.1 mM for the preparation of a calibration curve (solutions B-F, Table S1). 2 ml of solutions B-F were placed in a quartz cuvette and the absorbance was measured at 301 and 289 nm that is characteristic to the Fmoc moiety using UV spectrophotometer. A point graph was plotted to show the correlation between the concentration and the absorbance at 301 nm. Based on the calibration curve the correlation was determined by the equation of ($y = 4.865x + 0.0129$) where y is the absorbance at 301 nm and x is the concentration of 3 in mM.

### Measurement of the amount N-Cbz-O-Fmoc-hexanolamine (3) in the filtrate by UV absorbance analysis:

After the irradiation of **FHP-Linker,** the filtrate containing **3** was collected (4 ml of DCM in total) and dried overnight. The dry crude was dissolved in 1.5 ml of DCM using a volumetric pipette. In a 2 ml volumetric flask, 40 µl of this solution was placed and a flask filled with 20% of piperidine in DMF solution. The 2 ml of the solution was placed in a quartz cuvette and the absorbance at 301 and 289 nm was measured. The measured absorbance was later fitted into a calibration curve and the concentration of cleaved material was calculated

### Irradiation time effect on photo cleavage:

**FHP-Linker** (30 mg, loading 0.56 mmol/g) was suspended in DCM (2 ml) and placed in a quartz cuvette. Irradiation was performed using the same setup describe above but with a variable irradiation duration moving from 0 h to 6 h while maintaining the same stirring rate (1060 rpm). After each irradiation study, the solid support was collected in a fritted Sep-Pak, washed, and the Fmoc quantity was determined as described above. Loading was determined as described above the presented values are based on the absorbance at 301 and 289 nm. Few beads were taken for microscopic analysis to visualize the shape and integrity of the solid support (Figure S5).

### Determining the effect of stirring rate on cleavage efficiency:

**FHP-Linker** (30 mg, loading 0.56 mmol/g) was suspended in DCM (2 ml) and placed in a quartz cuvette. Irradiation was performed using the same setup described in the general photocleavage procedure section with a variable stirring rate with the same irradiation duration of 4 h. After each irradiation study, the solid support was collected in a fritted Sep-Pak, washed, and Fmoc quantity was determined as described above. Few beads were taken for microscopic analysis to visualize the shape and integrity of the solid support. (Figure S6).

### The effect of pre-grinding on photocleavage efficiency:

**FHP-Linker** (30 mg, loading 0.56 mmol/g) was placed in a quartz cuvette with a magnetic bar. The suspension was stirred for four hours without irradiation. After which the suspension was irradiated for 1 h while stirring at 1060 rpm. In a control experiment, **FHP-Linker** (30 mg, loading 0.56 mmol/g) was placed in a quartz cuvette with a magnetic bar and were irradiated for 1 h at 1060 rpm rate. After each irradiation, the resin was washed and Fmoc quantification was performed as described above. Few beads were taken for microscopic analysis to visualize the shape and integrity of the solid support. (Figure S7).

### The effect of pre-grinding on photocleavage efficiency in a shaker setup:

**FHP-Linker** (30 mg, loading 0.58 mmol/g) was placed in a quartz cuvette with a magnetic bar. The suspension was stirred for two hours at 1060 rpm without irradiation after which the cuvette was placed in a linear shaker and irradiated for 1 h using the setup describe above. After the irradiation, the efficiency of cleavage was determined by Fmoc quantification. In a control experiment, **FHP-Linker** (30 mg, loading 0.58 mmol/g) was placed in a quartz cuvette in the shaker and irradiated for 1 h using the setup describe above. After the irradiation, the efficiency of cleavage was determined by Fmoc quantification.

### Loading determination of the FAP-linker:

The Sep-Pak containing solid support **FAP-linker** after irradiation was stoppered and 1.5 ml of 20% of piperidine in DMF solution was added. The Sep-Pak was sealed, covered with aluminum foil, and left to shake for 2h on an oval shaker. In a 2 ml volumetric flask, 40 µl of this solution was placed and a flask filled with 20% of piperidine in DMF solution. The diluted solution was placed in a quartz cuvette and the absorbance was measured in a wavelength of (301 and 289 nm) using a Spectrophotometer and Fmoc quantity was determined as described above

### Irradiation time effect on photocleavage of FlAP-linker without stirring:

**FlAP-Linker** (20 mg) was suspended in DCM (2 ml) and placed in a quartz cuvette. The LED lamp was set to pass the beam directly into the solution of the cuvette. The irradiation was performed at 365 nm in a dark room without stirring for 2 to 8 h. After each experiment, the resin was filtered and washed. Few beads were taken for fluorescent microscopy analysis to visualize (Figure S2) and the degree of fluorescence of the solid support was quantified by mean fluorescence intensity (MFI) (Figure S3).

## Irradiation time effect on photocleavage of FlAP-linker with stirring:

**FlAP-Linker** (20 mg) was suspended in DCM (2 ml) and placed in quartz cuvette. The LED lamp was set to pass the beam directly into the solution of the cuvette. The irradiation was performed at 365 nm in a dark room with stirring at 1060 rpm for 2 to 8 h. After each experiment, the resin was filtered and washed. Few beads were taken for fluorescent microscopy analysis to visualize the shape and integrity of the solid support and the decrease in fluorescence (Figure S8).

## Determination of cleaved N-Cbz-O-Fmoc-hexanolamine (3) after irradiation (filtrate):

The following equation was used to calculate the amount of **3** in the filtrate after cleavage based on the UV measurements and fit to the calibration curve presented in Figure S4

$$n_{mmol} = C_{mM} * \alpha$$

$C_{mM}$ is the value calculated from the calibration curve, α is the dilution factor between the measured samples to the stock solution. The calculated amounts of **3** for all experiments are presented in Table S5.

## Surface area to volume ratio calculations of polystyrene beads before grinding and of the particles after grinding

Surface area to volume ratios of the polystyrene beads/particles were calculated before and after grinding based on the average diameter measured by microscope. In both cases, we assumed that the particles are spherical.

*Surface area to volume of beads before grinding* was calculated using the following equations (based on the image S6A) to be $0.075$ [$\mu m^{-1}$]

Beads diameter as calculated from microscope- d = 80 [μm]

Surface area of spherical beads- $A = 4\pi(\frac{d}{2})^2 = 20106$ [$\mu m^2$]

Volume of the beads- $V = \frac{4}{3}\pi(\frac{d}{2})^3 = 268083$ [$\mu m^2$]

Surface area- diameter ratio: $A{:}V = 0.075$ [$\mu m^{-1}$]

*Surface area to volume of the particles after grinding* was calculated using the following equations (based on the image S7B) to be $6.04$ [$\mu m^{-1}$] assuming spherical structure (The particles do not have defined geometrical structure):

Diameter as calculated from microscope by pixel size comparison - d = 1 [μm]

Surface area of spherical beads- $A = 4\pi(\frac{d}{2})^2 = 3.14$ [$\mu m^2$]

Volume of the beads- $V = \frac{4}{3}\pi(\frac{d}{2})^3 = 0.52$ [$\mu m^2$]

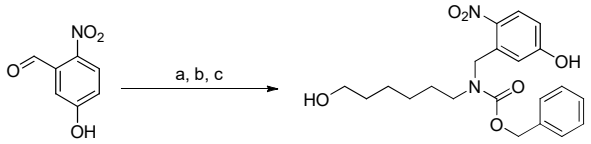
Surface area- diameter ratio: $A{:}V = 6.04$ [$\mu m^{-1}$]

*Grinding increase the surface area to*: Based on these calculation we determined that the increase in surface area/volume by grinding: $\frac{A{:}V_{powder}}{A{:}V_{beads}} = \frac{6.04\ [\mu m^{-1}]}{0.075\ [\mu m^{-1}]} = 80$

# Synthetic Schemes

## Linker Preparation and Solid Support Functionalization

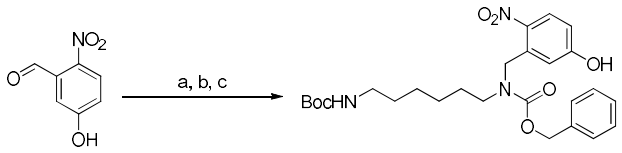
Synthesis of benzyl (5-hydroxy-2-nitrobenzyl) (6-hydroxyhexyl) carbamate (**1**)




**Scheme S1**. Synthesis of linker **(1)**. Reagents and conditions: **a)** 6-aminohexan-1-ol (1 equiv), toluene 2 h, 120 °C. **b)** $NaBH_4$ (1 equiv), MeOH, 30 min, 25 ° C. **c)** Cbz-Cl (2 equiv), 2. $Et_3N$ (2 equiv), $K_2CO_3$ (3 equiv), MeOH, 2 h, 25°C.

A solution of 5-hydroxy-2-nitrobenzaldehyde (2.502 g, 14.97 mmol) and 6-aminohexan-1-ol (1.754 g, 14.97 mmol) in dry toluene (70 ml) was stirred and refluxed at 120 °C with a Dean-Stark apparatus until approximately 1 ml of water was released. The solvent was then evaporated to furnish black foam. The crude imine was dissolved in MeOH (80 ml) and sodium borohydride ($NaBH_4$, 0.568 g, 14.97 mmol) was slowly added under bubbler control. After 30 minutes, acetone was slowly added (20 ml) and the solvent was evaporated to furnish yellowish foam. 17 ml of $Et_3N$ (32.93 mmol) and 4.25 ml Cbz-Cl (29.95 mmol) were added to a solution of the amine in MeOH (80 ml) and the mixture was stirred at ambient conditions. After 1 h, potassium carbonate ($K_2CO_3$, 6.20 g, 44.91 mmol) was added to the mixture and stirred for an additional 1 h. The solution was filtered through celite and solvents were evaporated. The crude was dissolved in DCM and washed successively with HCl (1 M) and water. The organic layer was dried over $MgSO_4$, filtered, and the solvent was evaporated. The crude was purified by silica chromatography $^1$H-NMR (500 MHz, $CDCl_3$, mixture of rotamers) 8.19-8.07 (m, 1H), 7.37 - 7.14 (m, 5H), 6.85 - 6.71 (m, 2H), 5.17 -5.05 (m, 2H), 4.91 (s, 2H), 3.63 -3.60 (m, 2H), 3.32-3.29 (m, 2H), 1.74-1.26 (m, 8H). $^{13}$C-NMR (500 MHz, CDCl3, mixture of rotamers): δ 171.4, 162.7, 157.0, 139.7, 137.4, 135.8, 128.9, 128.5, 128.3, 128.1, 128.0, 127.5, 114.9, 114.7, 114.1, 113.1, 67.7, 62.5, 60.5, 49.2, 48.4, 48.1, 32.0, 28.2, 27.7, 26.4, 26.2, 25.3, 25.1, 21.0, 14.2. HRMS calc for: C21H26N2O6Na $[M+Na]^+$: 425.1683; observed value- 425.1608. calc for L C21H27N2O6 $[M+H]^+$, 403.1869; observed value- 403.1858.

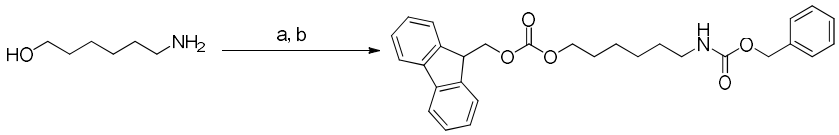
Synthesis of benzyl (6-(tert-butoxycarbonyl) amino) hexyl) (5-hydroxy-2- nitrobenzyl) carbamate (**2**)




**Scheme S2**: Synthesis of linker **(2)**. Reagents and conditions: **a)** 1 equiv $BocHN(CH_2)_6NH_2$, toluene, 2 h, 120 °C. **b)** 1 equiv $NaBH_4$, MeOH, 30 min, 25 °C. **c)** 2 equiv Cbz-Cl, 2. 2 equiv $Et_3N$, 3 equiv $K_2CO_3$, MeOH, 2 h, 25 °C.

BAP-linker **2** was synthesized according to the known procedure and analysis matches the previous report.[4d]

Synthesis of *N*-Cbz-*O*-Fmoc-hexanolamine (**3**) as standard for calibration curve preparation

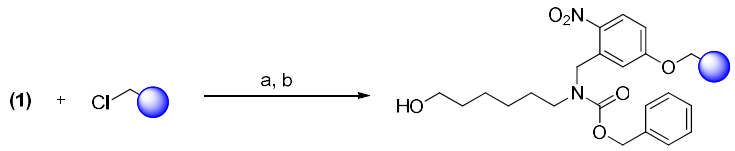



**Scheme S3**. Synthesis of **(3)** Reagents and conditions: **a)** 6-aminohexan-1-ol (1 equiv) MeOH, 30 min, 25 °C. Cbz-Cl (2 equiv), $Et_3N$ (2 equiv), $K_2CO_3$ (3 equiv), MeOH, 2 h, 25 °C. **b)** Fmoc-Cl, pyridine, DCM, 6h, 25 °C.

$Et_3N$ (4.57 ml, 32.93 mmol) and Cbz-Cl (4.25 ml, 29.94 mmol) were added to a solution of 6-aminohexan-1-ol (1.754 g, 14.97 mmol) in MeOH (80 ml) and the mixture was stirred at ambient conditions for 1 h. $K_2CO_3$ (6.20 g, 44.91 mmol) was added and the mixture was stirred for an additional 1 h. The solution was filtered through celite and solvents were evaporated. The crude was dissolved in DCM and washed successively with HCl (1 M) and water. The organic layer was dried over $MgSO_4$, filtered, and the solvent was evaporated. The crude N-Cbz-aminohexanol was purified by silica chromatography. 250 mg of N-Cbz-aminohexanol were dissolved in a 10 ml solution of pyridine 20% in DMF and the solution was added 280 mg of Fmoc-Cl. The reaction was mixed at room temperature overnight and the solvents were evaporated to give pure *N*-Cbz-*O*-Fmoc-hexanolamine **3** as white solid. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.31-7.41 (m, 9H), 5.08 (s, 2H), 4.64 (d, 2H), 4.48 (t, 1H), 4.31 (t, 2H), 3.21 (t, 2H), 1.73 (m, 2H), 1.58 (m, 2H), 1.41 (m, 2H). $^{13}$C-NMR (500 MHz, $CDCl_3$): 155.4, 154.2, 142.4, 140.4, 140.2, 136.5, 127.5, 127.0, 126.8, 126.5, 126.1, 126.0, 124.1, 123.7, 119.0, 118.9, 68.6, 67.1, 65.5, 64.1, 49.6, 45.7, 27.5, 25.2, 24.3.

Solid phase manipulations

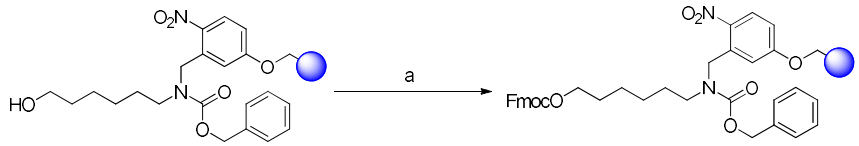
Synthesis of Hydroxy photo linker, **HP-Linker**.




**Scheme S4.** Synthesis of **HP-linker**. Reagents and conditions: **a)** Merrifield resin, TBAI (1.5 equiv), $Cs_2CO_3$ (1.5 equiv), DMF, 12 h, 60 °C. **b)** CsOAc, DMF, 12 h, 60 °C.

**HP-Linker** was prepared on a Merrifield resin (loading 0.564 mmol/g) following a previously described procedure.[1] To a suspension of Merrifield resin (1 g, 0.53 mmol) in DMF (30 ml), a solution of 1 (0.21 g, 0.522 mmol) in DCM (5 ml) was added followed by addition of Cesium carbonate ($Cs_2CO_3$, 0.25 g, 0.794 mmol) and tetrabutylammonium iodide (TBAI, 0.29 g, 0.8 mmol). The solution was allowed to rotate at 60 °C and 870 mbar on a rotavap overnight. The next morning, the resin was filtered and washed successively with DMF/Water (1/1), DMF, MeOH, DCM, MeOH, and DCM, and then allowed to swell in DCM for 1 h. The swollen resin was placed in a flask with DMF (60 ml) and Cesium acetate (CsOAc, 1.80 g, 9.47 mmol) was added. The suspension allowed to rotate at 60 °C and 870 mbar on a rotavap overnight. The resin was washed successively with DMF/Water (1/1), DMF, MeOH, DCM, MeOH, and DCM, the solvent was drained, and the resin dried under vacuum to give solid support functionalized with the linker.

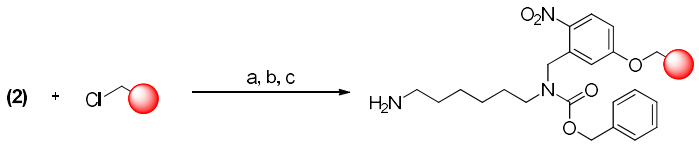
Preparation of Fmoc Labeled hydroxyl photo linker, **FHP-Linker**




**Scheme S5.** Synthesis of **FHP-linker**. Reagents and conditions: **a)** Fmoc-Cl, pyridine, DCM, 6h, 25 °C.

100 mg of **HP-Linker** were placed in a fritted syringe (10 ml). Dry DCM (8 ml) was added to swell the resin for 1 h. the DCM was drained and a solution of Fmoc-Cl (200 mg, 0.2 mmol) and pyridine (0.2 ml) in DCM (2 ml) was added to the resin. The reaction mixture was covered with aluminum foil and shaken for 6 h on an oval shaker. The solution drained and the resin washed with DCM, MeOH, and dried under vacuum

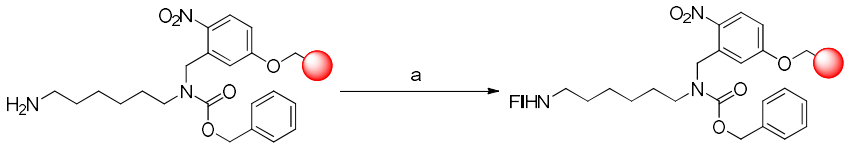
Solid Support Functionalization, synthesis of **AP-Linker**




**Scheme S6**. Synthesis of **AP-Linker**. Reagents and conditions: **a)** Merrifield resin, TBAI (1.5 equiv), CsCO3 (1.5 equiv), DMF, 12 h, 60 °C; **b)** CsOAc, DMF, 12 h, 60 °C; **c)** 10 % TFA in DCM, 30 min, 25 °C

**AP-Linker** was prepared using standard procedures. AP-Linker Loading determination and preparation of Fmoc Labelled Solid Support was performed as previously described. [4d]

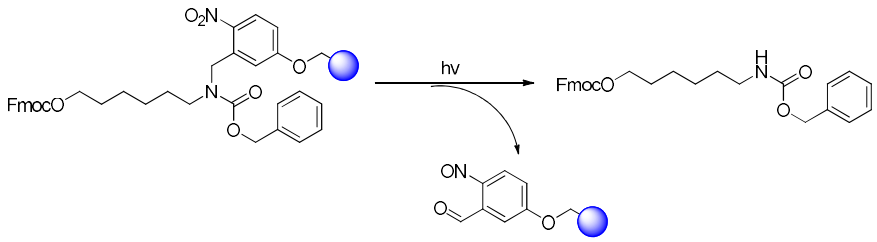
Preparation of Fluorescein Labeled Solid Support **FlAP-Linker**




**Scheme S7**: synthesis of **FLAP-Linker** Reagents and conditions: *N,N*-Diisopropylethylamine, *N,N'*-Diisopropylcarbodiimide, Hydroxy-benzotriazole, fluorescein. Overnight, RT

Labelling was performed according to a previously reported procedures.[14] 100 mg of **AP-Linker**, placed in Sep-Pak cartridge was added 1.7 ml DMF, 0.2 ml DCM, 46 µL *N,N*-diisopropylethylamine, 42 µL *N,N'*-diisopropylcarbodiimide, and 36 mg of hydroxy benzotriazole and 100 mg of fluorescein (CF). The Sep-Pak was covered with aluminum foil and the suspension was shaken at room temperature overnight. The resin was washed repeatedly with DMF, followed by DCM and MeOH. The obtained **FlAP-Linker** solid support was dried under vacuum and kept in dark.

Cleavage of *N*-Cbz-*O*-Fmoc-hexanolamine (**3**) from **FHP-Linker**



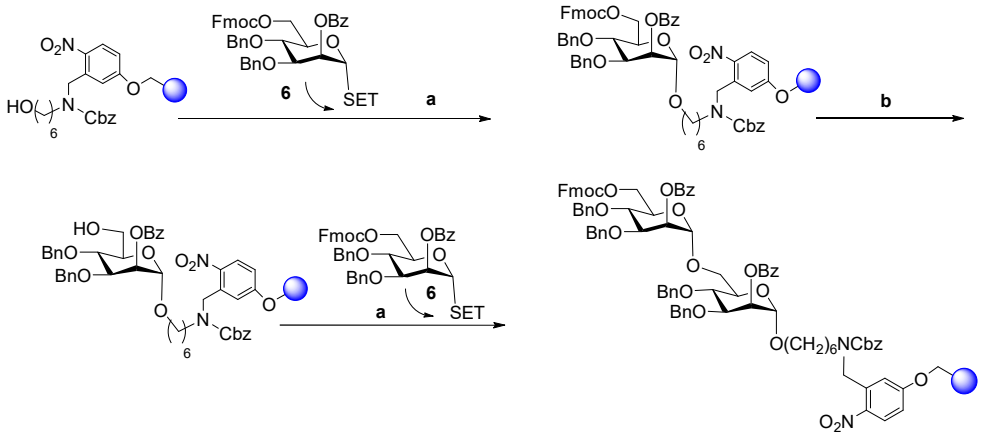

**Scheme S8:** FHP-linker cleavage with its products on solid support and in a filtrate.

A suspension of **FHP-linker** (100 mg, loading 0.564 mmol/g) and DCM (2 ml) in 1 cm quartz cuvette equipped with a magnetic bar was placed on magnetic stirrer in front of a LED lamp (Fig S1). The irradiation was performed at 365 nm in a dark room for different durations and with variable stirring rates as described later for each specific experiment. After the light source was turned off, the suspension was filtered through a fritted Sep-Pak cartridge (12 ml Capacity,

20 µm porosity Frits) and the solution was collected. An additional 2 ml of DCM was used to wash the cuvette the remaining solid support and also filtered through the same Sep-Pak. The combined DCM filtrate containing amino alcohol **3** was placed in a small 5 ml glass beaker and the DCM was allowed to evaporate. The crude material was characterized by NMR and HRMS. $^{1}$H-NMR (500 MHz, CDCl3): 7.31 - 7.41 (m, 9H), 7.67 (m, 2H), 7.86 (m, 2H), 5.17-5.05 (s, 2H,), 3.63 3.60 (m, 2H), 3.38-3.35 (m, 2H), 1.57-1.41 (m, 6H), 1.70-1.74 (m, 2H). $^{13}$C-NMR (500 MHz, CDCl3): 170.1, 154.2, 142.4, 140.2, 133.6, 127.5, 127.2, 127.0, 126.8, 126.1, 124.1, 119.0, 68.6, 67.0, 65.6, 59.3, 45.7, 30.9, 28.6, 27.5, 21.6, 20.0. HRMS calc for: C29H31NO5Na $[M+Na]^{+}$, 496.2100; found: 496.2115. The remaining solid support was further washed with DMF, DCM, MeOH, then dried under vacuum. After each irradiation, few beads were washed and taken for microscopic analysis to visualize the shape and integrity of the solid support.

# Synthesis, characterization and cleavage efficiency analysis of disaccharide 5

## Procedure for Automated glycan assemble of disaccharide 5




**Scheme S9**: Synthesis of disaccharide by AGA. Reagents and conditions: **a)** DMF, THF, DCM, TMSOTf in DCM **6**, NIS, TfOH, 40 min, -20 ° C. **b)** 20% Piperidine in DMF, 30 min, 25 ° C.

The fully protected α-1-6-dimannose **5** was synthesized on the **HP-Linker** using automated synthesizer glyconeer 2.1 (Scheme 2). **HP-Linker** (50 mg, loading 0.56 mmol/gr) was placed in the glyconeer 2.1 reaction vessel. The rest of the process was performed using typical automated glycan assembly (AGA) protocols. HP-Linker was swollen and washed using TMSOTf in DCM and glycosylation of Fmoc protected thiomannoside **6** (109 mg) was performed using NIS/TfOH (0.15 M) as activation reagents at -22 °C. After the glycosylation, Fmoc was removed using 20% piperidine in DMF and another glycosylation cycle using the exact conditions was performed.

## Automated glycan assembly working modules

The exact timing and quantity of solvents transferred to the reaction vessel in each step are controlled by the software and the exact composition of the solution is described for each module in the next section. The system is constantly pressurized using Argon gas so that the

specific solvent/reagent is transferred by timing the opening and closing of the appropriate valves (Table S7). AGA of Di-mannose **5** was performed in the following steps using the modules described in the method section below.

Module 1: Swelling the resin: The resin is washed with DMF, THF, DCM (six times each with 2 ml). The resin is swollen in 2 ml DCM

Module 2: Acidic Washing: The temperature of the reaction vessel was adjusted to –20 °C. A 0.5 ml solution of TMSOTf in DCM 40 ml is delivered to the reaction vessel. After one minute, the solution is drained. Finally, 2 ml DCM is added to the reaction vessel. And the resin is washed with DCM one time

Module 3: Glycosylation of Fmoc-Mannose-SEt **6**: Thioglycoside building block **6** is dissolved in the proper solvent mixture (2 ml for two glycosylation cycles) in the designated building block vial on the carousel. The reaction vessel is set to reach the initial glycosylation temperature (-22º C). During the adjustment of the temperature, the DCM in the reaction vessel is drained and half the solution of **6** (5.0 eq. in 1.0 ml DCM) is delivered from the building block vial to the reaction vessel. After the set temperature of -20 °C is reached, 1.0 ml solution of 1.5 g of NIS and 64 µl of TfOH in DCM dioxane (v/v, 2:1) is delivered to the reaction vessel. The glycosylation mixture is incubated for 5 min at -20 °C, and after reaching -20 °C the reaction mixture as incubated for an additional 20 min. Once incubation time is finished, the reaction mixture is drained to the fraction collector where it is collected in a designated tube. These reaction mixtures were analyzed by TLC to follow the activation of the thioglycosides. The resin in the reaction vessel is further washed with DCM (six times with 2 ml for 15 sec).

Module 4: Fmoc Deprotection: The resin is washed with DMF (six times with 2 ml for 25 s), swollen in 2 ml DMF and the temperature of the reaction vessel is adjusted to 25 °C. DMF is drained and a 2 ml solution of 20% piperidine in DMF was delivered to the reaction vessel. After 5 min the reaction solution is drained through the UV detector and the transmittance is recorded A fresh 2 ml solution of 20% piperidine in DMF is again delivered to the resin and incubated for another 5 min. after the second deprotection cycle, the resin was washed with DMF (six times with 2 ml for 15 sec) Online Fmoc quantification (**Figure S9**) UV transmittance of the Fmoc deprotection solution was measured at a wavelength of 301 nm after deprotection step.

Module 5: End wash: Rinses/purges all the manifold at 20º C for 20 min

## Cleavage of disaccharide **5** using the breaking beads approach

HP-Linker bearing disaccharide (25 mg, loading 0.56 mmol/g) were placed in a quartz cuvette with a magnetic bar. The suspension was stirred for four hours at 1060 rpm without irradiation. This suspension was irradiated for 1 h while maintaining the stirring rate of 1060 rpm. The light source was turned off and the suspension of solid support in DCM was collected with a plastic pipette and filtered through a fritted Sep-Pak cartridge (12 mL Capacity 100 pk, 20 µm porosity Frits). An additional 2 mL of DCM was used to collect the remaining solid support from the cuvette. This solution was filtered through the same Sep-Pak. The combined DCM filtrate containing the cleaved compound was placed in a 5 ml round flask and the DCM was evaporated to give **5** (14.22 mg, yield 74.3%). Fmoc quantification of the beads after cleavage shows that the yield is 77%, when taking into account that 30% of the beads' mass is the protected saccharide. The crude material was characterized to show that we have the compound

by $^1$H NMR, HSQC, COSY, HPLC. $^1$H NMR (500 MHz, $C_6D_6$) δ 8.44 – 8.39 (m, 2H), 8.39 – 8.36 (m, 2H), 7.53 – 7.52 (m, 4H), 7.45 – 7.42 (m, 1H), 7.34 – 7.25 (m, 14H), 7.19 – 7.17 (m, 2H), 7.13 – 6.99 (m, 18H), 6.96 – 6.93 (m, 1H), 6.09 – 6.08 (m, 1H), 5.95 – 5.94 (m, 1H), 5.18 (d, 1H), 5.11 (s, 1H), 5.03 – 5.01 (d, 1H), 4.95 – 4.93 (d, 1H), 4.81 – 4.78 (d, 1H), 4.64 – 4.62 (m, 1H), 4.58 – 4.55 (m, 2H), 4.50 – 4.46 (m, 1H), 4.38 – 4.36 (d, 1H), 4.34 – 4.32 (m, 1H), 4.29 – 4.27 (m, 1H), 4.25 – 4.19 (m, 4H), 4.18 – 4.14 (m, 1H), 4.13 – 4.10 (m, 1H), 4.09 – 4.01 (m, 4H), 3.85 – 3.80 (m, 2H), 3.59 – 3.55 (m, H), 3.19 – 3.14 (m, 1H), 2.99 – 2.95 (m, 1H),1.19 – 1.13 (m, 5H), 1.07 – 1.05 (m, 3H).C-NMR verified by HSQC: 131.0, 129.9, 129.9, 129.9, 129.9, 129.9, 128.8, 128.0, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.8, 127.8, 127.2, 127.2, 127.1, 125.5, 125.4, 125.3, 125.2, 119.9, 98.9, 98.0, 75.4, 75.3, 75.2. 75.1, 75.1, 75.0, 74.6, 74.0, 71.7, 71.6, 71.3, 71.2, 71.1 70.6, 70.5, 46.4, 40.3, 26.5, 26.4, 25.8, 23.2. HRMS calc for: $[M+Na]^+$, 1388.5553, found; 1388.5477. The Sep-Pak containing solid support after irradiation was stoppered and 1.5 ml of 20% piperidine in DMF was added. The Sep-Pak was sealed, covered with aluminium foil, and left to shake for 2 h on an oval shaker. 40 µl of this solution was placed in a 2 ml volumetric flask, and filled to volume with a 20% of piperidine in DMF solution. The diluted solution was placed in a quartz cuvette and the absorbance was measured at a wavelength of 301 nm. Loading of the Fmoc after cleavage was calculated based on the measured absorbance. The percentage of cleavage was calculated to be 77 % by comparing to theoretical loading before cleavage (Table S8).

### Cleavage of disaccharide **5** without stirring

HP-Linker bearing disaccharide (25 mg, loading 0.56 mmol/g) were placed in a quartz cuvette with a magnetic bar. The suspension was irradiated for 1 h without irradiation then. The light source was turned off and the suspension of solid support in DCM was collected with a plastic pipette and filtered through a fritted Sep-Pak cartridge (12 mL Capacity 100pk, 20 µm porosity Frits). Additional 2 mL of DCM was used to wash the cuvette remove the remaining solid support which also filtered through the same Sep-Pak the combined DCM filtrate containing the cleaved compound was placed in a 5 ml round flask and the DCM was allowed to evaporate to give **5** (0.8 mg, yield 4.2%). The Sep-Pak containing solid support after irradiation was stoppered and 1.5 ml of 20% piperidine in DMF was added. The Sep-Pak was sealed covered with aluminum foil and left to shake for 2h on an oval shaker. 40 µl of this solution was placed in a 2 ml volumetric flask and filled to volume with 20% of piperidine in DMF solution. The diluted solution was placed in a quartz cuvette and the absorbance was measured at a wavelength of 301 nm using a Spectrophotometer. Loading of the Fmoc after cleavage was calculated based on the measured absorbance. The percentage of cleavage was calculated to be 6 by comparing to theoretical loading before cleavage (Table S8).

## Tables

**Table S1: Concentrations and measured absorbance for calibration curve**

| Solution | Final concentration (mM) | Abs at 301 nm (l $mol^{-1}$ $cm^{-1}$) | Volume of standard solution (ml) | Final volume (ml) |
|---|---|---|---|---|
| B | 0.02 | 0.11 | 1 | 10 |
| C | 0.04 | 0.201 | 2 | 10 |
| D | 0.06 | 0.306 | 3 | 10 |
| E | 0.08 | 0.401 | 4 | 10 |
| F | 0.1 | 0.500 | 5 | 10 |

**Table S2: Effect of stirring duration on photo-cleavage**

| Irradiation duration (h) | Loading at 301 nm (mmol/g) | Loading at 289 nm (mmol/g) |
|---|---|---|
| 0 | 0.564 | 0.583 |
| 1 | 0.317±0.013 | 0.340±0.004 |
| 2 | 0.196±0.009 | 0.215±0.005 |
| 4 | 0.051±0.006 | 0.054±0.003 |
| 6 | 0.036±0.002 | 0.037±0.004 |

*loading was determined based on the absorbance at 301 and 289 nm.

**Table S3: Effect of mixing rate on photo-cleavage**

| Mixing rate (rpm) | Loading at 301 nm (mmol/g) | Loading at 289 nm (mmol/g) |
|---|---|---|
| 0 | 0.187±0.006 | 0.203±0.011 |
| 100 | 0.144±0.010 | 0.148±0.012 |
| 800 | 0.093±0.004 | 0.103±0.010 |
| 1060 | 0.051±0.006 | 0.054±0.003 |

**Table S4: Effect of pre-stirring on photo-cleavage**

| Stirrer time before irradiation (h) | Irradiation time (h) | Loading at 301 nm (mmol/g) | Loading at 289 nm (mmol/g) | %photo-cleavage | %Relative Fmoc-loading |
|---|---|---|---|---|---|
| 0 | 1 | 0.317±0.013 | 0.340±0.004 | 43.7 | 56.2 |
| 4 | 1 | 0.089±0.008 | 0.096±0.014 | 84.2 | 15.7 |

**Table S5: Absorbance of 3 after different irradiation time and calculated amount based on the calibration curve**

| Irradiation time (h) | Abs at 301 nm (l $mol^{-1}$ $cm^{-1}$) | n (mmol) |
|---|---|---|
| 1 | 0.461±0.004 | 0.0067 |
| 2 | 0.745±0.008 | 0.011 |
| 4 | 0.806±0.006 | 0.012 |
| 6 | 0.914±0.008 | 0.014 |
| Theoretical value for 100 % cleavage[a] | 1.119 | 0.017 |

*based on cleavage from 30 mg resin with a loading of 0.56 mmol/g.

**Table S6: The detailed MPLC gradient program:**

| Time (min) | % Hexane | % Ethyl acetate |
|---|---|---|
| 00 | 95 | 05 |
| 10:00 | 95 | 05 |
| 15:00 | 90 | 10 |
| 20:00 | 90 | 10 |
| 40:00 | 70 | 30 |
| 45:00 | 70 | 30 |
| 50:00 | 00 | 100 |
| 55:00 | 00 | 100 |
| 1:00:00 | 00 | 100 |
| 1:05:00 | 00 | 100 |

**Table S7: AGA manipulations**

| Step | Module | Temperature (°C) | Time (min) |
|---|---|---|---|
| Swelling | Swelling | 25 | 30 |
| Acid wash | Acid wash | -20 | 15 |
| Fmoc-Mannose-SEt | Glycosylation | -22 | 40 |
| Fmoc deprotection | Fmoc deprotection | 25 | 30 |
| Acid wash | Acid wash | -20 | 15 |
| Fmoc-Mannose-SEt | Glycosylation | -22 | 40 |
| End wash | End wash | 20 | 20 |

**Table S8: Effect of pre-stirring on photo-cleavage of Di-mannose 5**

| Stirrer time before irradiation (h) | Irradiation time (h) | Loading at 301 nm (mmol/g) | %photo-cleavage |
|---|---|---|---|
| 0 | 1 | 0.376 | 6% |
| 4 | 1 | 0.09 | 77% |

* We assumed that the loading after AGA should be 0.392 mmol/gr. Assuming 100% glycosylation means that 30% of the overall mass is the synthesized saccharide based on an initial loading of 0.56 mmol/g. Each irradiation was performed on 25 mg resin.

**Table S9: Effect of pre-stirring on photo-cleavage in a shaker**

| Stirrer time before irradiation (h) | Irradiation time (h) | Loading at 301 nm (mmol/g) | %photo-cleavage |
|---|---|---|---|
| 0 | 1 | 0.52 | 10.3% |
| 2 | 1 | 0.34 | 58.6% |

## Figures

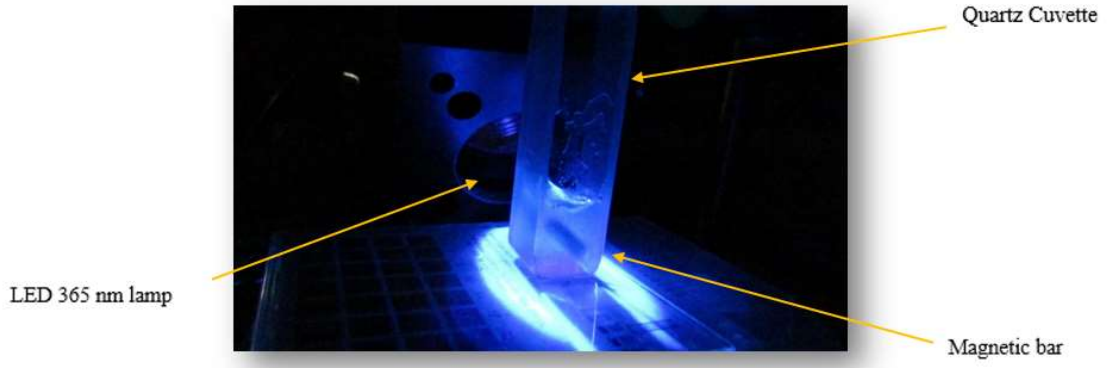



**Figure S1**: Setup of UV irradiation reactor that contains 1x1 [cm] quartz cuvette equipped with small magnetic bar and UV irradiation lamp at 365 nm.

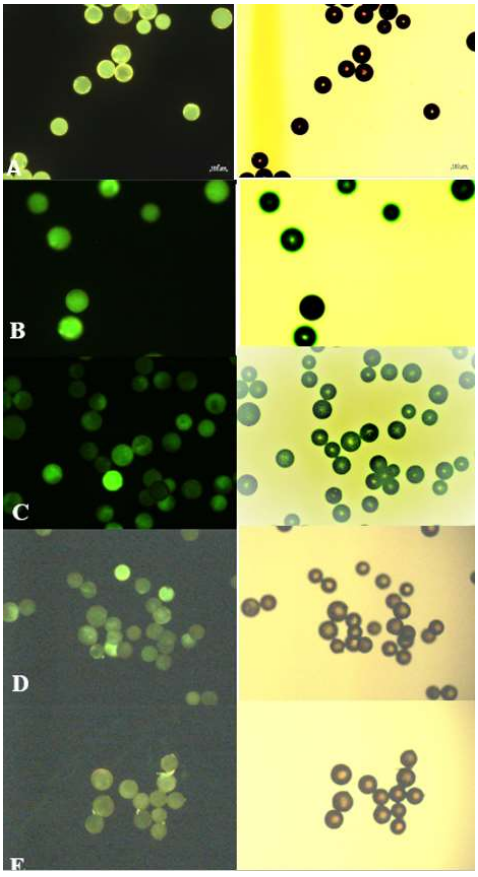



**Figure S2.** Fluorescent (left) and regular microscopy images (right) of **FlAP-Linker** before (A) and after 2 ,4 ,6 and 8 h irradiation (B, C, D, E respectively).

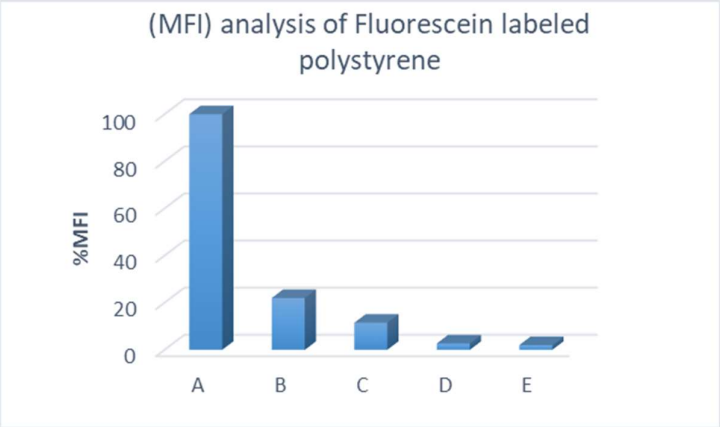



**Figure S3**: Mean fluorescence intensity analysis of Fluorescein labeled polystyrene before (A) and after 2 ,4 ,6 and 8 h irradiation (B, C, D, E respectively).

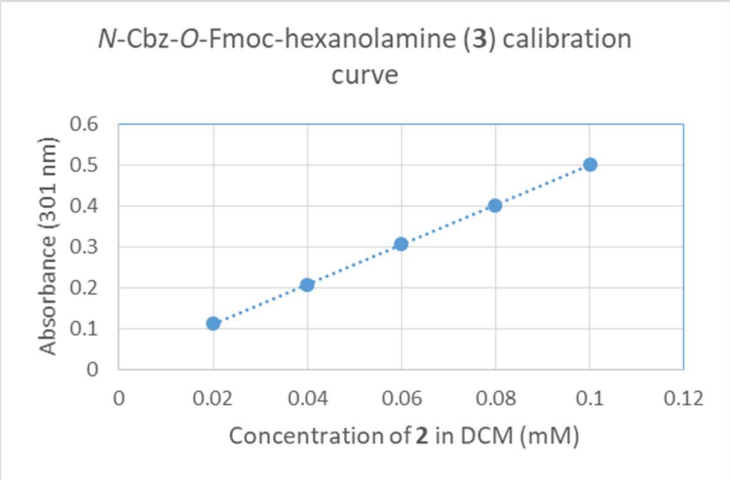



**Figure S4:** Calibration curve for **(3)**. The absorbance of solutions B-F was measures at 301 nm and a linear correlation between the absorbance and the concentration was measured.

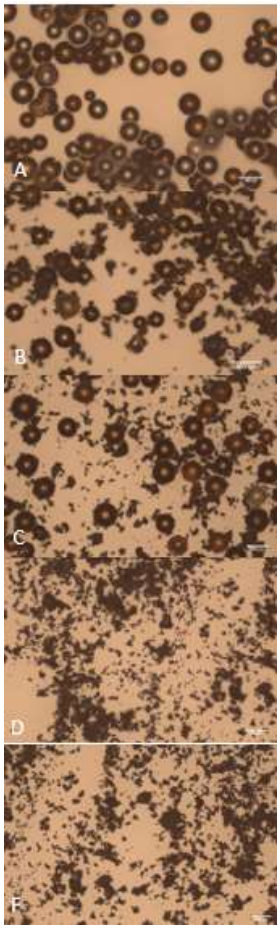



**Figure S5:** Microscope image of Fmoc labeled polystyrene beads before (A) and after 1,2 ,4 and 6 h irradiation (B, C, D, E respectively).

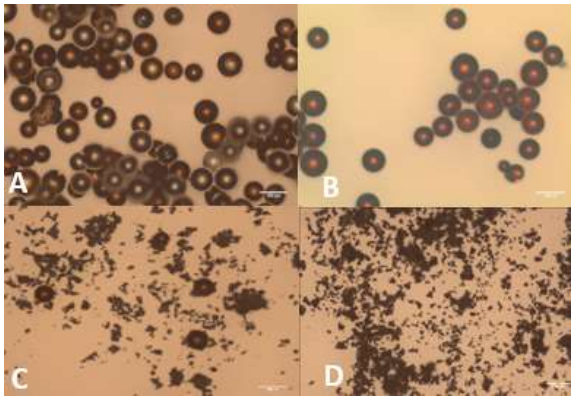



**Figure S6.** Microscope image of Fmoc labeled polystyrene beads before (A) and after stirring for 4 h at 100, 800 and 1060 rpm (B, C, D respectively).

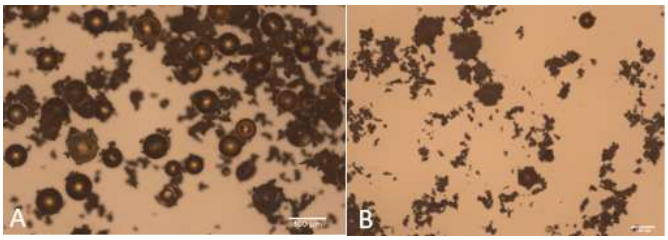



Figure S7: Microscope image of Fmoc labeled polystyrene beads after 1 h of irradiation without and with 4 h of stirring at 1060 rpm prior to irradiation (A, B respectively).

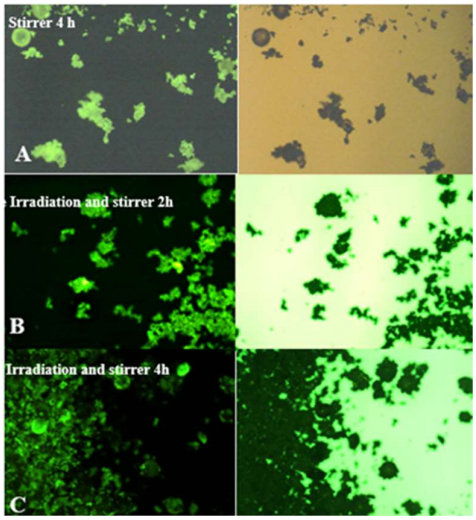



**Figure S8.** Fluorescent (left) and regular microscopy images (right) of **FlAP-Linker** before (A) and after 2 and 4h irradiation with irradiation (B, C respectively).

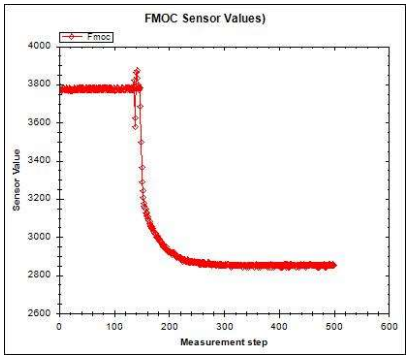



**Figure S9** Online Fmoc quantification of deprotection process in AGA measured at wavelength of 301 nm.

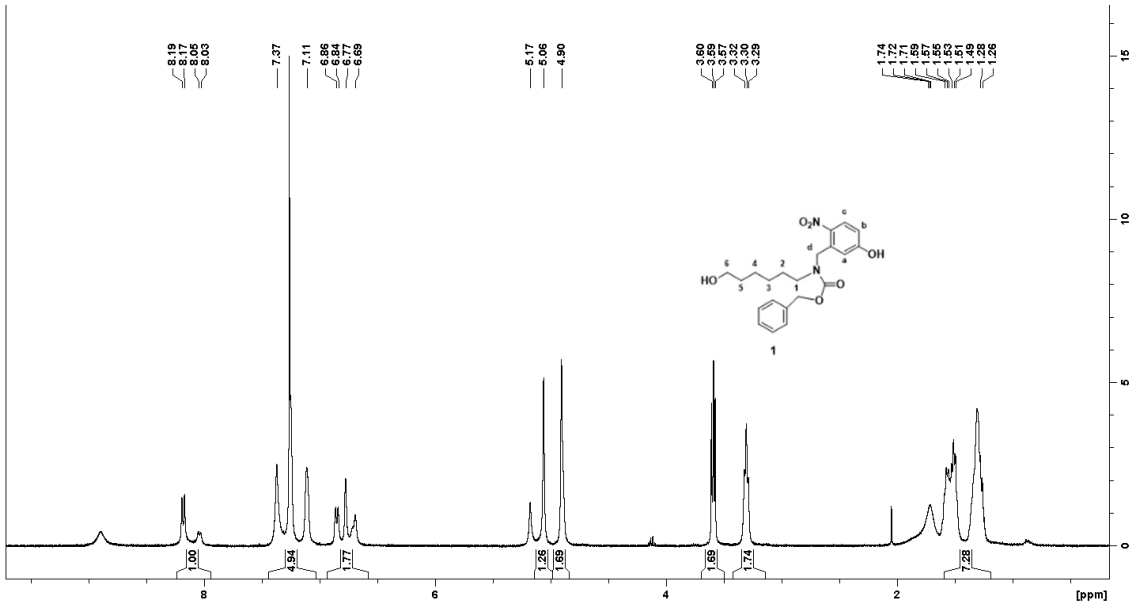



**Figure S10.** $^{1}$H-NMR spectrum of of benzyl (5-hydroxy-2-nitrobenzyl) (6-hydroxyhexyl) carbamate, (**1)** that was synthesized in solution (500 MHz) in $CDCl_3$.

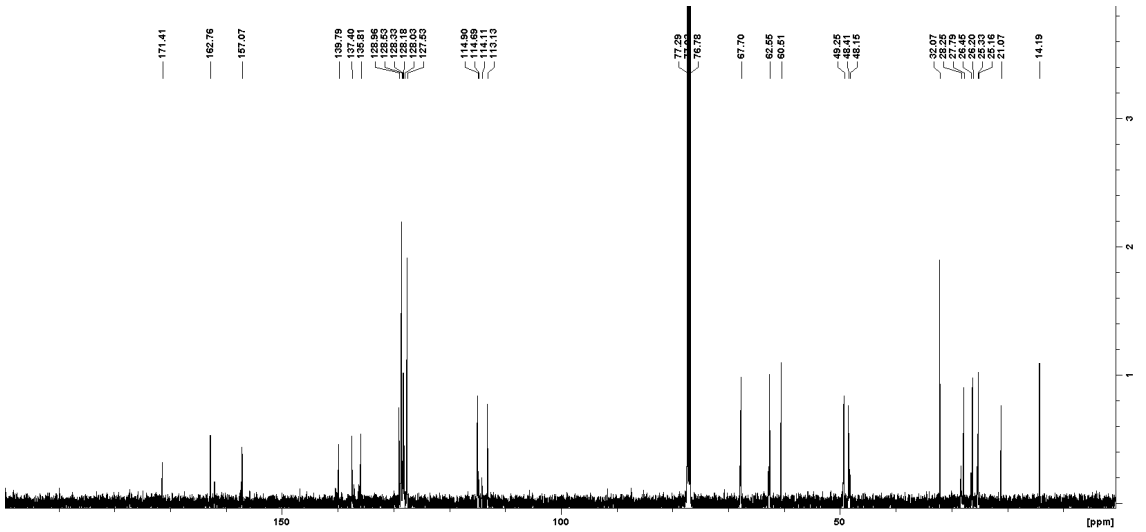



**Figure S11**. $^{13}$C-NMR spectrum of benzyl (5-hydroxy-2-nitrobenzyl) (6-hydroxyhexyl) carbamate **(1),** that was synthesized in solution (500 MHz) in $CDCl_3$.

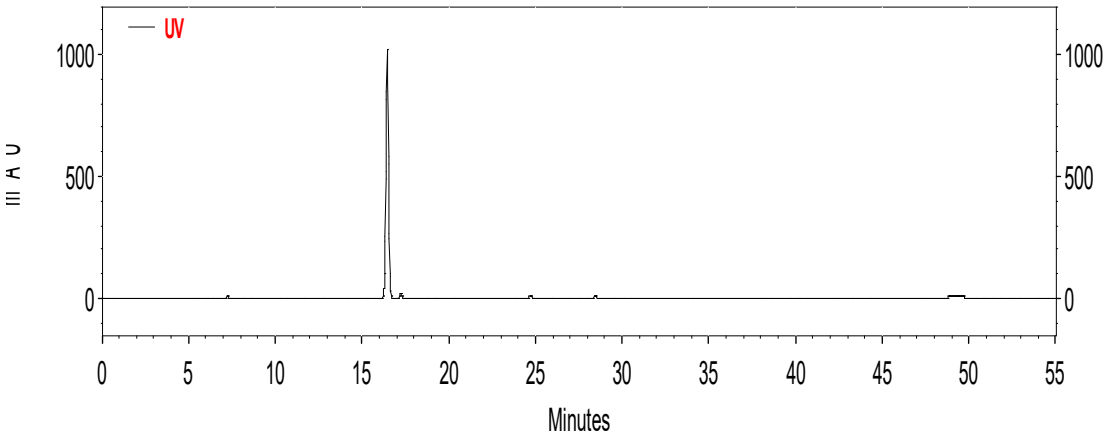



**Figure S12**. Analytical RP-HPLC spectrum of benzyl (5-hydroxy-2-nitrobenzyl) (6-hydroxyhexyl) carbamate **(1)**.

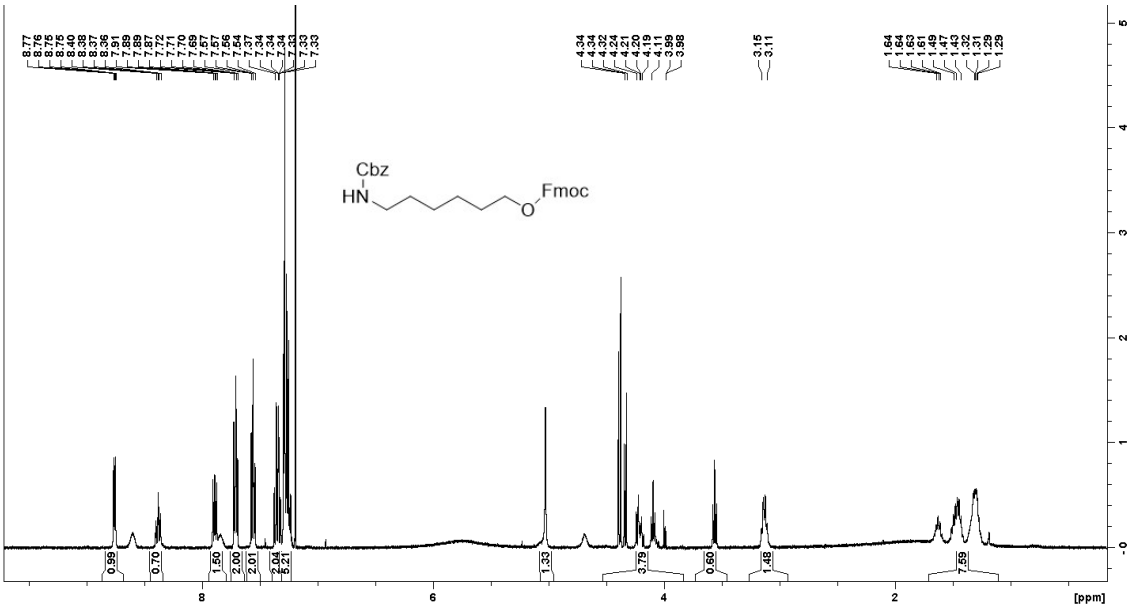



**Figure S13.** $^{1}$H-NMR spectrum of N-Cbz-O-Fmoc hexanolamine standard **(3)** that was synthesized in solution (500 MHz) in $CDCl_3$.

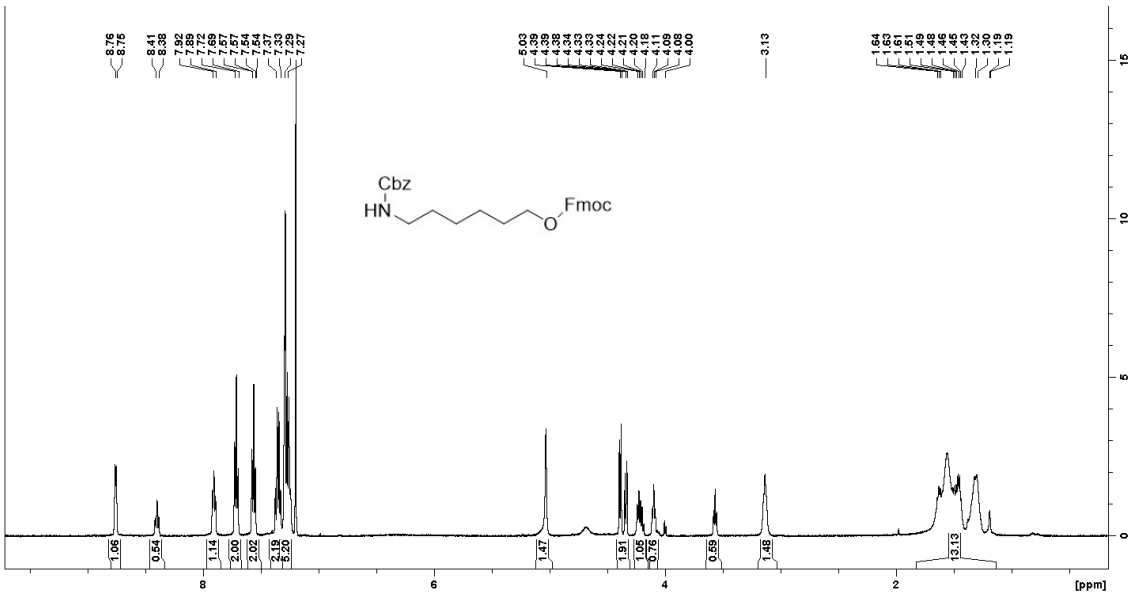



**Figure S14.** $^{1}$H-NMR spectrum of N-Cbz-O-Fmoc hexanolamine **(3)** that was cleaved off 100 mg of **FAP-Linker** without any purification (500 MHz) in $CDCl_3$.

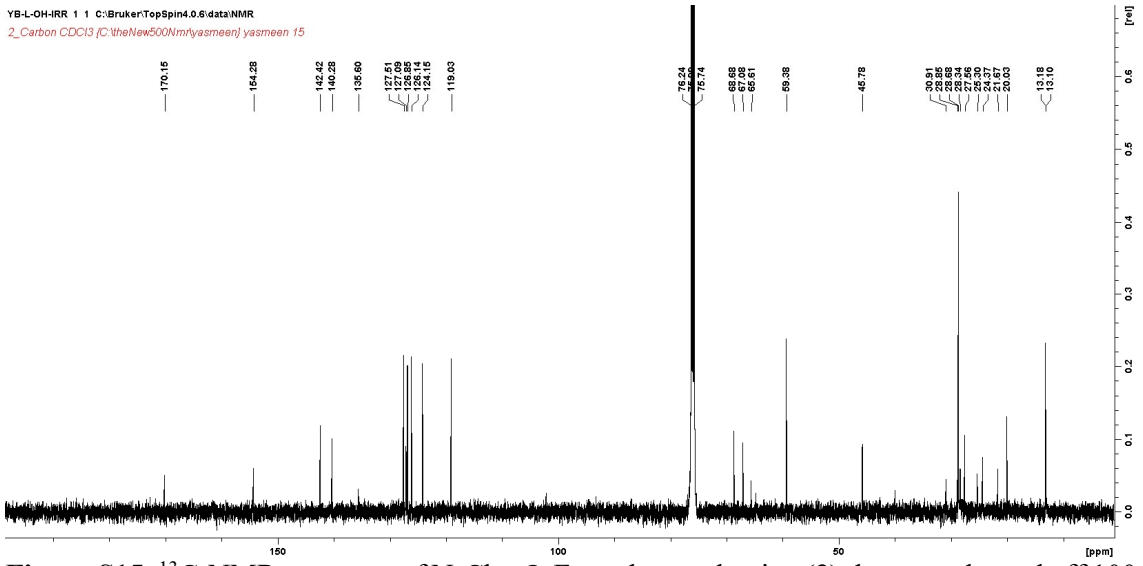



**Figure S15.** $^{13}$C-NMR spectrum of N-Cbz-O-Fmoc hexanolamine **(3)** that was cleaved off 100 mg of **FAP-Linker** without any purification in $CDCl_3$.

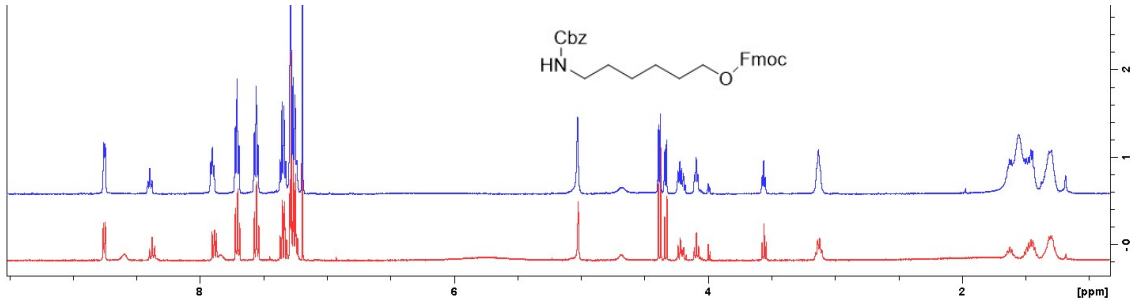



**Figure S16**. $^{1}$H-NMR spectra (500 MHz) in $CDCl_3$ of N-Cbz-O-Fmoc hexanolamine **(3)** that was either prepared synthetically in solution (red) or cleaved off 100 mg of **FAP-Linker** without any purification (Blue).

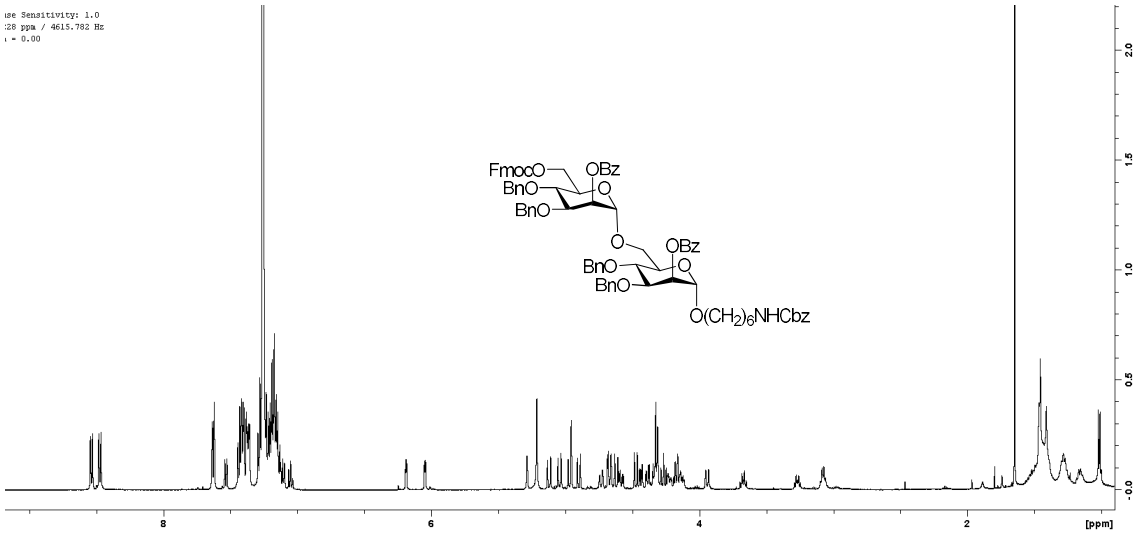



**Figure S17**. $^{1}$H -NMR spectrum (500 MHz) in $C_6D_6$ of dimannose **(5)** that was cleaved off 25 mg without any purification.

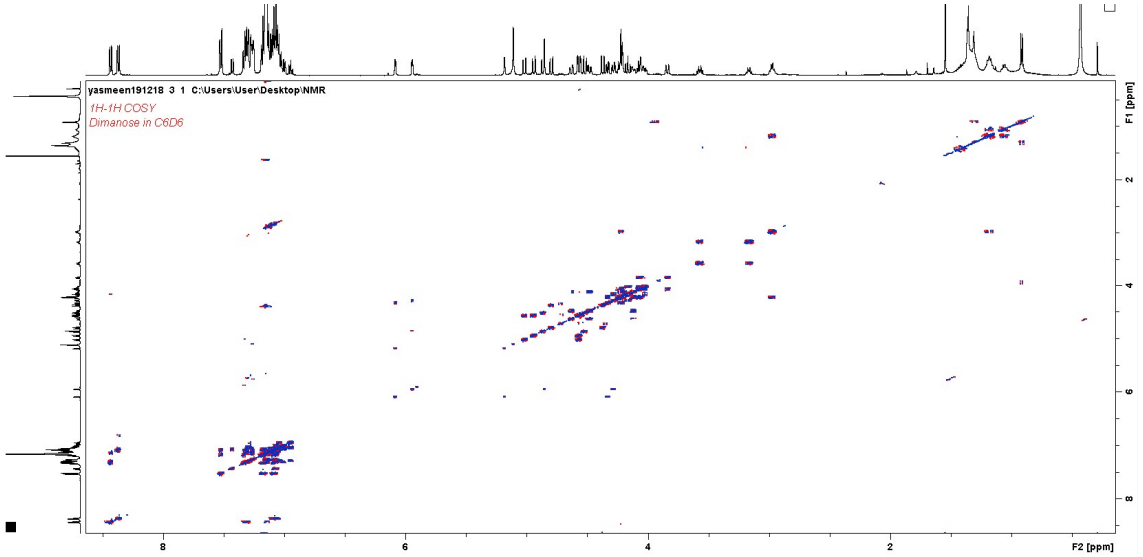



**Figure S18**. COSY NMR (500 MHz) in $C_6D_6$ of mannose **(5)** that was cleaved off 25 mg without any purification.

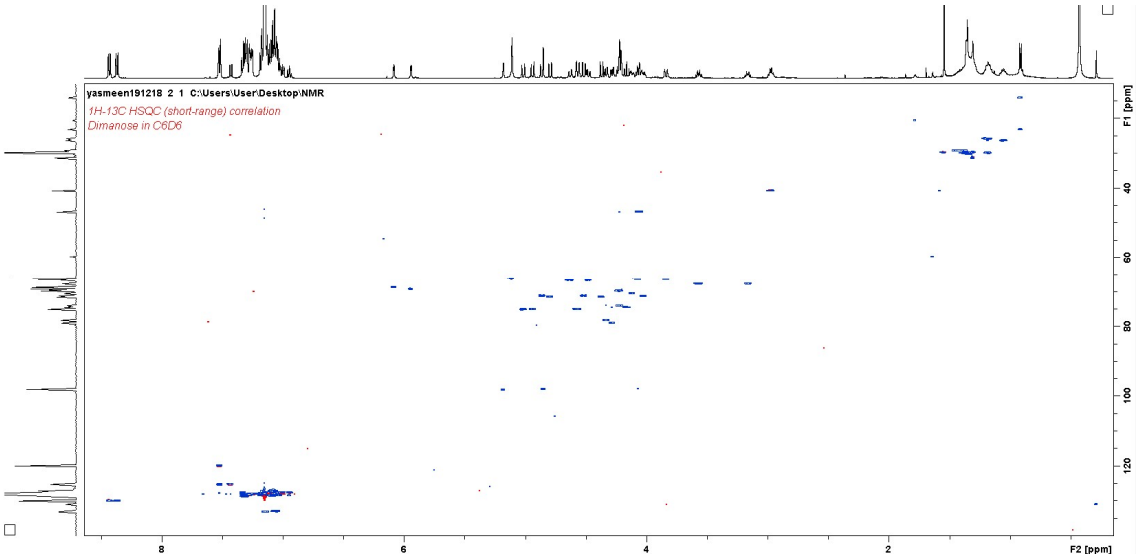



**Figure S19**. HSQC NMR (500 MHz) in $C_6D_6$ of dimannose **(5)** that was cleaved off 25 mg without any purification.

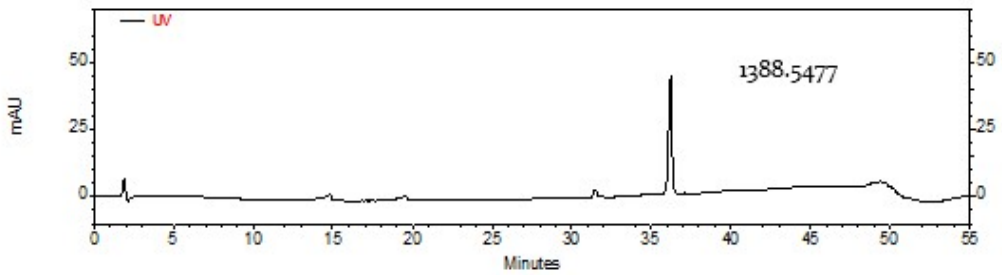



**Figure S20.** Analytical RP-HPLC of dimannoes **(5)** that was cleaved off 25 mg without any purification.

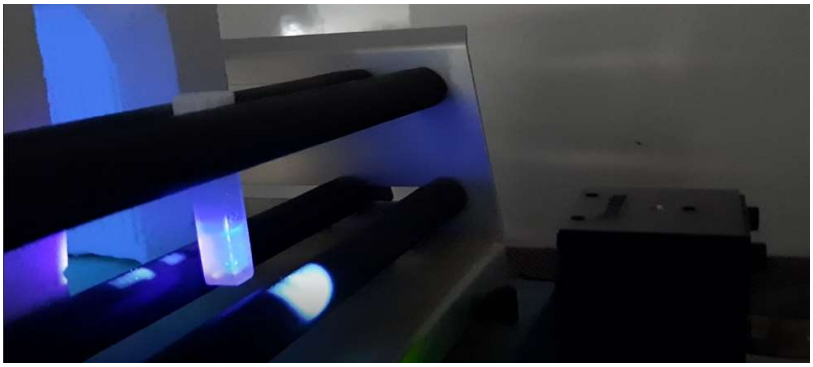


**Figure S21**: Setup of UV irradiation reactor that contains quartz cuvette equipped with small magnetic bar and UV irradiation lamp at 365 nm.in a shaker