Electronic Supplementary Information

Visible Light-Activatable Cyclodextrin-Conjugates for the Efficient Delivery of Nitric Oxide with Fluorescent Reporter and their Inclusion Complexes with Betaxolol

Mimimorena Seggio,[a] Sara Payamifar,[a,b,§] Aurore Fraix,[a] Eszter Kalydi,[c,d] Petr Kasal,[e] Ovidio Catanzano,[f] Claudia Conte,[g] Fabiana Quaglia[g,*] and Salvatore Sortino[a,*]

[a] *PhotoChemLab, Department of Drug and Health Sciences, University of Catania, Catania, Italy. Email: ssortino@unict.it*
[b] *Organic Chemistry Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, Iran.*
[c] *CycloLab Ltd., H-1097 Budapest, Illatos út 7, Hungary.*
[d] *Department of Pharmacognosy, Semmelweis University, Budapest, H-1085 Üllői út 26, Hungary.*
[e] *Department of Organic Chemistry, Charles University in Prague, Hlavova 2030/8 128 43 Prague 2 Czech Republic.*
[f] *Institute for Polymers, Composites and Biomaterials, CNR, Via Campi Flegrei 34, 80078 Pozzuoli, Napoli, Italy.*
[g] *Drug Delivery Laboratory, Department of Pharmacy, University of Napoli Federico II, Via Domenico Montesano 49, 80131, Napoli, Italy. Email: quaglia@unict.it*
[§] *S. P. carried out the work at the University of Catania during her stage from the IASBS.*

Synthetic Description

In Fig. S1, the synthesis scheme for βCD-NBFNO1 is shown. 6-Monodeoxy-6-monoamino free base (2) was obtained by precipitation of the corresponding hydrochloride salt with concentrated ammonia. The nitrobenzofurazanyl group was installed onto the CD scaffold without using any additional base in order to avoid the formation of NBF-related by-products; it is worth noticing that 4-chloro-7-nitrobenzofurazan would react with any additional base in the mixture thus creating new chromophoric species and complicating the purification process. The βCD-NBF1 derivative was isolated by chromatography. βCD-NBFNO1 was obtained by reacting βCD-NBF1, solubilized in a mixture of DMSO/CH_3COOH 1:1 with $NaNO_2$. The solution was stirred at 0 °C for 1 hour and at room temperature for 2 days. The reaction mixture was precipitated with acetone. The solid was filtered-off, and a dark yellow powder was obtained.

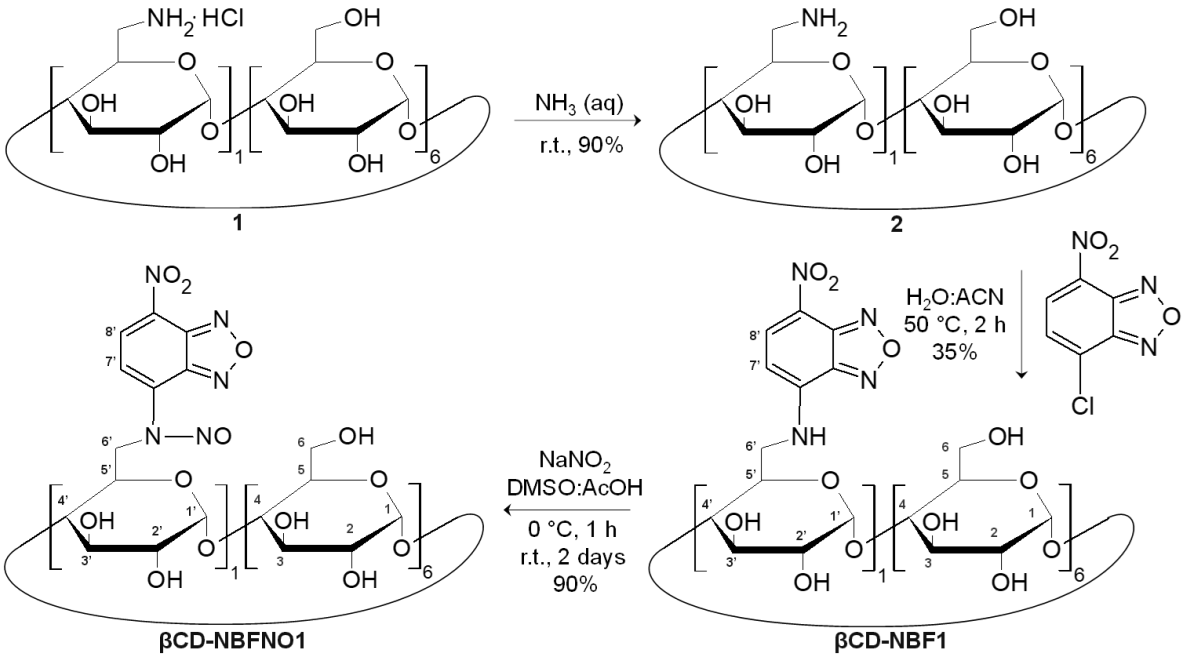


Figure S1: Synthesis scheme for βCD-NBFNO1.

In Fig. S2, the synthesis scheme for βCD-NBFNO2 is shown. Regioselective 2-*O*-monopropargylation of βCD (3) was achieved in DMSO with LiH and propargyl-bromide as previously reported.[1S] The conjugation of 2-*O*-monopropargyl-βCD (4) and azido-diethylene glycol-nitrobenzofuraran linker (5) was based on a copper-assisted azide–alkyne cycloaddition. The click reaction was performed in DMF mixture at 60 °C with copper(I) bromide as catalyst. The reaction crude was purified by preparative direct-phase chromatography on silica gel yielding βCD-NBF2 in good purity. βCD-NBFNO2 was obtained by reacting βCD-NBF2, solubilized in a mixture of DMSO/CH_3COOH 1:1 with $NaNO_2$. The solution was stirred at 0 °C for 1 hour and at room temperature for 2 days. The reaction mixture was precipitated with acetone. The solid was filtered-off, and a dark yellow powder was obtained.

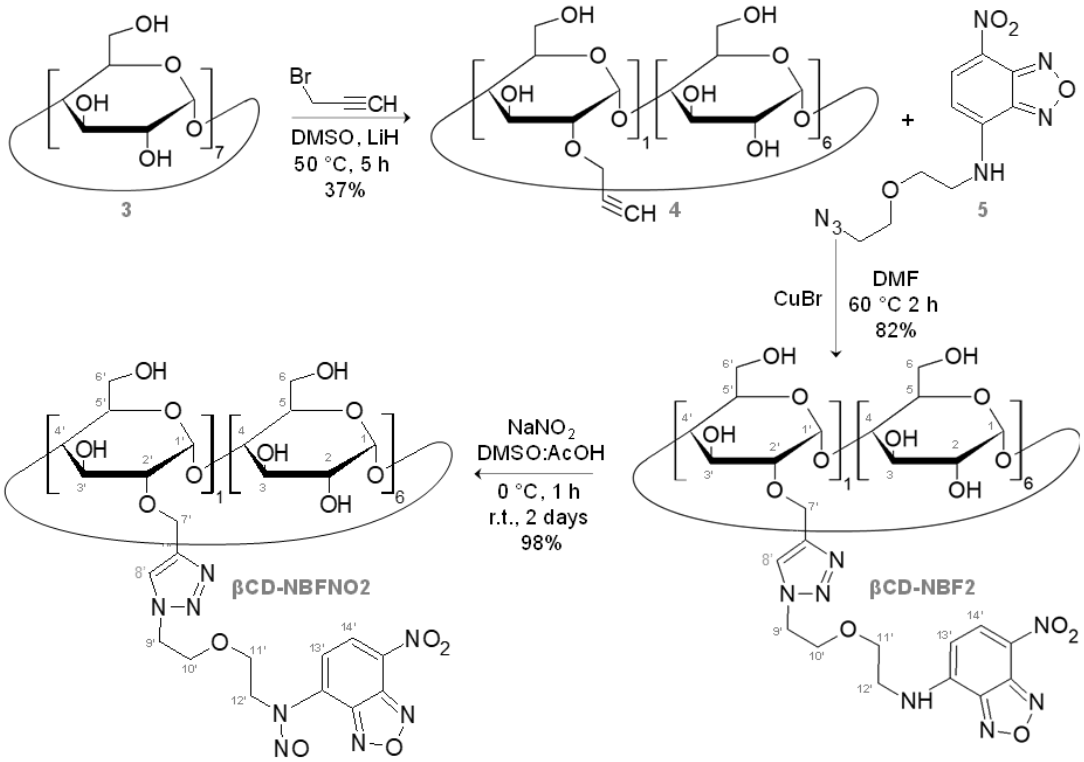


Figure S2: Synthesis scheme for βCD-NBFNO2.

The synthetic strategy for compound 5 is shown in Fig. S3. The preparation of the linker was divided in two parts. First 2-(2-azidoethoxy)ethan-1-amine (L4) was synthesized by modifications of existing synthetic procedures.[2S,3S] Compound 5 was prepared globally in 4 synthetic steps starting from the commercially available diethylene glycol (L1). The diol was exhaustively tosylated in DCM with potassium hydroxide (biphasic system) and the product, diethylene glycol di(*p*-toluenesulfonate) (L2), was effectively isolated by liquid-liquid extraction. Compound L2 was converted to the corresponding diazido diethylene glycol (L3) in DMF with excess of sodium azide. Isolation of the compound L3 was based on liquid-liquid extraction. Partially reduction of diazido diethylene glycol based on Staudinger reaction and *ad-hoc* developed work-up afforded the 2-(2-azidoethoxy)ethan-1-amine (L4) in good yield and purity. Chromophore 4-chloro-7-nitrobenzofurazan was finally reacted with compound L4 in absolute ethanol. Compound 5 was isolated by liquid-liquid extraction and purified by direct-phase chromatography on silica gel with DCM as eluent in isocratic elution.

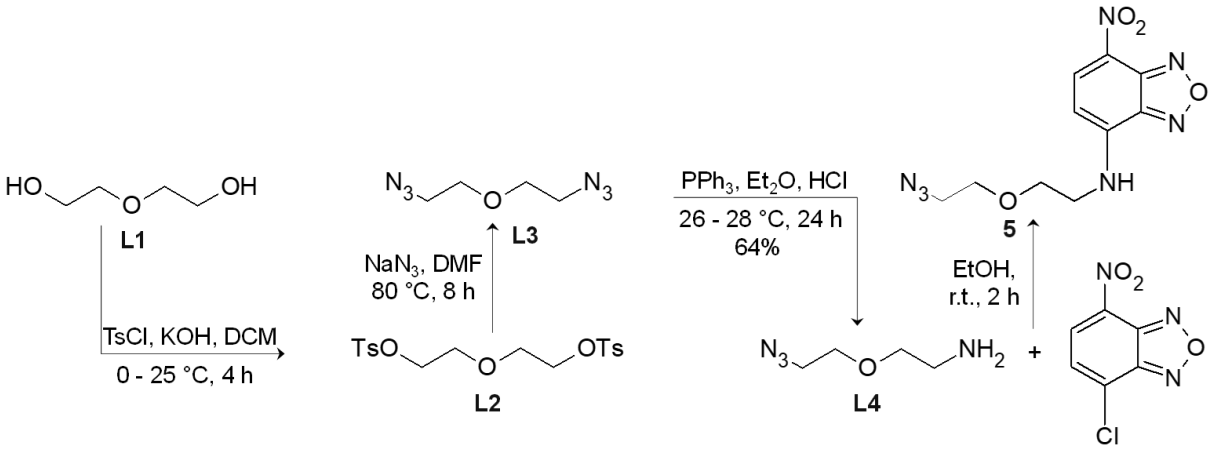


Figure S3: Synthesis scheme for compound 5.

6-Monodeoxy-6-monoamino-βCD free base (2)

6-Monodeoxy-6-monoamino-βCD hydrochloride (11.70 g, 10 mmol) was solubilized in water (50 mL) and the solution was added to ammonia 25% solution (80 mL) under vigorous stirring. The white precipitate was filtered on a sintered glass filter (porosity 3), the solid was washed with methanol (2 x 15 mL) and placed into a vacuum drying box overnight in the presence of P_2O_5 and KOH (~10 g, 88% yield).

m.p.: 203-205 °C (dec.). R_f: 0.26-0.29 in 1,4-dioxane:25% aqueous NH_3:1-propanol=10:7:3.

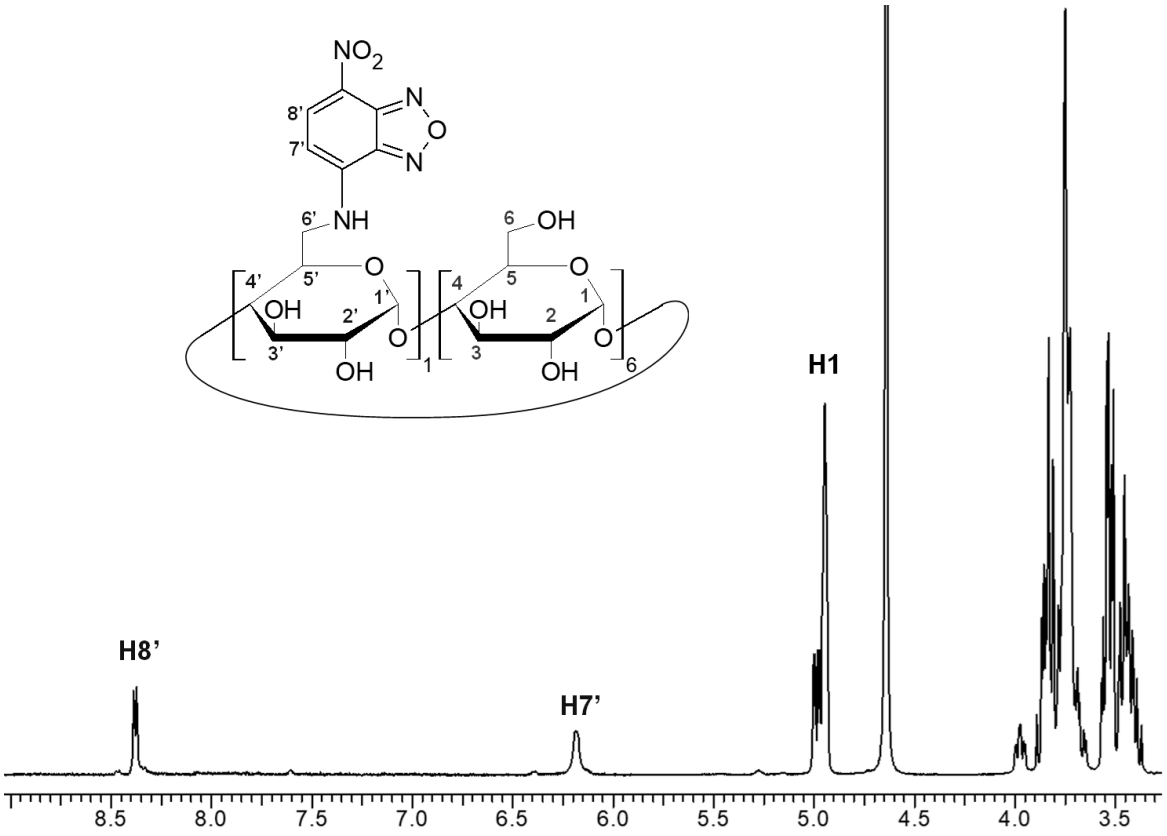
^{1}H-NMR (D_2O): δ(ppm) 5.10-5.05 (m, 7H, H1, H1'), 4.10-4.05 (m, 1H, H3'), 3.99-3.75 (bs, 25H, H3, H5, H6), 3.67-3.47 (bs, 15H, H2, H4, H6'), 3.26-3.20 (m, 1H, H6').

^{13}C-NMR (125 MHz, D_2O): δ(ppm) 101.94 (C1), 101.38 (C1'), 83.02 (C4'), 81.36 (C4), 81.30 (C4), 81.27 (C4), 81.24 (C4), 73.23 (C3), 73.00, 72.59, 72.17, 71.94, 68.00 (C3'), 60.45 (C6), 40.36 (C6').

βCD-NBF1

4-chloro-7-nitrobenzofurazan (NBF-Cl) (0.2 g, 1 mmol) dissolved in acetonitrile (5 mL) was added to an aqueous (50 mL) solution of (2) (1.13 g, 1 mmol) and the reaction mixture was heated at 50 °C for 2 h. The solvents were completely evaporated under reduced pressure (T=60 °C), the crude was dissolved in water (20 mL) and extracted with dichloromethane (2 x 20 mL). The aqueous phase was suspended with silica gel (5 g) and the mixture was evaporated under reduced pressure until dryness. This crude mixture thus preabsorbed onto silica was purified by chromatography over silica with CH_3OH:H_2O:HCOOH (0.05%) 9:1:0.5 as eluent in isocratic elution. The fractions were analyzed by TLC and those containing βCD-NBF1 were combined and concentrated under reduced pressure. The viscous solution was neutralized (NaOH 0.1 N) and precipitated with MeOH (50 mL). The obtained solid was filtered on a sintered glass filter (porosity 3), washed with methanol (2 x 15 mL) and placed into a vacuum drying box overnight in the presence of P_2O_5 and KOH (dark brown solid, 0.45 g, 35% yield).

R_f: 0.56, (9:1 MeOH:H_2O); ^{1}H-NMR (500 MHz DMSO-d_6): δ(ppm) 8.35 (m, 1H, H8'), 6.20 (bs, 1H, H7'), 5.11-4.94 (m, 7H, H1), 3.35-4.26 (bs, 42H, H2, H3, H4, H5, H6).

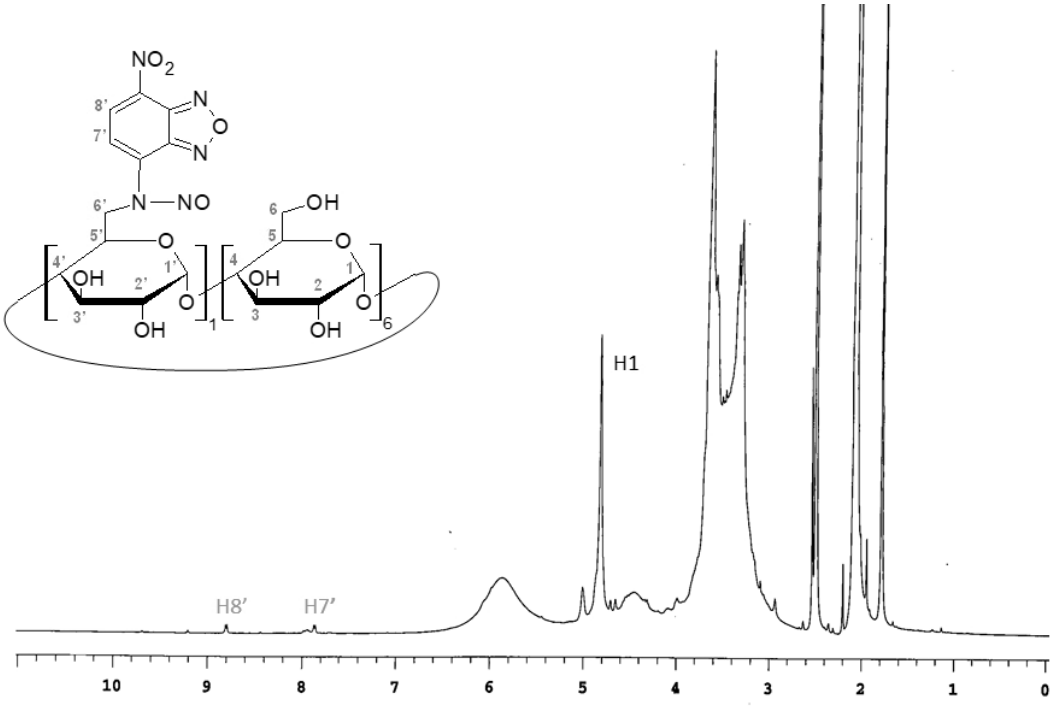


^{1}H NMR spectrum of βCD-NBF1 with partial assignment (500 MHz, DMSO, 298.15 K).

βCD-NBFNO1

βCD-NBF1 (50 mg, 0.04 mmol) was solubilized in a mixture of DMSO/CH_3COOH 1:1 (2 mL). After complete solubilization, the solution was cooled at 0 °C with an ice bath and $NaNO_2$ (100 mg, 1,45 mmol) was added; the solution was stirred at 0 °C for 1 hour and at room temperature for 2 days. The reaction mixture was precipitated with acetone (40 mL). The solid was filtered-off on a glass filter (porosity 3), extensively washed with acetone (2 x 10 mL) and dried until constant weight in a vacuum drying box (51 mg; 98% yield) and a dark yellow powder was obtained.

R_f = 0.70 (9:1 MeOH:H_2O);^{1}H-NMR (500 MHz DMSO-d_6): δ(ppm) 8.9 (d, 1H, H8'), 7.83 (d, 1H, H7'), 5.11-4.8 (m, 7H, H1), 2.9-3.95 (bs, 42H, H2, H3, H4, H5, H6).



^{1}H-NMR spectrum of βCD-NBFNO1 with partial assignment (DMSO-d_6, 500 MHz, 298.15 K).

2-O-Monopropargyl-βCD (4)

Lithium hydride (53 mg, 6.608 mmol) was added to βCD solution 1 (5 g, 4.405 mmol) in dry DMSO (75 mL). The resulting suspension was stirred under N_2 at room temperature until it became clear (12-24 h). Propargyl bromide (solution in toluene, 80% w/w, 491 μL, 4.405 mmol) and a catalytic amount of lithium iodide (~5 mg) were then added and the mixture was stirred at 50 °C in the absence of light for 5 h. TLC (10:5:2 CH_3CN:H_2O:25% aqueous NH_3) showed four spots with R_f values of 0.75, 0.65, 0.50, and 0.30, the last two corresponding to monopropargylated and nonpropargylated βCD, respectively. The solution was poured into acetone (800 mL), the precipitate was filtered on a sintered glass filter (porosity 4) and washed thoroughly with acetone. The resulting solid was transferred into a round-bottom flask and dissolved in a minimum volume of water. Silica gel (10 g) was added and the solvent was removed under vacuum until powdered residue was obtained. This crude mixture was applied on top of a column of silica (25 x 6 cm), and chromatography (10:5:2 CH_3CN:H_2O:25% aqueous NH_3) yielded, after freeze-drying, 2-*O*-monopropargyl-βCD (***4***) (1.912 g, 1.63 mmol, 37%) as white solid.

The material decomposes at 239-245 °C; [α]25D +126 (c 0.25, H_2O); R_f = 0.50 (10:5:2 CH_3CN–H_2O–25% aqueous NH_3); IR (KBr): 3397, 2923, 2117, 1646, 1156, 1081, 1029 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 5.98 (br d, 1H, OH), 5.88 (br s, 1H, OH), 5.79-5.69 (m, 10H, OH), 4.98 (d, 1H, 3J = 3.6 Hz, H1'), 4.84-4.82 (br s, 6H, H1), 4.54 (t, 1H, J = 5.6 Hz, OH), 4.50-4.45 (m, 8H, OH, CHC≡), 4.38 (dd, 1H, 2J = 15.8 Hz, 4J = 2.4 Hz, CHC≡), 3.78 (t, 1H, 3J = 9.8 Hz, H3'), 3.64-3.53 (m, 27H; H3, H5, H6a, H6b), 3.51 (t, 1H, 4J = 2.4 Hz, ≡CH), 3.43-3.40 (m, 2H, H2',H4'), 3.36-3.29 ppm (m, 12H, H2, H4); ^{13}C NMR (125 MHz, DMSO-d_6) δ 102.0-101.7 (C1), 100.1 (C1'), 82.2-81.4 (C4), 79.9 (C≡), 79.1 (C-2'), 77.8 (≡CH), 73.3-

71.7 (C2, C3, C), 72.6 (C3'), 60.1-59.7 (C6), 58.8 ppm ($CH_2C\equiv$); MALDI-TOF: [M+Na]+ calcd for $C_{45}H_{72}O_{35}Na$, 1195.4; found: 1195.58.

Compound 5

Diethylene glycol (L1) (50.0 g, 0.47 mol) was dissolved in CH_2Cl_2 (480 mL) and TsCl (180.0 g, 0.942 mol) was added. The solution was cooled to 0 °C and crushed KOH (211.0 g, 3.77 mol) was slowly added. The suspension was additionally stirred at 0 °C for 3 h. The reaction mixture was monitored by TLC using hexane:EtOAc 1:1 as eluent and detection was achieved with potassium permanganate solution. The mixture was warmed to room temperature and $CHCl_3$ (400 mL) was added. The mixture was extracted with water (3 x 400 mL) and the organic phase was dried with $MgSO_4$ (25 g). The desiccant was filtered off and the filtrate was evaporated on a rotary evaporator at 40 °C. The product was dried at room temperature using an oil rotary pump. Diethylene glycol ditosylate (L2) (169.6 g, 0.41 mol) was dissolved in DMF (800 mL) and NaN_3 (106.7 g, 1.64 mol) was added. The suspension was stirred at 80 °C for 8 h. The reaction mixture was monitored by TLC using hexane:EtOAc 1:1 mixture as eluent and detection was achieved with potassium permanganate solution. The suspension was cooled to room temperature and water (750 mL) was added. The solution was extracted with Et_2O (1600 mL). The organic phase was then extracted with water (3 x 1600 mL). It was verified by ^{1}H NMR that the mixture was free of DMF residues. The organic phase was then concentrated to a volume of approximately 800 mL on a rotary evaporator at room temperature To the solution was added 1 M HCl (800 mL), and the biphasic mixture was stirred vigorously. PPh_3 (123.0 g, 0.47 mol) was then added in small portions and the mixture was stirred overnight. The reaction mixture was monitored by TLC using hexane:EtOAc 1:1 mixture for the starting diazide (L3) and CH_2Cl_2:MeOH:25% aqueous NH_3 3:3:1 mixture for the product (L4); detection was achieved with potassium permanganate. The precipitated triphenylphosphine oxide was filtered off and washed with water. The organic phase was separated and the aqueous solution was subsequently extracted with Et_2O (3 x 500 mL). The aqueous solution was cooled to 0 °C and KOH (300 g) was slowly added. The basic aqueous solution was then extracted with CH_2Cl_2 (6 x 600 mL). The organic phase was then dried with $MgSO_4$ (18 g), the desiccant was filtered off and the filtrate was evaporated at 30 °C on a rotary evaporator. The product was dried at room temperature using an oil rotary pump. The product was obtained as an yellowish oil, in 64% yield (39 g). IR(KBr): 3357, 2860, 2101 ν(azide), 1595, 1440, 1344, 1269, 1120 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$):δ = 3.65 (t, *J* = 5.2 Hz, 2H, H-3), 3.52 (t, *J* = 5.1 Hz, 2H, H-2), 3.39 (t, *J* = 5.1 Hz, 2H, H-4), 2.88 (t, *J* = 5.1 Hz, 2H, H-1) ppm. ^{13}C NMR (100 MHz, $CDCl_3$):δ = 73.15 (C-2), 70.00 (C-3), 50.80 (C-4), 41.73 (C-1) ppm. ESI MS: for $C_4H_{10}N_4O$calcd: *m/z* 130.1, found 131.2 $[M+H]^+$. HRMS: for $C_4H_{10}N_4O$calcd: *m/z* 130.0855, found 131.0933 $[M+H]^+$, Δ 4.6 ppm. ^{1}H NMR spectrum was consistent with the literature (Klein et al.). NBF-Cl (0.8 g, 4 mmol) was solubilized in EtOH (20 mL) and slowly added to a EtOH solution (10 mL) of 2-(2-azidoethoxy)ethan-1-amine (L4) (1.3 g, 10 mmol) under vigorous stirring. The mixture was stirred at r.t. for 2 h. The reaction was monitored by TLC (DCM:hexane 8:2) and detection was achieved under UV-Lamp at 254 nm. The reaction mixture was evaporated under reduced pressure at 40 °C and the obtained oil was diluted with DCM (50 mL) and extracted with water (3 x 50 mL). The organic phase was extracted with

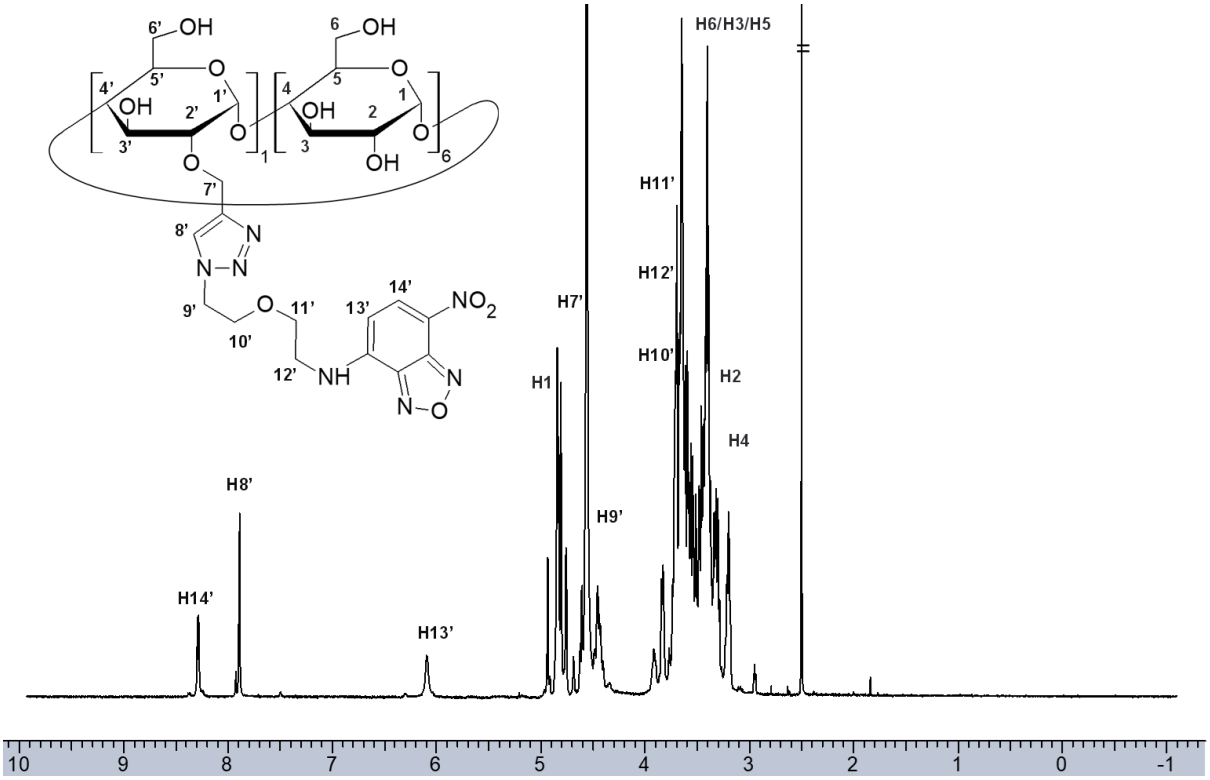
HCl 1 M (3 x 100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The obatined oil was purified by chromatography with DCM as eleunt in isocratic elution. the product was concentrated under reduced pressure and a dark oil was obtained (1.2 g, 42% yield).

R_f = 0.20 (8:2 DCM:Hexa$_n$e); ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 8.47 (d, 1H, H2), 6.72 (br s, 1H, NH), 6.23 (d, 1H, H3), 3.88 (t, 2H, H8), 3.76-3.74 (m, 4H, H7-H9), 3.45 (t, 2H, H10).

βCD-NBF2

Compound 5 (0.1 g, 0.35 mmol) was solubilized in DMF (5 mL) and added to a DMF solution (20 mL) of 2-*O*-monopropargyl-βCD (0.36 g, 0.31 mmol). CuBr (12 mg, 0.083 mmol) was added to the solution under vigorous stirring and the reaction mixture was heated at 60 °C for 2 h. The progress of the reaction was monitored by TLC (10:2.5:1 ACN:H_2O:25% aqueous NH_3). The crude reaction was filtered on a celite pad to remove copper-related material and the pad was extensively washed with DMF (3 x 10 mL). The filtrate was suspended with silica gel (5 g) and the solvent was removed under vacuum until a powdered residue was obtained. This crude mixture thus preabsorbed onto silica was purified by chromatography over silica with 10:2.5:1 ACN:H_2O:25% aqueous NH_3 as eluent in isocratic elution. The fractions were analyzed by TLC and those containing βCD-NBF2 were combined and concentrated under reduced pressure until dryness. The solid was solubilized in H_2O (5 mL) and dialyzed for 48 h against deionized water. The dialysate was finally concentrated until dryness under reduced pressure. The brown solid was placed into a vacuum drying box overnight in the presence of P_2O_5 and KOH (0.37 g, 82% yield).

R_f = 0.66 (9:1 MeOH:H_2O); ^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) 8.29 (d, 1H, H14'), 7.89 (s, 1H, H8'), 6.09 (br s, 1H, H13'), 4.94-4.69 (m, 7H, H1), 4.61 (overlapping with HDO signal, br s, 2H, H7'), 4.46 (m, 2H, H9'), 3.92-3.20 (m, 48H, H2, H3, H4, H5, H6, H10', H11', H12'); ^{13}C NMR (125 MHz, DMSO-d_6) assignment based on DEPT-edited HSQC spectrum δ 138.64 (C14'), 126.16 (C8'), 102.55-101.08 (C1), 81.79-80.27 (C4, C2), 74.09 (C5), 72.58 (C2), 72.57 (C10'), 72.18 (C3), 69.54 (C12'), 68.73 (C11'), 65.11 (C7'), 60.66 (C6), 51.22 (C9').

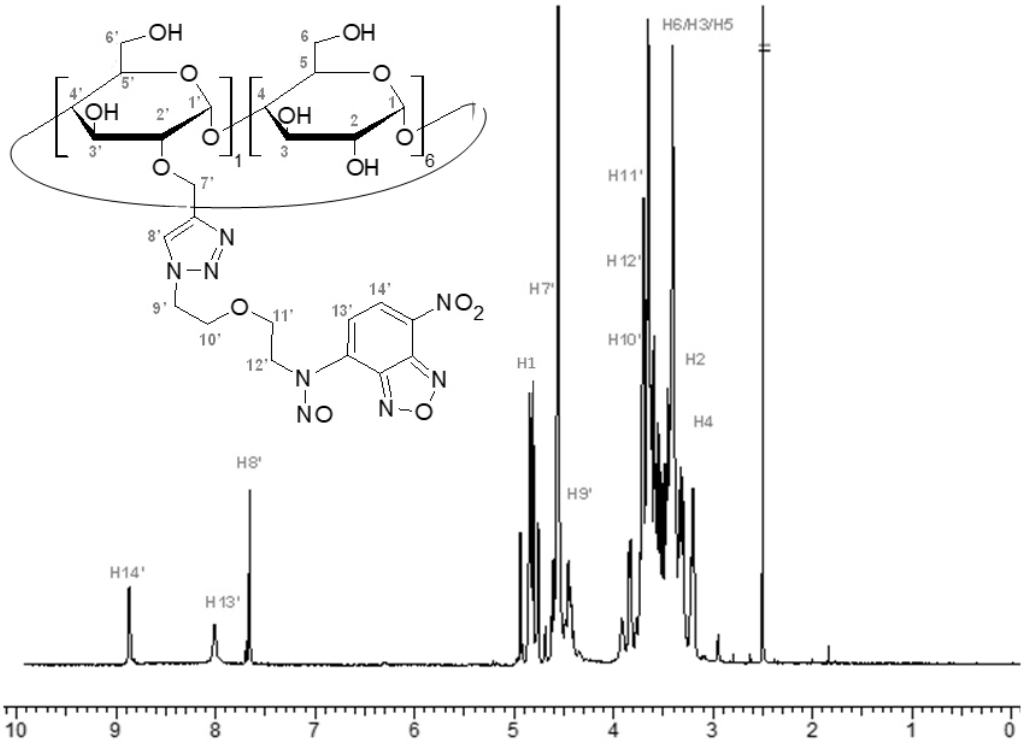


^{1}H-NMR spectrum of βCD-NBF2 with assignment (DMSO-d_6, 600 MHz, 298.15 K).

βCD-NBFNO2

βCD-NBF2 (56 mg, 0.04 mmol) was solubilized in a solution of DMSO/CH_3COOH 1:1 (2 mL). After complete solubilization, the solution was cooled at 0 °C with an ice bath and $NaNO_2$ (100 mg, 1,45 mmol) was added;. the solution was stirred at 0 °C for 1 hour and at room temperature for 2 days. The reaction mixture was precipitated with acetone (40 mL). The solid was filtered-off on a glass filter (porosity 3), extensively washed with acetone (2 x 10 mL) and dried until constant weight in a vacuum drying box (51 mg; 98% yield) and a dark yellow powder was obtained.

R_f = 0.85 (9:1 MeOH:H_2O); ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 8.8 (d, 1H, H14'), 8.03 (br s, 1H, H13'), 7.71 (s, 1H, H8'), 4.97-4.45 (m, 7H, H1), 4.51 (br s, 2H, H7'), 4.4 (m, 2H, H9'), 4.2-2.80 (m, 48H, H2, H3, H4, H5, H6, H10', H11', H12');



^{1}H-NMR spectrum of βCD-NBFNO2 with assignment (DMSO-d_6, 600 MHz, 298.15 K).

1S. Synthesis of 2I-*O*-propargylcyclomaltoheptaose and its peracetylated and hepta(6-*O*-silylated) derivatives, **2020**, In book: Carbohydrate Chemistry: Proven Synthetic Methods, Volume 5 (forthcoming) Publisher: CRC Press, Taylor & Francis Group.

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