ELECTRONIC SUPPLEMENTARY INFORMATION FOR:

Preorganized Macromolecular Gene Delivery Systems: Amphiphilic β-Cyclodextrin "Click Clusters"

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General Methods. Reagents and solvents were purchased from commercial sources and used without further purification. Optical rotations were measured at 20 ± 2 ºC in 1-cm or 1-dm tubes. ^{1}H (and ^{13}C NMR) spectra were recorded at 500 (125.7), 400 (100.6) and 300 (75.5) MHz. 2D COSY and HMQC experiments were carried out to assist on NMR assignments. Thin-layer chromatography (TLC) was carried out on aluminium sheets, silica gel 30F-245, with visualization by UV light and by charring with 10% H_2SO_4 or 0.2% ninhydrine in EtOH. Column chromatography was carried out on normal-phase Silica Gel (E. Merk, 230-400 mesh). MALDI-TOF mass spectra were acquired on a GSG System spectrometer operating in the positive-ion mode with an accelerating voltage of 28 keV. Samples were dissolved in water at mM concentration and mixed with a standard solution of 2,5-dihydroxybenzoic acid (DHB; 10 mg mL^{-1} in 10% aq EtOH, 2 μL) in 1:1 v/v relative proportions; 1 μL of the mixture was loaded onto the target plate, then allowed to air-dry at room temperature. Electrospray mass spectra were obtained for samples dissolved in MeCN, MeOH or H_2O-MeOH at mM concentrations. Elemental analyses were performed by the Instituto de Investigaciones Químicas, Sevilla, Spain.

Preparation of Complexes from Amphiphilic βCD Click Clusters and Plasmid pEGFP-N3. The plasmid pEGFP-N3, used for the preparation of the DNA complexes and for transfection assay is a plasmid of 4729 bp (base pairs). The quantities of amphiphilic βCD click clusters used were calculated according to the desired DNA concentration of 0.05 mg/mL (150 μM phosphate), the N/P ratio, the molar weight and the number of positive charges in the selected CD derivative. Experiments were performed for N/P 5, 10, 20 and 50. DNA was diluted in Milli-Q water to a final concentration of 150 μM phosphate, then the desired amount of CD

derivative was added from a 20 mg/mL stock solution (1:2 Me_2SO-water). The solution was previously diluted so that the desired N/P ratio was reached by mixing with an equal volume of the plasmid solution. In this way, the Me_2SO proportion was identical in all experiments (16.7% v/v). The preparation was vortexed for a few seconds and incubated for 30 minutes.

Vesicle Preparation: Vesicles of CDs were prepared by sonication. Typically, the amphiphilic βCD in HPLC-grade MeOH (50 μM) was dried by using a warm air stream to yield a thin film in a glass vial. The residual solvent was removed under high vacuum for 1-2 h. Milli-Q water (4 mL) was added and the sample solution was kept for 30 min at 50 ºC. The resulting suspension was sonicated for 30 min at rt to give small vesicles.

Particle Size and ζ Potential Measurements. The hydrodynamic diameters of the vesicles formed from the amphiphilic βCD click clusters as well as that of the corresponding CDplexes were measured by Dynamic Light Scattering at 633 nm on a Zetamaster S Dynamic Light Scattering instrument (Malvern Instruments) at 25 ºC and at a detection angle of 90º. All the measurements were performed in triplicate. Statistical analysis of the particle size was performed using a student's test (with two-sample unequal variance and two distribution tails). The ζ potential measurements were also performed using the same instrument at a detection angle of 90º in triplicate. Results are collected in Table S1.

Table S1. Hydrodynamic Diameter and ζ Potential for Micelles and CDplexes from Ampihphilic βCD Click Clucters.

Compound or CDplex	Hydrodynamic Diameter (nm)	Polydispersity Index (PI)	ζ Potential (mV)
11	125 ± 10	0.15 ± 0.03	17 ± 3
12	81± 21	0.25 ± 0.09	52 ± 4
13	210 ± 15	0.30 ± 0.02	59 ± 4
13:pDNA	103 ± 5	0.17 ± 0.08	48 ± 2
14	421 ± 12	0.09 ± 0.03	44 ± 2
14:pDNA	111 ± 10	0.25 ± 0.02	44 ± 6
15	482 ± 30	0.32± 0.228	57 ± 9
15:pDNA	155 ± 12	0.31 ± 0.02	33 ± 6
21	216 ± 15	0.33 ± 0.02	49 ± 4
21:pDNA	99 ± 6	0.23 ± 0.04	44 ± 3
22	336 ± 15	0.19 ± 0.09	70 ± 3
22:pDNA	120 ± 5	0.26 ± 0.01	50 ± 4

Cell Culture and DNA Transfection Assays. Wild type Chinese Hamster Ovary (CHO-k1; ATCC no. CCL-61) cells were grown in Dulbecco´s Modified Eagle´s

medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 2 mM Glutamine plus, 100 U/mL penicillin, and 0.1 μg/mL streptomycin. Prior to transfection, cells were seeded in 24 well plates and incubated for 24 h to reach a cell confluency of 80-90%. For transfection experiments, pEGFP-N3 plasmid (1 μg) was mixed with the corresponding CD derivative at N/P ratios of 5, 10, 20 and 50 at room temperature for 20 minutes in a final volume of 20 μl. The mixture was diluted to 0.5 ml with DMEM without serum or with 10% serum and added to each well. Untransfected cells and naked pEGFP-N3 were used as negative controls. As a positive control, a transfection experiment was performed using 2 μL of Lipofectamine™ 2000 (Invitrogen) and 1.0 μg of DNA, according to the manufacturer's instructions. Cells were incubated with the DNA-amphiphilic βCD mixtures for 5 h, then the transfection media was removed and cells were further grown in DMEM media plus 10% FBS for an additional period of 48 h. For results on transfection see Table 1 in the manuscript.

Fluorescence and Protein Assays. Transfected cells were washed three times with PBS and 600 μL of 0.5 % triton X-100 in PBS was added. The plates were shaken for 10 min at room temperature, the lysate was recovered and the fluorescence was quantified in a Shimadzu RF-5301PC fluorimeter with excitation of 460 nm (5 nm) and 510 nm (10 nm) emission wavelengths. Protein concentration was measured using the Bio-Rad Protein Assay (Hercules, CA, USA).

Statistical analysis. Results are expressed as mean ± SEM for the number of experiment indicated. The statistical significance of variations was evaluated using one-way Analysis of Variance (ANOVA). When significant effect was found, post hoc comparisons of the means were done using the *t* adjusted Tukey's test. A p value < 0.05 was considered significant. According to this criterion, differences between the data for Lipofectamine™ 2000 and compounds **14** and **22** in Figure 2b were found not significant. Similarly, differences between the transfection data for Lipofectamine™ 2000 and compound **14**-based CDplexes (see Figure 2c in the manuscript) were not significant.

Gel Electrophoresis Shift Assay. The DNA-amphiphilic βCD click cluster binding capability was analyzed by gel electrophoresis. The stock solutions of βCD derivatives were prepared in a 1:2 Me_2SO-water, while DNA was dissolved in Milli-Q water. pEGFP-N3 DNA (10 μL at 0.1 μg/μL) was mixed with an equal volume of βCD derivative using N/P ratios 0 to 100 and incubated for 30 min at room temperature

before the addition of loading buffer (2 μL). An aliquot (5 μL) of each sample was subjected to agarose gel electrophoresis (0.8% w/v) in TAE buffer (40 mM Tris-acetate, 1 mM EDTA). Electrophoresis was carried out at 7 V/cm and gels were stained after electrophoresis with ethidium bromide. Quantification of the band intensity was performed with the NIH Image Software.[1] A value of 1 has been assigned to the intensity of the band corresponding to the naked DNA.

DNase Protection Assays. pEGFP-N3 DNA (10 μL at 0.1 μg/μL) was mixed with an equal volume of stock solution of βCD derivative using N/P = 5 and incubated for 30 min at room temperature. To the mixture, 15 μL of a solution of DNase I (0.1 mg/mL in Tris HCl 50 mM pH 8, 2000 U/mg) were added and incubated for 1 h at 37 ºC. After the digestion, 20 μL of a 10 % SDS solution was added and the samples were incubated for 15 min at 65 ºC. Finally, an aliquot (20 μL) of each sample was subjected to agarose gel electrophoresis (0.8% w/v) in TAE buffer.

Materials. Heptakis(6-azido-6-deoxy)cyclomaltoheptaose (**1**),[2] *N*-(*tert*-butoxy-carbonylamino)propargylamine (**4**),[3] bis[2-(*tert*-butoxycarbonylamino)ethyl]-amine,[4] and heptakis[6-(2-aminoethylthio)-6-deoxy-2,3-di-*O*-hexanoyl]-cyclomaltoheptaose heptahydrochloride[5] were prepared according to literature procedures. pEGFP-N3 plasmid (Genbank Accession #: U57609) was obtained from Clontech Laboratories (Palo Alto, CA). This 4729 bp plasmid encodes for a red-shifted variant of wild-type GFP. Plasmid was purified from transfected bacteria using standard methods.[6] DNA concentration was measured using a fluorimetric method using the Hoechst 33258 dye.[7]

Heptakis(6-azido-6-deoxy-2,3-di-*O*-hexanoyl)cyclomaltoheptaose (2): To a solution of heptakis(6-azido-6-deoxy)cyclomaltoheptaose (**1**, 0.2 g, 0.15 mmol) in dry DMF (20 mL) under Ar at 0 ºC, DMAP (783 mg, 6.4 mmol, 3 eq.) was added. Hexanoic anhydride (2.0 ml, 8.5 mmol, 4 eq) was dropwise added, the reaction mixture was stirred at rt for 16 h, then MeOH (20 mL) was added. After 1 h, the solution was poured into ice-water (50 mL) and extracted with CH_2Cl_2 (4 x 50 mL). The organic phase was washed with 2 N H_2SO_4 (2 x 50 mL), water and saturated aqueous $NaHCO_3$ (4 x 50 mL), dried (Na_2SO_4), concentrated and purified by column chromatography (1:15 → 1:9 EtOAc-petroleum ether). Yield.: 286 mg (71%); R_f 0.42 (1:9 EtOAc-petroleum ether); $[\alpha]_D$ = +115.0 (*c* 1.0, CH_2Cl_2); IR: υ_{max} 2117 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ5.30 (t, 7 H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3), 5.06 (d, 7 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.80 (dd, 7 H, H-2), 4.00 (ddd, 7 H, $J_{4,5}$ = 9.2 Hz, $J_{5,6b}$ = 4.9 Hz, $J_{5,6a}$ = 2.7 Hz, H-5), 3.73 (t, 7 H, H-

4), 3.71 (dd, 7 H, $J_{6a,6b}$ = 13.6 Hz, H-6a), 3.63 (dd, 7 H, H-6b), 2.40-2.16 (m, 28 H, CH_2CO), 1.59 (m, 28 H, CH_2CH_2CO), 1.30 (m, 56 H, CH_2CH_3, $CH_2CH_2CH_3$), 0.91, 0.89 (2 t, 42 H, $^3J_{H,H}$ = 7.3 Hz, CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ173.2, 171.7 (CO), 96.4 (C-1), 76.7 (C-4), 70.8 (C-5), 70.1 (C-2, C-3), 51.6 (C-6), 34.0, 33.8 (CH_2CO), 31.4, 31.2 ($CH_2CH_2CH_3$), 24.6, 24.3 (CH_2CH_2CO), 22.3 (CH_2CH_3), 13.8 (CH_3). ESIMS: *m/z* 2706.3 $[M + Na]^+$. Anal. Calcd for $C_{126}H_{203}N_{21}O_{42}$: C 56.38, H 7.62, N 10.96. Found: C 56.33, H 7.23, N 10.93.

Heptakis(6-azido-6-deoxy-2,3-di-*O*-myristoyl)cyclomaltoheptaose (3). To a solution of heptakis(6-azido-6-deoxy)cyclomaltoheptaose (**1**, 100 mg, 76.3 μmol) in dry DMF (7 mL) under Ar at 0 ºC, DMAP (392 mg, 3.2 mmol, 3 eq) was added. Myristic anhydride (1.87 g, 4.3 mmol, 4 eq) was dropwise added, the reaction mixture was stirred at rt for 16 h, then MeOH-CH_2Cl_2 (95:5, 25 mL) was added. After 1 h, the solution was decanted and the resulting solid was dried. Yield.: 315 mg (97%); R_f 0.40 (1:8 EtOAc-petroleum ether); $[\alpha]_D$ = +66.1 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.28 (t, 7 H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, H-3), 5.02 (d, 7 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.77 (dd, 7 H, H-2), 3.98 (m, 7 H, H-5), 3.71 (t, 7 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.68 (m, 7 H, H-6a), 3.60 (dd, 7 H, $J_{6a,6b}$ = 13.5 Hz, $J_{5,6b}$ = 4.5 Hz, H-6b), 2.32, 2.21 (m, 28 H, CH_2CO), 1.53 (m, 28 H, CH_2CH_2CO), 1.25 (m, 280 H, CH_2), 0.86 (t, 42 H, $^3J_{H,H}$ = 7.1 Hz, CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 173.2, 171.8 (CO), 96.4 (C-1), 76.7 (C-4), 70.8 (C-5), 70.2 (C-2), 70.1 (C-3), 51.6 (C-6), 34.1, 33.9 (CH_2CO), 32.0 ($CH_2CH_2CH_3$), 29.9-29.2 (CH_2), 24.9, 24.8 (CH_2CH_2CO), 22.7 (CH_2CH_3), 14.0 (CH_3). MALDI-TOFMS: *m/z* 4523.7 $[M + H]^+$. Anal. Calcd for $C_{238}H_{427}N_{27}O_{42}$: C 67.18, H 10.11, N 6.91. Found: C 66.82, H 9.84, N 6.66.

3-Bis[2-(*tert*-butoxycarbonylamino)ethyl]aminopropine (5): To a solution of bis[2-(*tert*-butoxycarbonylamino)ethyl]amine (872 mg, 2.86 mmol) and Et_3N (1.2 mL, 8.6 mmol, 3.0 eq) in CH_3CN (15 mL), propargyl bromide was added (0.90 mL, 8.6 mmol, 3.0 eq) and the mixture was stirred for 2 days. The solvent was eliminated under vacuum and the residue was dissolved in CH_2Cl_2 (100 mL), washed with water (3 x 30 mL), dried ($MgSO_4$), and concentrated. The resulting residue was purified by column chromatography (30:1 CH_2Cl_2-MeOH). Yield: 567 mg (58%); R_f = 0.22 (50:1 CH_2Cl_2-MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.93 (bs, 2 H, NH), 3.42 (d, 2 H, $^4J_{H,H}$ = 2.4 Hz, $CH_2C{\equiv}CH$), 3.20 (q, 4 H, $^3J_{H,H}$ = 5.7 Hz, CH_2NHBoc), 2.64 (t, 4 H, CH_2CH_2NHBoc), 2.20 (t, 1 H, C≡CH), 1.45 (s, 18 H, CMe_3); ^{13}C NMR (75.5 MHz,

$CDCl_3$): δ 156.0 (CO), 79.2 (C_q), 77.9 (*C*≡CH), 73.4 (C≡*C*H), 52.8 (*C*$H_2$$CH_2$NHBoc), 41.8 (HC≡C*C*$H_2$), 38.0 (*C*$H_2$NHBoc), 28.4 (C*Me*$_3$). ESIMS: *m/z* 364.2 $[M + Na]^+$, 342.2 $[M + H]^+$. Anal. Calcd. for $C_{17}H_{31}N_3O_4$: C 59.80, H 9.15, N 12.31. Found: C 59.87, H 9.03, N 12.18.

Heptakis[6-(4-*tert*-butoxycarbonylaminomethyl-1*H*-1,2,3-triazol-1-yl)-6-deoxy] cyclomaltoheptaose (6): To a solution of heptakis (6-azido-6-deoxy)-cyclomaltoheptaose (**1**, 200 mg, 0.15 mmol) and *N*-(*tert*-butoxycarbonylamino)-propargylamine (**4**, 180 mg, 1.15 mmol, 1.1 eq) in DMF (6 mL), CuI (70 mg, 0.37 mmol, 0.3 eq) and water (6 mL) were added. The reaction mixture was heated for 2 h at 100 ºC. The solvent was eliminated under reduced pressure and the residue was purified by column chromatography (10:1:1 → 10:2:1 MeCN-H_2O-NH_4OH) to give **6**. Yield: 181 mg (50%); R_f 0.38 (10:2:1 MeCN-H_2O-NH_4OH); $[\alpha]_D$ = +17.0 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD, 323 K): δ 7.80 (s, 7 H, =CH), 5.18 (s, 7 H, H-1), 4.61 (m, 7 H, H-6a), 4.40 (bd, 7 H, $J_{6a,6b}$ = 8.6 Hz, H-6b), 4.20 (m, 21 H, C*H*$_2$NHBoc, H-5), 3.89 (t, 7 H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3), 3.48 (dd, 7 H, $J_{1,2}$ = 3.1 Hz, H-2), 3.35 (t, 7 H, $J_{4,5}$ = 8.7 Hz, H-4), 1.42 (s, 63 H, CMe_3); ^{13}C NMR (125.7 MHz, CD_3OD, 323 K): δ 158.0 (CO), 147.1 (C-4 triazole), 126.0 (C-5 triazole), 103.6 (C-1), 84.5 (C-4), 80.5 (C_q), 74.3 (C-3), 73.8 (C-2), 71.7 (C-5), 51.4 (C-6), 37.0 (*C*H_2NHBoc), 29.0 (C*Me*$_3$); ESIMS: *m/z* 1221 $[M + 2 Na]^{2+}$. Anal. Calcd. for $C_{98}H_{154}N_{28}O_{42}$: C 49.12, H 6.48, N 16.37. Found: C 49.08, H 6.33, N 16.24.

Heptakis[6-(4-(2,2-bis(*tert*-butoxycarbonylamino)ethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy]cyclomaltoheptaose (7). A solution of heptakis[6-azido-6-deoxy]cyclomaltoheptaose (**1**, 107 mg, 82 μmol), **5** (216 mg, 0.63 mmol, 1.1 eq) and CuI (33 mg, 0.17 mmol, 0.3 eq.) in DMF:H_2O (1:1, 6 mL) was heated for 2 h at 100 ºC. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (20:1:1 → 10:1:1 MeCN-H_2O-NH_4OH)**.** Yield: 204 mg (67%); R_f 0.24 (10:2:1 MeCN-H_2O-NH_4OH); $[\alpha]_D$ = +12.7 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD, 323 K): δ 7.89 (s, 7 H, =CH), 5.17 (d, 7 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.55 (m, 14 H, H-6a, H-6b), 4.30 (m, 7 H, H-5), 3.92 (t, 7 H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3), 3.77, 3.68 (2 d, 14 H, $^2J_{H,H}$ = 14.5 Hz, CH_2N), 3.44 (dd, 7 H, H-2), 3.31 (t, 7 H, $J_{4,5}$ = 9.1 Hz, H-4), 3.18-3.11 (m, 28 H, C*H*$_2$NHBoc), 2.64-2.56 (m, 28 H, C*H*$_2$$CH_2$NHBoc), 1.46 (s, 126 H, CMe_3); ^{13}C NMR (125.7 MHz, CD_3OD, 313 K): δ 158.2 (CO), 145.5 (C-4 triazole), 127.1 (C-5 triazole), 103.8 (C-1), 84.6 (C-4), 80.0 (C_q), 74.4 (C-3), 74.0 (C-2), 71.6 (C-

5), 54.5 (CH_2CH_2NHBoc), 51.5 (C-6), 49.0 (CH_2N), 39.5 (CH_2NHBoc), 29.0 (CMe_3); ESIMS: *m/z* 1849.3 $[M + Na + H]^{2+}$, 1240 $[M+ 2H + Na]^{3+}$. Anal. Calcd for $C_{161}H_{280}N_{42}O_{56}$: C 52.26, H 7.63, N 15.90. Found C 51.90, H 7.33, N 15.54 .

Heptakis[6-(4-*tert*-butoxycarbonylaminomethyl-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (8): To a solution of **2** (765 mg, 0.28 mmol) and *N*-*tert*-butoxycarbonylpropargylamine (**4**, 405 mg, 2.59 mmol, 1.3 eq) in acetone (10 mL), $CuI\cdot(EtO)_3P$ (71 mg, 200 μmol, 0.3 eq) and DIPEA (0.34 mL, 0.29 mmol, 1 eq) were added and the reaction mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure and the resulting residue was purified by column chromatography (50:1 → 20:1 CH_2Cl_2-MeOH). Yield: 871 mg (81%). R_f 0.47 (15:1 CH_2Cl_2-MeOH); $[\alpha]_D$ = +24.7 (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$, 313 K): δ 7.62 (s, 7 H, =CH), 5.45 (s, 7 H, H-1), 5.40 (s, 7 H, N*H*Boc), 5.38 (t, 7 H, $J_{2,3} = J_{3,4}$ = 8.0 Hz, H-3), 4.86 (bd, 7 H, $J_{6a,6b}$ = 13.7 Hz, H-6a), 4.75 (dd, 7 H, $J_{1,2}$ = 2.1 Hz, H-2), 4.57 (bs, 7 H, H-6b), 4.48 (bs, 7 H, H-5), 4.29 (dd, 7 H, $^2J_{H,H}$ = 15.2 Hz, $J_{CH,NH}$ = 5.4 Hz, C*H*aNHBoc), 4.18 (dd, 7 H, $J_{CH,NH}$ = 3.0 Hz, C*H*bNHBoc), 3.55 (t, 7 H, $J_{4,5}$ = 8.0 Hz, H-4), 2.36-2.13 (m, 28 H, CH_2CO), 1.57 (m, 28 H, CH_2CH_2CO), 1.40 (s, 63 H, CMe_3), 1.30 (m, 56 H, CH_2CH_3, $CH_2CH_2CH_3$), 0.90 (t, 42 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K): δ 172.9, 171.6 (CO ester), 155.8 (CO carbamate), 145.2 (C-4 triazole), 124.6 (C-5 triazole), 96.4 (C-1), 79.5 (C-4, C_q), 70.1 (C-3), 69.8 (C-5), 69.6 (C-2), 50.2 (C-6), 36.1 (CH_2NHBoc), 33.8 (CH_2CO), 31.4, 31.2 ($CH_2CH_2CH_3$), 28.3 (CMe_3), 24.3 (CH_2CH_2CO), 22.3 (CH_2CH_3), 13.8 (CH_3). ESIMS: *m/z* 1908.1 $[M + 2 Na]^{2+}$. Anal. Calcd for $C_{182}H_{294}N_{28}O_{56}$: C 57.98, H 7.86, N 10.40. Found: C 58.04, H 7.76, N 10.32.

Heptakis[6-(4-(2,2-bis-*tert*-butoxycarbonylamino)ethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (9). To a solution of **2** (156 mg, 58 μmol) and **5** (182 mg, 0.53 mmol, 1.3 eq) in acetone (10 mL), $CuI\cdot(EtO)_3P$ (15 mg, 41 μmol, 0.1 eq) was added and the reaction mixture was refluxed for 3 h. The solvent was evaporated under vacuum and the residue was purified by column chromatography (30:1 → 9:1 CH_2Cl_2-MeOH). Yield: 256 mg (87 %); R_f = 0.61 (9:1 CH_2Cl_2:MeOH); $[\alpha]_D$ = +25.9 (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$, 323 K): δ 7.73 (s, 7 H, =CH), 5.44 (d, 7 H, $J_{1,2}$ = 3.2 Hz, H-1), 5.36 (t, 7 H, $J_{2,3} = J_{3,4}$ = 8.7 Hz, H-3), 5.21 (bs, 14 H, N*H*Boc) 4.90 (bd, 7 H, $J_{6a,6b}$ = 13.6 Hz, H-6a), 4.75 (dd, 14 H, H-2, H-6b), 4.51 (m, 7 H, H-5), 3.78, 3.74 (2 d, 14 H, $^2J_{H,H}$ = 15.6 Hz, CH_2N), 3.53 (t, 7

H, $J_{4,5}$ = 8.5 Hz, H-4), 3.16 (q, 28 H, $^3J_{H,H}$ = 5.6 Hz, C*H*$_2$NHBoc), 2.56 (t, 28 H, C*H*$_2$CH$_2$NHBoc), 2.45-2.13 (m, 28 H, CH$_2$CO), 1.60 (m, 28 H, C*H*$_2$CH$_2$CO), 1.44 (bs, 126 H, CMe$_3$), 1.30-1.20 (m, 56 H, C*H*$_2$CH$_3$, C*H*$_2$CH$_2$CH$_3$), 0.94, 0.93 (2 t, 42 H, $^3J_{H,H}$ = 7.2 Hz, $^3J_{H,H}$ = 6.8 Hz, CH$_3$); ^{13}C NMR (100.6 MHz, CDCl$_3$, 323 K): δ 172.7, 171.5 (CO ester), 156.0 (CO carbamate), 143.6 (C-4 triazole), 125.4 (C-5 triazole), 96.5 (C-1), 78.8 (C-4, C$_q$), 70.0 (C-3), 69.7 (C-5), 69.3 (C-2), 53.0 (*C*H$_2$CH$_2$NHBoc), 50.0 (C-6), 48.0 (CH$_2$N), 38.5 (CH$_2$NHBoc), 33.8, 33.5 (*C*H$_2$CO), 31.1, 31.0 (*C*H$_2$CH$_2$CH$_3$), 28.3 (C*Me*$_3$), 24.1 (*C*H$_2$CH$_2$CO), 22.1 (*C*H$_2$CH$_3$), 13.6 (CH$_3$); ESIMS: *m/z* 2558.2 [M + 2 Na]$^{2+}$, 1713.6 [M + 3 Na]$^{3+}$. Anal. Calcd for $C_{245}H_{420}N_{42}O_{70}$: C 57.99, H 8.34, N 11.59. Found: C 57.72, H 8.20, N 11.24.

Heptakis[6-(4-(2,2-bis(*tert*-butoxycarbonylamino)ethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-myristoyl]cyclomaltoheptaose (10). A solution of heptakis[2,3-di-*O*-myristoyl-6-azido-6-deoxy]cyclomaltoheptaose (**3**, 50 mg, 11 μmol), **5** (34 mg, 0.1 mmol, 1.3 eq), CuI(EtO)$_3$P (3 mg, 8 μmol, 0.1 eq) and *N*-ethyldiisopropylamine (13 μL, 80 μmol, 1 eq) in acetone (5 mL) was refluxed for 6 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (30:1 → 9:1 CH$_2$Cl$_2$:MeOH) to give an amorphous solid. Yield: 61.3 mg (80%). $[\alpha]_D$ = +26.2 (*c* 0.9, CH$_2$Cl$_2$); R_f = 0.51 (9:1 CH$_2$Cl$_2$-MeOH); ^{1}H NMR (500 MHz, CDCl$_3$, 333 K): δ 7.28 (s, 7 H, =CH), 5.45 (s, 7 H, H-1), 5.38 (t, 7 H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), 5.19 (bs, 14 H, N*H*Boc), 4.90 (d, 7 H, $J_{6a,6b}$ = 13.7 Hz, H-6a), 4.75 (dd, 14 H, $J_{1,2}$ = 3.6 Hz, H-2, H-6b), 4.53 (m, 7 H, H-5), 3.78 (2d, 14 H, $^2J_{Ha,Hb}$ = 15.7 Hz, CH$_2$N), 3.55 (t, 7 H, $J_{4,5}$ = 8.1 Hz, H-4), 3.18 (bq, 28 H, $^3J_{H,H}$ = 5.2 Hz, C*H*$_2$NHBoc), 2.58 (bs, 28 H, C*H*$_2$CH$_2$NHBoc), 2.44-2.15 (m, 28 H, CH$_2$CO), 1.60-1.54 (m, 28 H, C*H*$_2$CH$_2$CO), 1.46 (s, 126 H, CMe$_3$), 1.38-1.29 (m, 280 H, (CH$_2$)$_{10}$), 0.92 (t, 42 H, $^3J_{H,H}$ = 6.5 Hz, CH$_3$); ^{13}C NMR (125.7 MHz, CDCl$_3$, 313 K): δ 172.9, 171.7 (CO ester), 156.2 (CO carbamate), 143.6 (C-4 triazole), 125.7 (C-5 triazole), 96.6 (C-1), 79.0 (C-4,C$_q$), 70.1 (C-3), 69.7 (C-5, C-2), 53.1 (C*H*$_2$CH$_2$NHBoc), 50.1 (C-6), 48.0 (CH$_2$N), 38.4 (*C*H$_2$NHBoc), 34.0, 33.7 (*C*H$_2$CO), 31.9 (CH$_3$CH$_2$*C*H$_2$), 29.8-28,9 (CH$_3$CH$_2$CH$_2$(*C*H$_2$)$_7$), 28.5 (C*Me*$_3$), 24.6 (*C*H$_2$CH$_2$CO), 22.5 (CH$_3$*C*H$_2$), 14.0 (CH$_3$); ESIMS: *m/z* 2228.6 [M + 2 Na + H]$^{3+}$, 1681.2 [M +K + 2 Na + H]$^{4+}$. Anal. Calcd for $C_{357}H_{644}N_{42}O_{70}$: C 64.53, H 9.77, N 8.85. Found C 64.41, H 9.62, N 8.58.

General Procedure for the Preparation of Unprotected Rigid Amphiphilic Click Clusters (11-15). Treatment of the corresponding hepta- o tetradecacarbamate

(**6-10,** 33 μmol) with TFA-CH_2Cl_2 (1:1, 2 mL) at rt for 2 h, followed by evaporation of the solvents and freeze-drying from a diluted HCl solution, gave pure **11-15.**

Heptakis[6-(4-aminomethyl-1*H*-1,2,3-triazol-1-yl)-6-deoxy]-cyclomalto-heptaose (11): Yield: 63.4 mg (99%); $[\alpha]_D$ = +25.6 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O, 323 K): δ 8.08 (s, 7 H, =CH), 5.08 (d, 7 H, $J_{1,2}$ = 2.5 Hz, H-1), 4.41 (dd, 7 H, $J_{6a,6b}$ = 14.2 Hz, $J_{5,6a}$ = 3.0 Hz, H-6a), 4.30 (dd, 7 H, $J_{5,6b}$ = 6.5 Hz, H-6b), 4.20 (ddd, 7 H, $J_{4,5}$ = 9.5 Hz, H-5), 4.11, 4.09 (2 d, 14 H, $^2J_{H,H}$ = 14.5 Hz, C*H*$_2$NH$_2$), 3.97 (t, 7 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 3.54 (dd, 7 H, H-2), 3.30 (t, 7 H, H-4); ^{13}C NMR (125.7 MHz, D_2O, 323 K): δ 140.1 (C-4 triazole), 127.5 (C-5 triazole), 102.9 (C-1), 82.5 (C-4), 72.6 (C-3), 72.1 (C-2), 70.2 (C-5), 50.7 (C-6), 34.3 (CH_2NH_2); ESIMS: *m/z* 879.3 $[M + 2 Na + H_2O]^{2+}$. Anal. Calcd for $C_{63}H_{105}Cl_7N_{28}O_{28}$: C 38.79, H 5.43, N 20.10. Found: C 38.47, H 5.34, N 20.03.

Heptakis[6-(4-(2,2-diaminoethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy]cyclomaltoheptaose (12). Yield: 102.4 mg, (99%); $[\alpha]_D$ = +17.0 (*c* 0.9, MeOH); ^{1}H NMR (500 MHz, CD_3OD, 313 K): δ = 8.02 (s, 7 H, =CH), 5.16 (d, 7 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.61 (dd, 7 H, $J_{6a,6b}$ = 15.3 Hz, $J_{5,6a}$ = 3.6 Hz, H-6a), 4.55 (dd, 7 H, $J_{5,6b}$ = 4.9 Hz, H-6b), 4.28 (m, 7 H, H-5), 3.97 (t, 7H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), 3.83, 3.76 (2d, 14 H, $^2J_{H,H}$ = 15.3 Hz, CH_2N), 3.42 (dd, 7 H, H-2), 3.26 (t, 7 H, $J_{4,5}$ = 9.3 Hz, H-4), 3.15 (t, 28 H, $^3J_{H,H}$ = 5.7 Hz, C*H*$_2$NH$_2$), 2.79 (t, 28 H, C*H*$_2$CH$_2$NH$_2$); ^{13}C NMR (125.7 MHz, CD_3OD, 313 K): δ = 143.3 (C-4 triazole), 128.0 (C-5 triazole), 103.5 (C-1), 84.2 (C-4), 74.0 (C-3), 73.8 (C-2), 71.1 (C-5), 51.8 (*C*H$_2$CH$_2$NH$_2$), 51.5 (C-6), 47.3 (*C*H$_2$N), 38.3 (*C*H$_2$NH$_2$); ESIMS: *m/z* 1149.7 $[M + 2H]^{2+}$, 767.1 $[M + 3H]^{3+}$. Anal. Calcd for $C_{91}H_{182}Cl_{14}N_{42}O_{28}$: C 38.91, H 6.53, N 20.94. Found C 38.52, H 6.39, N 20.61.

Heptakis[6-(4-aminomethyl-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (13): Yield: 106.4 mg (99%); $[\alpha]_D$ = +31.5 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD, 313 K): δ 7.98 (s, 7 H, =CH), 5.49 (t, 7 H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3), 5.46 (s, 7 H, H-1), 4.88 (d, 7 H, $J_{6a,6b}$ = 14.0 Hz, H-6a), 4.78 (dd, 7 H, $J_{1,2}$ = 2.1 Hz, H-2), 4.65 (s, 7 H, H-6b), 4.53 (bs, 7 H, H-5), 3.91 (s, 14 H, C*H*$_2$NH$_2$), 3.67 (t, 7 H, $J_{4,5}$ = 8.5 Hz, H-4), 2.47-2.21 (m, 28 H, CH_2CO), 1.64 (m, 28 H, C*H*$_2$CH$_2$CO), 1.36 (m, 56 H, C*H*$_2$CH$_3$, C*H*$_2$CH$_2$CH$_3$), 0.94 (t, 42 H, $^3J_{H,H}$ = 7.0 Hz, CH_3); ^{13}C NMR (125.7 MHz, CD_3OD, 313 K): δ174.3, 173.4 (CO ester), 145.5 (C-4 triazole), 126.8 (C-5 triazole), 98.1 (C-1), 78.8 (C-4), 71.5 (C-5), 71.1 (C-3, C-2), 51.6 (C-6), 36.5 (CH_2NH_2), 35.0, 34.9 (*C*H$_2$CO), 32.5, 32.4 (*C*H$_2$CH$_2$CH$_3$), 25.5

(CH_2CH_2CO), 23.4 (CH_2CH_3), 14.2 (CH_3); MS (ESI): *m/z* 1023.9 $[M + 3 H]^{3+}$, 1535.3 $[M + 2 H]^{2+}$; Anal. Calcd for $C_{147}H_{238}N_{28}O_{42}$: C 53.10, H 7.43, N 11.80. Found: C 52.77, H 7.24, N 11.69

Heptakis[6-(4-(2,2-diaminoethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (14): Yield: 136.4 mg (99%); $[\alpha]_D$ = +18.5 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD, 313 K): δ 8.11 (s, 7 H, =CH), 5.60 (t, 7 H, $J_{2,3} = J_{3,4}$ = 9.9 Hz, H-3), 5.59 (s, 7 H, H-1), 5.15 (dd, 7 H, $J_{6a,6b}$ = 15.2 Hz, $J_{5,6a}$ = 2.5 Hz, H-6a), 4.58 (dd, 7 H, $J_{5,6b}$ = 3.0 Hz, H-6b), 4.57 (m, 14 H, $J_{1,2}$ = 3.5 Hz, H-2, H-5), 3.91, 3.89 (2 d, 14 H, $^2J_{Ha,Hb}$ = 15.3 Hz, CH_2N), 3.54 (t, 7 H, $J_{4,5}$ = 8.9 Hz, H-4), 3.15 (t, 28 H, $^3J_{H,H}$ = 6.0 Hz, CH_2NH_2), 2.83 (t, 28 H, $CH_2CH_2NH_2$), 2.49-2.17 (m, 28 H, CH_2CO), 1.73-1.58 (m, 28 H, CH_2CH_2CO), 1.37 (m, 56 H, CH_2CH_3, $CH_2CH_2CH_3$), 0.94, 0.92 (2 t, 42 H, $^3J_{H,H}$ = 7.1 Hz, $^3J_{H,H}$ = 6.9 Hz, CH_3); ^{13}C NMR (125.7 MHz, CD_3OD, 313 K): δ175.7, 174.6 (CO ester), 144.6 (C-4 triazole), 129.7 (C-5 triazole), 99.2 (C-1), 79.4 (C-4), 73.0 (C-2), 72.9 (C-5), 71.7 (C-3), 53.0 ($CH_2CH_2NH_2$), 52.6 (C-6), 48.3 (CH_2N), 39.4 (CH_2NH_2), 36.3, 36.2 (CH_2CO), 33.9, 33.7 ($CH_2CH_2CH_3$), 26.8 (CH_2CH_2CO), 24.7 (CH_2CH_3), 15.6 (CH_3); ESIMS: *m/z* 1837 $[M + 2 H]^{2+}$, 1225.0 $[M + 3 H]^{3+}$, 919.0 $[M + 4 H]^{4+}$, 735.4 $[M + 5 H]^{5+}$. Anal. Calcd for $C_{175}H_{322}Cl_{14}N_{42}O_{42}$: C 50.25, H 7.76, N 14.06. Found: C 49.98, H 7.65, N 13.79.

Heptakis[6-(4-(2,2-diaminoethyl)aminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy-2,3-di-*O*-myristoyl]cyclomaltoheptaose (15). Yield: 178.8 mg (99%); $[\alpha]_D$ = +30.7 (*c* 0.9, CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD, 313 K): δ 8.12 (s, 7 H, =CH), 5.61 (t, 7 H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, H-3), 5.60 (s, 7 H, H-1), 5.13 (d, 7 H, $J_{6a,6b}$ = 13.9 Hz, H-6a), 4.97 (d, 7 H, H-6b), 4.57 (m, 14 H, H-2, H-5), 3.90 (2d, 14 H, $^2J_{H,H}$= 15.3 Hz, CH_2N), 3.55 (t, 7 H, $J_{4,5}$ = 9.2 Hz, H-4), 3.16 (t, 28 H, $^3J_{H,H}$ = 5.5 Hz, CH_2NH_2), 2.82 (t, 28 H, $CH_2CH_2NH_2$), 2.50-2.15 (m, 28 H, CH_2CO), 1.80-1.55 (m, 28 H, CH_2CH_2CO), 1.50-1.25 (m, 280 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 0.93 (t, 42 H, $^3J_{H,H}$ = 6.6 Hz, CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K): δ 172.9, 171.8 (CO ester), 142.0 (C-4 triazole), 126.9 (C-5 triazole), 96.6 (C-1), 76.7 (C-4), 70.4 (C-5, C-2), 69.1 (C-3), 50.4 ($CH_2CH_2NH_2$), 49.9 (C-6), 45.7 (CH_2N), 36.7 (CH_2NH_2), 33.6 (CH_2CO), 31.7, 29.8-29.1, 22.3 ($CH_3(CH_2)_{10}CH_2CH_2CO$), 24.6, 24.5 ($CH_2CH_2CO$), 13.0 ($CH_3$); ESIMS: *m/z* 2622.0 $[M + 2 H]^{2+}$, 1748.4 $[M + 3H]^{3+}$, 1311.7 $[M + 4 H]^{4+}$, 1049.7 $[M + 5 H]^{5+}$. Anal. Calcd for $C_{287}H_{546}Cl_{14}N_{42}O_{42}$: C 59.91, H 9.56, N 10.22. Found C 59.58, H 9.22, N 9.97.

Heptakis[6-(2-chloroacetamidoethylthio)-6-deoxy-2,3-di-*O*-hexanoyl]-cyclomaltoheptaose (17): To a solution of the heptahydrochloride **16** (500 mg, 0.16 mmol) in dry DMF (10 mL), under Ar atmosphere, DMAP (270 mg, 2.24 mmol, 2 eq) was added in portions and the mixture was stirred for 15 min. Chloroacetic anhydride (570 mg, 3.36 mmol, 3 eq) was added and the reaction was further stirred overnight at rt. The solvent was evaporated under reduced pressure and the residue was dissolved in CH_2Cl_2, washed with slightly acidified water (3 x 20 mL), saturated aqueous $NaHCO_3$ (3 x 20 mL) and water (3x 20 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (30:1 CH_2Cl_2-MeOH). Yield: 325 mg (59%); R_f = 0.53 (9:1 CH_2Cl_2-MeOH); $[\alpha]_D$ = +80.3 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz, 313 K) δ 5.35 (t, 7 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 5.14 (d, 7 H. $J_{1,2}$ = 4 Hz, H-1), 4.80 (dd, 7 H, H-2) 4.21 (m, 7 H, $J_{4,5}$ = 9.0 Hz, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 5.0 Hz, H-5) 4.11 (2 d, 14 H, $^2J_{H,H}$ = 15.0 Hz, CH_2Cl), 3.82 (t, 7H, H-4), 3.55 (m, 14 H, CH_2NH_{Cyst}), 3.16 (dd, 7H, $J_{6a,6b}$ = 14.0 Hz, H-6a), 3.10 (dd, 7H, H-6b), 2.85 (2dt, 14 H, $^2J_{H,H}$ = 13.5 Hz, $^3J_{H,H}$ = 6.5 Hz , CH_2S_{Cyst}), 2.43-2.17 (m, 28 H, CH_2CO), 1.70-1.57 (m, 28 H, CH_2CH_2CO), 1.36-1.29 (m, 56 H, $CH_2CH_2CH_3$, CH_2CH_3), 0.92 (2 t, 42 H, $^3J_{H,H}$ = 7.0 Hz, $^3J_{H,H}$ = 7.5 Hz, CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K) δ 173.3, 171.7 (CO ester), 166.5 (CO amide), 96.6 (C-1), 78.6 (C-4), 71.3 (C-5), 70.5(C-2) 70.3 (C-3), 42.7 (CH_2Cl), 39.4 (CH_2NH_{Cyst}), 34.1, 33.8 (CH_2CO, C-6), 32.9 (CH_2S_{Cyst}), 31.4, 31.3 ($CH_2CH_2CH_3$), 24.3 (CH_2CH_2CO), 22.3 (CH_2CH_3), 13.8 (CH_3); ESIMS: *m/z* 1175.3 $[M + 3\ Na]^{3+}$, 1752 $[M + 2\ Na]^{2+}$, 3477.7 $[M + Na]^+$. Anal. Calcd for $C_{154}H_{252}Cl_7N_7O_{49}S_7$: C 53.48, H 7.34, N 2.84, S 6.49. Found: C 53.37, H 7.21, N 2.65, S 6.33.

Heptakis[6-(2-azidoacetamidoethylthio)-6-deoxy-2,3-di-*O*-hexanoyl]-cyclomaltoheptaose (18): To a solution of **17** (109 mg, 31 μmol) in dry DMF (10 mL), under Ar atmosphere, NaN_3 (43 mg, 0.66 mmol, 3 eq) was added and the mixtures was stirred overnight. The solvent was eliminated under vacuum and the residue was dissolved in CH_2Cl_2 (50 mL), washed with H_2O (3 x 15 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by column chromatography (50:1→ 30:1 CH_2Cl_2:MeOH).Yield: 93.2 mg (89%); R_f = 0.48 (9:1 CH_2Cl_2:MeOH). $[\alpha]_D$ = +94.0 (*c* 1.0, CH_2Cl_2) ; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.34 (t, 7 H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3), 5.12 (d, 7 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.80 (dd, 7 H, H-2) 4.17 (m, 7 H, H-5) 4.02 (s, 14 H, CH_2N_3), 3.82 (t, 7 H, $J_{4,5}$ = 8.7 Hz, H-4) 3.51 (m, 14 H, CH_2NH_{Cyst}), 3.10 (m, 14 H, H-6a, H-6b), 2.82 (2 dt, 14 H, $^2J_{H,H}$ = 13.8 Hz, $^3J_{H,H}$ = 7.2 Hz , CH_2S_{Cyst}), 2.44-

2.12 (m, 28 H, CH_2CO) 1.62-1.55 (m, 28 H, CH_2CH_2CO), 1.34-1.30 (m, 56 H, $CH_2CH_2CH_3$, CH_2CH_3), 0.92 (2 t, 42 H, $^3J_{H,H}$ = 11.1 Hz, $^3J_{H,H}$ = 11.4 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 313 K) δ 173.7, 172.0 (CO ester), 167.7 (CO amide), 96.7 (C-1), 78.8 (C-4), 71.5 (C-5), 70.8 (C-2), 70.5 (C-3), 52.8 (CH_2N_3), 39.2 (CH_2NH_{Cyst}), 34.3, 34.1 (CH_2CO), 33.8 (C-6), 33.1 (CH_2S_{Cyst}), 31.7, 31.6 ($CH_2CH_2CH_3$), 24.7 (CH_2CH_2CO), 22.7 (CH_2CH_3), 14.2 (CH_3); ESIMS: *m/z* 1774.6 $[M + 2 Na]^{2+}$. Anal. Calcd for $C_{154}H_{252}N_{28}O_{49}S_7$: C 52.78, H 7.25, N 11.19, S 6.41. Found C 52.56, H 7.14, N 10.98, S 6.19.

Heptakis[6(2-(4-(*N-tert*-butoxycarbonyl)aminomethyl)-1*H*-1,2,3-triazol-1-yl)acetamidoethylthio)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (19): To a solution of **18** (45 mg, 13 μmol) and **4** (19 mg, 0.12 mmol, 1.3 eq) in acetone (5 mL), $(EtO)_3P{\cdot}CuI$ (3 mg, 9 μmol, 0.1 eq) and DIPEA (15 μL, 90 μmol, 1 eq) were added and the mixture was refluxed for 4 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (20:1 → 9:1 CH_2Cl_2:MeOH). Yield: 53 mg (89%); R_f = 0.4 (9:1 CH_2Cl_2:MeOH); $[\alpha]_D$ = +67.6 (*c* 1.0, CH_2Cl_2); ^1H NMR (CD_3OD, 500 MHz, 323 K) δ 7.90 (s, 7 H, =CH), 5.32 (t, 7 H, $J_{2,3} = J_{3,4}$ = 8.5 Hz, H-3), 5.15 (d, 7 H, $J_{1,2}$ = 3.6 Hz, H-1), 5.13 (s, 14 H, CH_2CONH), 4.87 (dd, 7 H, H-2), 4.34 (s, 14 H, CH_2NHBoc), 4.28 (m, 7 H, H-5), 3.84 (t, 7 H, $J_{4,5}$ = 8.0 Hz, H-4), 3.54 (m, 14 H, CH_2NH_{Cyst}), 3.40 (m, 7 H, H-6a), 3.08 (dd, 7 H, $J_{6a,6b}$ = 13.9 Hz, $J_{5,6b}$ = 7.0 Hz, H-6b), 2.88 (m, 14 H, CH_2S_{Cyst}), 2.50-2.22 (m, 28 H, CH_2CO), 1.70-1.57 (m, 28 H, CH_2CH_2CO), 1.42 (s, 63 H, CMe_3), 1.40-1.30 (m, 56 H, $CH_2CH_2CH_3$, CH_2CH_3), 0.96, 0.95 (2 t, 42 H, $^3J_{H,H}$ = 7.0 Hz, CH_3); ^{13}C NMR (125.7 MHz, CD_3OD): δ 174.5, 173.3 (CO ester), 167.9 (CO amide), 158.1 (CO carbamate), 147.4 (C-4 triazole), 125.7 (C-5 triazole), 98.3 (C-1), 80.6 (C-4, C_q), 72.9 (C-5), 72.1 (C-3), 71.6 (C-2), 53.4 (CH_2CONH), 40.7 (CH_2NH_{Cyst}), 37.0 (CH_2NHBoc) 35.1 (CH_2CO), 32.6, 32.5 ($CH_2CH_2CH_3$, CH_2S_{Cyst}, C-6), 28.9 (CMe_3), 25.5 (CH_2CH_2CO), 23.4 (CH_2CH_3), 14.4 (CH_3); MS ESI: (*m/z*) 4606.9 $[M + Na]^+$, 2317.9 $[M + 2Na]^{2+}$, 1552.2 $[M+ 3 Na]^{3+}$. Anal. Calcd for $C_{210}H_{343}N_{35}O_{63}S_7$: C 54.94, H 7.53 N 10.68, S 4.89. Found C 54.72, H 7.38 N 10.47, S 4.60.

Heptakis[6-(2-(4-(2,2-bis(*tert*-butoxycarbonylamino)ethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)acetamidoethylthio-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (20): To a solution of **18** (48 mg, 14 μmol) and **5** (44 mg, 0.13 mmol, 1.3 eq.) in acetone (5 mL), $CuI{\cdot}(EtO)_3P$ (4 mg, 10 μmol, 0.1 eq.) and

DIPEA (17 μL, 0.1 mmol, 1 eq.) were added and the reaction mixture was refluxed for 3 h. Solvent was eliminated under vacuum and the residue was purified by column chromatography (30:1 → 9:1 CH_2Cl_2-MeOH) to give **20**. Yield: 65 mg (79%); R_f = 0.36 (9:1 CH_2Cl_2:MeOH); $[\alpha]_D$ = +51.5 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, 313 K): δ 7.81 (s, 7 H, =CH), 5.34 (s, 14 H, N*H*Boc) 5.26 (t, 7 H, $J_{2,3} = J_{3,4}$ = 7.9 Hz, H-3), 5.10 (m, 21 H, C*H*$_2$CONH, H-1), 4.76 (bd, 7 H, H-2), 4.14 (bs, 7 H, H-5), 3.81 (bs, 14 H, C*H*$_2$N), 3.72 (bt, 7H, $J_{4,5}$ = 6.95 Hz, H-4), 3.44 (m, 14 H, C*H*$_2$NH$_{Cyst}$), 3.18 (bs, 14 H, C*H*$_2$NHBoc), 2.98 (bs, 7 H, H-6a), 2.80 (bs, 14 H, CH_2S_{Cyst}), 2.73 (bs, 7 H, H-6b), 2.58 (s, 14 H, C*H*$_2$CH$_2$NHBoc), 2.42-2.10 (m, 28 H, CH_2CO), 1.65-1.50 (m, 28 H, C*H*$_2$CH$_2$CO), 1.42 (s, 126 H, CMe_3), 1.38-1.20 (m, 56 H, C*H*$_2$CH$_2$CH$_3$, C*H*$_2$CH$_3$), 0.95-0.84 (2 t, 42 H, $^3J_{H,H}$ = 7.3 Hz, $^3J_{H,H}$ = 7.0 Hz, CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K): δ 173.4, 171.6 (CO ester), 166.0 (CO amide), 156.3 (CO carbamate) 144.1 (C-4 triazole), 125.1 (C-5 triazole), 96.6 (C-1), 79.1 (C-4, C_q), 71.4 (C-5), 70.4 (C-3, C-2), 53.3 (*C*H$_2$CONH), 52.4 (*C*H$_2$CH$_2$NHBoc), 48.3 (CH_2N), 39.4 (CH_2NH_{Cyst}), 38.4 (*C*H$_2$NHBoc) 34.0, 33.8 (*C*H$_2$CO), 32.9 (C-6, CH_2S_{Cyst}), 31.3, 31.2 (*C*H$_2$CH$_2$CH$_3$), 28.5 (C*Me*$_3$), 24.3 (*C*H$_2$CH$_2$CO), 22.3 (*C*H$_2$CH$_3$), 13.8 (CH_3); ESIMS: *m/z* 2969.7 [M + 2 Na]$^{2+}$, 2959.0 [M+ H + Na]$^{2+}$. Anal. Calcd for $C_{273}H_{469}N_{49}O_{77}S_7$: C 55.6, H 8.02, N 11.64, S 3.81. Found C 55.47, H 7.89, N 11.50, S 3.65.

General Procedure for the Preparation of Unprotected Flexible Amphiphilic Click Clusters (21, 22). Treatment of the corresponding hepta- or tetradecacarbamate (**19** or **20,** 19 μmol) with TFA-CH_2Cl_2 (1:1, 2 mL) at rt for 2 h, followed by evaporation of the solvents and freeze-drying from a diluted HCl solution, gave pure **21-22.**

Heptakis[6(2-(4-aminomethyl)-1*H*-1,2,3-triazol-1-yl)methylcarbamoyl)-ethylthio)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (21): Yield: 72 mg (91%); $[\alpha]_D$ = +22.1 (*c* 0.8, MeOH); ^{1}H NMR (500 MHz, CD_3OD, 323 K): δ 8.21 (s, 7 H, =CH), 5.34 (t, 7 H, $J_{2,3} = J_{3,4}$ = 7.9 Hz, H-3), 5.26 (s, 14 H, C*H*$_2$CONH), 5.16 (d, 7 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.85 (dd, 7 H, H-2), 4.33 (s, 14 H, C*H*$_2$NH$_2$), 4.29 (bs, 7 H, H-5), 3.87 (t, 7 H, $J_{4,5}$ = 7.9 Hz, H-4), 3.54 (m, 14 H, C*H*$_2$NH$_{Cyst}$), 3.42 (bd, 7 H, $J_{6a,6b}$ = 11.4 Hz, H-6a), 3.12 (dd, 7 H, $J_{5,6b}$ = 4.3 Hz, H-6b), 2.91 (m, 14 H, CH_2S_{Cyst}), 2.50-2.20 (m, 28 H, CH_2CO), 1.65 (m, 28 H, C*H*$_2$CH$_2$CO), 1.45-1.30 (m, 56 H, C*H*$_2$CH$_2$CH$_3$, C*H*$_2$CH$_3$), 1.00-0.92 (2 t, 42 H, $^3J_{H,H}$ = 7.3 Hz, $^3J_{H,H}$ = 6.9 Hz, CH_3); ^{13}C NMR (100.3 MHz, CD_3OD, 313 K): δ 173.3, 171.9 (CO ester), 166.3 (CO amide), 139.7 (C-4

triazole), 126.0 (C-5 triazole), 96.8 (C-1), 79.2 (C-4), 71.2 (C-5), 70.4 (C-3), 70.1 (C-2), 51.8 (CH_2CONH), 39.1 (CH_2NH_{Cyst}), 34.9 (CH_2NH_2), 33.6 (CH_2CO, CH_2S_{Cyst}, C-6), 31.5, 31.1 ($CH_2CH_2CH_3$), 24.1 (CH_2CH_2CO), 22.0 (CH_2CH_3), 13.0, 12.8 (CH_3). ESIMS: *m/z* 1976.6 $[M + 2\ Na]^{2+}$, 1317.8 $[M + 3\ Na]^{3+}$, 988.1 $[M + 2\ H + 2\ Na]^{4+}$. Anal. Calcd. for $C_{175}H_{294}N_{35}O_{49}Cl_7S_7$: C 50.71, H 7.15 N 11.83, S 5.42. Found C 50.35, H 6.89 N 11.56, S 5.11.

Heptakis[6(2-(4-(2,2-diaminoethylaminomethyl)-1*H*-1,2,3-triazol-1-yl)acetamidoethylthio)-6-deoxy-2,3-di-*O*-hexanoyl]cyclomaltoheptaose (22): Yield: 81.0 mg (91%). $[\alpha]_D$ = +39.6 (*c* 1.0 in MeOH); ^{1}H NMR (500 MHz, CD_3OD, 313 K): δ 8.05 (s, 7 H, =CH), 5.36 (t, 7 H, $J_{2,3} = J_{3,4}$ = 8.7 Hz, H-3) 5.21 (s, 14 H, CH_2CONH), 5.17 (d, 7 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.83 (dd, 7 H, H-2), 4.21 (bs, 7 H, H-5), 3.90 (bs, 21 H, CH_2N, H-4), 3.52 (m, 14 H, CH_2NH_{Cyst}), 3.15 (t, 14 H, CH_2NH_2, $^3J_{H,H}$ = 5.5 Hz), 3.11 (m, 7 H, H-6a), 2.88 (m, 21 H, CH_2S_{Cyst}, H-6b), 2.84 (t, 7 H, C$H_2CH_2NH_2$), 2.50-2.22 (m, 28 H, CH_2CO), 1.73-1.58 (m, 28 H, CH_2CH_2CO), 1.43-1.30 (m, 56 H, CH_2CH_3), 0.99-0.91 (2 t, 42 H, $^3J_{H,H}$ = 6.9 Hz, $^3J_{H,H}$ = 5.8 Hz, CH_3); ^{13}C NMR (125.7 MHz, CD_3OD, 313 K): δ 176.0, 174.7 (CO ester), 169.2 (CO amide), 145.4 (C-4 triazole), 128.5 (C-5 triazole), 99.5 (C-1), 81.8 (C-4), 74.5 (C-5), 73.0 (C-3, C-2), 54.4 (CH_2CONH), 53.4 ($CH_2CH_2NH_2$), 49.1 (CH_2N), 42.0 (CH_2NH_{Cyst}), 39.6 (CH_2NH_2) 36.4, 36.3 (CH_2CO), 33.8, 33.7 ($CH_2CH_2CH_3$), 32.0 (C-6, CH_2S_{Cyst}) 26.8 (CH_2CH_2CO), 24.7 (CH_2CH_3), 15.7, 15.6 (CH_3). Anal. Calcd. for $C_{203}H_{371}Cl_{14}N_{49}O_{49}S_7$: C 48.73, H 7.47, N 13.72, S 4.49. Found C 48.49, H 7.33, N 13.54, S 4.14.

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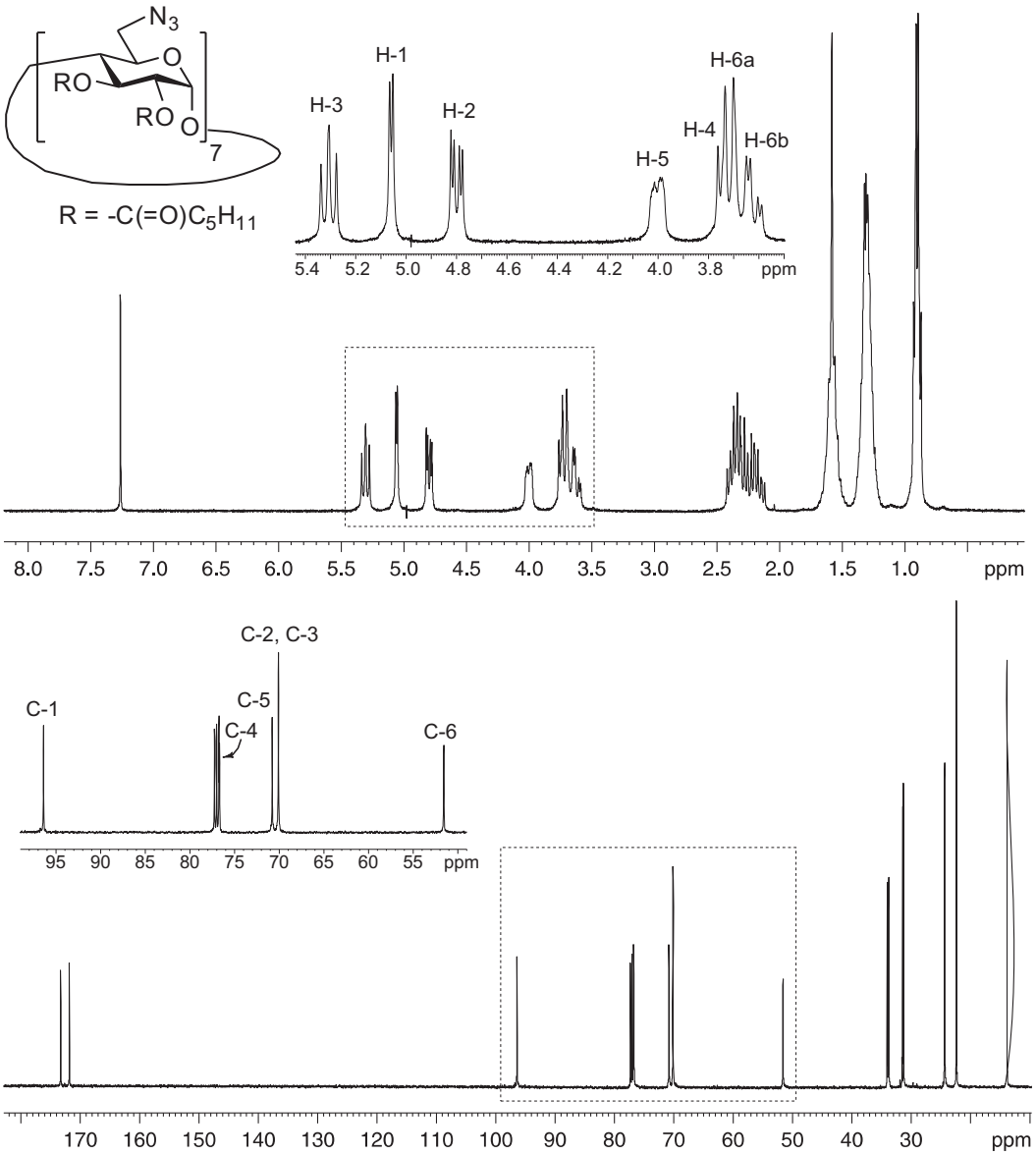

Figure S1. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, $CDCl_3$) of **2**.

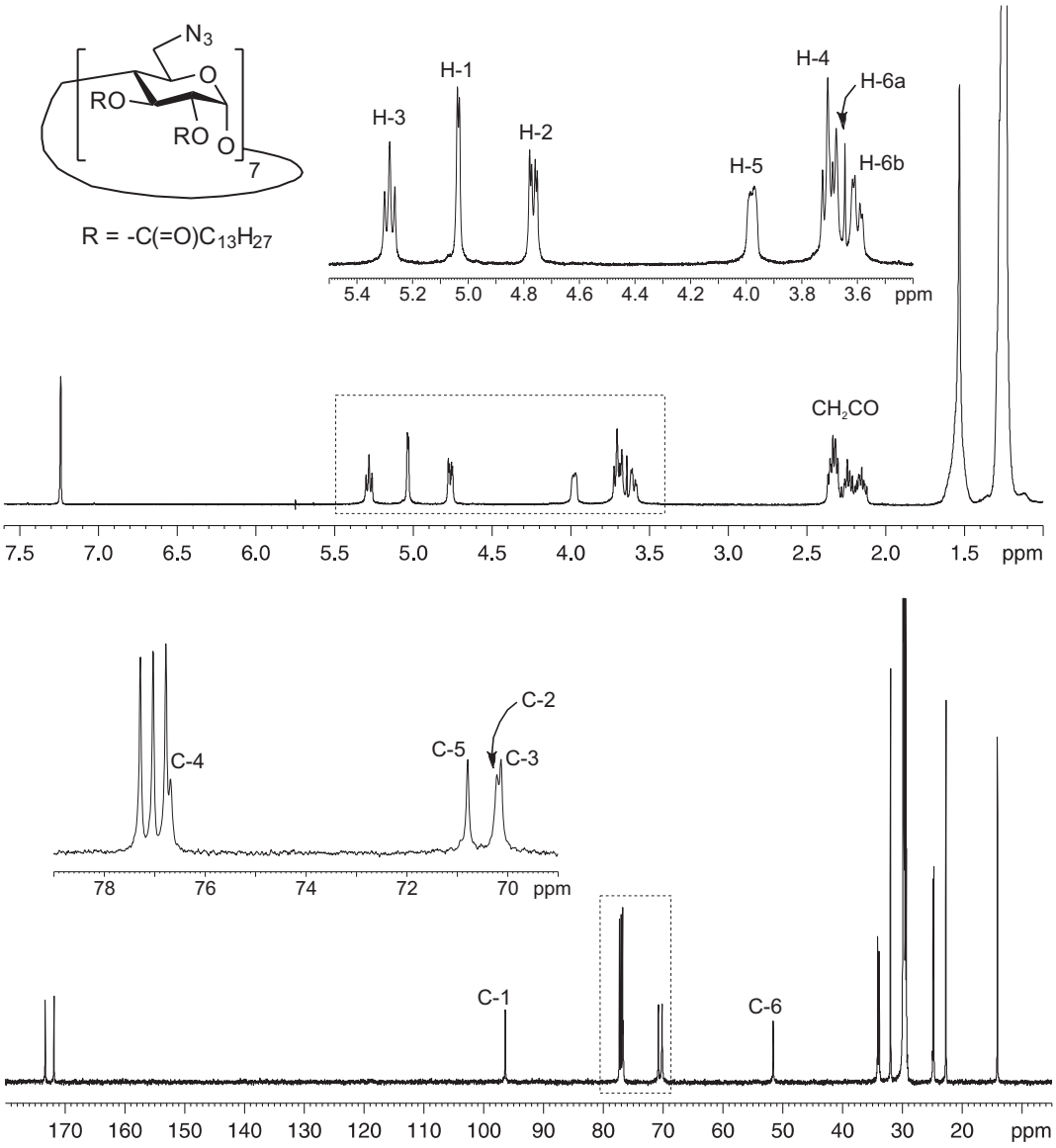

Figure S2. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, $CDCl_3$) of **3**.

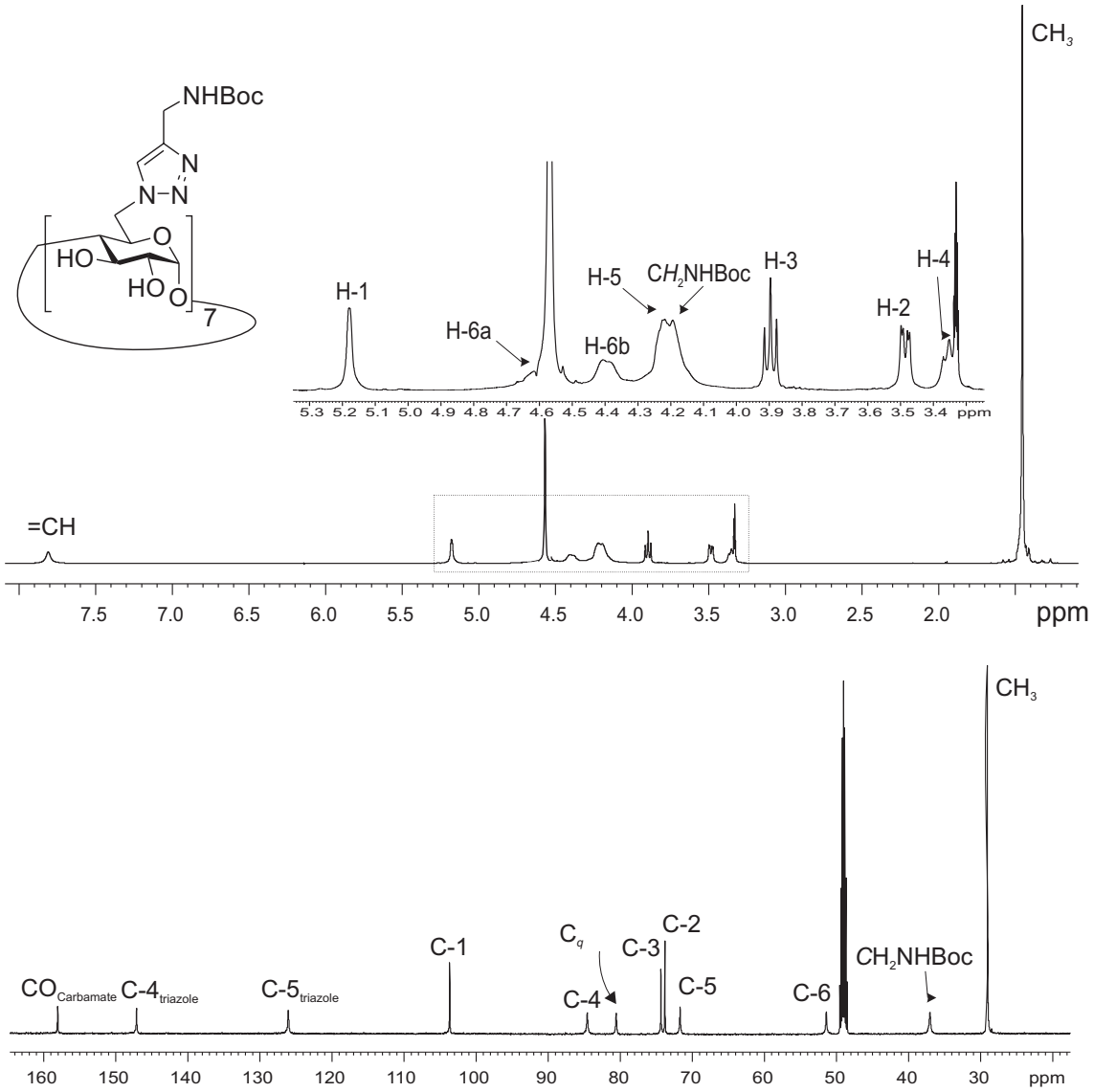

Figure S3. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD, 323 K) of **6**.

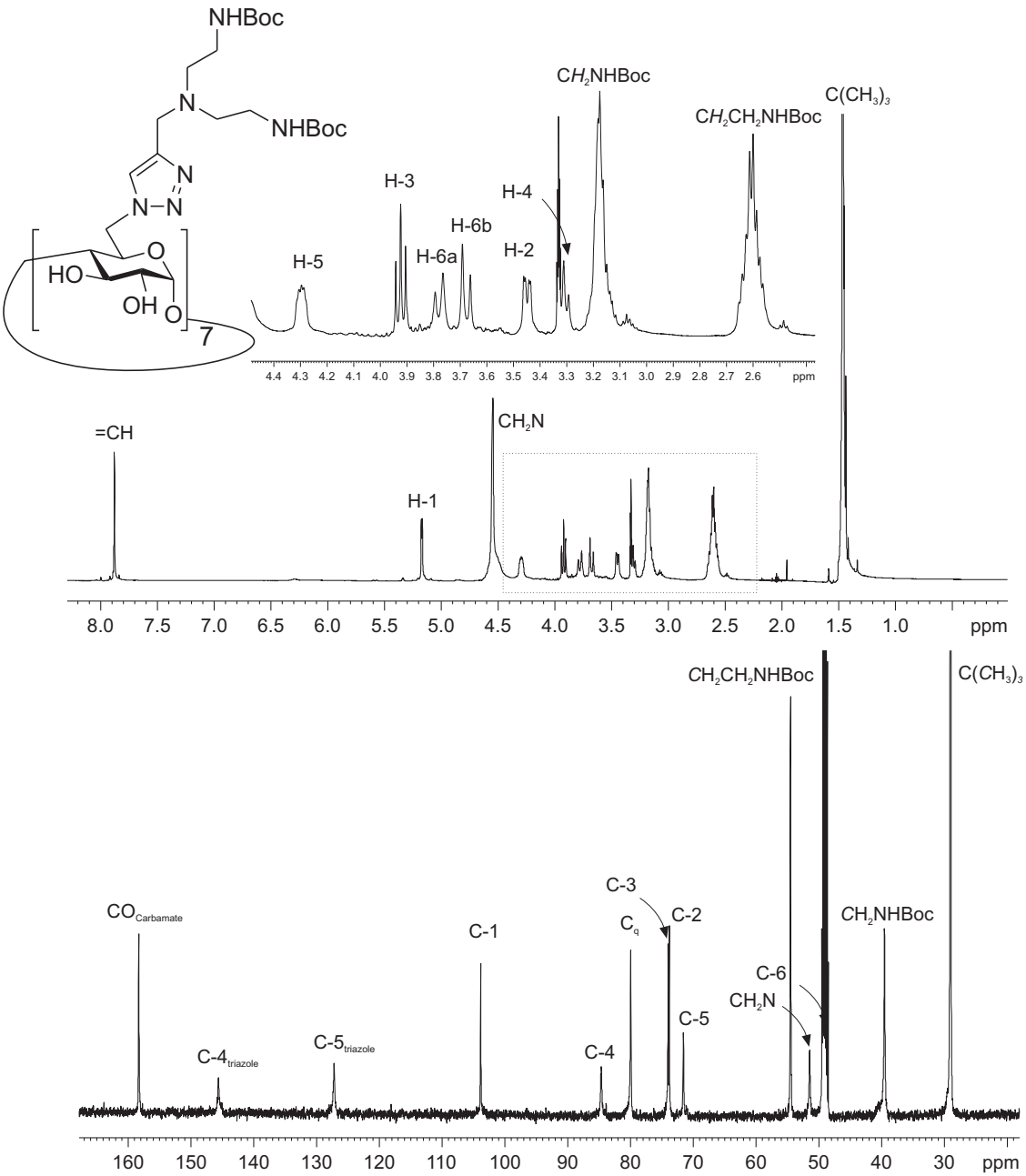

Figure S4. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD, 313 K) of **7**.

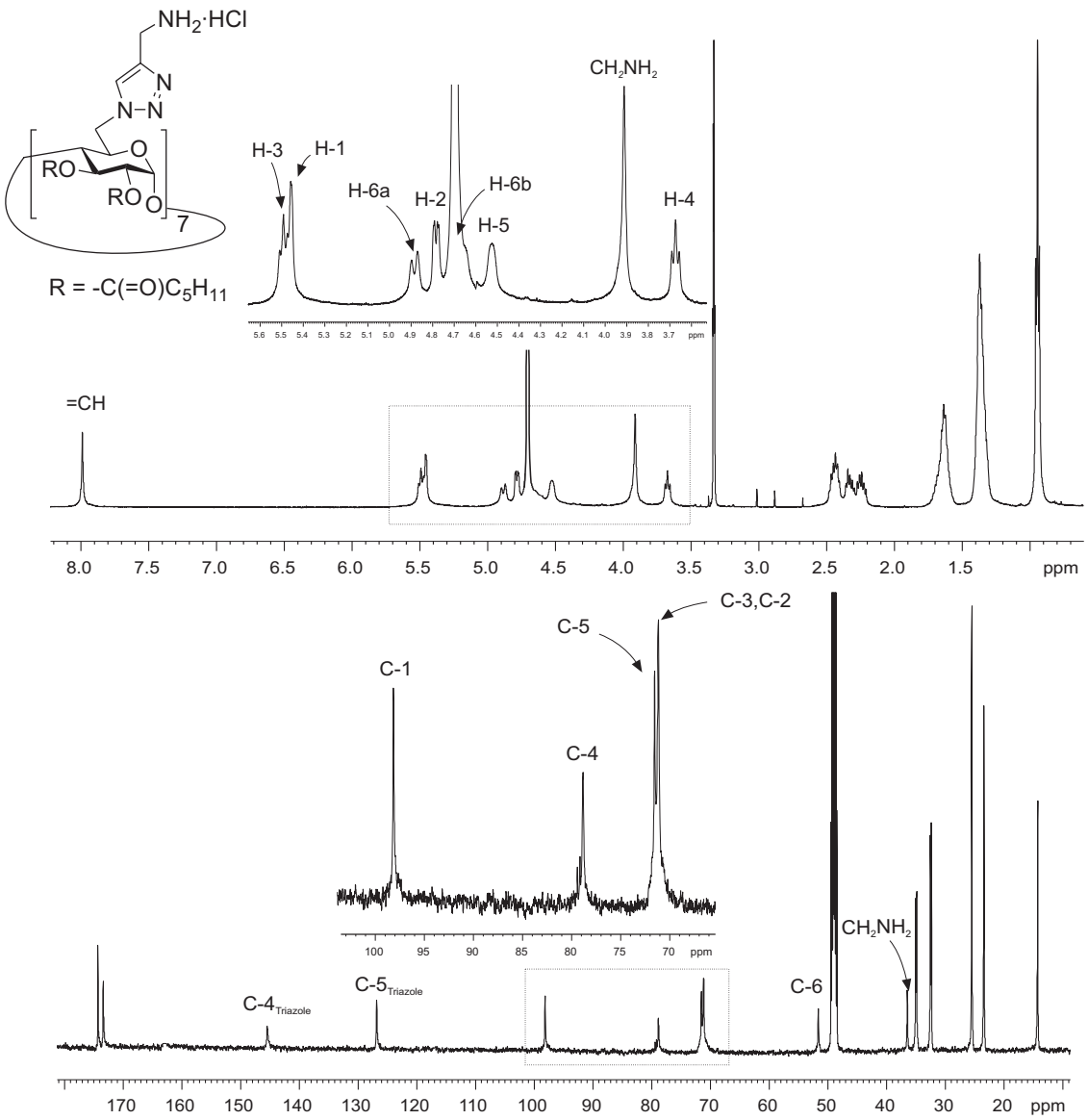

Figure S5. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, $CDCl_3$, 313 K) of **8**.

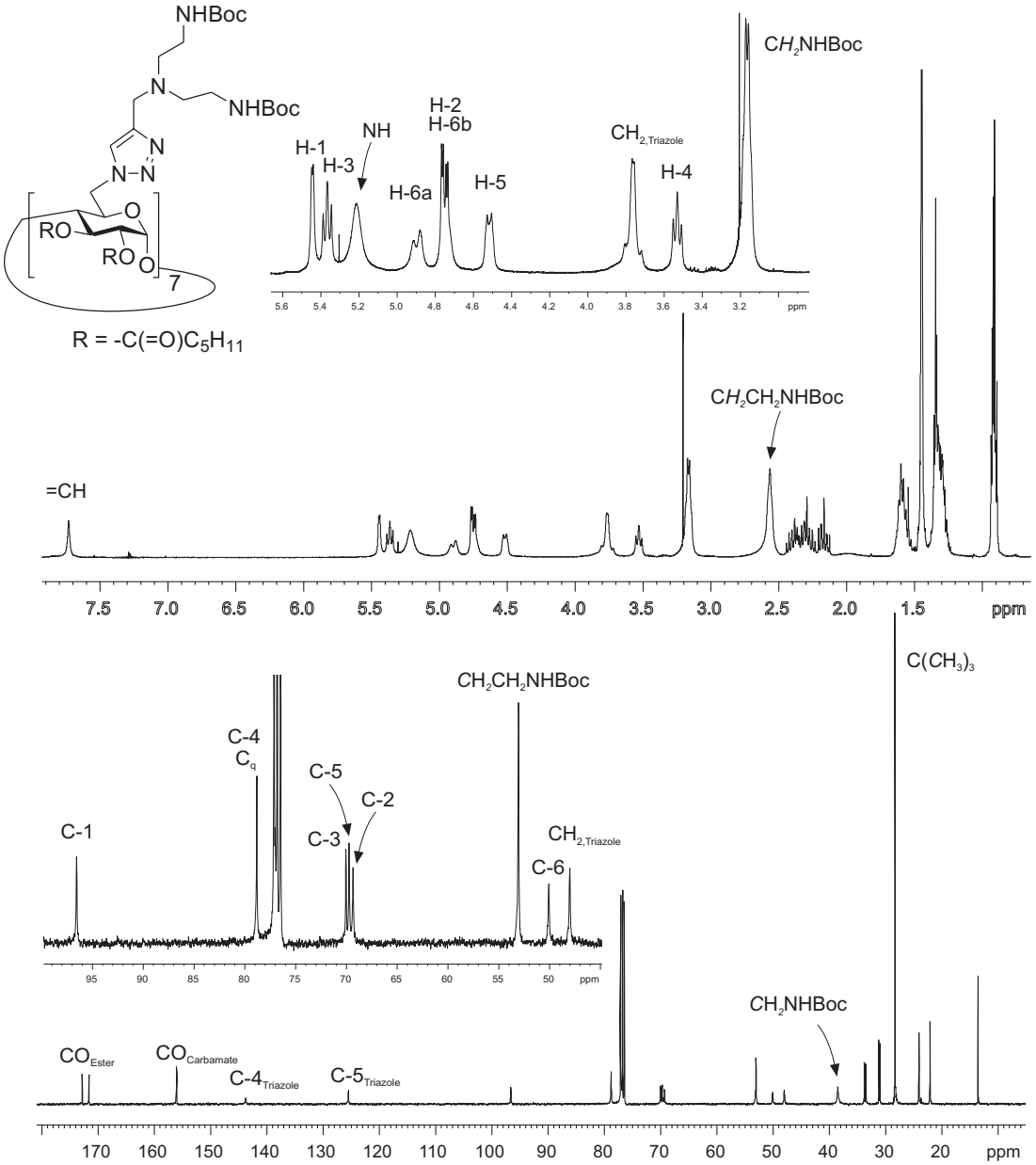

Figure S6. ^{1}H and ^{13}C NMR spectra (400 MHz, 100.6 MHz,, $CDCl_3$, 323 K) of **9**.

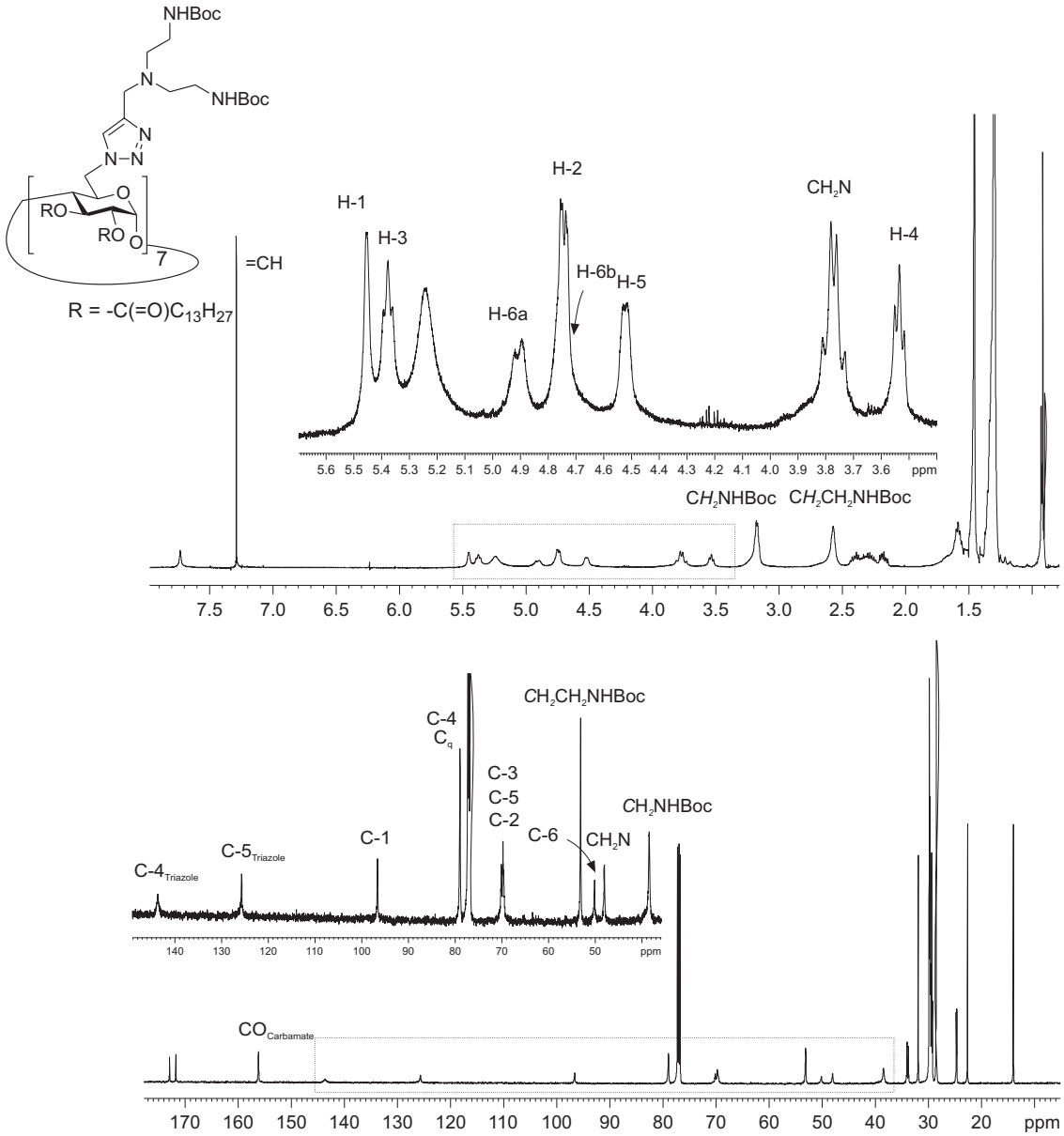

Figure S7. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD, 313 K) of **10**.

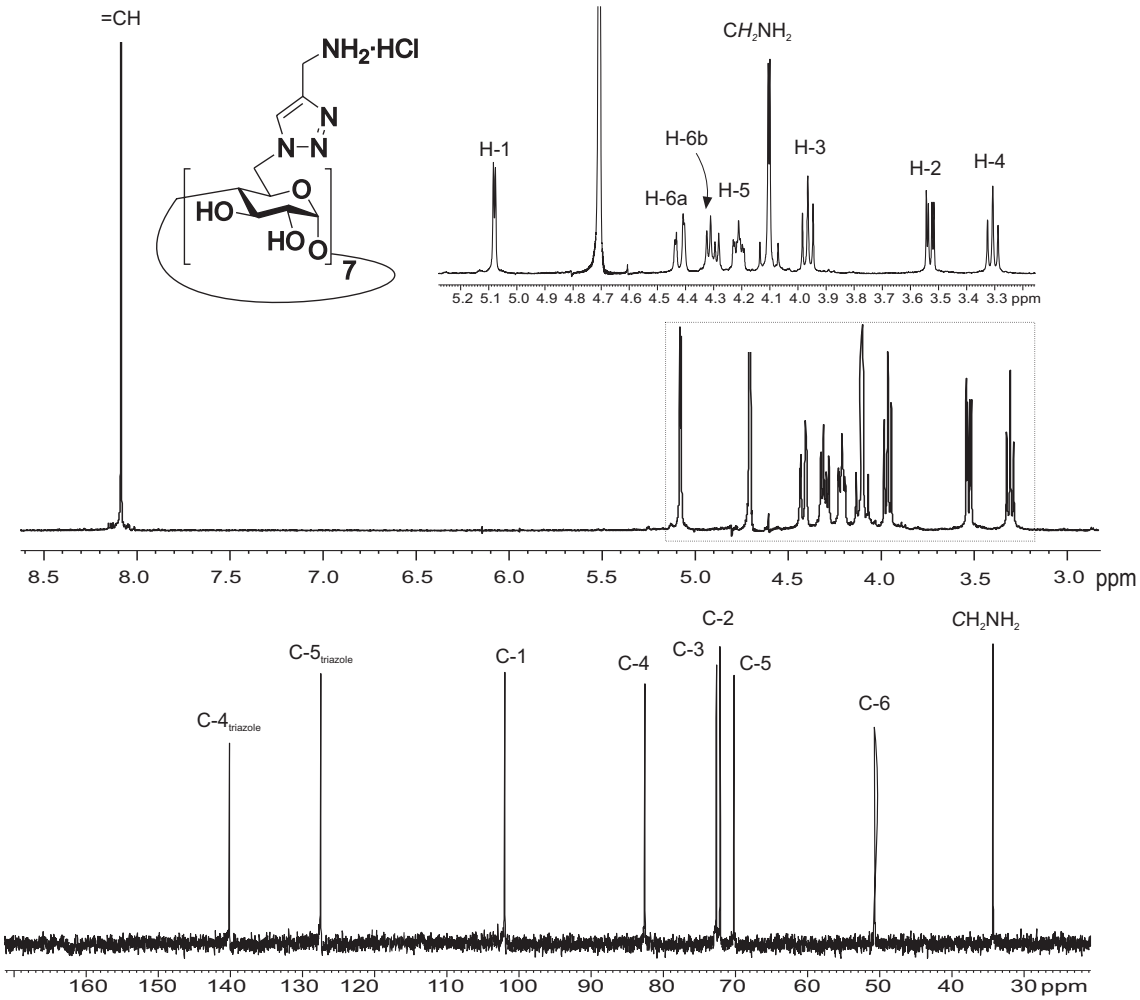

Figure S8. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, D_2O) of **11**.

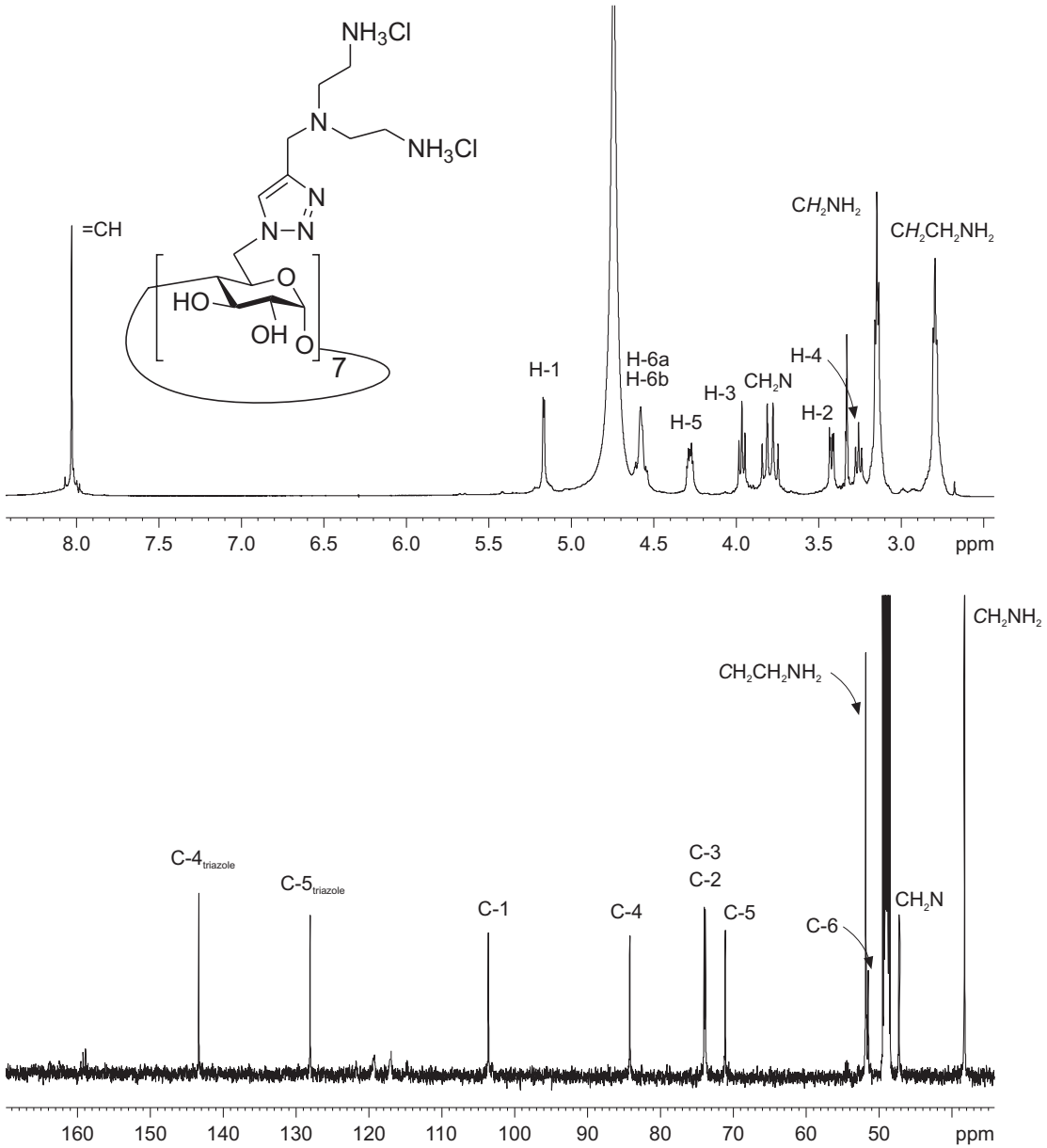

Figure S9. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD, 313 K) of **12**.

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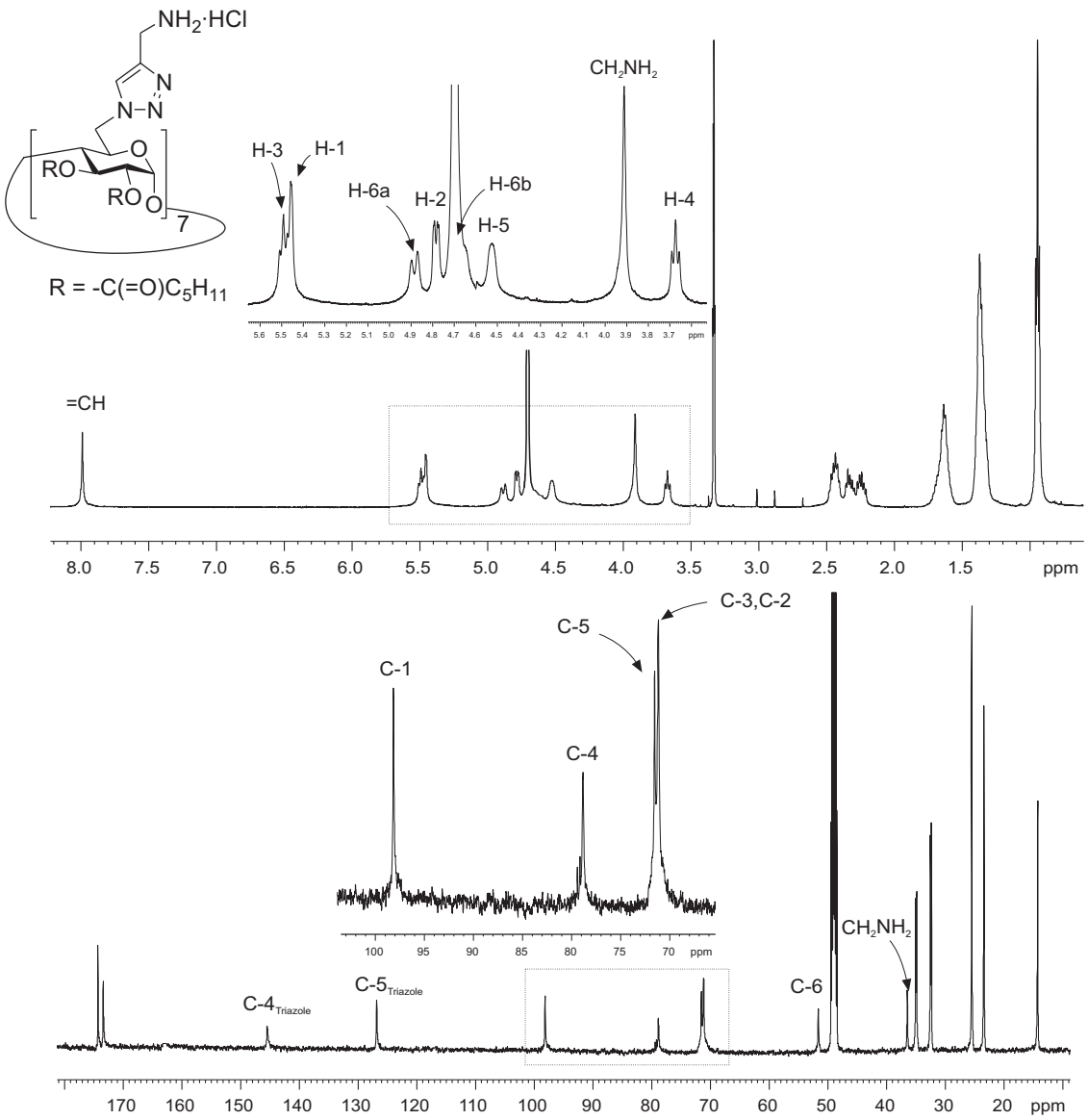

Figure S10. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD, 313 K) of **13**.

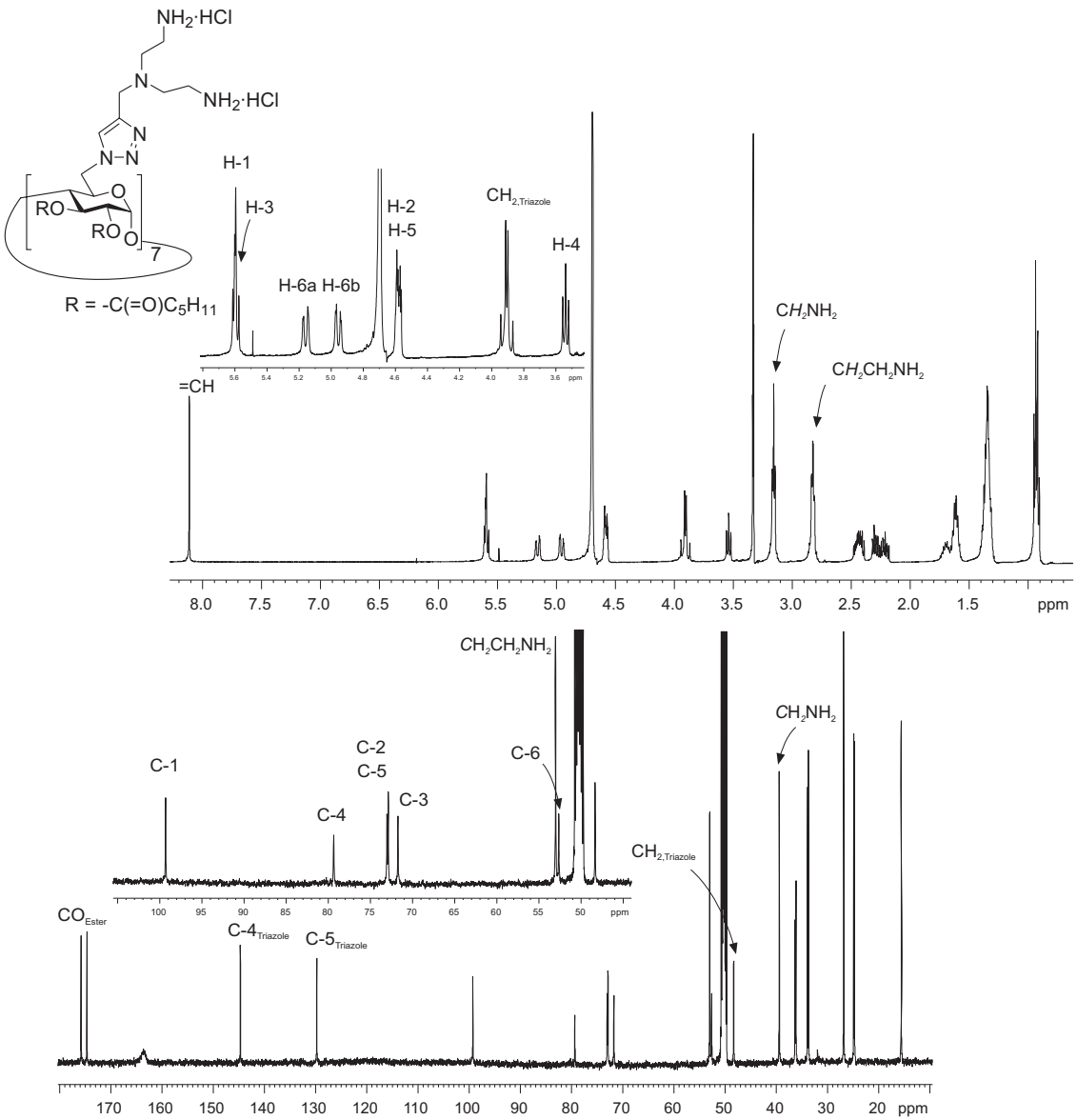

Figure S11. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD, 313 K) of **14**.

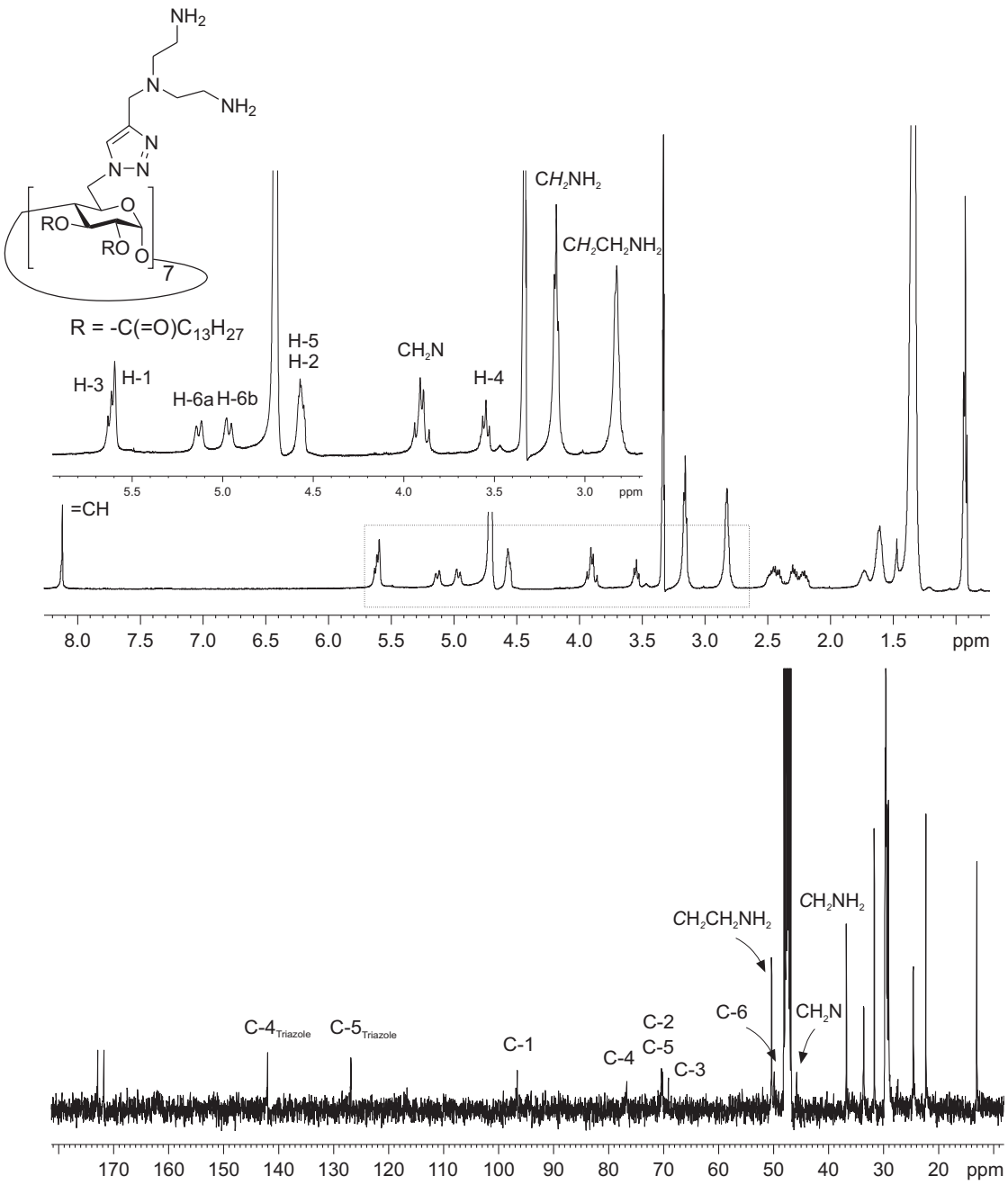

Figure S12. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD, 313 K) of **15**.

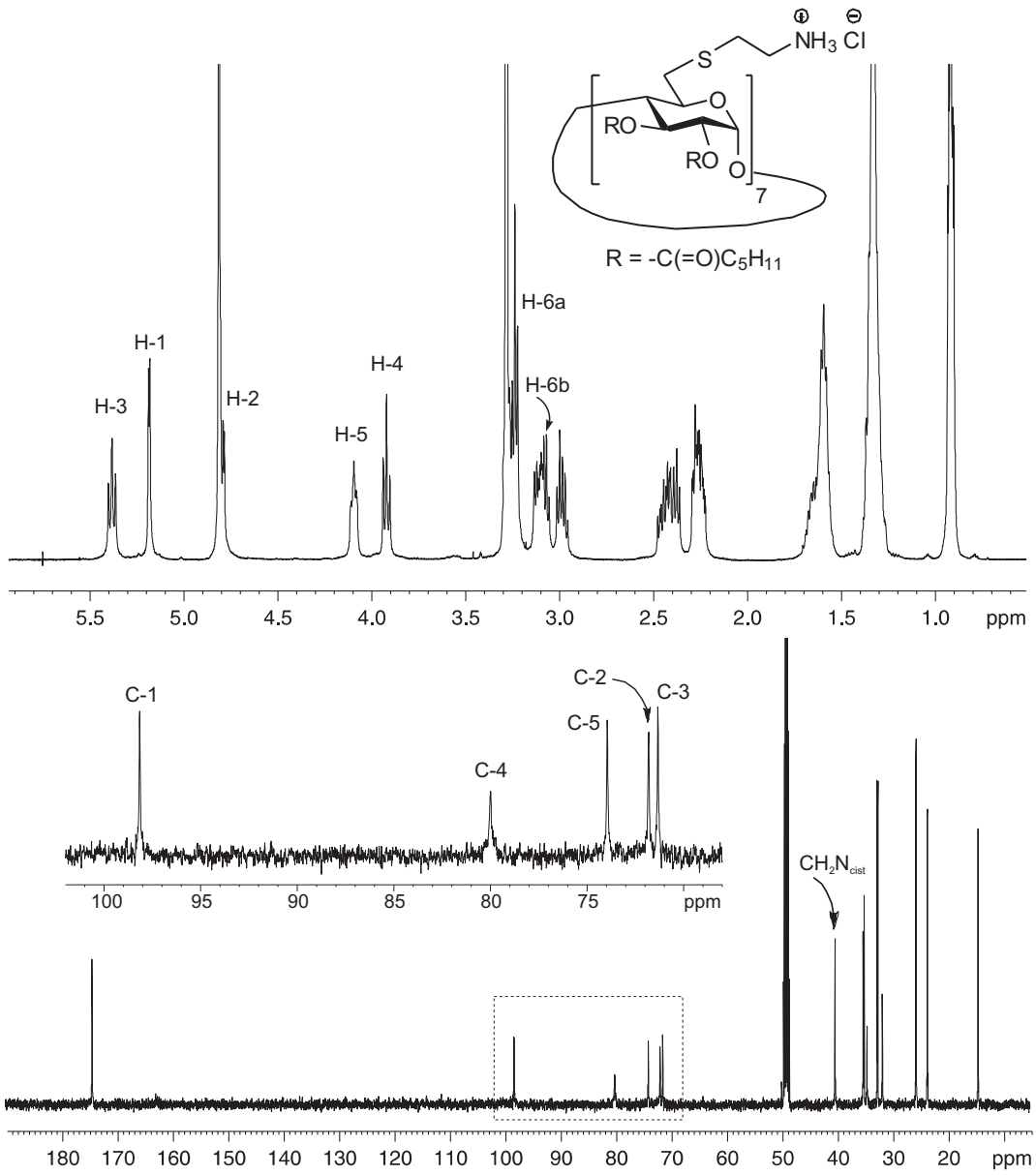

Figure S13. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD) of **16**.

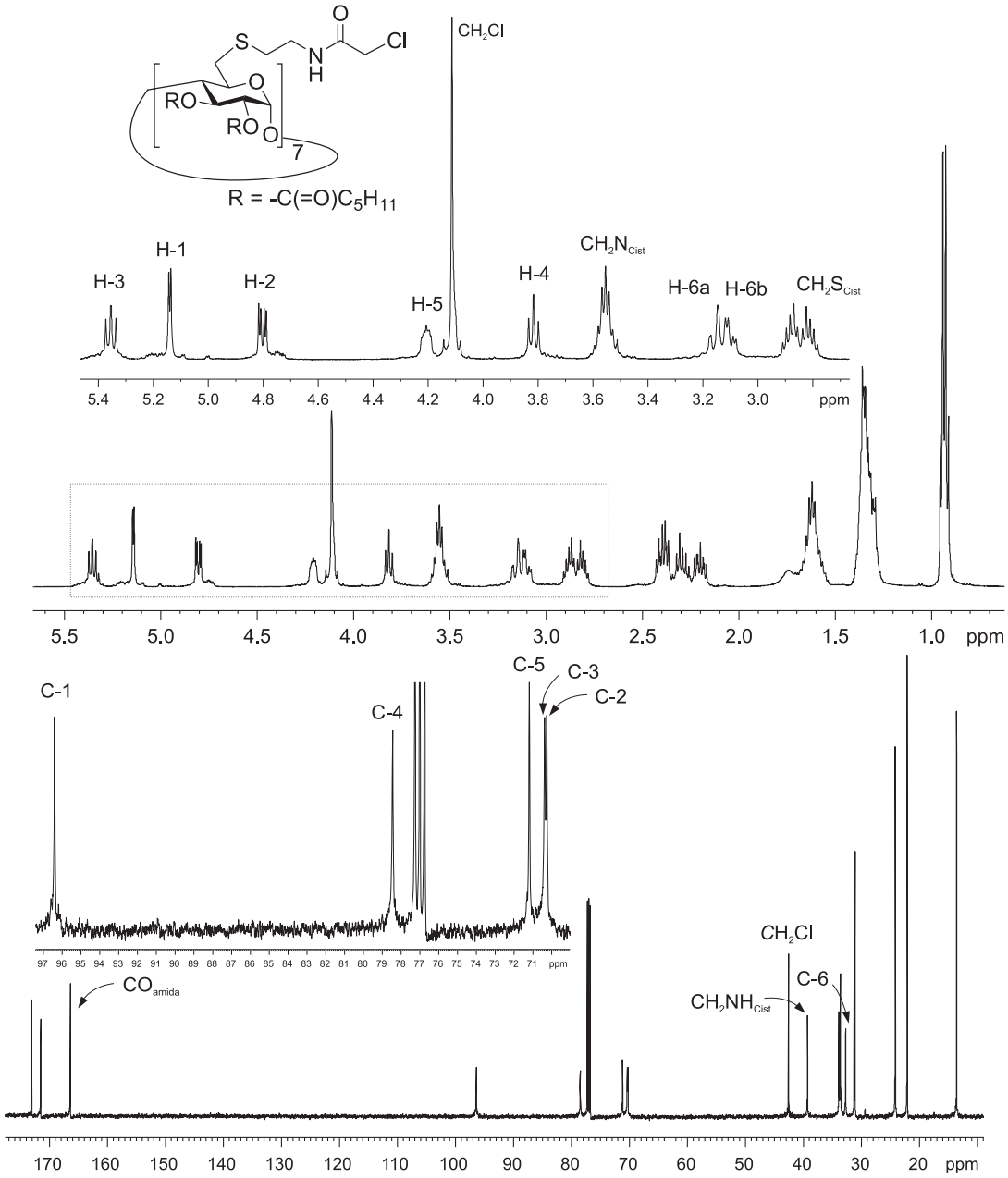

Figure S14. ^{1}H and ^{1} D TOCSY NMR spectra (500 MHz, $CDCl_3$,, 313 K) of **17**.

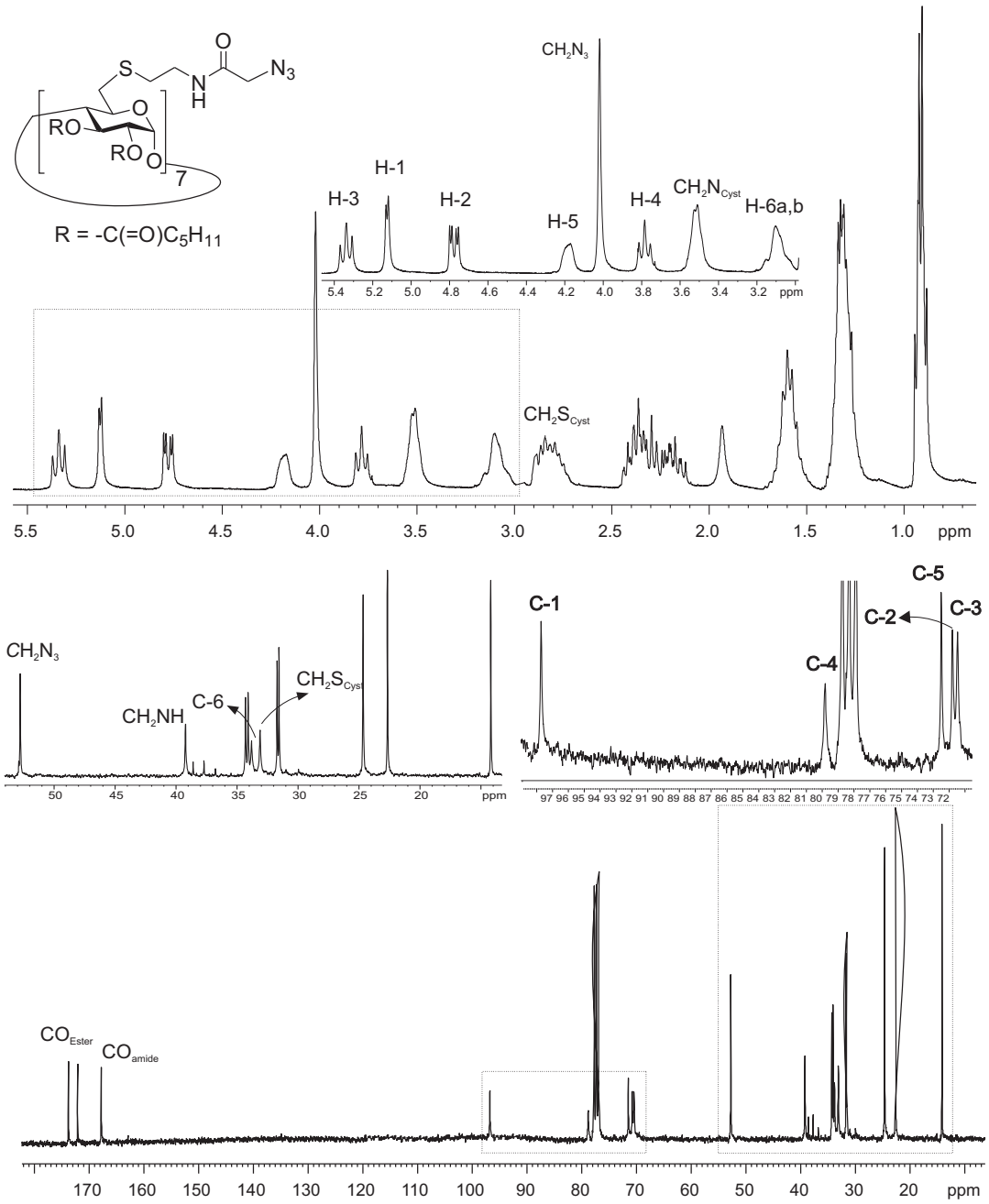

Figure S15. ^{1}H and ^{13}C NMR spectra (500 MHz, 75.5 MHz, $CDCl_3$) of **18**.

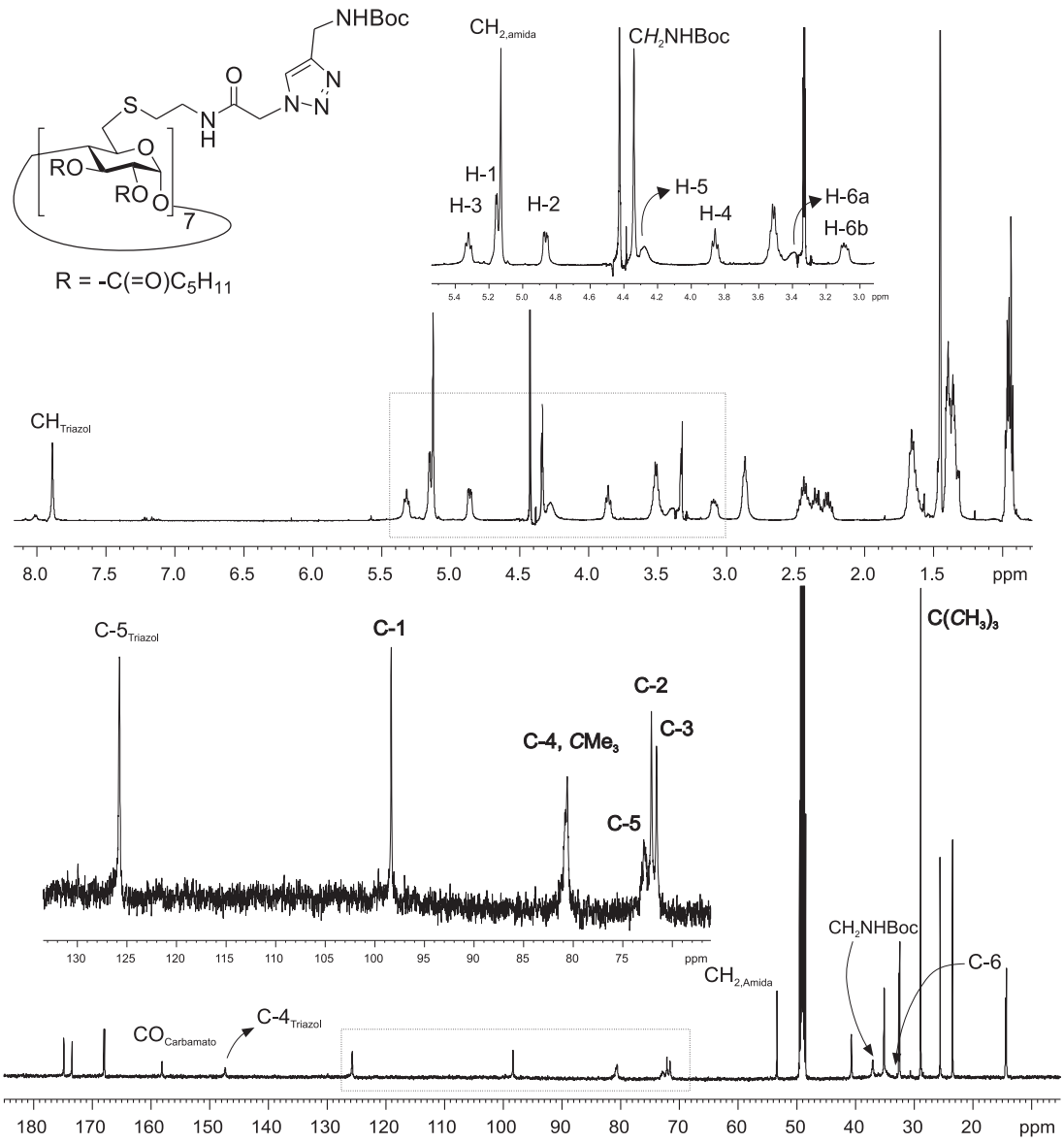

Figure S16. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD, 323 K) of **19**.

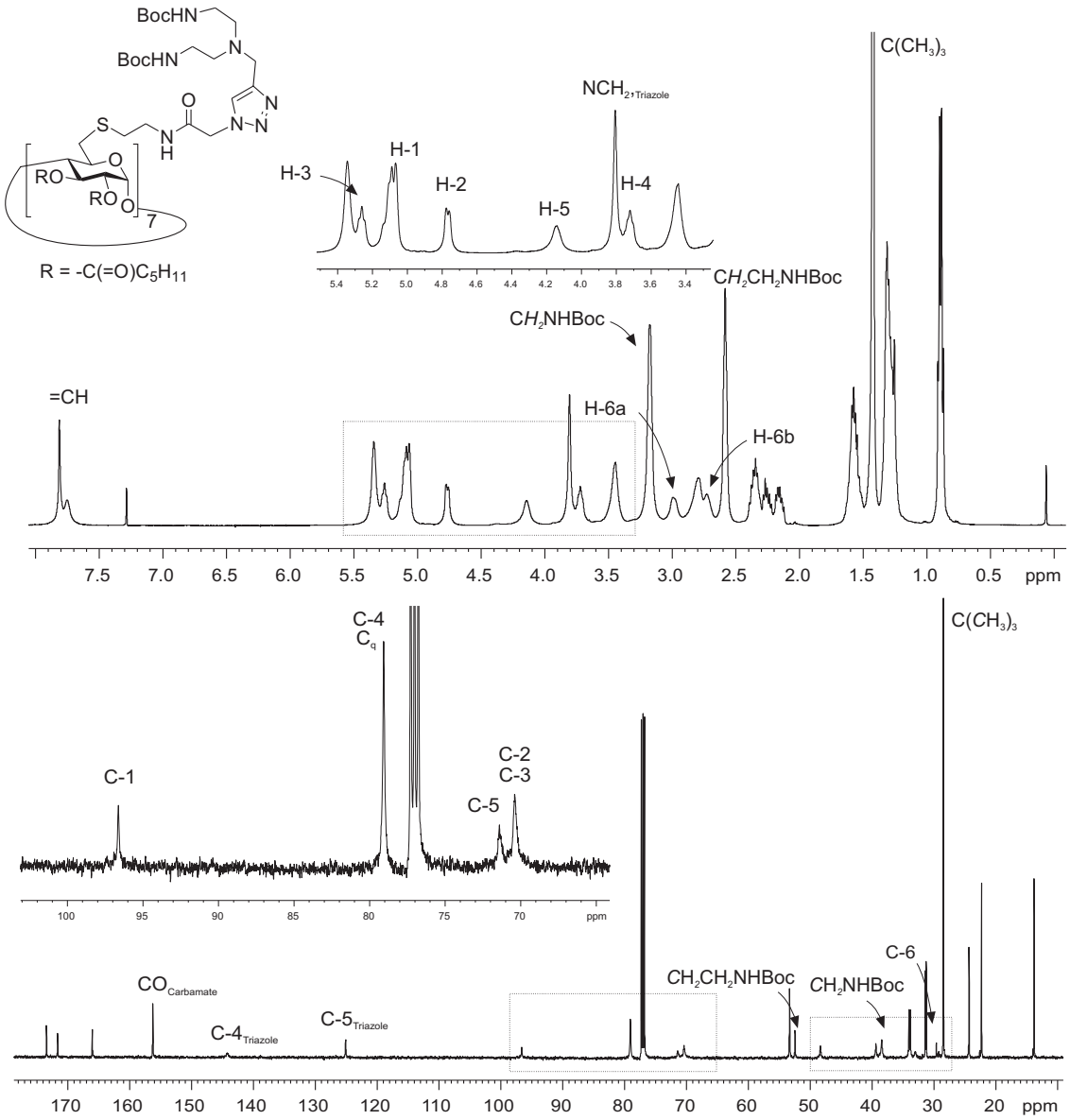

Figure S17. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, $CDCl_3$, 313 K) of **20**.

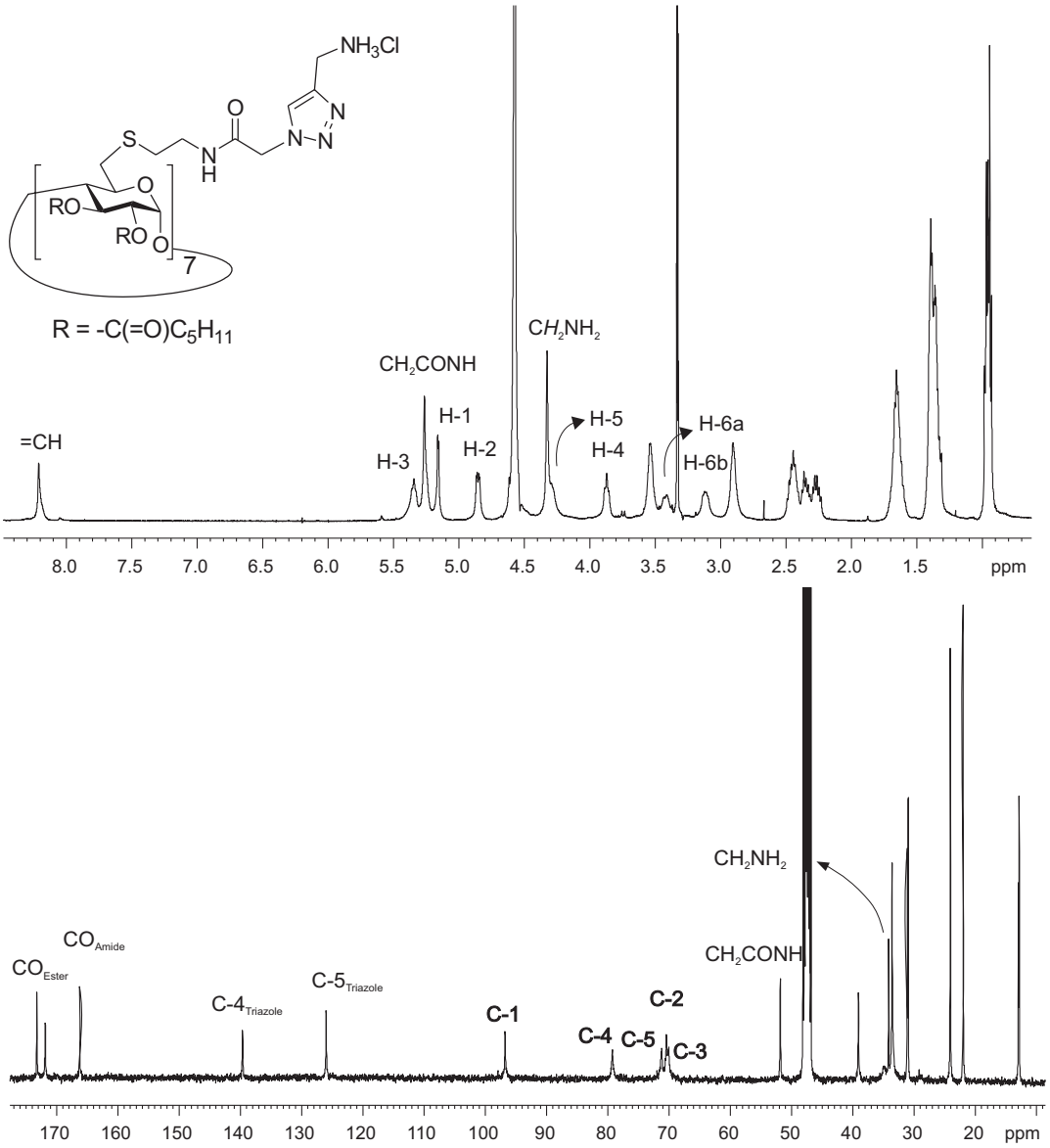

Figure S18. ^{1}H and ^{13}C NMR spectra (500 MHz, 100.3 MHz, CD_3OD, 323 K) of **21.**

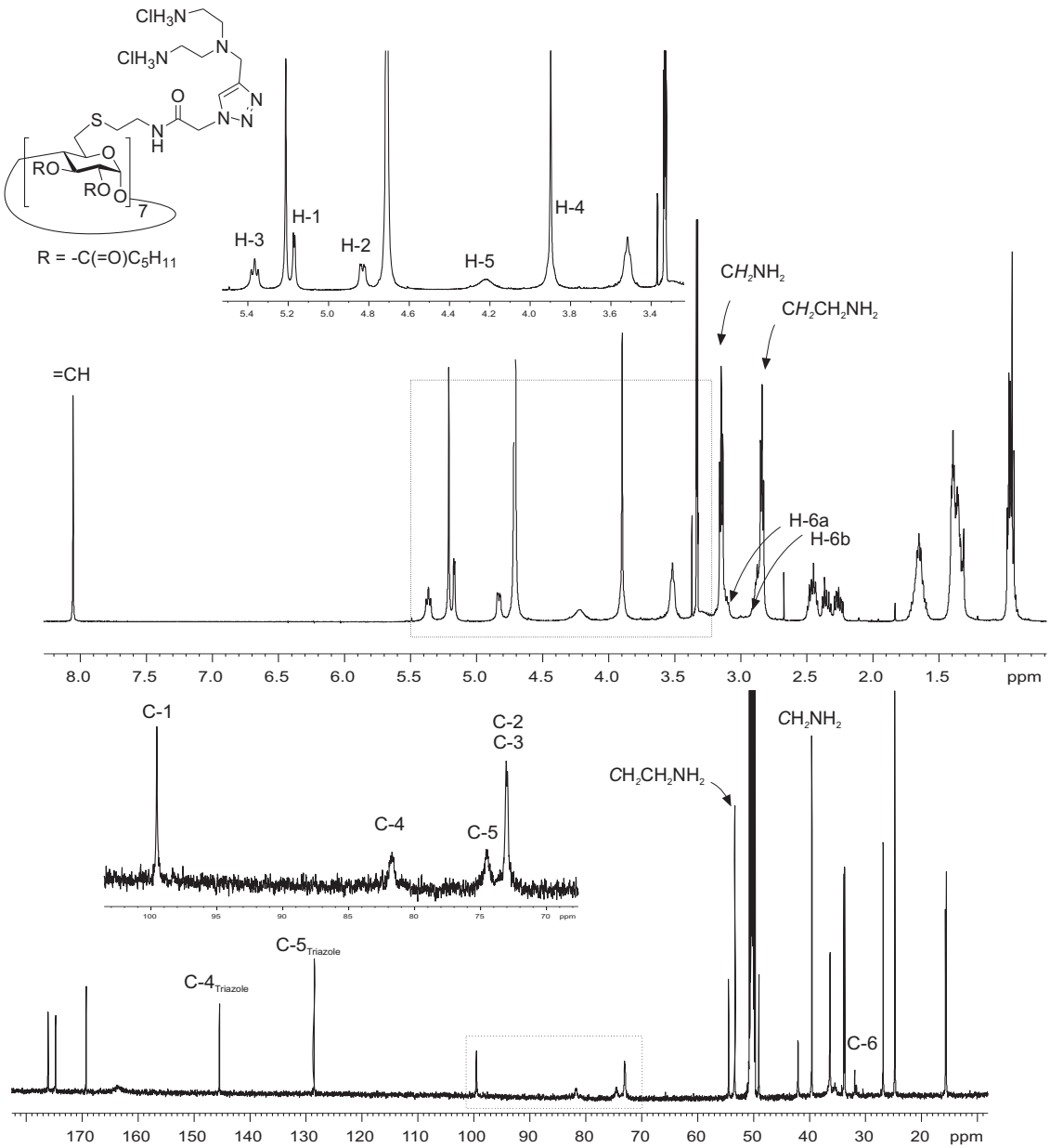

Figure S19. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD, 313 K) of **22**.

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