Electronic Supplementary Information

Selection of the biological activity of DNJ neoglycoconjugates through click length variation of the side chain

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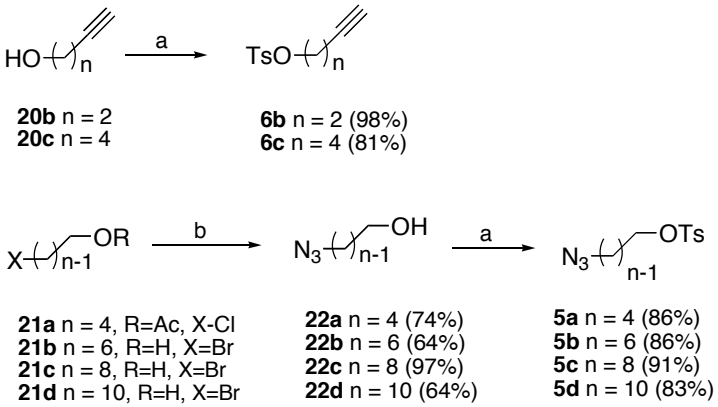
Synthesis of functionalised linkers

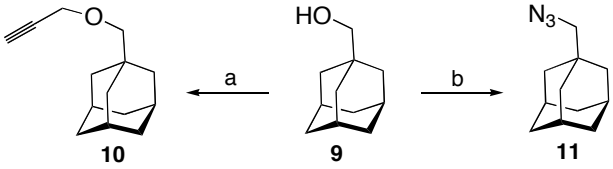
A series of linkers displaying different combinations of functionalities were synthesized. Starting from commercially available alcohols **20b** and **20c**, 4 and 6 carbon spacers **6b**[1] and **6c**[2] bearing a terminal alkyne on one end and a terminal tosylate on the other end were efficiently prepared through standard tosylation. The synthesis of tethers displaying a terminal azide was also achieved, in which commercial halogenoalcohols **21a-d** of various length were displaced with sodium azide to generate the azidoalcohols **22a-d.** Several aprotic solvents (acetone, acetonitrile, DMF) were screened for this reaction and DMF gave the best results. Subsequent tosylation furnished the tosyl azides **5a-d**[3] in good yield.[4]



Scheme S1. Reagents and conditions: a) TsCl, Et_3N, CH_2Cl_2, 0°C to RT; b) NaN_3, DMF, 80°C, 24 h; followed by K_2CO_3, CH_3OH, RT, 18h for **5a** only.

Synthesis of the adamantane precursors

Two adamantane precursors bearing either an *O*-propargyl moiety or a primary azide were used. Adamantanemethanol **9** was reacted with propargyl bromide to yield the propargylic derivative **10**[5] (58% yield). Tosylation of alcohol **9** followed by azide displacement gave the azidomethyl adamantane **11**[6] in 74% yield over 2 steps.

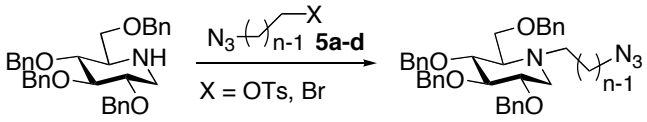


Scheme S2. Reagents and conditions: a) NaH, propargyl bromide, DMF, 0°C to RT, 18 h, 58%; b) TsCl, Et_3N, CH_2Cl_2, 0°C to RT then NaN_3, DMF, 80°C, 74% over two steps.

N-alkylation of DNJ

Planning to apply in the forthcoming step the click glycosylation conditions we have developed for unprotected sugars,[7] we first attempted the direct *N*-alkylation of unprotected DNJ **1** thus avoiding protecting groups manipulation. *N*-Alkylation through reductive amination with linear linkers bearing a terminal aldehyde[8] was first studied but afforded the desired *N*-alkyl DNJ derivatives only in modest yield, especially with longer chains.[9] Disappointing results were also obtained when switching to the *N*-alkylation of DNJ with alkyl tosylates or bromides.[10] These results prompted us to abandon this route and concentrate instead on the *N*-functionalisation of tetra-*O*-benzyl-deoxynojirimycin **4**. *N*-Alkylation of **4** with alkyl chains bearing an alkyne (propargyl bromide **6a** and **6b-6c**) or an azide moiety (**5a-**

5d) was examined and reaction parameters including temperature, solvent (acetonitrile, DMF) and base (NaH, Na_2CO_3, K_2CO_3) were screened (Table S1). Heating to 90°C was necessary for a good conversion and potassium carbonate proved to be the best base to perform the *N*-alkylation either starting from alkyl bromides or alkyl tosylates. However, since the preparation of bromides required one more synthetic step, the combination of alkyl tosylate and potassium carbonate was chosen as optimal conditions in which acetonitrile gave better results than DMF. In the case of the azido-containing chains **5a-5d**, chain length governed the yield for *N*-alkylation, lower conversion being observed for longer chains. This can be tentatively explained by the increased lipophilicity of the chain compared to the relative hydrophilicity of the DNJ derivative **4**.

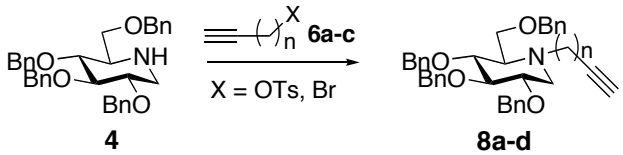


Scheme S3.

n	***N*-alkyl DNJ**	X	Base	Solvent	T (°C)	Yield (%)
4	**7a**	OTs	$NaHCO_3$	DMF	90	57
4	**7a**	Br	K_2CO_3	CH_3CN	Reflux	79
4	**7a**	OTs	K_2CO_3	CH_3CN	Reflux	80
6	**7b**	OTs	$NaHCO_3$	DMF	90	45
6	**7b**	Br	K_2CO_3	CH_3CN	Reflux	65
6	**7b**	OTs	K_2CO_3	CH_3CN	Reflux	69
8	**7c**	OTs	$NaHCO_3$	DMF	90	50
8	**7c**	Br	K_2CO_3	CH_3CN	Reflux	69
8	**7c**	OTs	K_2CO_3	CH_3CN	Reflux	70
10	**7d**	OTs	$NaHCO_3$	DMF	90	45
10	**7d**	Br	K_2CO_3	CH_3CN	Reflux	55
10	**7d**	OTs	K_2CO_3	CH_3CN	Reflux	55

Table S1 Yields and conditions for the alkylation of **4** with *azido* linkers

A similar study was conducted with alkyne derivatives (Table S2) showing that the use of potassium carbonate in refluxing acetonitrile gave slightly better yields than sodium hydrogenocarbonate in DMF (90°C).



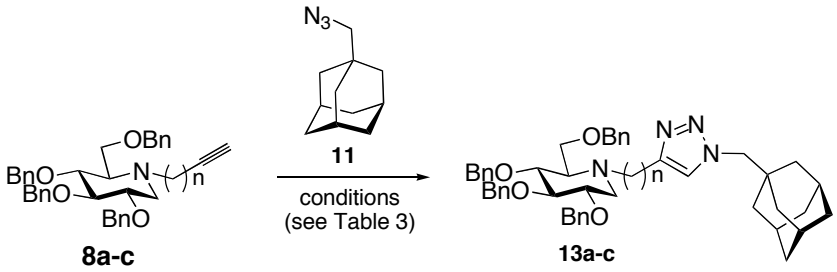
Scheme S4.

n	Product	X	Base	Solvent	T (°C)	Yield (%)
1	**8a**	Br	$NaHCO_3$	DMF	90	89
1	**8a**	Br	K_2CO_3	CH_3CN	Reflux	92
2	**8b**	OTs	$NaHCO_3$	DMF	90	64
2	**8b**	OTs	K_2CO_3	CH_3CN	Reflux	71
4	**8c**	OTs	$NaHCO_3$	DMF	90	61
4	**8c**	OTs	K_2CO_3	CH_3CN	Reflux	74

Table S2 Yields and conditions for the alkylation of **4** with *alkyne* linkers

Click coupling

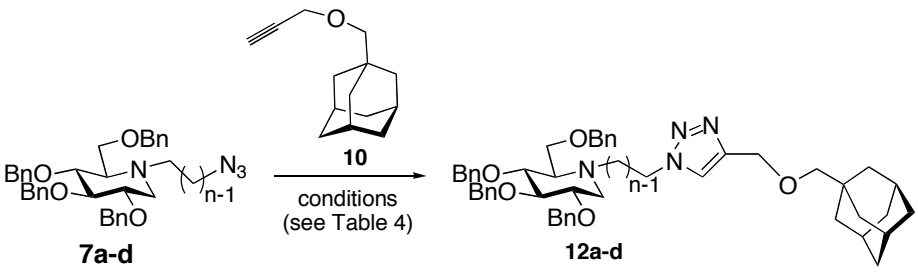
Copper-catalyzed Azide-Alkyne cycloaddition (CuAAC)[11] between the *N*-alkylated DNJ derivatives **7a-d** and **8a-c** and the adamantane partners **10** and **11** was then examined. Optimised click reaction conditions that were previously established in our laboratory regarding temperature (room temperature), substrate concentration (0.25M), the amounts of copper sulfate (5%) and sodium ascorbate (15 mol%)[7] were used in a dichloromethane/water (1:1) solvent mixture which proved better than the extensively used aqueous alcohol systems (tables S3 and S4).[12] Noteworthily, we observed that reaction in a screw-cap sealed tube allowed optimum conversion as undesired reoxidation of copper I to copper II was otherwise probably taking place during the reaction. These conditions were applied to the 1,3-cycloaddition of all complementary adamantane and DNJ partners.



Scheme S5.

n	Product	Screw-caped tube	Solvent (1:1)	Yield (%)
1	**13a**	No	CH_2Cl_2/H_2O	91
1	**13a**	Yes	CH_2Cl_2/H_2O	100
2	**13b**	No	CH_3OH/H_2O	71
2	**13b**	No	CH_2Cl_2/H_2O	77
2	**13b**	Yes	CH_2Cl_2/H_2O	99
4	**13c**	No	CH_2Cl_2/H_2O	94
4	**13c**	Yes	CH_2Cl_2/H_2O	99

Table S3 Yields and conditions for the cycloaddition of **8a-c** with **11**.



Scheme S6.

n	Product	Screw-caped tube	Solvent (1:1)	Yield (%)
4	**12a**	No	CH_2Cl_2/H_2O	64
4	**12a**	Yes	CH_2Cl_2/H_2O	86
6	**12b**	No	CH_2Cl_2/H_2O	64
6	**12b**	Yes	CH_2Cl_2/H_2O	93
8	**12c**	No	CH_2Cl_2/H_2O	77
8	**12c**	Yes	CH_2Cl_2/H_2O	97
10	**12d**	No	CH_2Cl_2/H_2O	62
10	**12d**	Yes	CH_2Cl_2/H_2O	96

Table S4 Yields and conditions for the cycloaddition of **7a-d** with **10**.

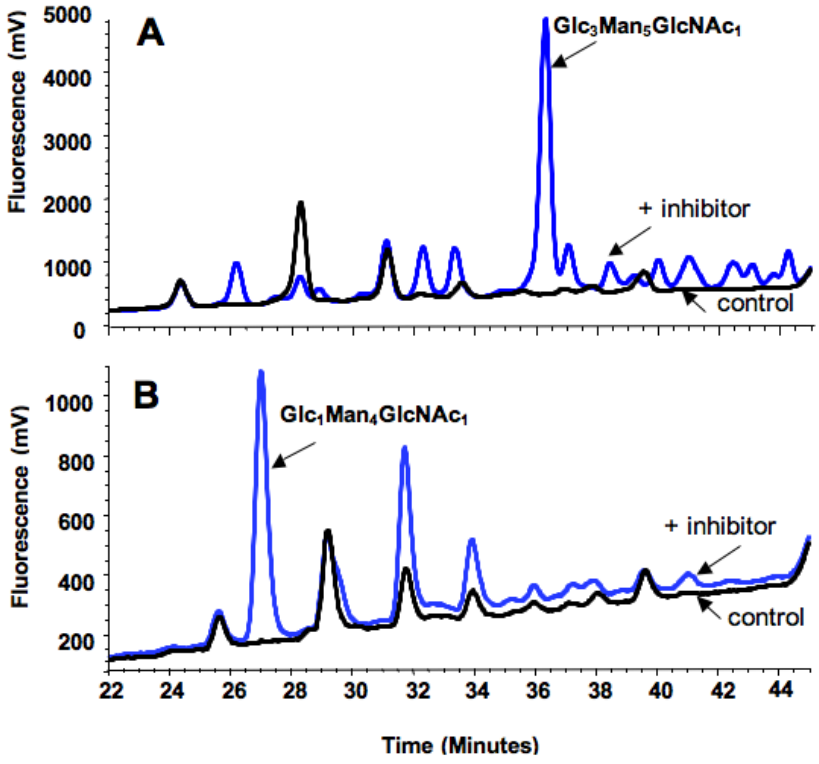


Figure S1. NP-HPLC analysis of FOS in HL60 cells following inhibitor treatment. Compounds were incubated for 24h with cells, FOS extracted, fluorescently labelled and separated by NP-HPLC as described in the text. A, representative profile for our compounds showing the $Glc_3Man_5GlcNAc_1$ species that was measured in response to high concentrations of inhibitor effecting glucosidase I activity; B, profile of our compounds following low concentration treatment (5 μM) showing major species $Glc_1Man_4GlcNAc_1$ as a result of measured glucosidase II inhibition. Data for peak area were used to construct Table 2 in the paper.

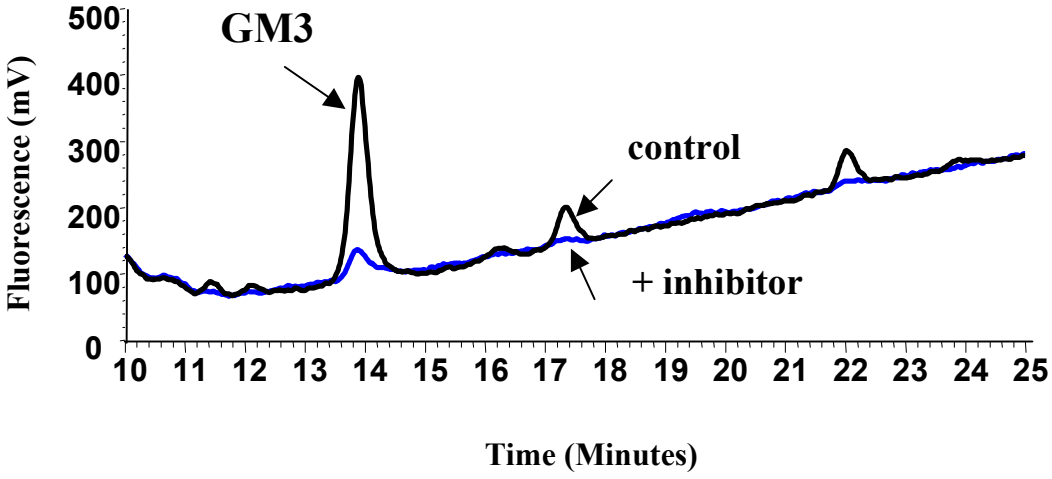


Figure S2. NP-HPLC analysis of GSL in HL60 cells following inhibitor treatment. Compounds were incubated for 3 days with cells, GSL extracted, ceramide glycanase treated and the released oligosaccharide fluorescently labelled and separated by NP-HPLC as described in the text. Representative profile showing the reduction in the major species,

ganglioside GM3, following treatment with our compounds. Data for peak area were used to construct Figure 4 in the paper.

Cytotoxicity assay

HL60 cells were seeded at densities of 500 cells / well in 96-well plates in 200 µl of supplemented media containing 0.01 % DMSO as control, and concentrations up to 200 µM of each compound (dissolved in DMSO) added for 3 days. Cell viability was assessed in triplicate using the Cell Titer-96 AQueous cellular proliferation assay kit according to manufacturer's (Promega, Southampton, UK) instructions.

Compound	CC_{50} (µM)
AMP-DNJ	>200
*N*B-DNJ	>200
14	>200
15	>200
16	>200
17	>200
18	>200
19	65 ± 8
20	19 ± 3

Table S5. Cytotoxicity of compounds in HL60 cells over a 3-day incubation time.

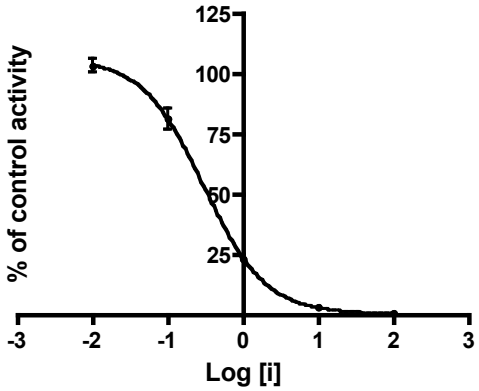


Figure S3. Representative Hill Slope plot of ß-glucocerebrosidase inhibition.

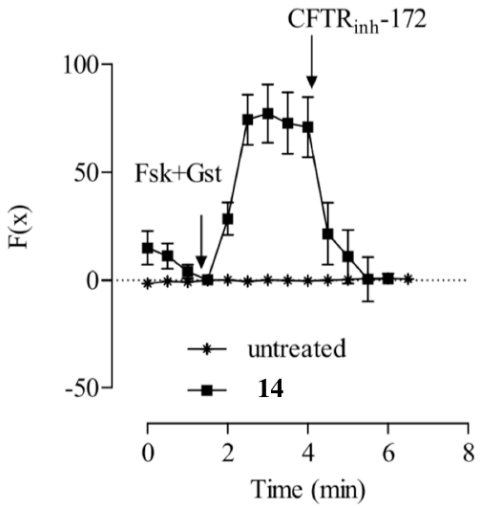


Figure S4. Functional evaluation of F508del-CFTR by DiSBAC2(3) assay in CFKM4 cells treated with 100µM of compounds during 2h. Examples of typical time courses obtained with untreated or **14**-treated cells. Data represent the mean (± SEM) of the relative fluorescence collected from 12 cells of a field. Mixture of forskolin (10 µM) + genistein (30 µM) is used to activate CFTR. CFTRinh-172 (10 µM) is used to inhibit CFTR.

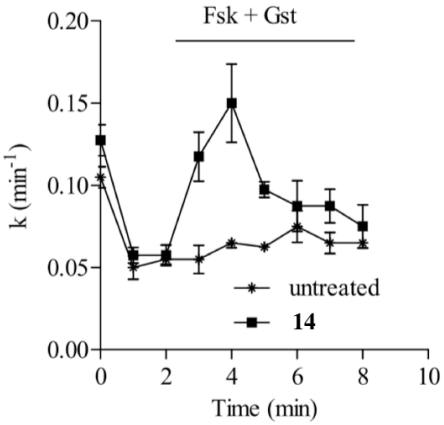


Figure S5. F508del-CFTR activity was assayed with iodide efflux technique in presence of forskolin (10 µM) + genistein (30 µM). Examples of iodide efflux curves as function of time on CF-KM4 cells treated or not by **14** (100 µM, 2h). Results are expressed as mean ± SEM of 4 experiments.

References and notes

1 K.M. Erixon, C.L. Dabalos; F.J. Leeper, *Org. Biomol. Chem.* **2008**, *6*, 3561-3572.

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4 In the case of the four carbon chain **5a**, a slightly more elaborate route, involving protection of the hydroxyl as an acetate (**22a**), followed by deprotection after the introduction the azido group, proved necessary due to competing THF formation when nucleophilic substitution was attempted directly from 4-chloro-butan-1-ol.

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8 These aldehydes were obtained by straightforward PCC oxydation of the corresponding alcohols and in refluxing dichloromethane.

9 Modest yields have often been reported for the direct reductive alkylation of DNJ. For an example, *see*: (a) I. Lundt, A.J. Steiner, A.E. Stütz, C. A. Tarling, S. Ully, S.G. Withers, T. M. Wrodnigg, *Bioorg. Med. Chem. Lett.* **2006**, *14*, 1737-1742; For a discussion on the optimization of the initially modest yielding alkylation of DNJ in the synthesis of AMP-DNJ, *see*: (b) T. Wennekes, B. Lang, M. Leeman, G.A. van der Marel, E. Smits, M. Weber, J. van Wiltenburg, M. Wolberg, J.M.F.G. Aerts, H.S. Overkleeft, *Org. Process Res. Dev.* **2008**, *12*, 414-423; For a recent report of the efficient reductive alkylation of DNJ with various aldehydes, *see*: (c) A.J. Rawlings, H. Lomas, A.W. Pilling, M.J.–R. Lee, D.S. Alonzi, J.S.S. Rountree, S.F. Jenkinson, G.W.J. Fleet, R.A. Dwek, J.H. Jones, T.D. Butters, *ChemBioChem* **2009**, *10*, 1101-1105.

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12 This solvent system has been described to promote CuAAC between organo-soluble azides and alkynes with more efficiency than water/alcohol mixtures: B.-Y. Lee, S.R. Park, H.B. Jeon, K.S. Kim, *Tetrahedron Lett.* **2006**, *47*, 5105-5109.

List of NMR characterisations,

Compound **7a**, ^{1}H, ^{13}C, COSY, HSQC

Compound **7b**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **7c**, ^{1}H, ^{13}C, COSY, DEPT, HSQC

Compound **7d**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **8a**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **8b**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **8c**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **12a**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **12b**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **12c**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **12d**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **13a**, ^{1}H, ^{13}C, COSY, HSQC, HMBC

Compound **13b**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **13c**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

Compound **14**, ^{1}H, ^{13}C, COSY, HSQC

Compound **15**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

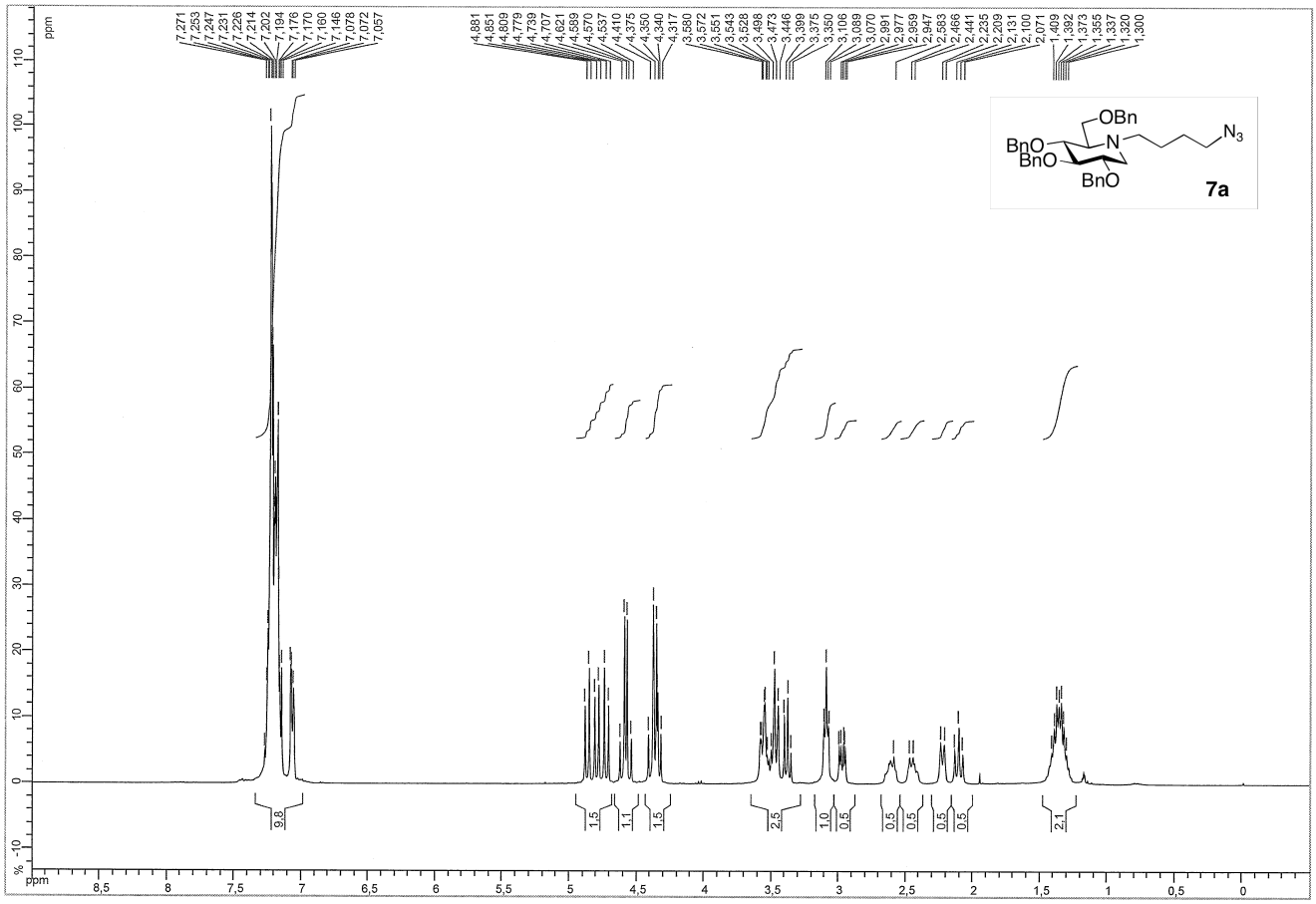
Compound **16**, ^{1}H, ^{13}C, COSY, DEPT, HSQC

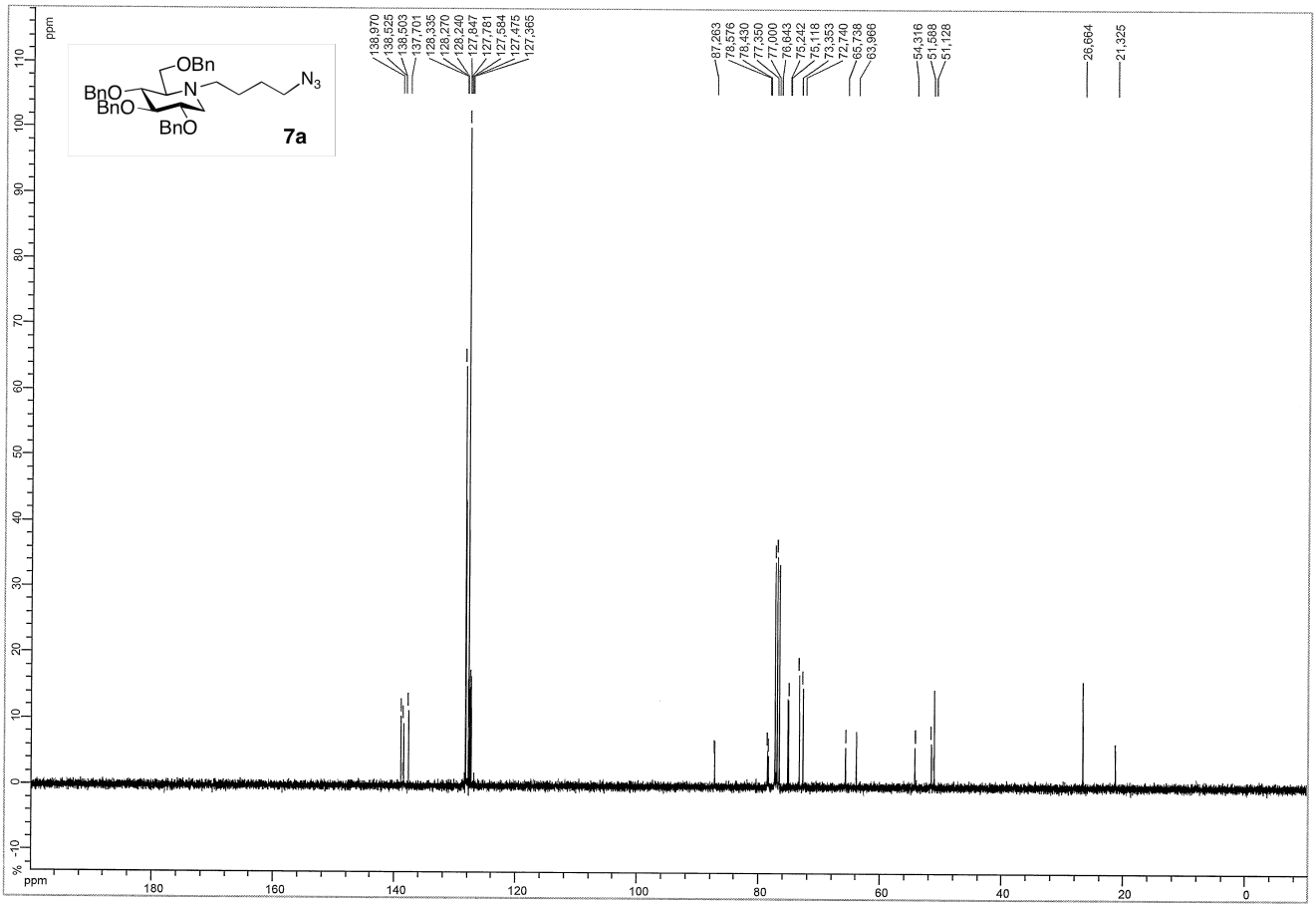
Compound **17**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

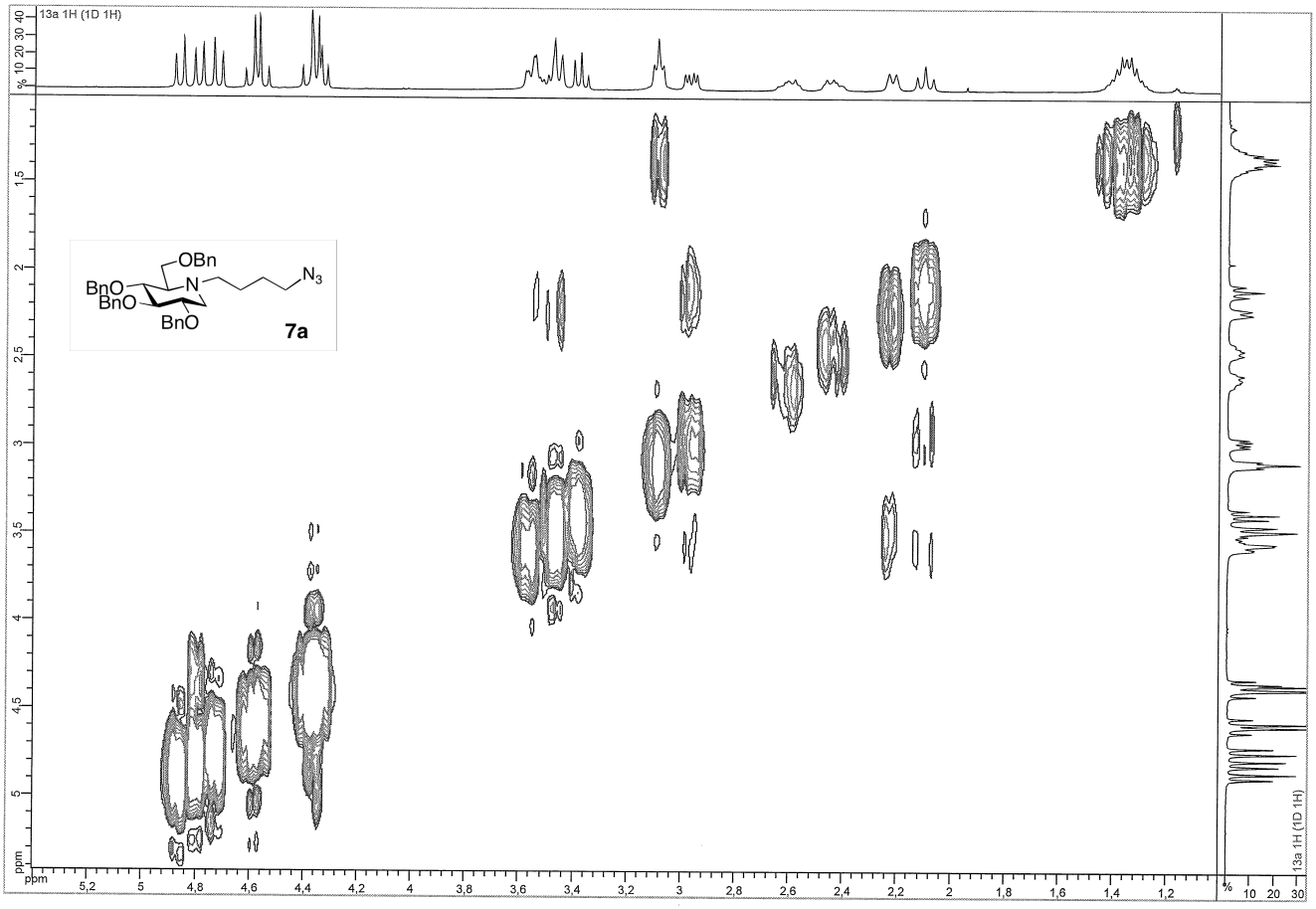
Compound **18**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

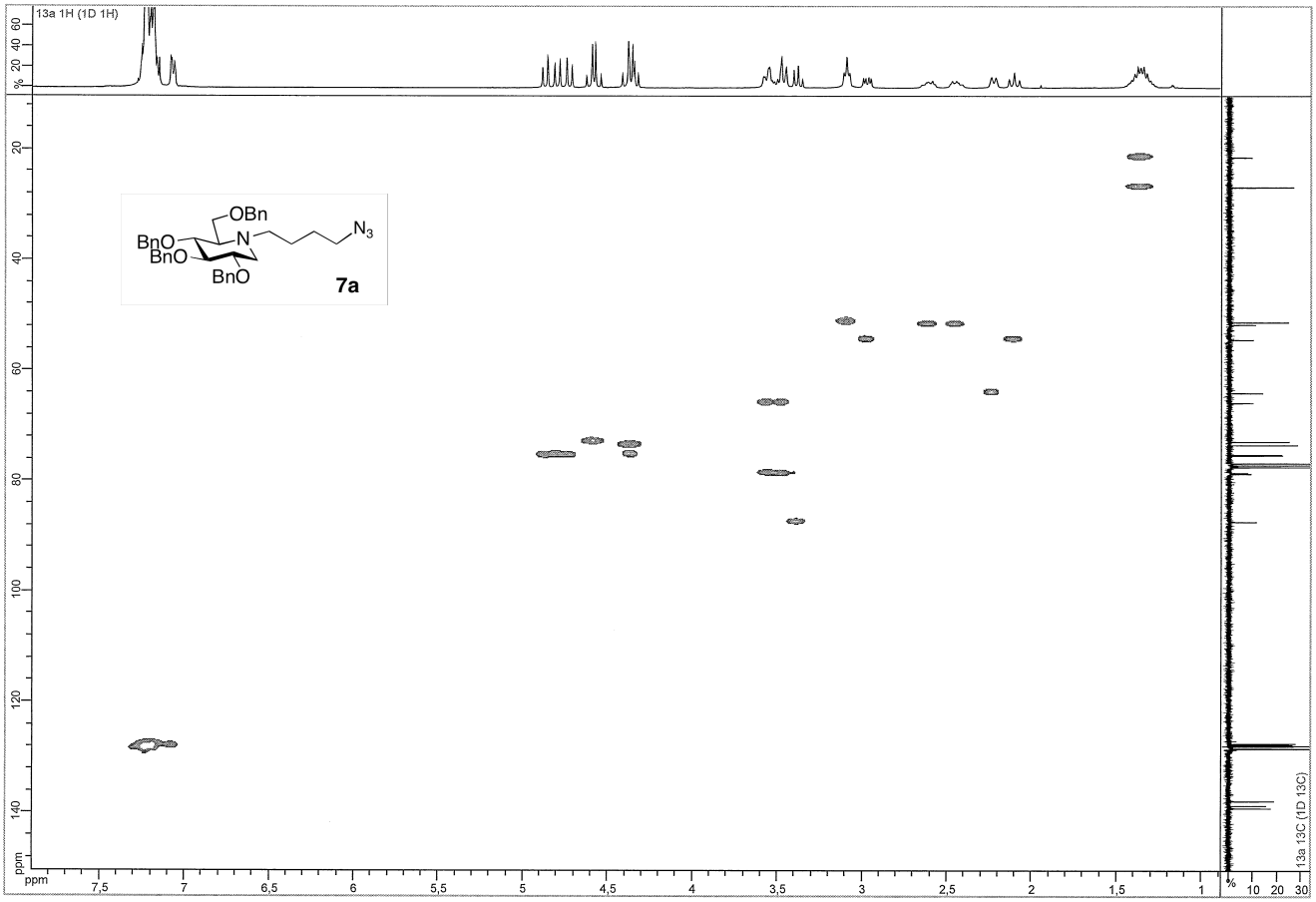
Compound **19**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

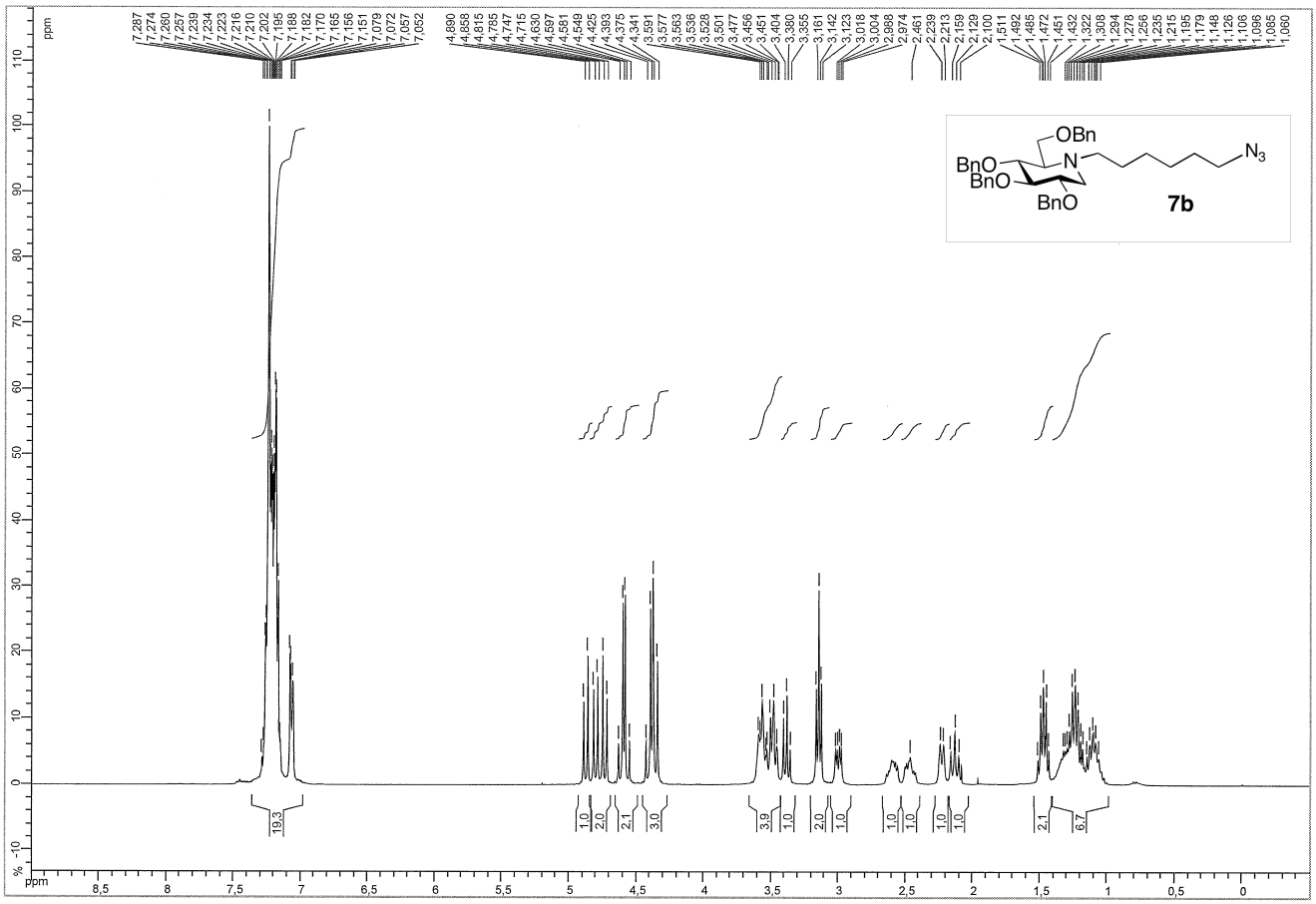
Compound **20**, ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC

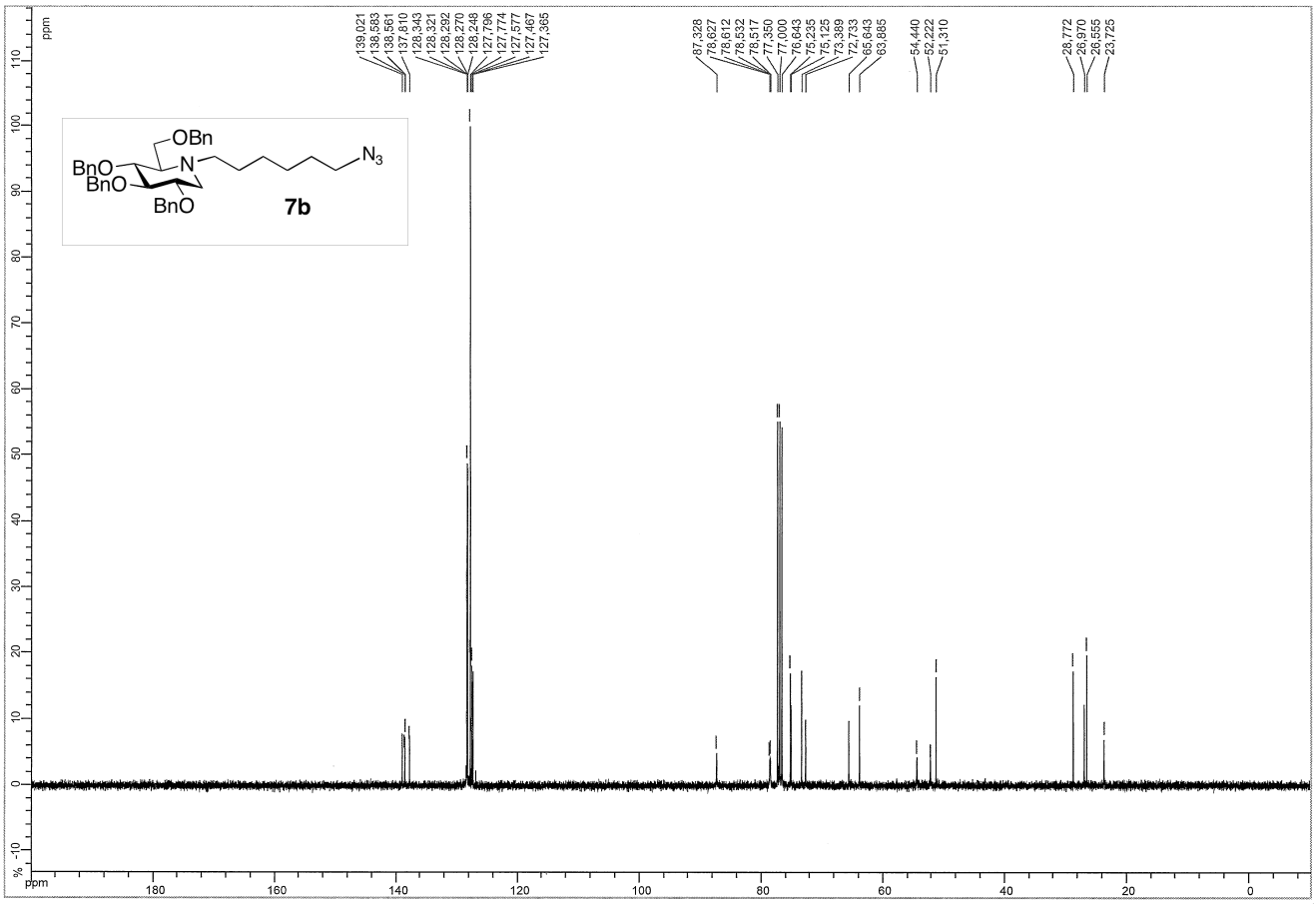


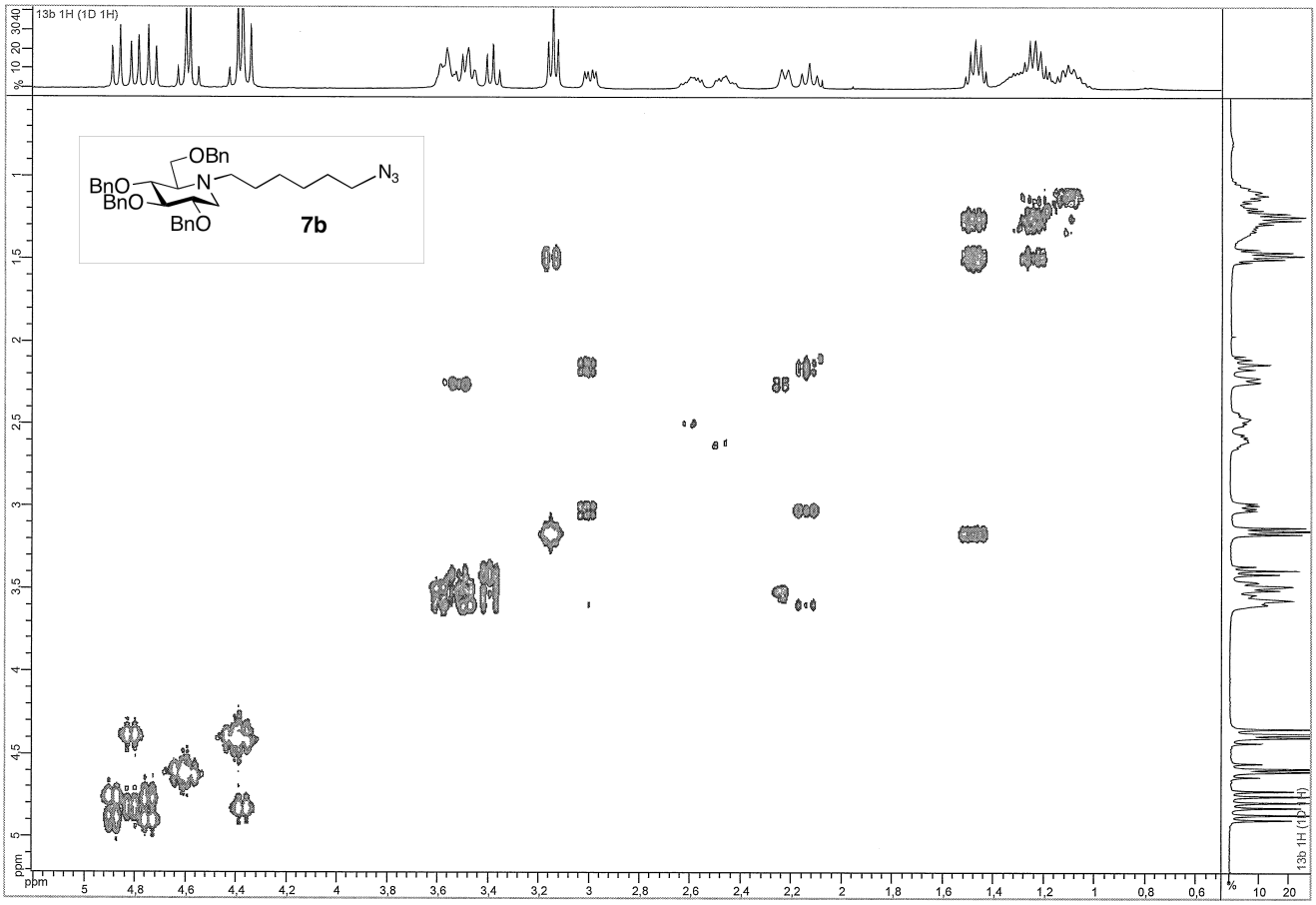


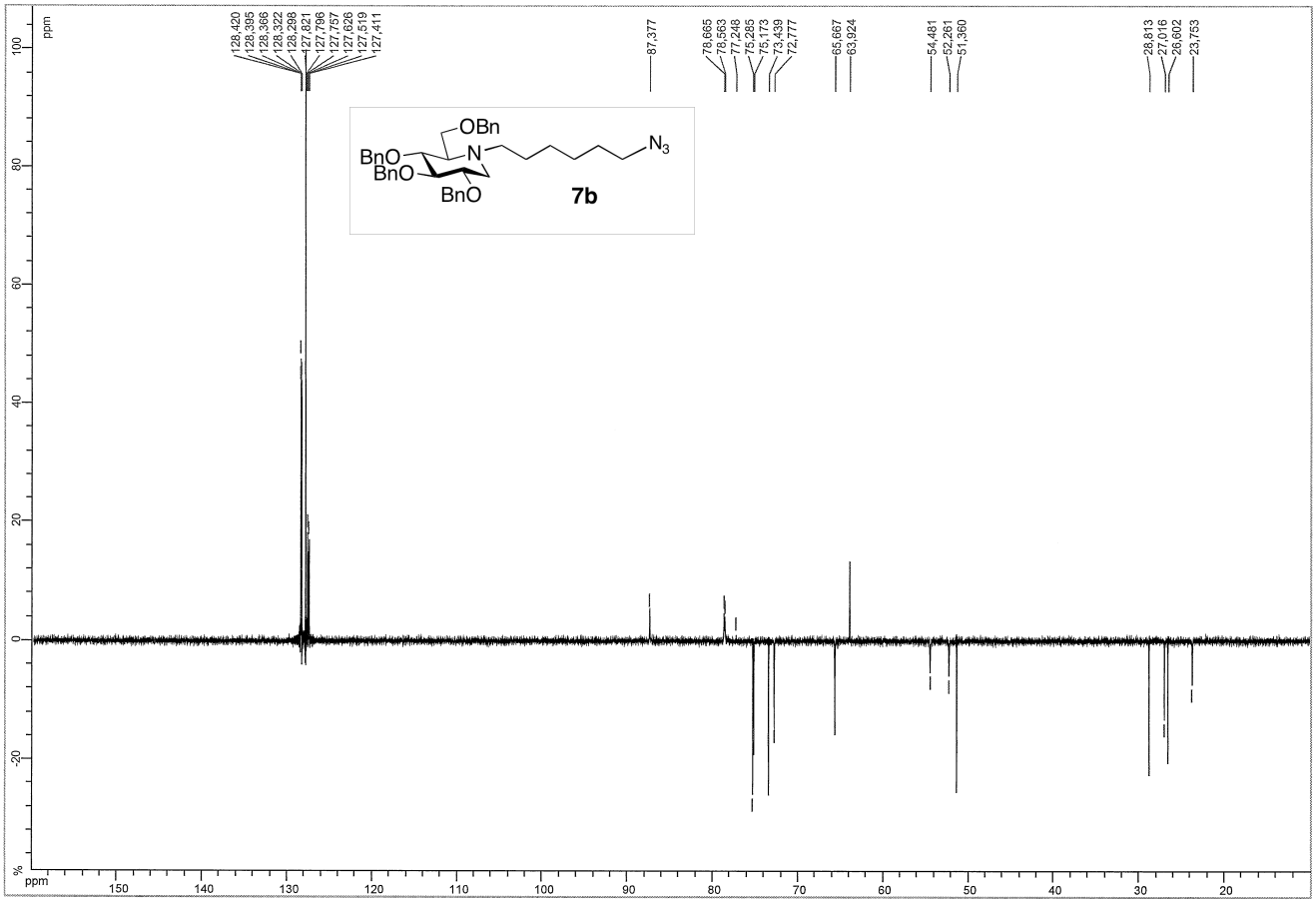


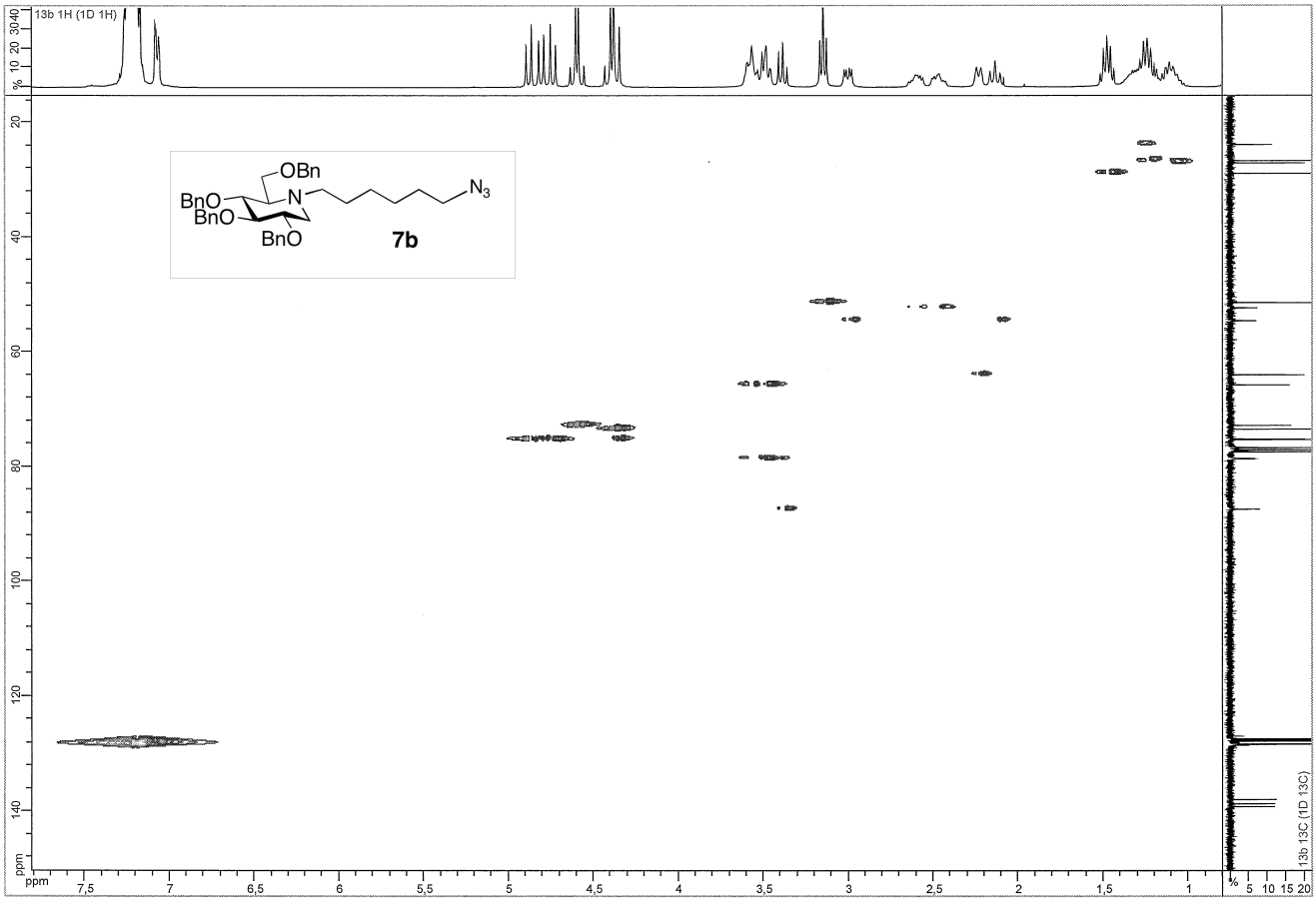


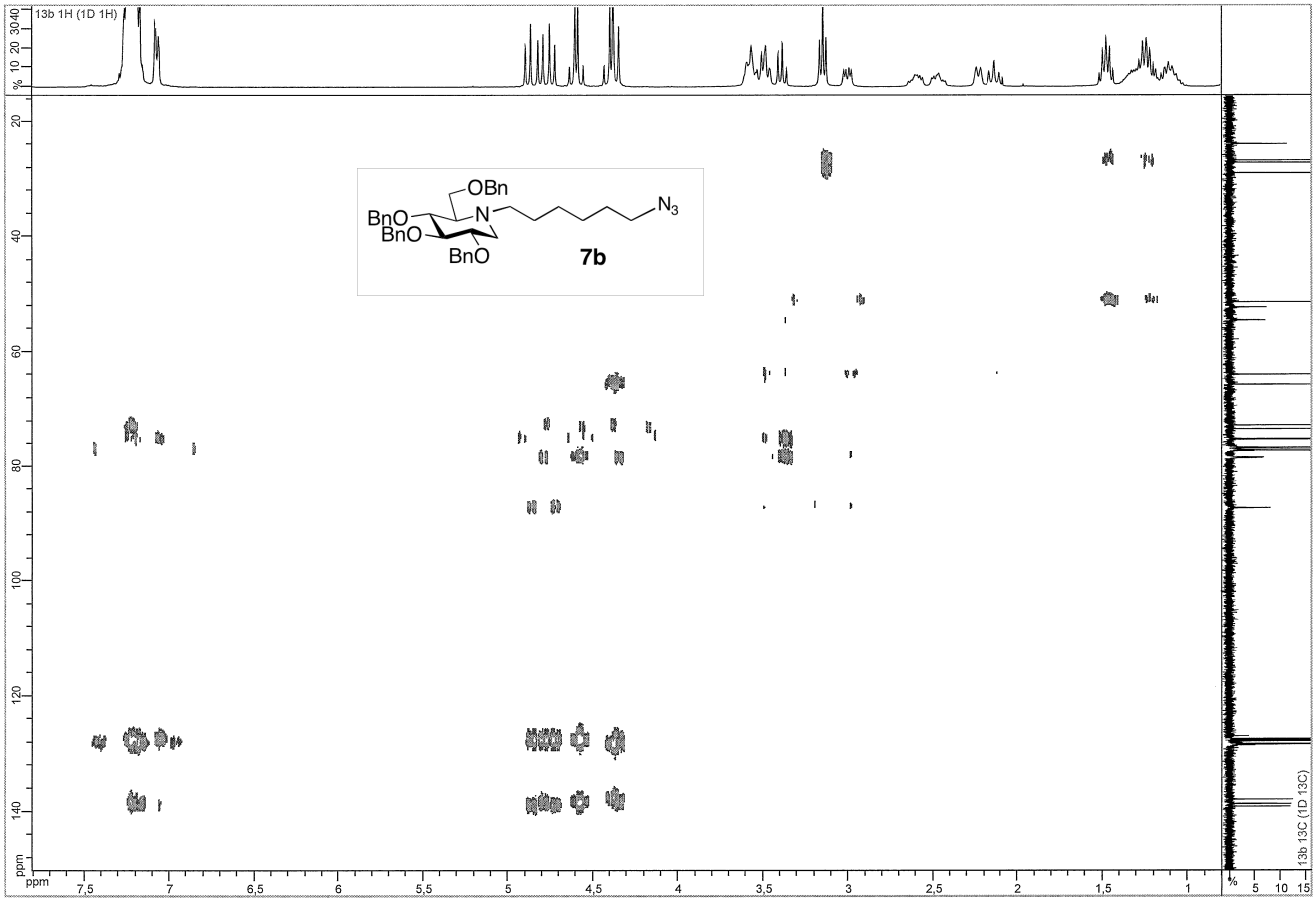


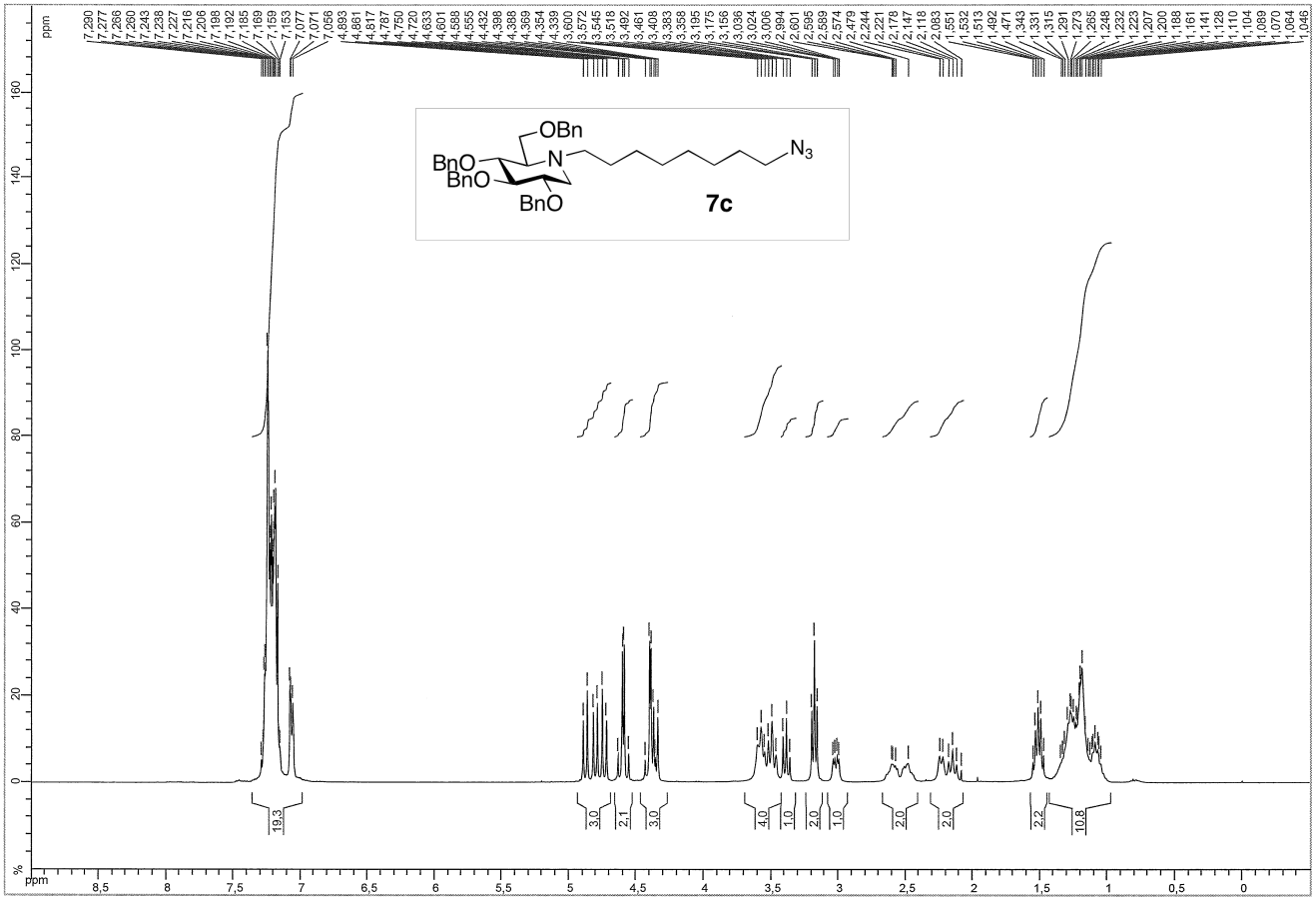


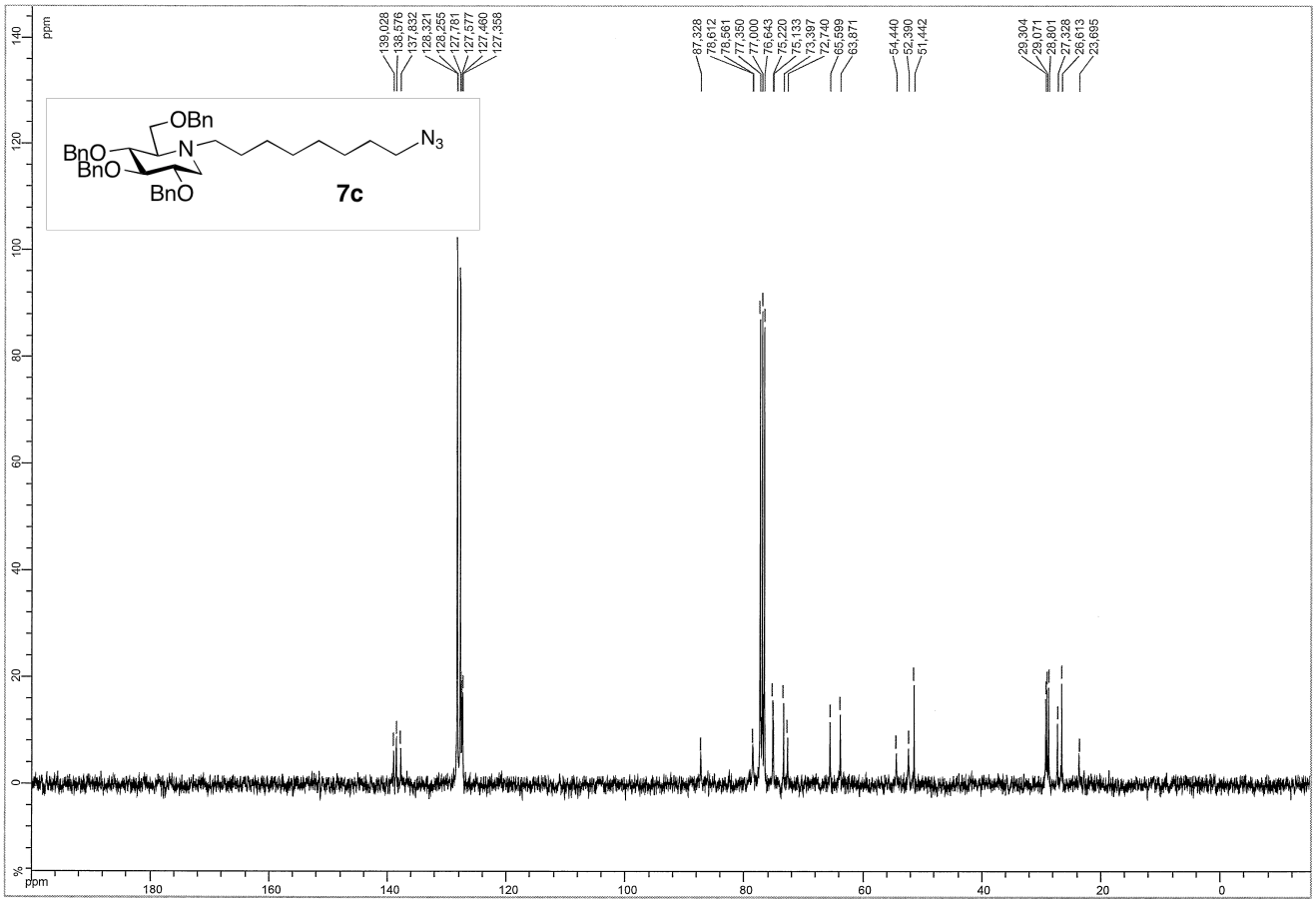


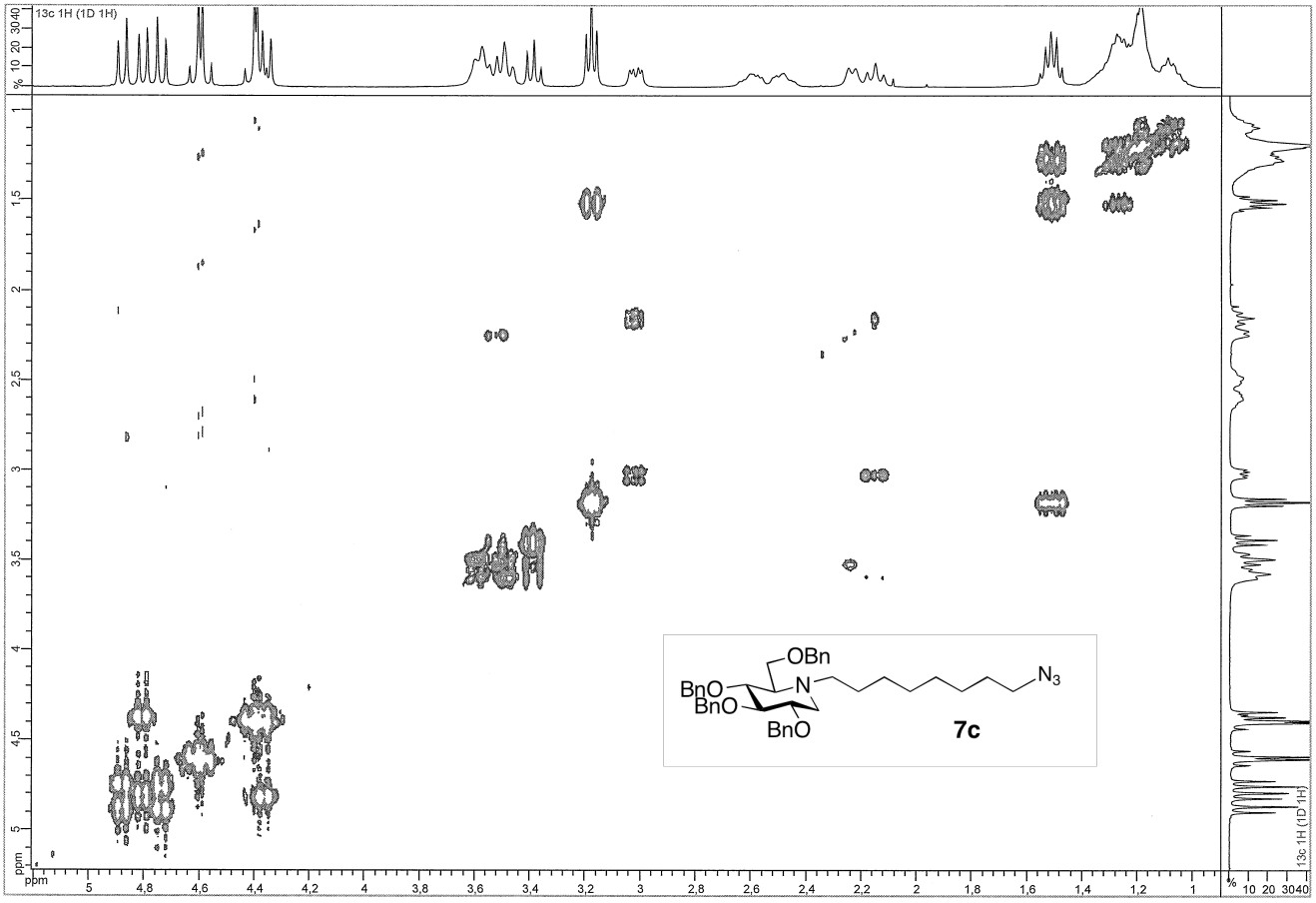


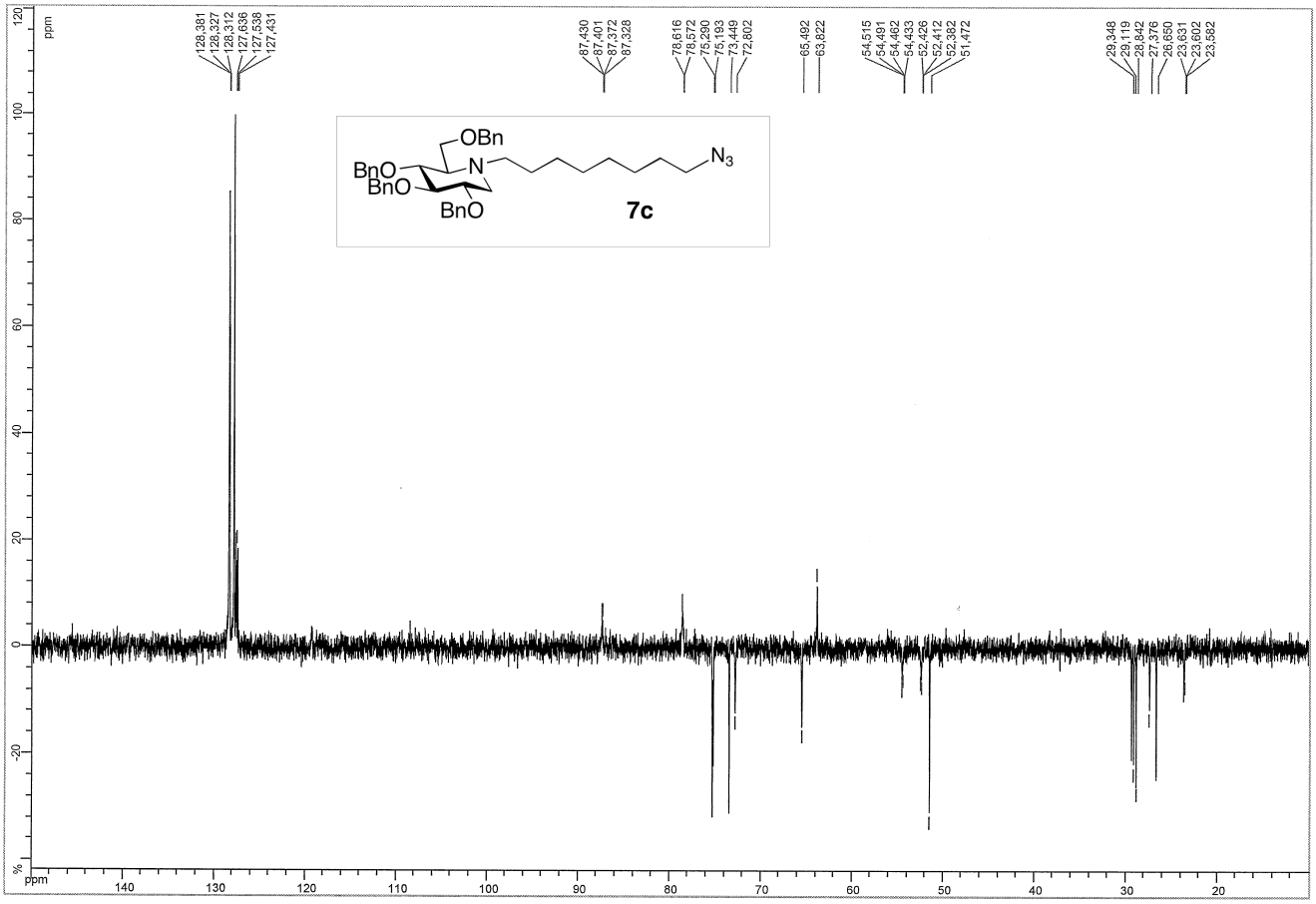


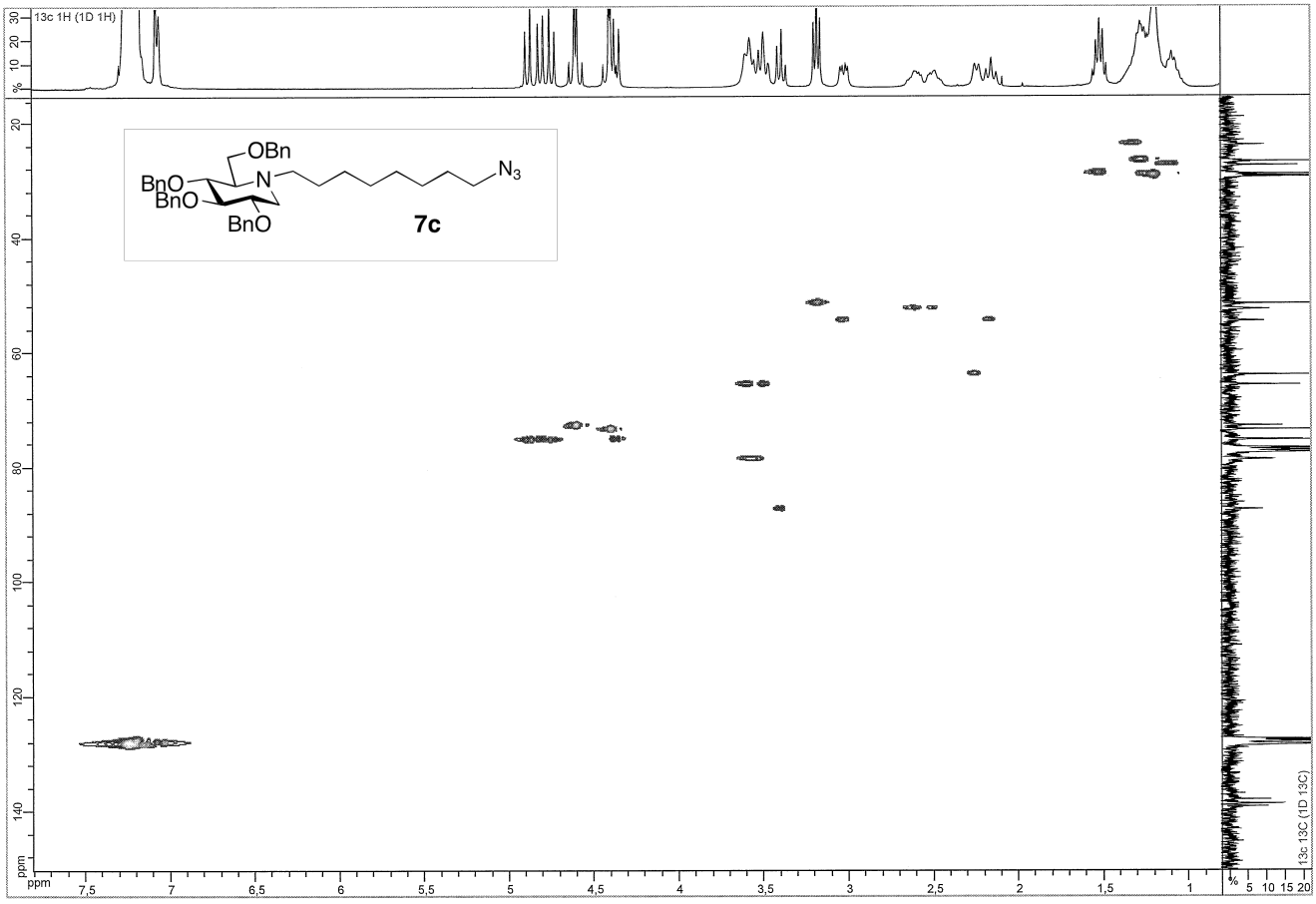


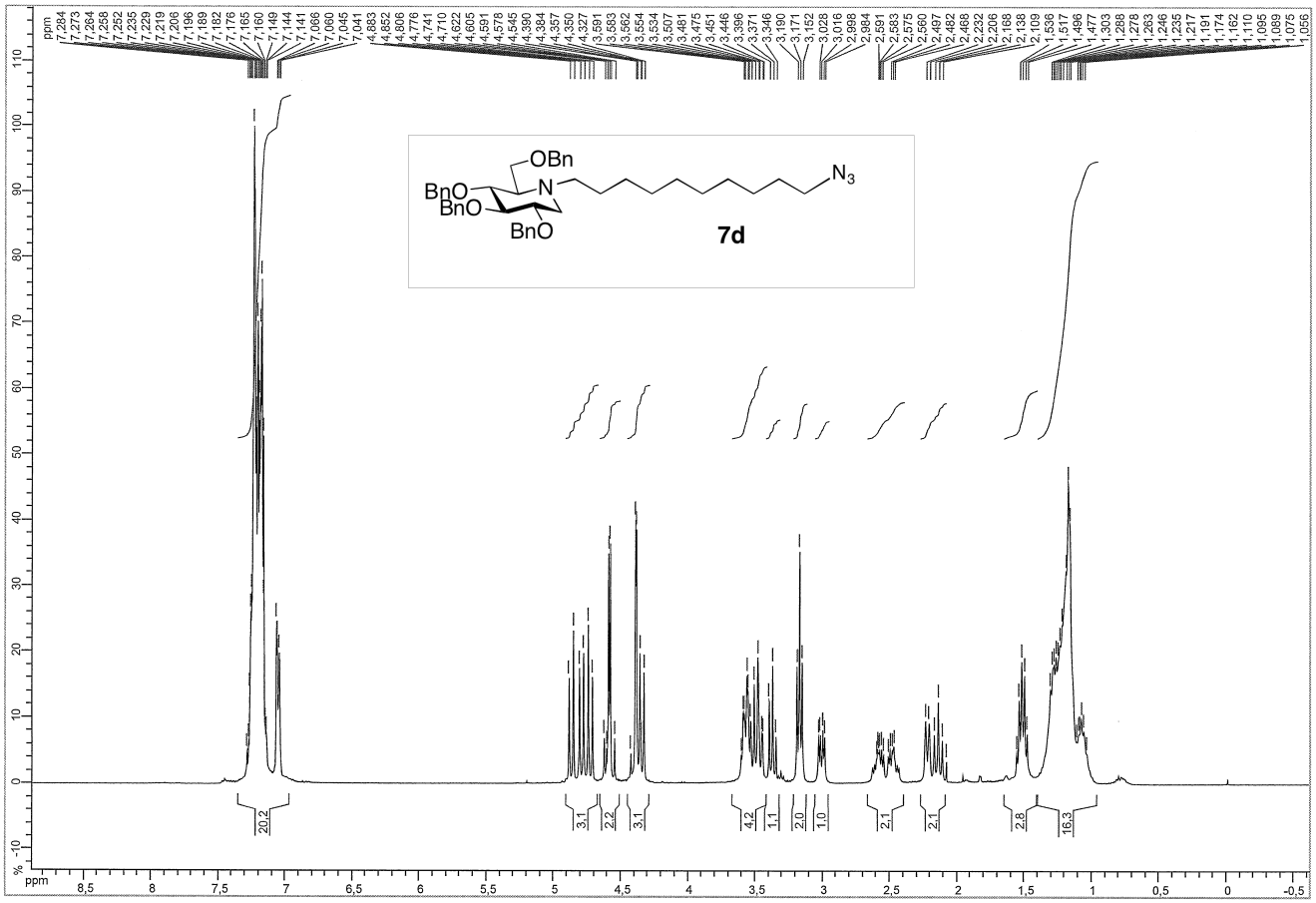


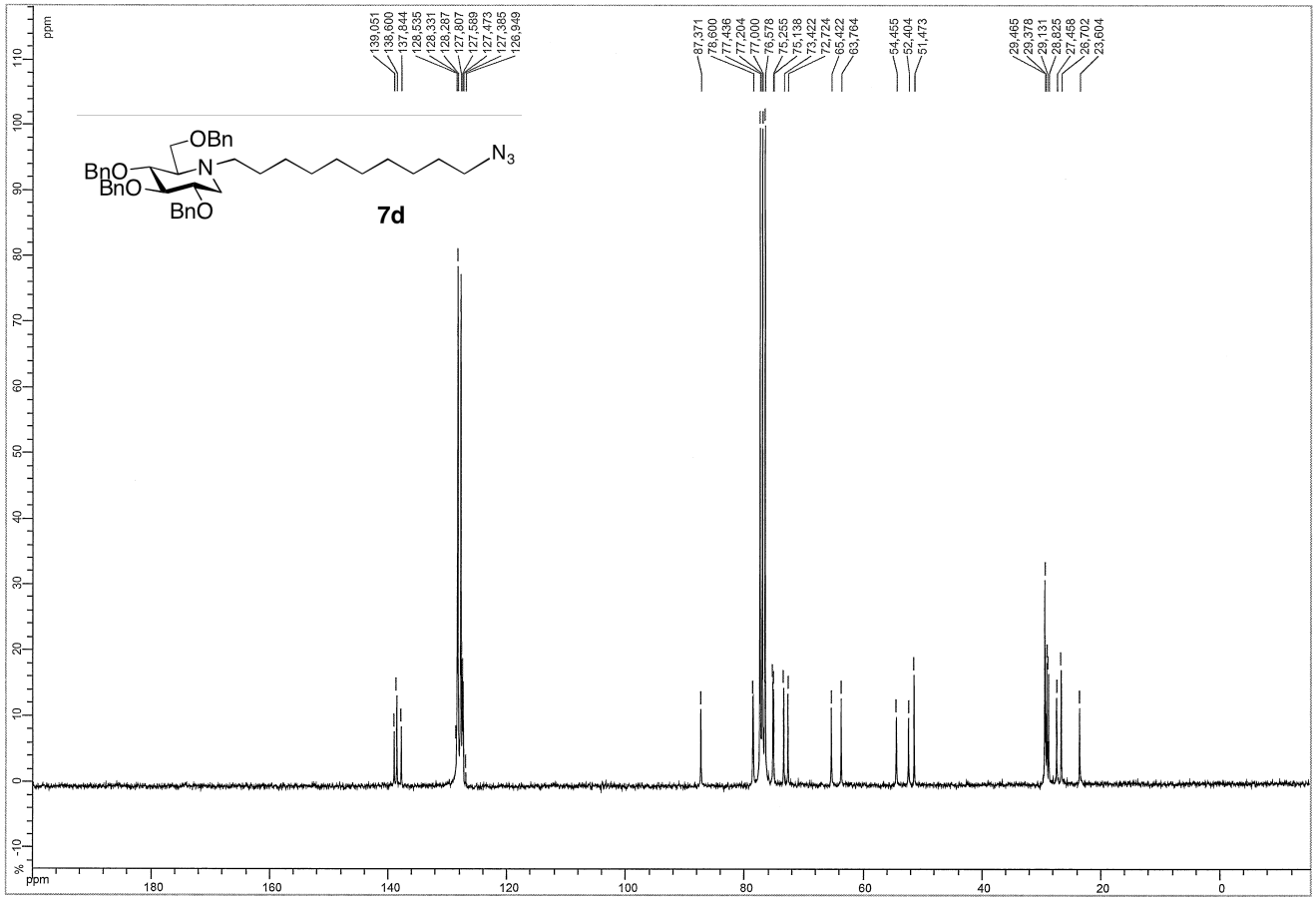


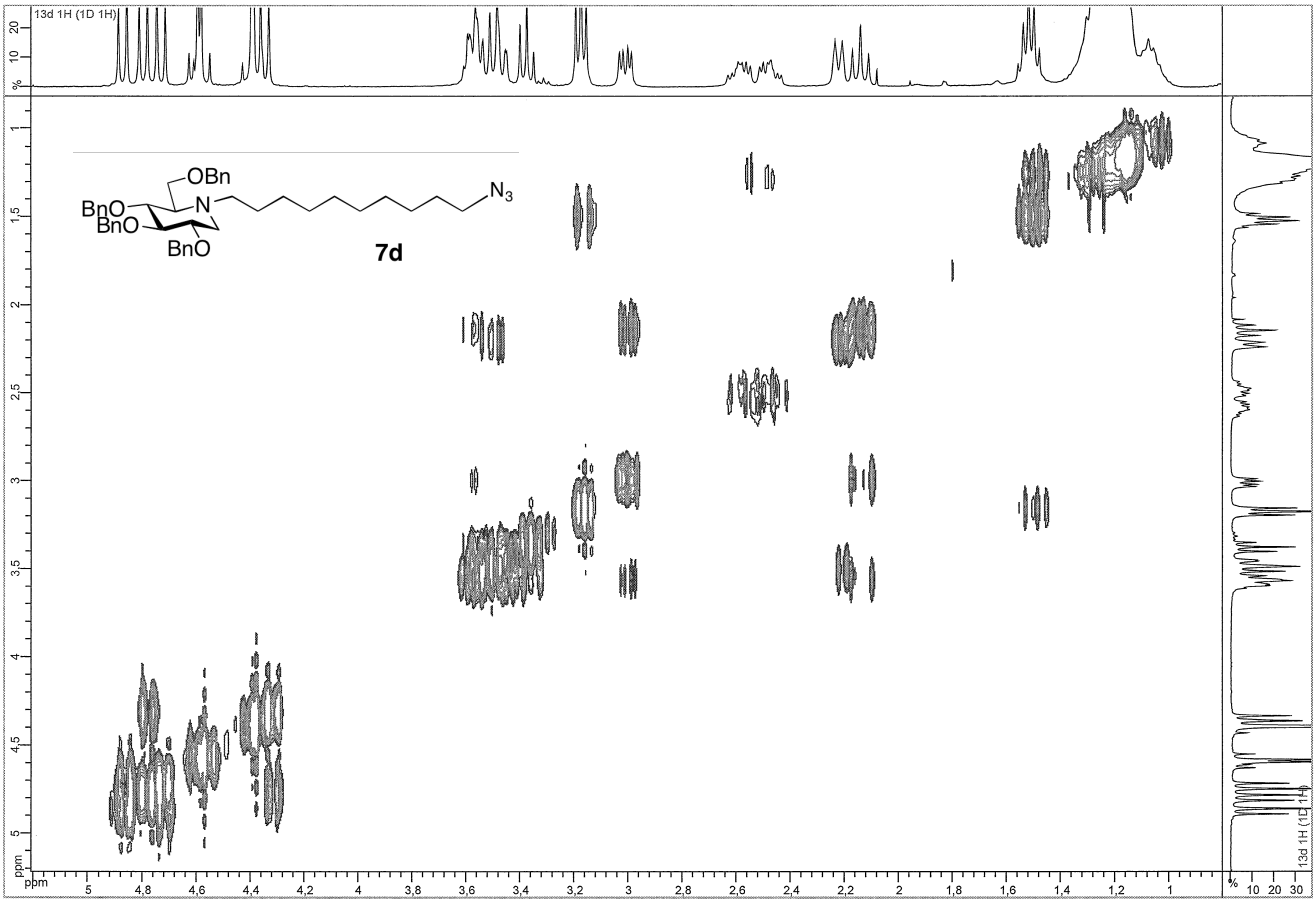


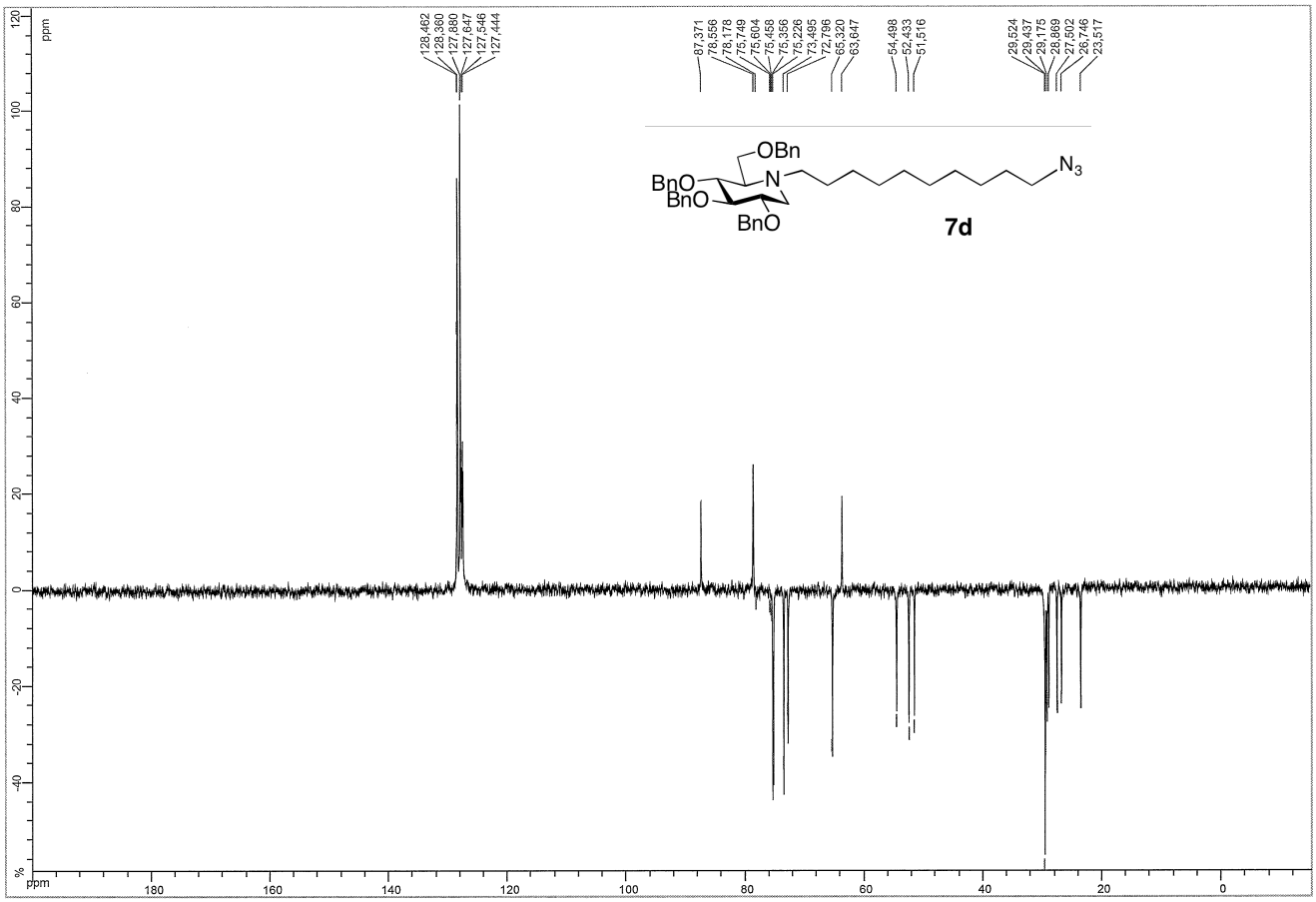


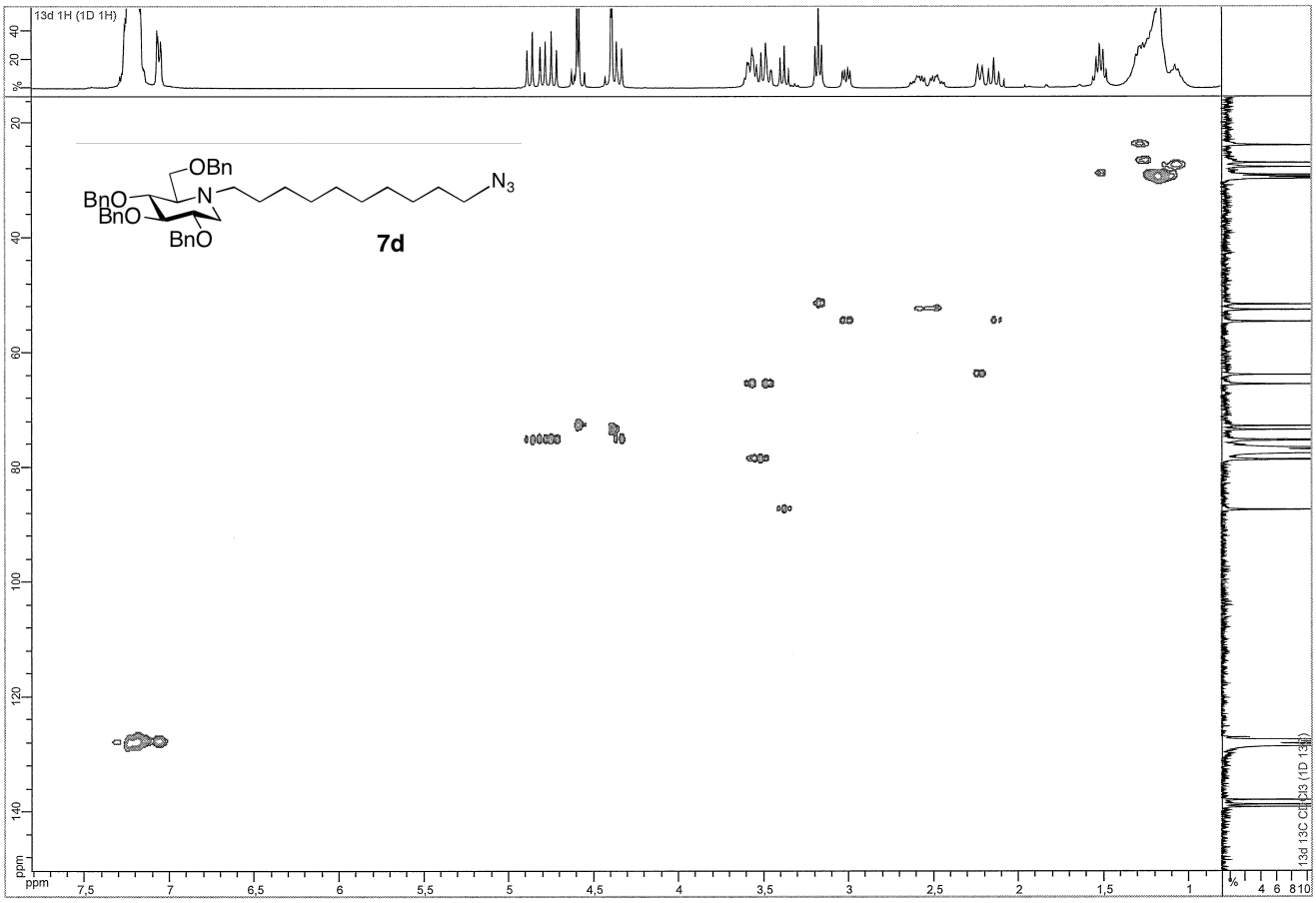


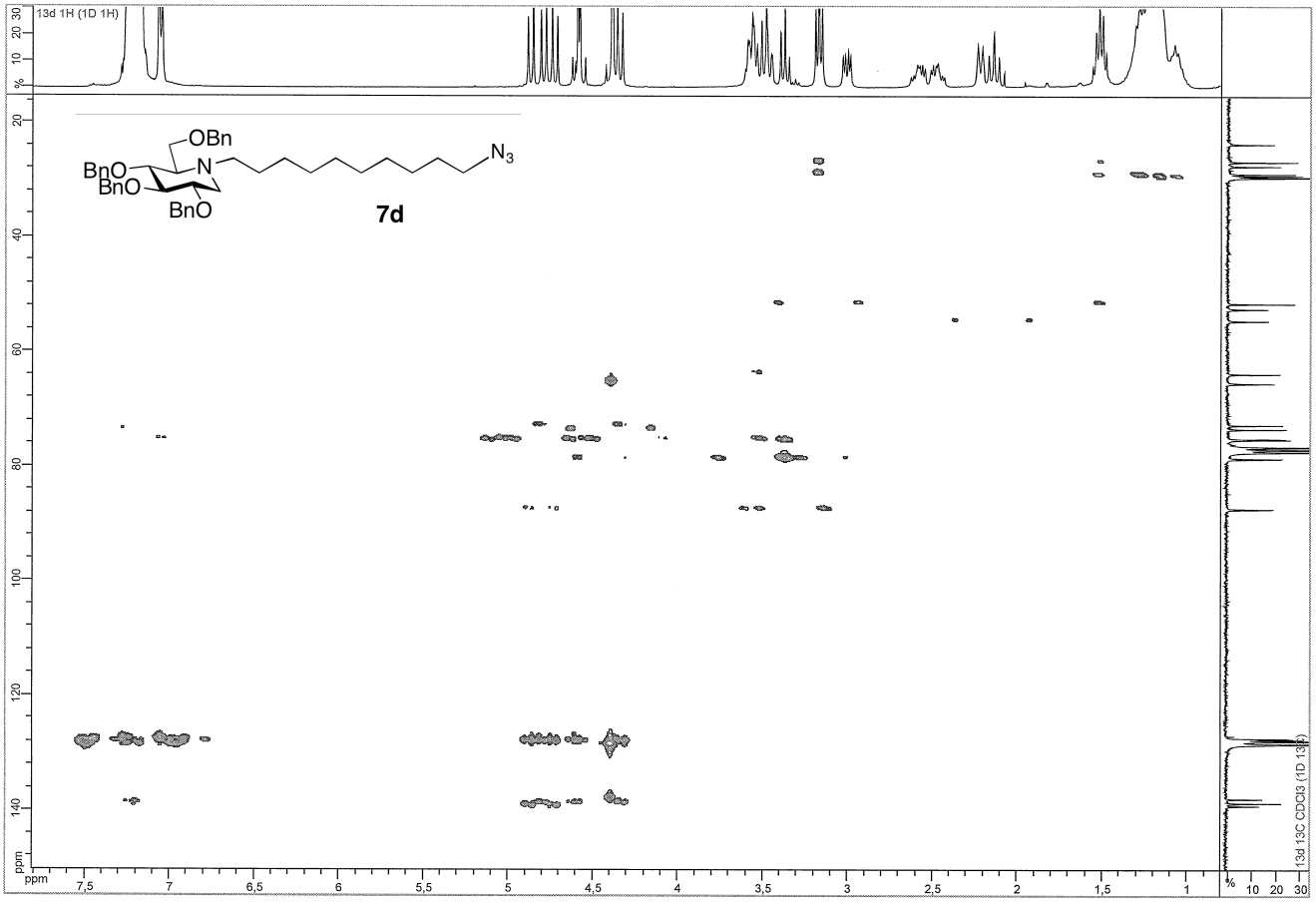


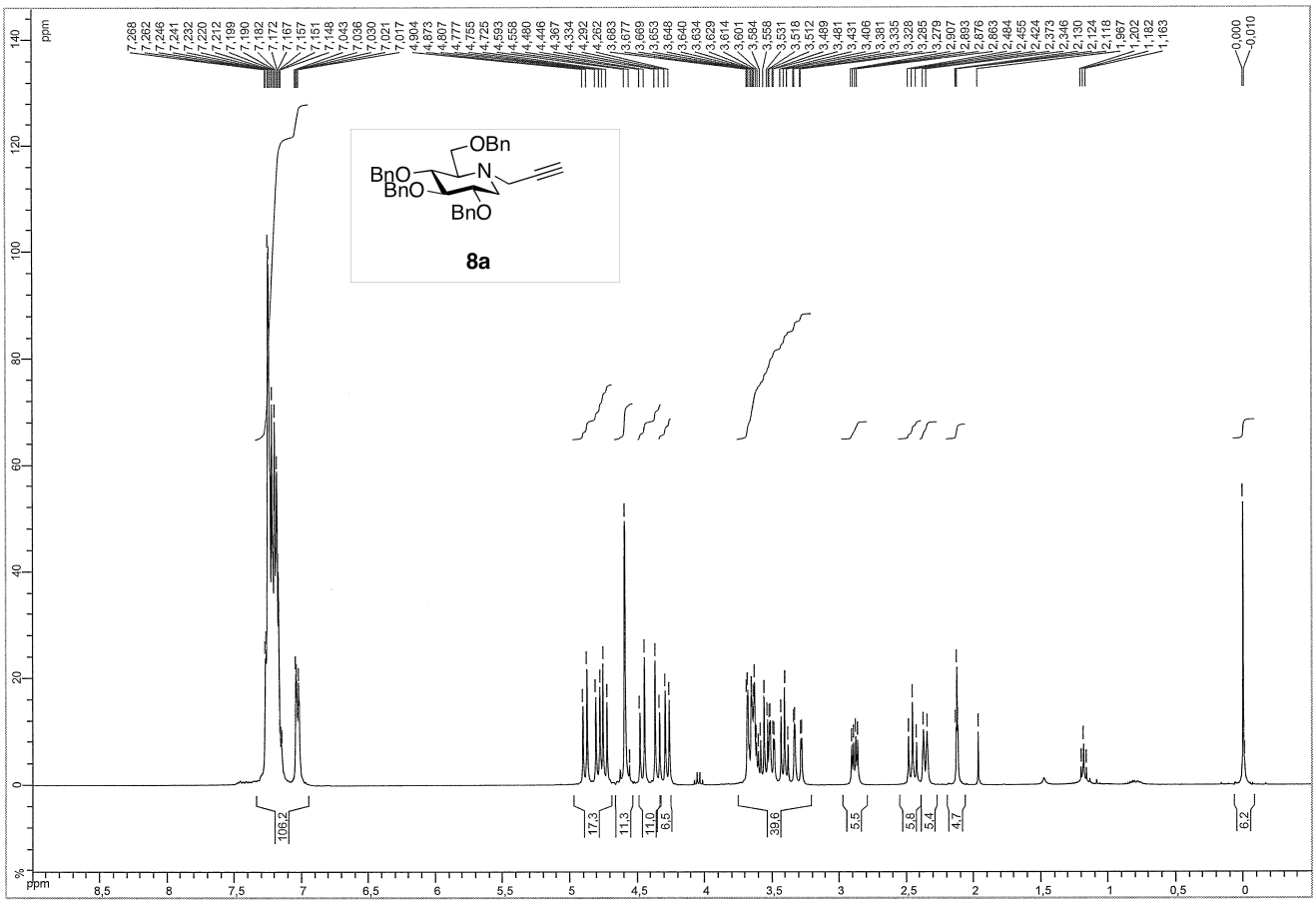


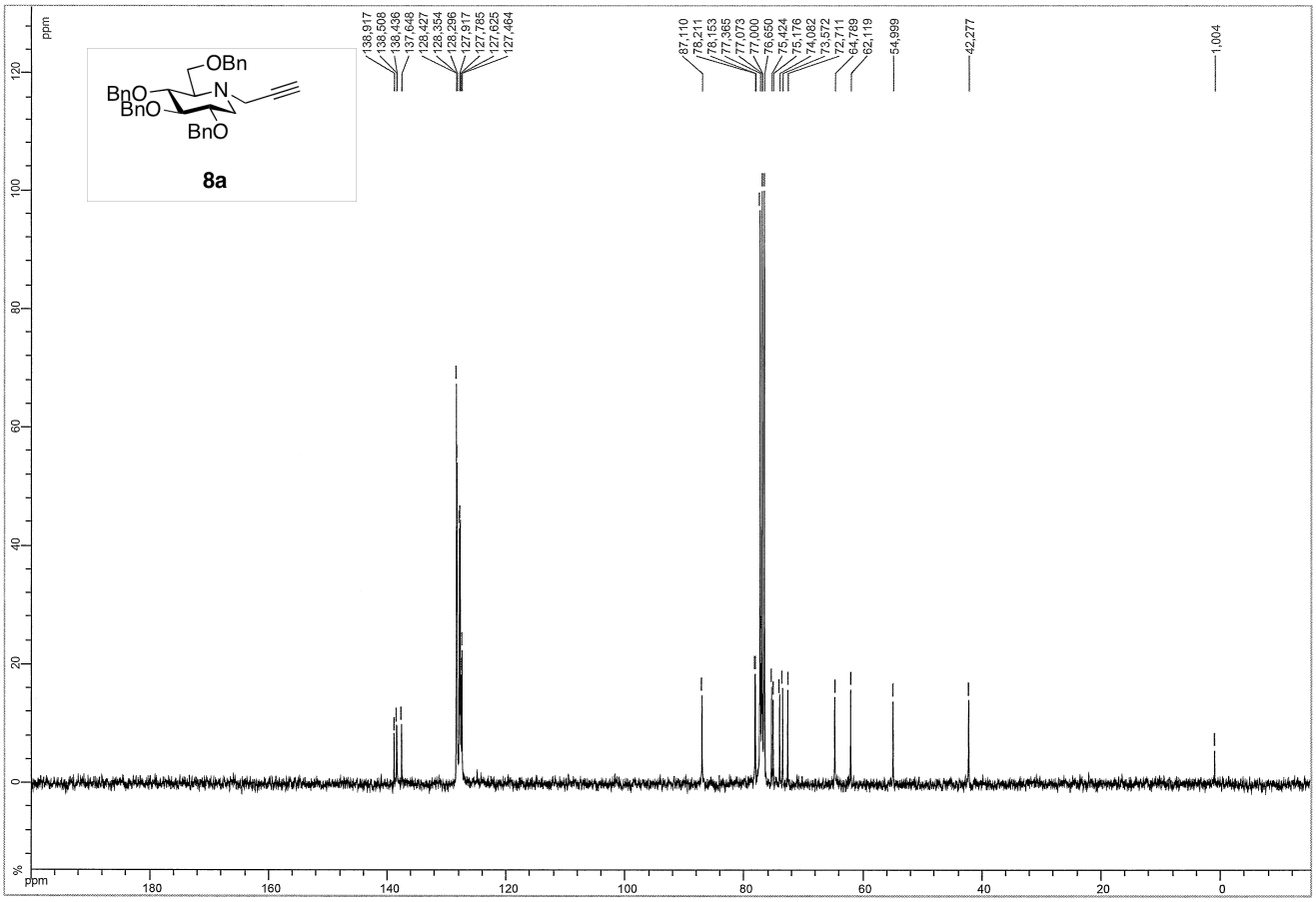


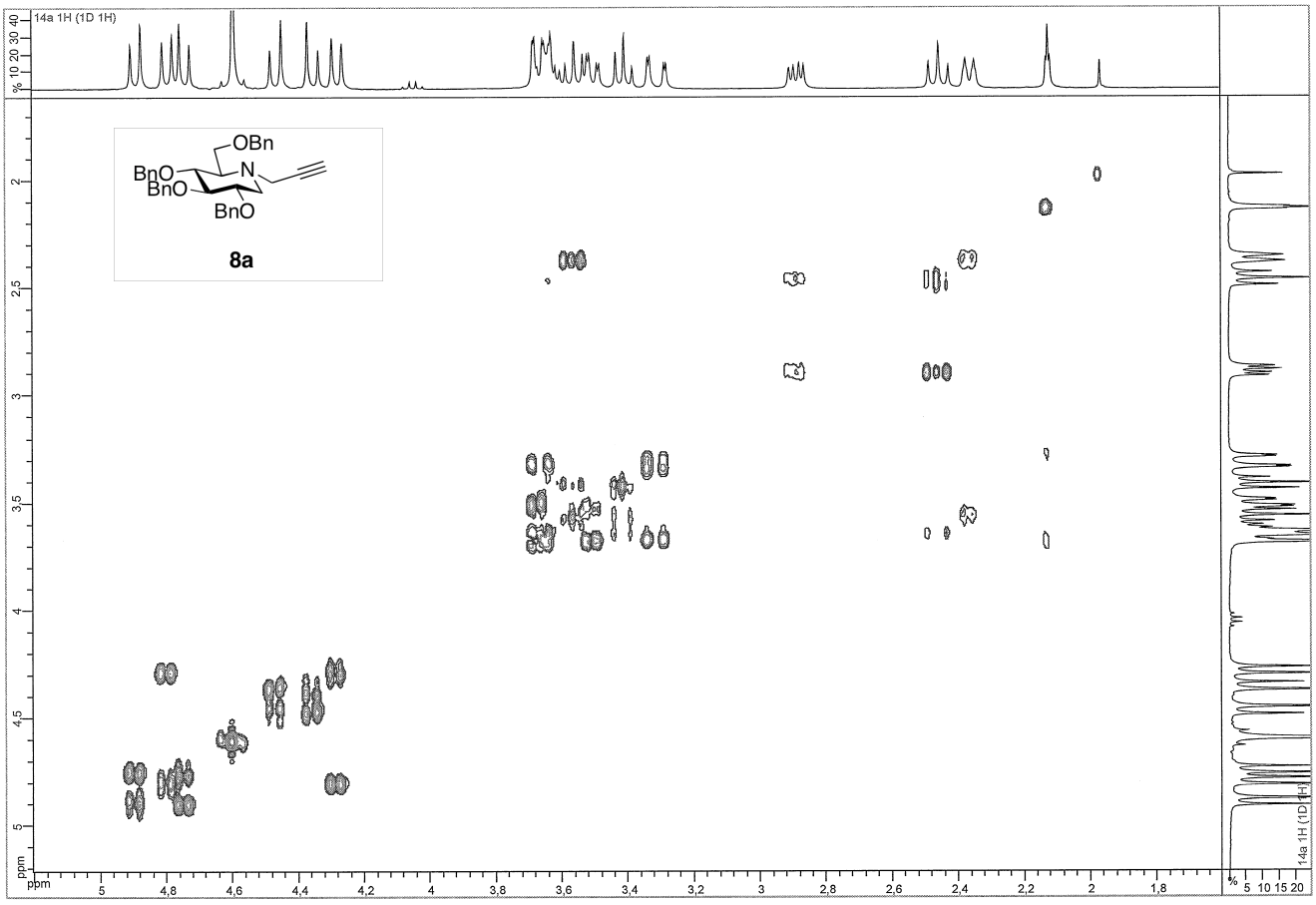


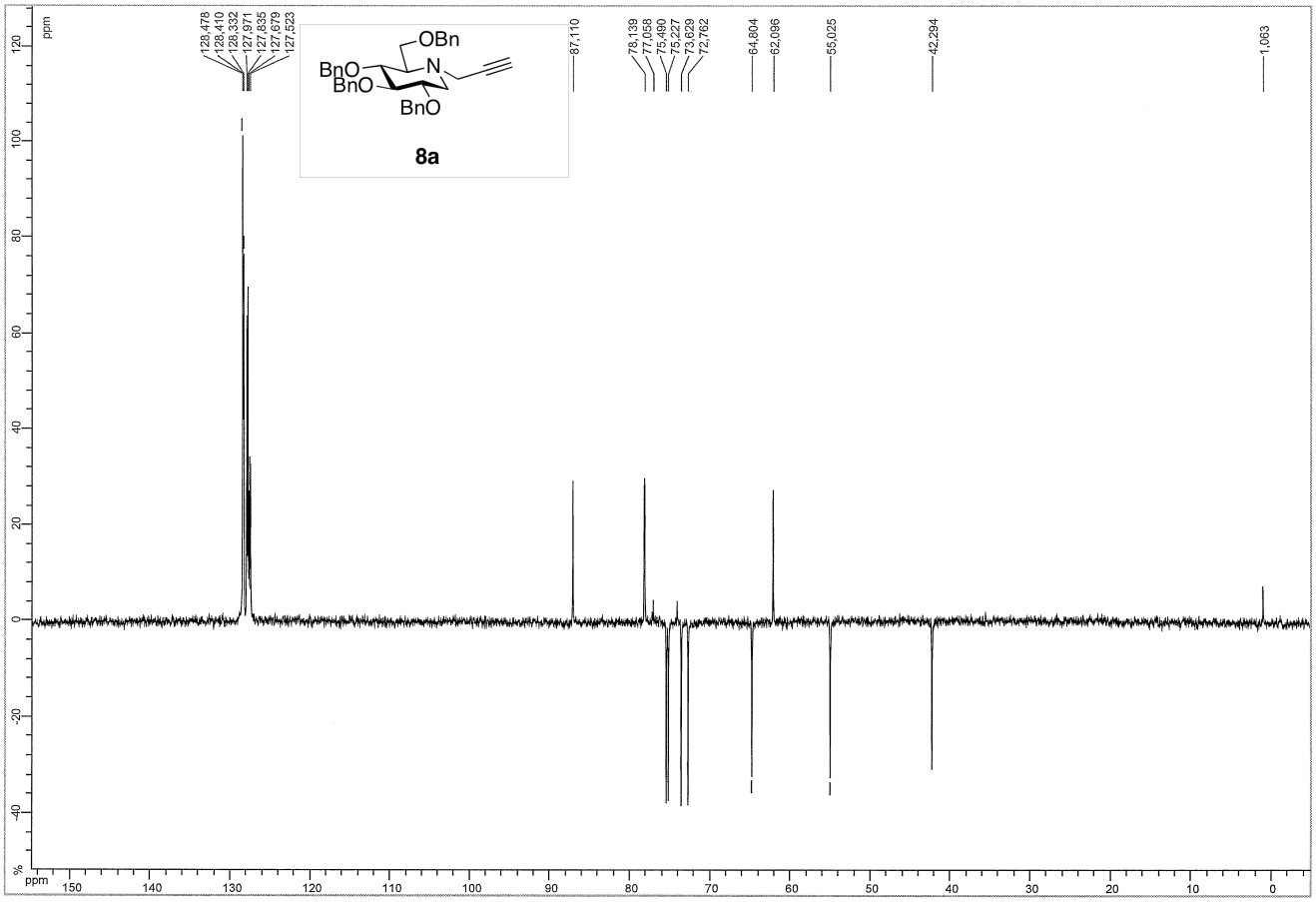


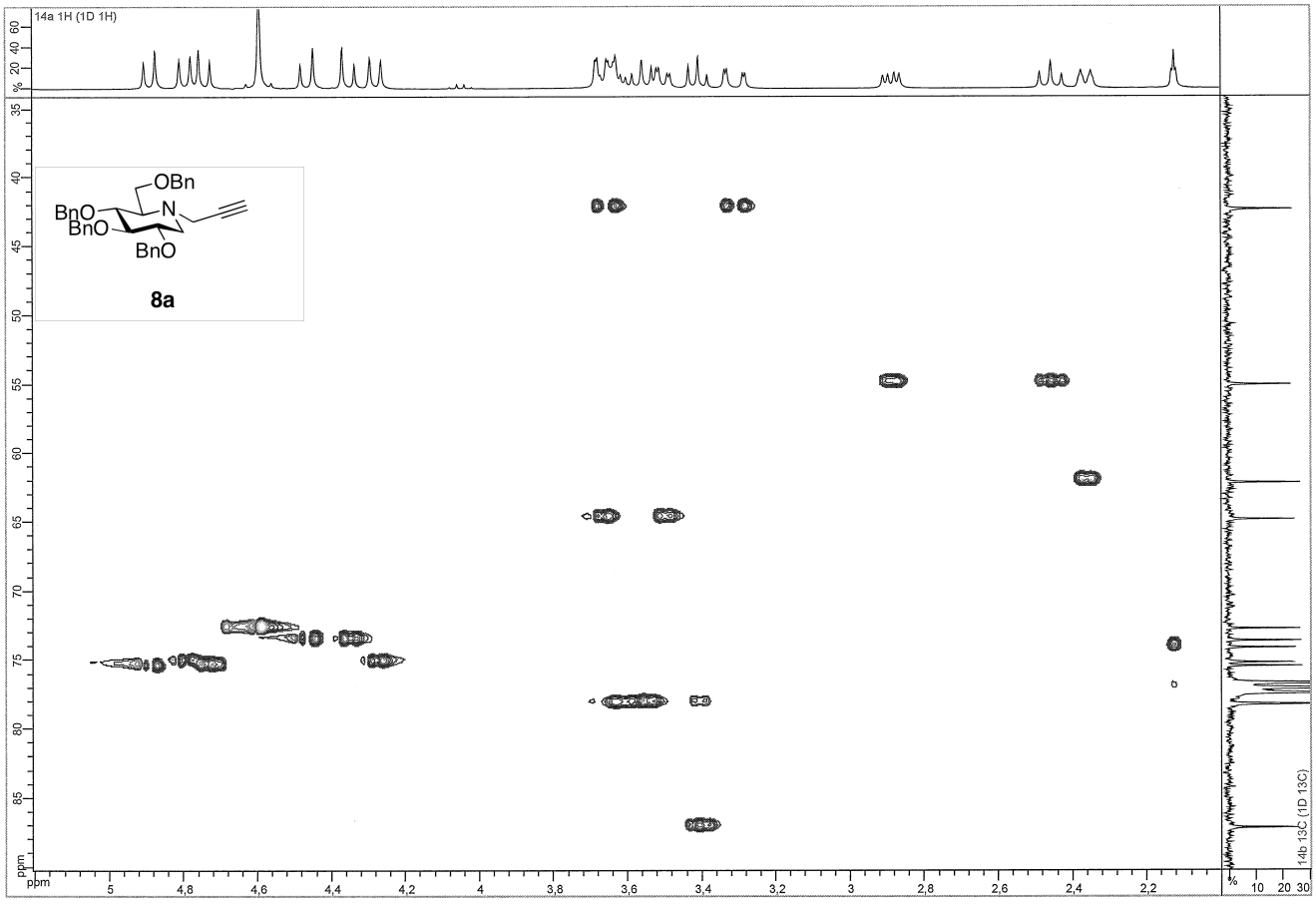


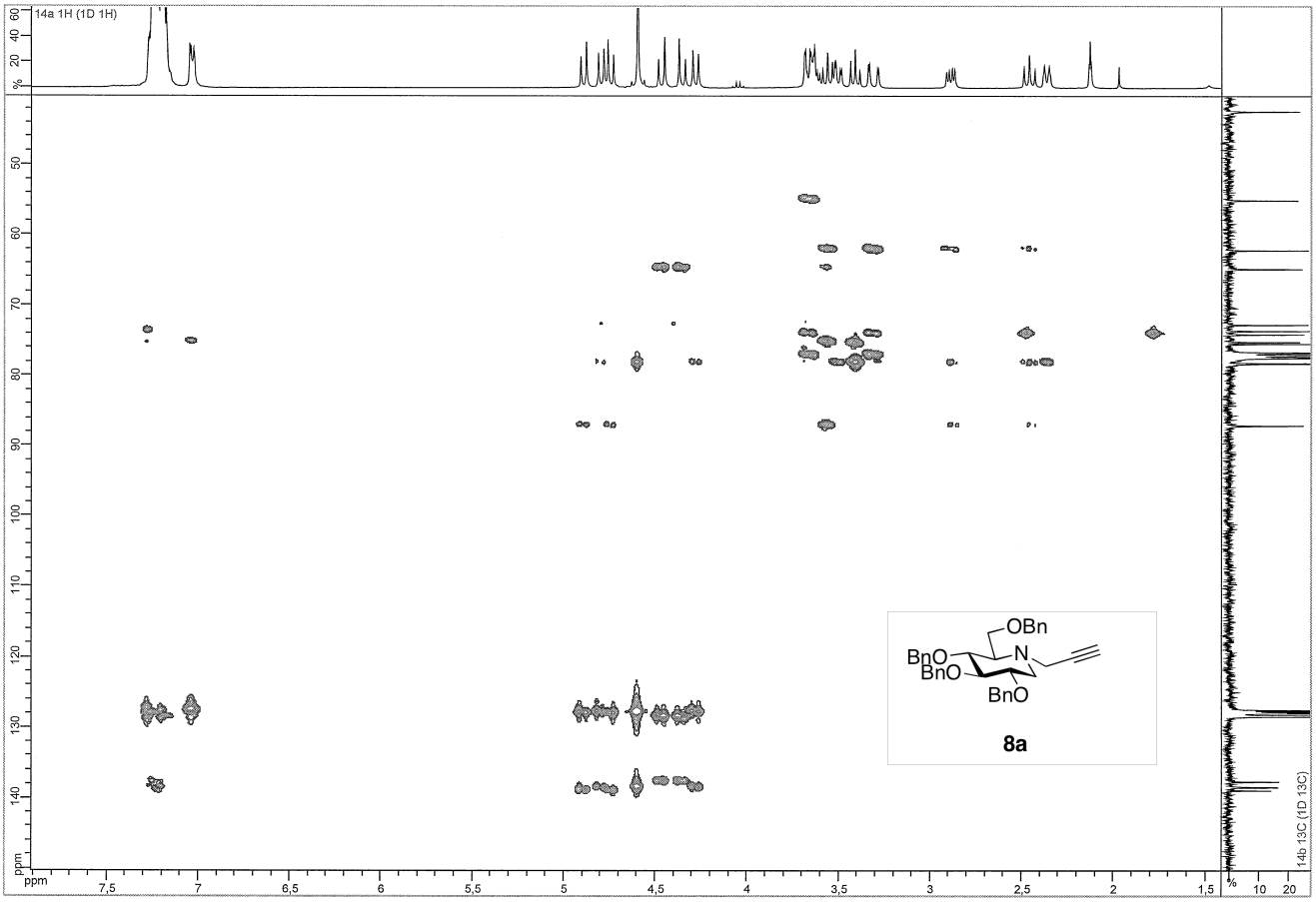


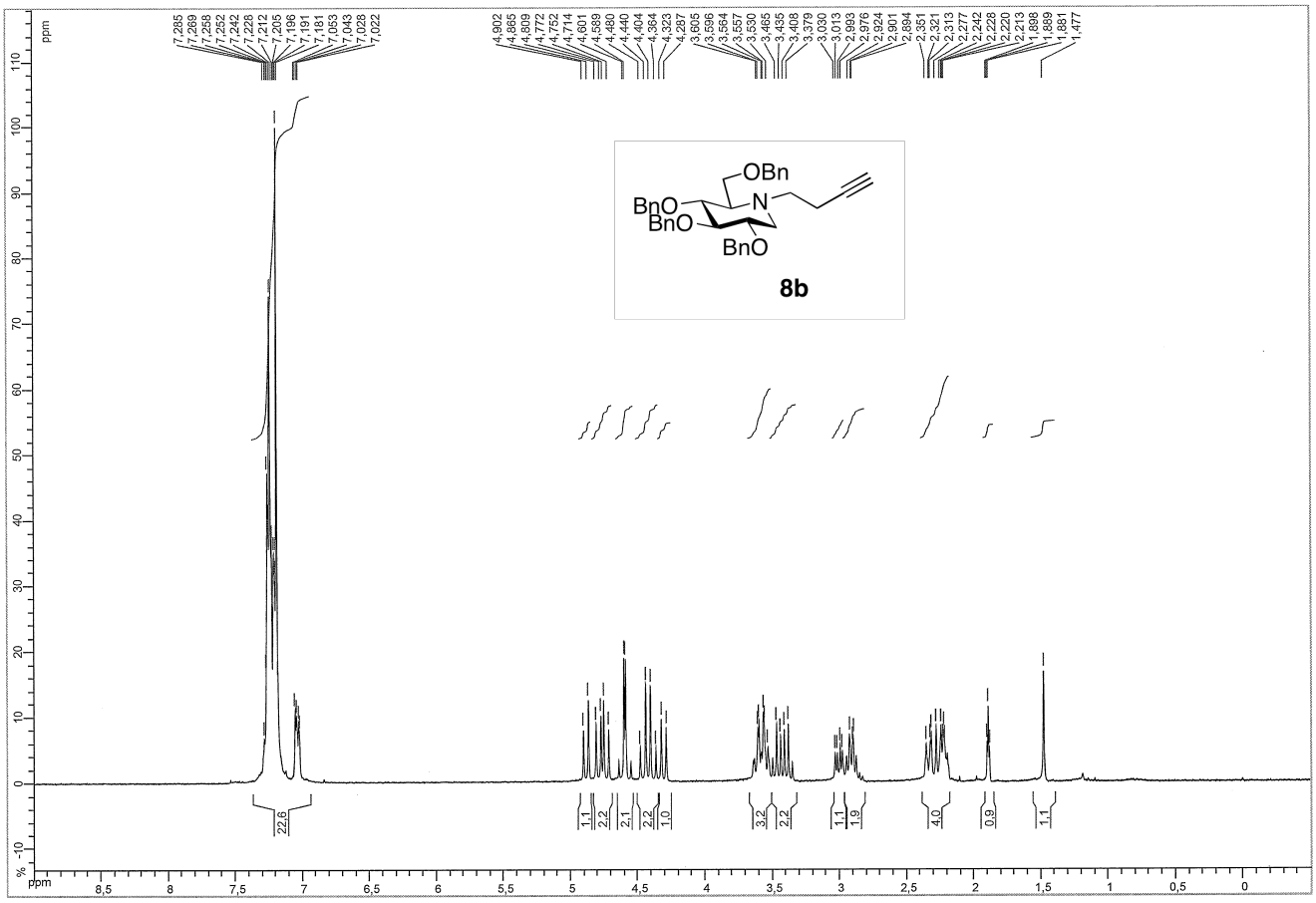


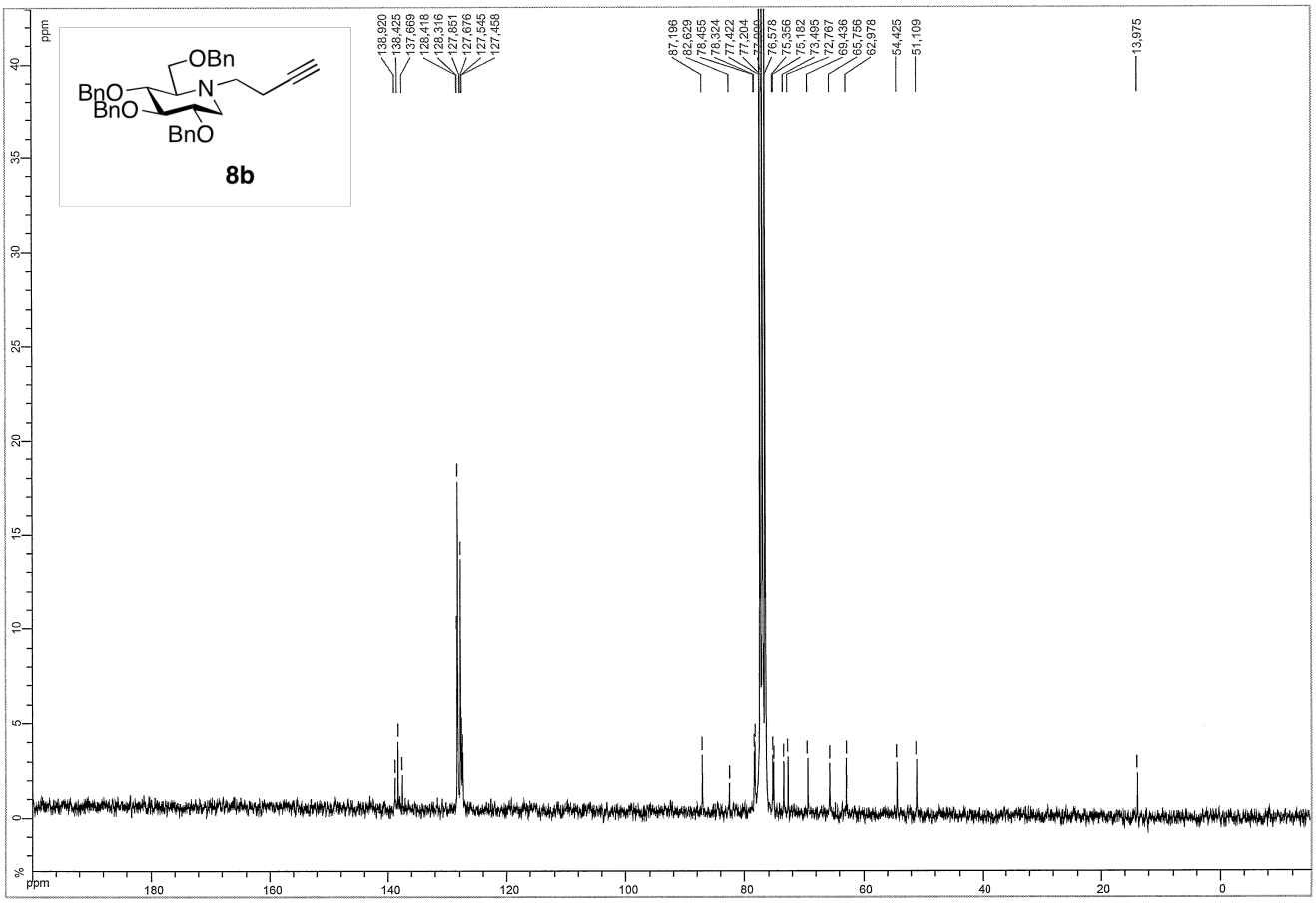


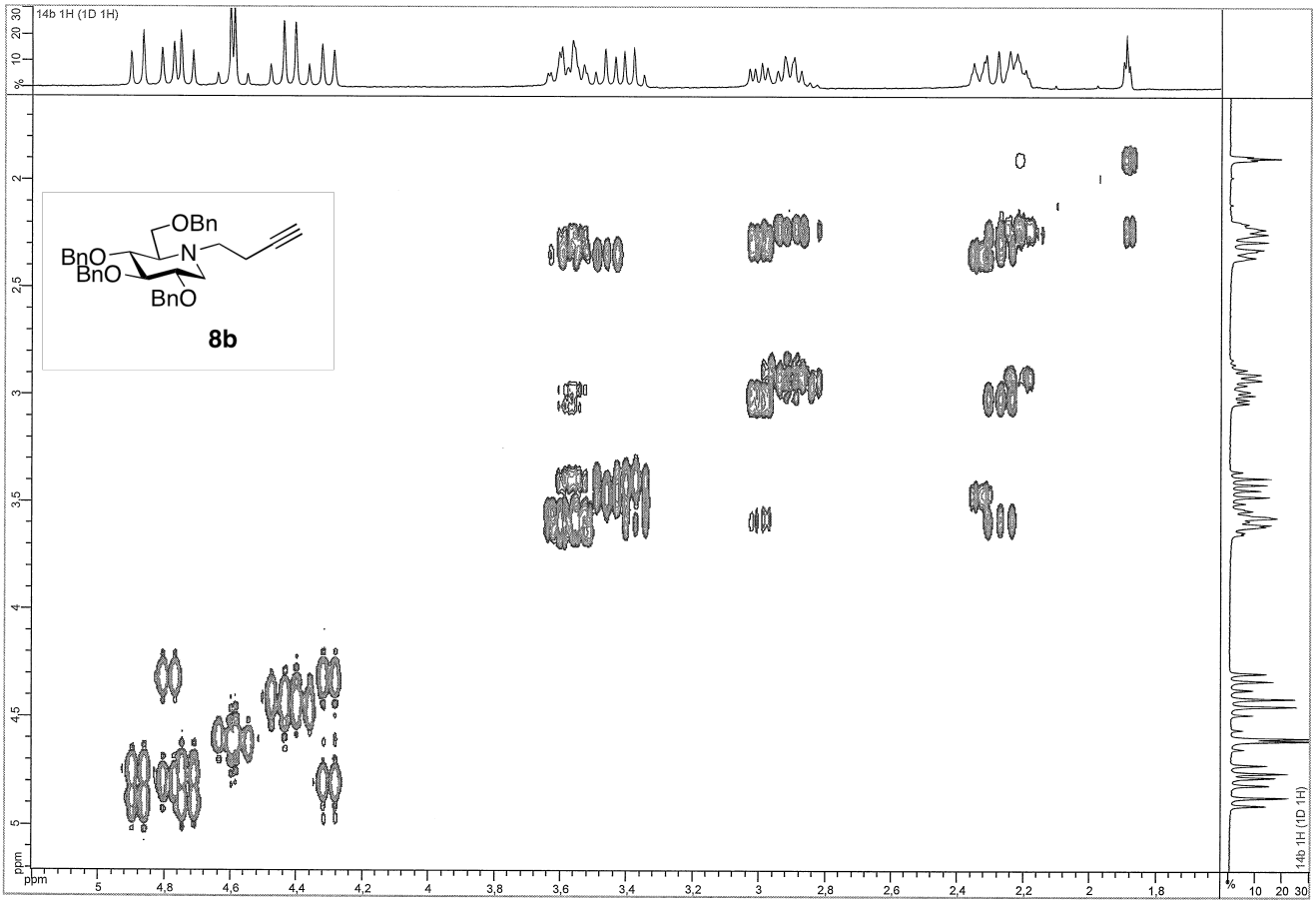


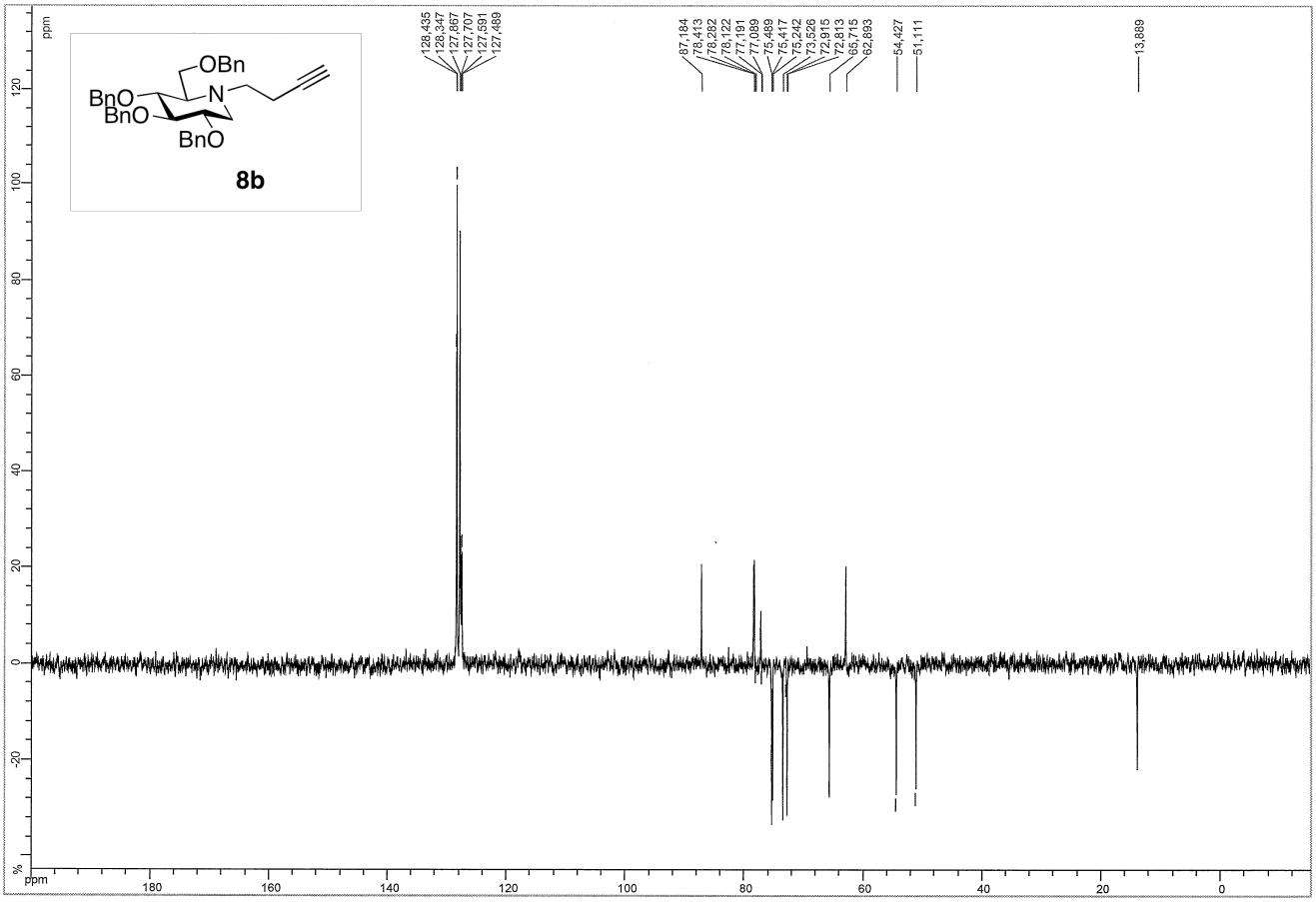


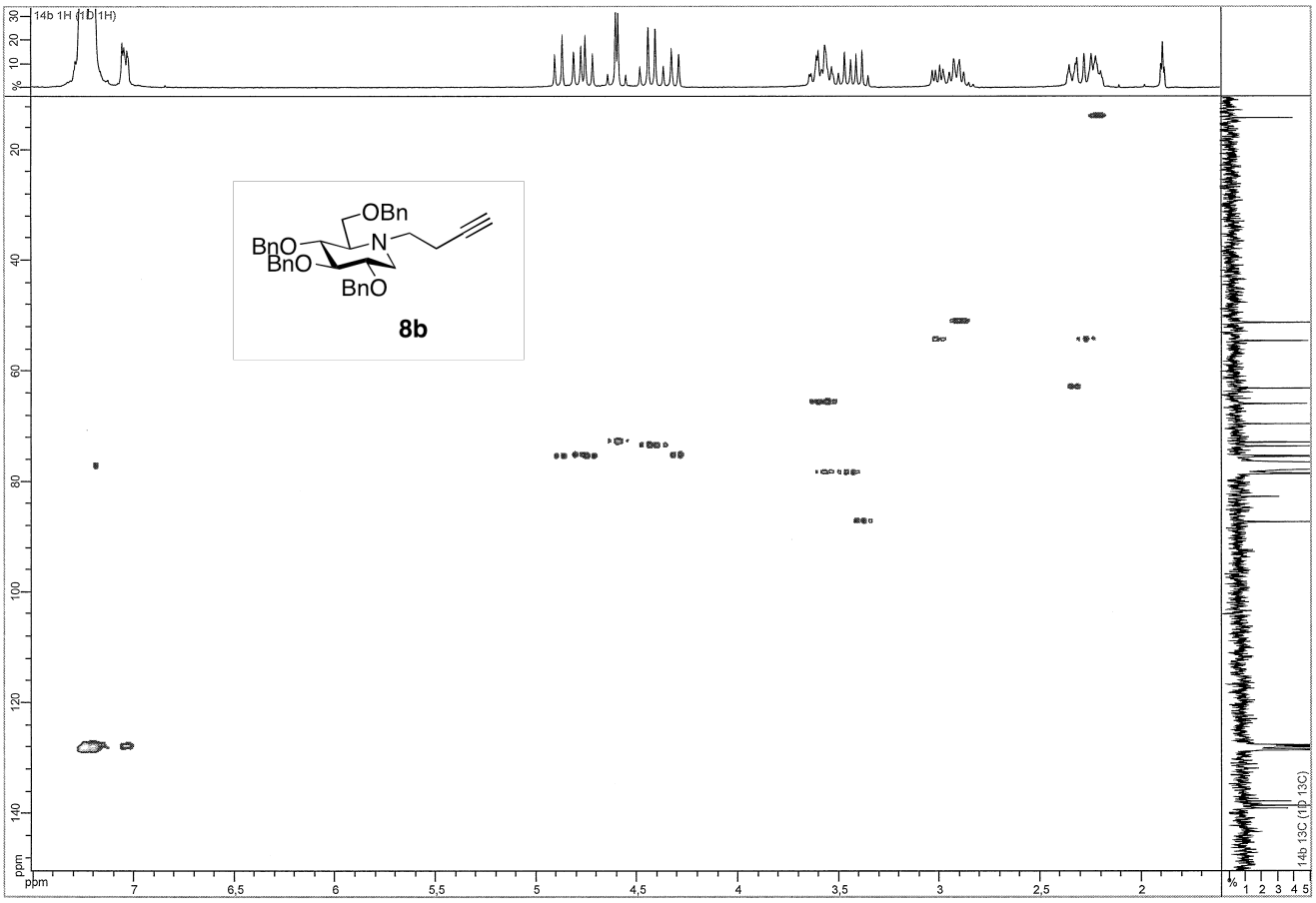


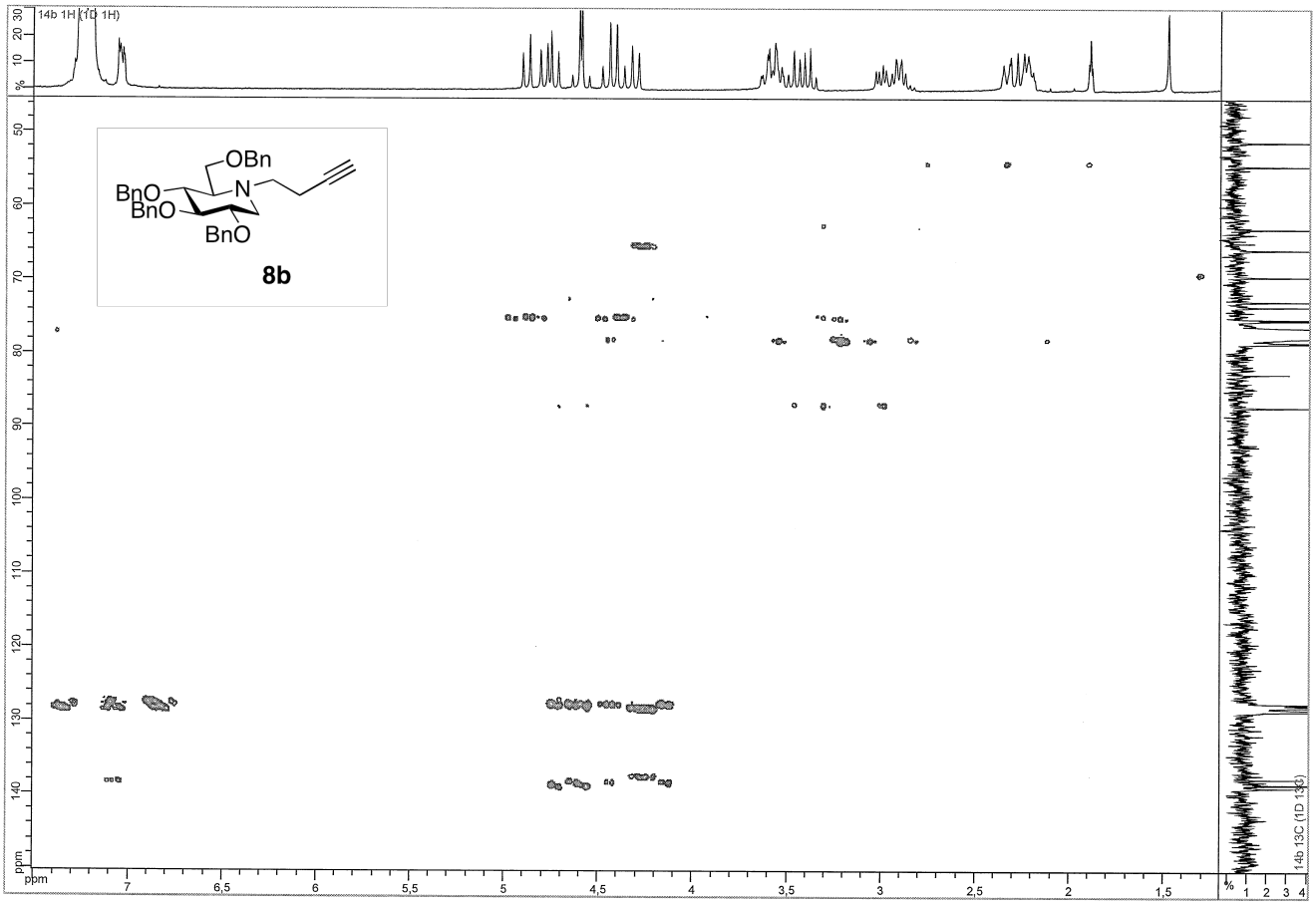


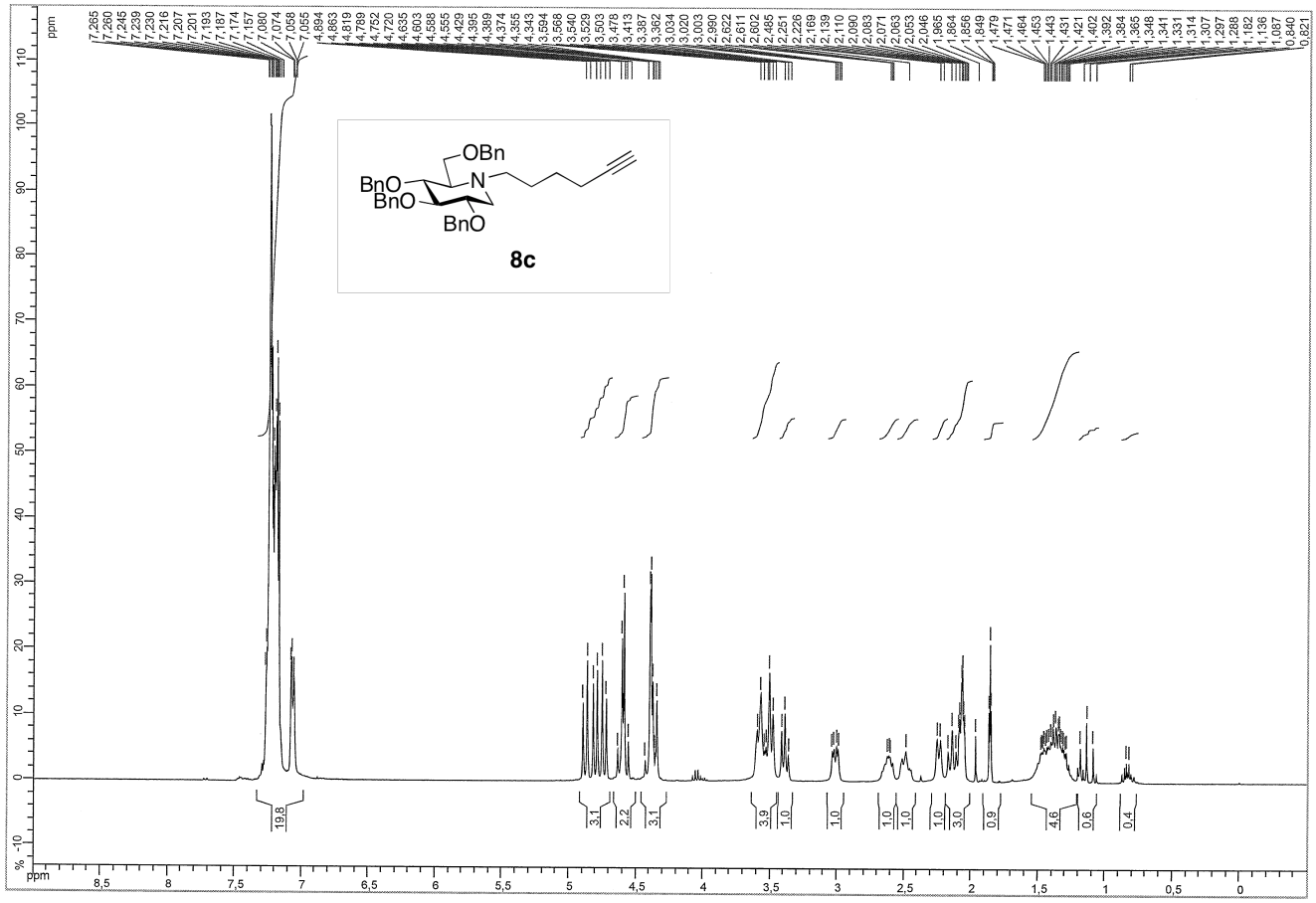


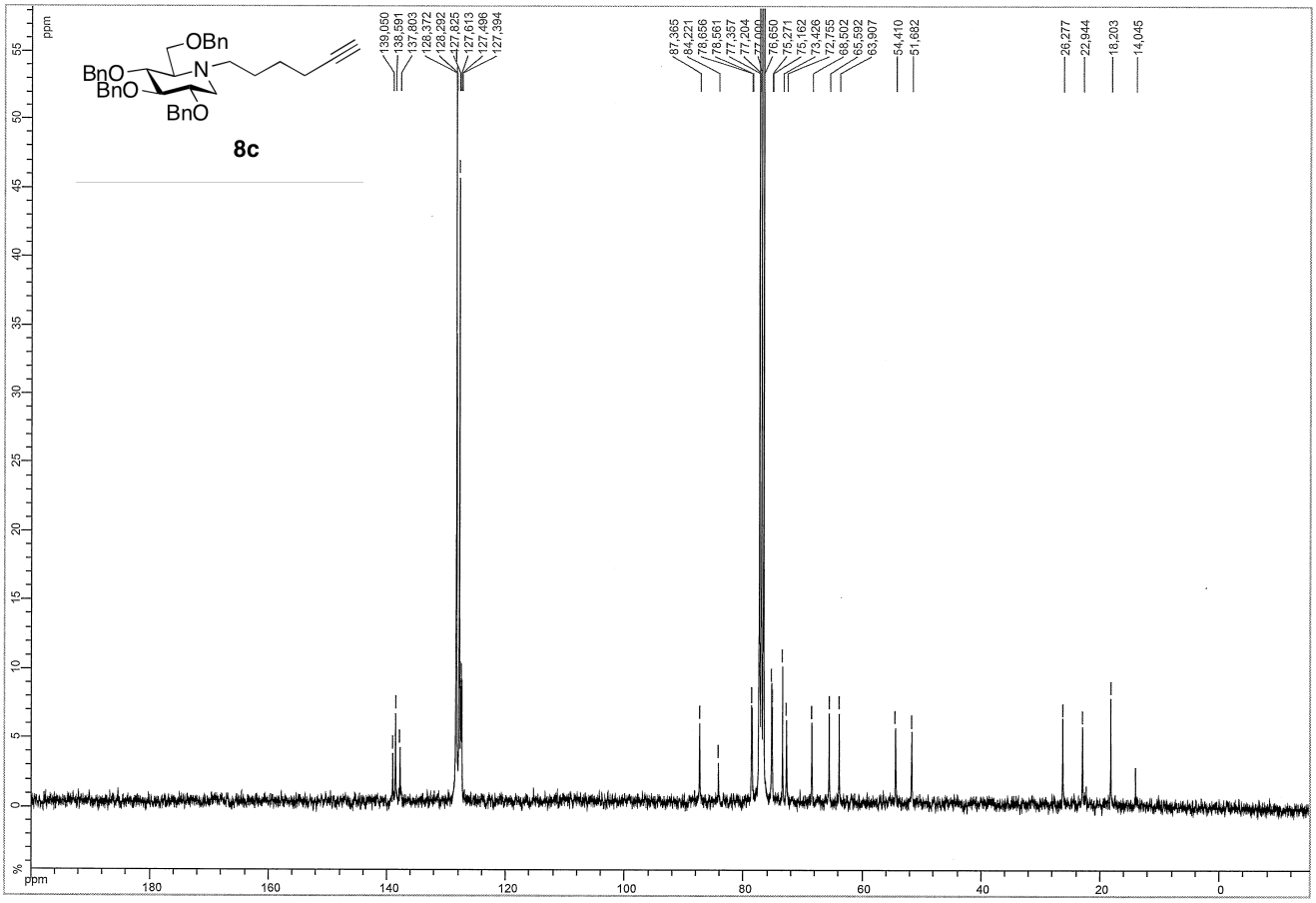


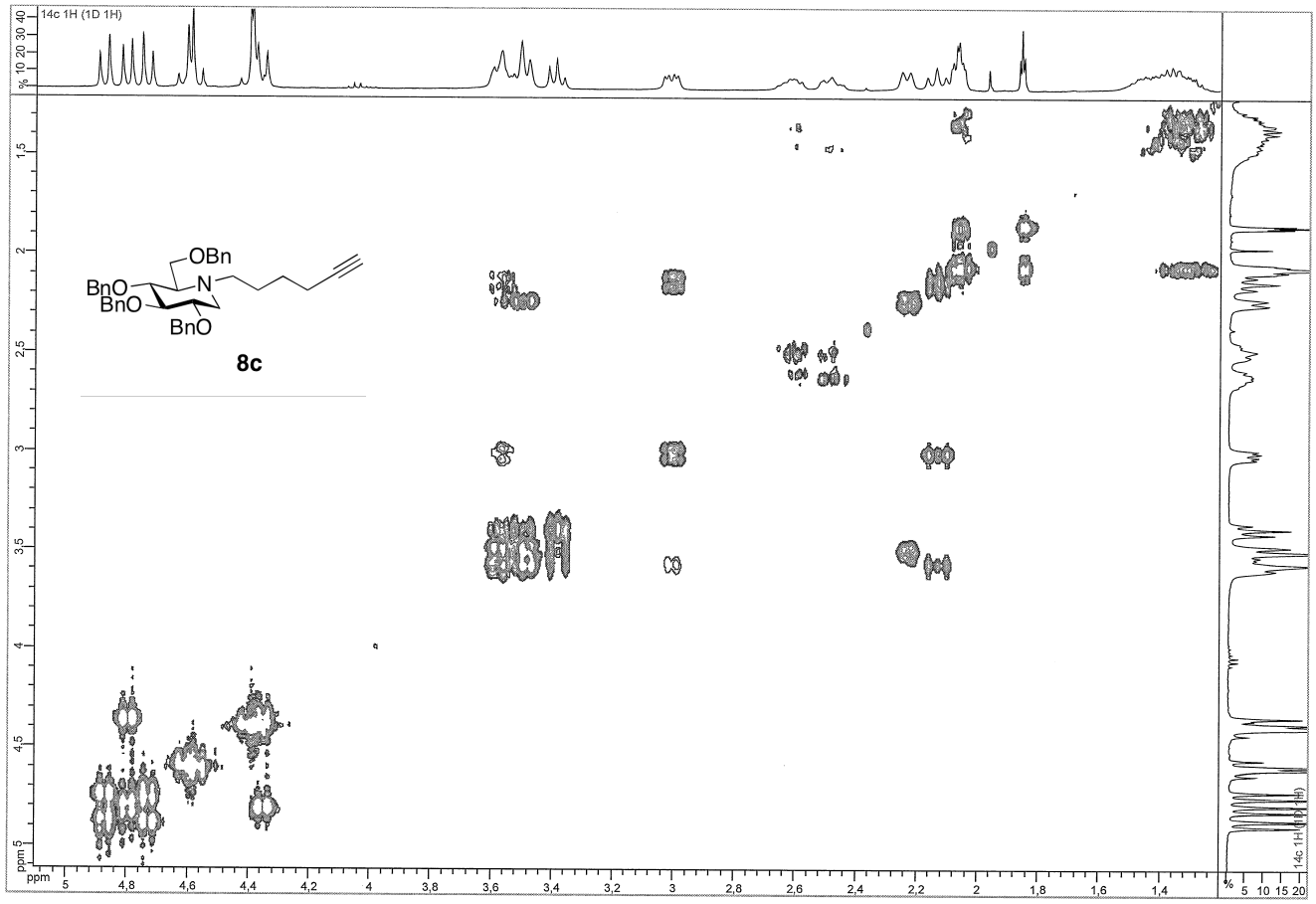


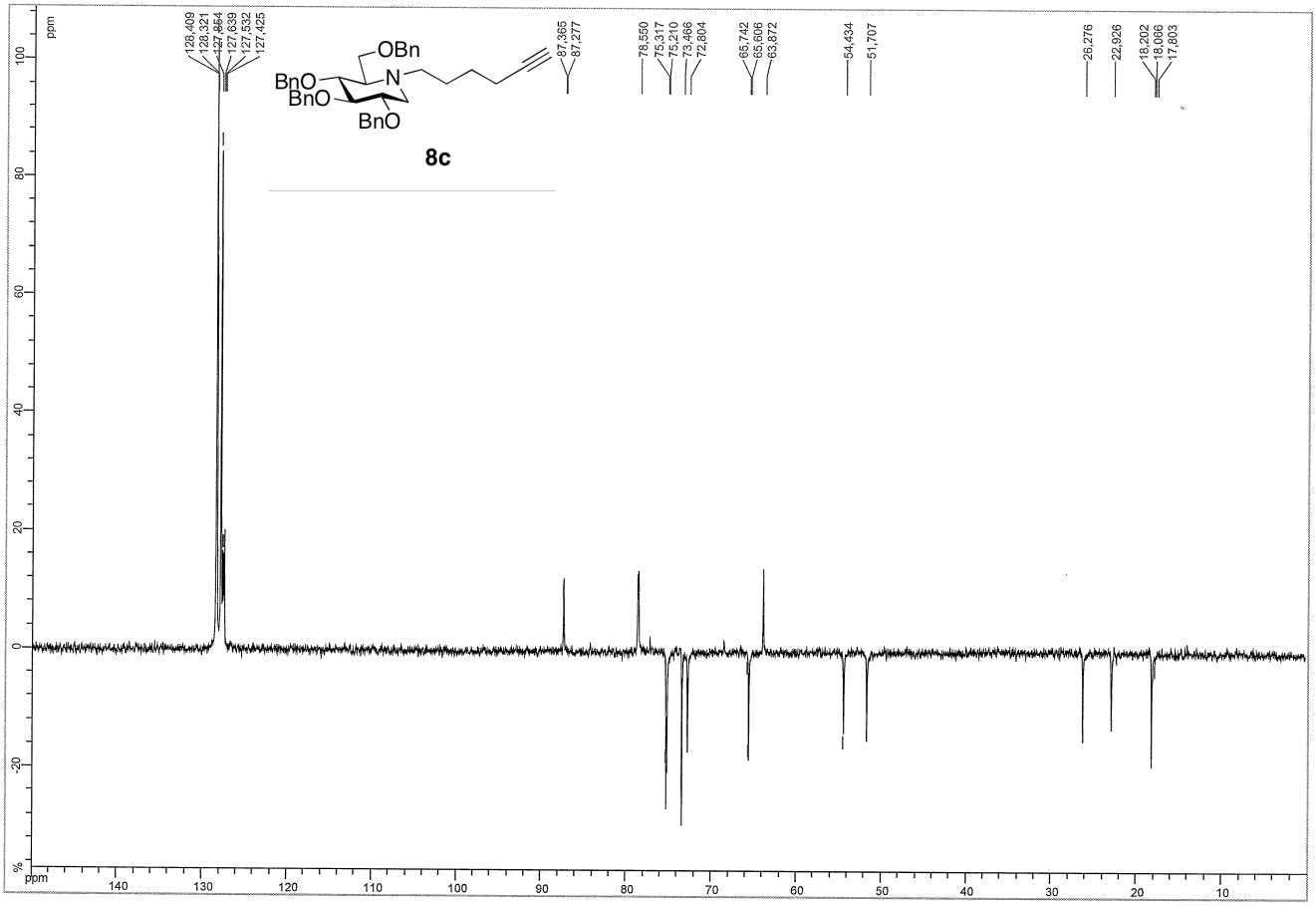


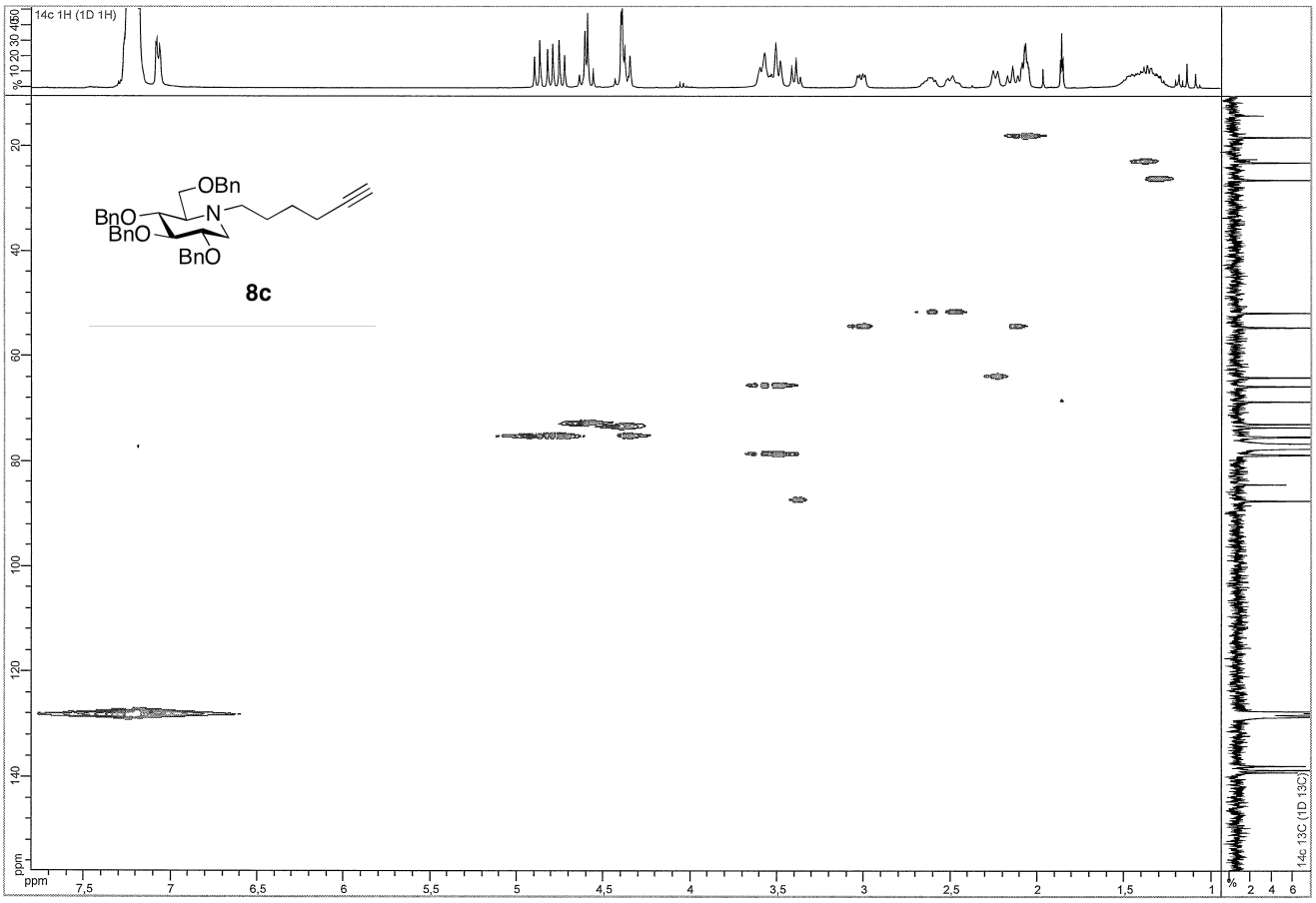


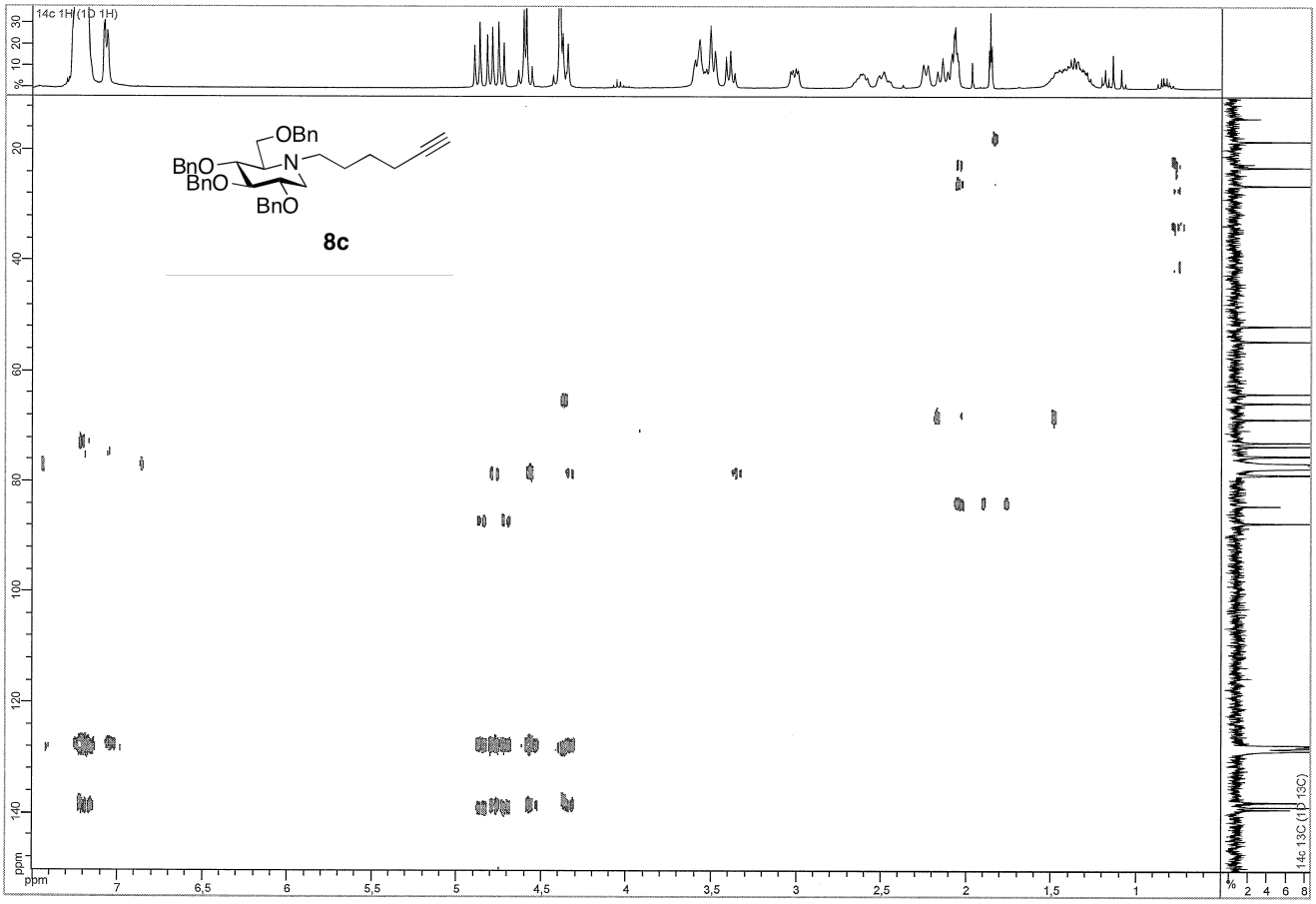


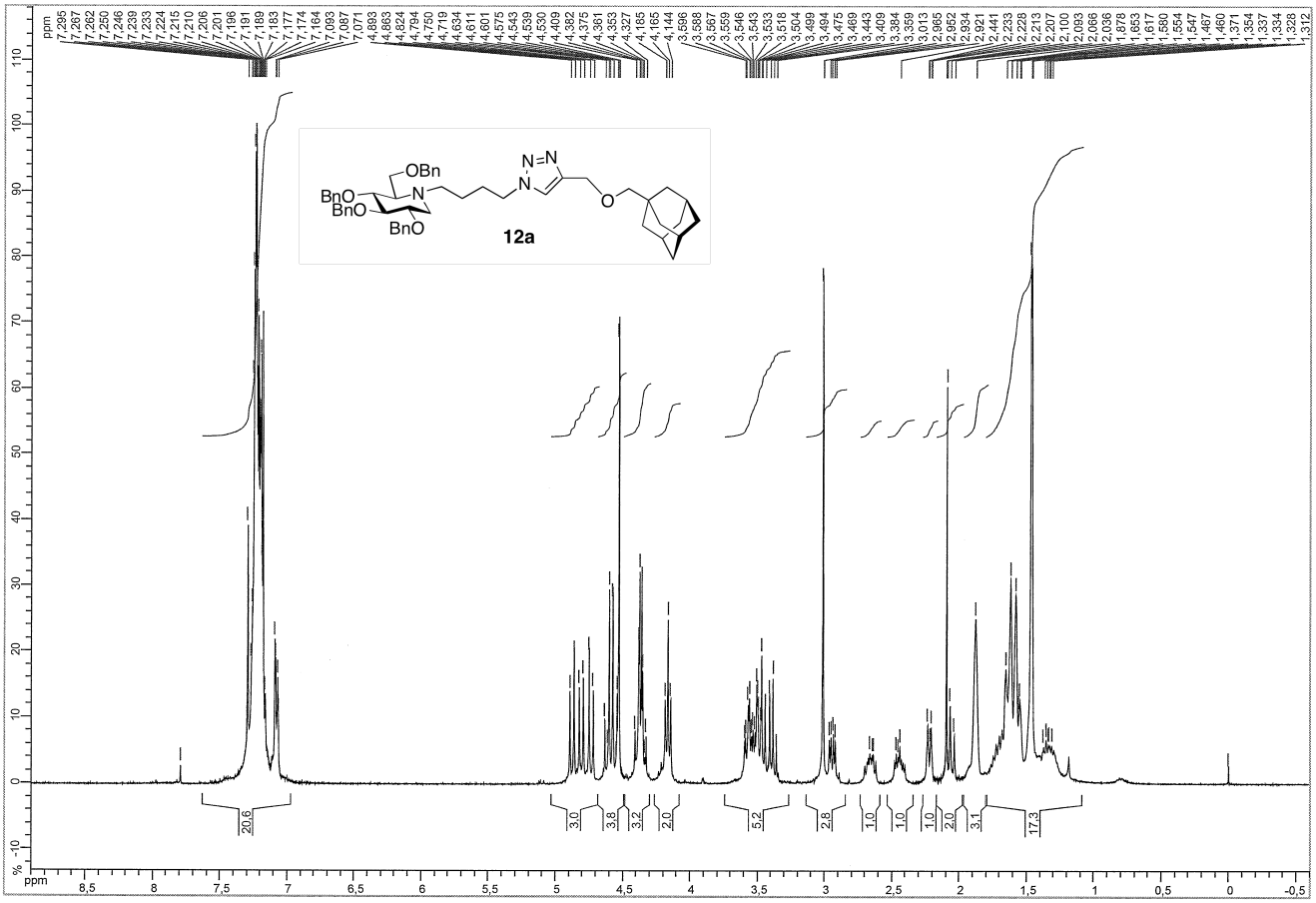


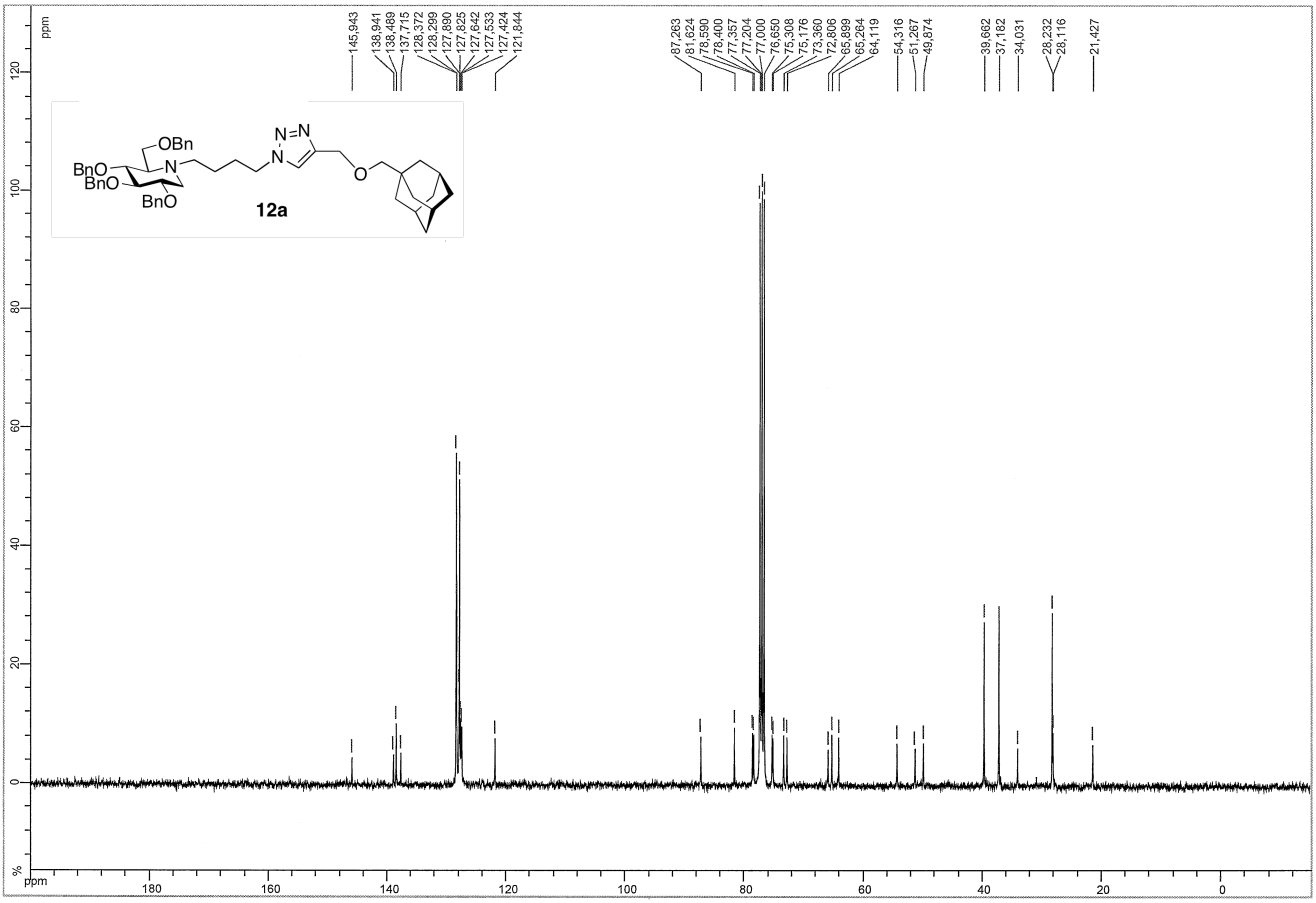


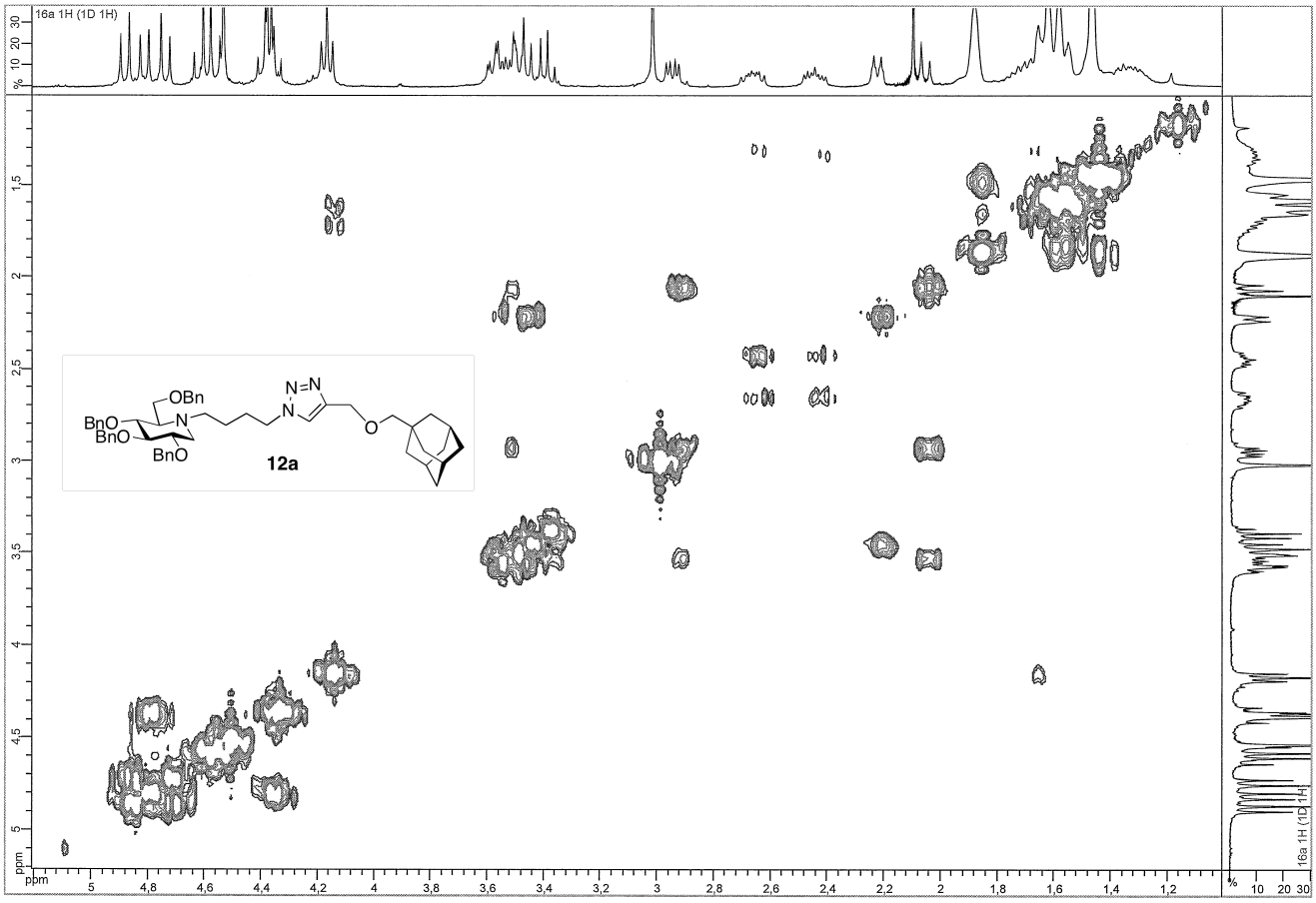


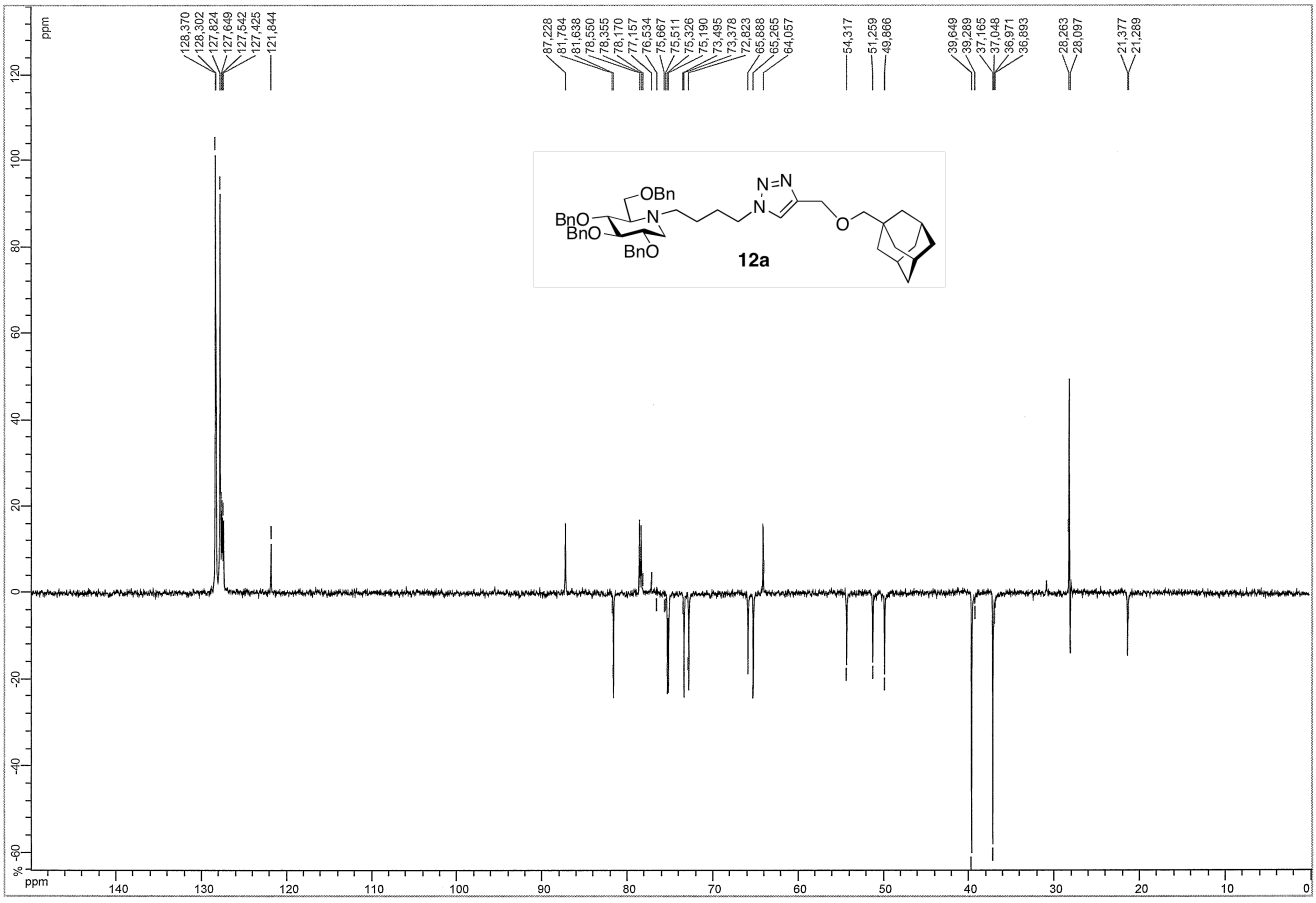


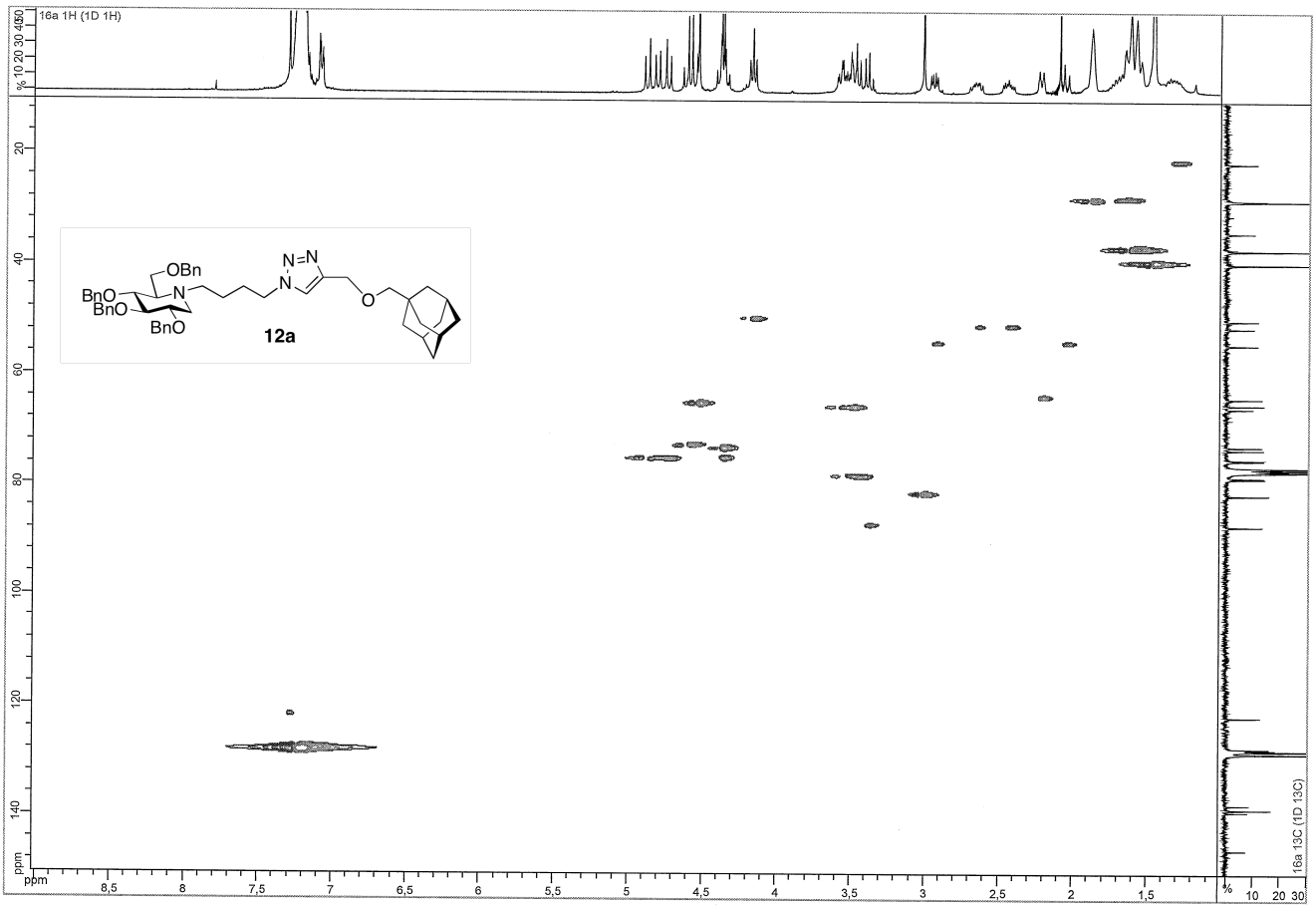


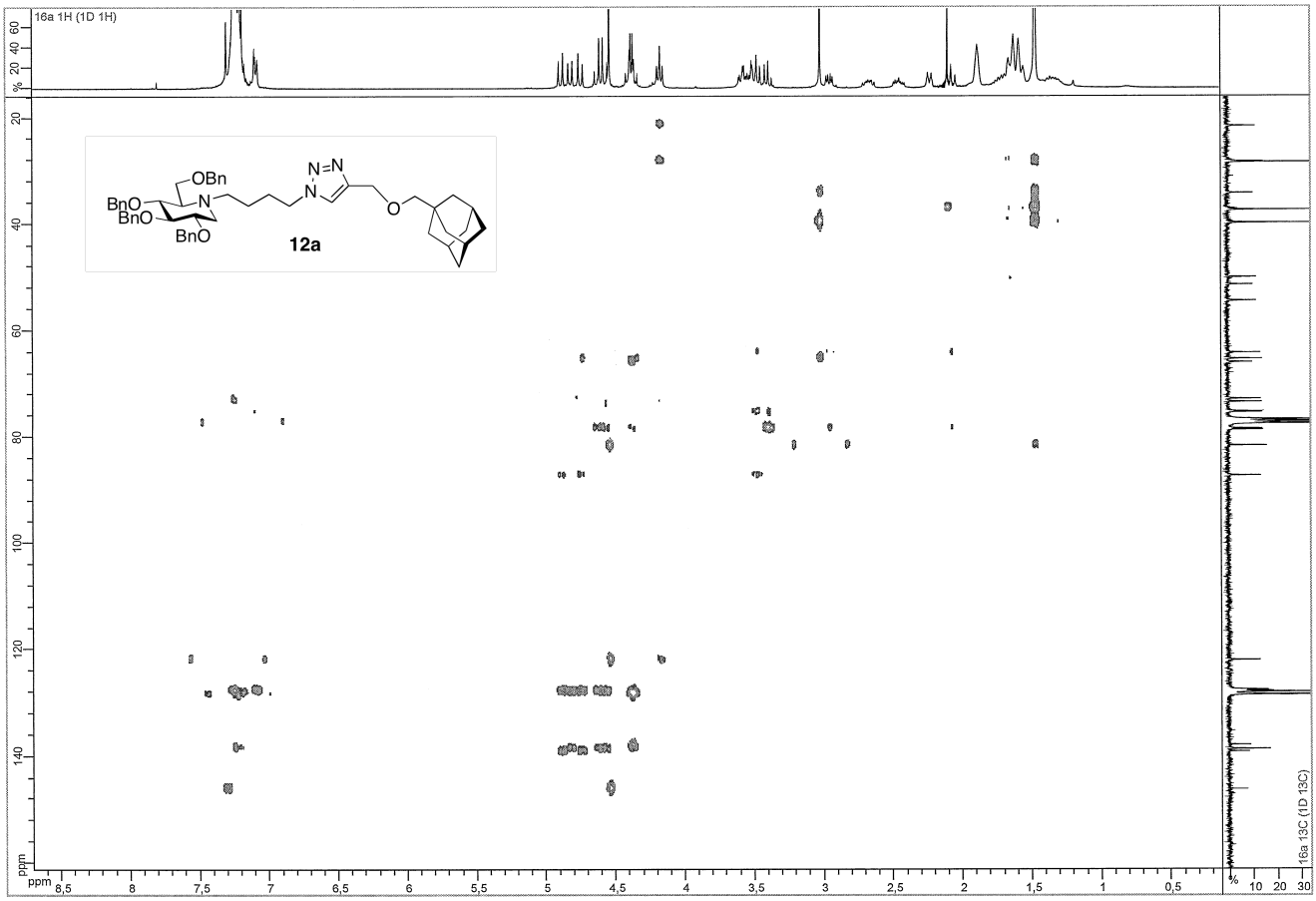


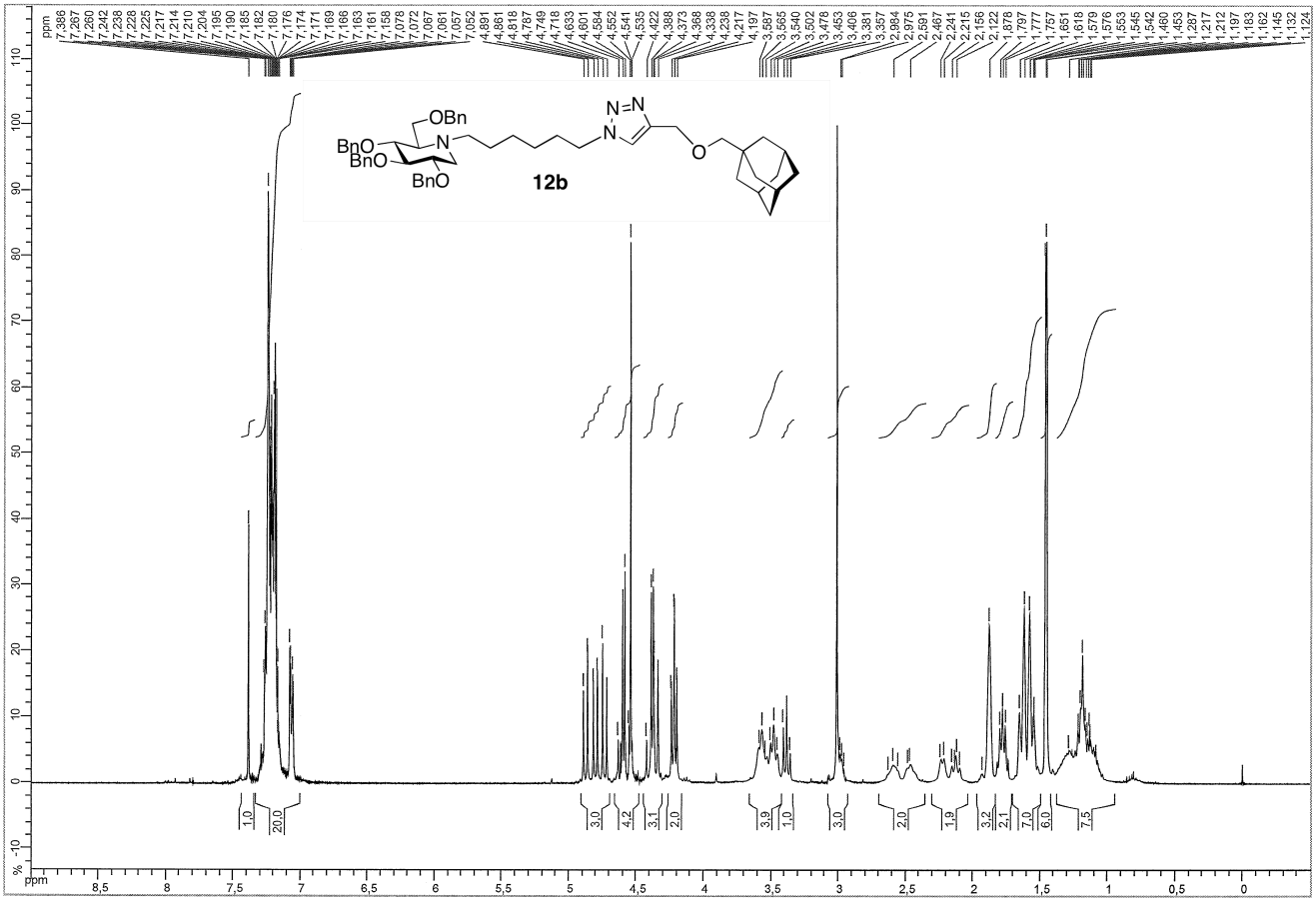


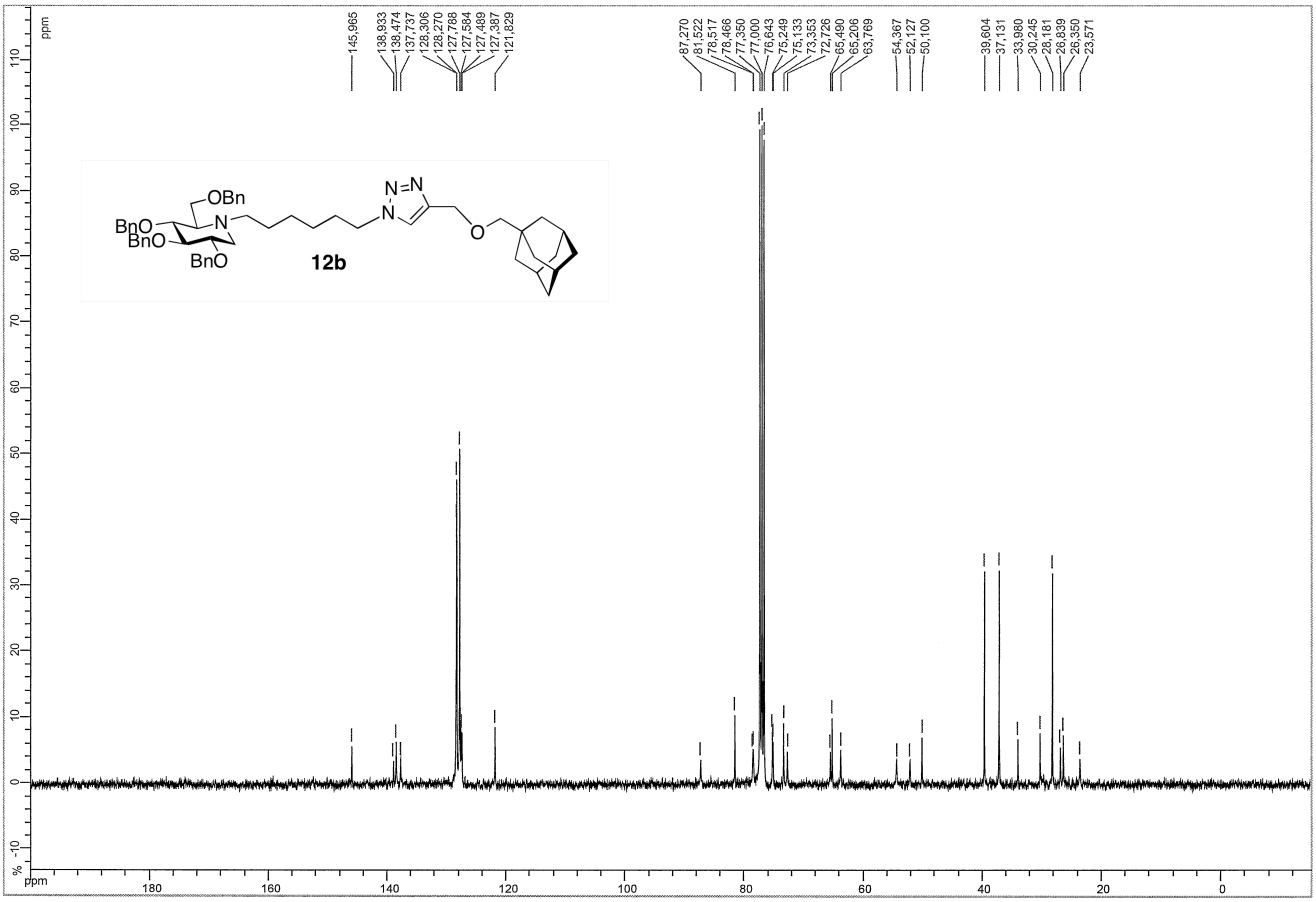


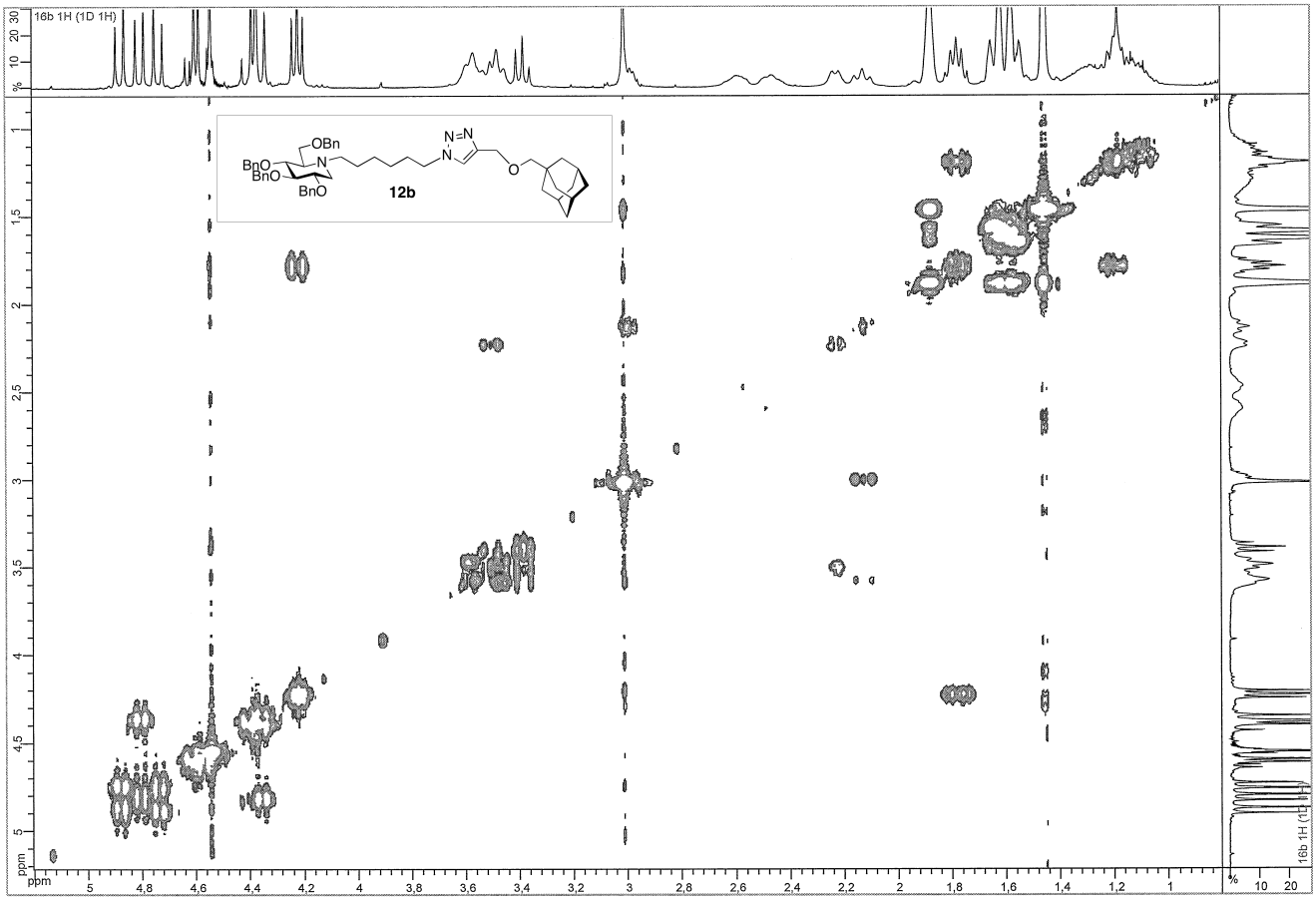


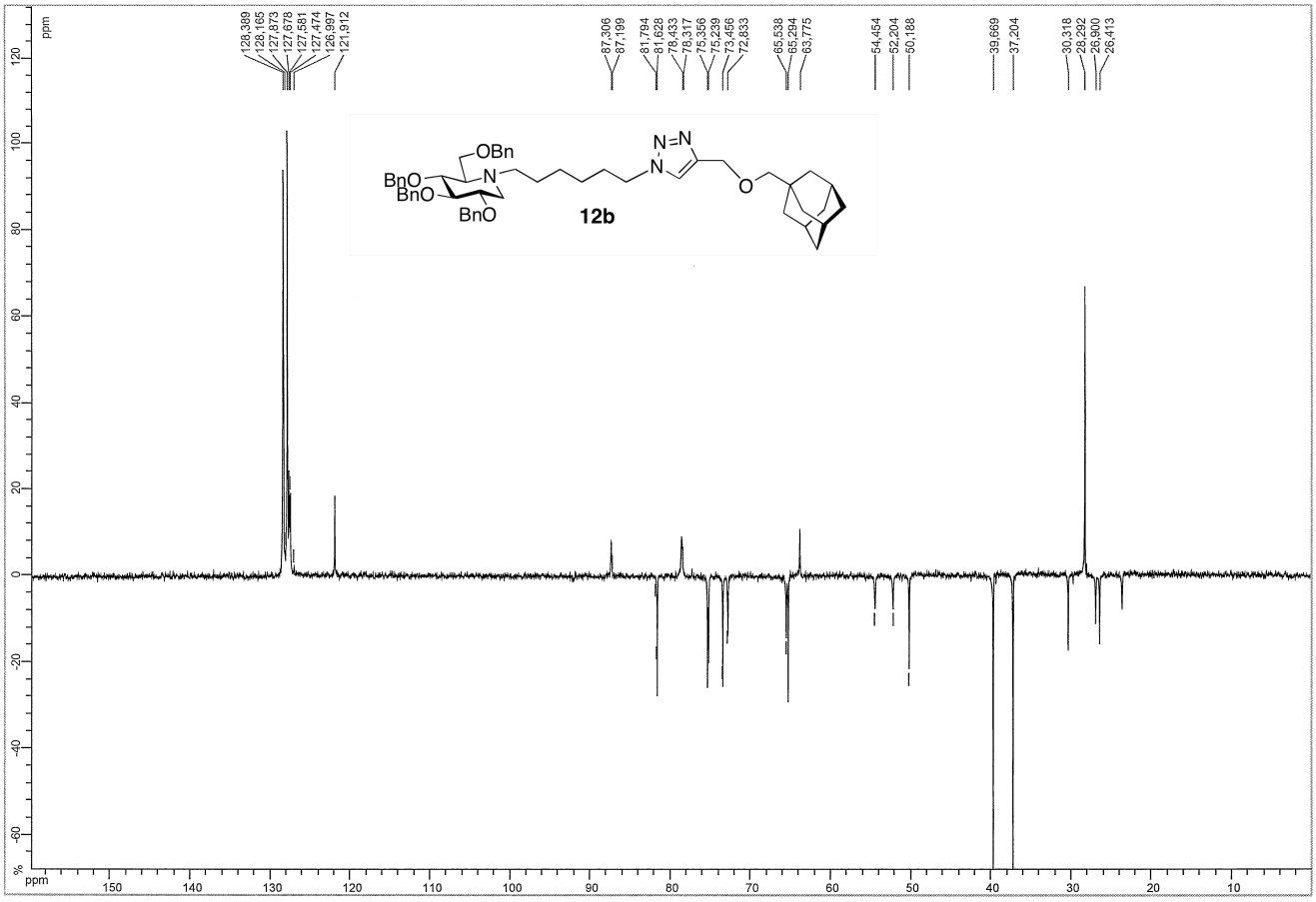


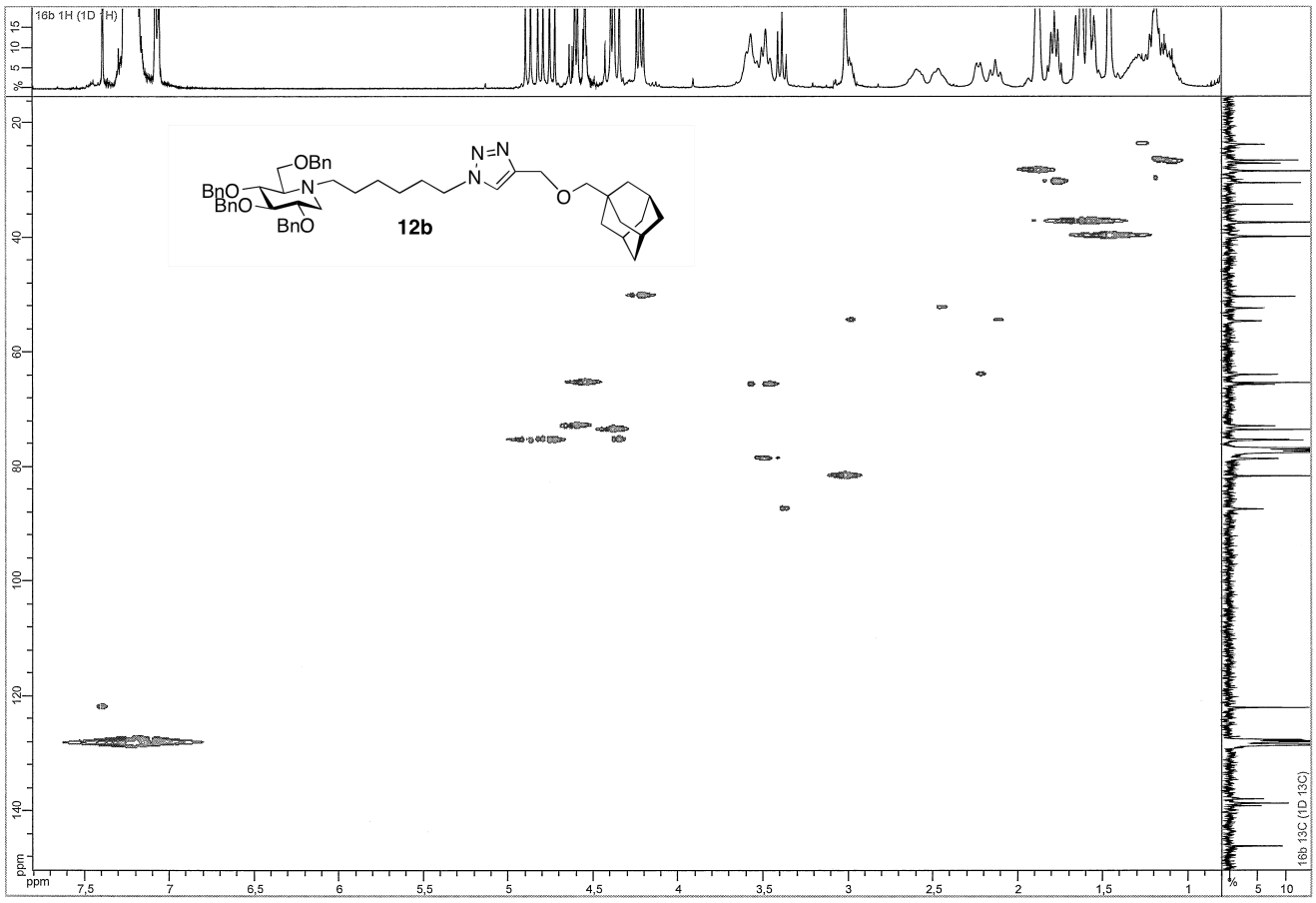


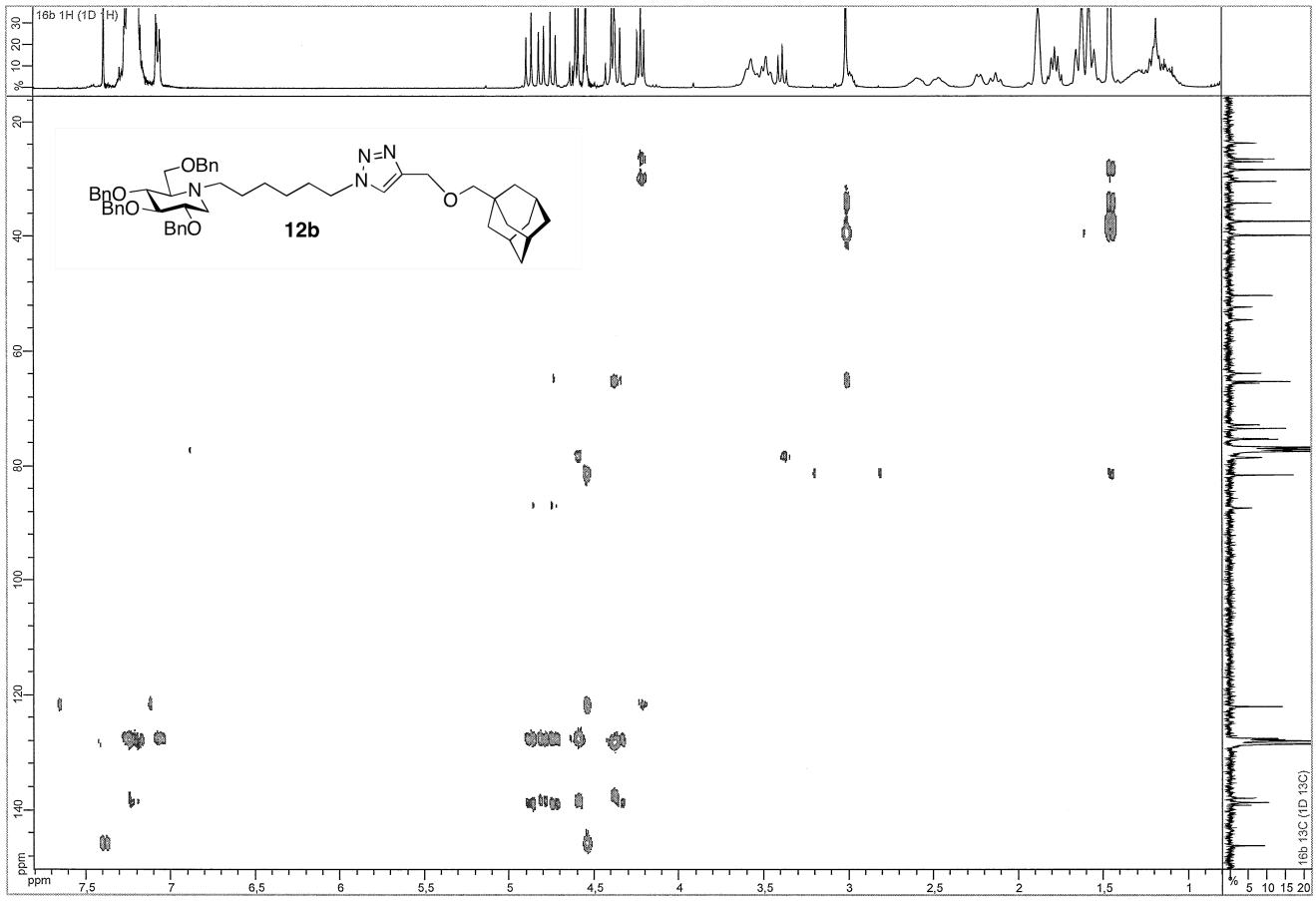


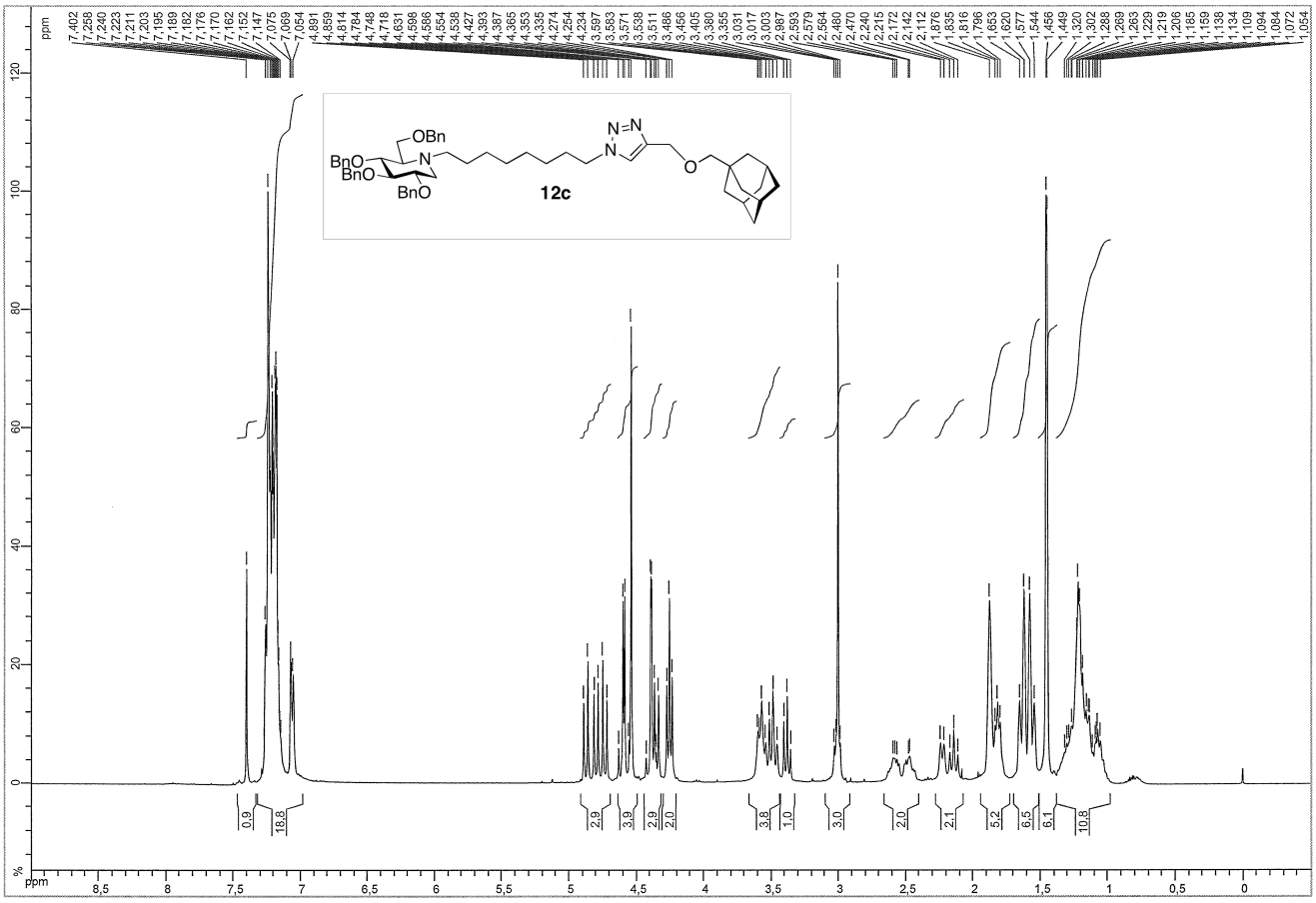


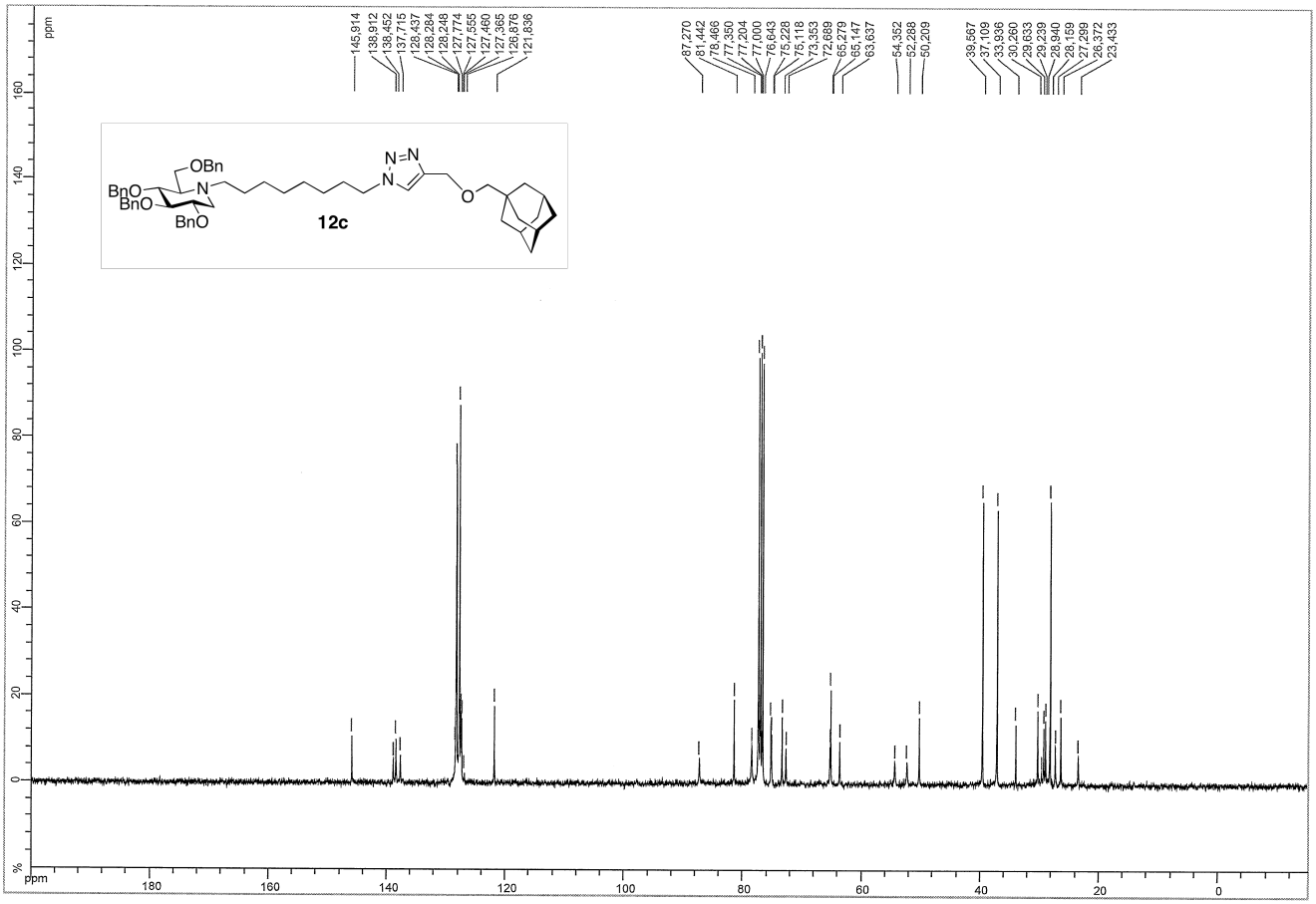


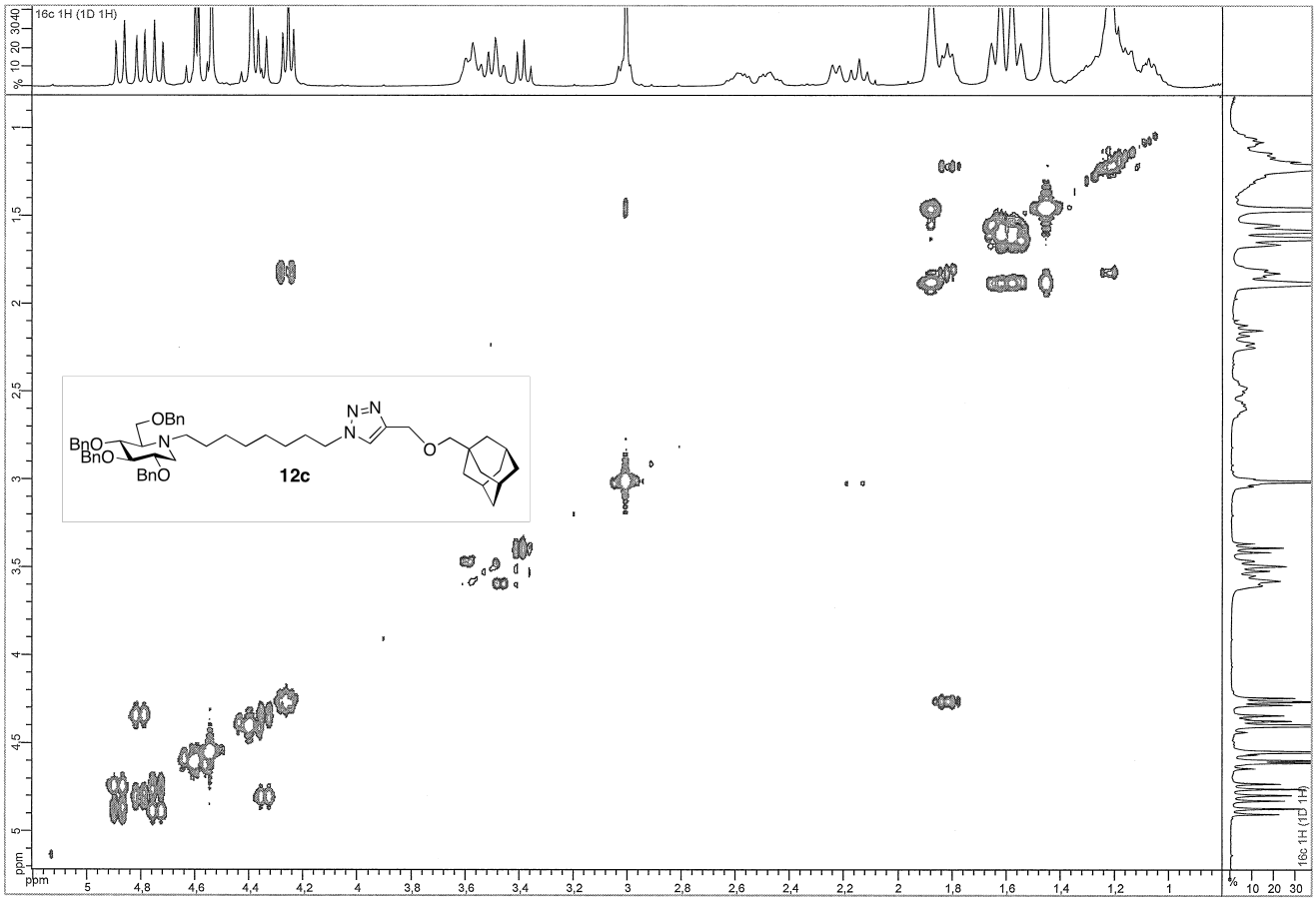


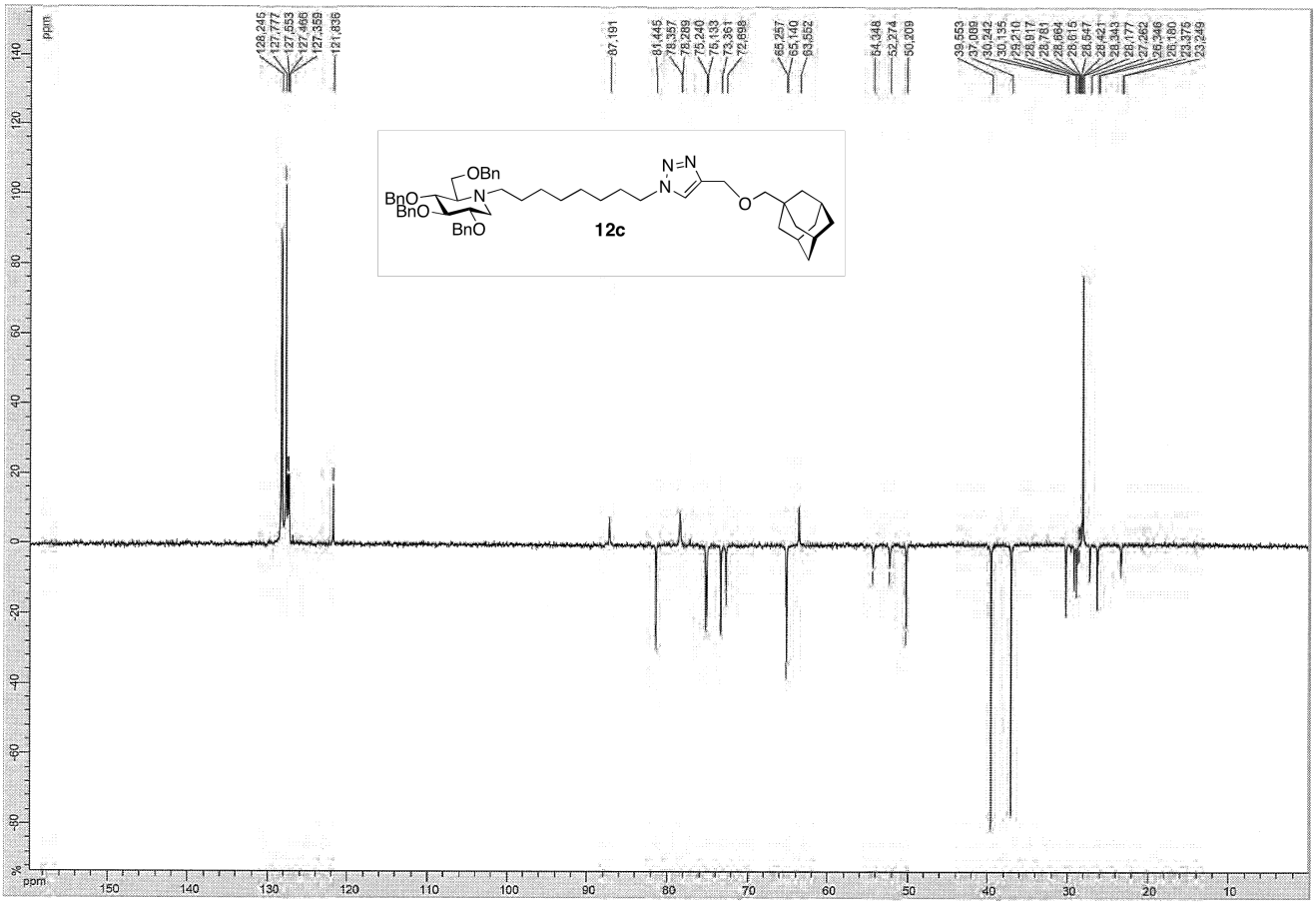


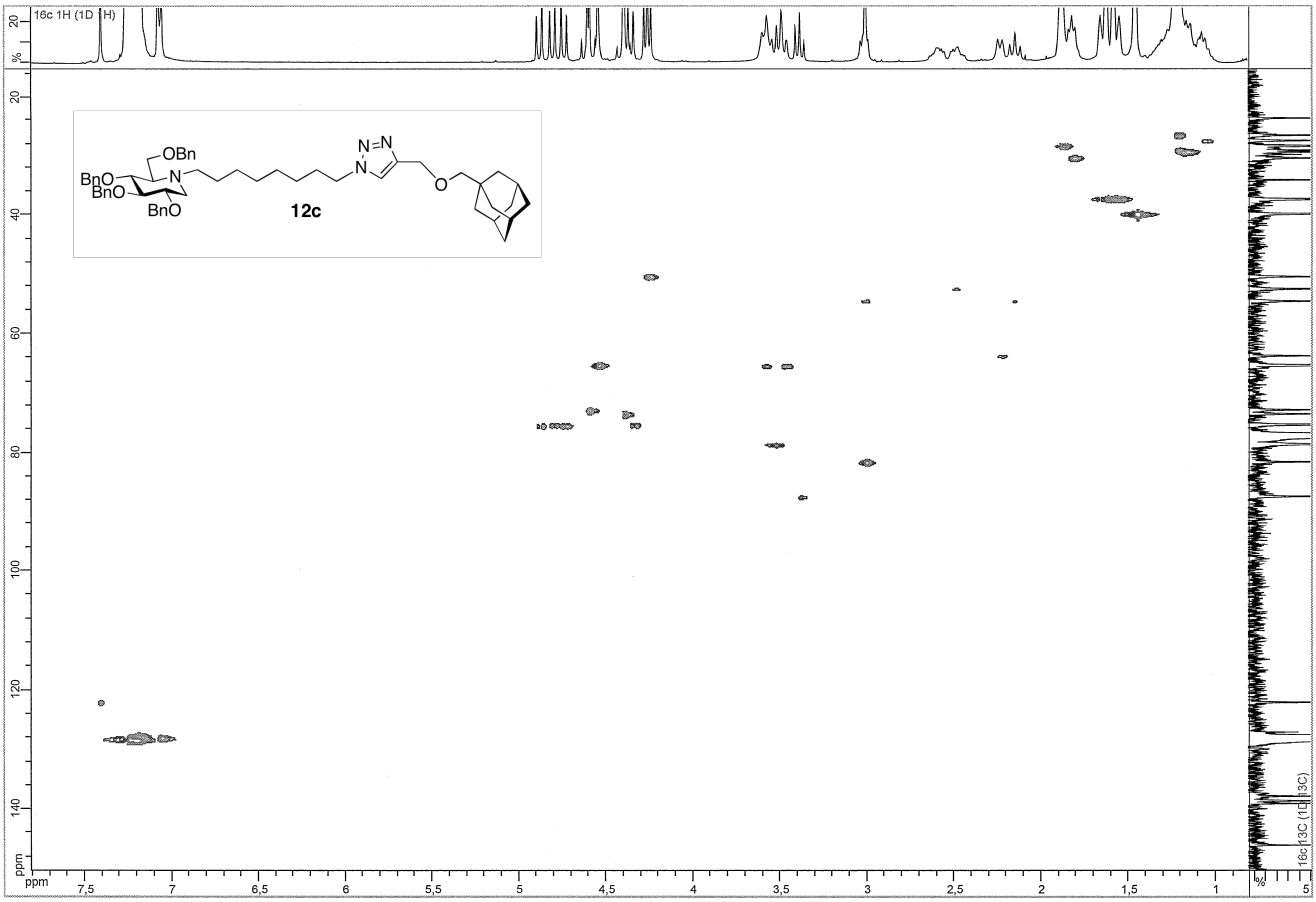


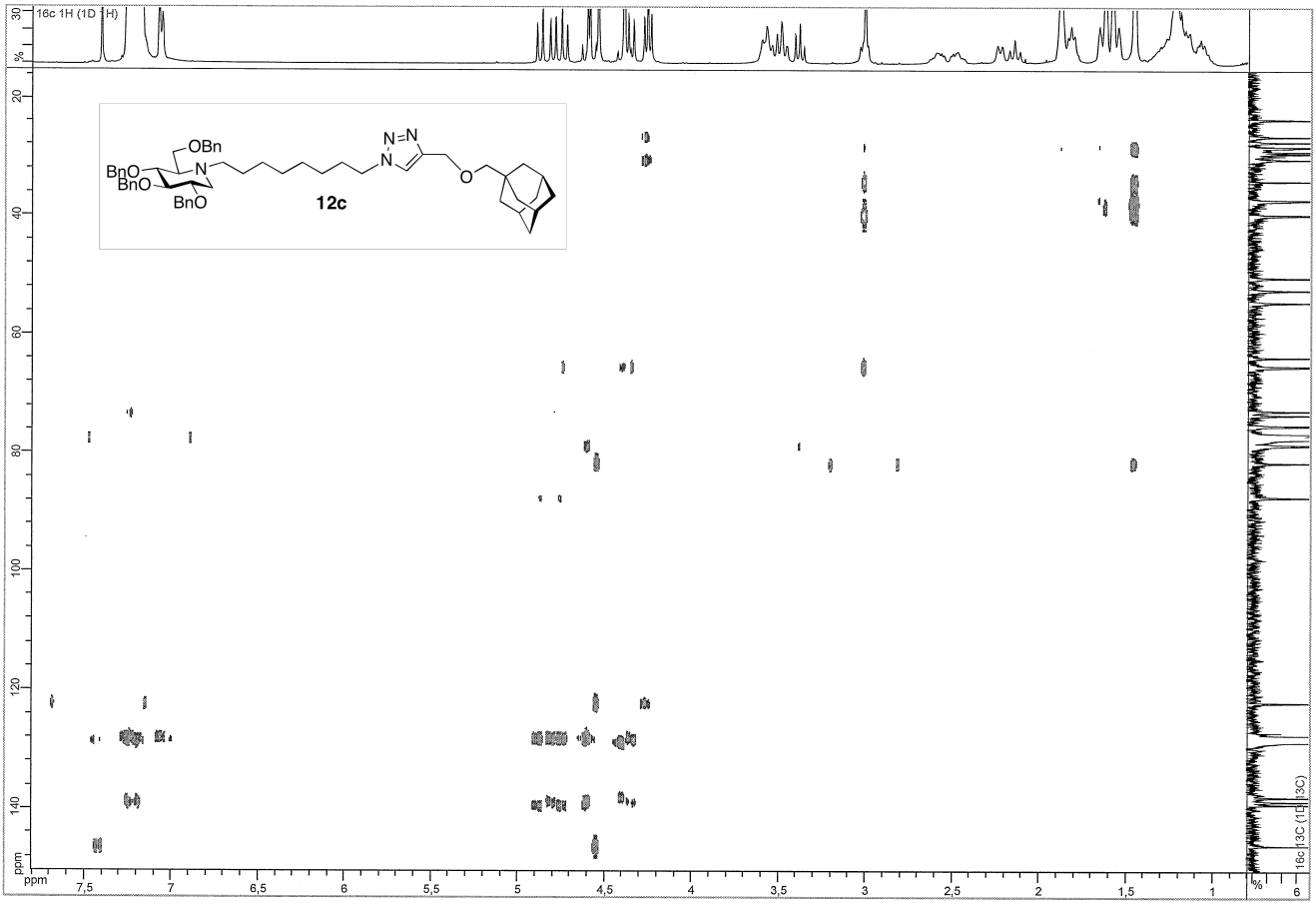


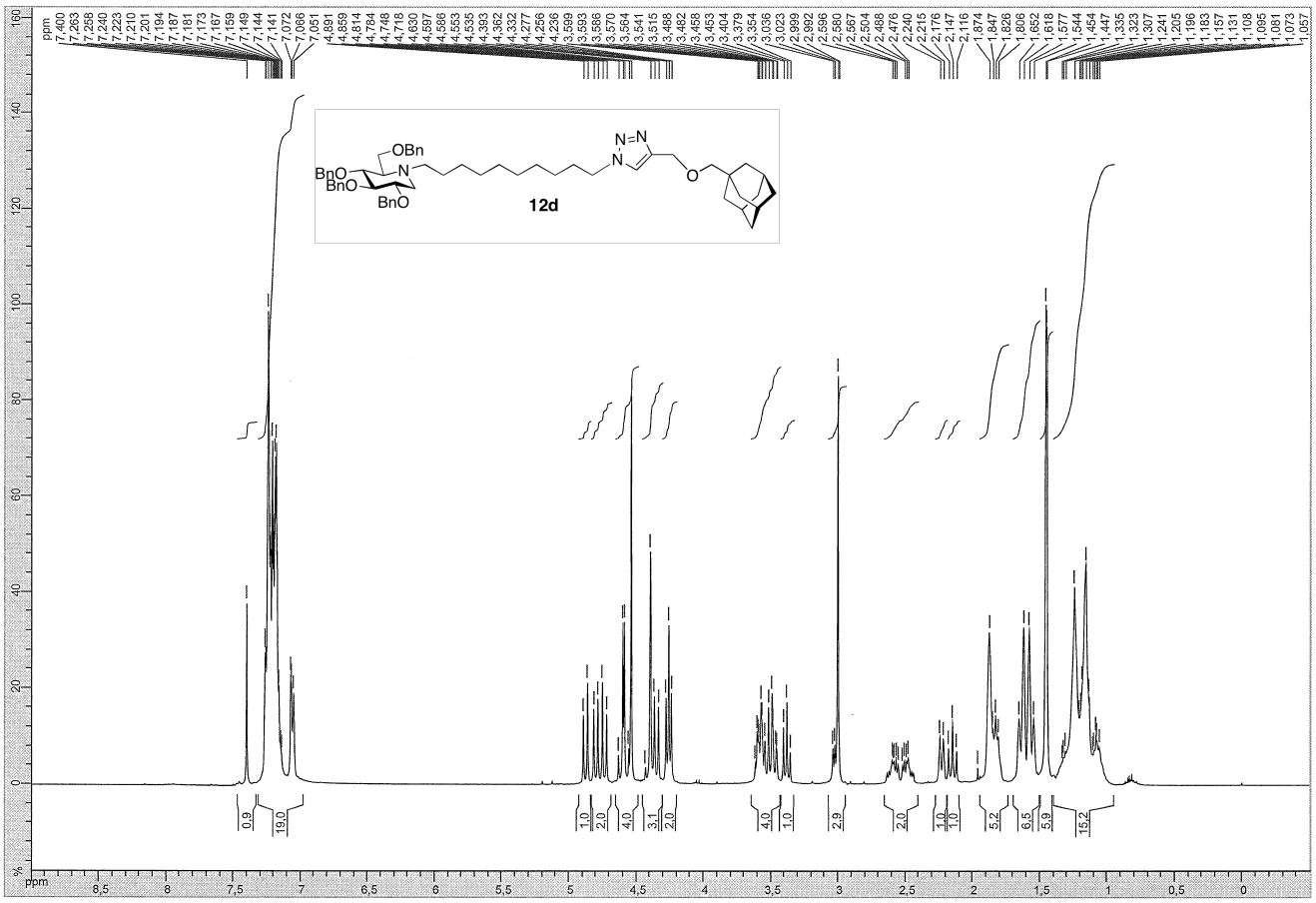


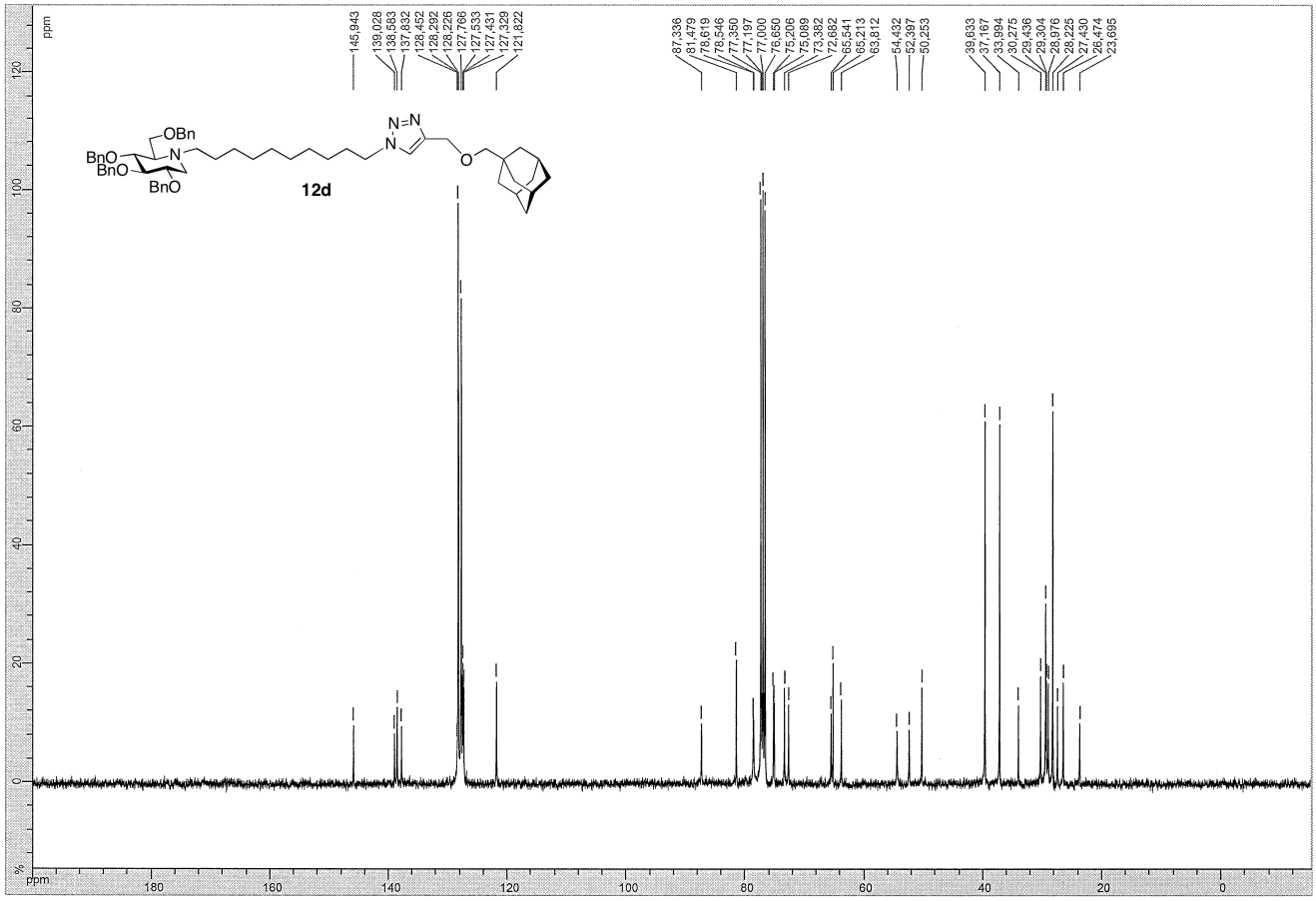


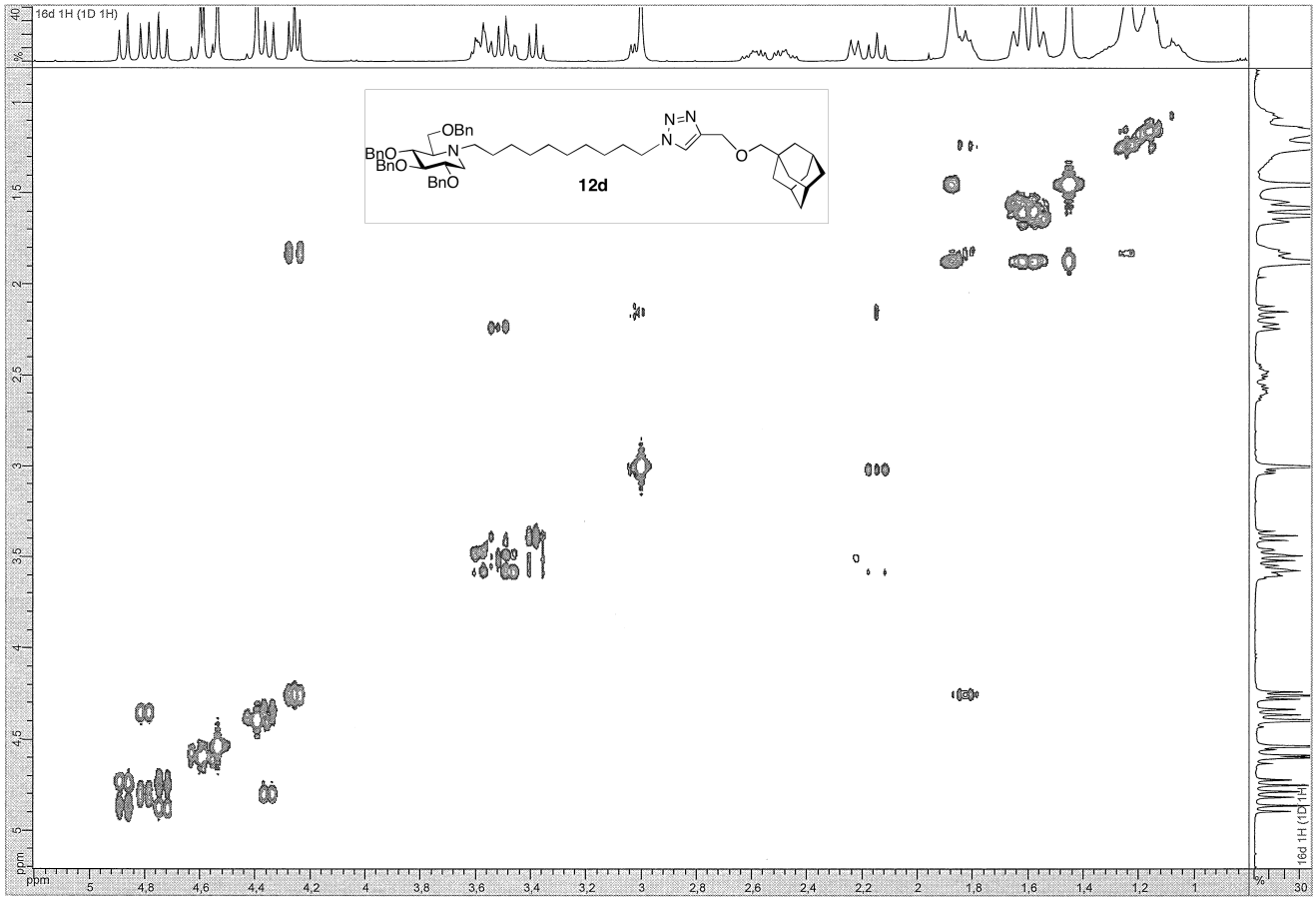


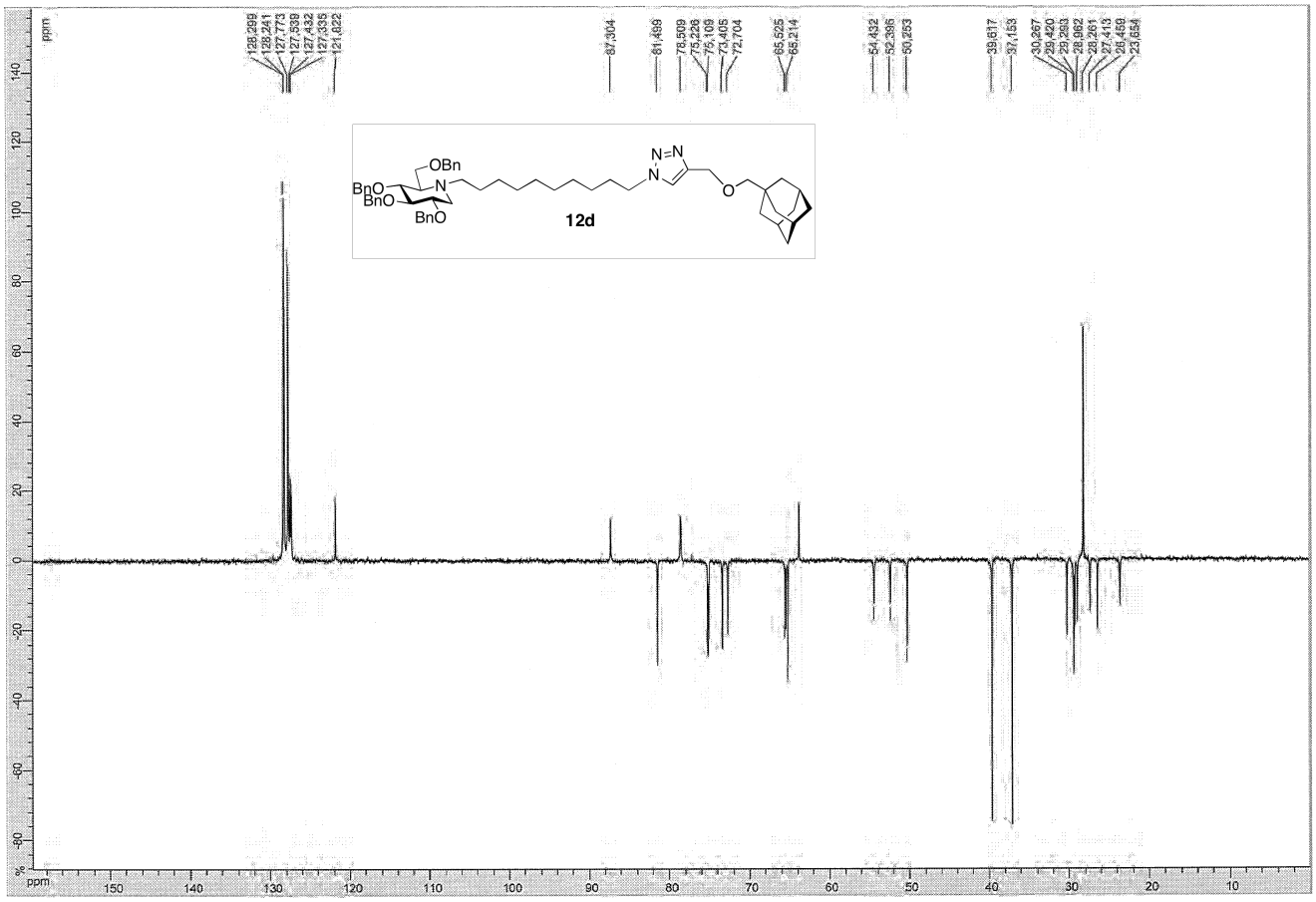


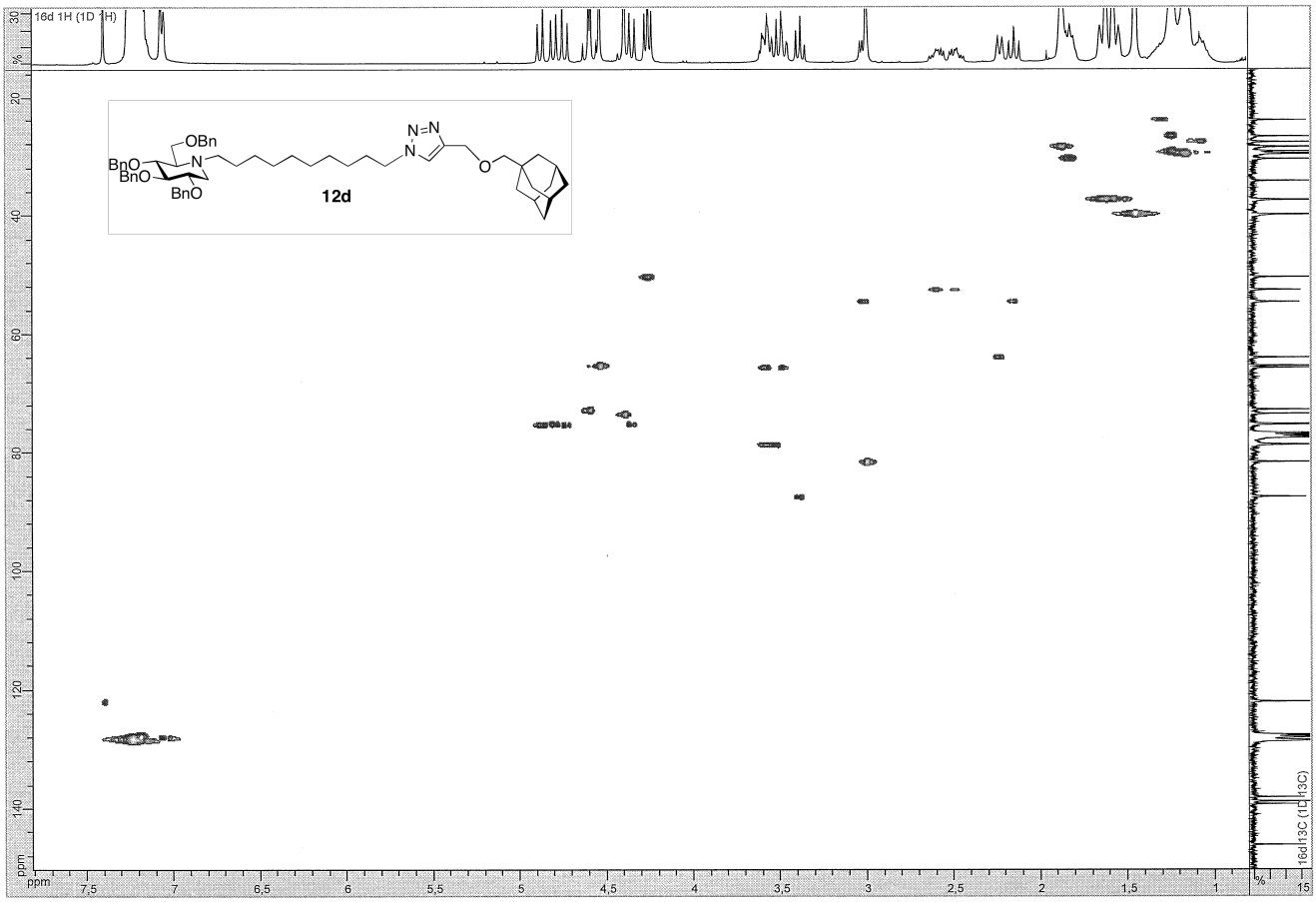


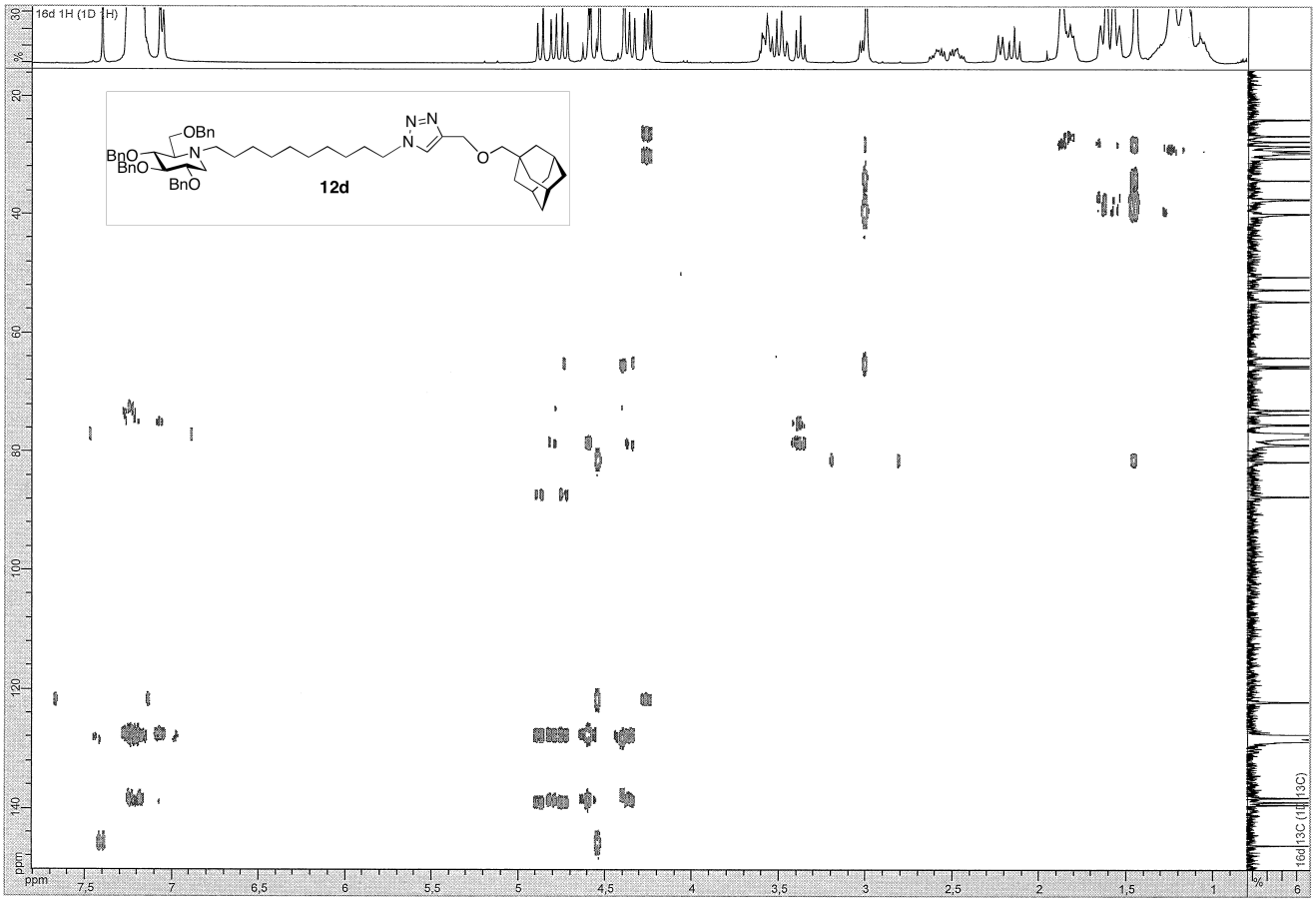


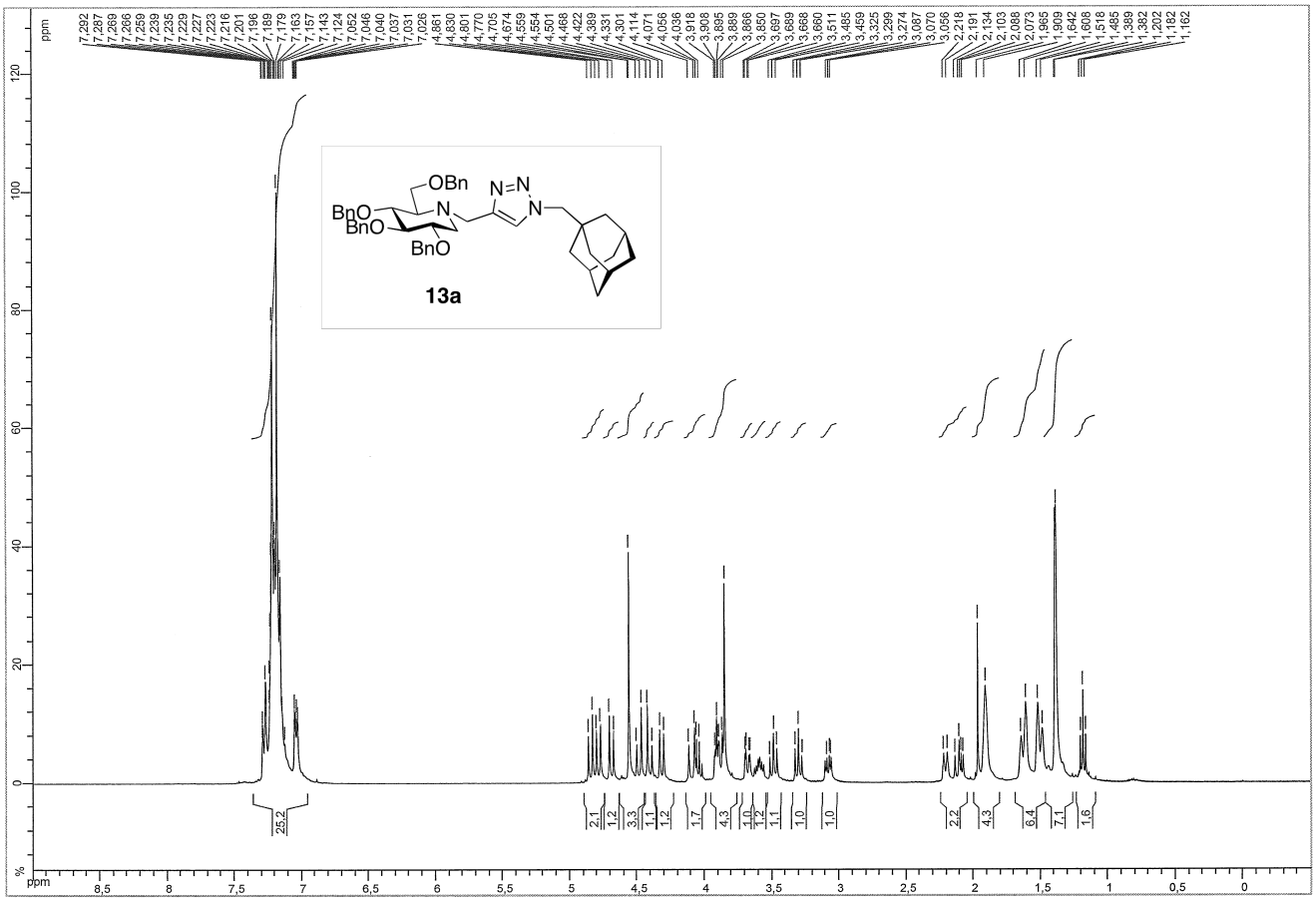


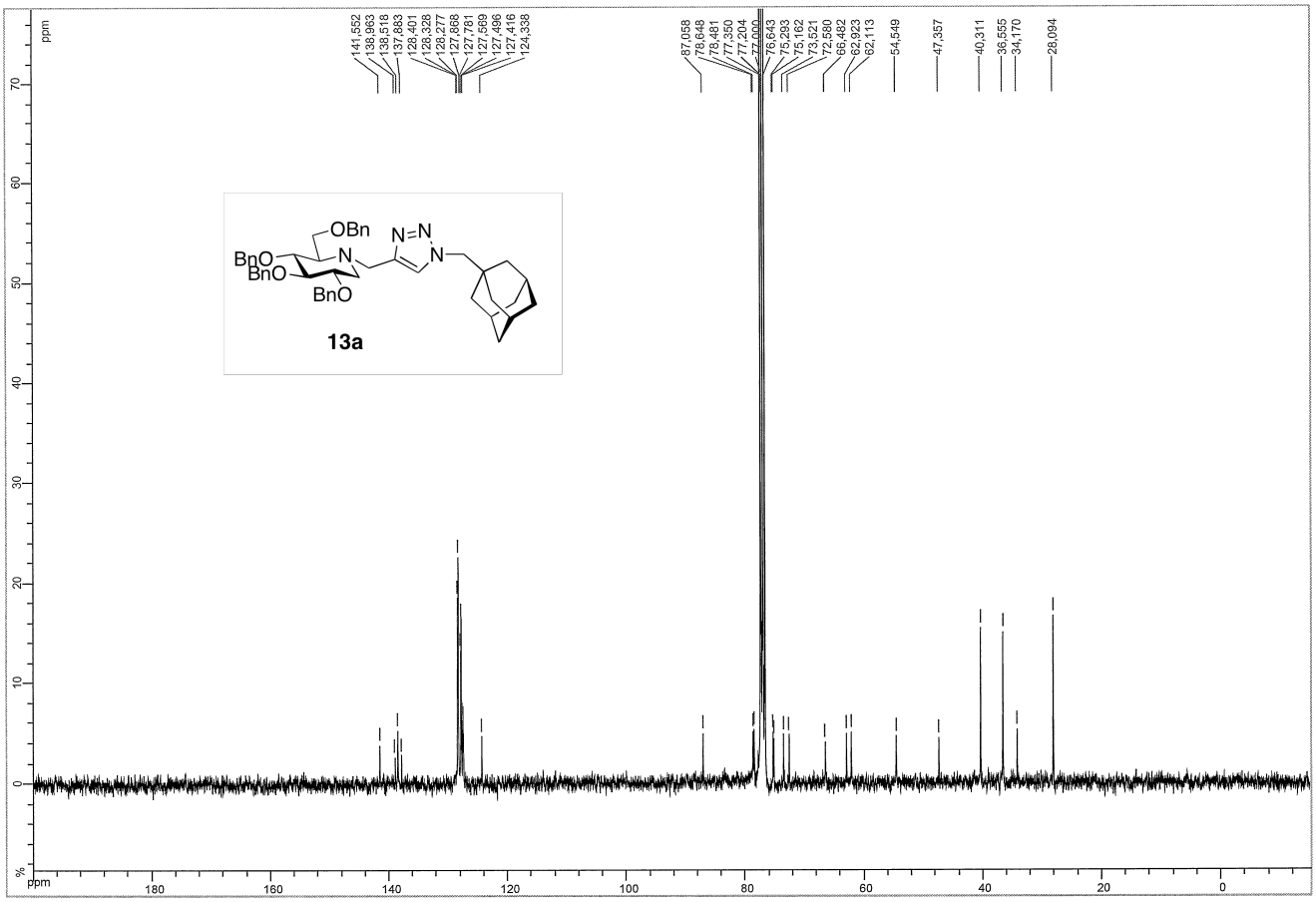


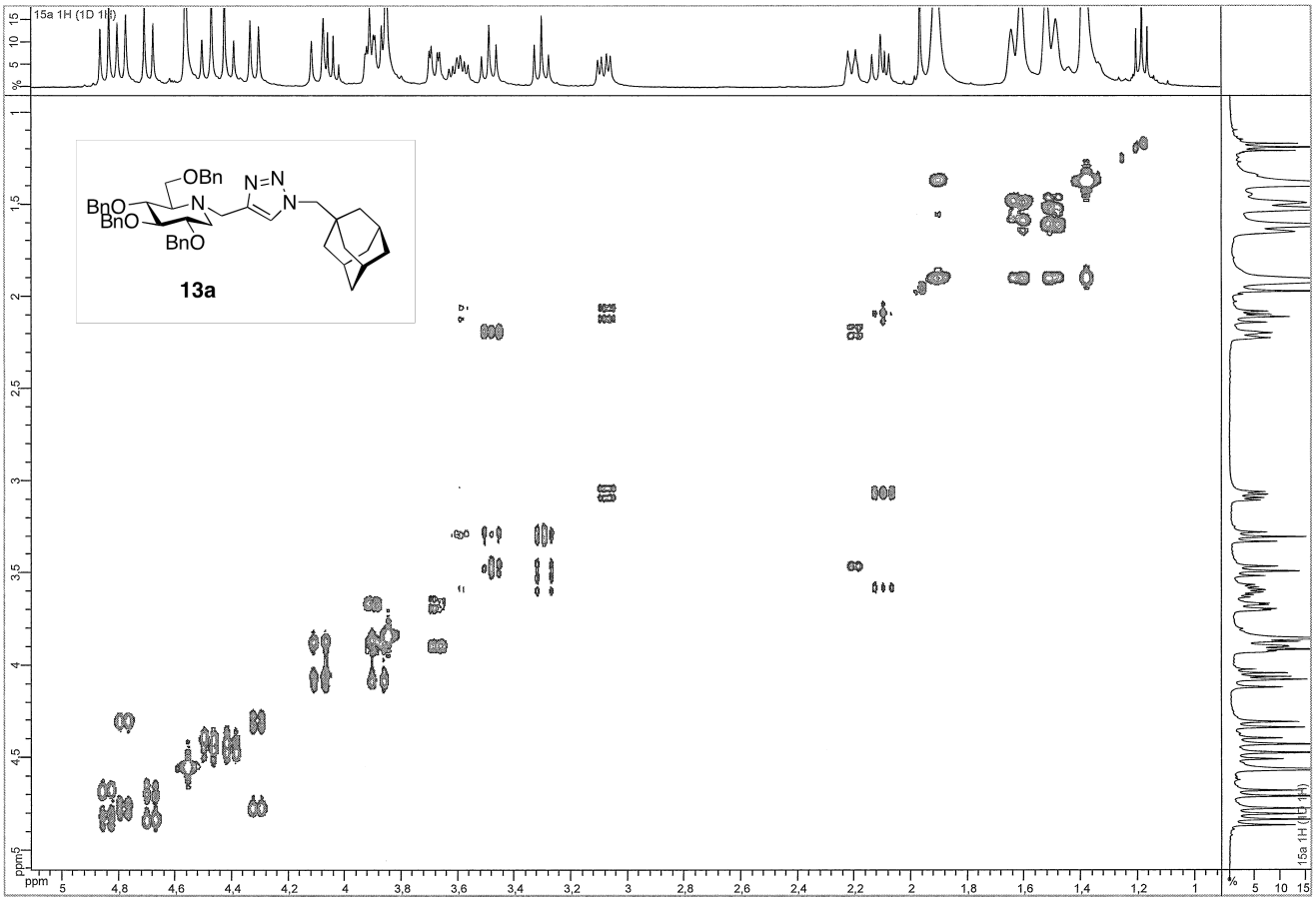


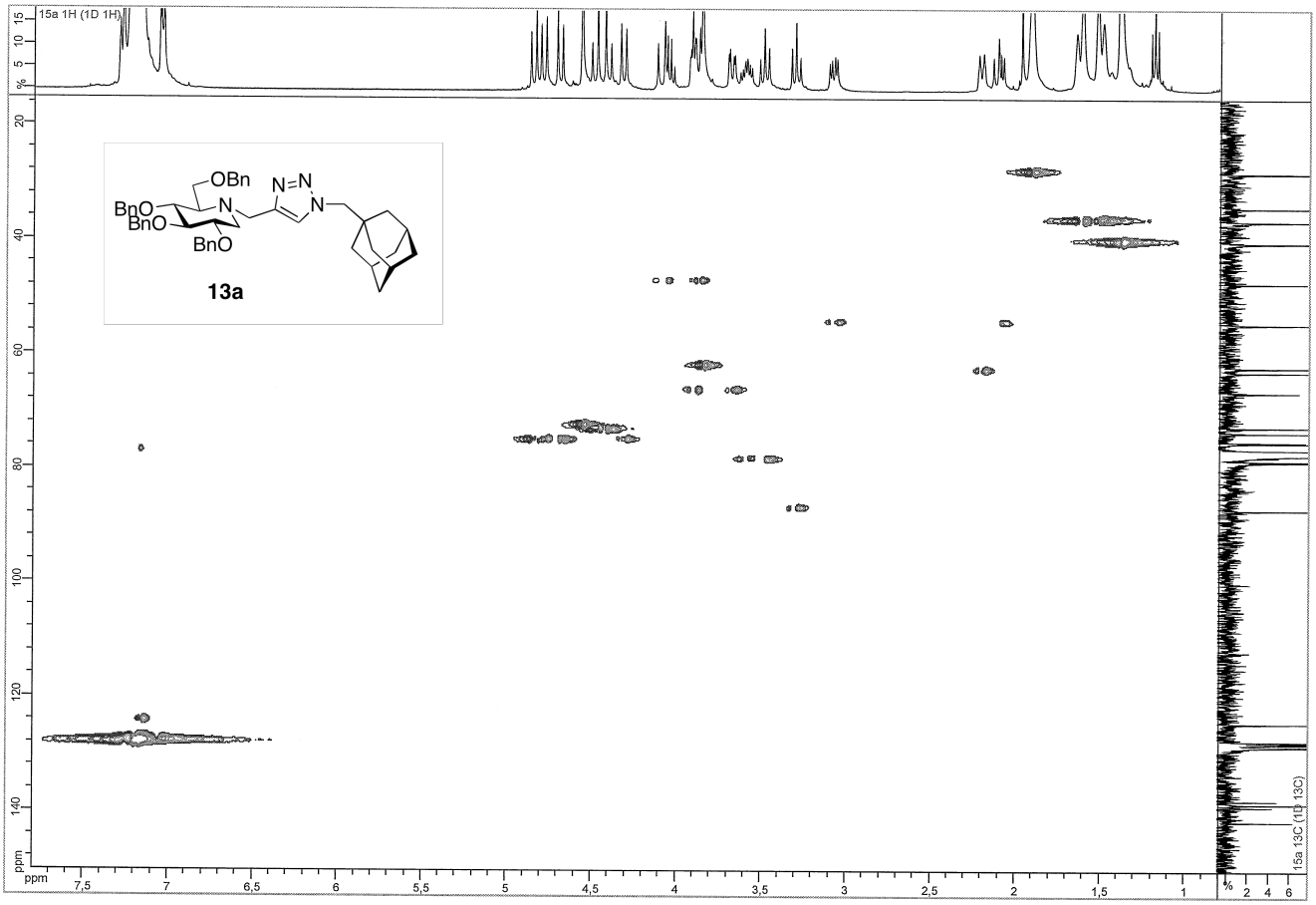


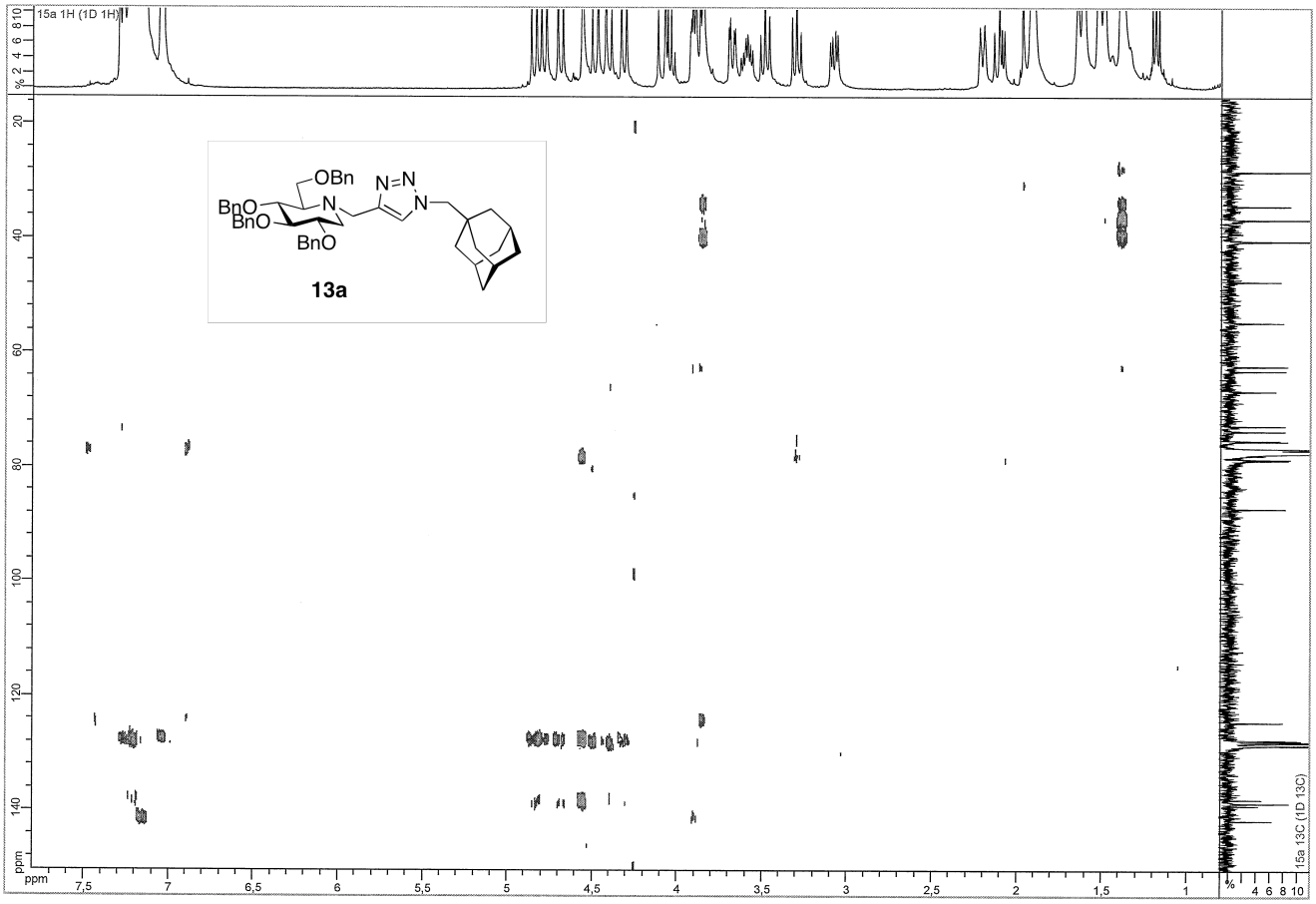








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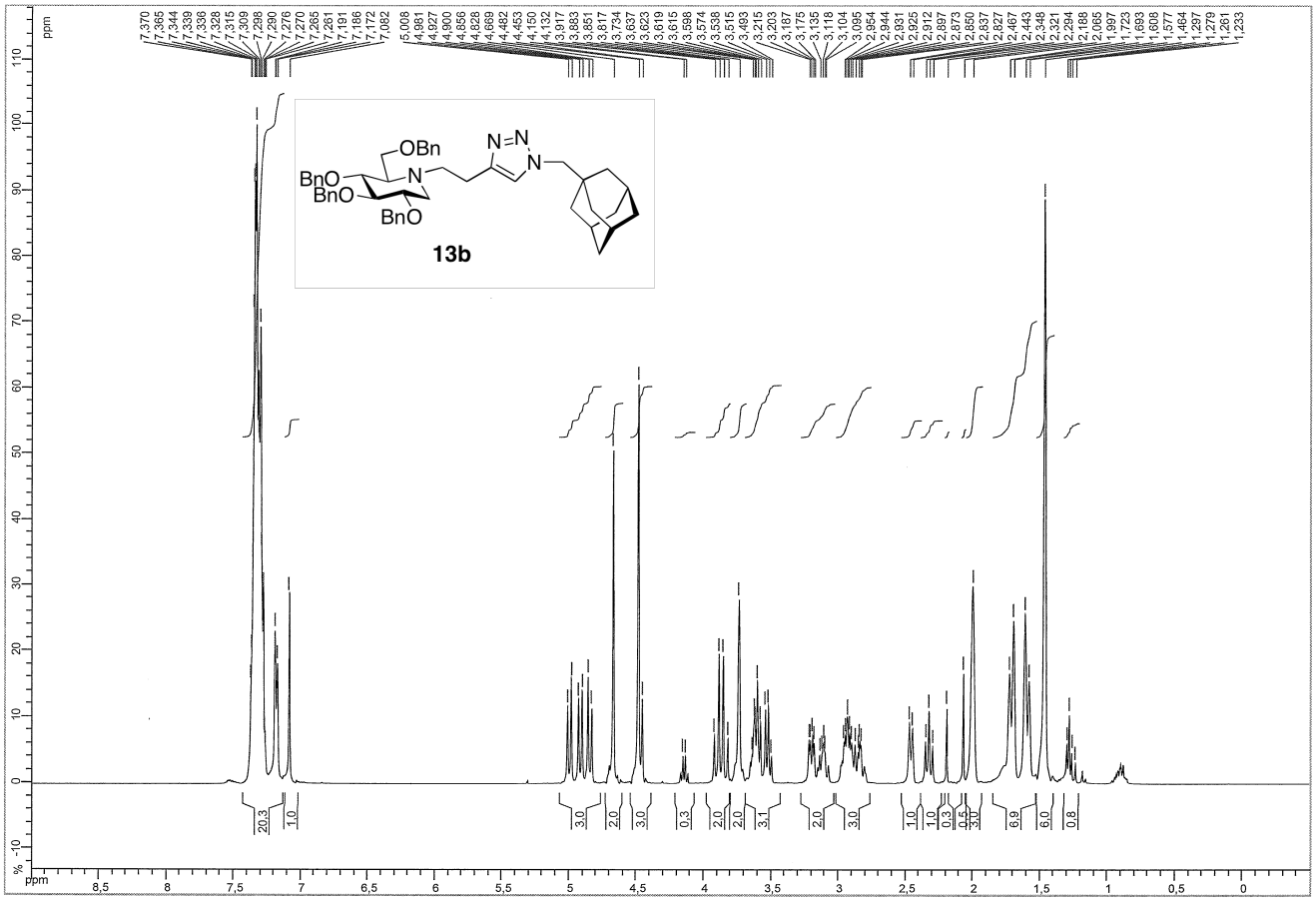

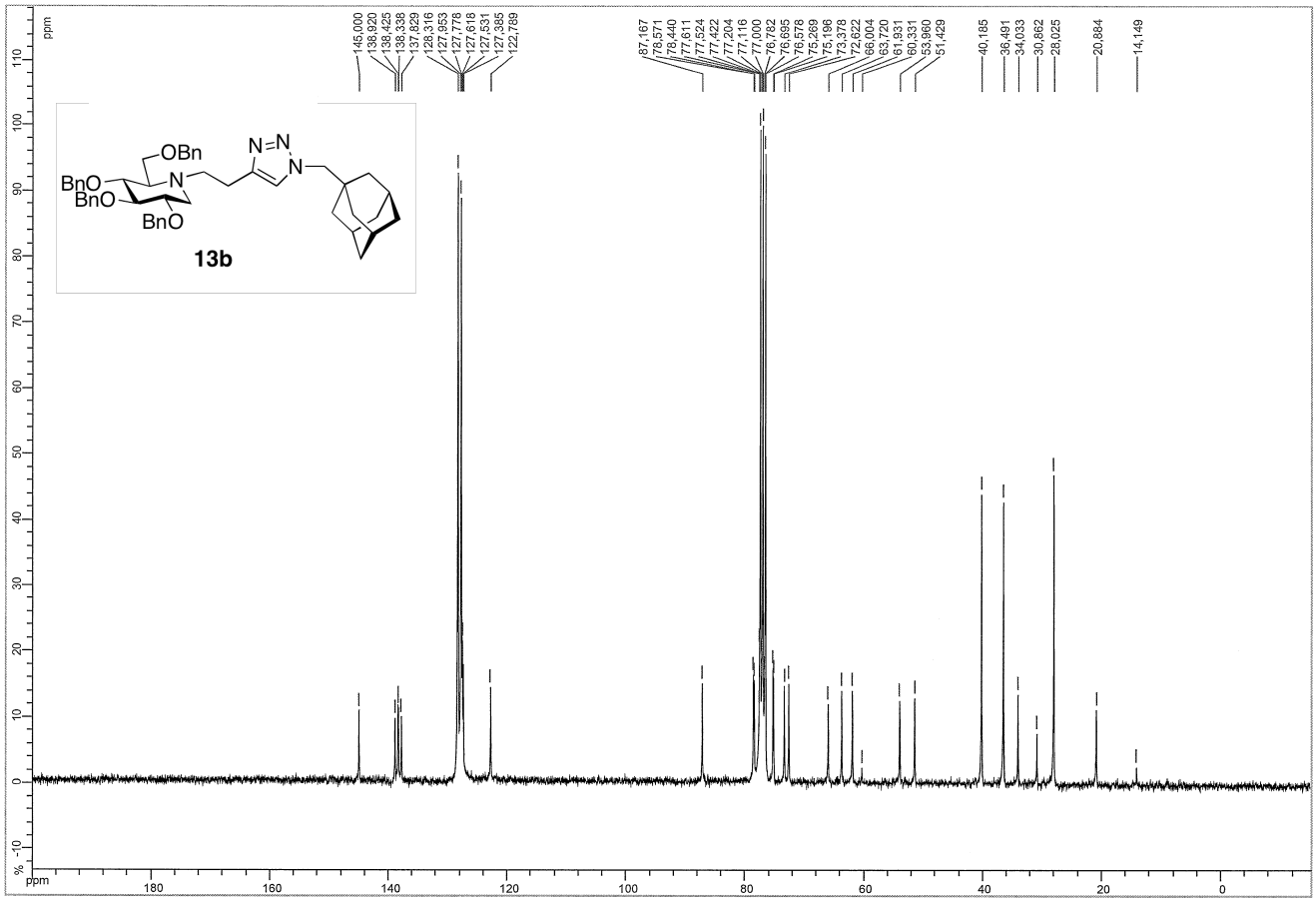

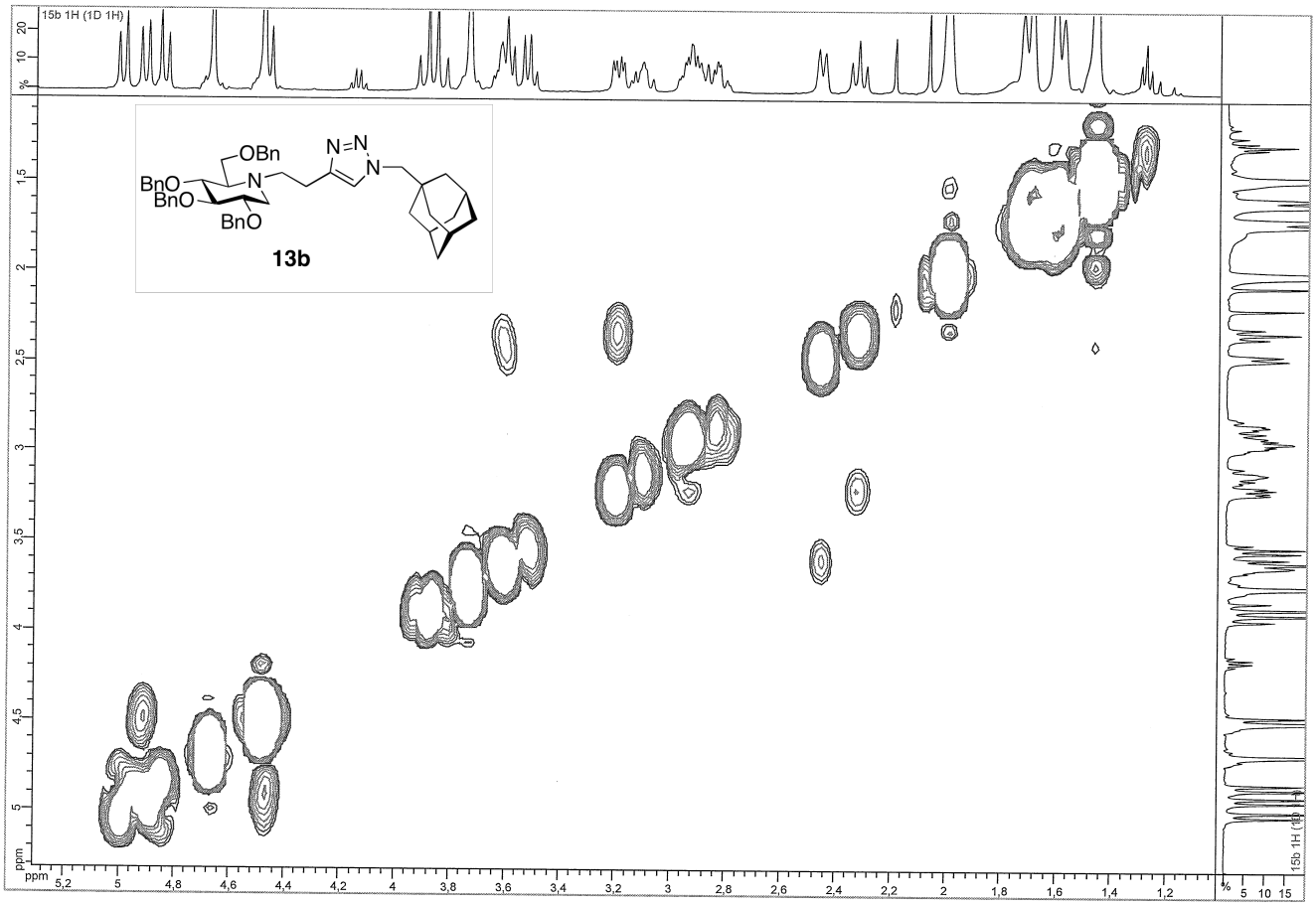

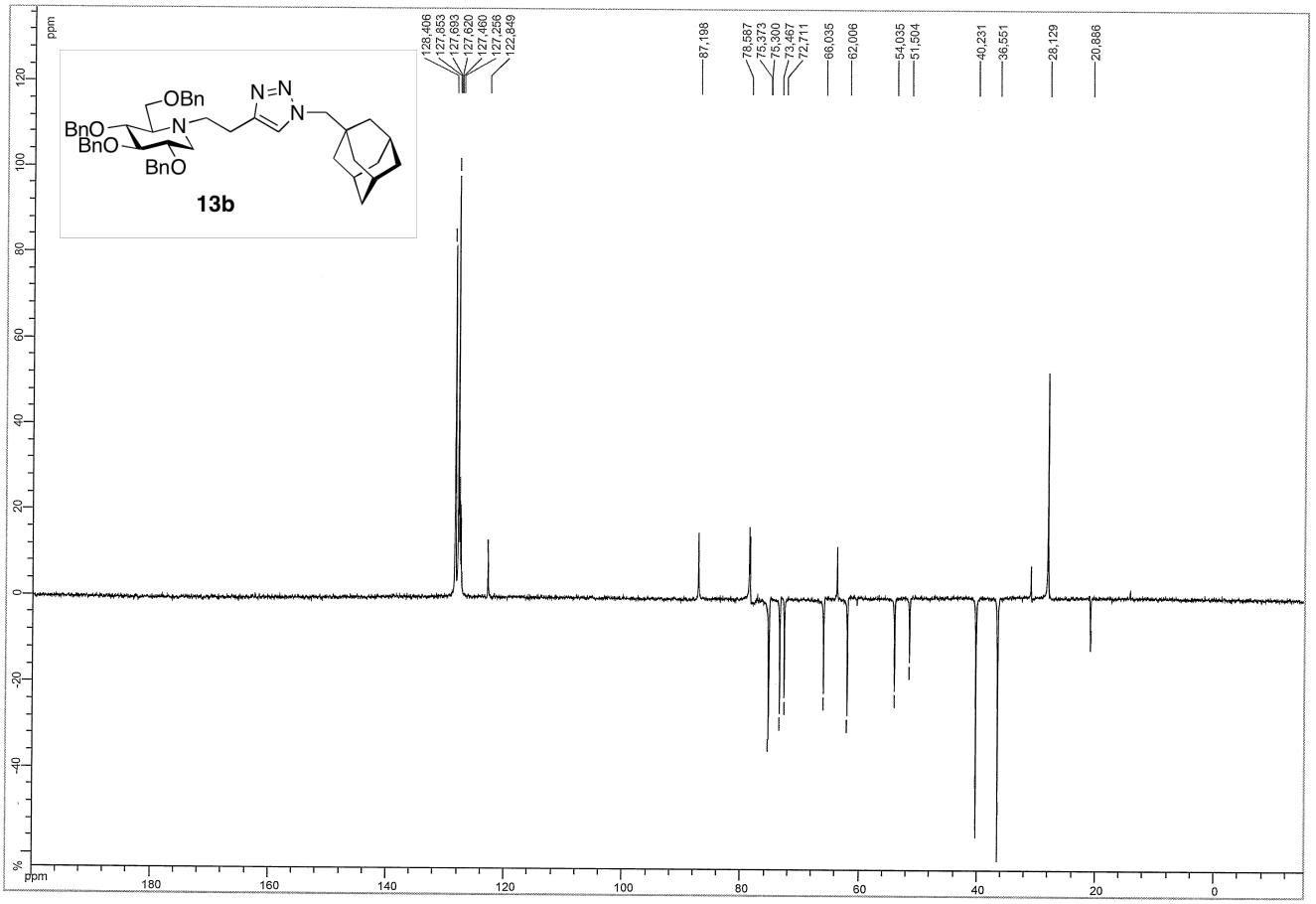

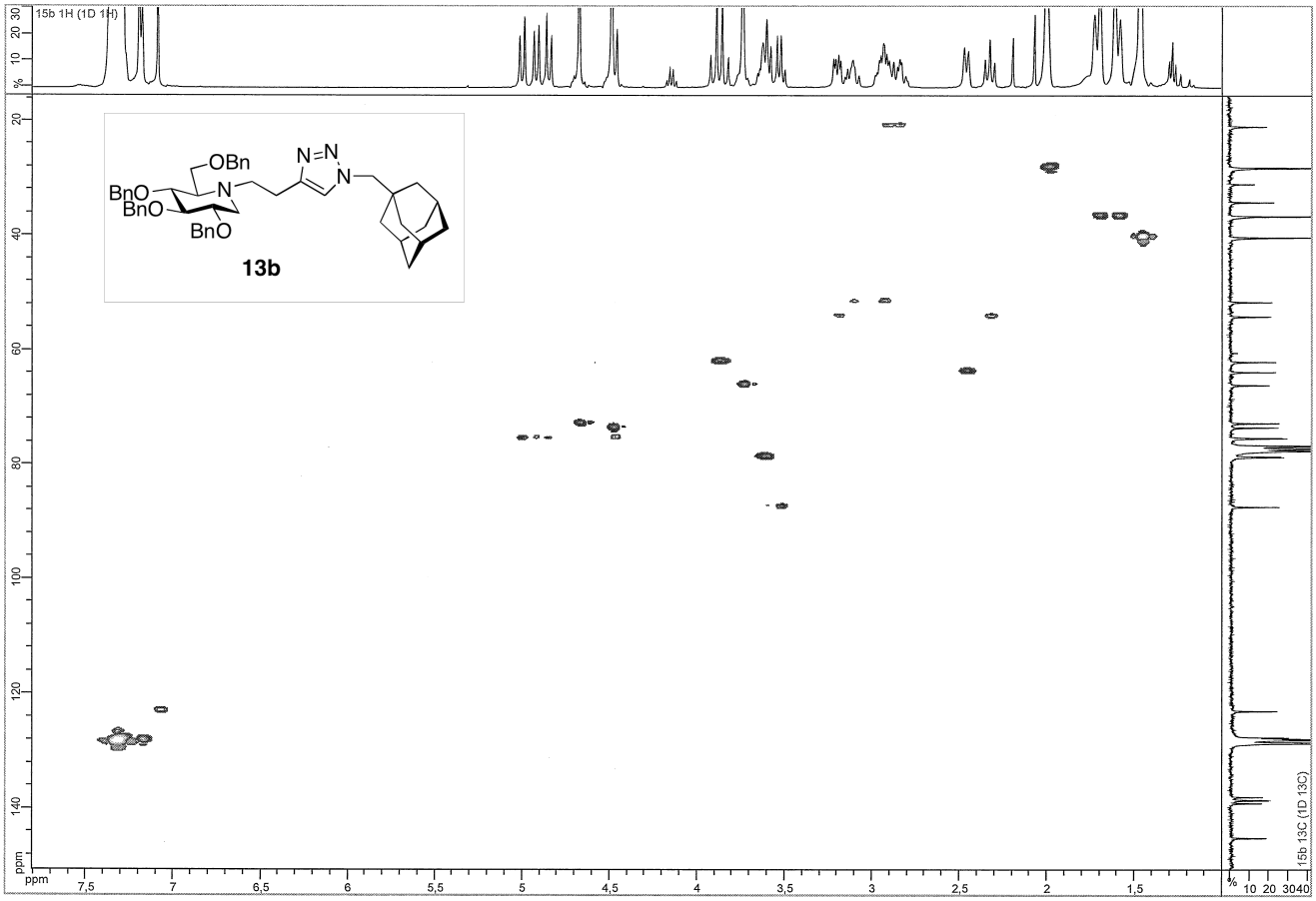

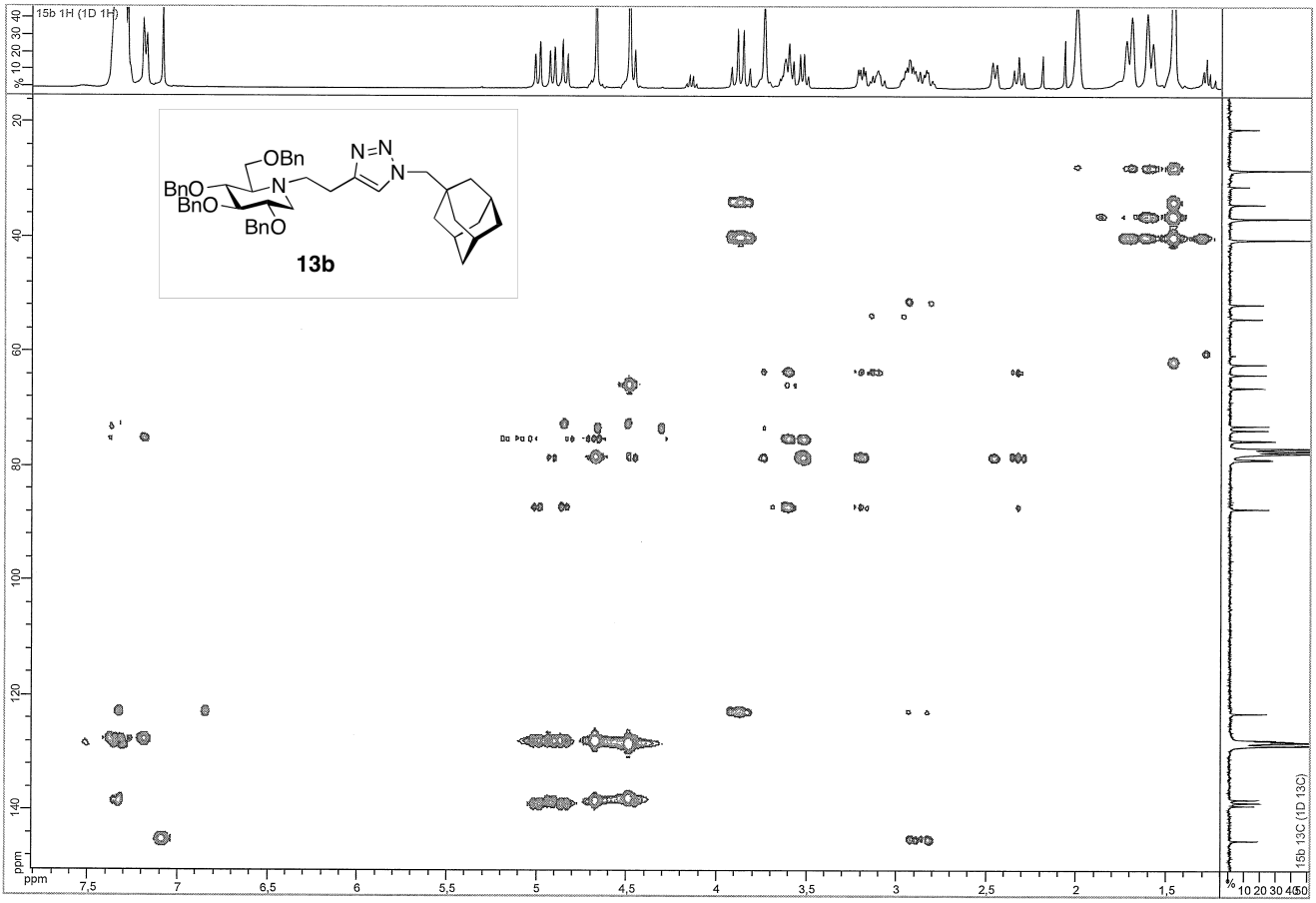

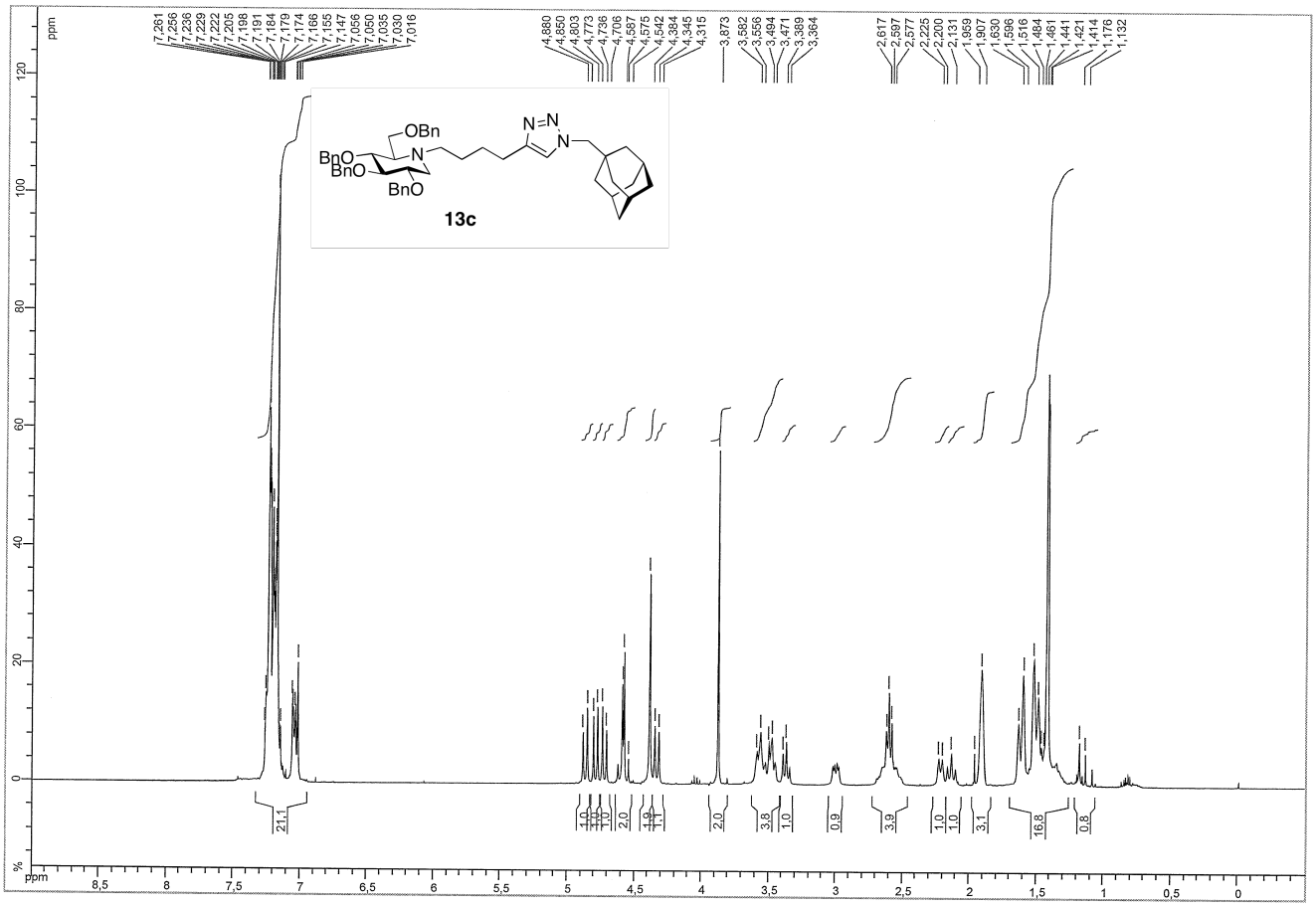

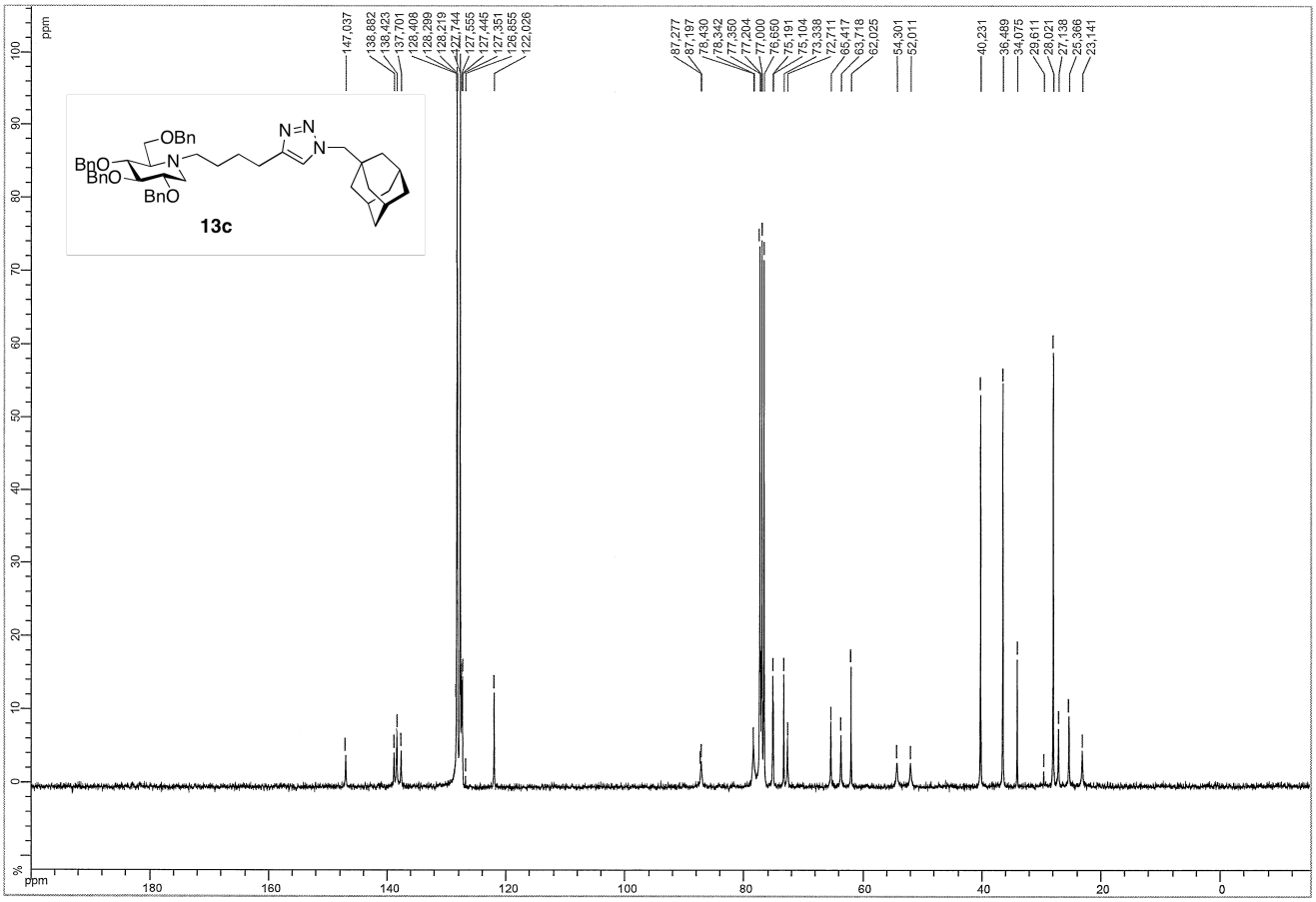

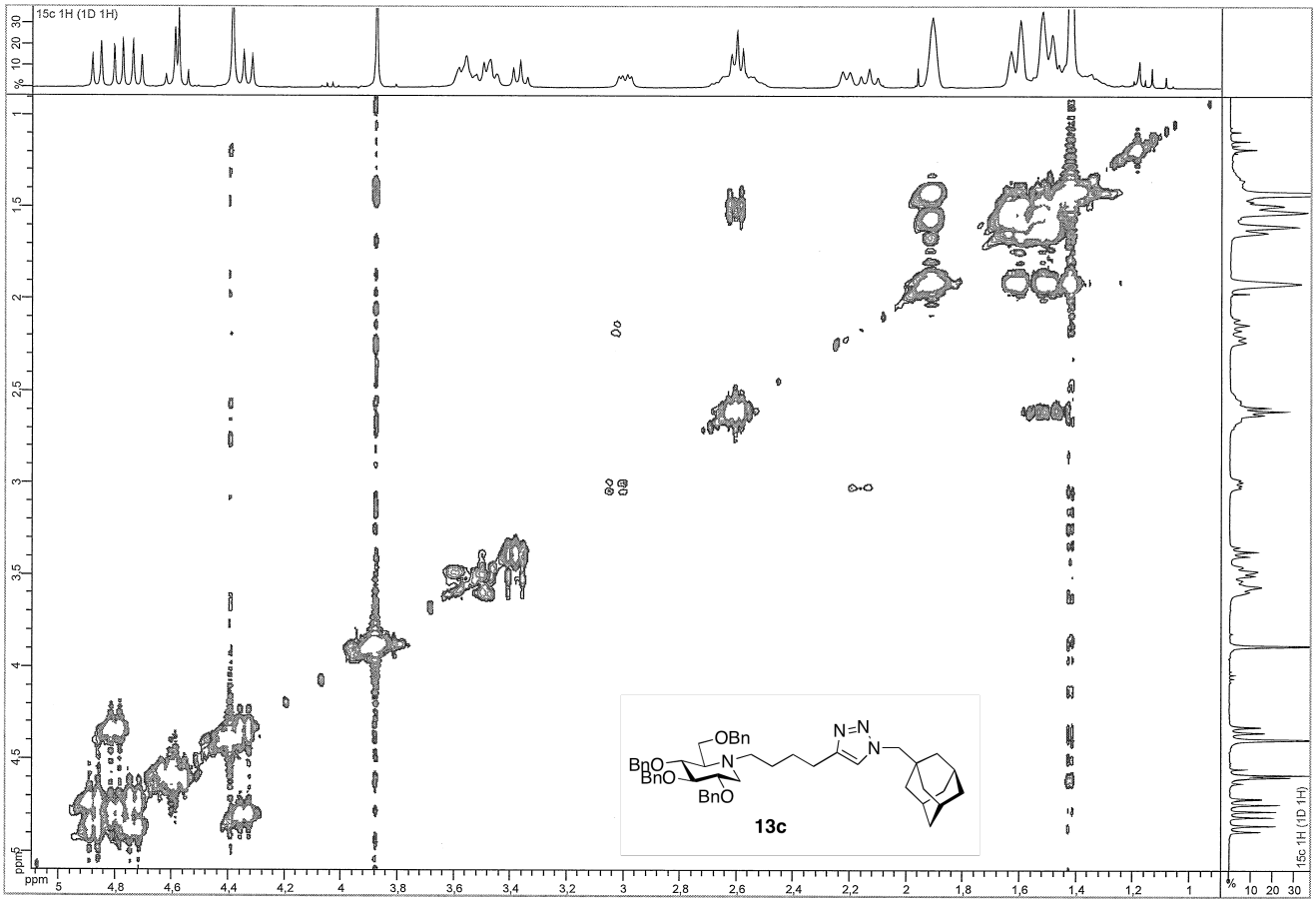

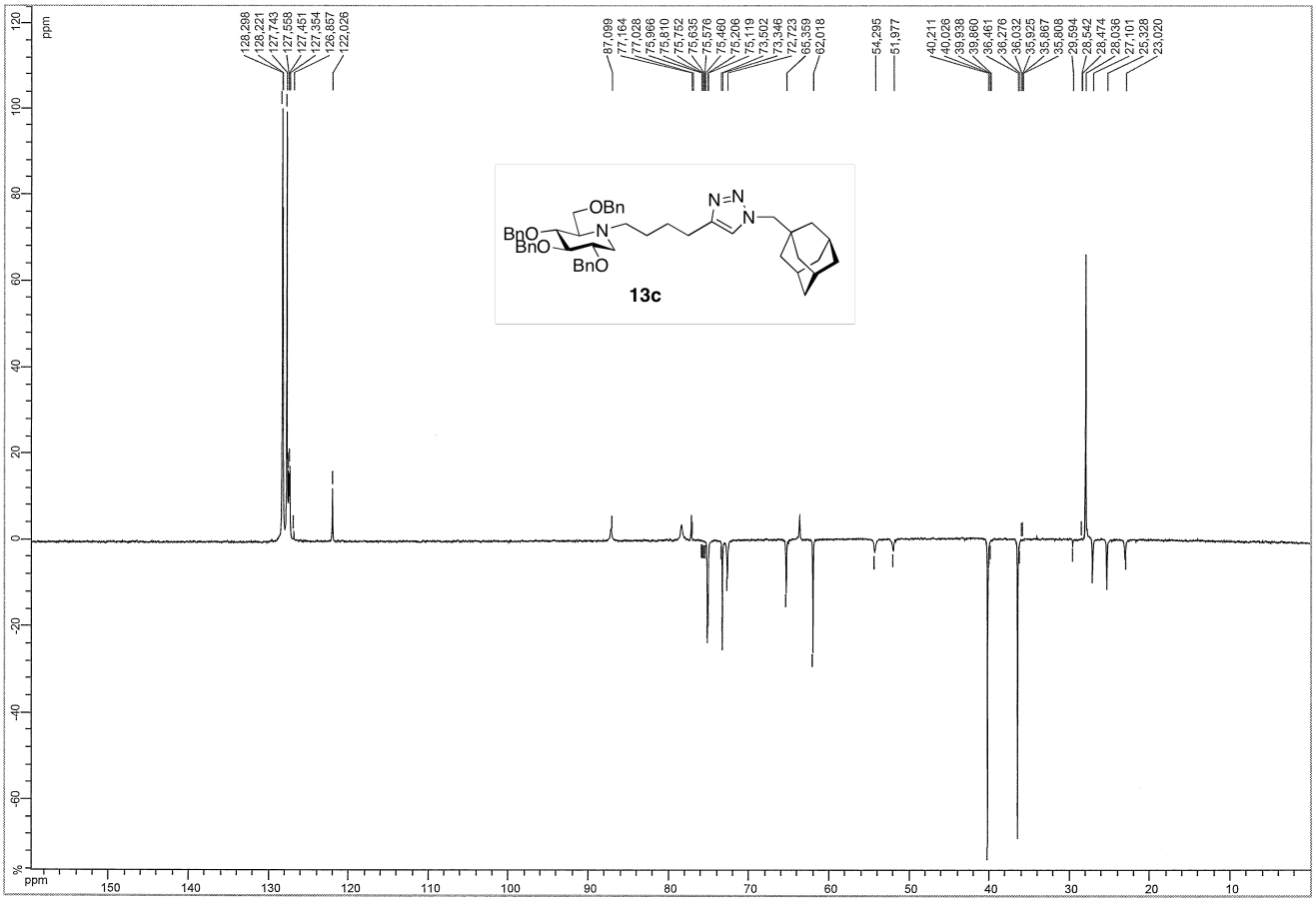

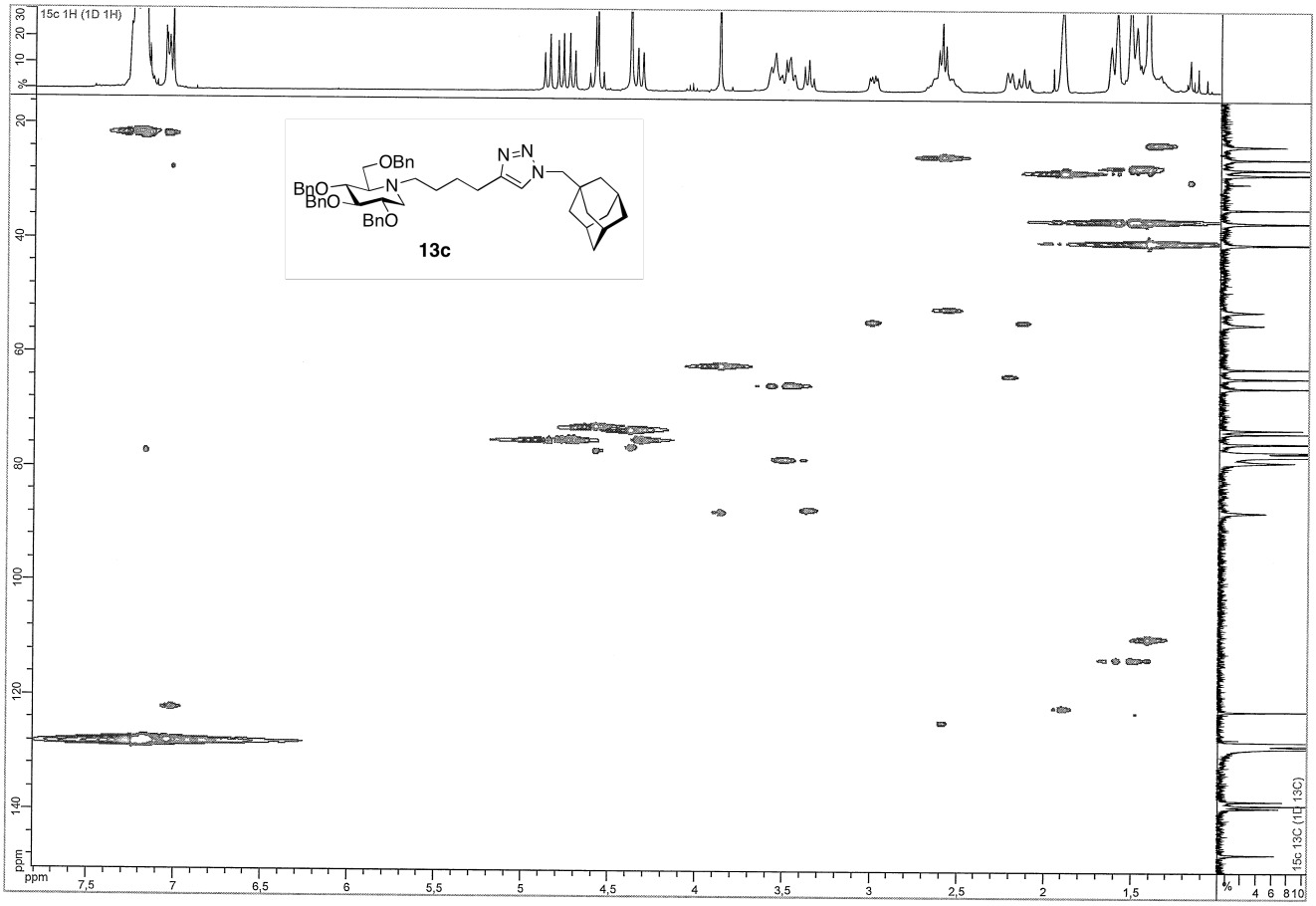

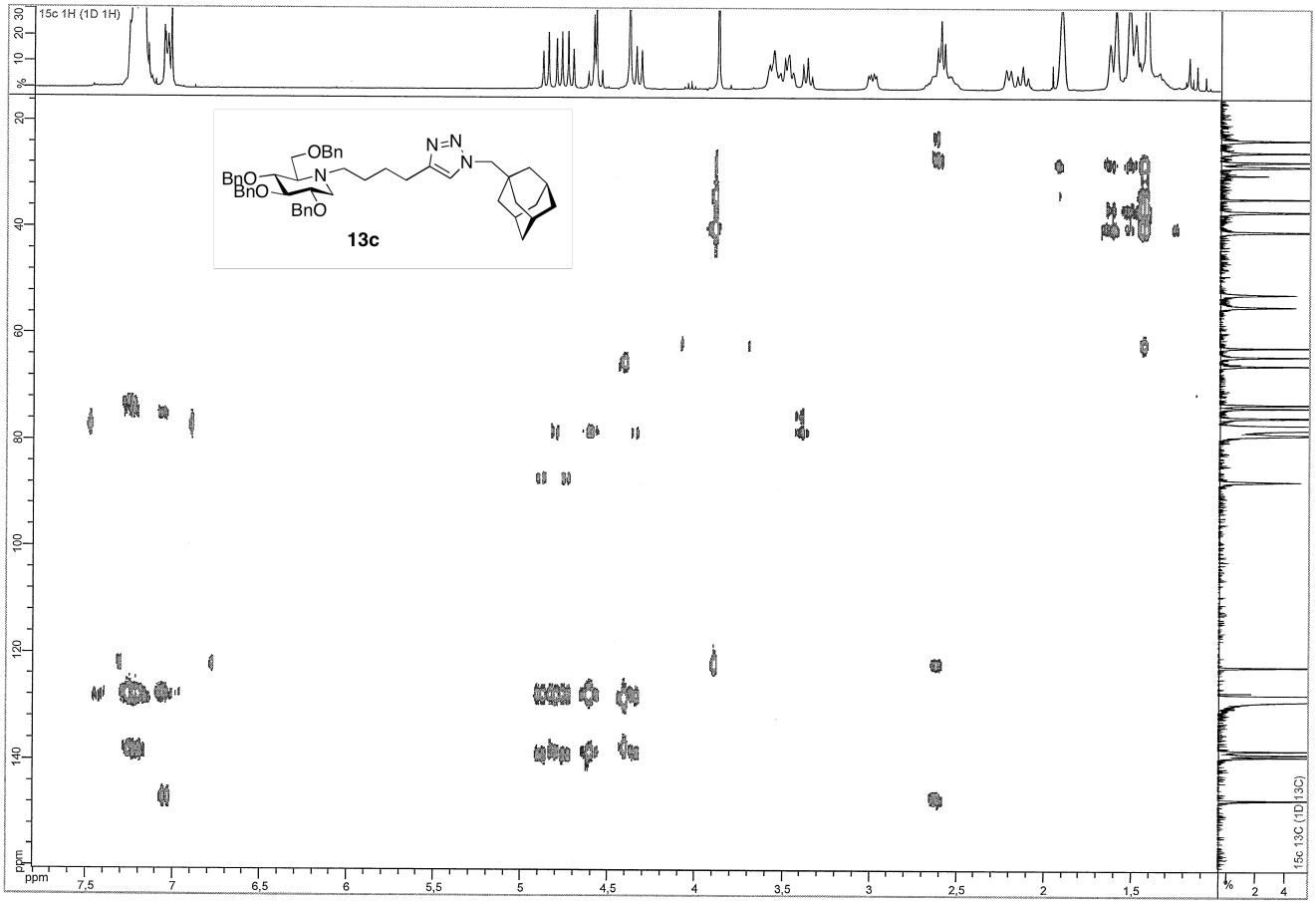

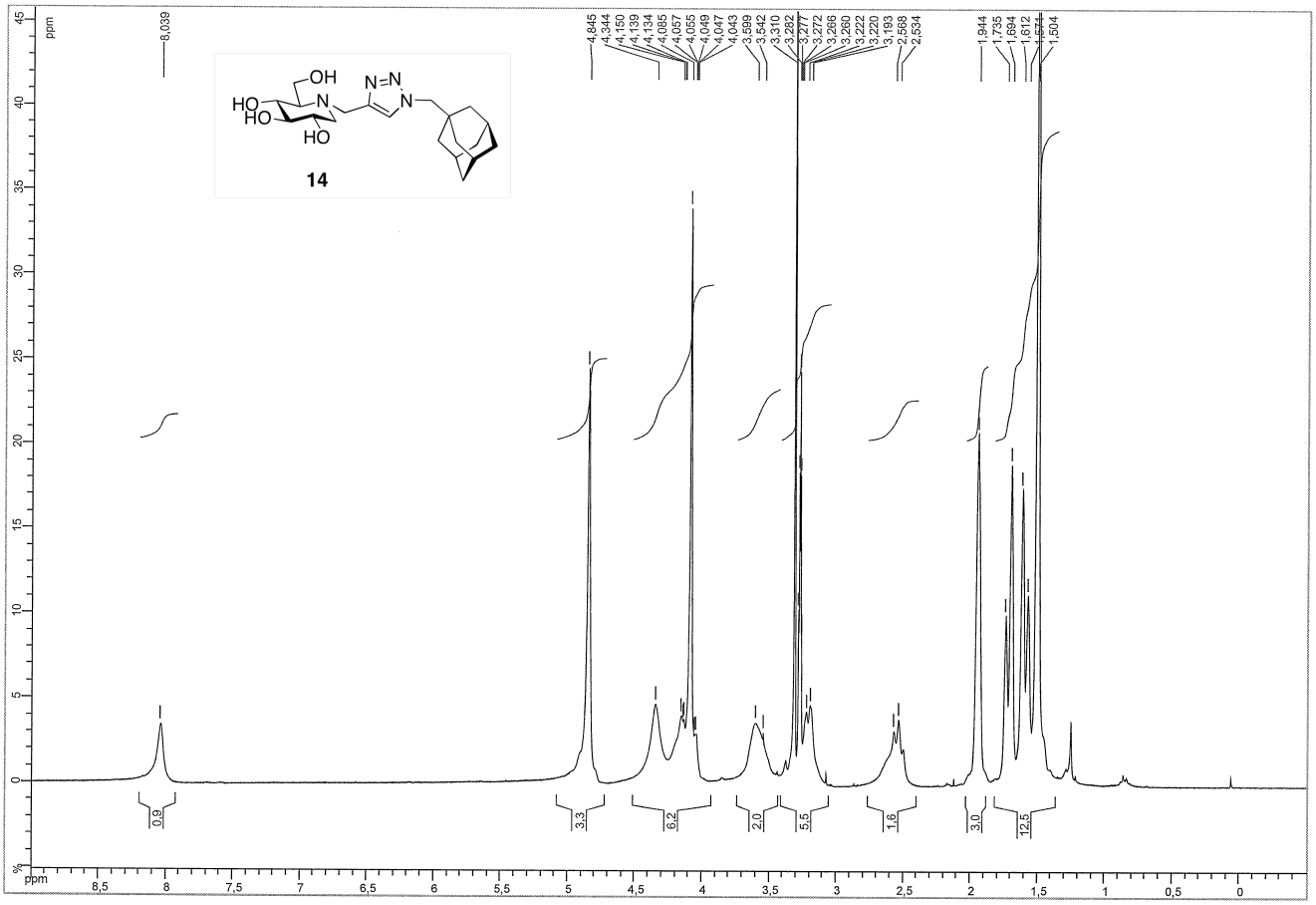

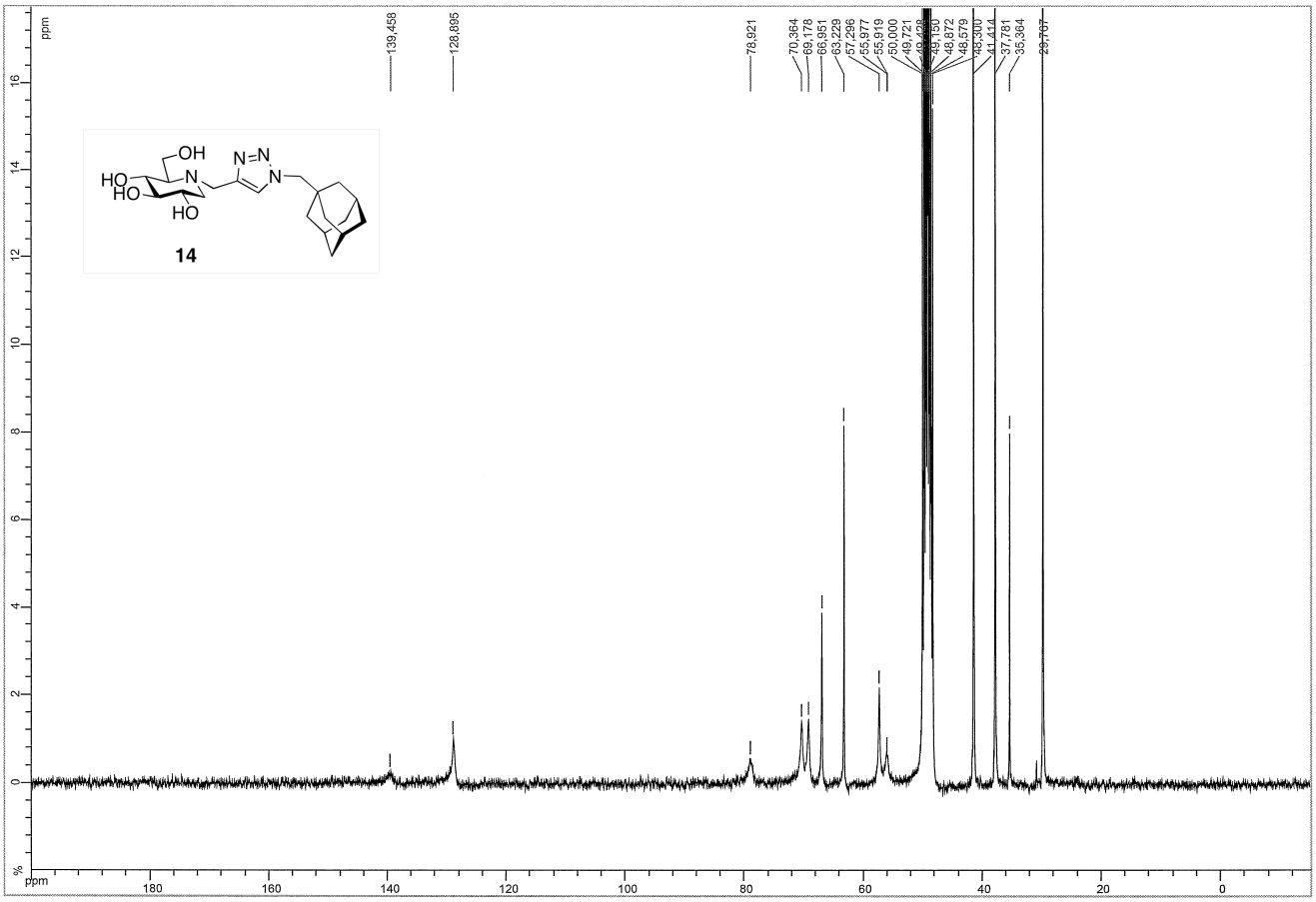

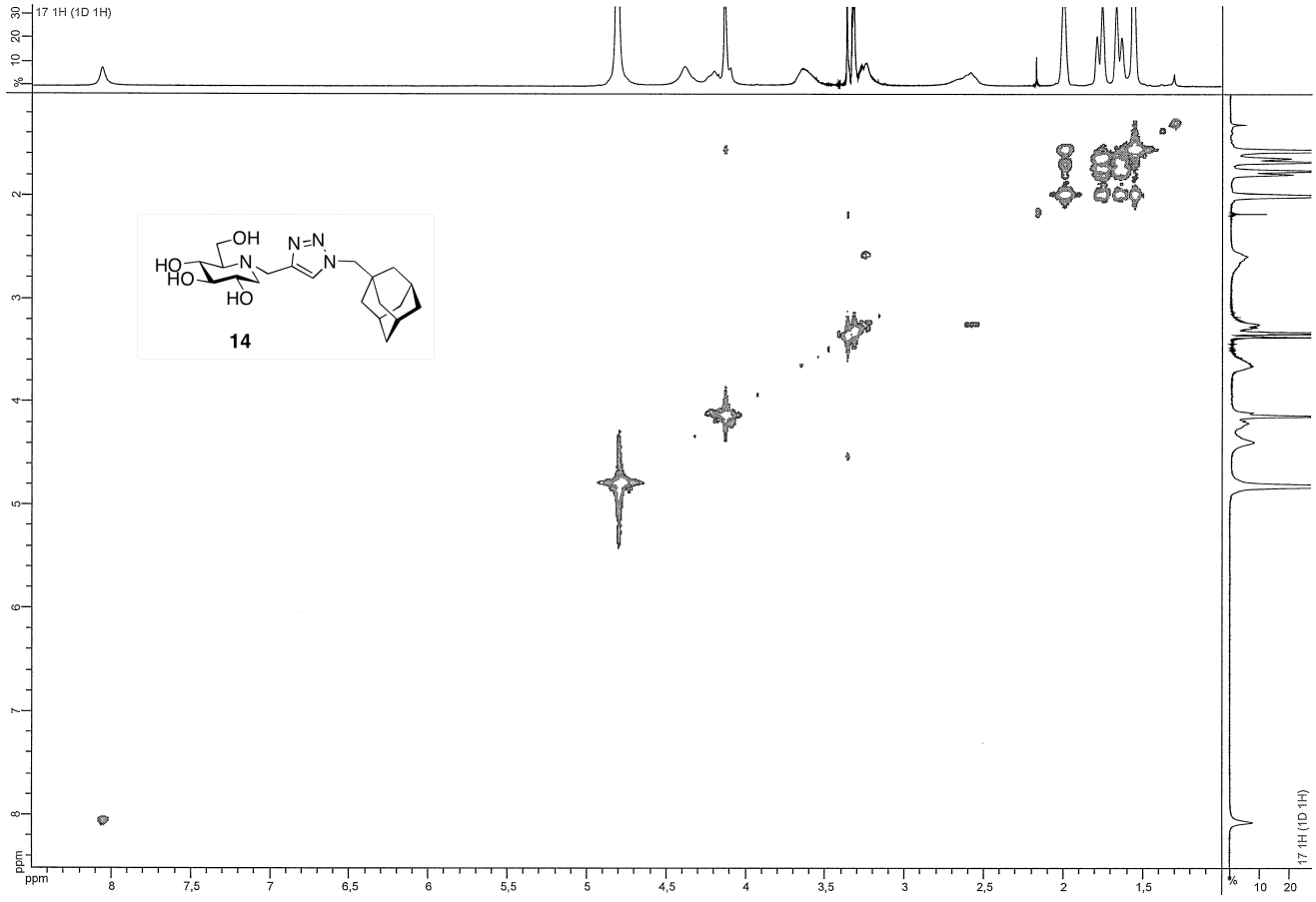

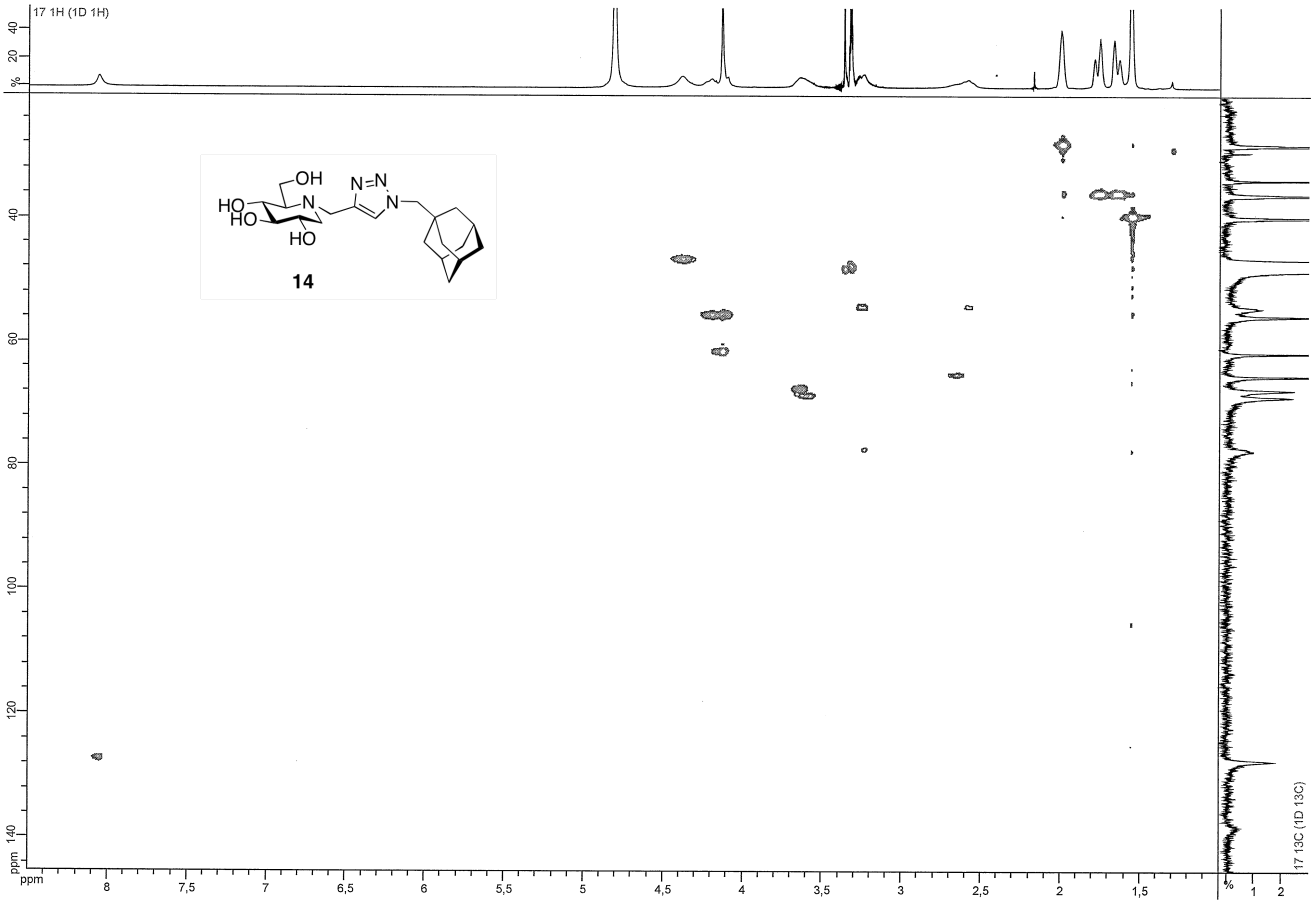

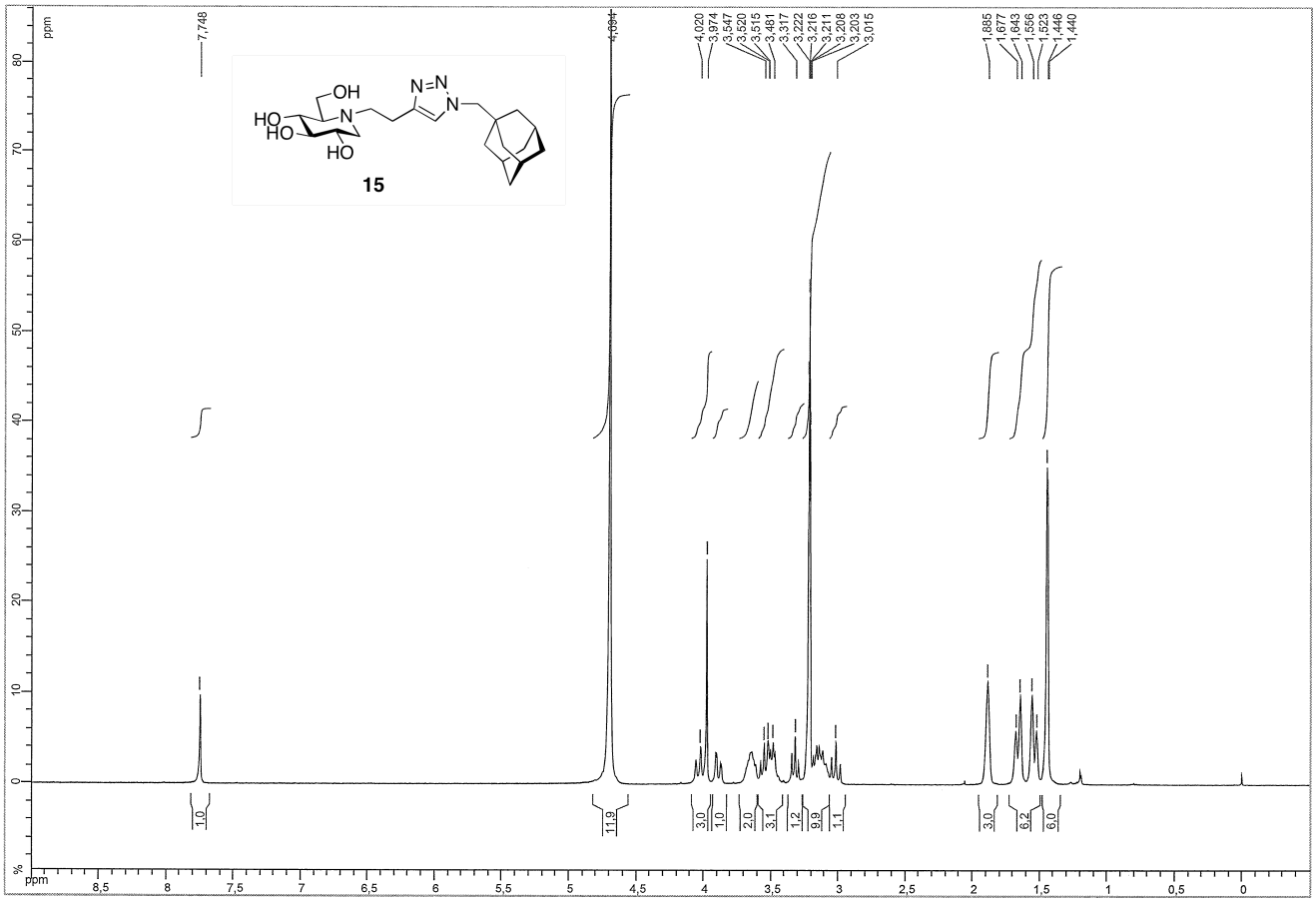

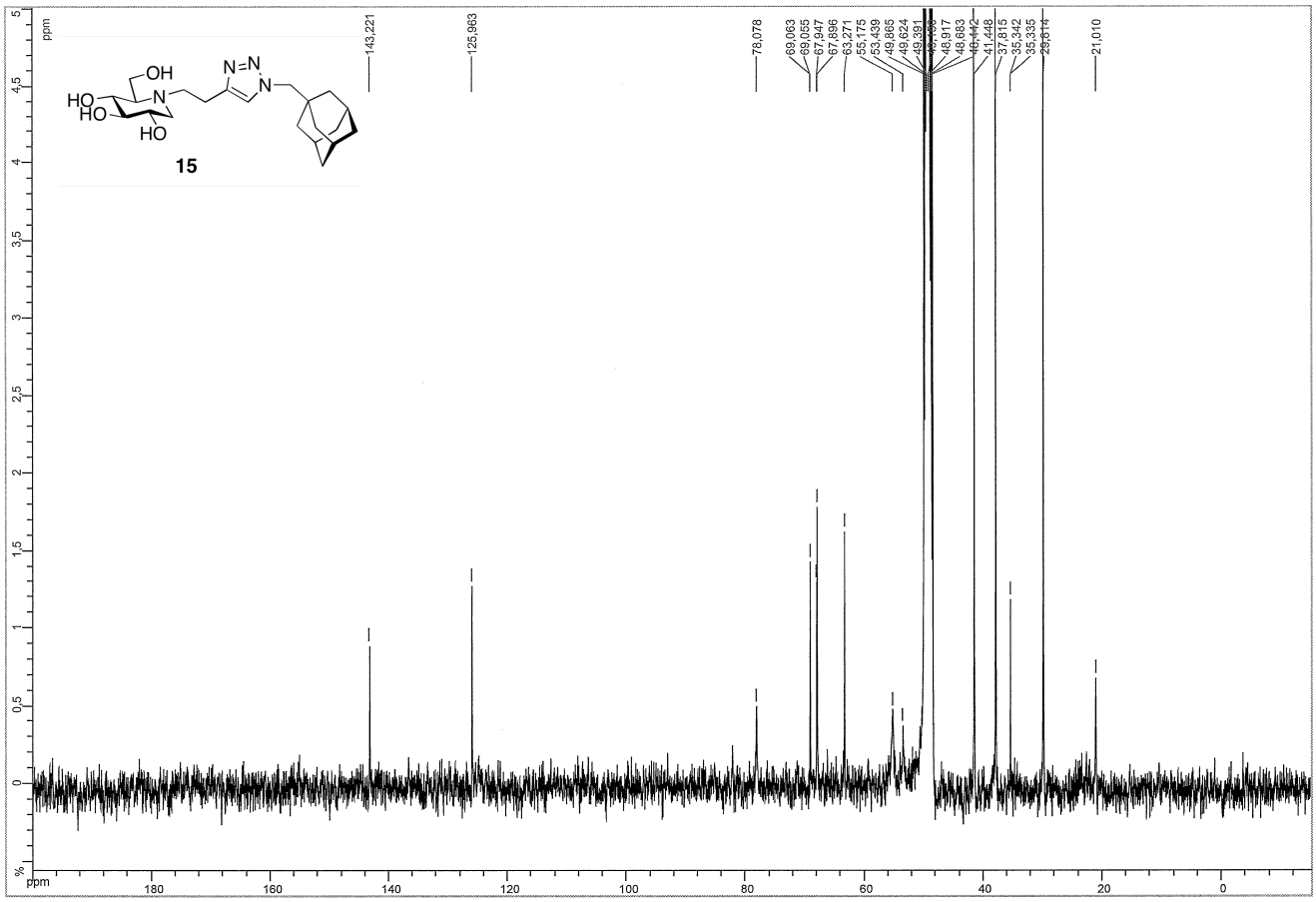

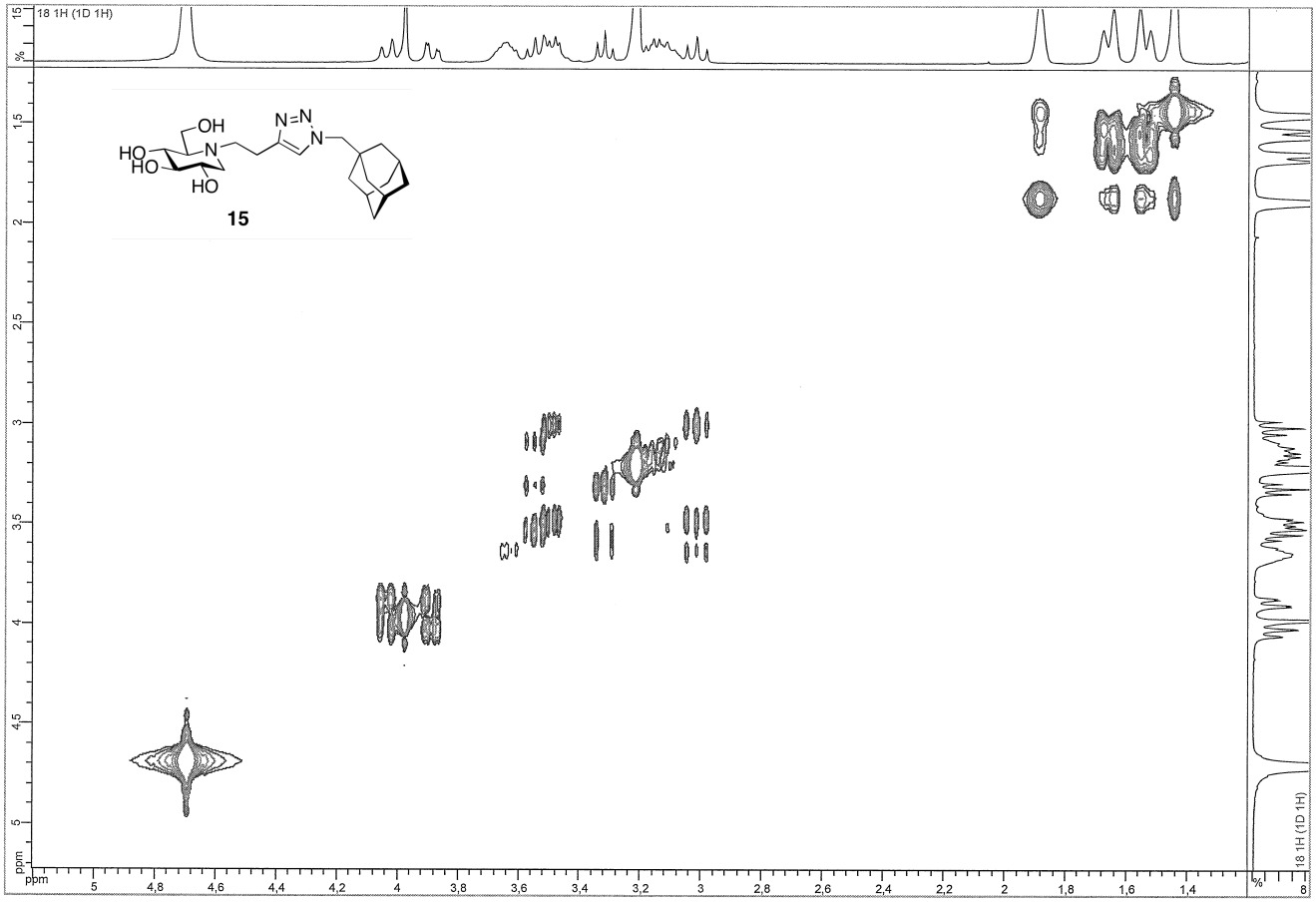

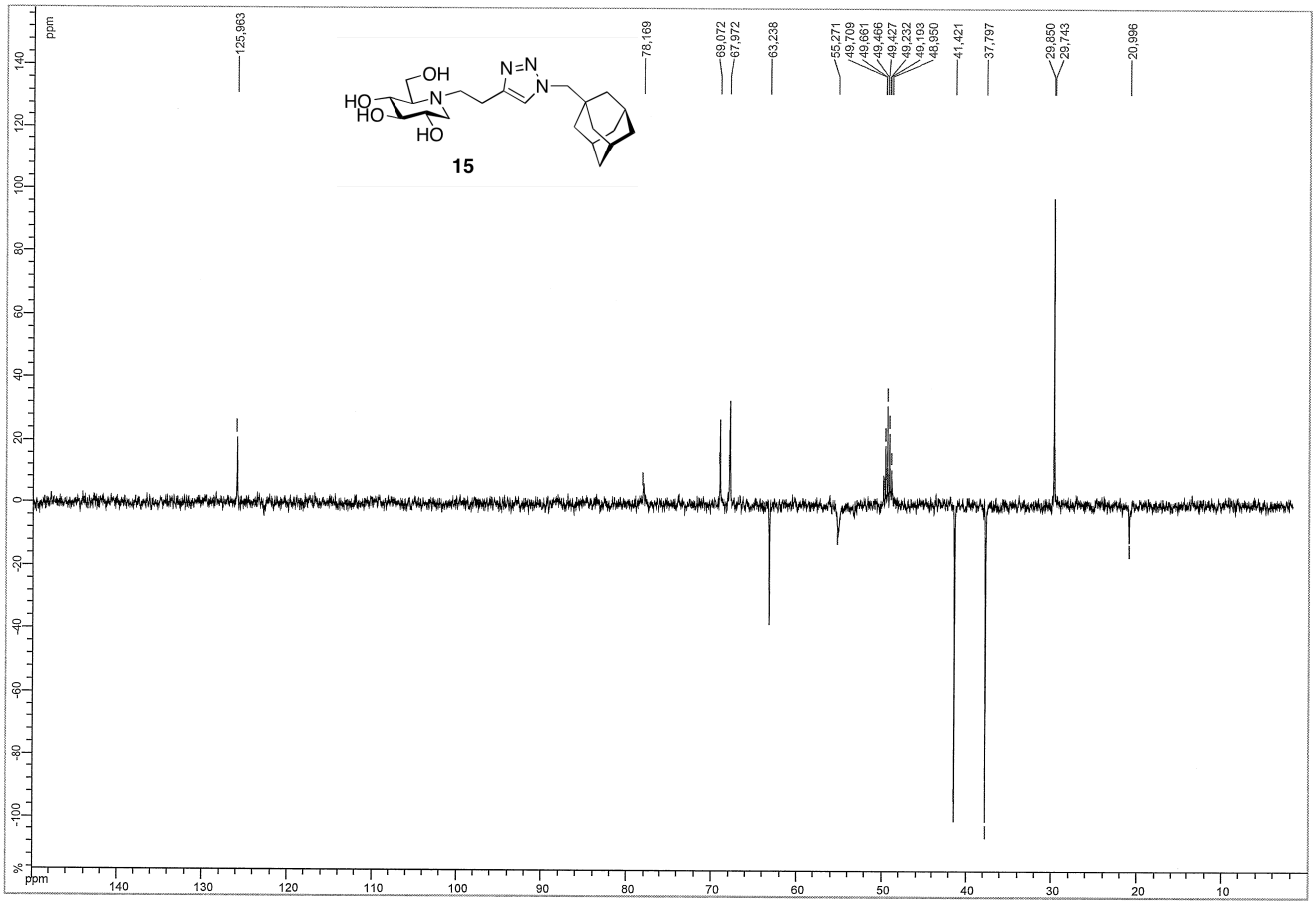

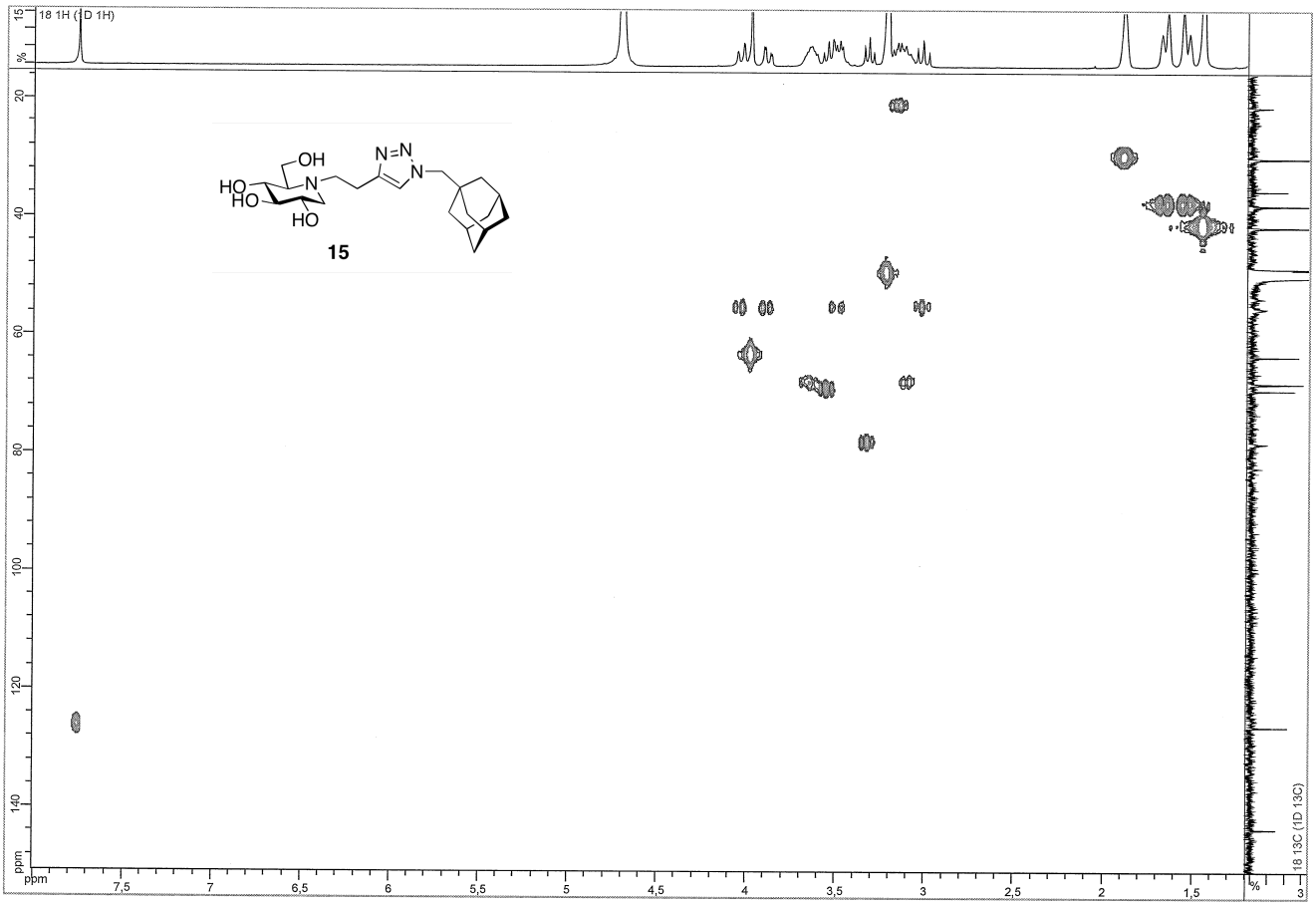

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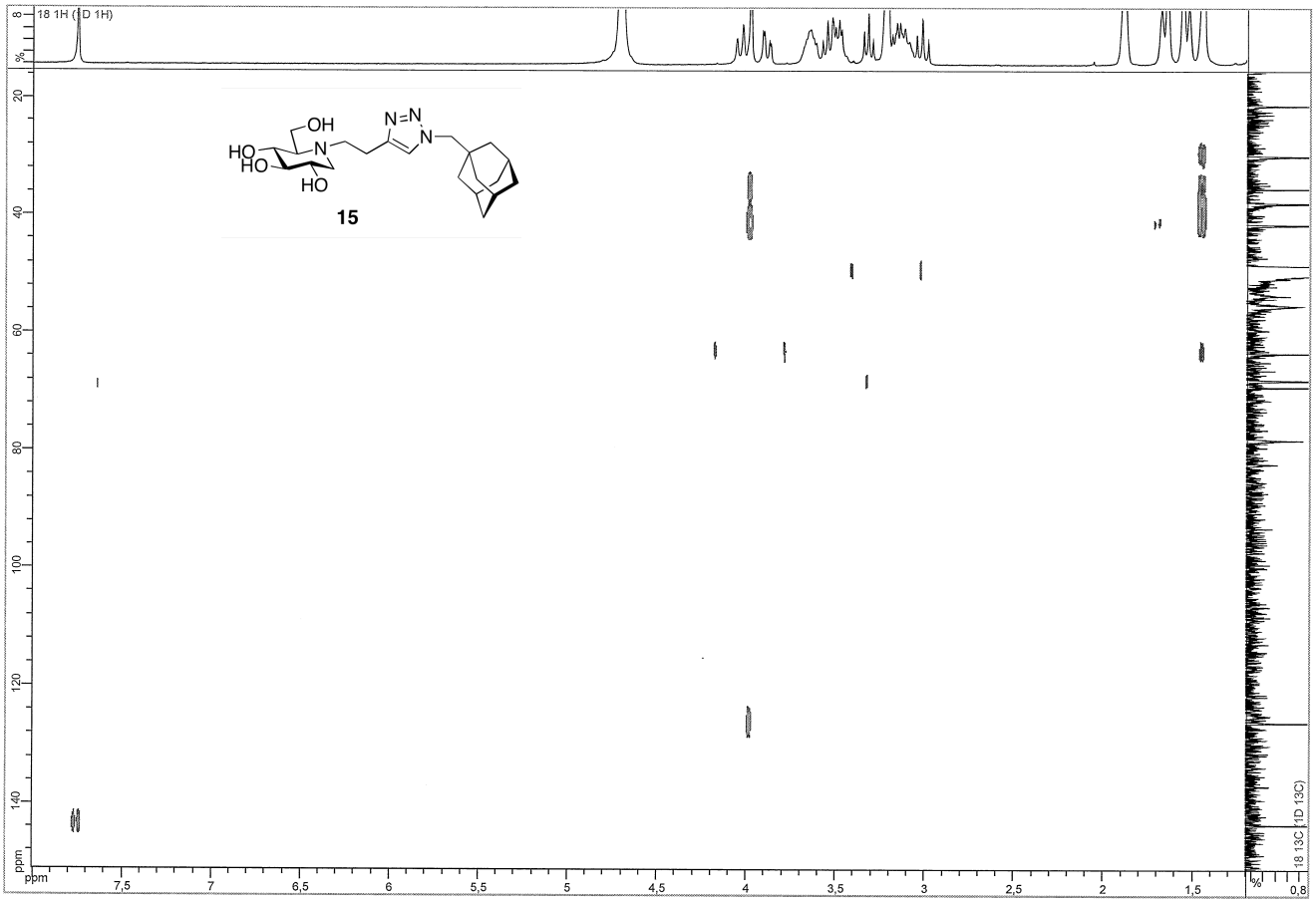

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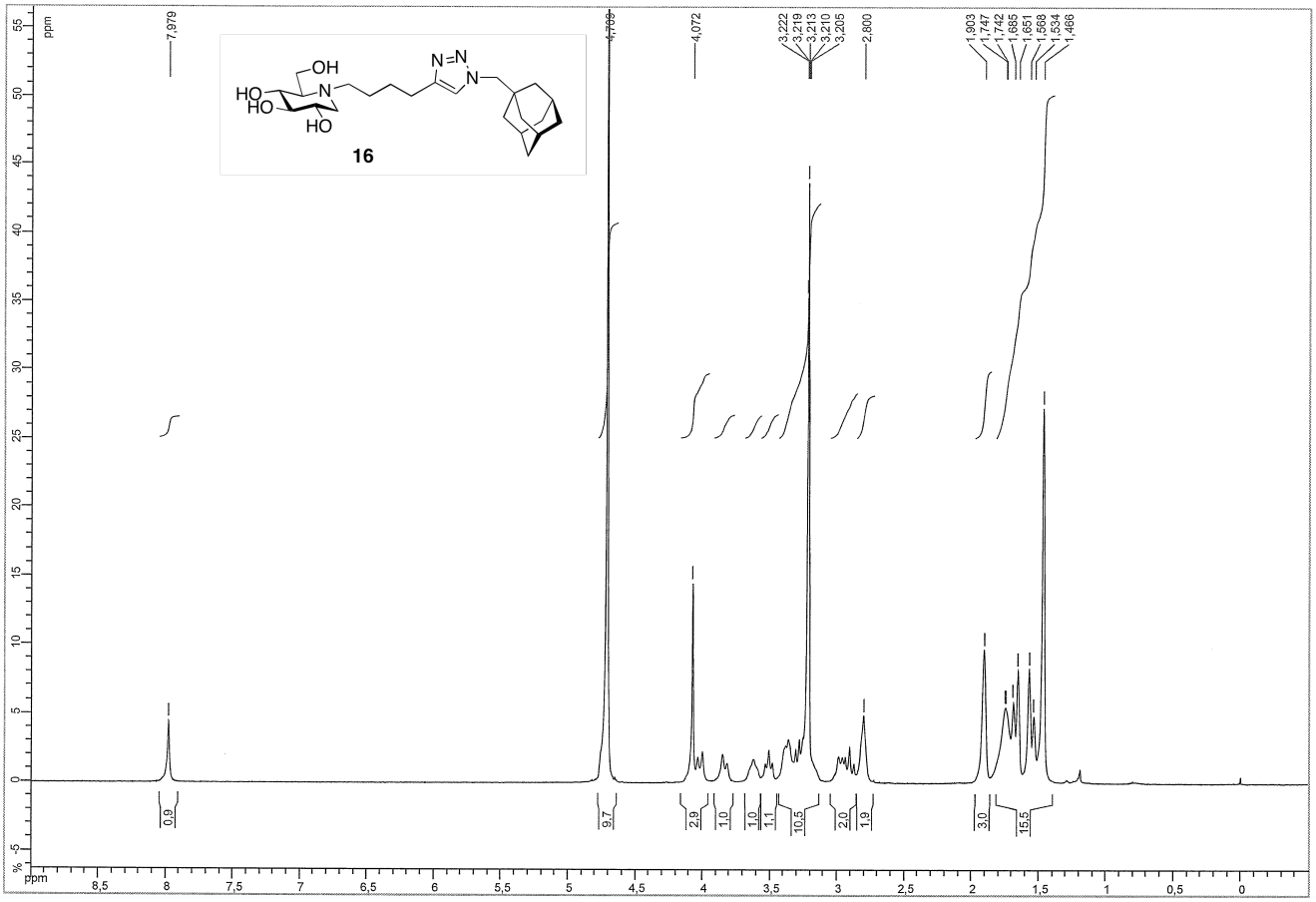

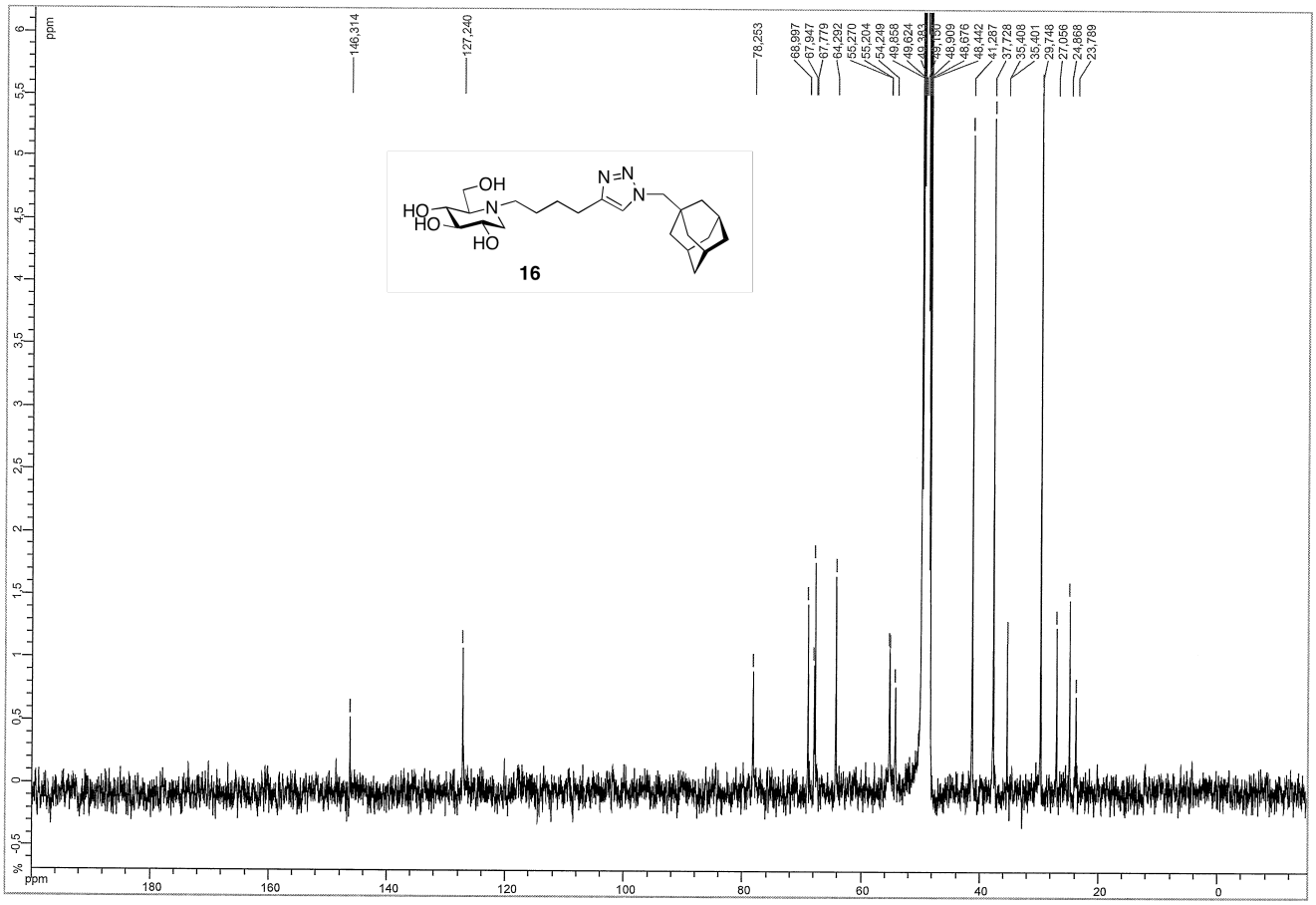

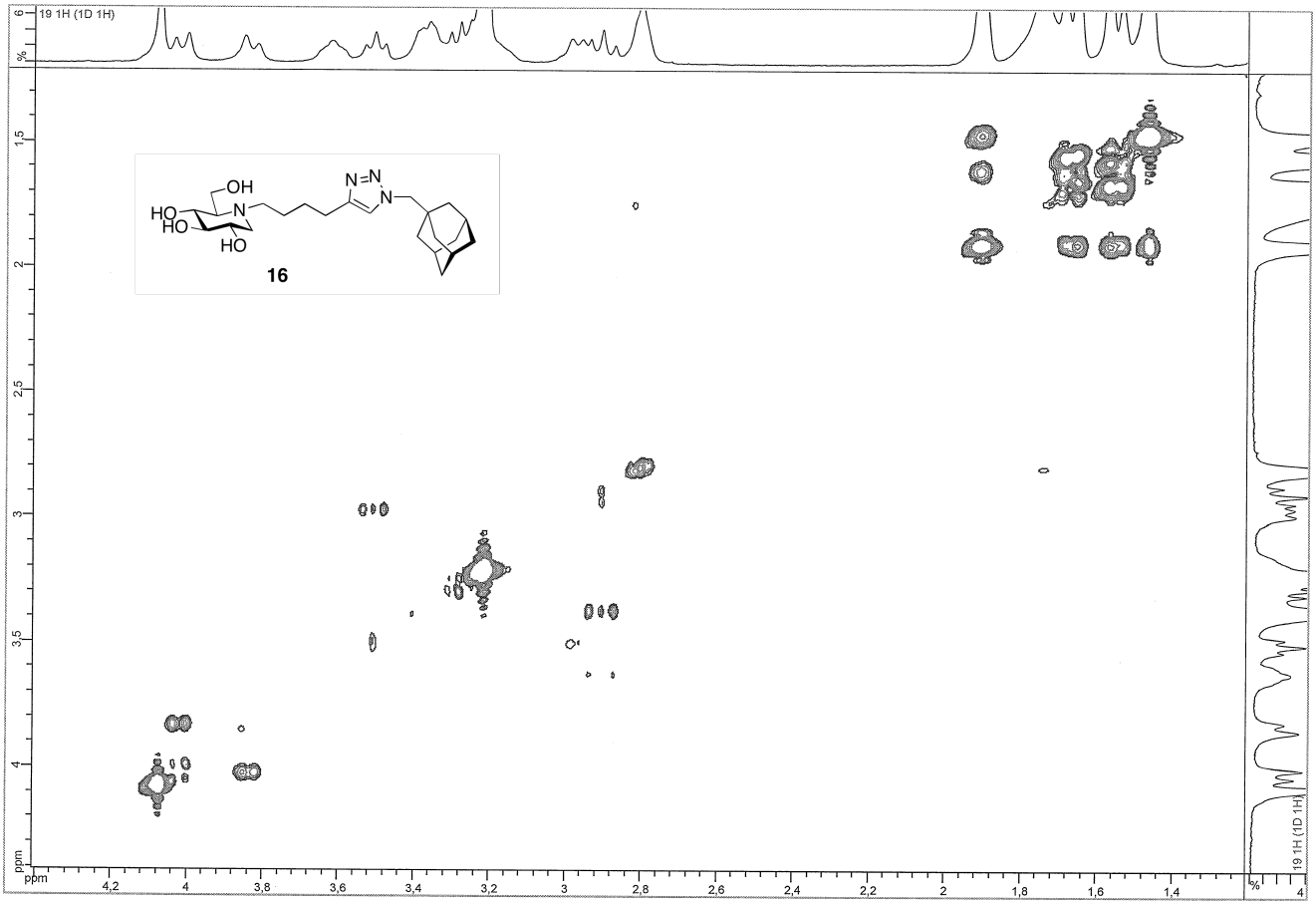

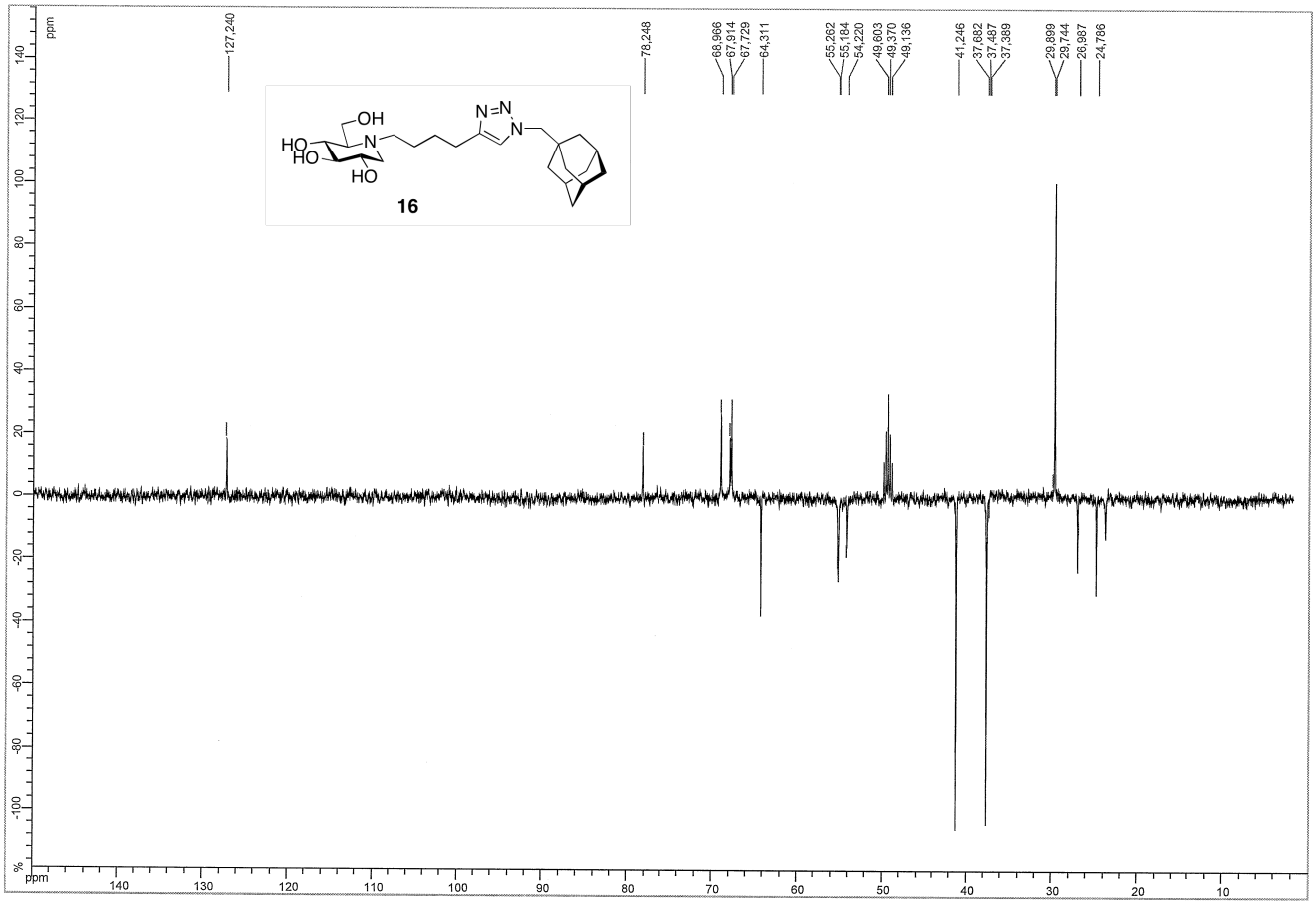

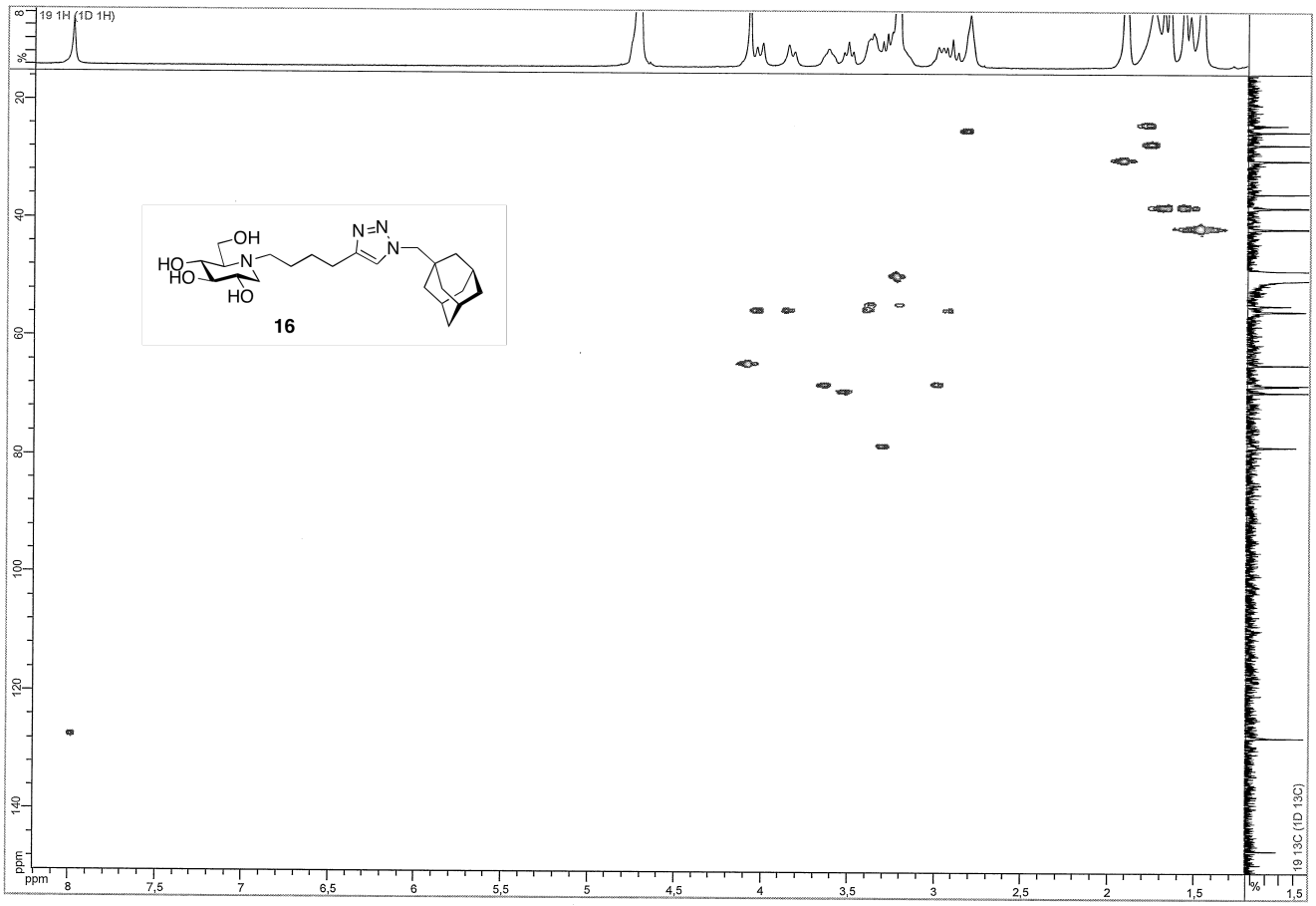

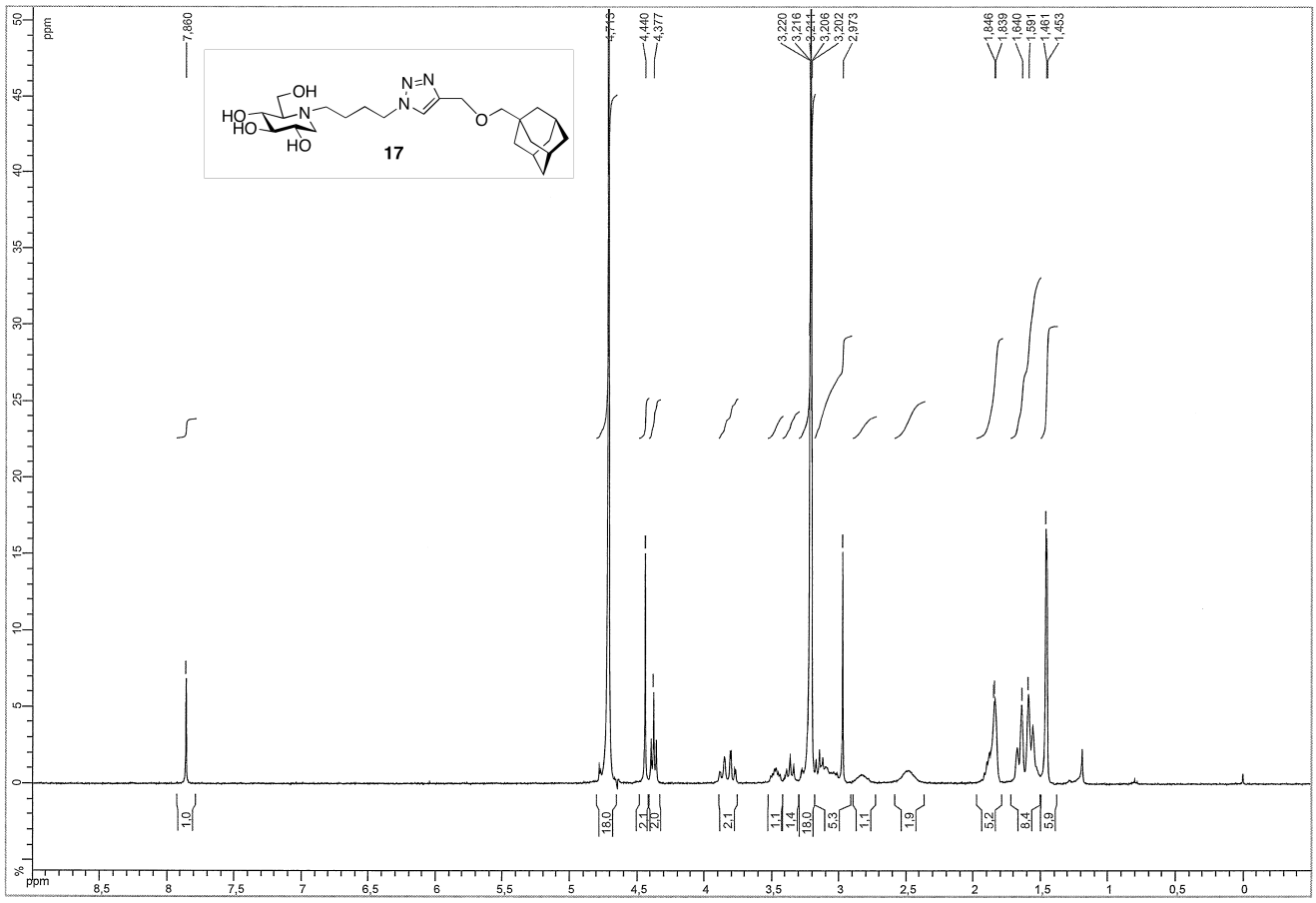

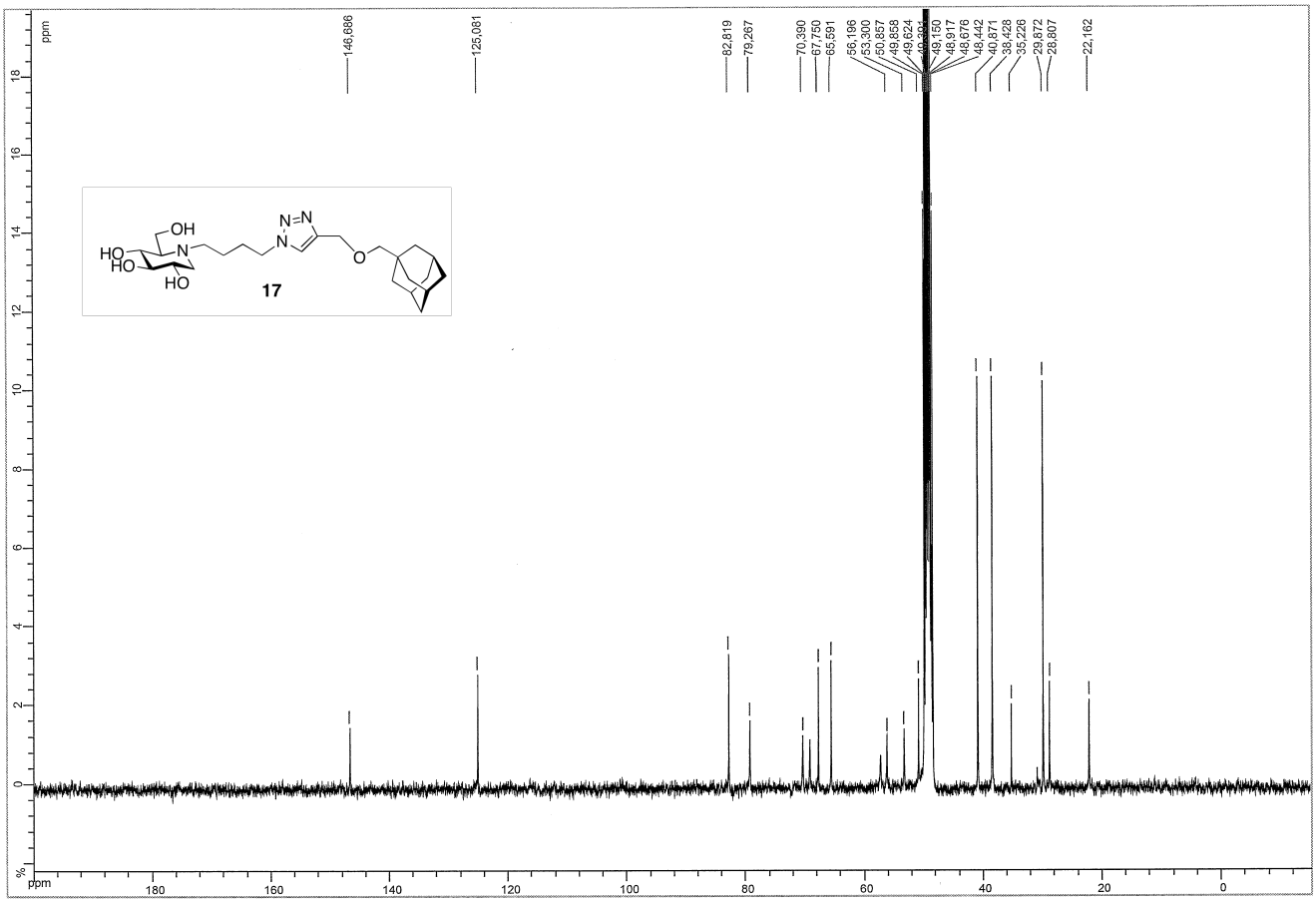

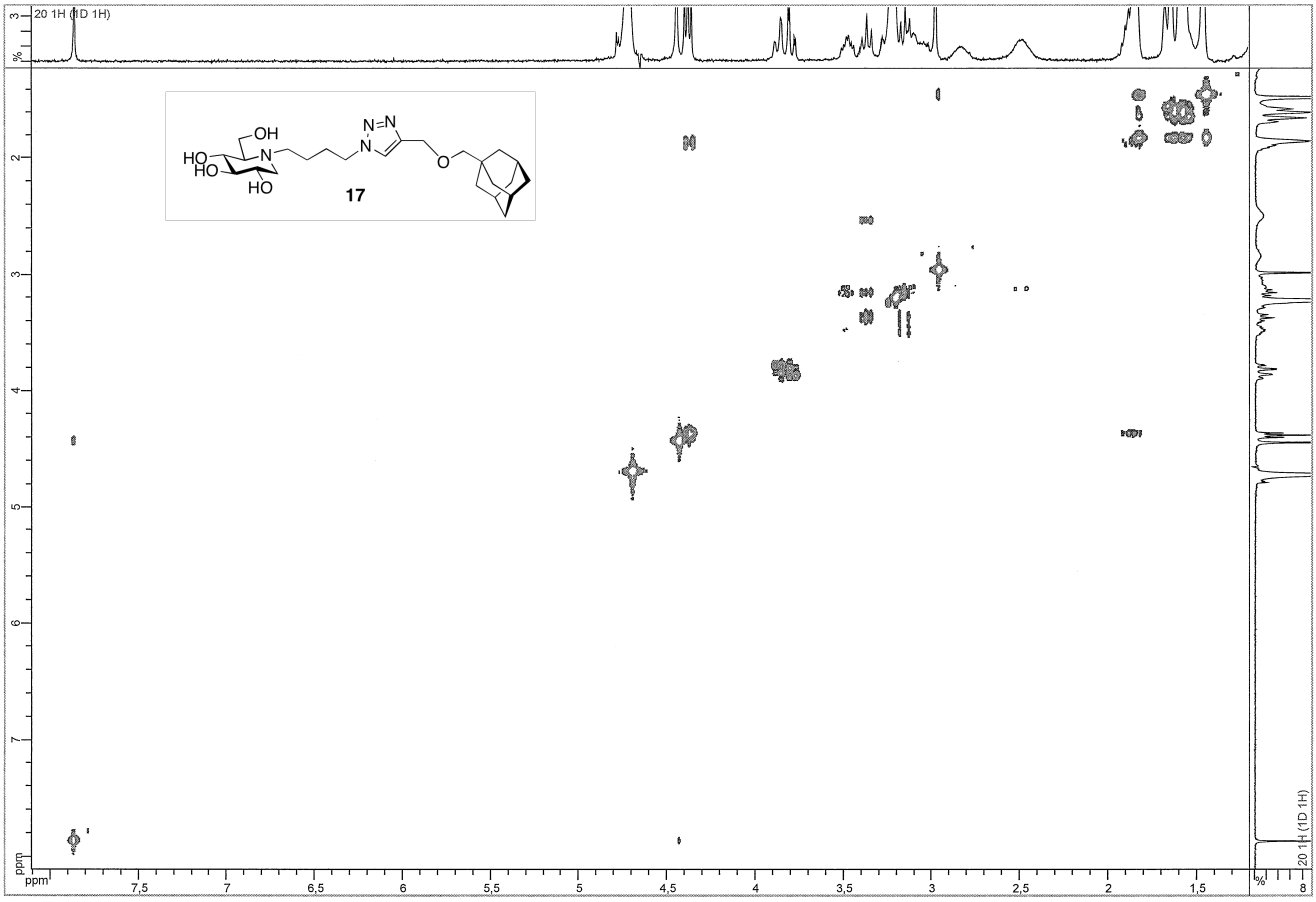

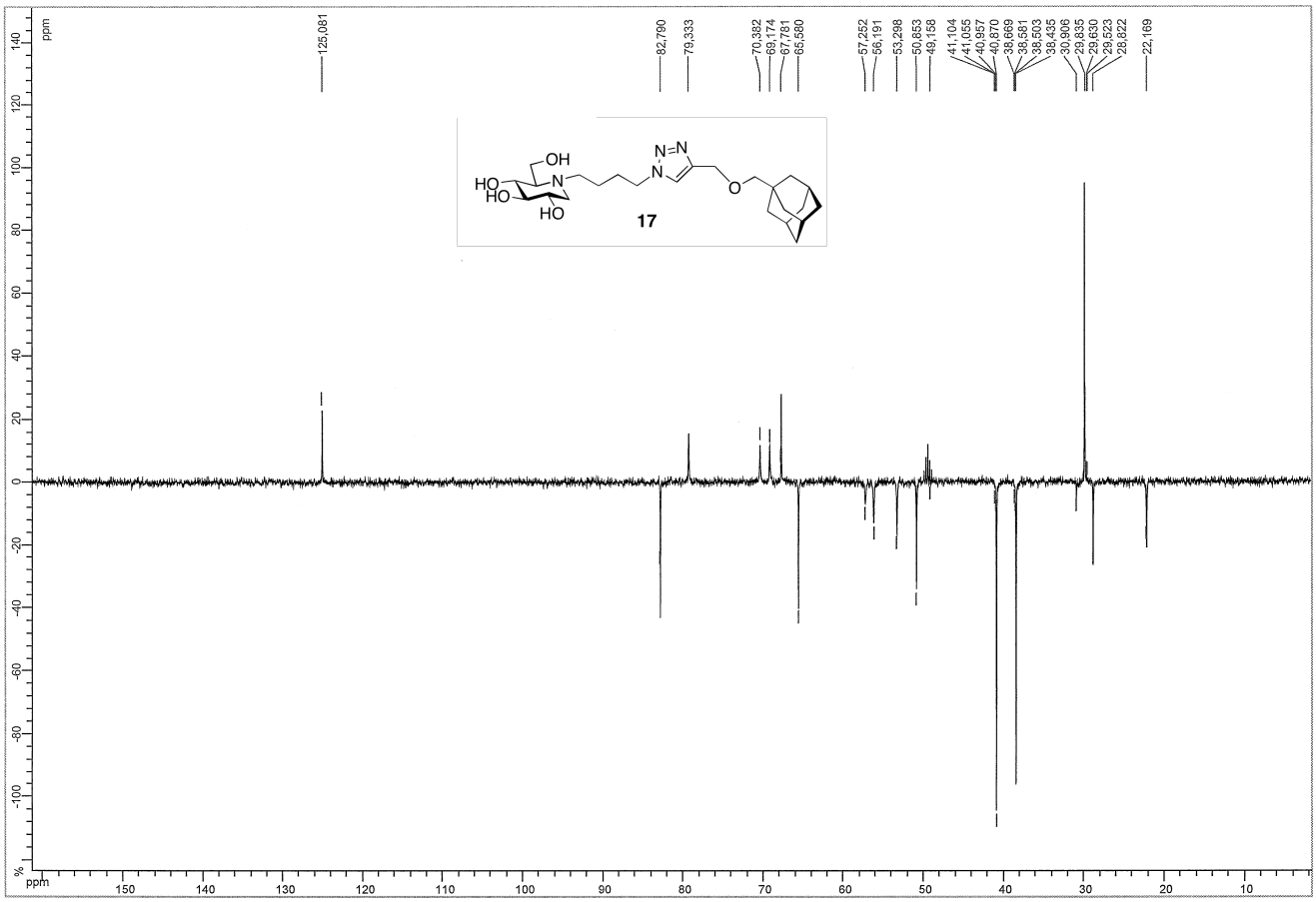

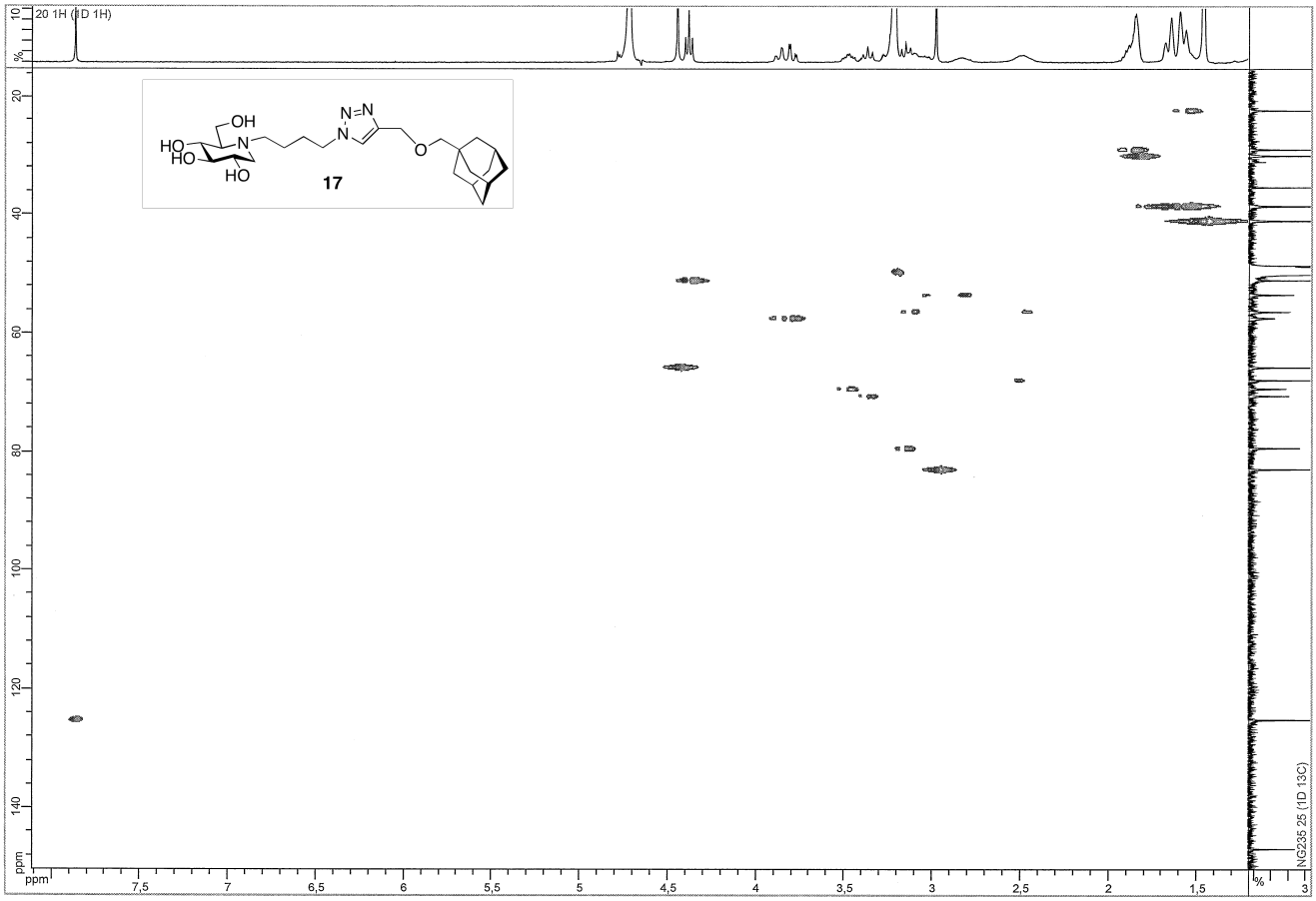

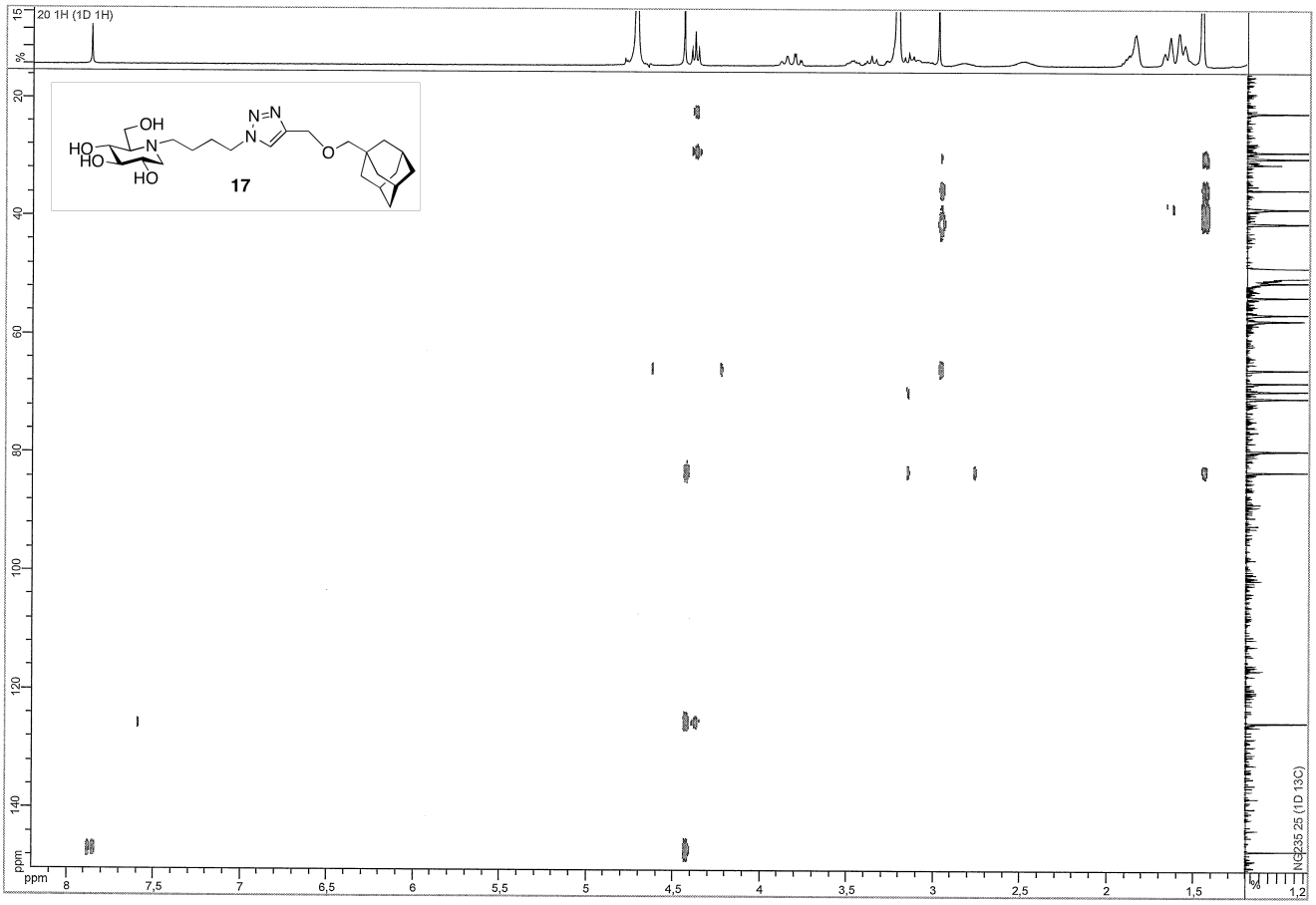

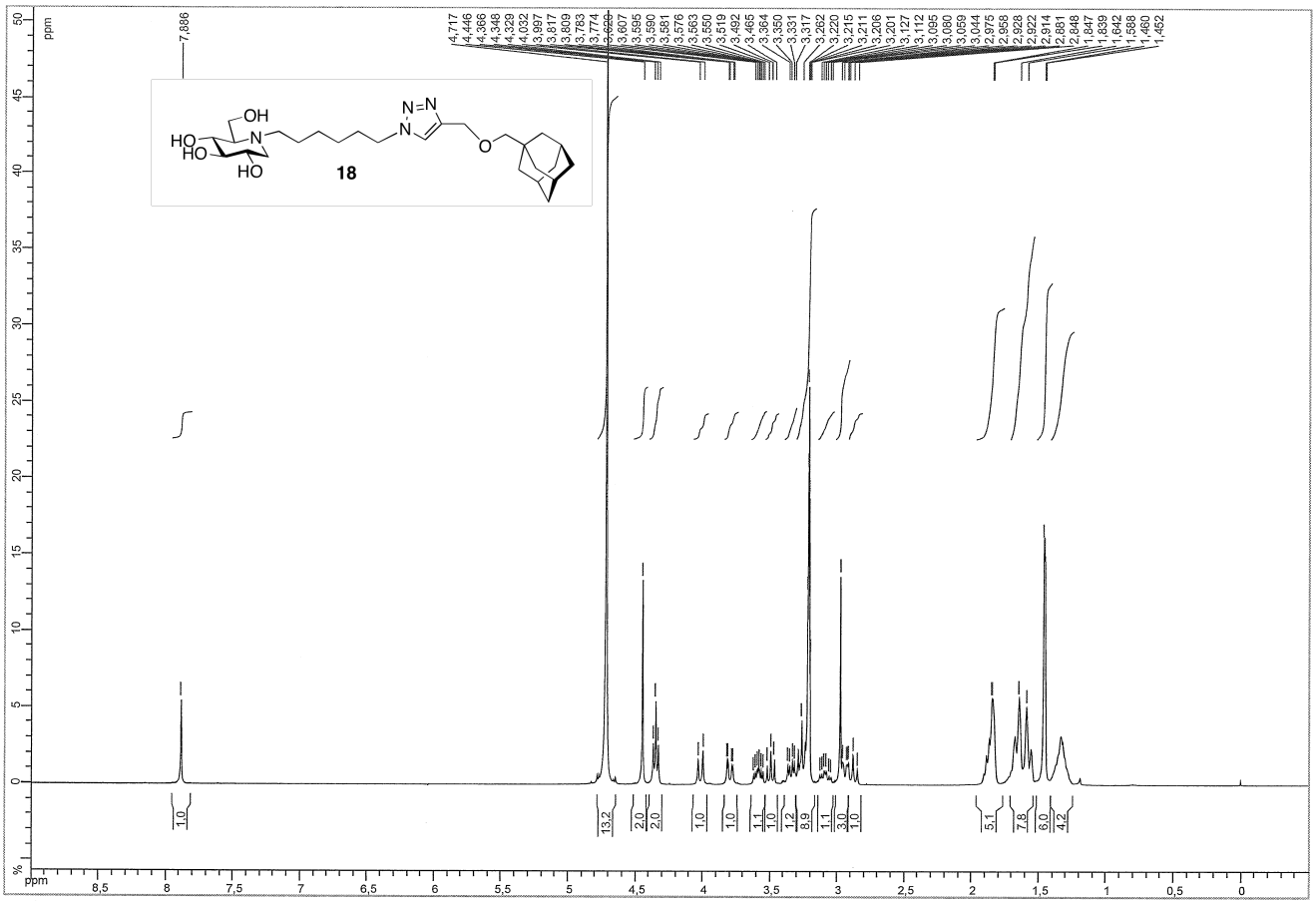

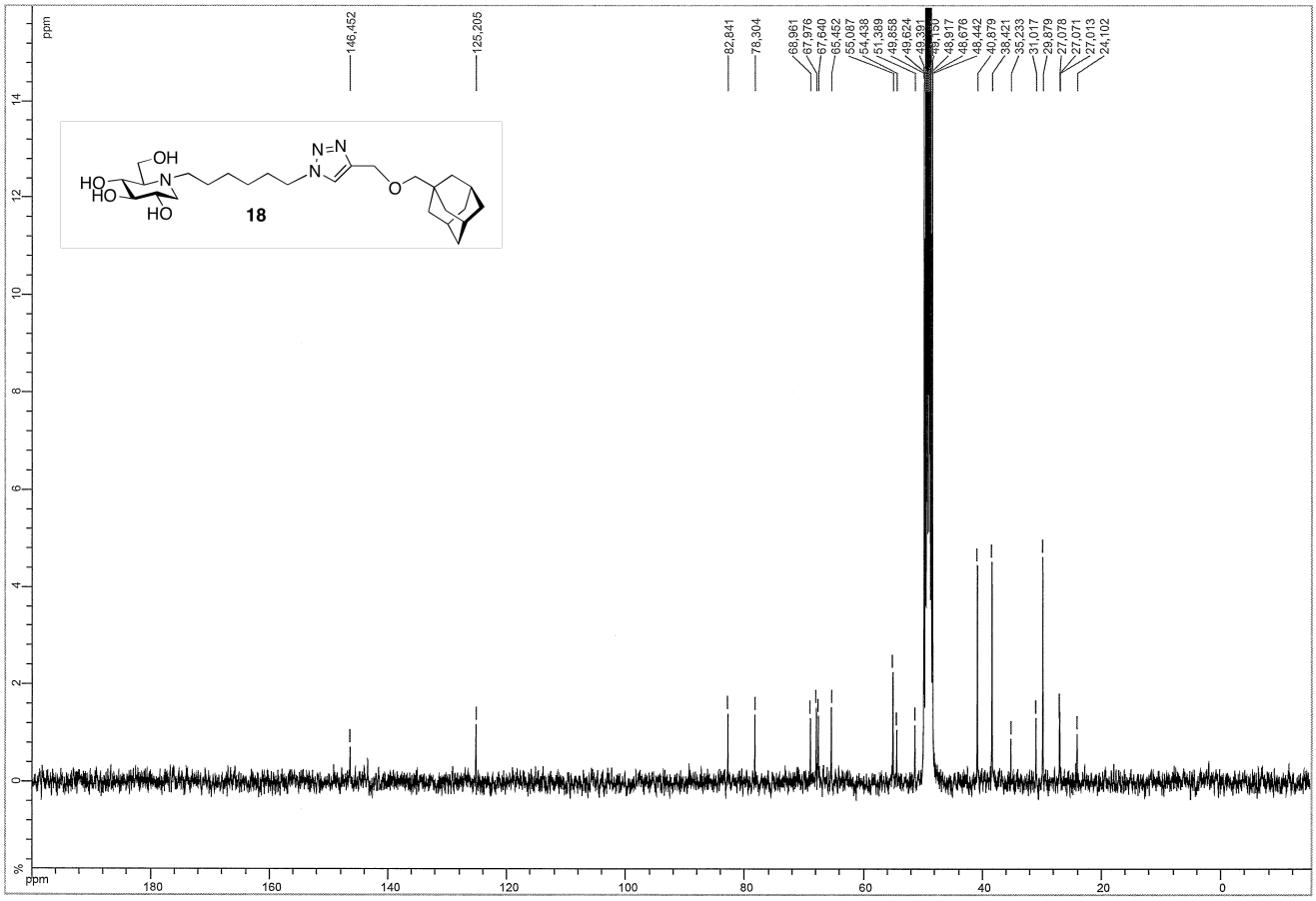

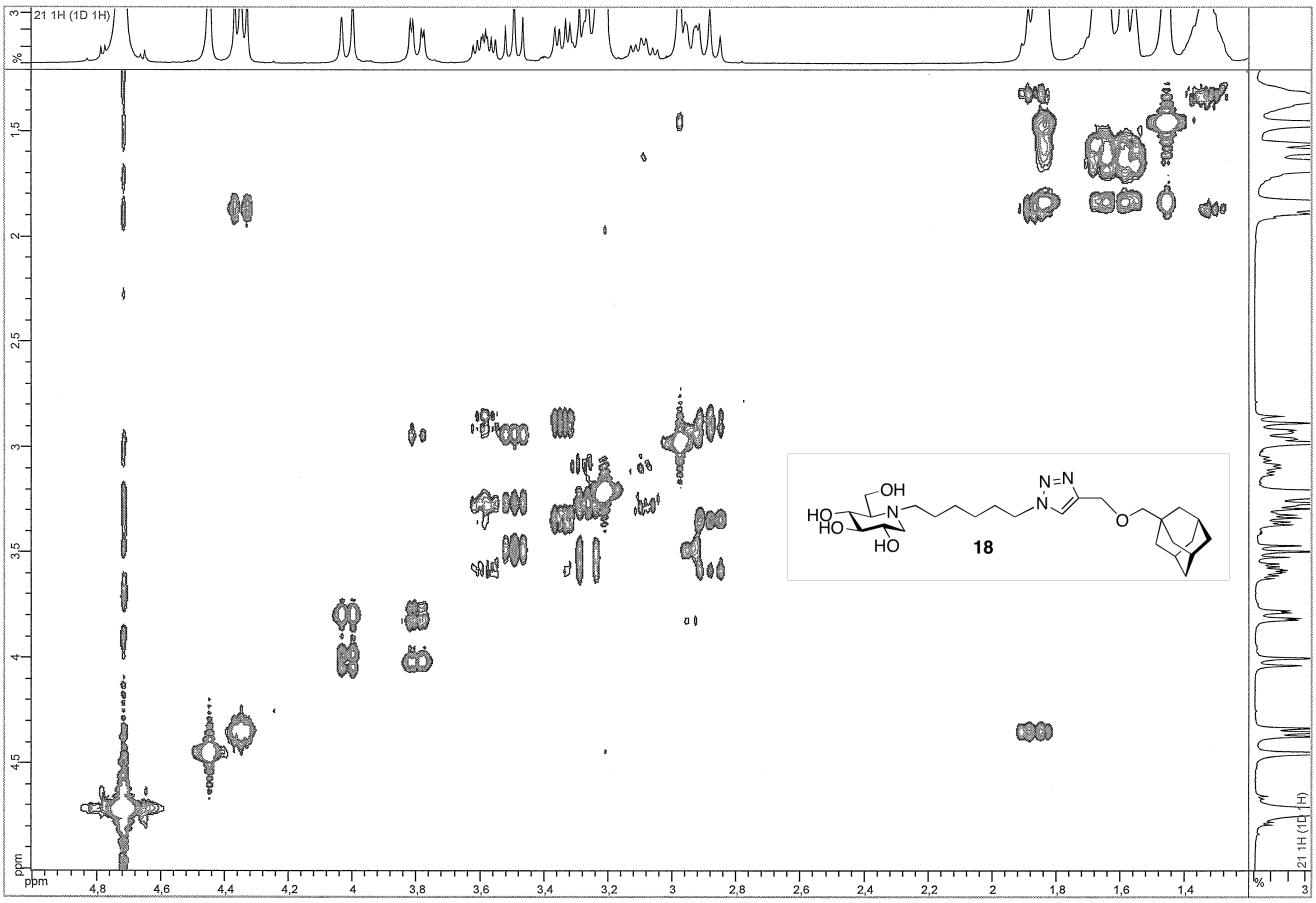

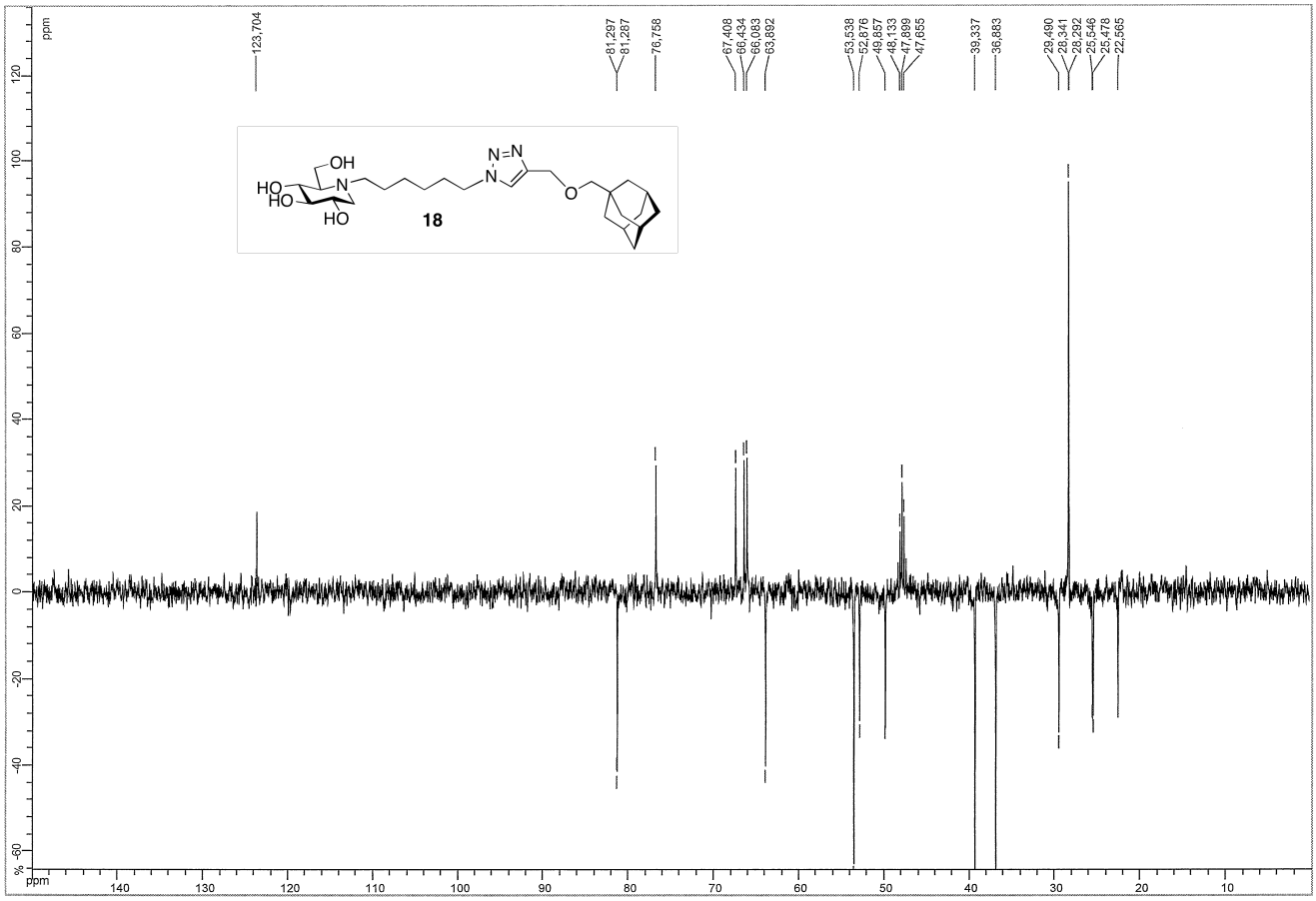

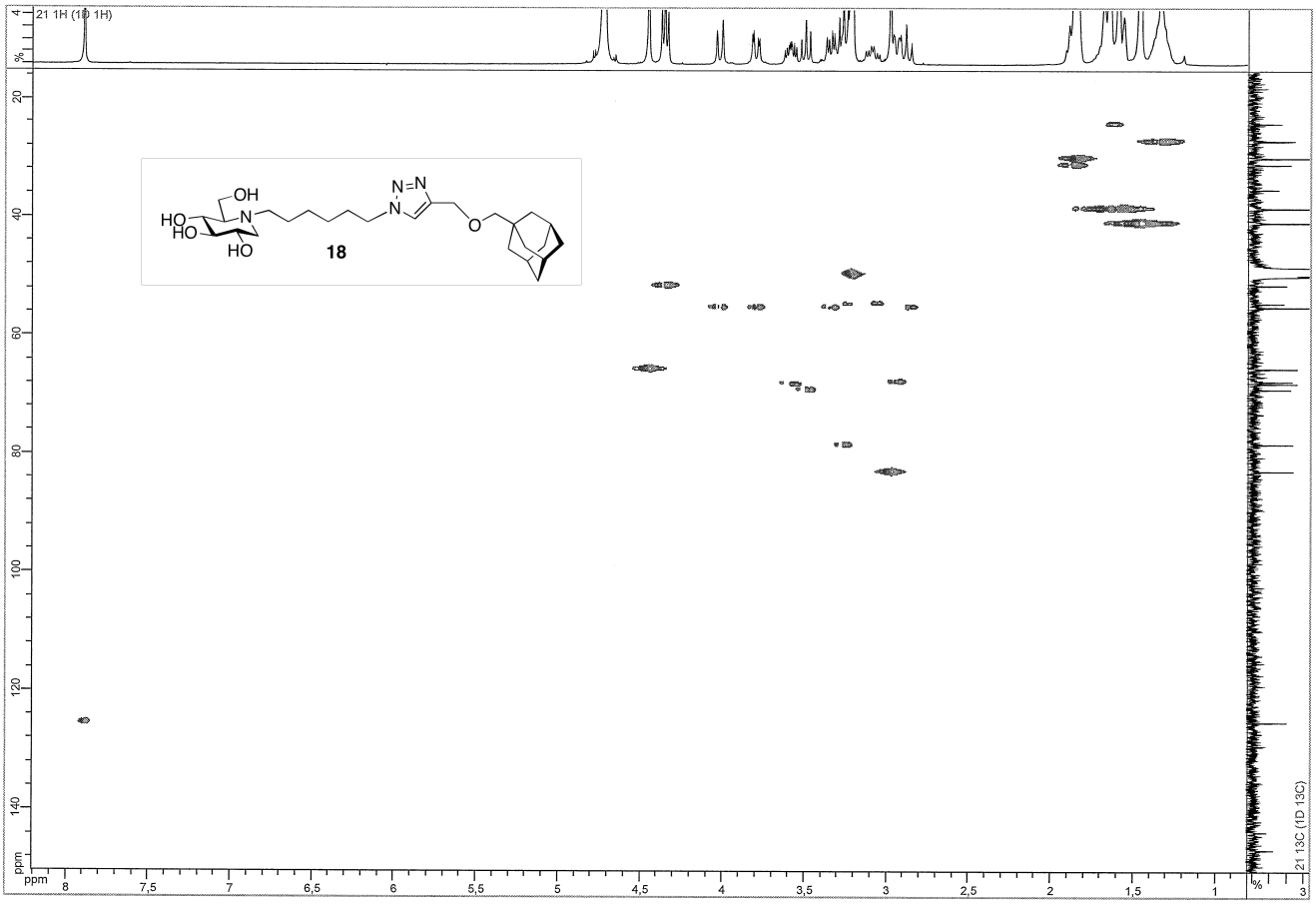

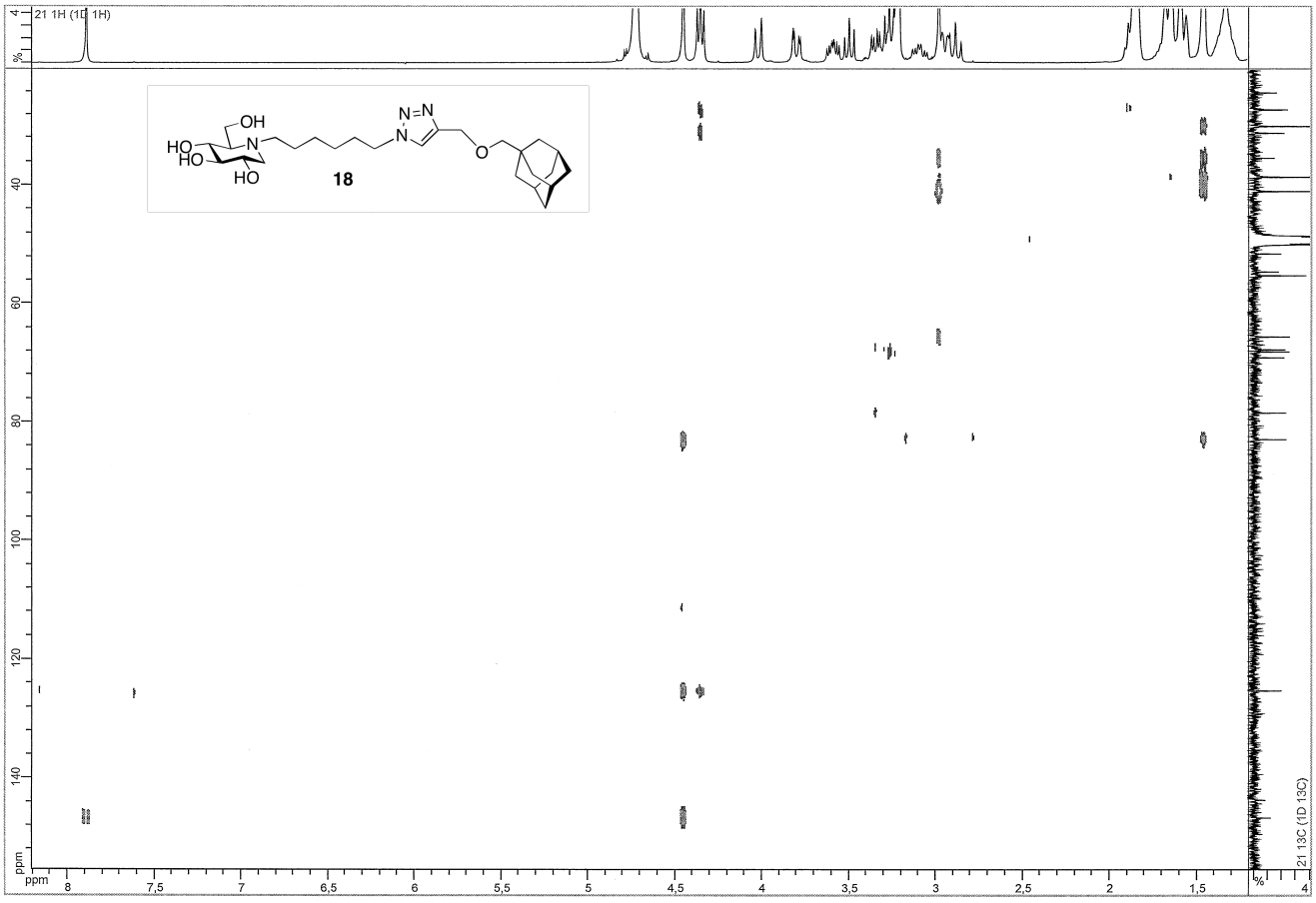

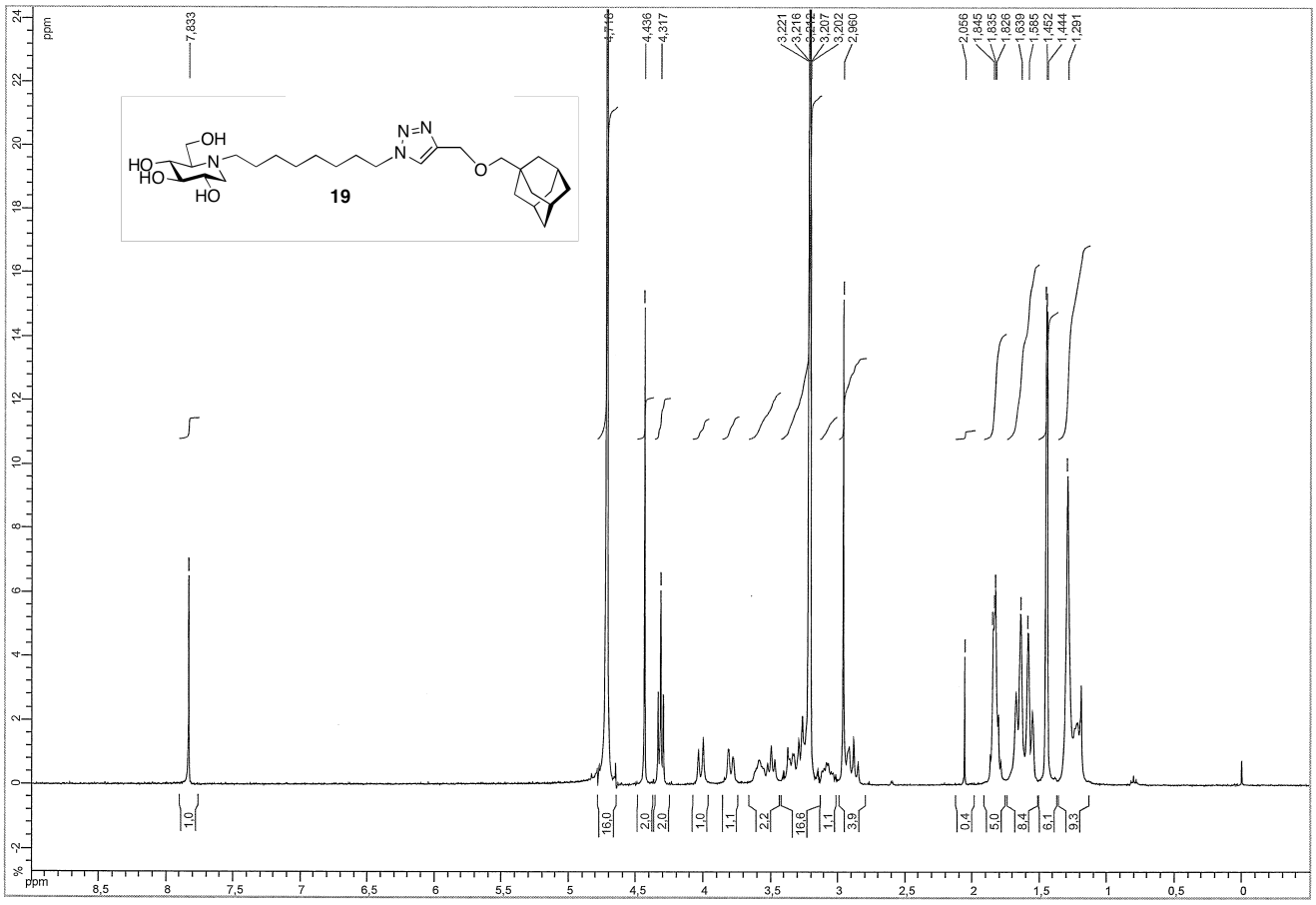

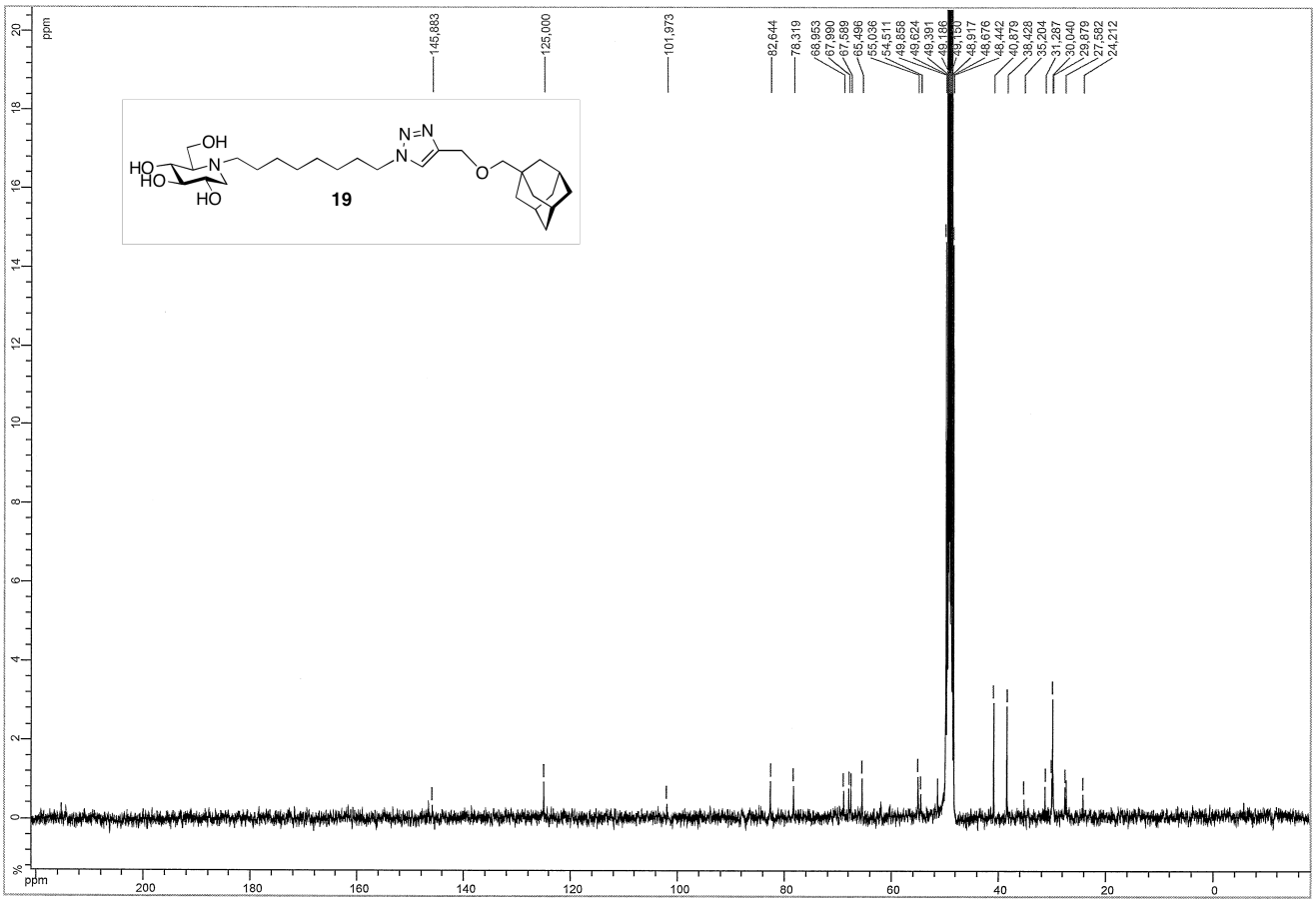

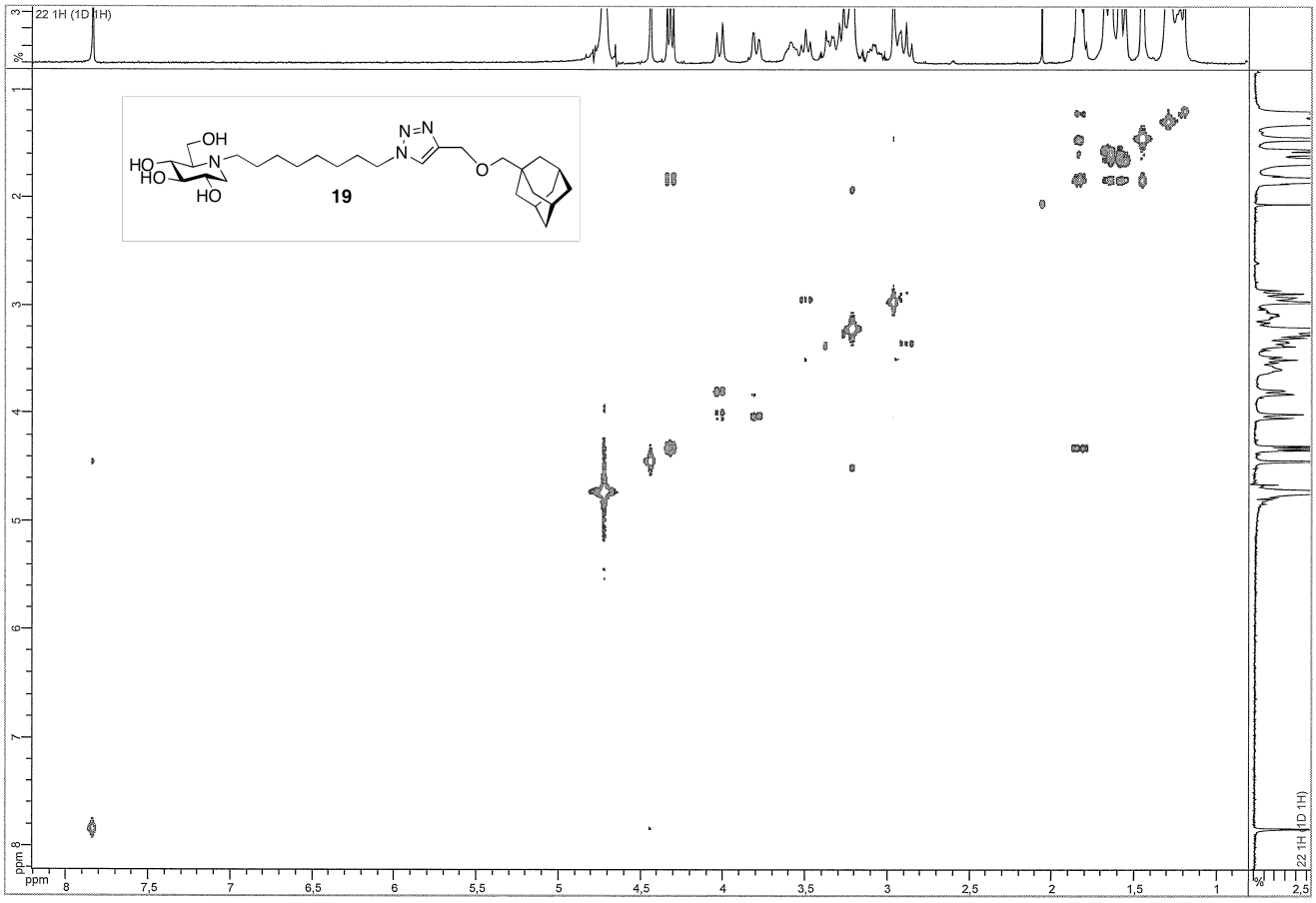

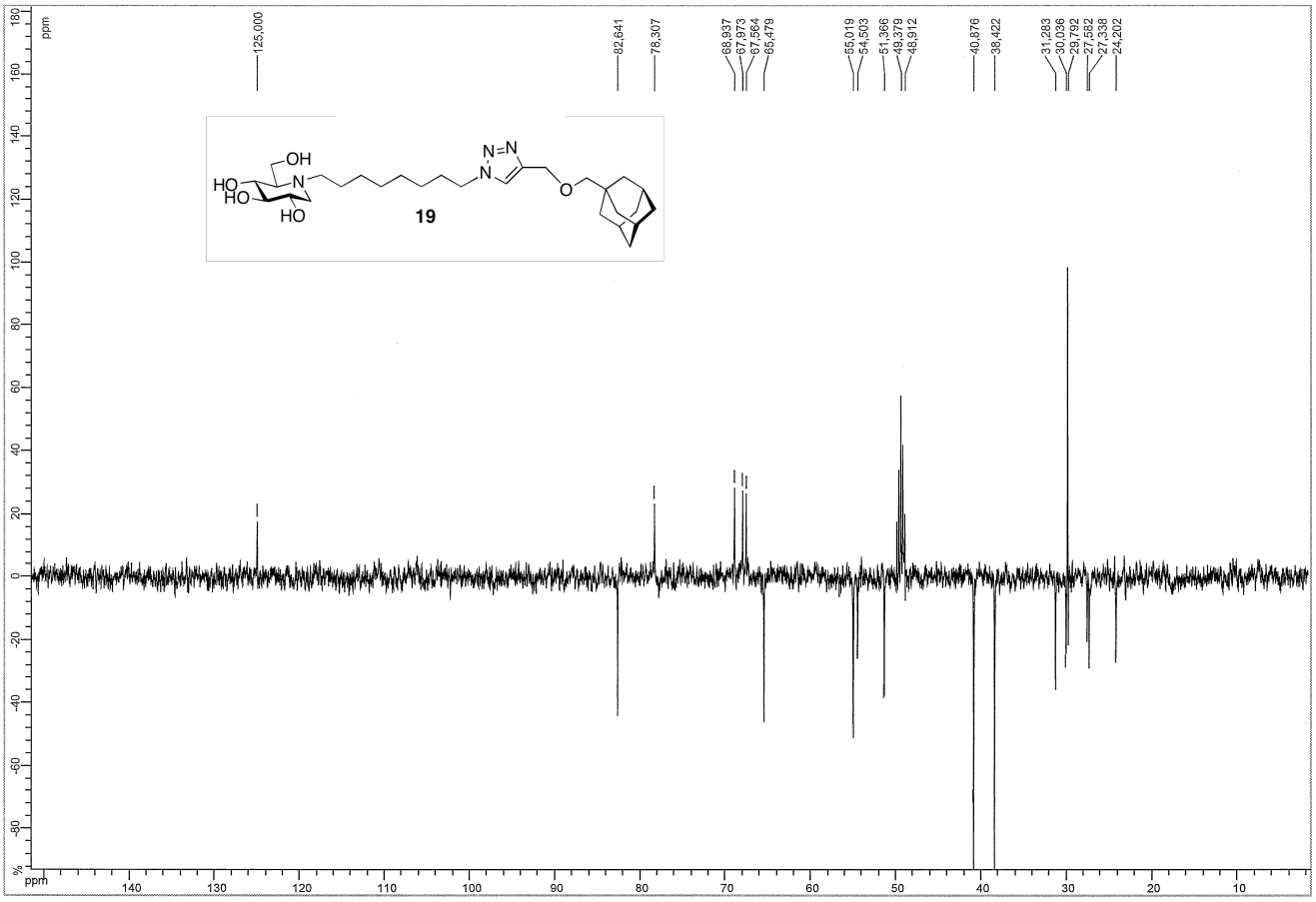

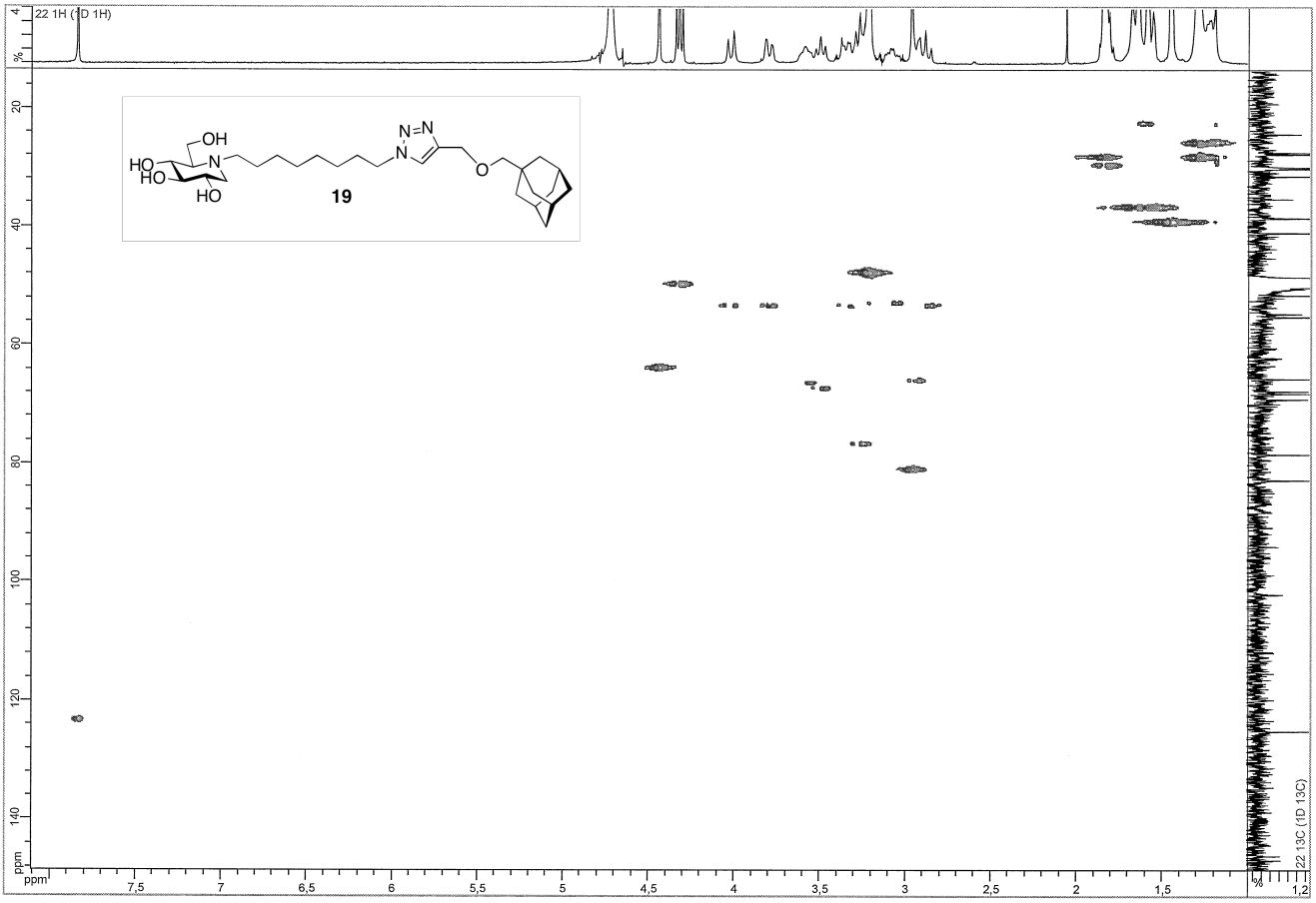
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HO
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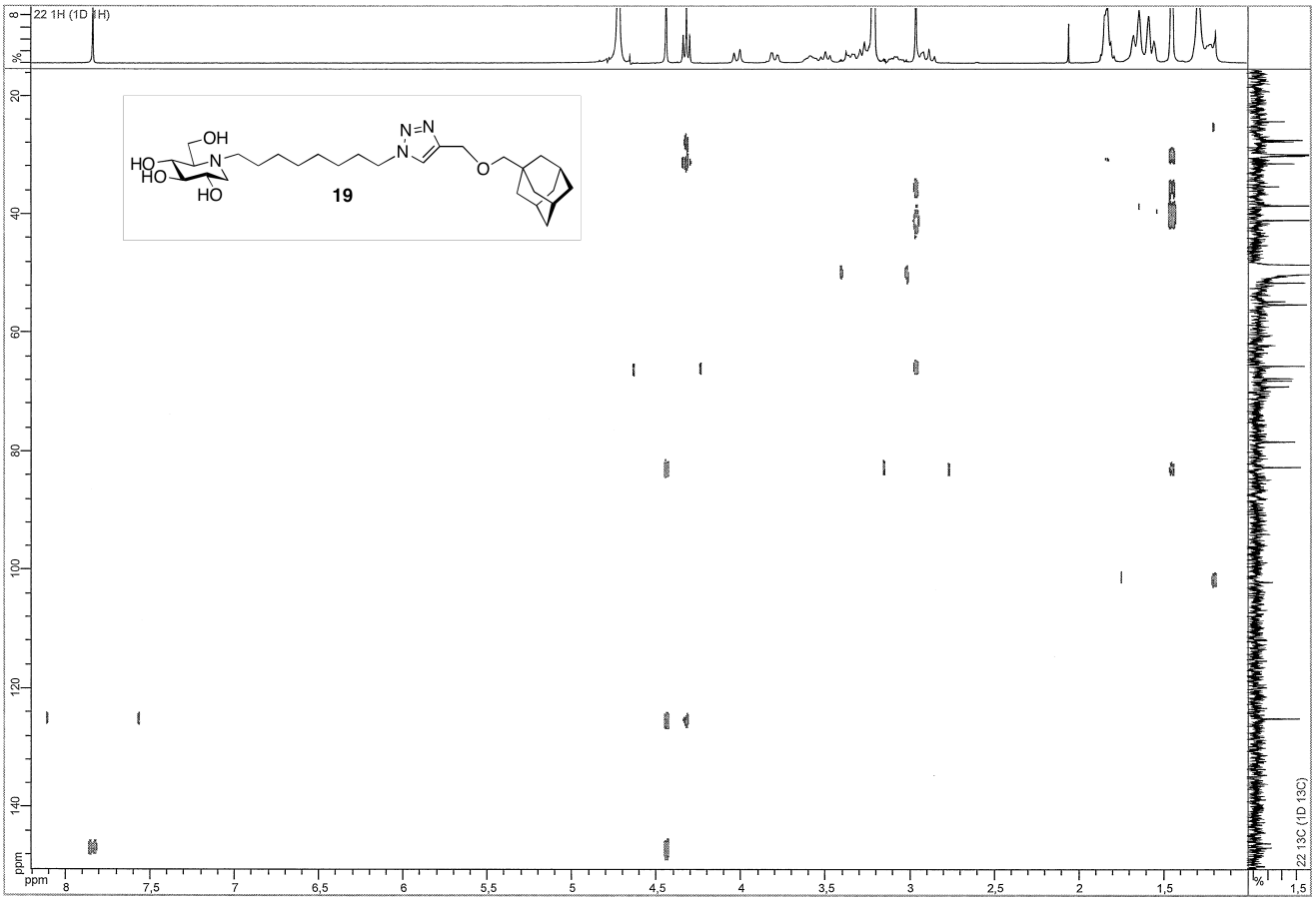
1,2
22 13C (1D 13C)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22 13C (1D 13C)