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Synthesis of triazolyl-linked polysialic acids

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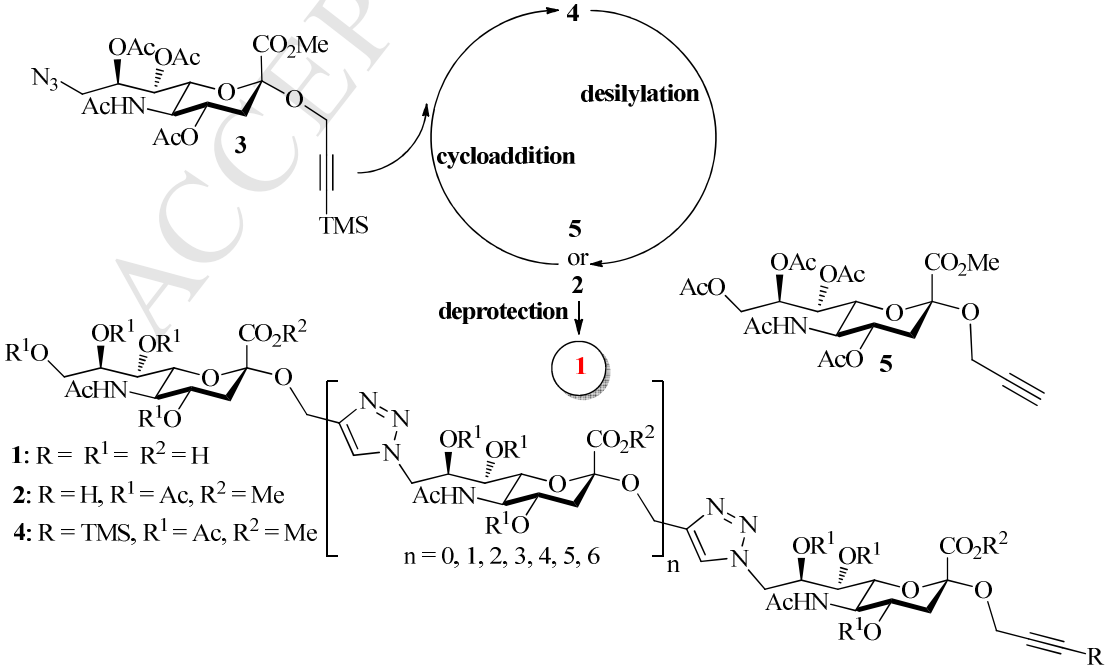
Abstract:

A new approach for the synthesis of triazolyl-linked α-(2-9) oligosialic acids by iterative copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) and desilylation has been developed. By using propargyl α-sialoside **5** and trimethylsilylated-propargyl 9-azido-α-sialoside **3** as building blocks, triazolyl-linked α-(2-9) di- to octa-sialic acids were constructed in good to moderate yields. Finally, the pseudo-α-(2-9)-oligosialic acids were obtained very smoothly via *O*-deprotection. The oligosialic acids with a triazolyl-linkage represent a new type of pseudooligosialic acids. This protocol may find applications in the preparation of oligosialic acid analogues with biological importance.

Keywords: polysialic acid; click reaction; triazole; cycloaddition; carbohydrate

Graphical abstract:



1. Introduction

Protein or lipid glycosylation is an omnipresent and life-governing process. Sialic acids, a family of sugar units with a nine-carbon backbone that are typically attached to the outermost ends of sugar chains,[1] exert a multitude of biological and pathological effects such as cellular adhesion, inflammatory response, cell signaling, and cell differentiation.[2] Polysialic acid (PSA) is a type of linear homopolymer of sialic acid. Three kinds of polysialic acids have been identified in nature, these include α(2-8), α(2-9), and alternating α(2-8) and α(2-9) linkages,[3] as shown in Figure 1.

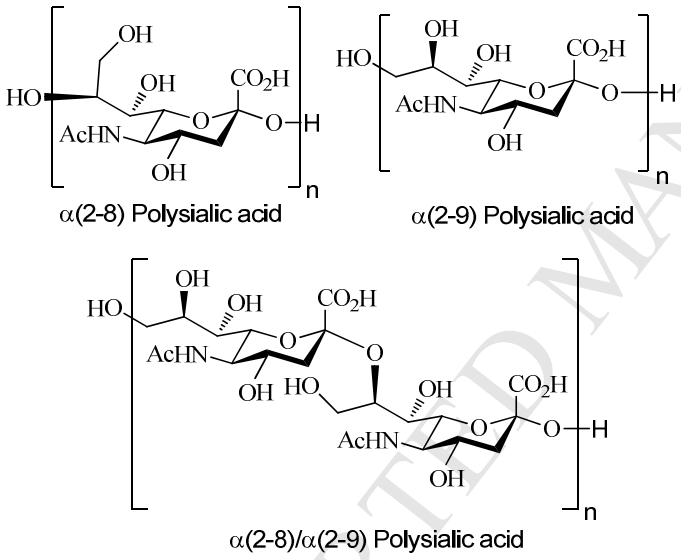


Figure 1. Structures of polysialic acids.

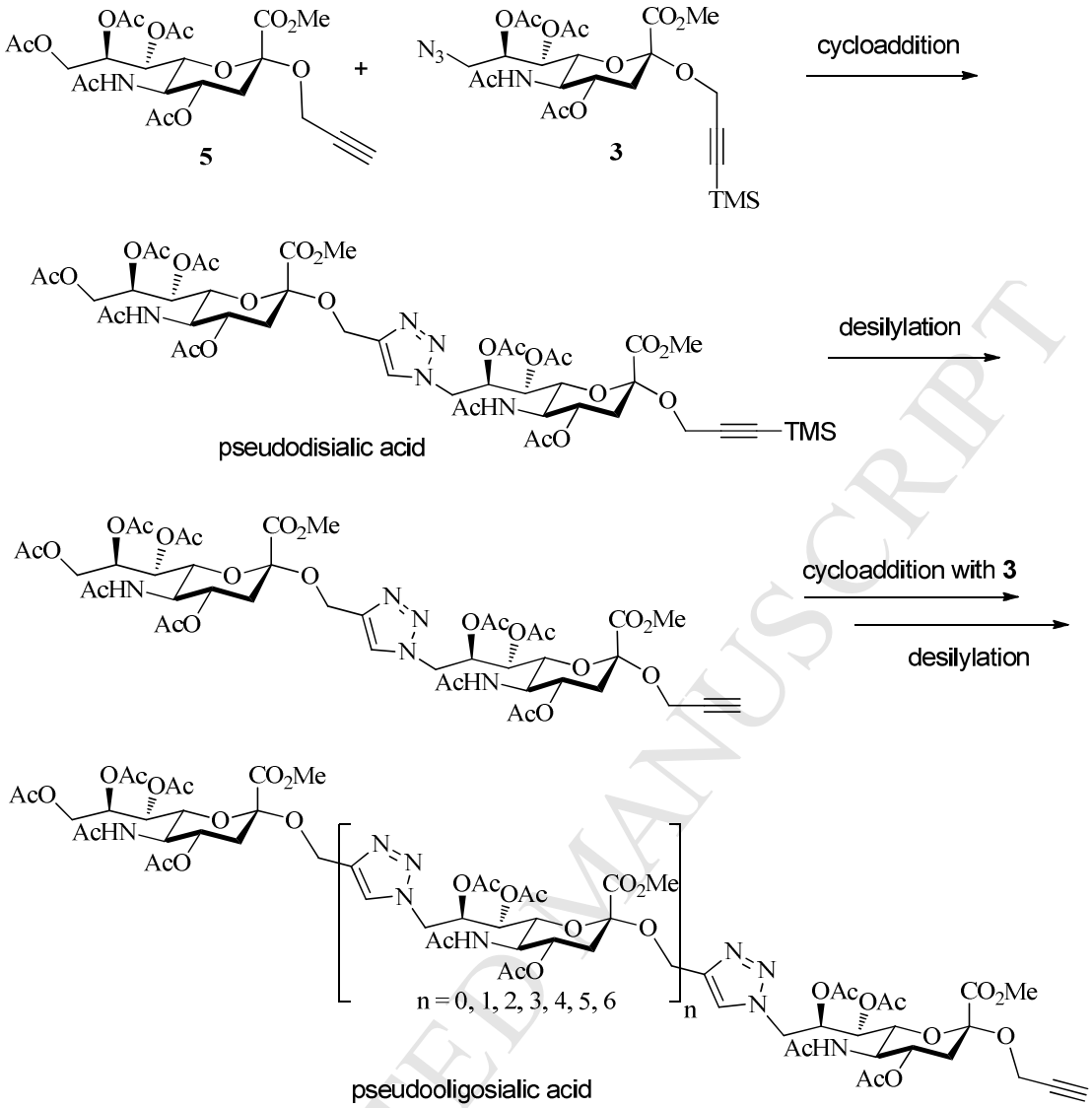
Due to its outermost location, PSA plays a vital role in biological processes such as embryogenesis, neural cell growth, differentiation, cell-cell mediating, and membrane transport.[4] In addition, many studies suggest that polysialic acids, present on the surface of *N. meningitidis* B and C,[5] human embryonal carcinoma cells,[6] mouse neurobrastoma cells,[7] and sea urchin sperm flagella *et al.*,[8] serve as bacterial or cancer cell shields against the attack systems. Moreover, PSA can also be used in biomedical engineering and tissue engineering.[9] Therefore, polysialic acids are considered either good targets for the development of vaccines or tools for probing the interactions between polysaccharides and their receptors.[10] However, polysialic acids are often heterogeneous or contaminated from natural sources and susceptible to degradation by chemical

hydrolysis or glycosyl hydrolases.[10b,11] The establishment of an effective method to synthesize pure polysialic acids/pseudopolysialic acids with the well-defined structure will not only simplify the complexity of polysialic acid-based vaccines, but also benefit a better understanding of the functions of polysialic acids.

Considerable efforts have been aroused in the synthesis of both PSA and PSA analogues. Some methods have been developed for the synthesis of homooligosialic acids with α(2-8), α(2-9), and alternating α(2-8)/α(2-9) intersialyl linkages.[12] *S*-Linked α(2-8) and alternating α(2-8)/α(2-9) oligosialic acids resistant to hydrolysis have also been prepared.[13] On the other hand, the [3+2] Huisgen cyclization of an azide with an alkyne to form a triazole has recently emerged as a powerful conjugate strategy for the preparation of neoglycoconjugates with biomedical interest,[14] in which the formed triazole ring can improve the solubility in water and take part in hydrogen bonding and dipole interactions.[15] By using the iterative copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction strategy,[16] herein we report the synthesis of triazolyl-linked α(2-9) oligosialic acids.

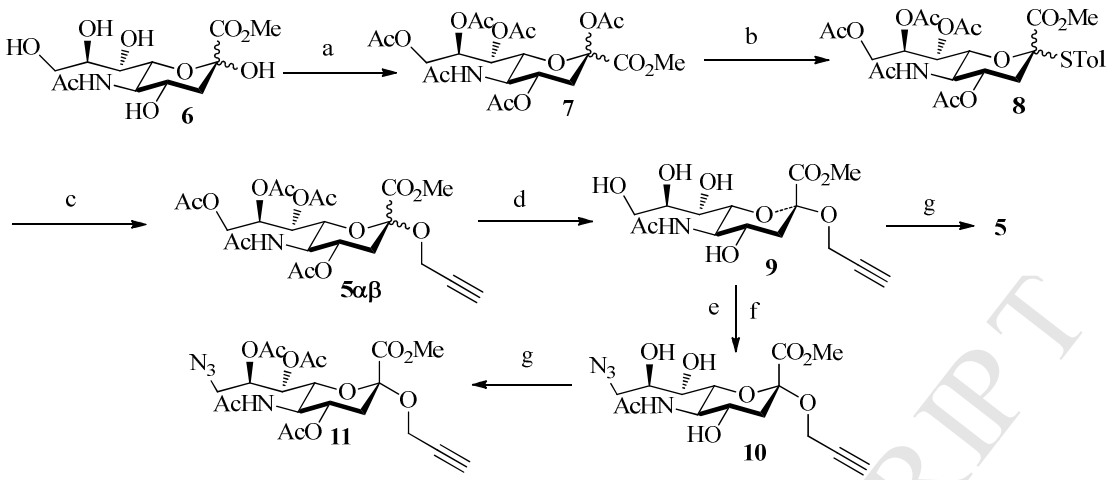
2. Results and discussion

To achieve triazolyl-linked α(2-9) oligosialic acids, sialic acid building blocks **5** and **3** were chosen. As shown in Scheme 1, compound **5** is coupled with **3** using CuAAC reaction to afford the pseudodisialic acid. The temporarily silyl protective group can be removed to yield the desilylated product, which is followed by the cycloaddition reaction to produce the pseudotrisialic acid. The repetition of cycloaddition-desilylation reactions allows rapid assembly of triazolyl-linked oligosialic acids. In this synthetic strategy, *O*-peracetylated propargyl α-sialoside **5** and *O*-peracetylated trimethylsilylated-propargyl 9-azido-α-sialoside **3** are the key building blocks. The trimethylsilyl protection of alkyne was a prerequisite devoid of polymerization. The use of *O*-acetyl protection is to facilitate the preparation of the building blocks, the coupling reactions, and the isolation of the coupling products. Ethynyl α-sialoside might be the most proper building blocks in view of the similarity and resistance to chemical and enzymatic degradation, but it is difficult to obtain. Thus, a longer propargyl linker, which might not impair the functions of polysaccharides,[14f] was used.



Scheme 1. The synthetic strategy of triazolyl-linked α(2-9) oligosialic acids.

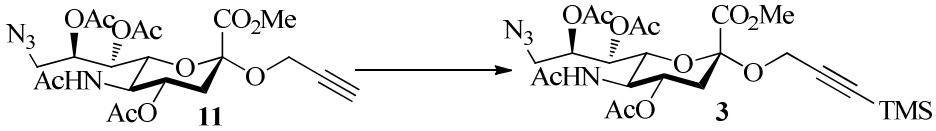
The synthesis of building block **5** and intermediate **11** followed the reported procedure with some modifications (Scheme 2).[17] The peracetylated sialic acid **7**[18] was treated with *p*-thiocresol to give thiosialoside **8** in 87% yield. Promoted by NIS/TfOH, glycosyl donor **8** underwent the sialylation reaction with propargyl alcohol smoothly to afford compound **5αβ** in 89% yield (α/β = 2/1, inseparable mixture). Deacetylation of **5αβ** obtained the desired α-isomer **9** after column chromatography, which was acetylated to provide **5**. Treatment of **9** with TsCl, which was followed by the azido-substitution with NaN_3 generated compound **10**. Acetylation of **10** gave compound **11** in 75% yield.



Scheme 2. Reagents and conditions: a) Pyr., Ac_2O, DMAP, r.t., 95%; b) BF_3•Et_2O, TolSH, CH_2Cl_2, r.t. overnight, 87%; c) NIS/TfOH, propargyl alcohol, CH_2Cl_2, 4 Å MS, 89%; d) NaOMe/MeOH, r.t. 47%; e) TsCl, Pyr., 4 Å MS, 0 ^oC; f) NaN_3, 15-crown-5, DMF, H_2O, 65 ^oC, 42% over two steps; g) Pyr., Ac_2O, DMAP, r.t., 75% for **11**, 85% for **5**.

Next, we turn our attention to the synthesis of silylalkyne **3**. There are many methods for silylation of terminal alkynes.[19] Due to the presence of ester group in compound **11**, mild trimethylsilylation conditions are needed. The silylation conditions varying both the reagents and solvents were investigated at different temperatures, and the results were summarized in Table 1. When TMSOTf/Et_3N[16b] (Entry 1) or Et_2NTMS[20] (Entry 2) was employed in THF using $ZnCl_2$ as the catalyst, there was no reaction observed. When trifluoromethyltrimethylsilane ($TMSCF_3$)/KF or CsF (Entries 3 and 4) was used,[21] the silylalkyne **3** was obtained in low yield (< 25%). We inferred that the fluoride could react with SiO_2. Therefore, when Teflon tube was used instead of glassware, the yield was improved significantly (70%, Entry 5). Similarly, 4Å molecular sieves also made this reaction not occur in Teflon tube (Entry 6). With the increase of the reaction temperature, the yield was decreased (< 50%, Entry 7), resulting from the decomposition of silylalkyne **3** at higher temperatures. So we got the optimized silylation reaction conditions: $TMSCF_3$/CsF/anhydrous 1,4-dioxane/30 ^oC in Teflon tube.

Table 1. Preparation of **3** and optimization of the silylation conditions of **11**.



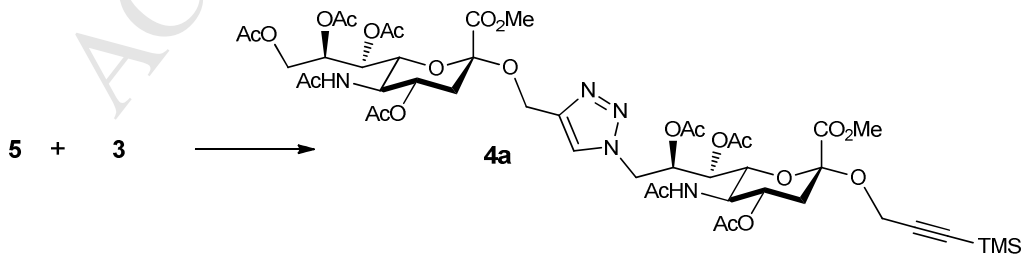
Entry	Reagent	Solvent	T (^oC)	Yield

1	$ZnCl_2$/TMSOTf/ Et_3N	THF	r.t. to 60	no reaction
2	$ZnCl_2$/Et_2NTMS	THF	r.t. to 60	no reaction
3	$TMSCF_3$/KF	DMF	r.t.	< 25%
4[c]	$TMSCF_3$/CsF	1,4-dioxane	r.t.	< 20%
5[a,c]	$TMSCF_3$/CsF	1,4-dioxane	30	70%
6[a,b,c]	$TMSCF_3$/CsF	1,4-dioxane	30	no reaction
7[a,c]	$TMSCF_3$/CsF	1,4-dioxane	50	< 50%

[a]In Teflon tube. [b]With 4Å molecular sieves. [c]Conditions: $TMSCF_3$ (3.0 equiv.), CsF (0.4 equiv.), 1,4-dioxane (2.0 mL).

With these building blocks in hand, the copper(I)-catalyzed azide-alkyne cycloaddition reaction of propargyl α-sialoside **5** and trimethylsilylated-propargyl 9-azido-α-sialoside **3** was investigated (Table 2). When $CuSO_4$/sodium ascorbate/DMF was used, the reaction failed to provide the desired product **4a** (Entry 1). Under the catalysis of $CuSO_4$/sodium ascorbate in *t*-BuOH/CH_2Cl_2/H_2O or DMF/H_2O, the thin layer chromatography (TLC) monitoring showed that most of the starting materials disappeared and some polymeric products generated (Entries 2 and 3). This is because the silylalkynes **3** and **4a**, which are water-sensitive, were desilylated under these conditions. When using CuI (0.2 eq.)/DIPEA/DMF at 50 °C, the reaction proceeded and the dimeric product **4a** was isolated in 48% yield (Entry 4). The yield was improved as the solvent was changed to acetonitrile (Entry 5, 65% yield). However, the microwave irradiation provided no gains in the yield (Entry 6, 54% yield). With the decrease of the reaction temperature, the yield was further increased (Entry 7, 78% yield).

Table 2. CuAAC reaction of monosaccharide building blocks **5** and **3**.

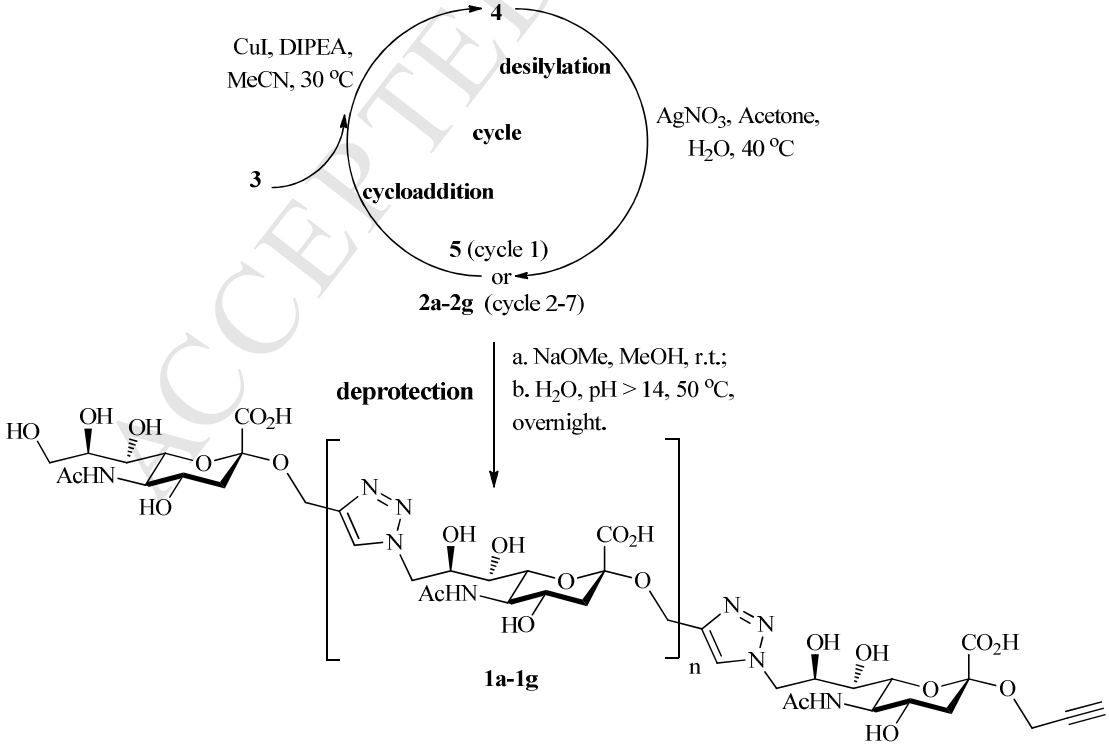


Entry	Catalyst	Solvent	Temperature	Yield
1	$CuSO_4$(0.15 eq.)/sodium ascorbate	DMF	50 °C	no reaction

2	$CuSO_4$ (0.15 eq.)/sodium ascorbate	DMF/H_2O	50 °C	polymers
3	$CuSO_4$(0.15 eq.)/sodium ascorbate	*t*-BuOH/CH_2Cl_2/H_2O	50 °C	polymers
4	CuI (0.2 eq.)/DIPEA	DMF	50 °C	48%
5	CuI (0.2 eq.)/DIPEA	MeCN	50 °C	65%
6	CuI (0.2 eq.)/DIPEA	MeCN	50 °C，MW 150W	54%
7	CuI (0.2 eq.)/DIPEA	MeCN	30 °C	78%

Subsequently, we optimized the reaction conditions of desilylation. After surveying the efficiency of reaction with TBAF/THF/MeCN,[22] AgF/Et_3N/MeCN[23] or $AgNO_3$/H_2O/acetone,[24] we were pleased to discover that the treatment of dimer **4a** with $AgNO_3$ in H_2O and acetone at 40 °C afforded the desired desilylated pseudodisaccharide **2a** in quantitative yield. As the coupling reaction and the desilylation reaction were merged in one-pot, some higher oligomers could be produced, resulting from the further reaction of the desilylated product with the residual azido-containing building block. Therefore, the desired oligomeric product needed to be purified by column chromatography.

Table 3. Iterative CuAAC reaction and desilylation to produce pseudo-α(2-9)-oligosialic acids.



Cycle	n	Cycloaddition and Desilylation	Deprotection

		Product	Yield	Product	yield
1	0	**2a**	78%	**1a**	78%
2	1	**2b**	76%	**1b**	89%
3	2	**2c**	60%	**1c**	88%
4	3	**2d**	77%	**1d**	99%
5	4	**2e**	68%	**1e**	87%
6	5	**2f**	56%	**1f**	94%
7	6	**2g**	47%	**1g**	96%

Thus, the same cycloaddition-desilylation sequence was repeated over several times (up to seven cycles) with gradually decreased efficiency. This is because a higher trimethylsilylated-propargyl oligomer (with one more sialic acid than the desired) was formed due to the further cycloaddition reaction of the partially desilylated product with **3**. As the sugar chain becomes longer, the side-product (higher oligomer) would be produced in an increased amount. Oligomers **2a-g** were deprotected smoothly under the basic conditions to afford the corresponding triazolyl-linked α-(2-9) di- to octa- oligosialic acids **1a**-**1g**. Based on this protocol, all synthetic compounds **2a-g** and **1a-g** were obtained in excellent to moderate yields. The isolated yield of each cycle was summarized in Table 3. The structures of **2a-g** and **1a-g** were unambiguously identified by their ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectra.

3. Conclusion

In conclusion, we have developed an efficient way to synthesize α-(2-9)-oligosialic acid analogues linked by a triazole ring using the Cu(I)-catalyzed azide-alkyne cycloaddition strategy. By using propargyl α-sialoside **5** and trimethylsilylated-propargyl 9-azido-α-sialoside **3** as building blocks, triazolyl-linked α-(2-9) di- to octa- oligosialic acids were assembled in good to moderate yields via the iterative cycloaddition-desilylation operation. The subsequent *O*-deprotection afforded the pseudo-α-(2-9)-oligosialic acids in high yields and with high purity. To the best our knowledge, the triazolyl linkage was introduced by us for the assembly of pseudooligosialic acids for the first time. The disclosed protocol may find applications in the preparation of α(2-8) and alternating α(2-8)/α(2-9) oligosialic acid analogues which will facilitate the understanding of functions of polysialic acids and the related drug discovery.

4. Experimental

4.1. General

All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Dichloromethane (CH_2Cl_2), pyridine, and acetonitrile were distilled over calcium hydride (CaH_2). Methanol was distilled from magnesium. DMF and 1,4-dioxane were stirred with calcium hydride (CaH_2) and distilled under reduced pressure. All reactions were carried out under anhydrous conditions with freshly distilled solvents, unless otherwise noted. Reactions were monitored by analytical thin-layer chromatography on silica gel 60-F_{254} precoated on alunminum plates (E. Merck.). Spots were detected under UV (254 nm) and/or by staining with acidic ceric ammonium molybdate. Solvents were evaporated under reduced pressure and below 40 ^{o}C (bath). Column chromatography was performed on silica gel (200-300 mesh, TsingDao Ocean or 230-400 mesh, Merck), reversed phase column chromatography was performed on LiChroprep RP-18 (40-63 μm, Merck). ^{1}H NMR spectra were recorded on a Bruker 400M or 600M spectrometers at 25 ^{o}C. Chemical shifts (in ppm) were referenced to tetramethylsilane (δ = 0 ppm) in deuterated chloroform. ^{13}C NMR spectra were obtained by using the same NMR spectrometers and were calibrated with $CDCl_3$ (δ = 77.00 ppm). Mass spectra were recorded using a Bruker APEX IV FT-MS（7.0 T）spectrometer.

4.2. General procedure for cycloaddition-desilylation reactions

To a mixed solution of **5** or **2a-f** (1.0 eq.) and **3** (1.0-1.1 eq.) in dried MeCN, CuI (0.2 eq.) and DIPEA (2.0 eq.) was added. The reaction mixture was stirred at 30 ^{o}C. After the disappearance of **5** or **2a-f** (detected by TLC) the solvent was evaporated. The silylalkyne products **4a-g** were purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 25:1). Products **4a-g** were dissolved in acetone (3.0 mL) and water (0.1 mL). To the reaction mixture was added $AgNO_3$ (0.5 eq.), and the mixture was stirred for 1 h at 40 °C. Subsequently, the reaction was quenched by saturated aq. NH_4Cl and the solvent was evaporated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH) to afford **2a-g**.

4.3. General procedure for deprotection

To a solution of **2a-2g** in methanol (1.0 mL) was added NaOMe (81.0 mg, 1.5 mmol). After stirring for 0.5 h, the reaction mixture was added H_2O (0.1 mL) and stirred overnight at 50 ^{o}C. The resulting solution was neutralized with hydrochloric acid (1 M) to pH = 4-5, and evaporated to

dryness. The residue was purified by reversed-phase column chromatography to afford the deprotected products **1a-g**.

4.4. Methyl (2-(3-trimethylsilyl-2-propynyl) 5-acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyrano sid)onate (3)

To a solution of **11**[17] (600.0 mg, 1.17 mmol) in dry 1,4-dioxane in a Teflon tube was added $TMSCF_3$ (0.6 mL) and cesium fluoride (20.0 mg) under N_2 atmosphere at 30 ^oC. After stirring for 0.5 h, the mixture was evaporated, and the residue was purified by column chromatography on silica gel (petroleum ether/acetone, 3:1) to afford compound **3** as a white solid (476.0 mg, 70% yield). $[\alpha]_D^{30}$ -0.81 (*c* 2.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.34-5.30 (m, 2H, H-7, H-8), 5.15 (d, *J* = 9.4 Hz, 1H, NH), 4.85 (ddd, *J* = 12.0, 9.9, 4.7 Hz, 1H, H-4), 4.43 (d, *J* = 15.6 Hz, 1H, HC≡CCH_2), 4.12-4.03 (m, 3H, HC≡CCH_2, H-5, H-6), 3.82 (s, 3H, OCH_3), 3.59-3.56 (m, 1H, H-9b), 3.30-3.25 (m, 1H, H-9a), 2.64 (dd, *J* = 12.8, 4.6 Hz, 1H, H-3b), 2.18 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.01 (t, *J* = 12.6 Hz, 1H, H-3a), 1.89 (s, 3H, NHCOCH_3), 0.17 (s, 9H, (CH_3)$_3$Si); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.98, 170.25, 170.19, 170.12, 167.74, 100.26, 98.22, 91.57, 72.92, 69.75, 68.80, 67.88, 53.60, 52.88, 50.88, 49.33, 37.81, 23.16, 21.00, 20.81, -0.27. HRMS (ESI) Calcd. for $C_{24}H_{40}N_5O_{11}Si$ ($[M + NH_4]^+$): 602.2488; found: 602.2486.

4.5. Protected disialic acid (2a)

The reaction of **5**[17] (27.2 mg, 51.4 μmol, 1.0 eq.) and **3** (30.0 mg, 51.4 μmol, 1.0 eq.) was performed for 2 h as described in the general procedure for cycloaddition-desilylation, affording **2a** (elution with CH_2Cl_2/MeOH (25/1); 41.6 mg, 78% yield; R_f = 0.48, MeOH/EtOAc, 1:8, v/v) as white solids. $[\alpha]_D^{30}$ -0.93 (*c* 3.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 1H, NCH), 5.48-5.43 (m, 2H, H-8, H-8'), 5.36 (d, *J* = 9.8 Hz, 1H, NH), 5.33 (dd, *J* = 8.5, 2.0 Hz, 1H, H-7'), 5.29 (dd, *J* = 6.4, 1.6 Hz, 1H, H-7), 5.24 (d, *J* = 9.6 Hz, 1H, NH), 4.91-4.83 (m, 4H, H-4, H-4', OCH_2C), 4.53 (d, *J* = 12.0 Hz, 1H, H-9b), 4.43 (dd, *J* = 15.7, 2.4 Hz, 1H, HC≡CCH_2), 4.40 (dd, *J* = 14.6, 7.9 Hz, 1H, H-9b'), 4.34 (dd, *J* = 12.4, 2.7 Hz, 1H, H-9a'), 4.23 (dd, *J* = 15.7, 2.2 Hz, 1H, HC≡CCH_2), 4.16-4.06 (m, 5H, H-9a, H-5, H-5', H-6, H-6'), 3.83 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 2.67 (dd, *J* = 12.8, 4.7 Hz, 1H, H-3e), 2.62 (dd, *J* = 12.8, 4.5 Hz, 1H, H-3e'), 2.46 (t, *J* = 2.4 Hz, 1H, *H*C≡C), 2.21 (s, 3H, $COCH_3$), 2.17 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 2.07 (s, 3H,

$COCH_3$), 2.04 (s, 6H, $2COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.01-1.94 (m, 2H, H-3a, H-3a'), 1.89 (s, 3H, NHCOCH_3), 1.88 (s, 3H, NHCOCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.96, 170.93, 170.71, 170.67, 170.30, 170.21, 170.14, 170.08, 169.86, 168.17, 167.69, 143.97, 124.02, 98.63, 98.37, 78.84, 74.69, 73.23, 72.60, 70.17, 68.93, 68.66, 68.41, 67.35, 62.49, 58.50, 52.98, 52.89, 50.08, 49.39, 49.34, 37.90, 37.68, 23.16, 23.11, 21.10, 20.89, 20.81, 20.74; HRMS (ESI) Calcd. for $C_{44}H_{60}N_5O_{24}$ ($[M + H]^+$): 1042.3623; found: 1042.3657.

4.6. Protected trisialic acid (2b)

The reaction of **2a** (33.9 mg, 32.6 μmol) and **3** (24.6 mg, 35.8 μmol, 1.1 eq.) was performed for 4 h as described in the general procedure for cycloaddition-desilylation, affording **2b** (elution with CH_2Cl_2/MeOH (20/1); 38.4 mg, 76% yield; R_f = 0.48, MeOH/EtOAc, 1:8, v/v) as white solids. $[\alpha]_D^{30}$ –1.04 (*c* 3.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.72 (s, 1H), 5.68 (d, *J* = 9.0 Hz, 1H), 5.49-5.28 (m, 8H), 4.95-4.84 (m, 7H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.45-4.39 (m, 3H), 4.35 (dd, *J* = 12.3, 1.6 Hz, 1H), 4.24 (dd, *J* = 15.7, 2.0 Hz, 1H), 4.18-4.06 (m, 7H), 3.81 (s, 3H), 3.79 (s, 3H), 3.76 (s, 3H), 2.69-2.59 (m, 3H), 2.46 (br.s, 1H), 2.22 (s, 3H), 2.20 (s, 3H), 2.16 (s, 6H), 2.07-2.02 (m, 18H), 2.00-1.94 (m, 3H), 1.88-1.87 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.94, 170.79, 170.73, 170.69, 170.62, 170.34, 170.29, 170.25, 170.10, 169.91, 169.78, 168.17, 168.12, 167.73, 143.90, 143.81, 124.16, 123.97, 98.81, 98.58, 98.34, 78.86, 74.71, 73.23, 72.60, 70.38, 70.15, 68.93, 68.74, 68.53, 68.47, 68.32, 67.34, 62.45, 58.42, 52.96, 52.87, 50.10, 49.39, 49.19, 37.88, 37.64, 23.14, 23.10, 23.06, 21.10, 20.91, 20.88, 20.80, 20.74; HRMS (ESI) Calcd. for $C_{65}H_{88}N_9O_{35}$ ($[M + H]^+$): 1554.5377; found: 1554.5416.

4.7. Protected tetrasialic acid (2c)

The reaction of **2b** (38.4 mg, 24.7 μmol) and **3** (15.9 mg, 27.2 μmol, 1.1 eq.) was performed for 8 h as described in the general procedure for cycloaddition-desilylation, affording **2c** (elution with CH_2Cl_2/MeOH (15/1); 30.4 mg, 60% yield; R_f = 0.48, MeOH/EtOAc, 1:7, v/v) as white solids. $[\alpha]_D^{30}$ -0.27 (*c* 3.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.74 (s, 1H), 7.72 (s, 1H), 5.76 (br.d, *J* = 9.4 Hz, 1H), 5.58 (br.d, *J* = 9.6 Hz, 1H), 5.51-5.43 (m, 4H), 5.37-5.27 (m, 6H), 4.95-4.85 (m, 10H), 4.63-4.53 (m, 3H), 4.46-4.38 (m, 4H), 4.35 (dd, *J* = 12.5, 2.7 Hz, 1H), 4.24 (dd, *J* = 15.7, 2.4 Hz, 1H), 4.16-4.06 (m, 9H), 3.80 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H), 3.75 (s, 3H), 2.69-2.56 (m, 4H), 2.46 (t, *J* = 2.4 Hz, 1H), 2.21- 2.20 (m, 9H), 2.16 (s, 6H), 2.05-2.03 (m,

21H), 2.00-1.93 (m, 4H), 1.88 (br.s, 12H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.99, 170.90, 170.86, 170.80, 170.71, 170.69, 170.41, 170.34, 170.18, 170.12, 169.96, 169.92, 169.88, 168.16, 168.14, 168.12, 167.78, 143.92, 143.82, 143.71, 124.25, 124.18, 124.05, 98.80, 98.78, 98.57, 98.34, 78.92, 74.79, 73.23, 73.16, 72.59, 70.47, 70.41, 70.16, 68.91, 68.85, 68.78, 68.75, 68.46, 68.44, 68.32, 67.32, 62.46, 58.46, 58.40, 58.37, 53.01, 52.90, 50.12, 50.08, 50.02, 49.37, 49.26, 49.13, 37.86, 37.65, 29.66, 23.15, 23.11, 23.08, 23.06, 21.13, 21.03, 20.93, 20.91, 20.83, 20.77; HRMS (ESI) Calcd. for $C_{86}H_{116}N_{13}O_{46}$ ($[M + H]^+$): 2066.7132; found: 2066.7181.

4.8. Protected pentasialic acid (2d)

The reaction of **2c** (40.5 mg, 19.6 μmol) and **3** (12.0 mg, 20.6 μmol, 1.05 eq.) was performed for 12 h as described in the general procedure for cycloaddition-desilylation, affording **2d** (elution with CH_2Cl_2/MeOH (12/1); 38.9 mg, 77% yield; R_f = 0.39, MeOH/EtOAc, 1:3, v/v) as white solids. $[\alpha]_D^{30}$ –1.15 (*c* 2.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75-7.72 (m, 4H), 5.87 (d, *J* = 9.2 Hz, 1H), 5.79 (d, *J* = 9.1 Hz, 1H), 5.70 (d, *J* = 9.2 Hz, 1H), 5.57-5.44 (m, 6H), 5.37-5.29 (m, 6H), 4.92-4.86 (m, 14H), 4.63-4.53 (m, 4H), 4.45-4.42 (m, 5H), 4.35 (dd, *J* = 12.4, 2.6 Hz, 1H), 4.24 (dd, *J* = 15.7, 2.4 Hz, 1H), 4.20-4.07 (m, 12H), 3.80-3.74 (m, 15H), 2.68-2.59 (m, 5H), 2.47 (t, *J* = 2.4 Hz, 1H), 2.21-2.20 (m, 12H), 2.16 (s, 6H), 2.05-2.02 (m, 30H), 2.00-1.97 (m, 5H), 1.88-1.87 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.99, 170.88, 170.84, 170.80, 170.72, 170.70, 170.67, 170.43, 170.34, 170.18, 170.11, 169.94, 169.89, 168.17, 168.14, 168.12, 167.79, 143.92, 143.71, 124.20, 124.04, 98.80, 98.77, 98.58, 98.34, 78.88, 74.78, 73.22, 73.16, 72.59, 70.45, 68.91, 68.85, 68.78, 68.76, 68.47, 68.44, 68.34, 67.32, 62.47, 60.39, 58.47, 58.37, 54.03, 53.01, 52.91, 52.89, 50.11, 49.38, 49.28, 49.23, 49.13, 37.87, 37.65, 23.16, 23.11, 23.08, 21.14, 21.03, 20.95, 20.92, 20.86, 20.83, 20.77. HRMS (ESI) Calcd. for $C_{107}H_{144}N_{17}O_{57}$ ($[M + H]^+$): 2578.8886; found: 2578.8902.

4.9. Protected hexasialic acid (2e)

The reaction of **2d** (32.1 mg, 12.4 μmol) and **3** (7.3 mg, 12.4 μmol, 1.0 eq.) was performed for 12 h as described in the general procedure for cycloaddition-desilylation, affording **2e** (elution with CH_2Cl_2/MeOH (10/1); 26.2 mg, 68% yield; R_f = 0.35, MeOH/EtOAc, 1:3, v/v) as white solids. $[\alpha]_D^{30}$ -0.73 (*c* 3.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77 (m, 5H), 6.00-5.89 (m, 4H), 5.66-5.44 (m, 8H), 5.35-5.30 (m, 6H), 4.92-4.88 (m, 16H), 4.62-4.35 (m, 12H), 4.26-4.07 (m,

14H), 3.80-3.74 (m, 18H), 2.69-2.60 (m, 6H), 2.51 (s, 1H), 2.21-2.20 (m, 15H), 2.15 (s, 6H), 2.07-1.98 (m, 42H), 1.88(s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.97, 170.96, 170.82, 170.76, 170.68, 170.54, 170.50, 170.41, 170.24, 170.11, 170.00, 169.96, 168.14, 168.12, 167.77, 143.78, 124.25, 98.79, 98.76, 98.56, 98.32, 74.84, 73.20, 73.13, 72.57, 70.53, 70.19, 68.89, 68.85, 68.77, 68.47, 68.42, 68.32, 67.32, 62.46, 58.44, 58.33, 53.00, 52.92, 52.87, 50.13, 49.31, 49.16, 49.06, 37.83, 37.62, 23.11, 23.06, 23.02, 21.12, 20.92, 20.89, 20.84, 20.81, 20.75; HRMS (ESI) Calcd. for $C_{128}H_{171}N_{21}O_{68}K_2$ ($[M + 2K]^{2+}$): 1564.5089; found: 1564.5160.

4.10. Protected heptasialic acid (2f)

The reaction of **2e** (25.6 mg, 8.3 μmol) and **3** (4.9 mg, 8.3 μmol, 1.0 eq.) was performed for 12 h as described in the general procedure for cycloaddition-desilylation, affording **2f** (elution with CH_2Cl_2/MeOH (8/1); 16.8 mg, 56% yield; R_f = 0.48, MeOH/EtOAc, 2:5, v/v) as white solids. $[\alpha]_D^{30}$ -0.31 (*c* 3.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (m, 6H), 5.93-5.84 (m, 5H), 5.59-5.44 (m, 8H), 5.33-5.30 (m, 7H), 4.93-4.88 (m, 19H), 4.61-4.52 (m, 5H), 4.44-4.34 (m, 9H), 4.26-4.07 (m, 17H), 3.80-3.74 (m, 21H), 2.68-2.63 (m, 7H), 2.50 (s, 1H), 2.20 (m, 18H), 2.15 (m, 6H), 2.07-1.96 (m, 49H), 1.88 (br.s, 21H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.95, 170.83, 170.78, 170.75, 170.66, 170.46, 170.40, 170.33, 170.16, 170.07, 169.90, 168.14, 167.77, 143.82, 132.25, 130.88, 129.97, 129.67, 128.79, 124.25, 98.75, 98.56, 98.32, 74.83, 73.20, 73.14, 72.57, 70.40, 68.90, 68.87, 68.77, 68.49, 68.41, 68.35, 67.31, 65.53, 62.46, 58.34, 53.01, 52.88, 50.17, 49.34, 49.20, 49.09, 37.85, 37.64, 23.14, 23.09, 23.06, 22.63, 21.13, 20.95, 20.90, 20.85, 20.81, 20.76. HRMS (ESI) Calcd. for $C_{149}H_{201}N_{25}O_{79}$ ($[M + 2H]^{2+}$): 1802.1239.; found: 1802.2267.

4.11. Protected octasialic acid (2g)

The reaction of **2f** (49.0 mg, 13.6 μmol) and **3** (8.3 mg, 14.3 μmol, 1.05 eq.) was performed for 12 h as described in the general procedure for cycloaddition-desilylation, affording **2g** (elution with CH_2Cl_2/MeOH (8/1); 26.1 mg, 47% yield; R_f = 0.39, MeOH/EtOAc, 2:5, v/v) as white solids. $[\alpha]_D^{30}$ -0.97 (*c* 3.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76-7.73 (m, 7H), 5.98-5.85 (m, 6H), 5.48-5.43 (m, 8H), 5.35-5.29 (m, 9H), 4.93-4.85 (m, 22H), 4.62-4.60 (m, 6H), 4.47-4.33 (m, 10H), 4.27-4.06 (m, 19H), 3.80-3.73 (m, 24H), 2.64-2.62 (m, 8H), 2.49 (s, 1H), 2.21-2.15 (m, 27H), 2.07-1.97 (m, 56H), 1.88 (br.s, 27H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.97, 170.80, 170.76, 170.67, 170.50, 170.45, 170.36, 170.19, 170.09, 169.93, 168.16, 167.78, 167.70, 143.74, 132.26,

130.89, 129.98, 129.68, 128.80, 124.24, 98.79, 98.76, 98.57, 98.33, 74.82, 73.19, 72.58, 70.43, 70.15, 68.88, 68.78, 68.44, 68.35, 67.33, 65.54, 62.47, 58.34, 53.01, 52.91, 52.87, 50.14, 49.34, 49.19, 49.09, 37.64, 23.05, 20.94, 20.91, 20.85, 20.82, 20.77. MS (MALDI-TOF) m/z Calcd. for $C_{170}H_{227}N_{29}O_{90}Na$ $[M + Na]^+$: 4138.40; found: 4139.24.

4.12. Triazolyl-linked α(2-9)-disialic acid (1a)

The deprotection of **2a** (25.4 mg, 24.4 μmol) was performed as described in the general procedure for deprotection, affording **1a** as white solids (13.7 mg, 78% yield). $[\alpha]_D^{30}$ -0.15 (*c* 2.6, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 8.06 (s, 1H), 4.90 (d, *J* = 12.0 Hz, 1H), 4.84-4.74 (m, 1H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.54 (dd, *J* = 14.6, 8.0 Hz, 1H), 4.33 (d, *J* = 15.6 Hz, 1H), 4.25 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.20 (d, *J* = 15.6 Hz, 1H), 3.94-3.80 (m, 4H), 3.75-3.58 (m, 6H), 3.43 (dd, *J* = 9.0, 1.4 Hz, 1H), 2.77-2.69 (m, 2H), 2.03 (s, 3H), 2.02 (s, 3H), 1.71-1.64 (m, 2H); ^{13}C NMR (100 MHz, D_2O): δ 175.10, 175.03, 173.23, 172.73, 143.77, 126.07, 100.79, 100.67, 72.69, 72.54, 71.68, 70.03, 69.25, 68.25, 68.99, 62.63, 57.34, 52.92, 52.58, 51.84, 51.76, 40.27, 22.04; HRMS (ESI) Calcd. for $C_{28}H_{42}N_5O_{17}$ ($[M + H]^+$): 720.2576; found: 720.2569.

4.13. Triazolyl-linked α(2-9)-trisialic acid (1b)

The deprotection of **2b** (21.2 mg, 13.6 μmol) was performed as described in the general procedure for deprotection, affording **1b** as white solids (13.6 mg, 89% yield). $[\alpha]_D^{30}$ -3.23 (*c* 2.9, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 8.07 (s, 1H), 8.05 (s, 1H), 4.90 (d, *J* = 12.0 Hz, 1H), 4.88 (d, *J* = 12.0 Hz, 1H), 4.82-4.75 (m, 2H), 4.62-4.52 (m, 4H), 4.33 (dd, *J* = 15.7, 2.3 Hz, 1H), 4.26-4.19 (m, 3H), 3.88-3.81 (m, 5H), 3.76-3.62 (m, 7H), 3.59 (d, *J* = 10.0 Hz, 1H), 3.43-3.38 (m, 2H), 2.76-2.70 (m, 3H), 2.03 (s, 3H), 2.01 (s, 6H), 1.67 (t, *J* = 12.0 Hz, 3H); ^{13}C NMR (150 MHz, D_2O): δ 175.83, 175.80, 175.76, 173.98, 173.92, 173.48, 144.49, 126.98, 126.88, 101.54, 101.50, 101.41, 80.35, 76.07, 73.42, 73.28, 73.13, 72.40, 70.71, 70.45, 69.96, 69.93, 69.01, 68.98, 68.84, 68.75, 63.34, 58.07, 53.63, 53.54, 53.35, 52.58, 52.50, 41.03, 39.45, 22.76; HRMS (ESI) Calcd. for $C_{42}H_{61}N_9O_{25}Na$ ($[M + Na]^+$): 1114.3671; found: 1114.3677.

4.14. Triazolyl-linked α(2-9)-tetrasialic acid (1c)

The deprotection of **2c** (29.4 mg, 14.2 μmol) was performed as described in the general procedure for deprotection, affording **1c** as white solids (18.4 mg, 88% yield). $[\alpha]_D^{30}$ -6.68 (*c* 1.6, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 8.07-8.06 (m, 3H), 4.91-4.87 (m, 3H), 4.84-4.75 (m, 3H),

4.62-4.52 (m, 6H), 4.33 (d, *J* = 15.7 Hz, 1H), 4.26-4.20 (m, 4H), 3.88-3.81 (m, 6H), 3.77-3.63 (m, 9H), 3.59 (d, *J* = 10.4 Hz, 1H), 3.44-3.37 (m, 3H), 2.76-2.70 (m, 4H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.01 (s, 3H), 1.68 (t, *J* = 12.0 Hz, 4H); ^{13}C NMR (150 MHz, D_2O): *δ* 175.83, 175.81, 175.77, 173.98, 173.93, 173.49, 144.47, 127.01, 126.88, 101.54, 101.50, 101.41, 79.92, 73.43, 73.28, 73.12, 72.41, 70.74, 70.41, 69.97, 69.89, 69.01, 68.99, 68.83, 68.76, 63.35, 58.07, 53.64, 53.51, 53.34, 52.59, 52.50, 41.02, 22.78. HRMS (ESI) Calcd. for $C_{56}H_{82}N_{13}O_{33}$ ($[M + H]^+$): 1464.5132; found: 1464.5173.

4.15. Triazolyl-linked α(2-9)-pentasialic acid (1d)

The deprotection of **2d** (32.9 mg, 12.8 *μ*mol) was performed as described in the general procedure for deprotection, affording **1d** as white solids (23.4 mg, 99% yield). $[\alpha]_D^{30}$ -4.89 (*c* 3.0, H_2O); ^{1}H NMR (600 MHz, D_2O): *δ* 8.10 (m, 4H), 4.92-4.89 (m, 4H), 4.84-4.75 (m, 4H), 4.64-4.54 (m, 8H), 4.35 (d, *J* = 15.7 Hz, 1H), 4.28-4.22 (m, 5H), 3.90-3.64 (m, 18H), 3.61 (d, *J* = 9.3 Hz, 1H), 3.46-3.37 (m, 4H), 2.78-2.72 (m, 5H), 2.06-2.02 (m, 15H), 1.70 (t, *J* = 12.0 Hz, 5H); ^{13}C NMR (150 MHz, D_2O): *δ* 175.86, 175.83, 175.79, 173.93, 173.48, 171.80, 144.62, 127.10, 101.57, 79.97, 73.44, 73.29, 73.13, 72.48, 72.43, 70.83, 70.76, 70.53, 70.47, 69.99, 69.96, 69.92, 69.00, 68.83, 68.76, 63.40, 58.11, 53.69, 53.58, 53.44, 53.37, 53.30, 52.64, 52.61, 41.03, 22.91, 22.87, 22.82; HRMS (ESI) Calcd for $C_{70}H_{101}N_{17}O_{41}Na_2$ ($[M + 2Na]^{2+}$): 940.8068; found: 940.8051.

4.16. Triazolyl-linked α(2-9)-hexasialic acid (1e)

The deprotection of **2e** (33.6 mg, 10.9 *μ*mol) was performed as described in the general procedure for deprotection, affording **1e** as white solids (20.9 mg, 87% yield). $[\alpha]_D^{30}$ -11.39 (*c* 2.5, H_2O); ^{1}H NMR (600 MHz, D_2O): *δ* 8.08-8.07 (m, 5H), 4.91-4.88 (m, 5H), 4.84-4.75 (m, 5H), 4.63-4.53 (m, 10H), 4.40 (d, *J* = 15.7 Hz, 1H), 4.27-4.20 (m, 5H), 3.89-3.59 (m, 23H), 3.45-3.36 (m, 5H), 2.77-2.72 (m, 6H), 2.04-2.01 (m, 18H), 1.68 (t, *J* = 12.0 Hz, 6H). ^{13}C NMR (150 MHz, D_2O): *δ* 182.22, 175.85, 175.82, 175.78, 173.99, 173.94, 173.49, 171.80, 144.47, 127.04, 126.89, 101.49, 79.94, 73.43, 73.29, 73.12, 72.43, 70.77, 70.45, 70.37, 69.98, 69.90, 69.83, 69.09, 68.99, 68.83, 68.76, 63.37, 58.07, 53.66, 53.53, 53.48, 53.38, 52.61, 52.52, 41.02, 22.81. HRMS (ESI) Calcd. for $C_{84}H_{121}N_{21}O_{49}Na_2$ ($[M + 2Na]^{2+}$): 1126.8698; found: 1126.8745.

4.17. Triazolyl-linked α(2-9)-heptasialic acid (1f)

The deprotection of **2f** (35.0 mg, 9.7 *μ*mol) was performed as described in the general

procedure for deprotection, affording **1f** as white solids (23.6 mg, 94% yield). $[\alpha]_D^{30}$ -10.10 (*c* 3.1, H_2O); ^1H NMR (400 MHz, D_2O): δ 8.13 (m, 6H), 4.90-4.74 (m, 14H), 4.62 (m, 10H), 4.36-4.20 (m, 8H), 3.90-3.59 (m, 25H), 3.45-3.36 (m, 6H), 2.81-2.72 (m, 7H), 2.04-2.01 (m, 21H), 1.72-1.70 (m, 7H); ^{13}C NMR (150 MHz, D_2O): δ 175.81, 175.77, 173.88, 144.83, 143.25, 130.19, 127.18, 126.08, 103.39, 101.58, 73.43, 73.29, 73.12, 72.42, 70.74, 70.40, 70.31, 69.98, 69.89, 69.80, 69.01, 68.99, 68.83, 68.76, 63.36, 58.09, 53.67, 53.53, 53.47, 53.35, 52.61, 52.51, 41.02, 22.80. MS (MALDI-TOF) m/z Calcd. for $C_{98}H_{139}N_{25}O_{57}$ $[M - 2H]^{2-}$: 1288.94; found: 1288.55.

4.18. Triazolyl-linked α(2-9)-octasialic acid (1g)

The deprotection of **2g** (35.3 mg, 8.58 μmol) was performed as described in the general procedure for deprotection, affording **1g** as white solids (24.4 mg, 96% yield). $[\alpha]_D^{30}$ -12.45 (*c* 3.2, H_2O); ^1H NMR (400 MHz, D_2O): δ 8.08 (br.s, 7H), 4.92-4.89 (m, 7H), 4.80-4.75 (m, 2H), 4.64-4.55 (m, 15H), 4.37-4.21 (m, 10H), 3.91-3.60 (m, 31H), 3.47-3.37 (m, 7H), 2.79-2.74 (m, 8H), 2.05-2.02 (m, 24H), 1.70 (t, *J* = 12.0 Hz, 8H); ^{13}C NMR (150 MHz, D_2O): δ 175.80, 173.99, 173.93, 173.49, 144.39, 127.04, 101.54, 101.46, 101.40, 73.40, 73.26, 73.09, 70.86, 70.42, 70.19, 70.04, 69.91, 69.83, 69.72, 69.00, 68.83, 68.76, 63.44, 63.28, 58.09, 53.54, 53.44, 53.32, 52.68, 52.54, 41.12, 41.02, 40.91, 23.06, 22.88, 22.70, 22.53. MS (MALDI-TOF) m/z Calcd. for $C_{112}H_{162}N_{29}O_{65}$ $[M + H]^+$: 2953.02; found: 2953.00.

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Supplementary data

Copies of ^1H NMR and ^{13}C NMR spectra of the reported compounds. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.07.099.

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Electronic Supplementary Information

Synthesis of triazolyl-linked polysialic acids

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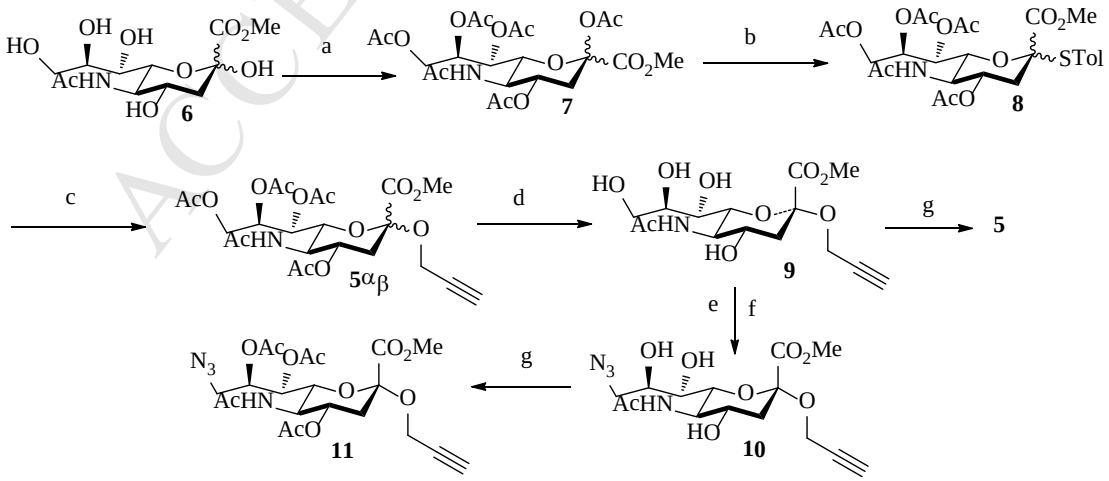
Contents

General procedures. All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Dichloromethane (CH_2Cl_2), pyridine, and acetonitrile were distilled over calcium hydride (CaH_2). Methanol was distilled from magnesium. DMF and 1,4-dioxane were stirred with calcium hydride (CaH_2) and distilled under reduced pressure. All reactions were carried out under anhydrous conditions with freshly distilled solvents, unless otherwise noted. Reactions were monitored by analytical thin-layer chromatography on silica gel 60-F_{254} precoated on alunminum plates (E. Merck.). Spots were detected under UV (254 nm) and/or by staining with acidic ceric ammonium molybdate. Solvents were evaporated under reduced pressure and below 40 ^{o}C (bath). Column chromatography was performed on silica gel (200-300 mesh, TsingDao Ocean or 230-400 mesh, Merck), reversed phase column chromatography was performed on LiChroprep RP-18 (40-63 μm, Merck). ^{1}H NMR spectra were recorded on a Bruker 400M or 600M spectrometers at 25 ^{o}C. Chemical shifts (in ppm) were referenced to tetramethylsilane (δ = 0 ppm) in deuterated chloroform. ^{13}C NMR spectra were obtained by using the same NMR spectrometers and were calibrated with $CDCl_3$ (δ = 77.00 ppm). Mass spectra were recorded using a Bruker APEX IV FT-MS（7.0 T）spectrometer.



Methyl (5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onate (7).

To a solution of **6** (1.0 g) in dry pyridine (4.0 mL) was added Ac_2O (3.0 mL) and DMAP (50.0 mg) at 0 ^oC. After stirring at room temperature for 3 h, the solvents were evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with H_2O (20 mL), saturated aq. $NaHCO_3$ and brine (20 mL). The organic layer was dried over Na_2SO_4. After filtration, the solution was evaporated to afford **7** as a yellow foam (1.5 g, 95%). α isomer: ^1H NMR (400 MHz, $CDCl_3$): δ 5.38 (dd, J = 5.2, 1.8 Hz, 1H, H-7), 5.30-5.22 (m, 2H, H-8, NH), 5.07 (ddd, J = 6.6, 5.3, 2.6 Hz, 1H, H-4), 4.49 (dd, J = 12.4, 2.5 Hz, 1H, H-9b), 4.14-4.09 (m, 3H, H-5, H-6, H-9a), 3.80 (s, 3H, OCH_3), 2.55 (dd, J = 13.5, 4.9 Hz, 1H, H-3e), 2.18 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$), 2.14-2.10 (m, 1H, H-3a), 2.07 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.90 (s, 3H, $NHCOCH_3$). The ^1H NMR data is consistent with the literature.[1]

Methyl (2-*p*-methylphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-thio-nonulopyranosid)onate (8).

To a solution of **7** (16.90 g, 31.7 mmol) and *p*-methylthiophenol (7.50 g, 60.4 mmol, 2.0 eq.) in dry CH_2Cl_2 (50 mL) was added $BF_3{\cdot}Et_2O$ (6.5 mL, 1.5 eq.). The resulting solution was stirred at r.t. overnight. The mixture was diluted with CH_2Cl_2 (200 mL) and washed with H_2O (20 mL), saturated aq. $NaHCO_3$ and brine (20 mL). The organic layer was dried over Na_2SO_4. After filtration, the solution was evaporated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 3/1 to 0/1) to afford **8** as a white foam (16.5 g, 87%). α isomer: ^1H NMR (400 MHz, $CDCl_3$): δ 7.39 (d, J = 7.6 Hz, 2H, ArH), 7.14 (d, J = 7.6 Hz, 2H, ArH), 5.30-5.24 (m, 2H, H-7, H-8), 5.09 (d, J = 9.7 Hz, 1H, NH), 4.84 (td, J = 10.5, 3.8 Hz, 1H, H-4), 4.41 (d, J = 12.1 Hz, 1H, H-9b), 4.21 (dd, J = 12.7, 6.0 Hz, 1H, H-9a), 3.97

[1] Marra, A. and Sinay, P. *Carbohydr. Res.* **1989**, *187*, 35-42

(q, *J* = 10.8 Hz, 1H, H-5), 3.87 (d, *J* = 11.0 Hz, 1H, H-6), 3.61 (s, 3H, OCH_3), 2.78 (dd, *J* = 12.3, 4.5 Hz, 1H, H-3e), 2.36 (s, 3H, ArCH_3), 2.14 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 2.00-1.94 (m, 1H, H-3a), 1.86 (s, 3H, NHCOCH_3). The ^{1}H NMR data is consistent with the literature.[2]

Methyl (2-propynyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onate (5αβ).

To a suspension of 4 Å molecular sieves (3.0 g), compound **8** (16.50 g, 27.6 mmol) and propargyl alcohol (6.2 mL) in dry CH_2Cl_2 (30 mL) was added NIS (14.30 g, 63.5 mmol, 2.3 eq.) and TfOH (0.93 mL, 11.0 mmol, 0.3 eq.) at 0 ^{o}C. After stirring for 1 h at 0 ^{o}C, the mixture was filtered through a pad of celite and the celite was washed thoroughly with CH_2Cl_2. The filtrate was washed with saturated aq. $NaHCO_3$, saturated aq. $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/acetone, 3:1) to afford **5αβ** as white foams (13.0, 89%). The α isomer is compound **5**：^{1}H NMR (400 MHz, $CDCl_3$): δ 5.43-5.40 (m, 1H, H-8), 5.31 (d, *J* = 9.0 Hz, 1H, NH), 5.14-5.12 (m, 1H, H-7), 4.91-4.84 (m, 1H, H-4), 4.41 (dd, *J* = 15.7, 2.4 Hz, 1H, HC≡CCH_2), 4.29 (dd, *J* = 12.4, 2.7 Hz, 1H, H-9b), 4.17 (dd, *J* = 15.6, 2.4 Hz, 1H, HC≡CCH_2), 4.11-4.05 (m, 3H, H-5, H-6, H-9a), 3.82 (s, 3H, OCH_3), 2.64 (dd, *J* = 12.8, 4.6 Hz, 1H, H-3e), 2.44 (t, *J* = 2.4 Hz, 1H, *H*C≡C), 2.16 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.99 (t, *J* = 12.4 Hz, 1H, H-3a), 1.88 (s, 3H, NHCOCH_3). The ^{1}H NMR data is consistent with the literature.[3]

Methyl (2-propynyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onate (9).

To a solution of **5αβ** (11.1 g, 21.0 mmol) in dry MeOH (100 mL) was added 1 M

[2] Ye, X.-S.; Huang, X. and Wong, C.-H. *Chem. Commun.* **2001**, 974–975.
[3] Shelke, S. V.; Cutting, B.; Jiang, X. H.; Ernst, B. *Angew. Chem. Int. Ed.* **2010**, *49*, 5721-5725.

NaOMe/MeOH (20 mL). The resulting solution was stirred at room temperature for 0.5 h. The reaction mixture was neutralized with Amberlyst 15 (H+) ion-exchange resin and filtered through a pad of celite. The celite was thoroughly washed with MeOH, and the combined filtrates were concentrated under vacuum. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 14:1) to afford pure α isomer **9** as white solids (3.54 g, 47%). ^{1}H NMR (400 MHz, MeOD): δ 4.37 (ABq, J = 12.0, 2.5 Hz, 2H, HC≡CCH_2), 3.86 (s, 3H, OCH_3), 3.85-3.77 (m, 2H, H-8, H-9b), 3.71-3.64 (m, 2H, H-4, H-9a), 3.60 (dd, J = 10.4, 1.4 Hz, 1H, H-6), 3.52 (dd, J = 9.0, 1.1 Hz, 1H, H-7), 2.88 (t, J = 2.0 Hz, 1H, HC≡C), 2.72 (dd, J = 12.8 ,4.6 Hz, 1H, H-3e), 2.02 (s, 3H, NHCOCH_3), 1.77 (t, J = 12.4 Hz, 1H, H-3a). The ^{1}H NMR data is consistent with the literature.[3]

Methyl [2-propynyl 5-acetamido-3,5-dideoxy-9-*O*-(4-toluenesulfonyl)-D-glycero-α-D-galacto-2-nonulopyranosid]onate (9Ts).

To a solution of 4Å molecular sieves (3.0 g) and **9** (3.24 g, 8.98 mmol) in dry pyridine (15 mL) was added *p*-TsCl (2.91 g, 15.3 mmol, 1.7 eq.) and DMAP (200 mg) at 0 ^{o}C. After stirring for 2 h, the reaction was quenched with methanol (20 mL). After removal of the sieves and solvent, the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 30:1) to afford the product as white solids (5.50 g). ^{1}H NMR (400 MHz, MeOD): δ 7.82 (d, J = 8.3 Hz, 2H, ArH), 7.46 (d, J = 8.1 Hz, 2H, ArH), 4.33 (dd, J = 8.2, 1.7 Hz, 1H, H-9b), 4.29 (ABq, J = 11.3, 2.4 Hz, 2H, HC≡CCH_2), 4.11 (dd, J = 9.9, 6.0 Hz, 1H, H-9a), 4.02-3.98 (m, 1H, H-8), 3.83 (s, 3H, OCH_3), 3.72 (t, J = 10.1 Hz, 1H, H-5), 3.66 (td, J = 10.5, 4.5 Hz, 1H, H-4), 3.57 (d, J = 10.4 Hz, 1H, H-6), 3.47 (d, J = 8.7 Hz, 1H, H-7), 2.86 (t, J = 2.1 Hz, 1H, HC≡C), 2.69 (dd, J = 12.9, 4.6 Hz, 1H, H-3e), 2.47 (s, 3H, ArCH_3), 2.01 (s, 3H, NHCOCH_3), 1.72 (t, J = 12.1 Hz, 1H, H-3a). The ^{1}H NMR data is consistent with the literature.[3]

Methyl (2-propynyl 5-acetamido-9-azido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)on

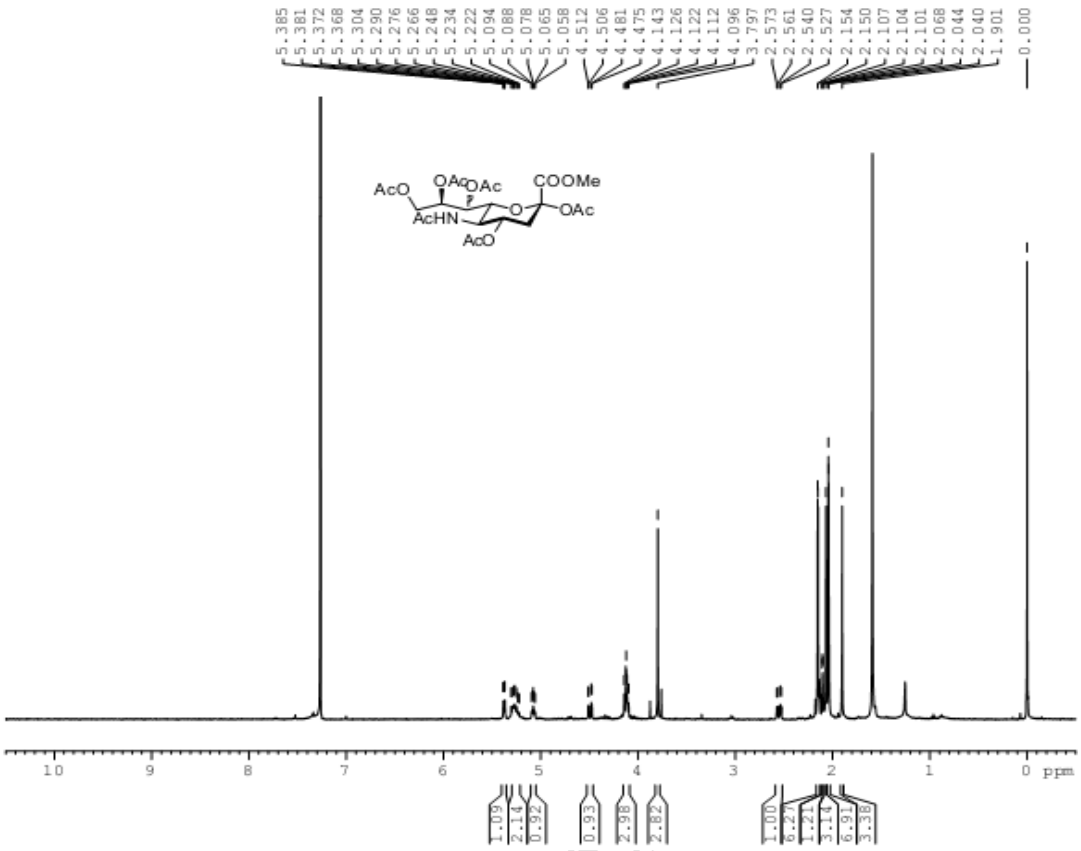
ate (10).

To a solution of **9-Ts** (5.50 g) in DMF (25 mL) was added water (1 mL), 15-crown-5 (0.2 mL) and NaN_3 (2.10 g, 5eq.). The resulting solution was stirred at 65 ^oC overnight. The mixture was filtered through a pad of celite and the filtrate was evaporated to dryness. Purification by column chromatography on silica gel (CH_2Cl_2/MeOH, 25:1) afforded **10** as white foams (1.46 g, 42% from **9**). ^1H NMR (400 MHz, MeOD): δ 4.37 (ABq, J = 10.7, 2.4 Hz, 2H, HC≡CCH_2), 4.02-3.98 (m, 1H, H-8), 3.86 (s, 3H, OCH_3), 3.79 (t, J = 10.2 Hz, 1H, H-5), 3.68 (dd, J = 11.7, 4.6 Hz, 1H, H-4), 3.63 (dd, J = 10.5, 1.4 Hz, 1H, H-6), 3.56 (dd, J = 12.8, 2.6 Hz, 1H, H-9b), 3.48 (dd, J = 9.0, 1.2 Hz, 1H, H-7), 3.41 (t, J = 6.2 Hz, 1H, H-9a), 2.86 (t, J = 2.4 Hz, 1H, *H*C≡C), 2.71 (dd, J = 12.8, 4.6 Hz, 1H, H-3e), 2.03 (s, 3H, NHCOCH_3), 1.75 (t, J = 12.0 Hz, 1H, H-3a). The ^1H NMR data is consistent with the literature.[3]

Methyl (2-propynyl 5-acetamido-4,7,8-tri-*O*-acetyl-9-azido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onate (11).

To a solution of **10** (1.46 g, 37.8 mmol) in dry pyridine (10 mL) was added Ac_2O (7.5 mL) and DMAP (50.0 mg) at 0 ^oC. The reaction mixture was stirred at room temperature for 3 h. The solvents were evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/acetone, 3:1) to afford **11** as white foams (1.47 g, 75%). $[\alpha]_D^{30}$ -0.29 (*c* 2.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.36-5.30 (m, 2H, H-7, H-8), 5.15 (d, J = 9.6 Hz, 1H, NH), 4.87 (ddd, J = 12.0, 9.9, 4.5 Hz, 1H, H-4), 4.40 (dd, J = 15.6, 2.5 Hz, 1H, HC≡CCH_2), 4.16 (dd, J = 15.6, 2.4 Hz, 1H, HC≡CCH_2), 4.11-4.06 (m, 2H, H-5, H-6), 3.83 (s, 3H, OCH_3), 3.57 (dd, J = 13.2, 2.2 Hz, 1H, H-9b), 3.28 (dd, J = 13.4, 5.2 Hz, 1H, H-9a), 2.64 (dd, J = 12.8, 4.6 Hz, 1H, H-3e), 2.46 (t, J = 2.5 Hz, 1H, *H*C≡C), 2.19 (s, 3H, $COCH_3$), 2.17 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.99 (t, J = 12.6 Hz, 1H, H-3a), 1.89 (s, 3H, NHCOCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.98, 170.26, 170.19, 170.11, 167.75, 98.12, 78.81, 74.56, 72.86, 69.48, 68.71, 67.78, 52.90,

52.81, 50.90, 49.28, 37.79, 23.16, 21.02, 20.84, 20.82. The ^{1}H NMR data is consistent with the literature.[3]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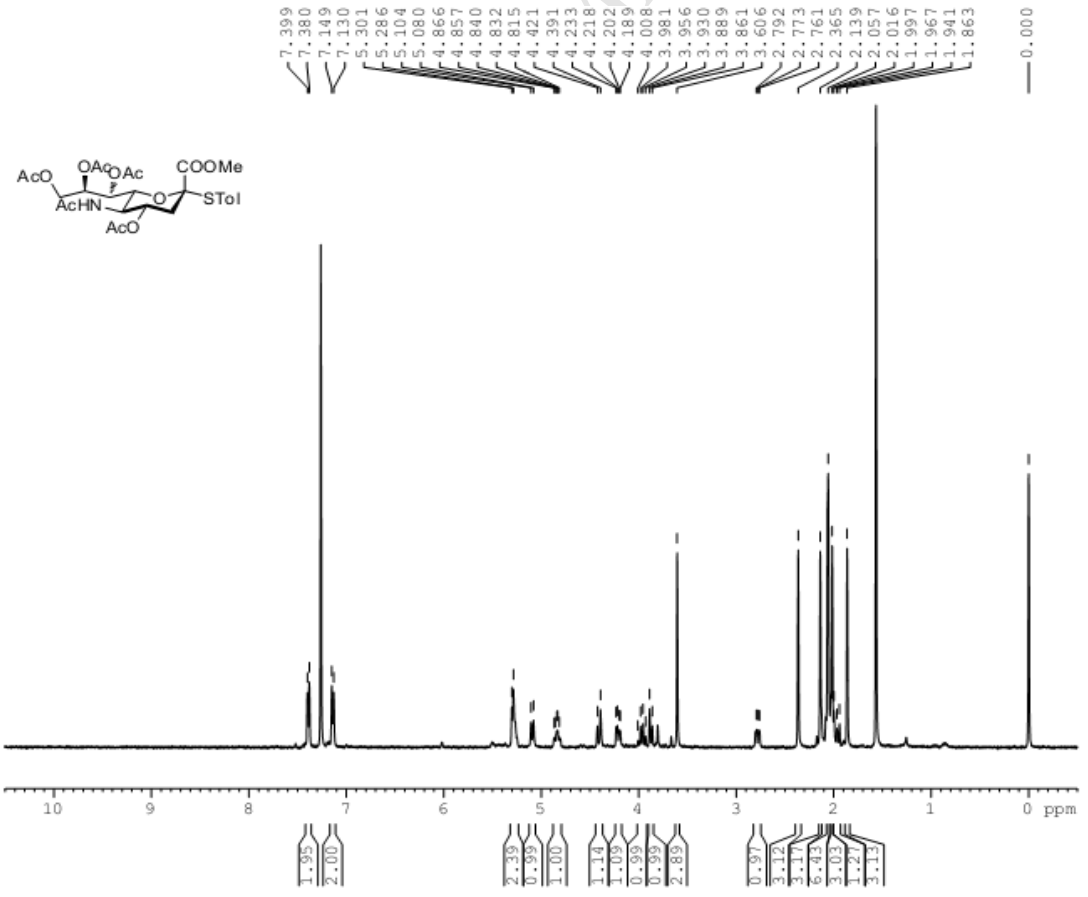
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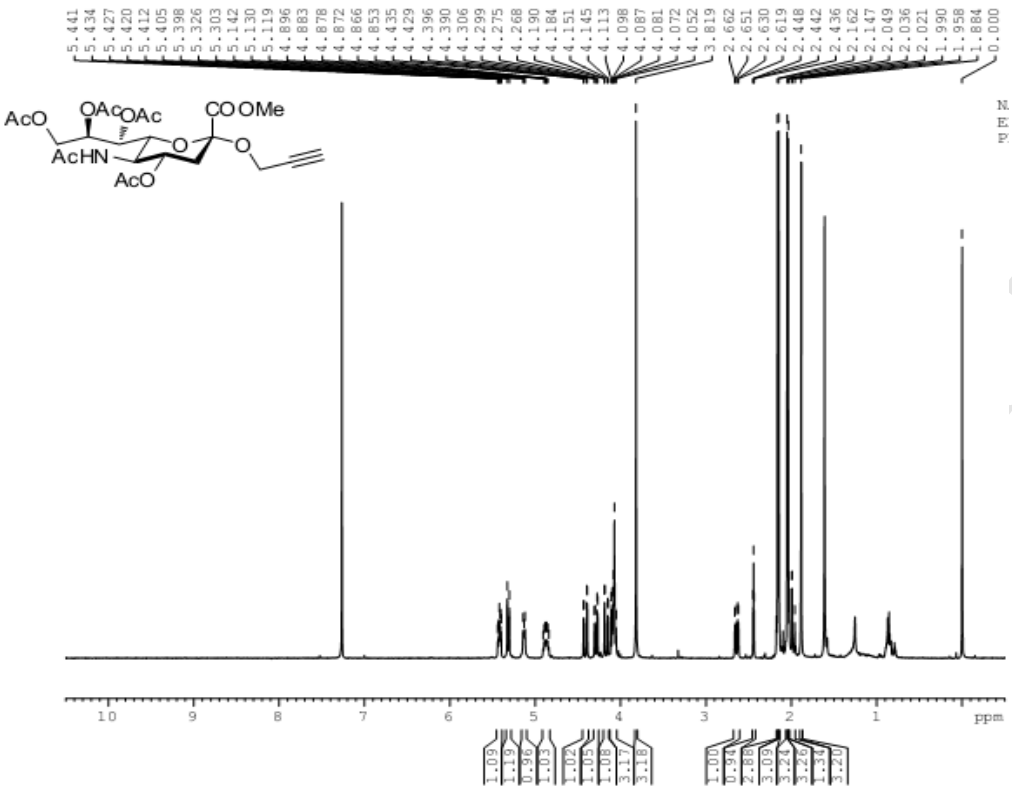

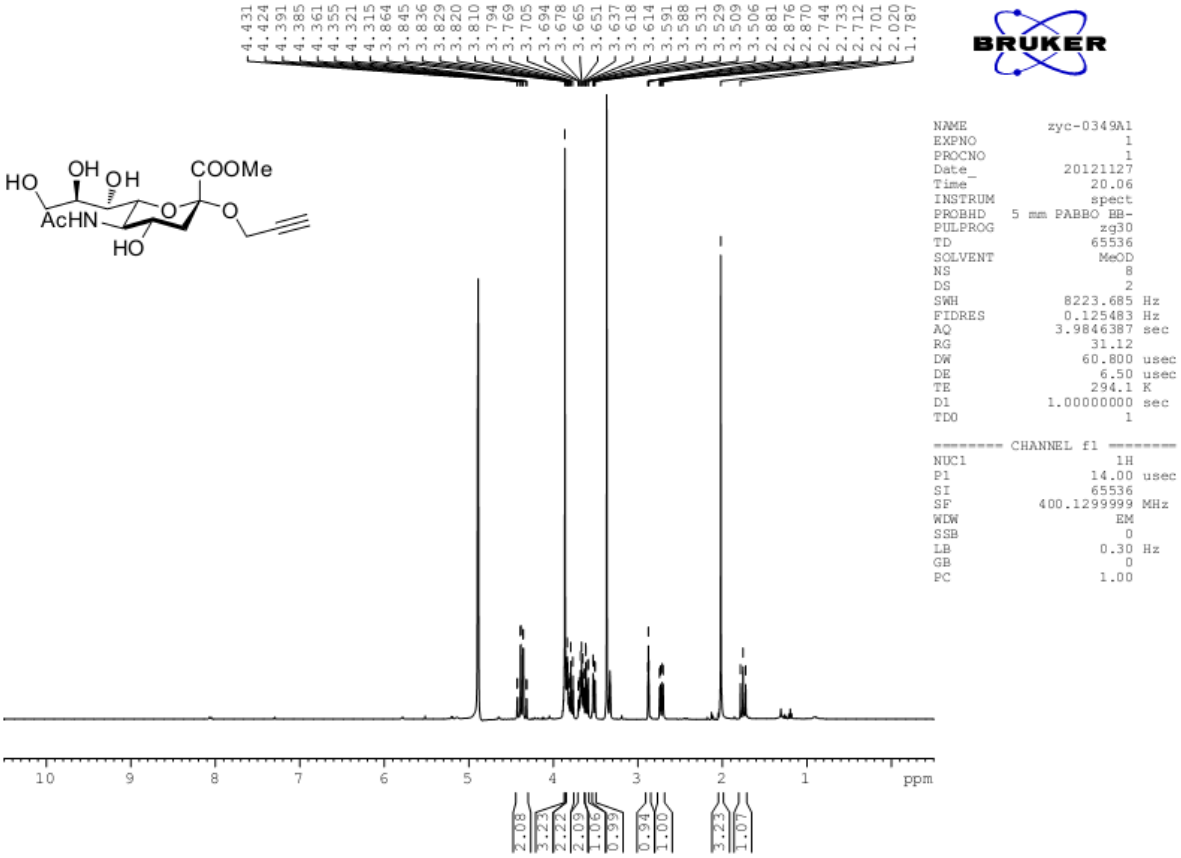


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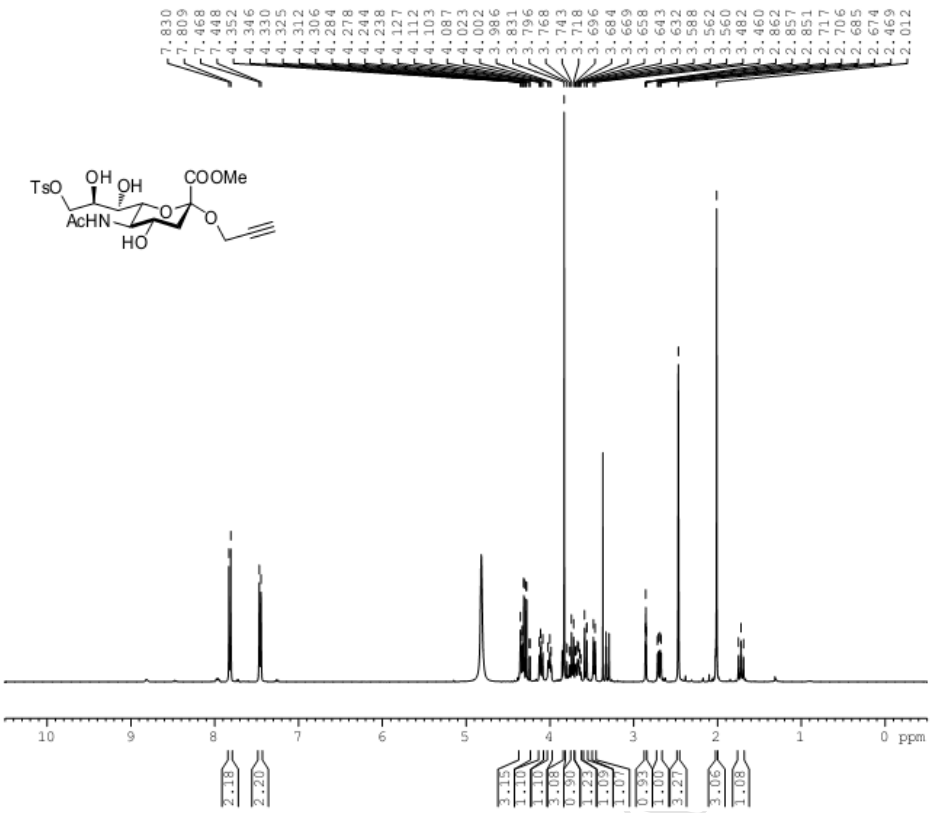
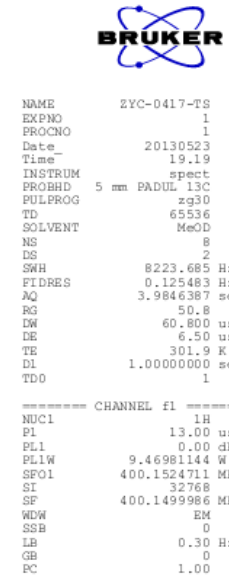
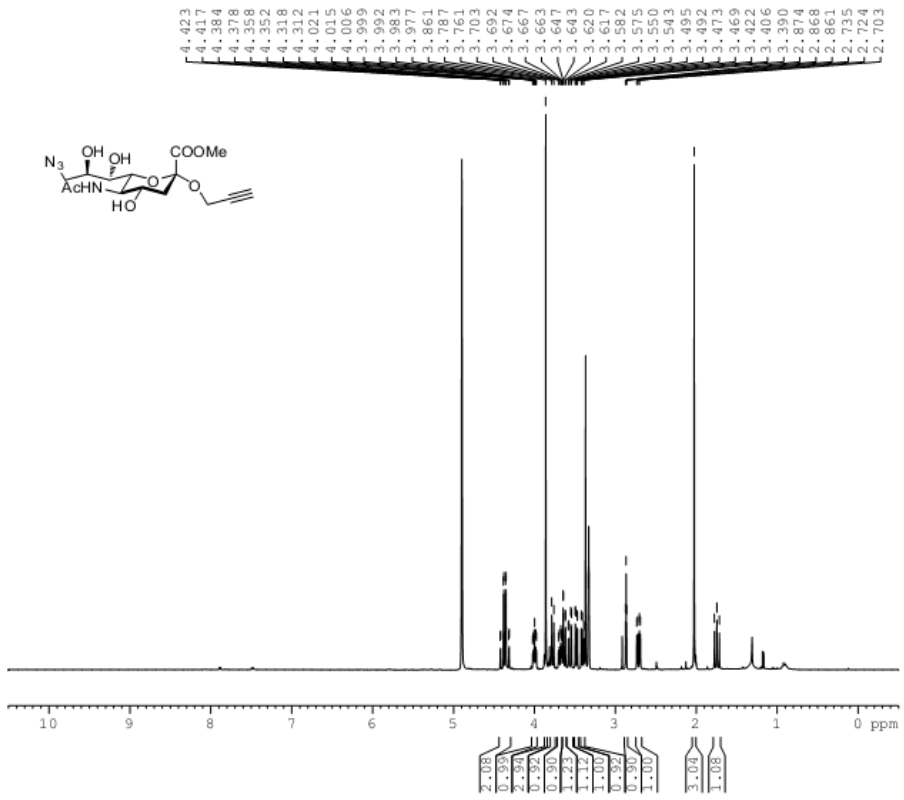
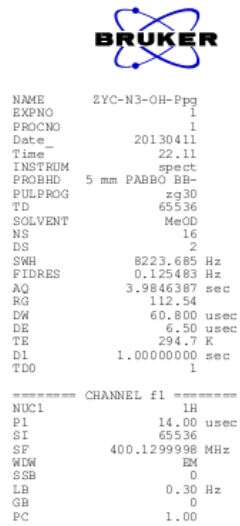
PROBHD   5 mm PABBO BB-
PULPROG              zg30
TD                  65536
SOLVENT              MeOD
NS                       8
DS                       2
SWH              8223.685 Hz
FIDRES           0.125483 Hz
AQ               3.9846387 sec
RG                   31.12
DW                  60.800 usec
DE                   6.50 usec
TE                  294.1 K
D1              1.00000000 sec
TD0                      1

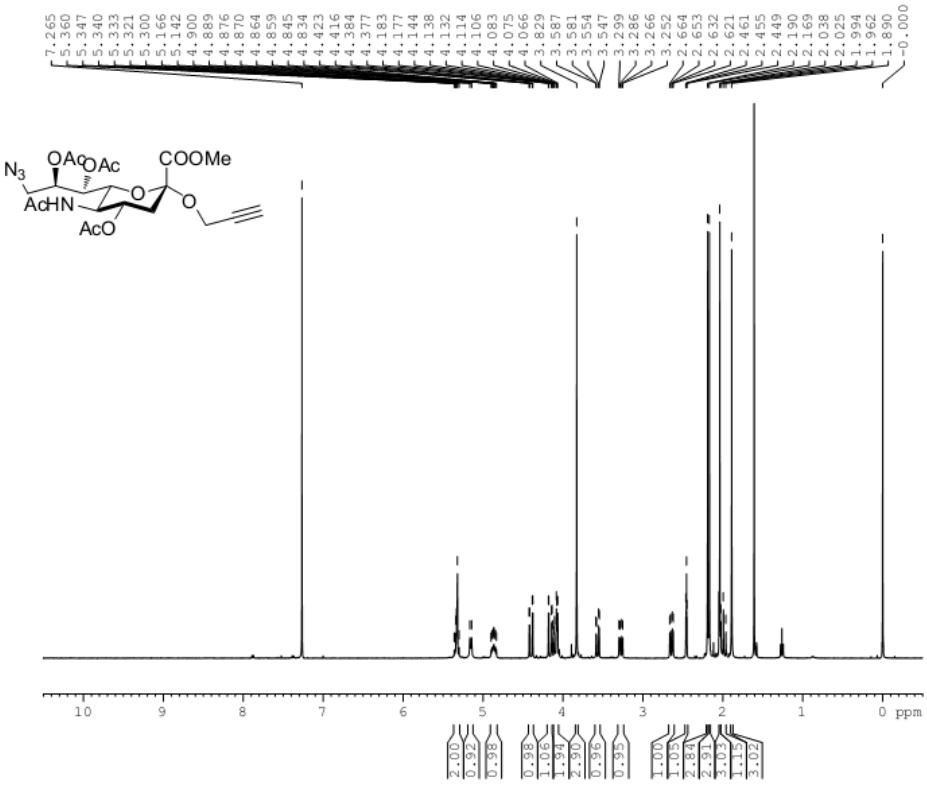
======== CHANNEL f1 ========
NUC1                    1H
P1                  14.00 usec
SI                   65536
SF           400.1299999 MHz
WDW                     EM
SSB                      0
LB                  0.30 Hz
GB                       0
PC                   1.00
```


7.830
7.809
7.468
7.448
4.352
4.346
4.330
4.325
4.312
4.306
4.284
4.278
4.244
4.238
4.127
4.112
4.103
4.087
4.023
4.002
3.986
3.831
3.796
3.768
3.743
3.718
3.696
3.684
3.669
3.658
3.643
3.632
3.588
3.562
3.560
3.482
3.460
2.862
2.857
2.851
2.717
2.706
2.685
2.674
2.469
2.012
TsO
OH
OH
COOMe
AcHN
O
O
HO
10
9
8
7
6
5
4
3
2
1
0 ppm
2.18
2.20
3.15
1.10
1.10
3.08
0.90
1.22
1.05
1.07
0.97
1.00
3.27
3.06
1.08


BRUKER
NAME ZYC-0417-TS
EXPNO 1
PROCNO 1
Date_ 20130523
Time 19.19
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 8
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 50.8
DW 60.800 usec
DE 6.50 usec
TE 301.9 K
D1 1.00000000 sec
TD0 1
======= CHANNEL f1 =======
NUC1 1H
P1 13.00 usec
PL1 0.00 dB
PL1W 9.46981144 W
SFO1 400.1524711 MHz
SI 32768
SF 400.1499986 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

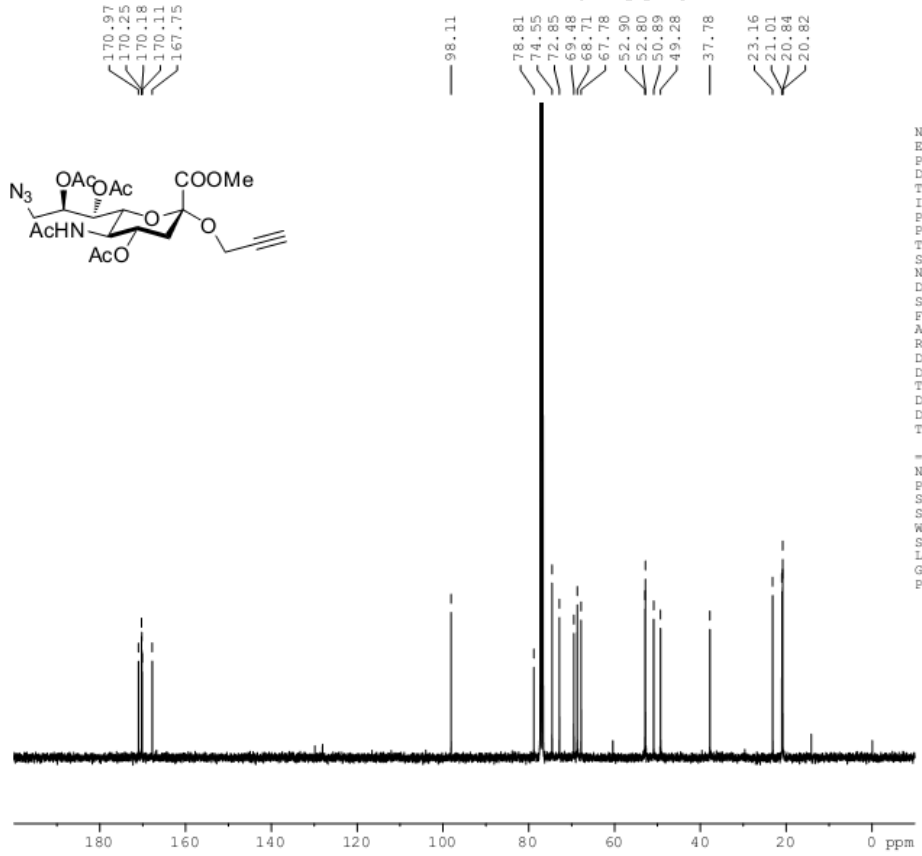

4.423
4.417
4.384
4.378
4.358
4.352
4.318
4.312
4.021
4.015
4.006
3.999
3.992
3.983
3.977
3.861
3.787
3.761
3.703
3.692
3.674
3.667
3.663
3.647
3.643
3.620
3.617
3.582
3.575
3.550
3.543
3.495
3.492
3.473
3.469
3.422
3.406
3.390
2.874
2.868
2.861
2.735
2.724
2.703
N3
OH
OH
COOMe
AcHN
O
O
HO
10
9
8
7
6
5
4
3
2
1
0 ppm
2.08
0.99
2.39
0.91
0.90
1.12
1.00
0.92
0.90
1.00
3.04
1.08


BRUKER
NAME ZYC-N3-OH-Ppg
EXPNO 1
PROCNO 1
Date_ 20130411
Time 22.11
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 112.54
DW 60.800 usec
DE 6.50 usec
TE 294.7 K
D1 1.00000000 sec
TD0 1
======= CHANNEL f1 =======
NUC1 1H
P1 14.00 usec
SI 65536
SF 400.1299998 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



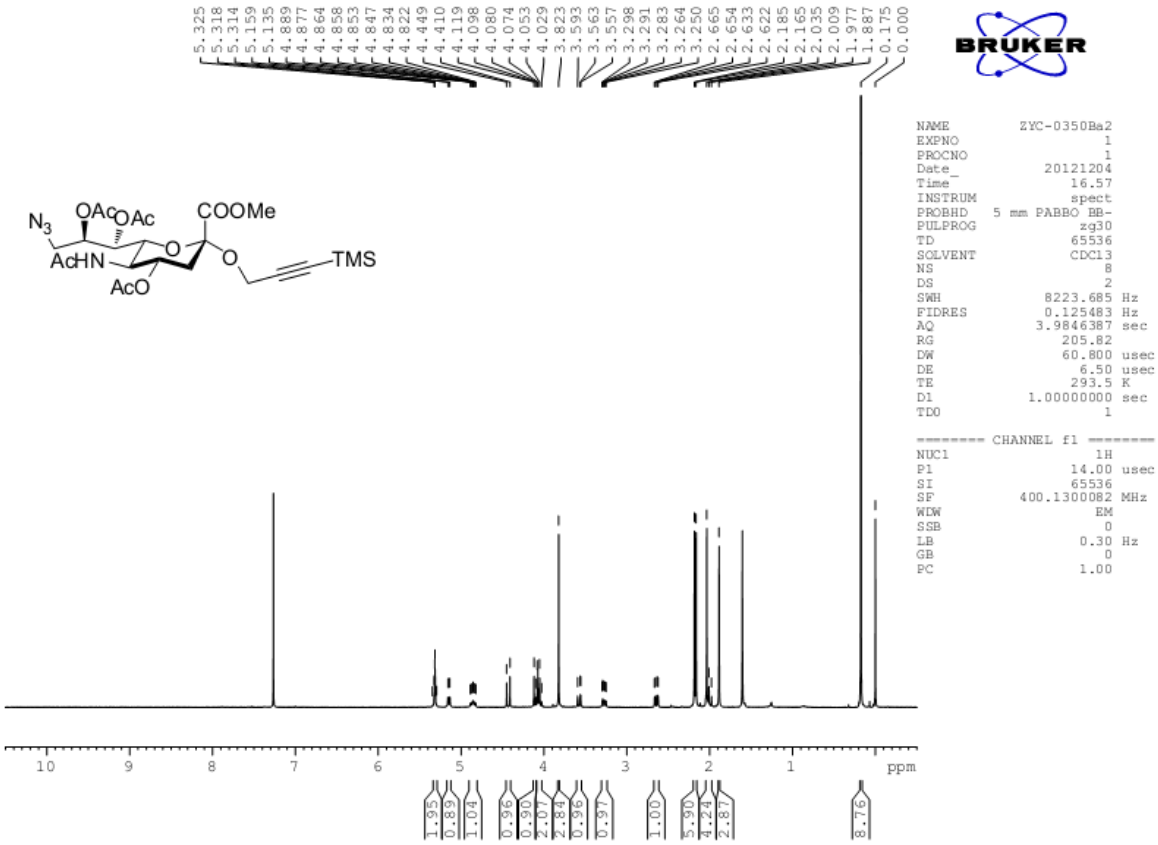
BRUKER

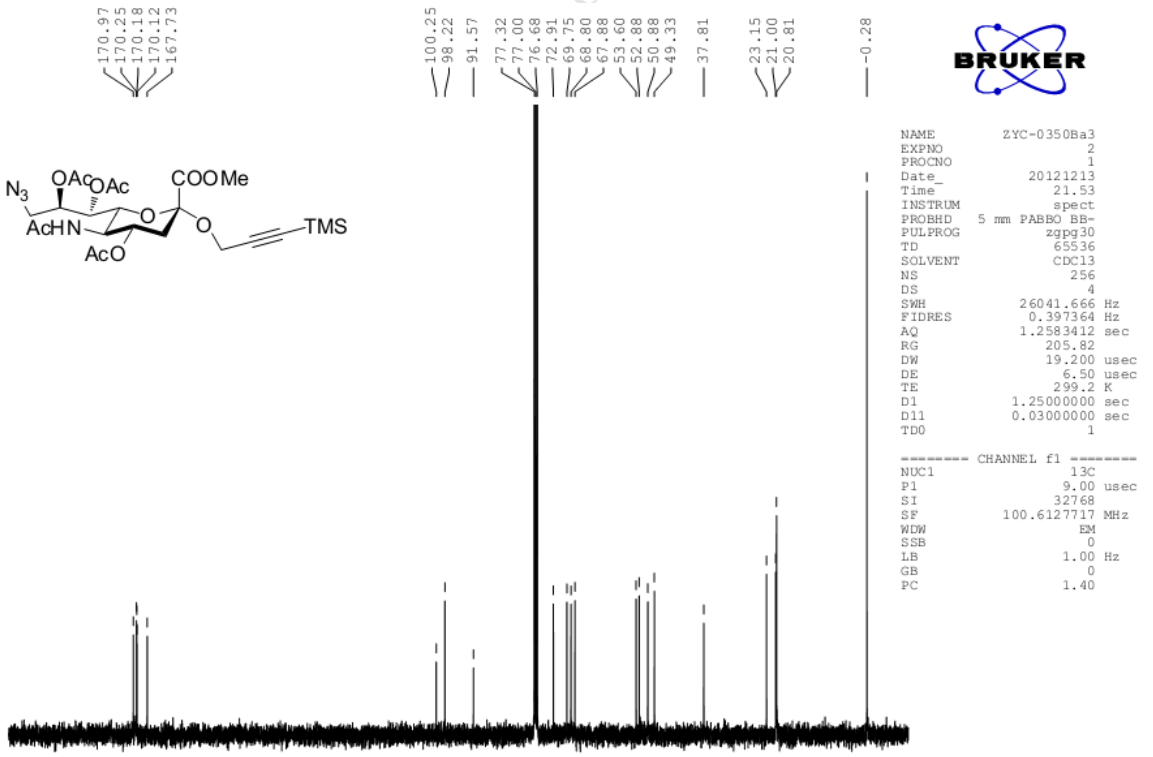
NAME	ZYC-0350Ac
EXPNO	1
PROCNO	1
Date_	20121204
Time	17.02
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	2
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	205.82
DW	60.800 usec
DE	6.50 usec
TE	293.6 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	14.00 usec
SI	65536
SF	400.1300081 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

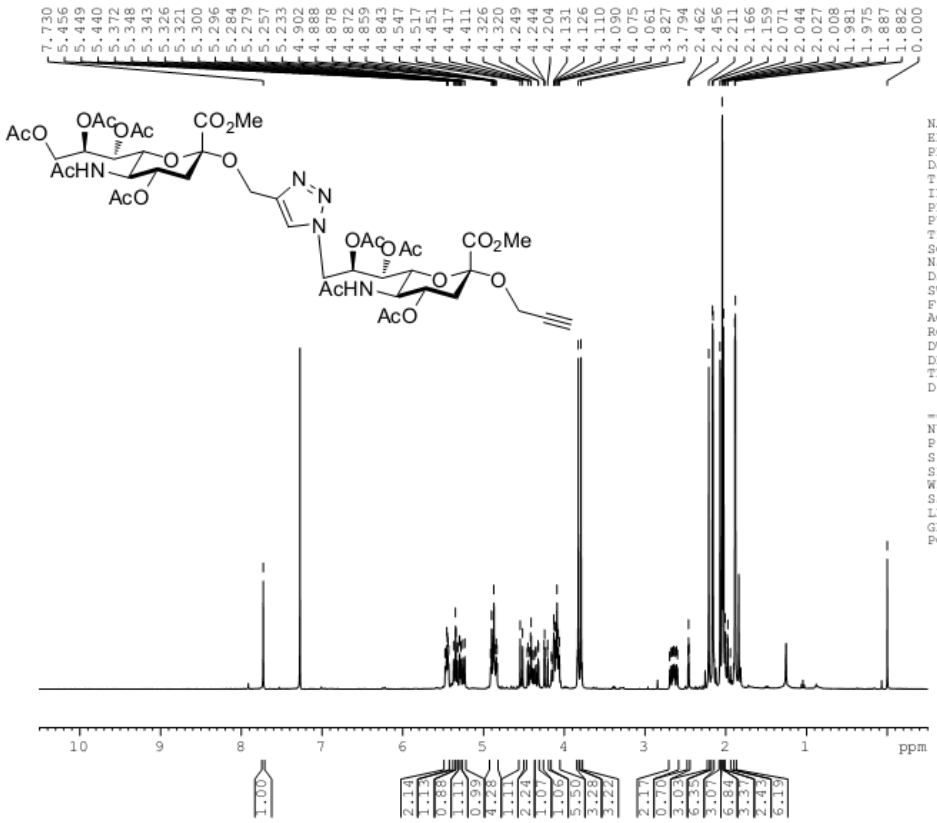


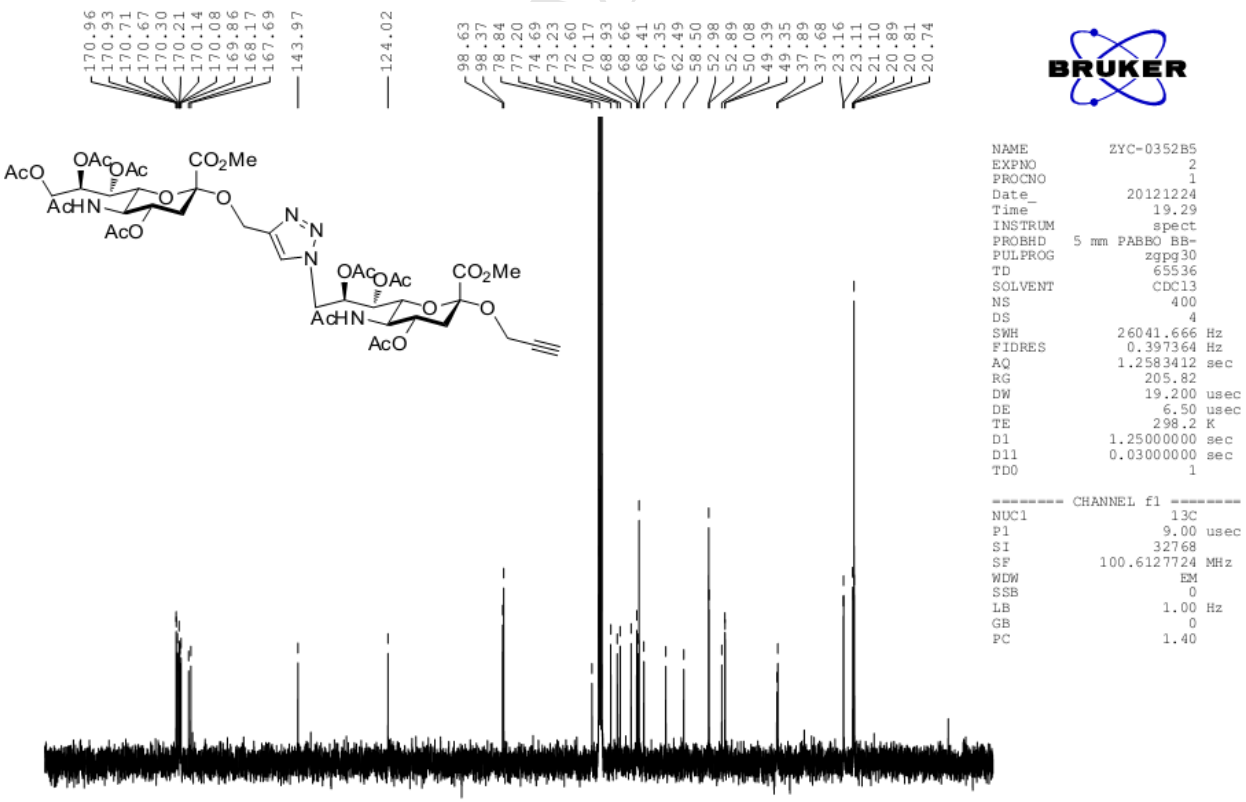
BRUKER

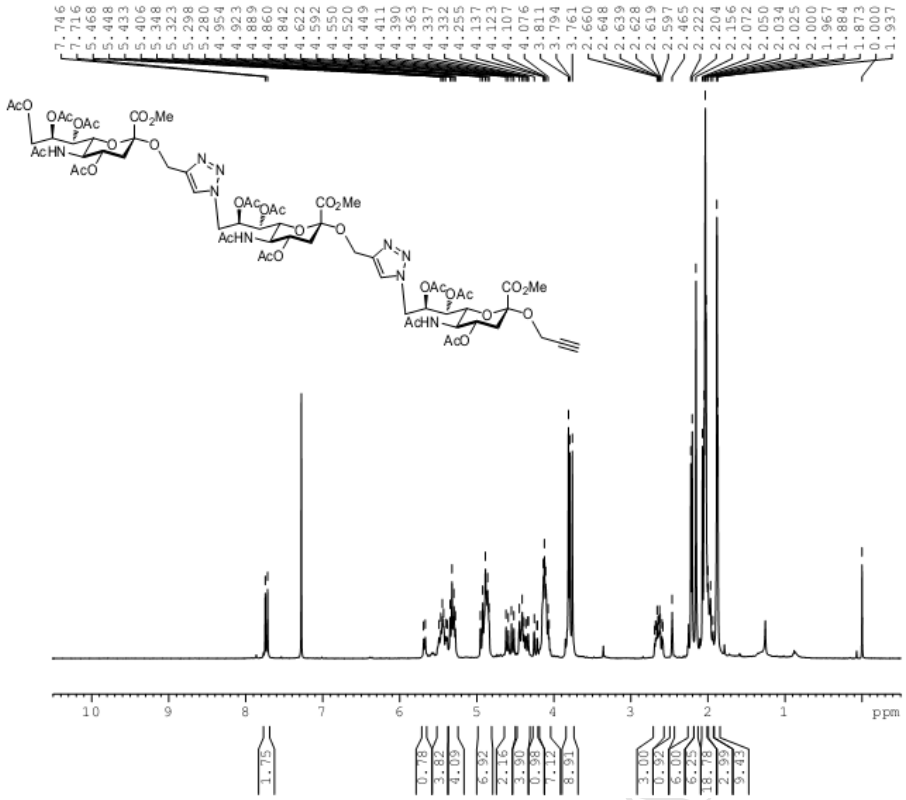
NAME	ZYC-0350Ac1
EXPNO	2
PROCNO	1
Date_	20121207
Time	19.39
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	1024
DS	4
SWH	26041.666 Hz
FIDRES	0.397364 Hz
AQ	1.2583412 sec
RG	205.82
DW	19.200 usec
DE	6.50 usec
TE	296.2 K
D1	1.25000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	9.00 usec
SI	32768
SF	100.6127732 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40


5.325
5.318
5.314
5.159
5.135
4.889
4.877
4.864
4.858
4.853
4.847
4.834
4.822
4.449
4.410
4.119
4.098
4.080
4.074
4.053
4.029
3.823
3.593
3.563
3.557
3.298
3.291
3.283
3.264
3.250
2.665
2.654
2.633
2.622
2.185
2.165
2.035
2.009
1.977
1.887
0.175
0.000
BRUKER
N3
OAc
OAc
COOMe
AcHN
O
O
TMS
AcO
NAME ZYC-0350Ba2
EXPNO 1
PROCNO 1
Date_ 20121204
Time 16.57
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 205.82
DW 60.800 usec
DE 6.50 usec
TE 293.5 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 14.00 usec
SI 65536
SF 400.1300082 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
10
9
8
7
6
5
4
3
2
1
ppm
1.95
0.89
1.04
0.96
0.90
2.07
2.84
0.96
0.97
1.00
5.90
4.24
2.87
8.76

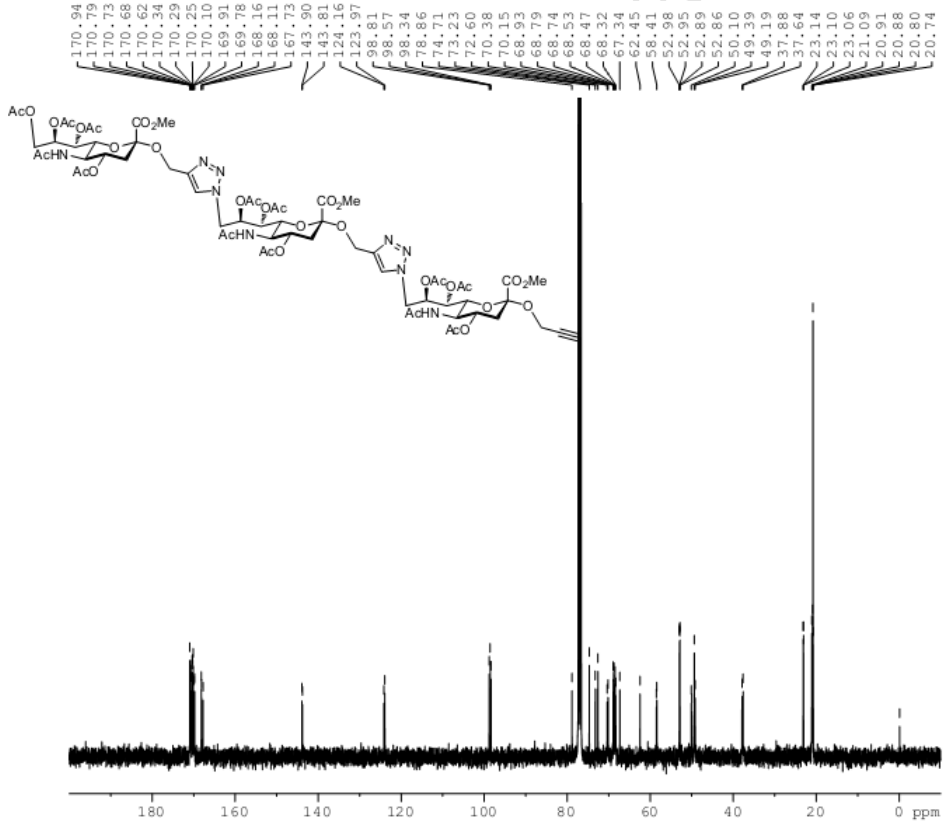

170.97
170.25
170.18
170.12
167.73
100.25
98.22
91.57
77.32
77.00
76.68
72.91
69.75
68.80
67.88
53.60
52.88
50.88
49.33
37.81
23.15
21.00
20.81
-0.28
BRUKER
N3
OAc
OAc
COOMe
AcHN
O
O
TMS
AcO
NAME ZYC-0350Ba3
EXPNO 2
PROCNO 1
Date_ 20121213
Time 21.53
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 256
DS 4
SWH 26041.666 Hz
FIDRES 0.397364 Hz
AQ 1.2583412 sec
RG 205.82
DW 19.200 usec
DE 6.50 usec
TE 299.2 K
D1 1.25000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 9.00 usec
SI 32768
SF 100.6127717 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40


7.730
5.456
5.449
5.440
5.372
5.348
5.343
5.326
5.321
5.300
5.296
5.284
5.279
5.257
5.233
4.902
4.888
4.878
4.872
4.859
4.843
4.547
4.517
4.451
4.417
4.411
4.326
4.320
4.249
4.244
4.204
4.131
4.126
4.110
4.090
4.075
4.061
3.827
3.794
2.462
2.456
2.211
2.166
2.159
2.071
2.044
2.027
2.008
1.981
1.975
1.887
1.882
0.000
BRUKER
AcO
OAc
OAc
CO2Me
AcHN
AcO
N
N
N
OAc
OAc
CO2Me
AcHN
AcO
NAME ZYC-0352B5
EXPNO 1
PROCNO 1
Date_ 20121225
Time 20.34
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 119.06
DW 60.800 usec
DE 6.50 usec
TE 295.7 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 14.60 usec
SI 65536
SF 400.1300034 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
10
9
8
7
6
5
4
3
2
1
ppm
1.00
2.14
1.13
0.88
1.11
0.99
4.28
1.11
2.24
1.07
1.06
5.50
3.28
3.22
2.17
0.70
3.03
6.35
3.07
6.84
3.33
2.43
6.19


170.96
170.93
170.71
170.67
170.30
170.21
170.14
170.08
169.86
168.17
167.69
143.97
124.02
98.63
98.37
78.84
77.20
74.69
73.23
72.60
70.17
68.93
68.66
68.41
67.35
62.49
58.50
52.98
52.89
50.08
49.39
49.35
37.89
37.68
23.16
23.11
21.10
20.89
20.81
20.74
BRUKER
AcO
OAc
OAc
CO2Me
AcHN
AcO
N
N
N
OAc
OAc
CO2Me
AcHN
AcO
NAME ZYC-0352B5
EXPNO 2
PROCNO 1
Date_ 20121224
Time 19.29
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 400
DS 4
SWH 26041.666 Hz
FIDRES 0.397364 Hz
AQ 1.2583412 sec
RG 205.82
DW 19.200 usec
DE 6.50 usec
TE 298.2 K
D1 1.25000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 9.00 usec
SI 32768
SF 100.6127724 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

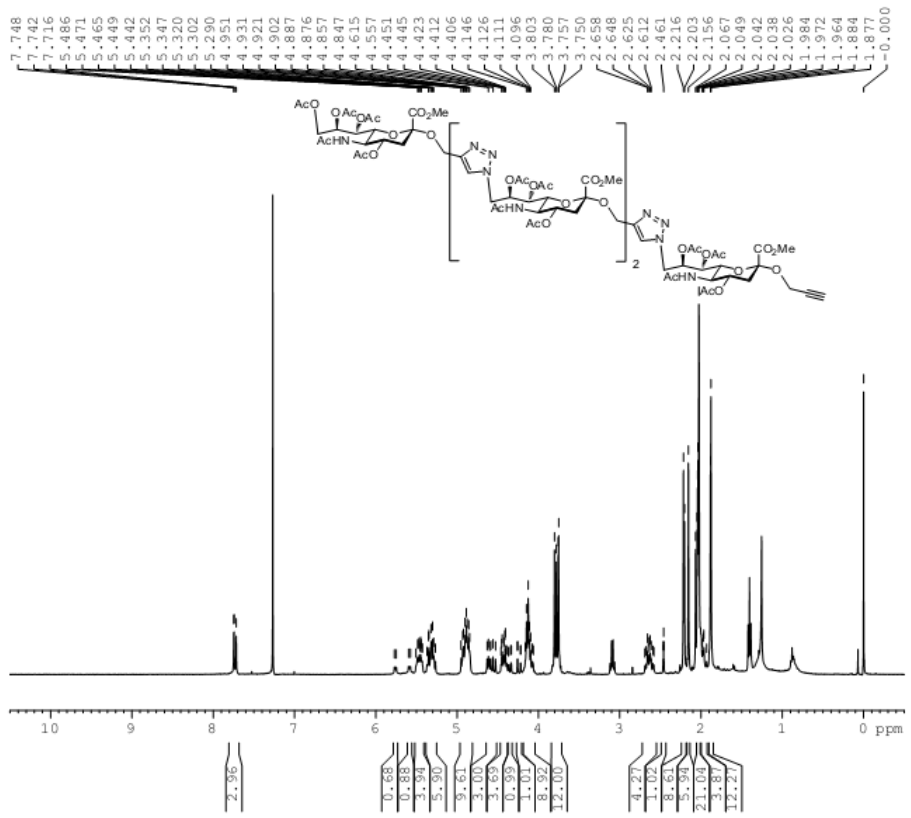


NAME ZYC-3
EXPNO 1
PROCNO 1



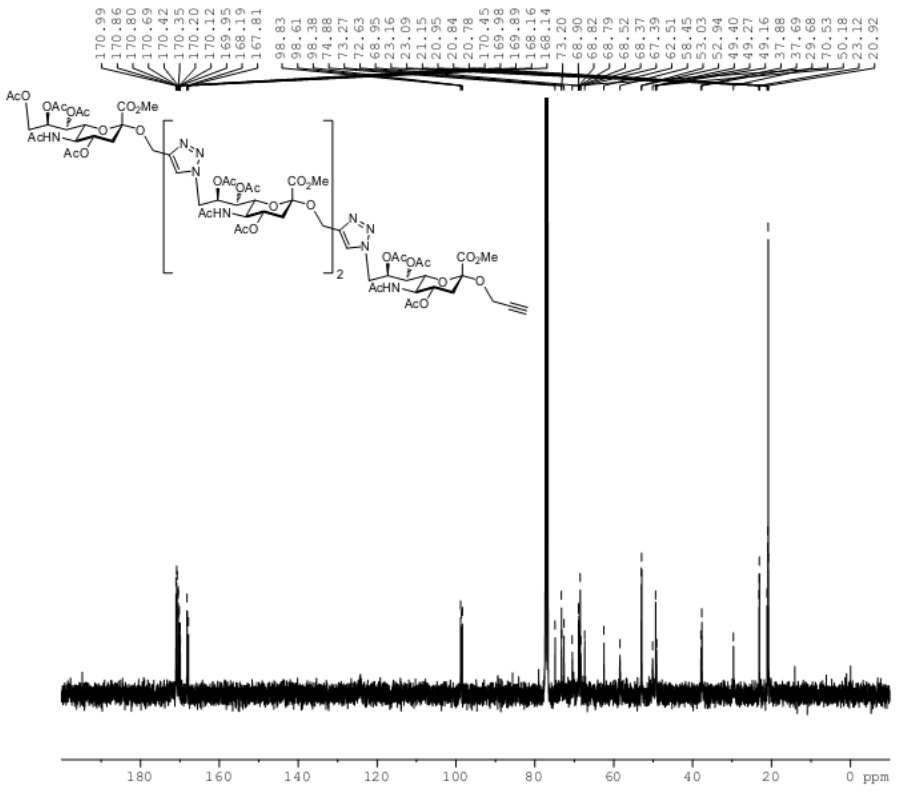
NAME ZYC-0364A5
EXPNO 2
PROCNO 1
Date_ 20130301
Time 14.25
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDC13
NS 1273
DS 4
SWH 28409.092 Hz
FIDRES 0.433488 Hz
AQ 1.1534836 sec
RG 183.47
DW 17.600 usec
DE 6.50 usec
TE 299.5 K
D1 1.20000005 sec
D11 0.03000000 sec

======== CHANNEL f1 ========
NUC1 13C
P1 9.00 usec
SI 32768
SF 100.6127725 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



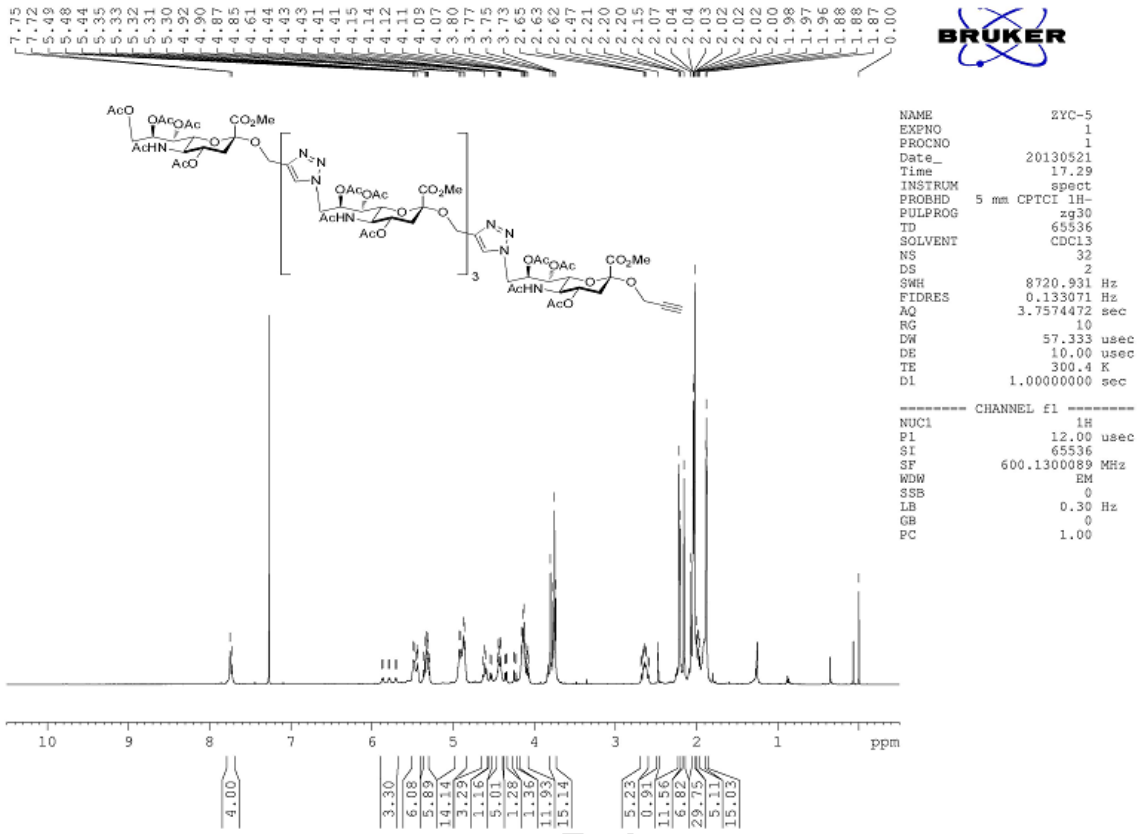
```
NAME                ZYC-4
EXPNO                  11
PROCNO                  1
Date_            20130301
Time                16.38
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                      8
DS                      2
SWH           8223.685 Hz
FIDRES        0.125483 Hz
AQ            3.9846387 sec
RG                161.46
DW               60.800 usec
DE                6.50 usec
TE                298.9 K
D1           1.00000000 sec
TD0                     1

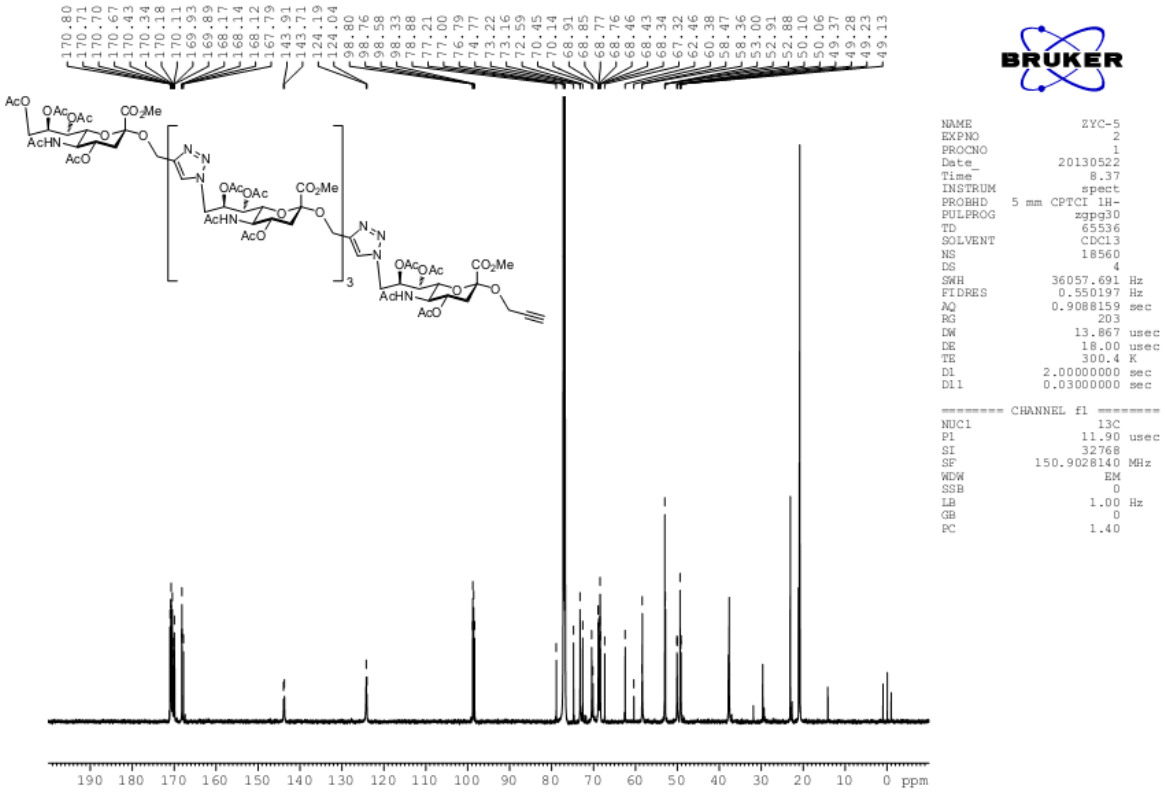
======== CHANNEL f1 ========
NUC1                   1H
P1                 14.00 usec
SI                 65536
SF          400.1300075 MHz
WDW                    EM
SSB                     0
LB                 0.30 Hz
GB                      0
PC                 1.00
```

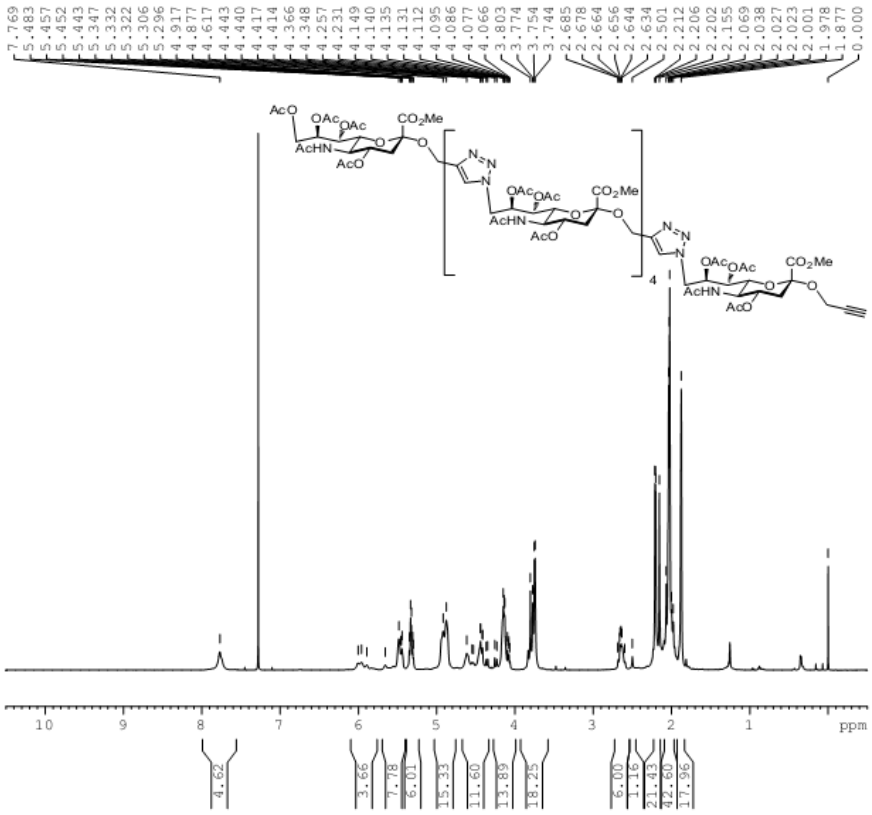


```
NAME            ZYC-0408Ba
EXPNO                    2
PROCNO                   1
Date_             20130424
Time                 22.11
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                   1024
DS                      4
SWH           26041.666 Hz
FIDRES        0.397364 Hz
AQ            1.2583412 sec
RG                205.82
DW               19.200 usec
DE                6.50 usec
TE                298.2 K
D1           1.25000000 sec
D11          0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                 9.00 usec
SI                 32768
SF          100.6127690 MHz
WDW                    EM
SSB                     0
LB                 1.00 Hz
GB                      0
PC                 1.40
```

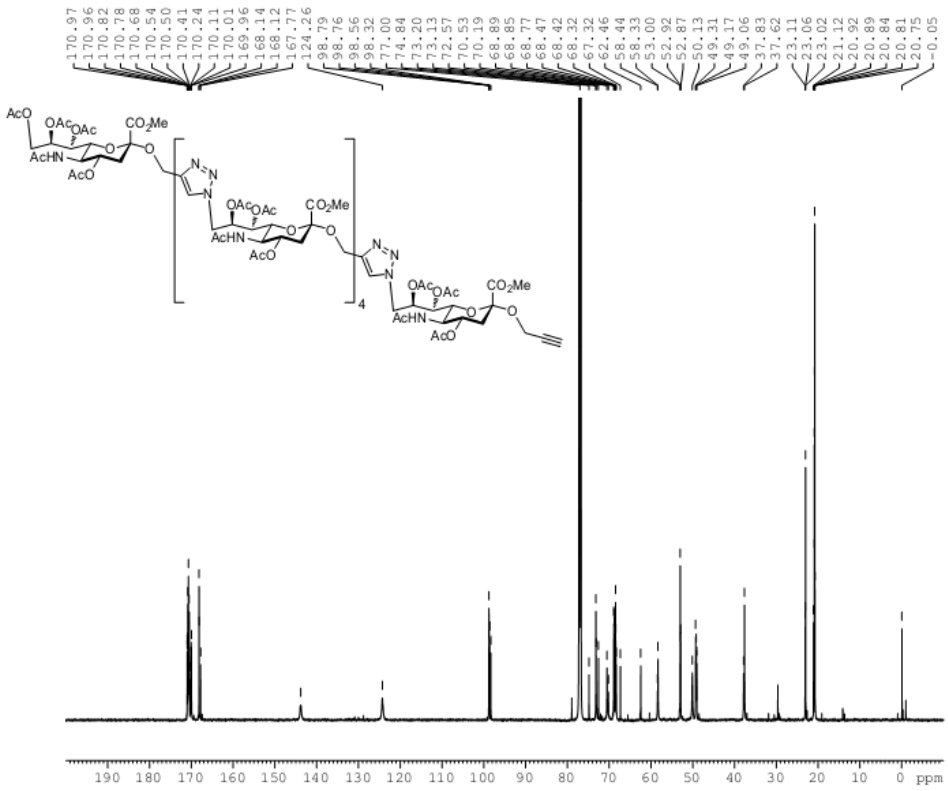

7.75 7.72 5.49 5.48 5.44 5.35 5.33 5.32 5.31 5.30 4.92 4.90 4.87 4.85 4.61 4.44 4.43 4.43 4.41 4.41 4.15 4.14 4.12 4.11 4.09 4.07 3.80 3.77 3.75 3.73 2.65 2.63 2.62 2.47 2.21 2.20 2.20 2.15 2.07 2.04 2.03 2.02 2.02 2.02 2.00 1.98 1.97 1.96 1.88 1.88 1.87 0.00
BRUKER
NAME ZYC-5
EXPNO 1
PROCNO 1
Date_ 20130521
Time 17.29
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 32
DS 2
SWH 8720.931 Hz
FIDRES 0.133071 Hz
AQ 3.7574472 sec
RG 10
DW 57.333 usec
DE 10.00 usec
TE 300.4 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
SI 65536
SF 600.1300089 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
10 9 8 7 6 5 4 3 2 1 ppm
4.00 3.30 6.08 5.89 14.14 3.29 1.16 5.01 1.28 1.36 11.93 15.14 5.23 0.91 11.56 6.82 29.75 5.11 15.03


170.80 170.71 170.70 170.67 170.43 170.34 170.18 170.11 169.93 169.89 168.17 168.14 168.12 167.79 143.91 143.71 124.19 124.04 98.80 98.76 98.58 98.33 78.88 77.21 77.00 76.79 74.77 73.22 73.16 72.59 70.45 70.14 68.91 68.85 68.77 68.76 68.46 68.43 68.34 67.32 62.46 60.38 58.47 58.36 53.00 52.91 52.88 50.10 50.06 49.37 49.28 49.23 49.13
BRUKER
NAME ZYC-5
EXPNO 2
PROCNO 1
Date_ 20130522
Time 8.37
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 18560
DS 4
SWH 36057.691 Hz
FIDRES 0.550197 Hz
AQ 0.9088159 sec
RG 203
DW 13.867 usec
DE 18.00 usec
TE 300.4 K
D1 2.00000000 sec
D11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 11.90 usec
SI 32768
SF 150.9028140 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm



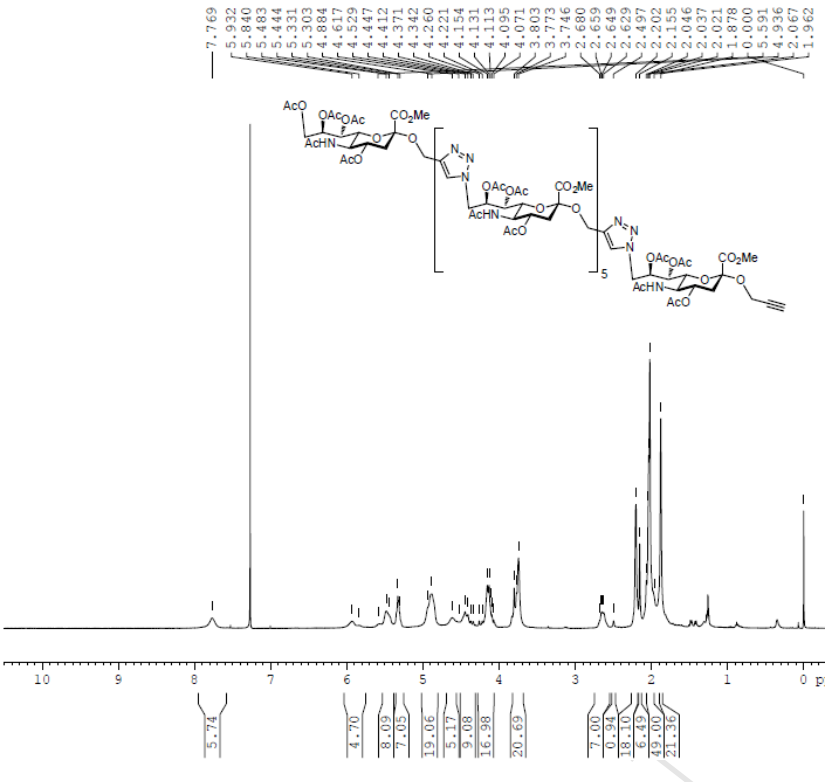
```
NAME                ZYC-6
EXPNO                   1
PROCNO                  1
Date_            20130710
Time_               17.39
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    64
DS                     2
SWH             8561.644 Hz
FIDRES          0.130640 Hz
AQ              3.8273523 sec
RG                     9
DW                58.400 usec
DE                10.00 usec
TE                300.4 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                12.00 usec
SI                 65536
SF          600.1300054 MHz
WDW                   EM
SSB                    0
LB                0.30 Hz
GB                     0
PC                 1.00
```



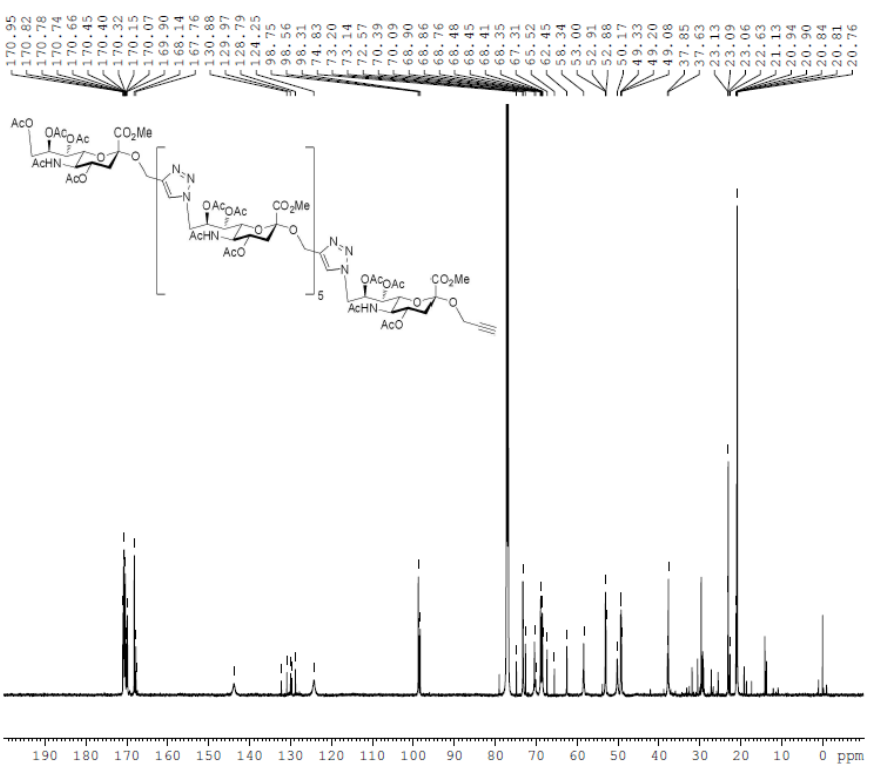
```
NAME                ZYC-6
EXPNO                   2
PROCNO                  1
Date_            20130711
Time_                8.54
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                 25728
DS                     4
SWH            36057.691 Hz
FIDRES          0.550197 Hz
AQ              0.9088159 sec
RG                   203
DW                13.867 usec
DE                18.00 usec
TE                300.4 K
D1             2.00000000 sec
D11            0.03000000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                11.90 usec
SI                 32768
SF          150.9028162 MHz
WDW                   EM
SSB                    0
LB                1.00 Hz
GB                     0
PC                 1.40
```



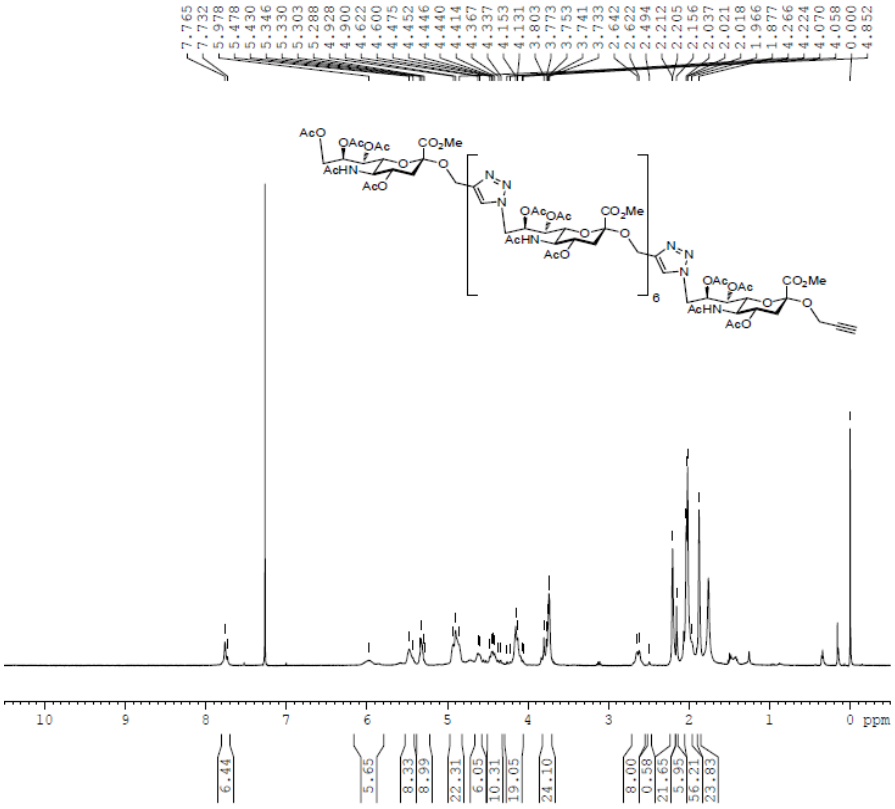
BRUKER

NAME	ZYC-7
EXPNO	1
PROCNO	1
Date_	20130717
Time	16.36
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	0
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	133.41
DW	60.800 usec
DE	6.50 usec
TE	298.9 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.60 usec
SI	65536
SF	400.1300054 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



BRUKER

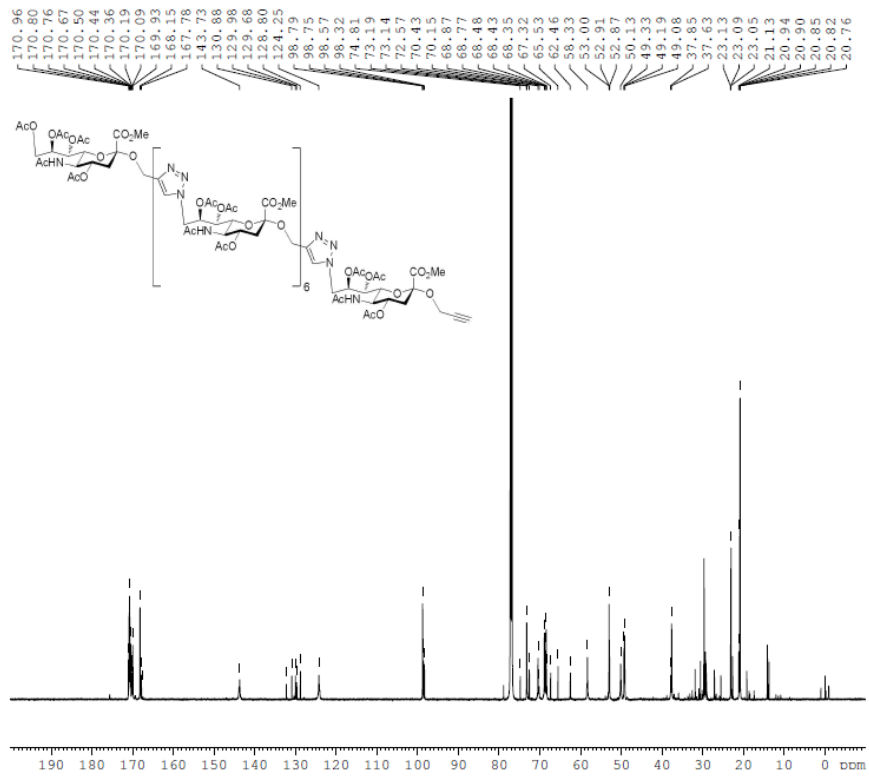
NAME	ZYC-7A-600
EXPNO	2
PROCNO	1
Date_	20131110
Time	15.19
INSTRUM	spect
PROBHD	5 mm CPTCI 1H-
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	25600
DS	4
SWH	36057.691 Hz
FIDRES	0.550197 Hz
AQ	0.9088159 sec
RG	203
DW	13.867 usec
DE	18.00 usec
TE	300.4 K
D1	2.00000000 sec
D11	0.03000000 sec
-------- CHANNEL f1 --------	
NUC1	13C
P1	11.90 usec
SI	32768
SF	150.9028165 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40



BRUKER

```
NAME                ZYC-8
EXPNO                   1
PROCNO                  1
Date_            20130712
Time                18.10
INSTRUM             spect
PROBHD    5 mm PABBO BB-
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                      8
DS                      2
SWH           8223.685 Hz
FIDRES        0.125483 Hz
AQ            3.9846387 sec
RG                    183
DW               60.800 usec
DE                6.50 usec
TE                297.2 K
D1           1.00000000 sec
TD0                     1

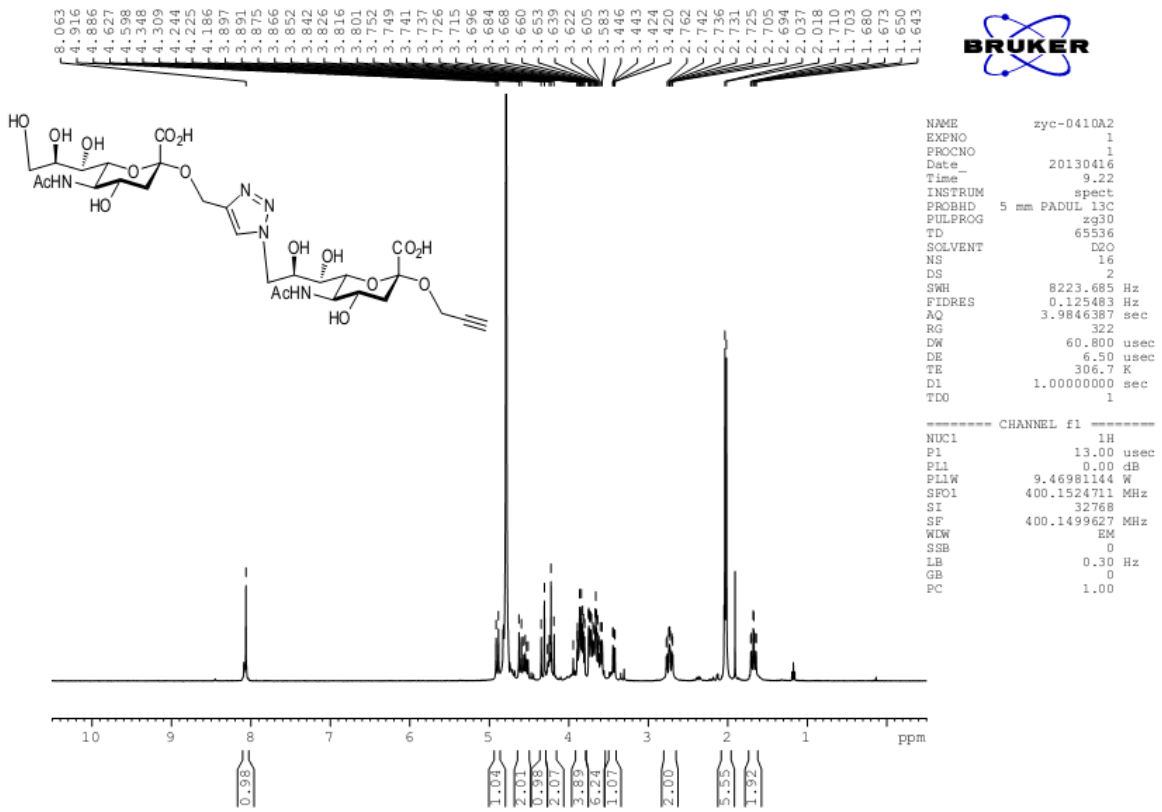
======== CHANNEL f1 ========
NUC1                   1H
P1                14.00 usec
SI                  65536
SF          400.1300078 MHz
WDW                    EM
SSB                     0
LB                 0.30 Hz
GB                      0
PC                   1.00
```

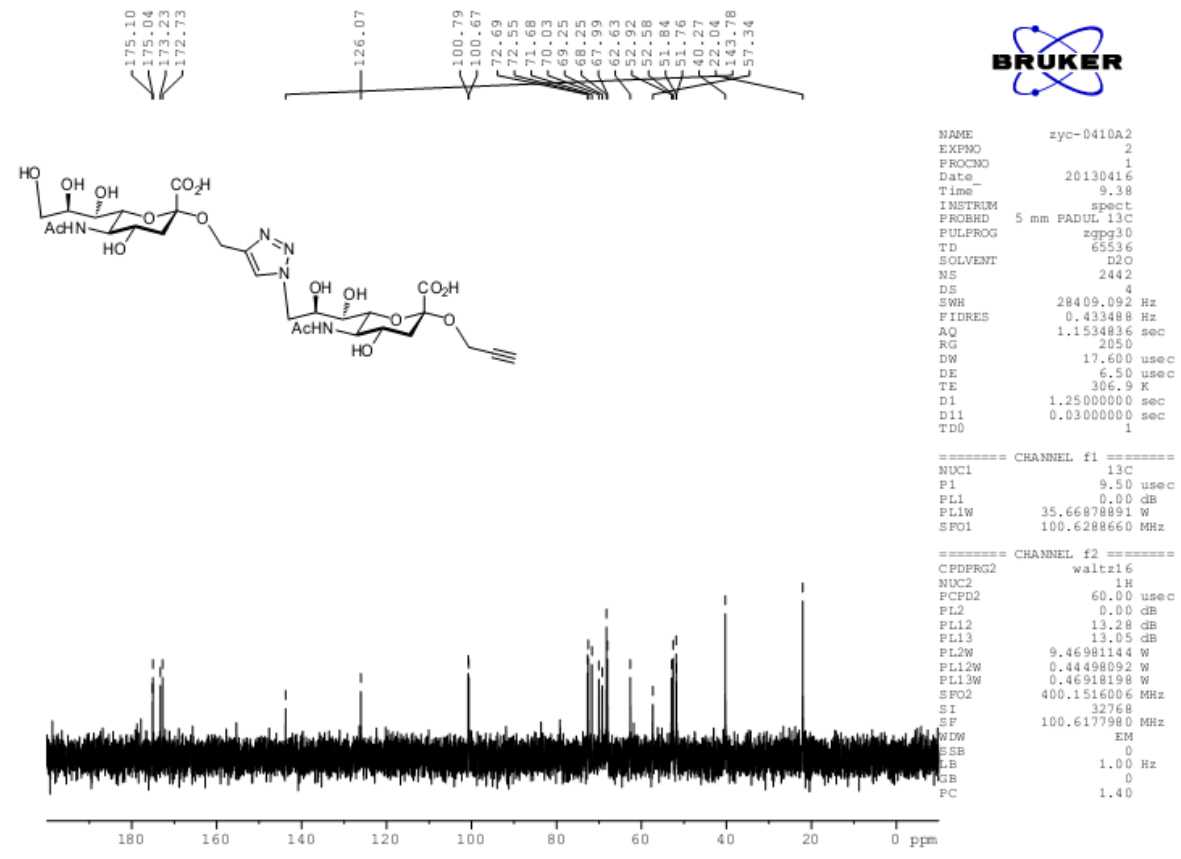


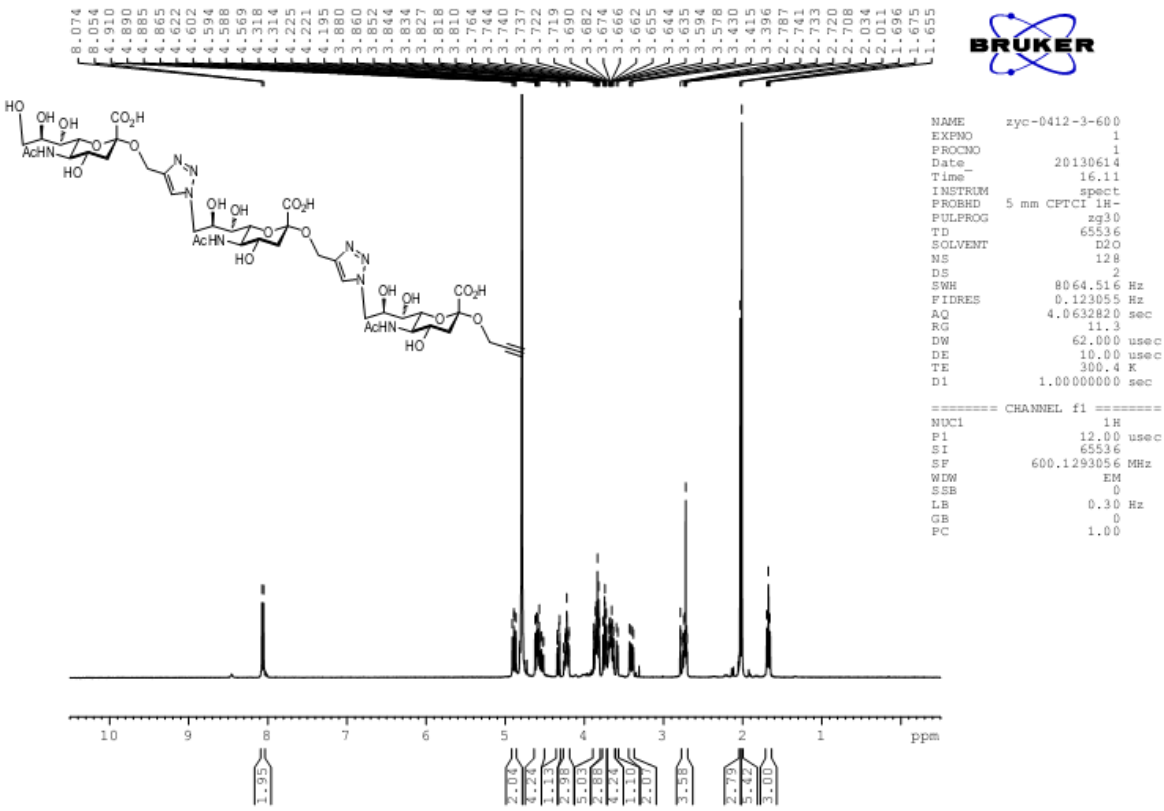
BRUKER

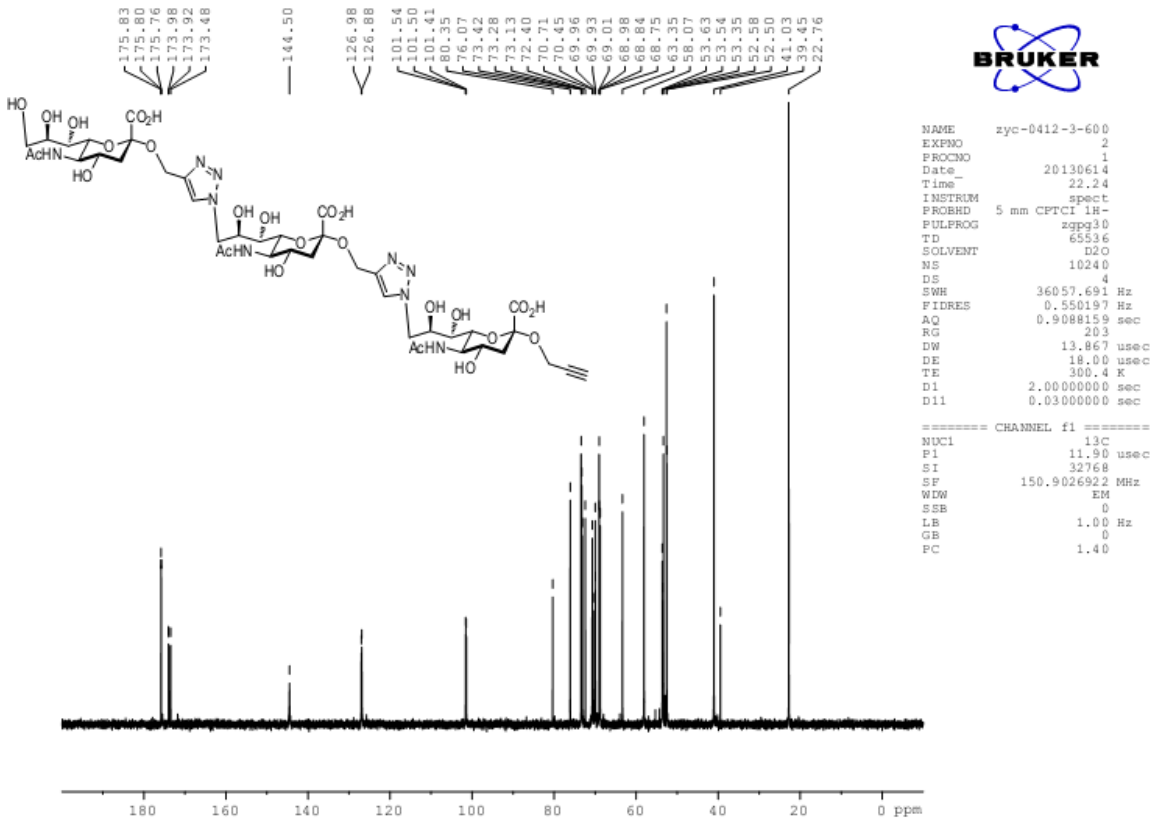
```
NAME            ZYC-8A-600
EXPNO                    2
PROCNO                   1
Date_             20131102
Time                 14.38
INSTRUM              spect
PROBHD    5 mm CPTCI 1H-
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                   25600
DS                       4
SWH           36057.691 Hz
FIDRES         0.550197 Hz
AQ             0.9088159 sec
RG                     203
DW                13.867 usec
DE                 18.00 usec
TE                 300.4 K
D1            2.00000000 sec
D11           0.03000000 sec

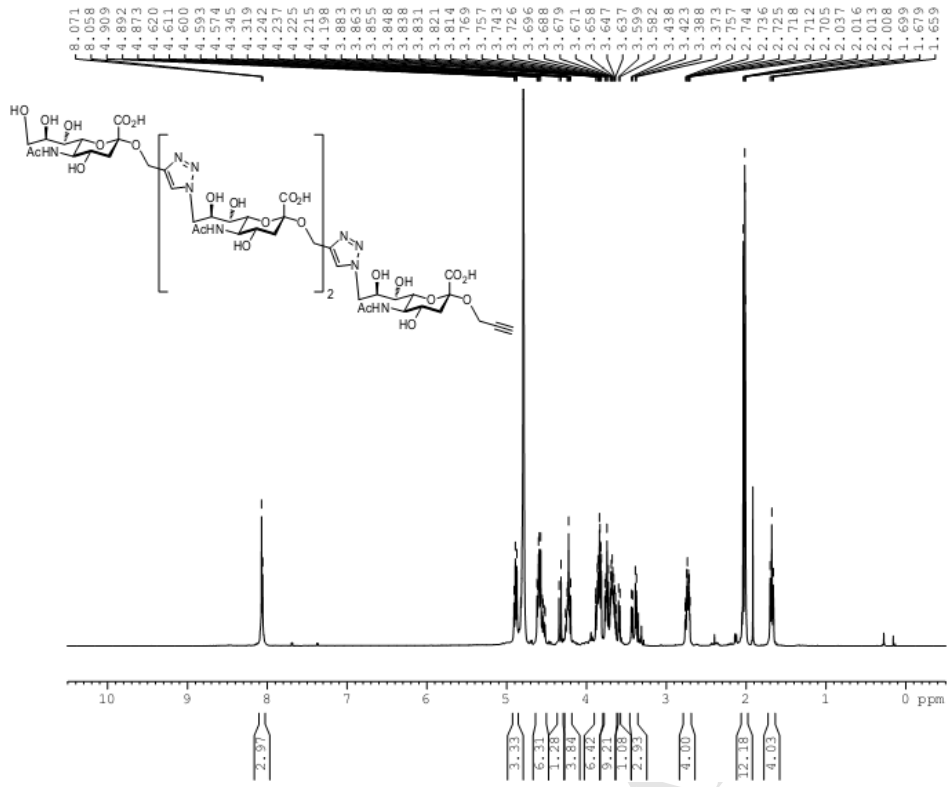
======== CHANNEL f1 ========
NUC1                   13C
P1                 11.90 usec
SI                   32768
SF          150.9028153 MHz
WDW                    EM
SSB                     0
LB                 1.00 Hz
GB                      0
PC                   1.40
```


8.063
4.916
4.886
4.627
4.598
4.348
4.309
4.244
4.225
4.186
3.897
3.891
3.875
3.866
3.852
3.842
3.826
3.816
3.801
3.752
3.749
3.741
3.737
3.726
3.715
3.696
3.684
3.668
3.660
3.653
3.639
3.622
3.605
3.583
3.446
3.443
3.424
3.420
2.762
2.742
2.736
2.731
2.725
2.705
2.694
2.037
2.018
1.710
1.703
1.680
1.673
1.650
1.643
BRUKER
HO
OH
OH
CO2H
AcHN
HO
N
N
N
OH
OH
CO2H
AcHN
HO
O
NAME zyc-0410A2
EXPNO 1
PROCNO 1
Date_ 20130416
Time 9.22
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg30
TD 65536
SOLVENT D2O
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 322
DW 60.800 usec
DE 6.50 usec
TE 306.7 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 13.00 usec
PL1 0.00 dB
PL1W 9.46981144 W
SFO1 400.1524711 MHz
SI 32768
SF 400.1499627 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
10
9
8
7
6
5
4
3
2
1
ppm
0.98
1.04
2.01
0.98
2.07
3.89
6.24
1.07
2.00
5.55
1.92


175.10
175.04
173.23
172.73
126.07
100.79
100.67
72.69
72.55
71.68
70.03
69.25
68.25
67.99
62.63
52.92
52.58
51.84
51.76
40.27
22.04
143.78
57.34
BRUKER
HO
OH
OH
CO2H
AcHN
HO
N
N
N
OH
OH
CO2H
AcHN
HO
O
NAME zyc-0410A2
EXPNO 2
PROCNO 1
Date_ 20130416
Time 9.38
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 2442
DS 4
SWH 28409.092 Hz
FIDRES 0.433488 Hz
AQ 1.1534836 sec
RG 2050
DW 17.600 usec
DE 6.50 usec
TE 306.9 K
D1 1.25000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 9.50 usec
PL1 0.00 dB
PL1W 35.66878891 W
SFO1 100.6288660 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 60.00 usec
PL2 0.00 dB
PL12 13.28 dB
PL13 13.05 dB
PL2W 9.46981144 W
PL12W 0.44498092 W
PL13W 0.46918198 W
SFO2 400.1516006 MHz
SI 32768
SF 100.6177980 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
180
160
140
120
100
80
60
40
20
0 ppm

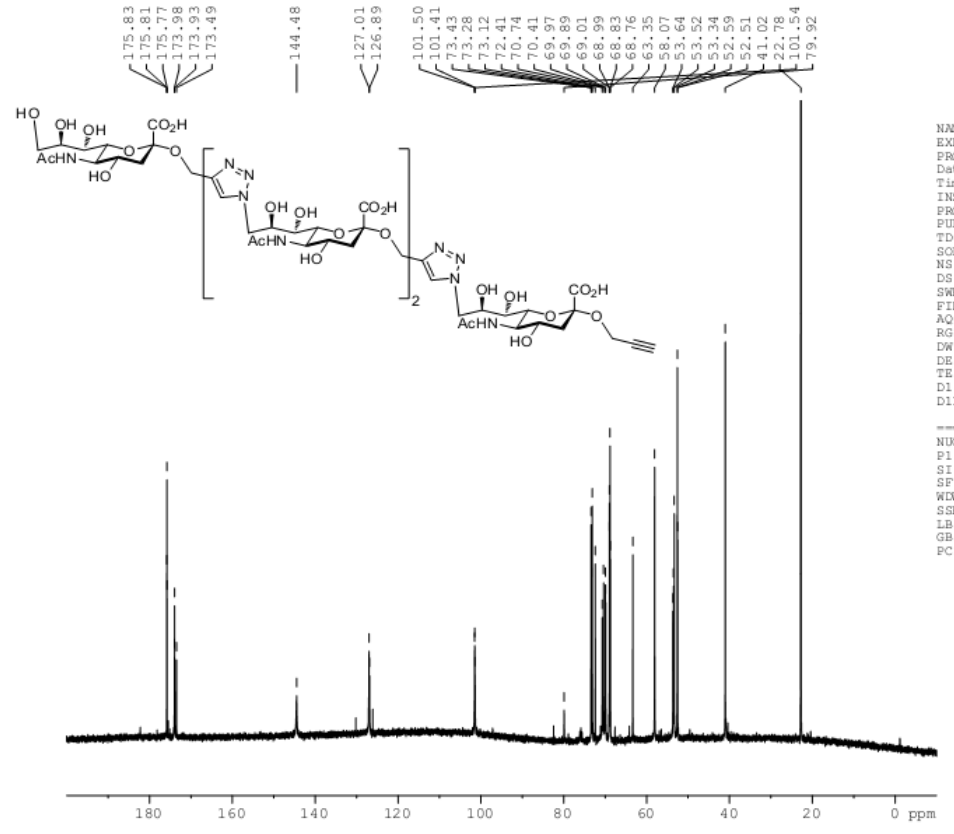

8.074
8.054
4.910
4.890
4.885
4.865
4.622
4.602
4.594
4.588
4.569
4.318
4.314
4.225
4.221
4.195
3.880
3.860
3.852
3.844
3.834
3.827
3.818
3.810
3.764
3.744
3.740
3.737
3.722
3.719
3.690
3.682
3.674
3.666
3.662
3.655
3.644
3.635
3.594
3.578
3.430
3.415
3.396
2.787
2.741
2.733
2.720
2.708
2.034
2.011
1.696
1.675
1.655
BRUKER
NAME zyc-0412-3-600
EXPNO 1
PROCNO 1
Date_ 20130614
Time_ 16.11
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 65536
SOLVENT D2O
NS 128
DS 2
SWH 8064.516 Hz
FIDRES 0.123055 Hz
AQ 4.0632820 sec
RG 11.3
DW 62.000 usec
DE 10.00 usec
TE 300.4 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
SI 65536
SF 600.1293056 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
10 9 8 7 6 5 4 3 2 1 ppm
1.95 2.04 2.24 1.13 2.98 5.03 2.86 4.24 1.10 2.07 3.58 2.79 5.42 3.00


175.83
175.80
175.76
173.98
173.92
173.48
144.50
126.98
126.88
101.54
101.50
101.41
80.35
76.07
73.42
73.28
73.13
72.40
70.71
70.45
69.96
69.93
69.01
68.98
68.84
68.75
63.35
58.07
53.63
53.54
53.35
52.58
52.50
41.03
39.45
22.76
BRUKER
NAME zyc-0412-3-600
EXPNO 2
PROCNO 1
Date_ 20130614
Time_ 22.24
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 10240
DS 4
SWH 36057.691 Hz
FIDRES 0.550197 Hz
AQ 0.9088159 sec
RG 203
DW 13.867 usec
DE 18.00 usec
TE 300.4 K
D1 2.00000000 sec
D11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 11.90 usec
SI 32768
SF 150.9026922 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
180 160 140 120 100 80 60 40 20 0 ppm



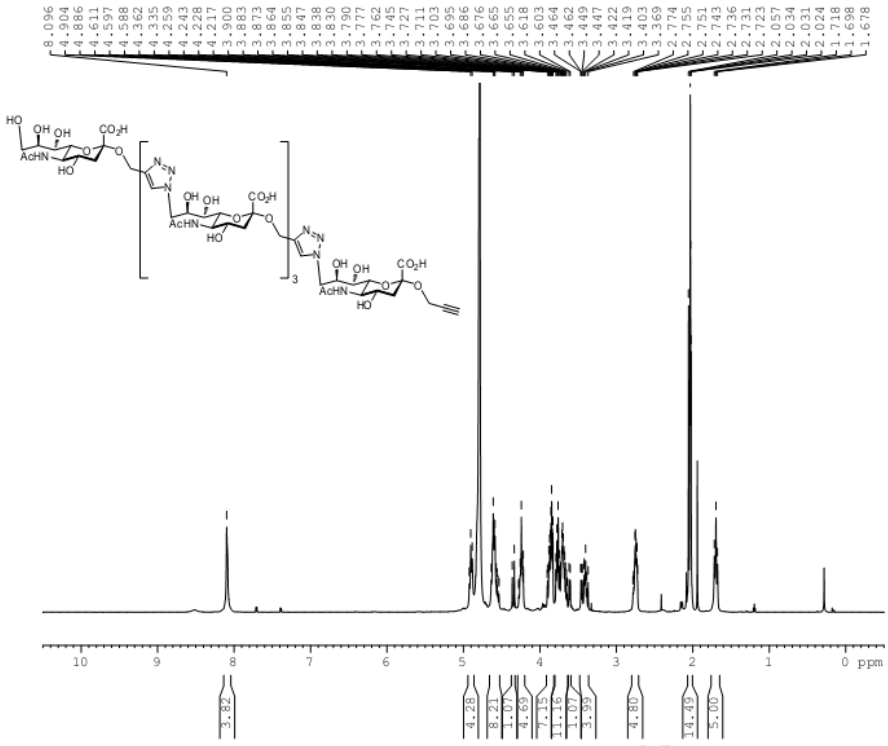

```
NAME          ZYC-4-COOH
EXPNO                  1
PROCNO                 1
Date_           20130503
Time               9.24
INSTRUM           spect
PROBHD   5 mm CPTCI 1H-
PULPROG            zg30
TD                65536
SOLVENT             D2O
NS                   32
DS                    2
SWH           12335.526 Hz
FIDRES        0.188225 Hz
AQ            2.6564426 sec
RG                 11.3
DW               40.533 usec
DE               10.00 usec
TE               300.4 K
D1          1.00000000 sec

======== CHANNEL f1 ========
NUC1                1H
P1              12.00 usec
SI                65536
SF          600.1299478 MHz
WDW                EM
SSB                 0
LB               0.30 Hz
GB                  0
PC               1.00
```



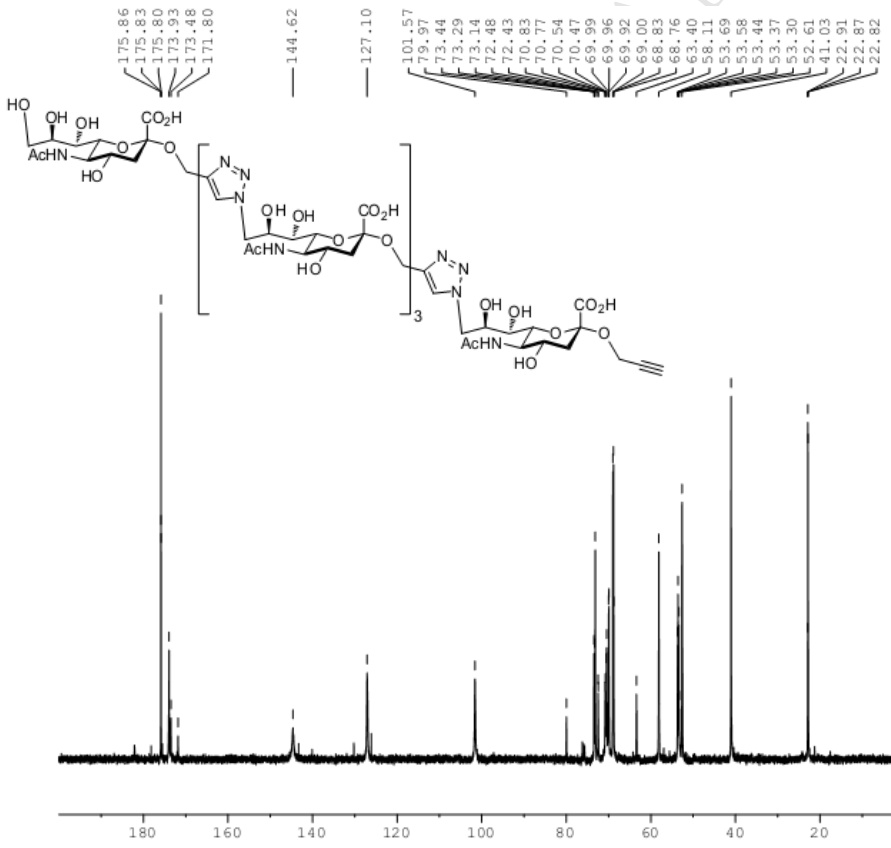

```
NAME       ZYC-0415B-600
EXPNO                  2
PROCNO                 1
Date_           20130503
Time               9.36
INSTRUM           spect
PROBHD   5 mm CPTCI 1H-
PULPROG          zgpg30
TD                65536
SOLVENT             D2O
NS                 6688
DS                    4
SWH           36057.691 Hz
FIDRES        0.550197 Hz
AQ            0.9088159 sec
RG                  203
DW               13.867 usec
DE               18.00 usec
TE               300.4 K
D1          2.00000000 sec
D11         0.03000000 sec

======== CHANNEL f1 ========
NUC1               13C
P1              11.90 usec
SI                32768
SF          150.9026921 MHz
WDW                EM
SSB                 0
LB               1.00 Hz
GB                  0
PC               1.40
```



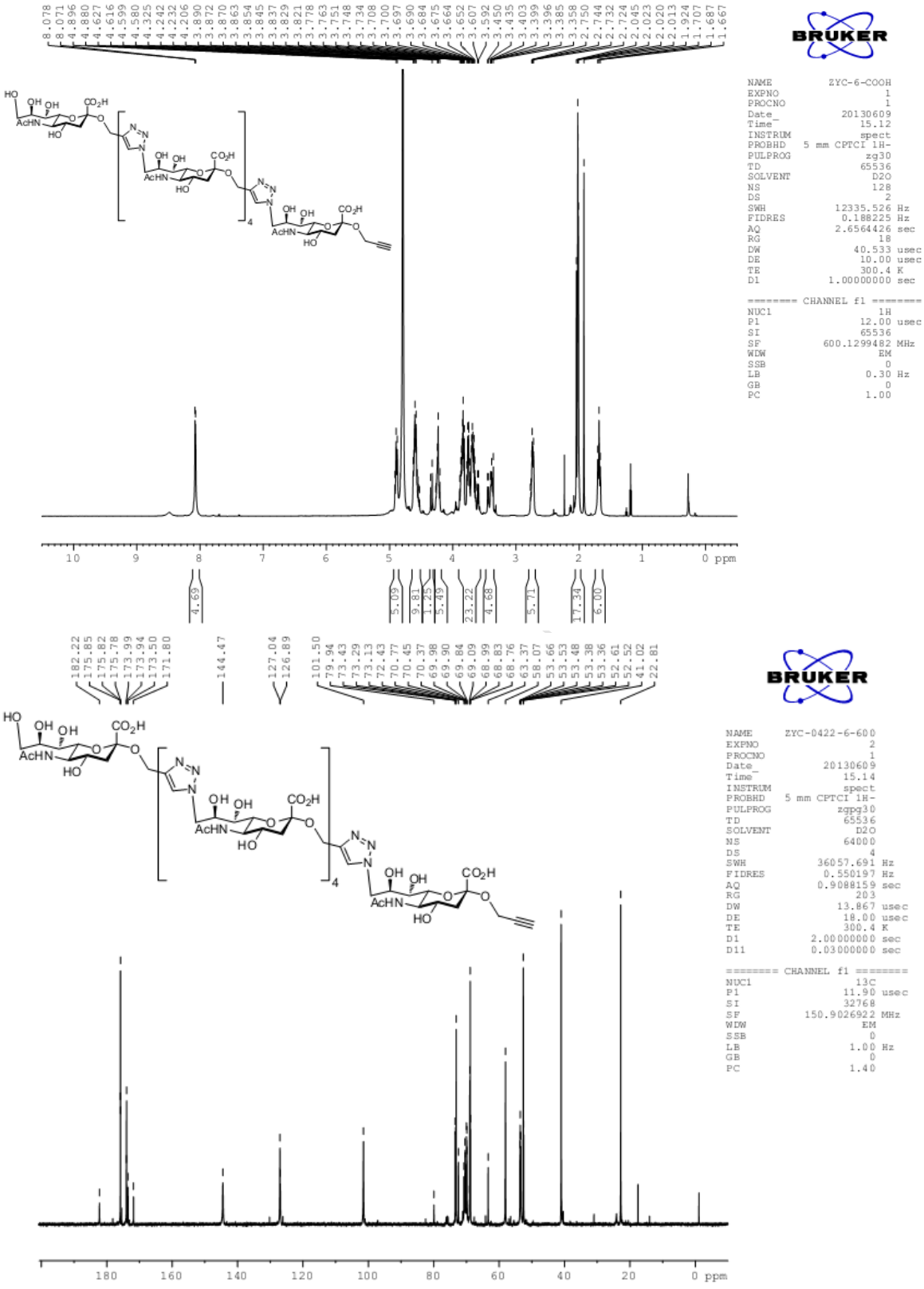

```
NAME          zyc-5-COOH
EXPNO                  1
PROCNO                 1
Date_           20130704
Time_             15.50
INSTRUM           spect
PROBHD   5 mm CPTCI 1H-
PULPROG           zg30
TD               65536
SOLVENT            D2O
NS                 128
DS                   2
SWH           9259.259 Hz
FIDRES        0.141285 Hz
AQ           3.5389941 sec
RG                20.2
DW              54.000 usec
DE              10.00 usec
TE              300.4 K
D1           1.00000000 sec

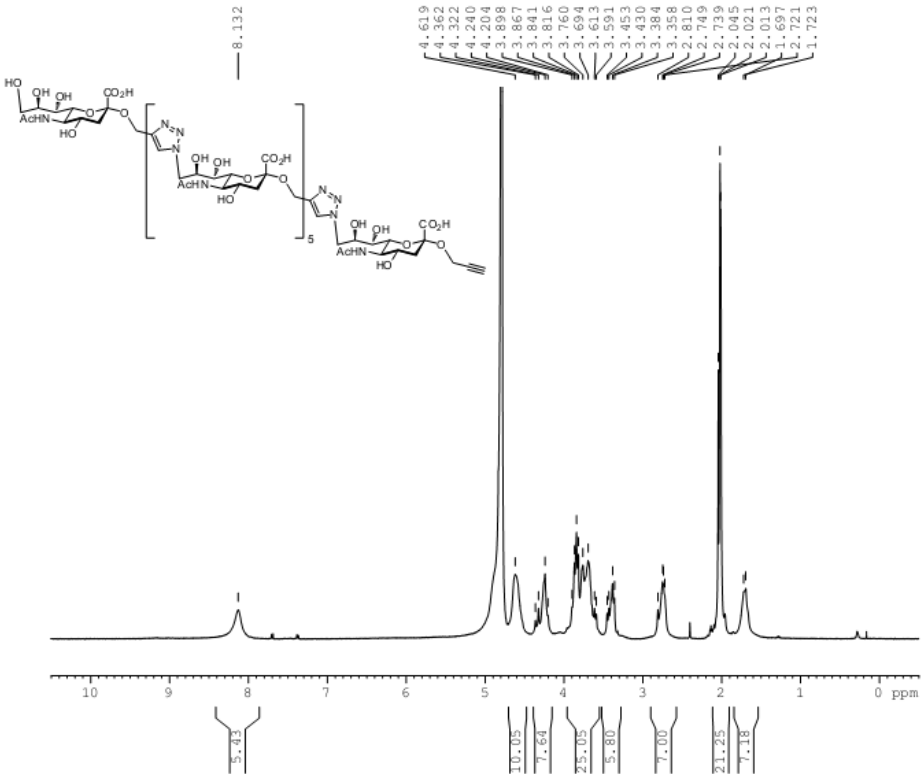
======== CHANNEL f1 ========
NUC1               1H
P1              12.00 usec
SI              65536
SF         600.1299483 MHz
WDW                EM
SSB                 0
LB              0.30 Hz
GB                  0
PC               1.00
```




```
NAME       ZYC-0426-5-600
EXPNO                   2
PROCNO                  1
Date_            20130704
Time_              15.59
INSTRUM            spect
PROBHD    5 mm CPTCI 1H-
PULPROG          zgpg30
TD                65536
SOLVENT             D2O
NS                20992
DS                    4
SWH           36057.691 Hz
FIDRES         0.550197 Hz
AQ             0.9088159 sec
RG                  203
DW               13.867 usec
DE               18.00 usec
TE               300.4 K
D1            2.00000000 sec
D11           0.03000000 sec

======== CHANNEL f1 ========
NUC1               13C
P1              11.90 usec
SI              32768
SF         150.9026922 MHz
WDW                EM
SSB                 0
LB              1.00 Hz
GB                  0
PC               1.40
```

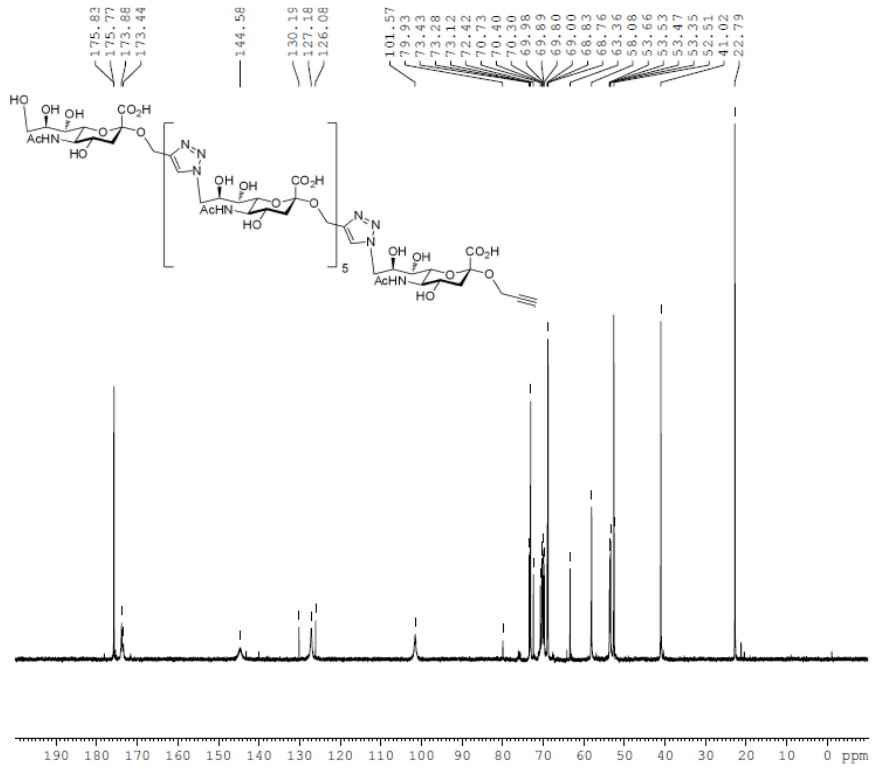

8.078
8.071
4.896
4.880
4.627
4.616
4.599
4.580
4.325
4.242
4.232
4.206
3.890
3.872
3.870
3.863
3.854
3.845
3.837
3.829
3.821
3.778
3.763
3.751
3.748
3.734
3.708
3.700
3.697
3.690
3.684
3.675
3.664
3.652
3.607
3.592
3.450
3.435
3.403
3.399
3.396
3.385
3.358
2.750
2.744
2.732
2.724
2.045
2.023
2.020
2.013
1.924
1.707
1.687
1.667
BRUKER
NAME ZYC-6-COOH
EXPNO 1
PROCNO 1
Date_ 20130609
Time_ 15.12
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 65536
SOLVENT D2O
NS 128
DS 2
SWH 12335.526 Hz
FIDRES 0.188225 Hz
AQ 2.6564426 sec
RG 18
DW 40.533 usec
DE 10.00 usec
TE 300.4 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
SI 65536
SF 600.1299482 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
10 9 8 7 6 5 4 3 2 1 0 ppm
4.69
5.09
9.81
1.25
5.49
23.22
4.68
5.71
17.34
6.00
182.22
175.85
175.82
175.78
173.99
173.94
173.50
171.80
144.47
127.04
126.89
101.50
79.94
73.43
73.29
73.13
72.43
70.77
70.45
70.37
69.98
69.90
69.84
69.09
68.99
68.83
68.76
63.37
58.07
53.66
53.53
53.48
53.38
53.36
52.61
52.52
41.02
22.81
BRUKER
NAME ZYC-0422-6-600
EXPNO 2
PROCNO 1
Date_ 20130609
Time_ 15.14
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 64000
DS 4
SWH 36057.691 Hz
FIDRES 0.550197 Hz
AQ 0.9088159 sec
RG 203
DW 13.867 usec
DE 18.00 usec
TE 300.4 K
D1 2.00000000 sec
D11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 11.90 usec
SI 32768
SF 150.9026922 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
180 160 140 120 100 80 60 40 20 0 ppm



BRUKER

```
NAME            ZYC-7-COOH
EXPNO                    1
PROCNO                   1
Date_             20130712
Time                 18.05
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT                D2O
NS                       8
DS                       2
SWH               8223.685 Hz
FIDRES            0.125483 Hz
AQ                3.9846387 sec
RG                    89.6
DW                  60.800 usec
DE                   6.50 usec
TE                 297.3 K
D1                1.00000000 sec
TD0                      1

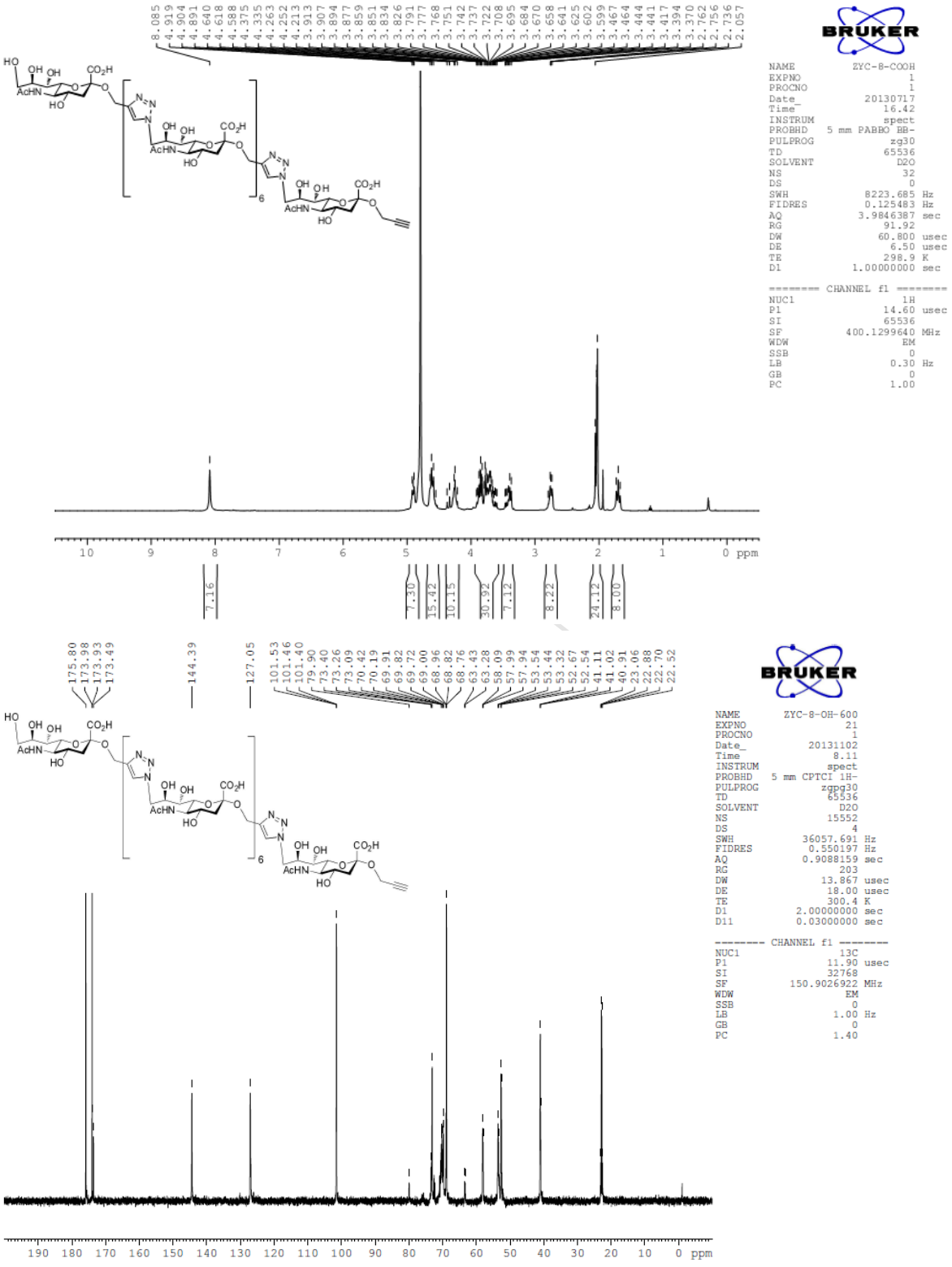
======== CHANNEL f1 ========
NUC1                    1H
P1                  14.00 usec
SI                   65536
SF            400.1299662 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```



BRUKER

```
NAME          ZYC-7OH-600
EXPNO                   2
PROCNO                  1
Date_            20131108
Time                14.19
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG            zgpg30
TD                  65536
SOLVENT               D2O
NS                  35200
DS                      4
SWH              36057.691 Hz
FIDRES            0.550197 Hz
AQ                0.9088159 sec
RG                    203
DW                 13.867 usec
DE                 18.00 usec
TE                 300.4 K
D1               2.00000000 sec
D11              0.03000000 sec

-------- CHANNEL f1 --------
NUC1                   13C
P1                 11.90 usec
SI                  32768
SF           150.9026922 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```


8.085
4.919
4.904
4.891
4.640
4.618
4.588
4.375
4.335
4.263
4.252
4.213
3.913
3.907
3.894
3.877
3.859
3.851
3.834
3.826
3.791
3.777
3.768
3.751
3.742
3.737
3.722
3.708
3.695
3.684
3.670
3.658
3.641
3.625
3.602
3.599
3.467
3.464
3.444
3.441
3.417
3.394
3.370
2.762
2.756
2.736
2.057
BRUKER
NAME ZYC-8-COOH
EXPNO 1
PROCNO 1
Date_ 20130717
Time 16.42
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT D2O
NS 32
DS 0
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 91.92
DW 60.800 usec
DE 6.50 usec
TE 298.9 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 14.60 usec
SI 65536
SF 400.1299640 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
10 9 8 7 6 5 4 3 2 1 0 ppm
7.16
7.30
15.42
10.15
30.92
7.12
8.22
24.12
8.00
175.80
173.98
173.93
173.49
144.39
127.05
101.53
101.46
101.40
79.90
73.40
73.26
73.09
70.42
70.19
69.91
69.82
69.72
69.00
68.96
68.82
68.76
63.43
63.28
58.09
57.99
57.94
53.54
53.44
53.32
52.67
52.54
41.11
41.02
40.91
23.06
22.88
22.70
22.52
BRUKER
NAME ZYC-8-OH-600
EXPNO 21
PROCNO 1
Date_ 20131102
Time 8.11
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 15552
DS 4
SWH 36057.691 Hz
FIDRES 0.550197 Hz
AQ 0.9088159 sec
RG 203
DW 13.867 usec
DE 18.00 usec
TE 300.4 K
D1 2.00000000 sec
D11 0.03000000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 11.90 usec
SI 32768
SF 150.9026922 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm