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Note

Studies toward the synthesis of linear triazole linked pseudo oligosaccharides and the use of ferrocene as analytical probe

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ABSTRACT

Three different building blocks have been synthesised and used for the synthesis of linear triazole linked pseudo oligosaccharides with copper(I)-catalysed cycloaddition (CuAAC). Ethynylferrocene has been used as analytical probe to improve the UV/Vis properties and HPLC methods have been used and optimised for the analysis of the pseudo oligosaccharides. The smallest ones have been isolated and characterised by analytical HPLC, NMR, ESI-MS and elemental analysis.

1. Introduction

The regiospecific biorthogonal copper(I) catalysed alkyne azide cycloaddition (CuAAC)[1,2] has been widely used as a tool for the synthesis of complex carbohydrate structures such as glycodendrimers,[3,4] glycoclusters,[5–7] polymer based glycoconjugates[8,9] or carbohydrate functionalised surfaces.[10–12] Most applications aim to study multivalent protein–carbohydrate interactions which are involved in multiple biological mechanisms like cellular recognition, cell–cell communication or molecular targeting.[13–15] However, carbohydrate structures have also been found to be promising compounds, in material sciences especially with biotechnical and biomedical applications in mind. Different CuAAC based approaches have been investigated in the past years such as the modification of existing biopolymers like cellulose,[16] where azides were introduced in the C-6 position of the glucose moieties and were afterwards modified with small organic alkines via CuAAC. Others are connecting alkine modified polymeric structures to azido-cyclodextrins, resulting in bigger, star-shaped polymeric structures.[17] Another approach is to build up alkine-functionalised synthetic polymers, *e.g.* based on alkine-modified methacrylates,[18,19] which can be modified with azido sugars after the polymerisation step. There are also reports on the synthesis of linear oligomeric structures built in a step by step approach.[20,21] To our knowledge, there are only a few reports up to now, on carbohydrate based pseudo poly- or oligomeric structures built from bifunctional carbohydrate monomers containing both azide and alkine moieties. For example there are reports on crystal lattice supported oligomerisation of such bifunctional monomers resulting in linear oligomeric structures.[22,23] There have also been reports on the synthesis of cyclic and linear pseudo oligomeric structures as potential therapeutic agents against the parasite Trypanosoma cruzi, which causes Chaga's disease.[24,25] Herein we want to present approaches toward the synthesis of linear triazole linked pseudo oligomeric carbohydrate structures by a one pot approach (Scheme 1).

To investigate the synthesis of linear oligomeric structures three different building blocks (**4, 5** and **7**) based on N-acetylglucosamin (GlcNAc, **1**) have been synthesised. We decided to use GlcNAc (**1**) because of its polymeric form chitin, which can be found in nature quite frequently *e.g.* in cell wall of fungi or exoskeleton of insects. Chitin exhibits interesting traits like high stability and low solubility. These properties make chitin an attractive polymer structure as new biomaterial in medicinal applications.[26] In analogy to the natural biopolymer a possible GlcNAc based oligomeric structure could also bear interesting properties. The synthetic approach allows additional modifications *e.g.* bearing a prodrug bound to a cleavable linker, which can be cleaved yielding a bioactive compound. This would be interesting for localised disease treatments. The building blocks used should either be commercially available or easily accessible (Scheme 2).

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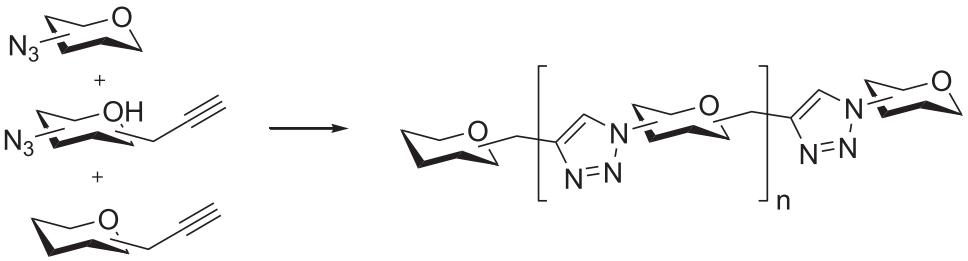


Scheme 1. General concept of the synthesis of linear triazole based oligosaccharides.

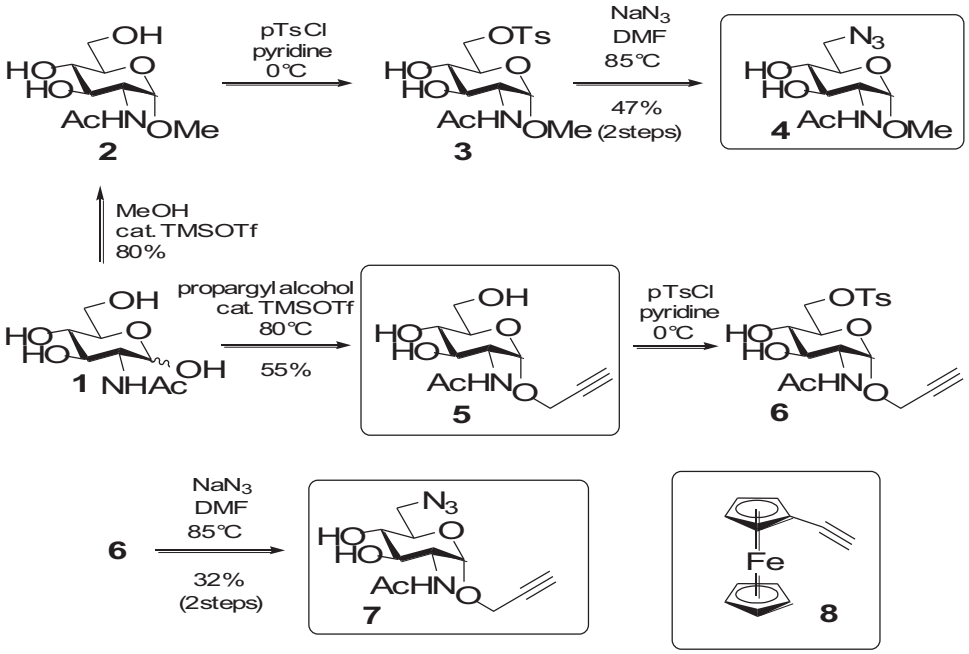
Compound **4** was synthesised according to literature procedures[27] starting from the Fischer glycoside **2** in two steps by tosylation followed by substitution reaction with NaN_3. Fischer glycosylation of GlcNAc **1** with propargylalcohol leads to compound **5**,[28] which again can be converted to the azide **7** by tosylation followed by substitution. Ethynylferrocene **10** has been used from the MCAT company stock. These protecting group free strategy on the one hand gave fast access to useable building blocks, and on the other hand resulted in anomeric alpha compounds (propargyl or methyl glycosides) and azides in 6-Position what limits the comparability to the natural biopolymers like chitin which shows a β–1,4 connection. Nonetheless we based our approach on these easy accessible compounds.

In a first approach we investigated the CuAAC reaction between **4** and **5** (Scheme 3). Both building blocks were used in equimolar amounts; the first reactions were performed once in a solvent mixture containing DCM/MeOH/H_2O in a 3:10:3 ratio and once in pure water, in both cases with 0.05 equiv. $CuSO_4$, 0.25 equiv. sodium ascorbate and 0.02 equiv. of TBTA. The reaction mixture was stirred at 60 °C for one hour and monitored by TLC, indicating complete reaction after 50 minutes. Then the solvents were evaporated and the residue was subjected to column chromatography. Although the desired compound **9** could be isolated in >90% yield (purity of the isolated compound > 90%) the work up of the very polar compound was very laborious and material consuming; thus we decided to perform an additional acetylation step after removal of the solvent in pyridine. As expected the peracetylated product **10** was much easier to purify and therefore we decided to go on with the additional acetylation step in further experiments.

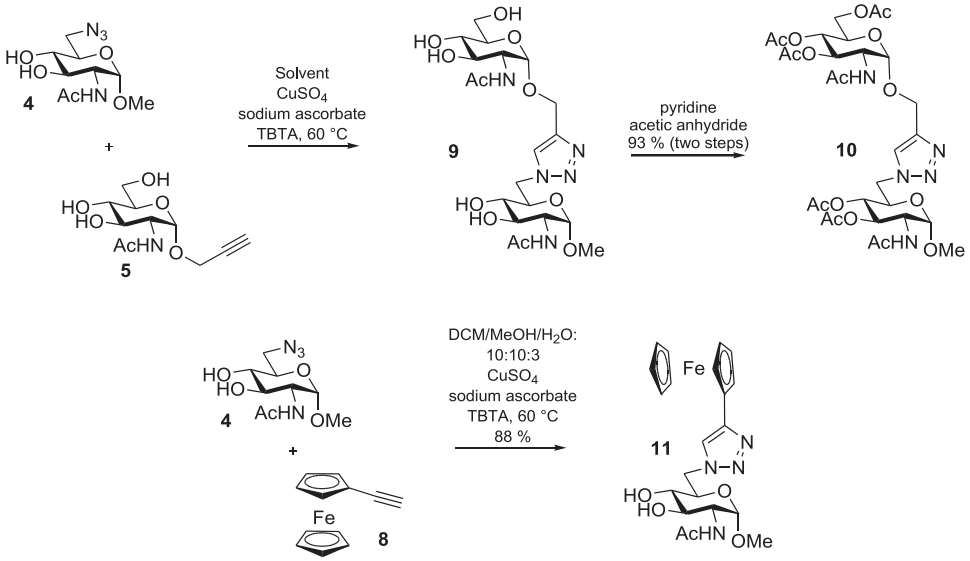
Next we tried the reaction of azide **4** with ferrocene **8** in order to investigate **8** as an analytical probe, which should simplify the work up without an additional acetylation step and increase the UV/Vis activity to extend the analytical options. Once more, the building blocks **4** and **8** were used in equimolar amounts with DCM/MeOH/H_2O in a 10:10:3 ratio as solvent system and the same $CuSO_4$, sodium ascorbate and TBTA amounts as before. The reaction mixture was stirred at 60 °C for one hour and monitored by TLC indicating complete reaction after 20 minutes. The mixture was concentrated and purified by column chromatography to give **11** in 88% yield. Though both reactions have been finally worked up after one hour TLC monitoring shows a faster product formation for the reaction of **8** with **4** compared to the reaction of **5** with **4** indicating a higher reactivity for compound **8** what is an important information for the later discussion on the formation of the oligomeric structures.

Compounds **10** and **11** have both been analysed by HPLC, ESI-MS, NMR and CHN analysis. Especially HPLC analysis was very important, since for further oligomer synthesis experiments this method should be used as main analytical tool.

Moreover, interesting information for the characterisation of the longer oligomeric compounds could be obtained from NMR spectroscopy of compounds **10** and **11**. The signals have been assigned with additional apostrophes for each sugar ring starting from the methylglycosidic moiety.



Scheme 2. Synthesis of the building blocks **4**, **5** and **7**; ethynylferrocene **8.**



Scheme 3. First synthetic approaches.

For compound **10** we found a significantly higher coupling constant for coupling between the H-6 protons (14.5 Hz) compared to the coupling between the H-6' protons (~12 Hz). In compound **11** we found the same tendency for the coupling between the H-6 protons (coupling > 14 Hz) confirming the influence of the triazole substituent in 6-position. Another interesting observation are the differences in the chemical shifts of the H-1/H-1' protons or the C-1/C-1' carbons respectively. In compound **10** the H-1 proton shows a chemical shift of 4.68 ppm, whereas the H-1' proton has a chemical shift of 4.90 ppm. In the carbon spectrum we can observe an inverse effect. Here the C-1 carbon comes at lower field with a chemical shift of 98.1 ppm whereas the C-1' has a chemical shift of 96.7 ppm. These observations can also be explained by the influences of the different aglycons (Me or CH_2triazole). These characteristic differences will be quite useful for the characterisation of the longer oligomeric structures.

Following this successful trials, we continued toward the synthesis of longer oligomeric structures. Therefore we investigated reactions of compounds **4**, **5** and **7** or **4**, **8** and **7**. Tables 1 and 2 show the results of the HPLC investigations; Fig. 1 depicts identified oligomeric structures. The data given indicate the ratio of the oligomeric structures (sum of all oligomeric peak integrals set to 100%) after work up. The ratios of the reaction partners have been varied from 1:1:1 to 1:8:1. From the 1:1:1 ratio reactions compounds **12** and **13** have been isolated by flash chromatography and analysed by HPLC, NMR and ESI-MS. The oligomeric structures **14**–**17** have been identified by mass spectrometry. All higher oligomeric structures given in Tables 1 and 2 have been assigned to their supposed HPLC peaks.

In both reaction systems it was necessary to adjust the HPLC gradient from the very broad 10–90% to the more flat gradients of 20–55% in the case of the acetylated oligosaccharides (Table 1) and 15–35% in the case of the ferrocenoyloligosaccharides (Table 2) to get a better interpretability. The adjusted gradient chromatograms showed a much better peak resolution and were used for the evaluation of the oligomeric structures (**10**–**17**, Figs. 2 and 3).

In the case of the acetylated oligosaccharides the amount of side products increases with the increasing amounts of **7**, whereas in the case of the ferrocenoyloligosaccharides the formation of side products seems quite low according to the HPLC chromatogramms. Different reasons can be discussed here. On the one hand all side products not connected to a ferrocene will pass quite fast through the RP-column of the HPLC leading to an increasing peak response below the retention time of one minute, what actually can be observed for the reactions with increasing amounts of **7**. On the other hand the higher reactivity of the ferrocene **8** compared to the pure propargyl glycoside **5** could lead to higher amounts of starting Fe-monomers consisting of a ferrocene **8** and a sugar **7** moiety which react further to higher oligomers, till they react with compound **4** to the final oligomeric structures. Additionally, the function of the ferrocene as analytical probe gives of course higher UV-detection responses for the ferrocenoyl oligosaccharides than the possible side products that have not been functionalised by the ferrocene.

In case of the acetylated oligosaccharides the formation of a comparable starting dimer consisting of a **5** and **7** is not supported, due to the more similar reactivity of the reaction partners. Therefore higher amounts of oligomeric structures involving different amounts of only one or two similar monomers are possible.

The last differing observation between the two reaction systems are the different oligomer ratios. For the acetylated oligosaccharides one can find a clear tendency leading to higher oligomeric structures as major products. Starting from dimer **10** as the main product in case of the 1:1:1 ratio reaction we already find the tetramer **14** in case of the 1:4:1, and pentamer **16** in the case of the 1:8:1 ratio reactions (Fig. 2). However, in case of the ferrocenoyl oligosaccharides this effect is less distinct. Starting from the Fe-monomer **11** as main product in the case of the 1:1:1 ratio reaction, even in the case of the 1:8:1 ratio reaction one only finds the Fe-dimer **13** as main product (Fig. 3). Despite that, the distribution between the oligomers in case of the ferrocenoyl oligosaccharides is much higher for the 1:8:1 ratio reaction compared to the acetylated ones. Also here the higher reactivity of the ferrocene **8** can be used as the best way to explain these effects.

In conclusion we have shown that it is possible to obtain linear oligomeric structures by using azido, alkyne and azido-alkyne building blocks in a one pot like CuAAC reaction system. Though we concentrated on the analysis of the oligomeric structures **10**–**17** (and higher ones) and have not identified all the possible side reactions and side products, the discussed results were satisfactory. Further steps toward larger polymeric structures shall be investigated in future. One should also consider the potential to use this method in an automatised fashion on solid support (Supporting Information available: HPLC chromatograms and NMR spectra).

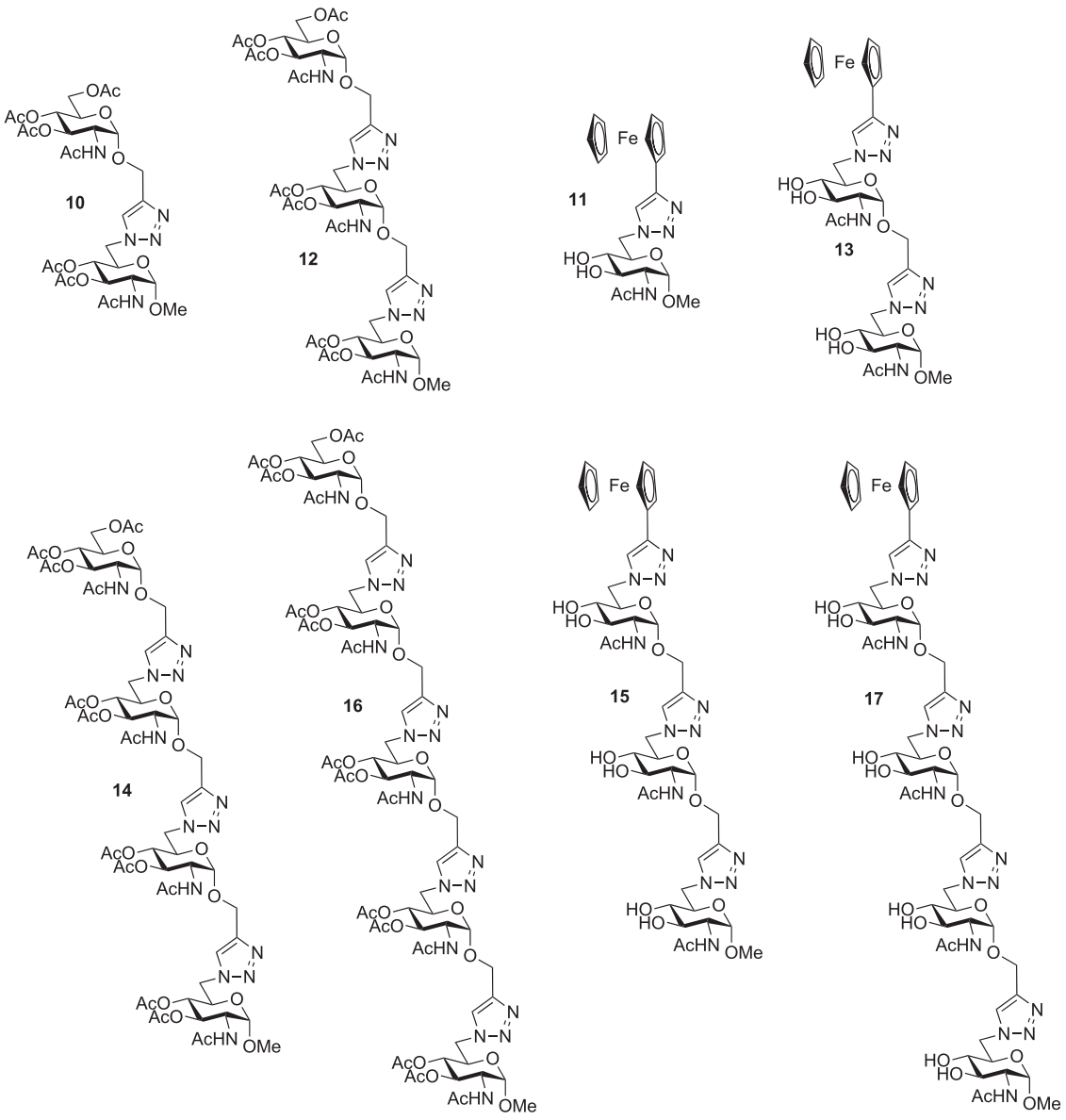


Fig. 1. With ESI-MS identified oligomeric structures.

2. Experimental section

2.1. General methods

TLC was carried out on Silica Gel 60 F254 (Merck, layer thickness 0.2 mm) with detection by UV light (254 nm) and/or by charring with 15% sulphuric acid in ethanol. Flash column chromatography (FC) was performed on M&N Silica Gel 60 (0.063–0.200 mm). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 400. Chemical shifts are reported in ppm relative to solvent signals ($CDCl_3$: δH = 7.26 ppm, δC = 77.0 ppm; DMSO-d6: δH = 2.49 ppm, δC = 39.7 ppm; CD_3OD: δH = 4.78 ppm, δC = 49.3 ppm). Signals were assigned by first-order analysis and assignments were supported, where feasible, by two-dimensional 1H, ^{1}H and ^{1}H, ^{13}C correlation

Table 1
Results HPLC analysis of the CuAAC reactions between **6**, **7** and **9**

Ratio **4**:**7**:**5**	Method	Dimer **10**	Trimer **12**	Tetramer **14**	Pentamer **16**	Hexamer	Heptamer	Octamer
1:1:1	10–90%	4.475 min/36.0%	4.772 min/30.6%	4.961 min/15.7%	5.094 min/11.0%	5.193 min/6.7%	–	–
1:1:1	20–55%	4.386 min/35.4%	5.830 min/22.8%	6.773 min/14.4%	7.442 min/13.4%	7.953 min/8.0%	8.352 min/6.0%	–
1:2:1	10–90%	4.438 min/27.2%	4.739 min/27.5%	4.929 min/20.3%	5.064 min/14.6%	5.164 min/10.4%	–	–
1:2:1	20–55%	4.473 min/23.6%	5.838 min/21.2%	6.753 min/17.0%	7.416 min/16.9%	7.926 min/11.3%	8.322 min/10.0%	–
1:4:1	10–90%	4.490 min/14.2%	4.773 min/25.9%	4.961 min/22.9%	5.093 min/15.5%	5.188 min/10.9%	5.259 min/10.6%	–
1:4:1	20–55%	4.466 min/13.7%	5.862 min/15.6%	6.776 min/21.8%	7.437 min/19.0%	7.951 min/14.0%	8.342 min/10.7%	8.662 min/5.2%
1:8:1	10–90%	–	–	–	–	–	–	–
1:8:1	20–55%	4.472 min/6.8%	5.838 min/13.1%	6.736 min/24.3%	7.394 min/28.4%	7.917 min/21.0%	8.388 min/6.4%	–

Table 2
HPLC results of the reactions between **4**, **8** and **7**

Ratio **4**:**7**:**8**	Method	Fe-mono **11**	Fe-dimer **13**	Fe-trimer **15**	Fe-Tetramer **17**	Fe-Pentamer (time/%)	Fe-Hexamer	Fe-Heptamer	Fe-Octamer
1:1:1	10–90%	4.838 min/52.6%	4.316 min/31.3%	4.061 min/10.5%	3.933 min/5.6%	–	–	–	
1:1:1	15–35%	9.877 min/51.2%	7.859 min/32.9%	6.693 min/8.4%	6.106 min/5.0%	5.727 min/2.4%	–	–	
1:2:1	10–90%	4.837 min/41.6%	4.316 min/35.3%	4.060 min/15.8%	3.928 min/8.2%	–	–	–	
1:2:1	15–35%	9.876 min/38.9%	7.854 min/35.0%	6.698 min/11.2%	6.113 min/7.5%	5.736 min/4.7%	5.482 min/2.7%	–	
1:4:1	10–90%	4.822 min/25.7%	4.305 min/29.9%	4.050 min/17.9%	3.914 min/12.2%	3.827 min/8.6%	3.760 min/5.6%	–	
1:4:1	15–35%	9.864 min/28.7%	7.850 min/34.3%	6.692 min/13.9%	6.104 min/10.9%	5.724 min/7.7%	5.464 min/5.0%	–	
1:8:1	10–90%	4.840 min/22.4%	4.320 min/28.8%	4.062 min/24.0%	3.927 min/12.4%	3.835 min/12.5%	–	–	
1:8:1	15–35%	9.874 min/21.6%	7.863 min/28.0%	6.706 min/13.6%	6.118 min/12.8%	5.739 min/8.7%	5.480 min/6.2%	5.296 min/4.9%	5.159 min/4.1%

spectroscopy. Coupling constants are reported in Hz. Carbohydrate ring protons have been assigned as described above.

Mass spectra were recorded on a Bruker Amazon SL spectrometer. Elemental analysis was performed on an elementar CHNS vario EL instrument.

RP-HPLC was performed on a Agilent 1100 series HPLC. Phenomenex Aqua columns (5u, C18, 128 A, 2 × 150 mm, flow 1 ml/min) were used. As eluent was used a gradient of water (eluent A) in acetonitrile (eluent B).

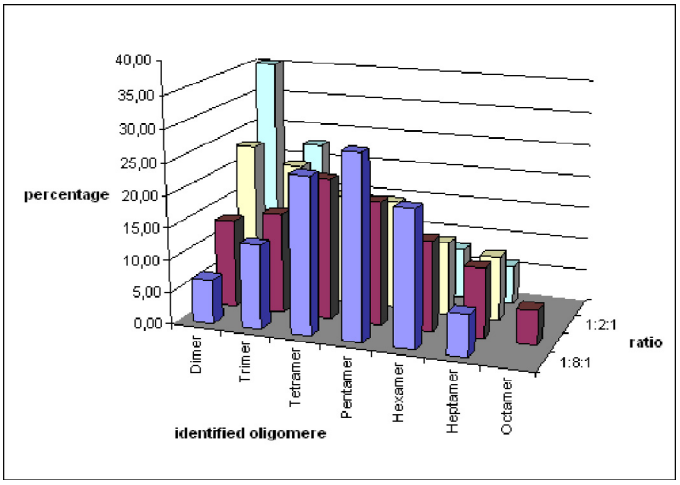


Fig. 2. Graphical illustration of the oligomeric structure ratios of the reactions between **4**, **5** and **7.**

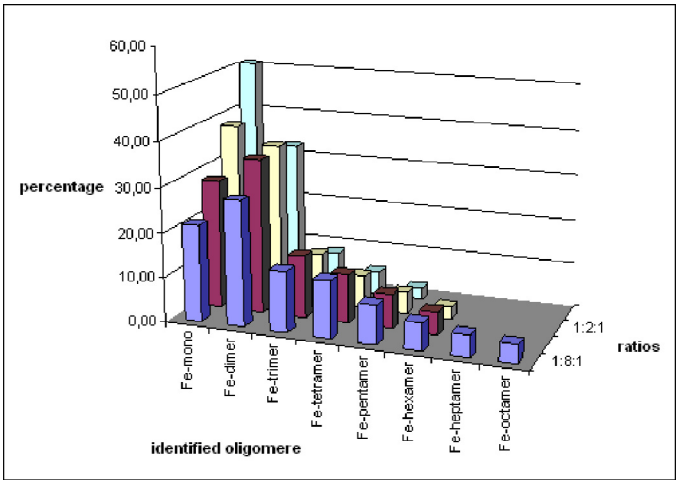


Fig. 3. Graphical illustration of the oligomeric structure ratios of the reactions between **4**, **8** and **7.**

Chemicals and reagents were purchased from Acros, Sigma-Aldrich or ABCR or have been used from the MCAT company stock (www.mcat.de) and were used without further purification.

2.2. Synthesis of propargyl-6-azido-6-deoxy-α-D-glucopyranoside 7

To a solution of propargylglycoside **5** (46.3 mmol, 12 g) in dry pyridine (80 mL), TsCl (53.2 mmol, 10.15 g) dissolved in dry pyridine (40 mL) was added drop wise over a period of 15 minutes at 0 °C. The mixture was stirred for 4 h at rt, concentrated and after addition of DCM (200 mL) was washed two times with 2N HCl (100 mL), saturated $NaHCO_3$ solution (100 mL) and brine (100 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was dissolved in DMF (250 mL) and NaN_3 (88 mmol, 5.7 g) was added. The mixture was stirred for 20 h at 85 °C. After addition of DCM (200 mL) and water (200 mL) the mixture was saturated with NaCl and extracted three times with THF (100 mL). The organic phase was dried (Na_2SO_4) and concentrated. Purification by column chromatography (DCM to DCM/MeOH 6:1) yielded **7** (4.6 g, 35%) as a white solid.

^{1}H-NMR (400.1 MHz, DMSO d6): δ = 7.82 (d, J = 9.4, 1H, NH), 5.31 (d, J = 5.9, 1H, 4-OH), 4,86 (d, J = 6.0, 1H, 3-OH), 4.84 (d, J = 3.6, 1H, H-1), 4.22 (dq, J = 16, 2.4, 2H, CH_2), 3.72 (ddd, J = 11.5, 8.3, 3.4, 1H, H-2), 3.58 (ddd, J = 9.3, 5.9, 2.2, 1H, H-5), 3.53–3.39 (m, 3H, H-3, H-6a, H-6b), 3.16 (d't', J = 9.5, 6.0, 1H, H-4), 1.83 (s, 3H, C(O)CH_3);

^{13}C-NMR (100.6 MHz, $CDCl_3$): δ = 169.5 (C(O)CH_3), 95.7 (C-1), 79.4 and 77.5 (alkyne Cs), 71.6 (C-3), 71.4 (C-4), 70.1 (C-5), 54.1 (CH_2), 53.4 (C-2), 51.0 (C-6), 22.5 (CH_3);

(ESI-MS): *m/z* $[M+H]^+$: 285.14;

Anal. Calcd for $C_{11}H_{16}N_4O_5$: C, 46.48; H, 5.67; N, 19.71; Found: C, 46.73; H, 5.76; N, 19.31;

2.3. General procedure for the synthesis of linear triazole linked pseudo oligosaccharides

Azido sugar compound **4** (0.385 mmol), propargyl glycoside **5** (0.385 mmol) and azidopropargyl sugar **7** (different amounts, see Table 1) were dissolved in H_2O (10 mL). $CuSO_4$ (0.019 mmol), sodium ascorbate (0.096 mmol) and TBTA (7 µmol) were added. The mixture was stirred at 60 °C for one hour, evaporated and pyridine (5 mL) and acetic acid anhydride (5 mL) were added to the residue. The mixture was stirred overnight resulting in acetylation. Then 2N HCl (50 mL) was added and the mixture was extracted three times with DCM (3 × 40 mL). The combined organic layers were washed with a sat. $NaHCO_3$ solution and brine were dried (Na_2SO_4) and the solvent was evaporated. Purification by flash chromatography (EtOAc to EtOAc/MeOH 10:1) yielded the different fractions of oligomers and mixtures (see Table 1); some reactions were analysed only by HPLC and mass spectra.

2.3.1. Dimer 10

Retention time (RP-HPLC, 10–90% B in A in 12 min): 4.49 min; retention time (RP-HPLC, 20–55% B in A in 15 min): 4.47 min; **^{1}H-NMR** (400.1 MHz, $CDCl_3$): δ = 7.66 (s, 1 H, triazole-H), 5.93 (d, *J* = 9.4, 1H, NH), 5.69 (d, *J* = 9.5, 1H, NH), 5.30 - 5.10 (m, 3 H, H-3, H-3', H-4'), 4.91 ('t', *J* = 9.2, 1H, H-4), 4.90 (d, *J* = 3.2, 1H, H-1'), 4.85 (m, 1H, CH_2), 4.68 (d, *J* = 3.5, 1H, H-1), 4.67 (m, 1H, CH_2), 4.61 (dd, *J* = 14.5, 2.7, 1H, H-6a), 4.38 (dd, *J* = 14.5, 8.5, 1H, H-6b), 4.36-4,27 (m, 2H, H-2, H-2'), 4.24 (dd, *J* = 12.1, 4.4, 1 H, H-6a'), 4.16 (ddd, *J* = 10.1, 8.4, 2.5, 1H, H-5), 4.10 (dd, *J* = 12.3, 2.5, 1H, H-6b'), 4.03 (ddd, *J* = 9.5, 4.2. 2.4, 1H, H-5'), 3.14 (s, 3H, OCH_3), 2.10 (s, 3H, $C(O)CH_3$), 2.09 (s, 3H, $C(O)CH_3$), 2.08 (s, 3H, $C(O)CH_3$), 2.01 (s, 3H, $C(O)CH_3$), 1.99 (s, 3H, $C(O)CH_3$), 1.94 (s, 3H, $C(O)CH_3$), 1.92 (s, 3H, $C(O)CH_3$);

^{13}C-NMR (100.6 MHz, $CDCl_3$): δ = 171.2 ($C(O)CH_3$), 171.1 ($C(O)CH_3$), 170.7 ($C(O)CH_3$), 170.2 ($C(O)CH_3$), 170.0 ($C(O)CH_3$), 169.9 ($C(O)CH_3$), 169.2 ($C(O)CH_3$), 143.3 (quaternary triazole C), 124.5 (triazole CH), 98.1 (C-1), 96.7 (C-1'), 71.2 and 70.9 (2C, C-3, C-3'), 69,6 (C-4), 68.2 (2C, C-5, C-5'), 68.1 (C-4'), 62.0 (C-6'), 60.9 (CH_2), 55.4 (OCH_3), 51.9 and 51.7 (2C, C-2, C-2'), 50.9 (C-6), 23.1 (CH_3), 23.0 (CH_3), 22.1 (CH_3), 20.7 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 20.5 (CH_3);

(ESI-MS): *m/z* $[M+H]^+$: 730.18;

Anal. Calcd for $C_{30}H_{43}N_5O16$: C, 49.38; H, 5.94; N, 9.60; Found: C, 49.19; H, 6.05; N, 9.24;

2.3.2. Trimer 12

Retention time (RP-HPLC, 10–90% B in A in 12 min): 4.79 min; retention time (RP-HPLC, 20–55% B in A in 15 min): 5.84 min; **^{1}H-NMR** (400.1 MHz, $CDCl_3$): δ = 7.73 (s, 1 H, triazole-H), 7.64 (s, 1 H, triazole-H), 6.33 (d, *J* = 9.4, 1H, NH), 5.99 (d, *J* = 9.4, 1H, NH), 5.75 (d, *J* = 9.5, 1H, NH), 5.37-5.19 (m, 3H, H-3, H-3', H-3''), 5.13 ('t', = 9.8, 1H, H-4''), 4.98 - 4.85 (m, 4H, H-1', H-1'', H-4, H-4'), 4.82 (m, 1H, CH_2), 4.74 (d, *J* = 3.7, 1H, H-1), 4.70 (m, 1H, CH_2), 4.62 (m, 2H, H-6a, H-6a'), 4.46-4,24 (m, 6H, H-2, H-2', H-2'', H-5, H-6b, H-6b'), 4.21 (dd, *J* = 12.4, 4.5, 1H, H-6a''), 4.15 (ddd, *J* = 10.3, 8.3, 2.4, 1H, H-5'), 4.09 (dd, *J* = 12.7, 2.4, 1H, H-6b''), 4.02 (ddd, *J* = 9.9, 4.1. 2.5, 1H, H-5''), 3.15 (s, 3H, OCH_3), 2.08 (s, 3H, $C(O)CH_3$), 2.08 (s, 3H, $C(O)CH_3$), 2.07 (s, 3H, $C(O)CH_3$), 2.04 (s, 3H, $C(O)CH_3$), 1.99 (s, 3H, $C(O)CH_3$), 1.98 (s, 3H, $C(O)CH_3$), 1.98 (s, 3H, $C(O)CH_3$), 1.96 (s, 3H, $C(O)CH_3$) 1.92 (s, 3H, $C(O)CH_3$) 1.91 (s, 3H, $C(O)CH_3$).

^{13}C-NMR (100.6 MHz, $CDCl_3$): δ = 171.3 ($C(O)CH_3$), 171.2 ($C(O)CH_3$), 171.1 ($C(O)CH_3$), 171.1 ($C(O)CH_3$), 170.8 ($C(O)CH_3$), 170.5 ($C(O)CH_3$), 170.4 ($C(O)CH_3$), 170.1 ($C(O)CH_3$), 169.7 ($C(O)CH_3$), 169.4 ($C(O)CH_3$), 143.5 (quaternary triazole C), 143.1 (quaternary triazole C), 124.6 (triazole CH), 124.5 (triazole CH), 98.1 (C-1), 96.7 (C-1'), 96.6 (C-1''), 71.1, 70.9 and 70.8 (3C, C-3, C-3', C-3''), 69,7 and 69.5 (2C, C-4, C-4'), 68.5, 68.1 and 68.0 (3C, C-5, C-5', C-5''), 68.3 (C-4''), 62.0 (C-6''), 61.0 (CH_2), 60.9 (CH_2), 55.5 (OCH_3), 51.9, 51.7 and 51.6 (2C, C-2, C-2', C-2''), 50.9 (C-6), 23.1 (CH_3), 23.0 (2C, 2 x CH_3), 20.8-20.5 (7C, 7 x CH_3).

(ESI-MS): *m/z* $[M+H]^+$: 1098.29.

2.3.3. Tetramer (mass identified in mixture) 14

Retention time (RP-HPLC, 10–90% B in A in 12 min): 4.97 min; retention time (RP-HPLC, 20–55% B in A in 15 min): 6.78 min; (ESI-MS): *m/z* $[M+H]^+$: 1466.54.

2.3.4. Pentamer (mass identified in mixture) 16

Retention time (RP-HPLC, 10–90% B in A in 12 min): 5.09 min; retention time (RP-HPLC, 20–55% in 15 min): 7.43 min; (ESI-MS): *m/z* $[M+H]^+$: 1834.64.

2.3.5. Hexamer (supposed HPLC peak)

Retention time (RP-HPLC, 10–90% B in A in 12 min): 5.18 min; retention time (RP-HPLC, 20–55% B in A in 15 min): 7.93 min.

2.3.6. Heptamer (supposed HPLC peak)

Retention time (RP-HPLC, 10–90% B in A in 12 min): 5.26 min; retention time (RP-HPLC, 20–55% B in A in 15 min): 8.35 min.

2.3.7. Octamer (supposed HPLC peak)

Retention time (RP-HPLC, 20–55% B in A in 15 min): 8.66 min.

2.4. General procedure for the synthesis of linear triazole linked pseudo oligosaccharides with ferrocene as analytical probe

Azido sugar **4** (0.385 mmol), ethynylferrocene **8** (0.385 mmol) and azidopropargyl sugar **7** (different amounts, see Table 2) were dissolved in 10 mL of a 10:10:3 mixture of DCM, MeOH and H_2O. $CuSO_4$ (0.019 mmol), sodium ascorbate (0.096 mmol) and TBTA (7 µmol) were added. The mixture was stirred at 60 °C. After one hour the mixture was diluted with H_2O (50 mL) and then extracted three times with DCM (3 × 40 mL). The organic layer was washed with a sat. $NaHCO_3$ solution and brine were dried (Na_2SO_4) and the solvent was evaporated. Purification by flash chromatography (EtOAc to EtOAc/MeOH 10:1) yielded the different fractions of oligomeres and mixtures (see Table 2); some experiments were analysed only by HPLC and ESI-MS.

2.4.1. Fe-monomer 11

Retention time (RP-HPLC, 10–90% B in A in 12 min): 4.84 min; retention time (RP-HPLC, 15–35% B in A in 15 min): 9.87 min.

^{1}H-NMR (400.1 MHz, DMSO-d6): δ = 8.13 (s, 1 H, triazole-H), 7.75 (d, *J* = 8.2, 1H, NH), 5.48 (d, *J* = 5.8, 1H, 3-OH), 4.89 (d, *J* = 5.9, 1H, 4-OH), 4.75 (m, 1 H, H-6a), 4.74 (m, 2H, Cp-H), 4.55 (d, *J* = 3.4, 1 H, H-1), 4.41 (dd, *J* = 14.2, 9.1, 1H, H-6b), 4.29 (m, 2H, Cp-H), 4.00 (s, 5H, Cp-H), 3.72 (m, 2H, H-2, H-4), 3.48 (m, 1H, H-3), 3.11 (ddd, *J* = 14.4, 8.9, 5.8, 1H, H-5), 3.00 (s, 3 H, OCH_3), 1.82 (s, 3H, $C(O)CH_3$).

^{13}C-NMR (100.6 MHz, $CDCl_3$): δ = 169.4 ($C(O)CH_3$), 145.1 (quaternary triazole C), 121.4 (triazole CH), 97.9 (C-1), 72.2 (C-5), 70.6 (C-3), 70.5 (C-4), 69.1 (Cp-Cs), 68.1 (Cp-Cs), 66.2 (Cp-C), 55.5 (OCH_3), 54.0 (C-2), 50.9 (C-6), 22.5 (CH_3).

(ESI-MS): *m/z* $[M+H]^+$: 471.05.

2.4.2. Fe-dimer 13

Retention time (RP-HPLC, 10–90% B in A in 12 min): 4.30 min; retention time (RP-HPLC, 15–35% B in A in 15 min): 7.85 min.

^{3}C-NMR (100.6 MHz, $CDCl_3$): δ = 169.5 ($C(O)CH_3$), 169.4 ($C(O)CH_3$), 145.2 (quaternary triazole C), 142.9 (quaternary triazole C), 125.0 (triazole CH), 121.3 (triazole CH), 97.9 (C-1a), 95.7 (C-1b), 76.0-50.9 (carbohydrate ring Cs and Cp-Cs), 22.6 (CH_3), 22.5 (CH_3).

(ESI-MS): *m/z* $[M+H]^+$: 755.15.

2.4.3. Fe-trimer (mass identified in mixture) 15

Retention time (RP-HPLC, 10–90% B in A in 12 min): 4.06 min; **retention time (RP-HPLC, 15–35% B in A in 15 min): 6.69 min; (ESI-MS): *m/z*** $[M+H]^+$: 1039.38.

2.4.4. Fe-tetramer (mass identified in mixture) 17

Retention time (RP-HPLC, B in A 10–90% in 12 min): 3.92 min; **retention time (RP-HPLC, 15–35% B in A in 15 min): 6.10 min; (ESI-MS): *m/z*** $[M+H]^+$: 1323.45.

2.4.5. Fe-pentamer (supposed HPLC peak)

Retention time (RP-HPLC, 10–90% B in A in 12 min): 3.83 min; retention time (RP-HPLC, 15–35% in 15 min): 5.73 min.

2.4.6. Fe-hexamer (supposed HPLC peak)

Retention time (RP-HPLC, 10–90% B in A in 12 min): 3.76 min; retention time (RP-HPLC, 15–35% in 15 min): 5.48 min.

2.4.7. Fe-heptamer (supposed HPLC peak)
Retention time (RP-HPLC, 15–35% B in A in 15 min): 5.30 min.

2.4.8. Fe-octamer (supposed HPLC peak)
Retention time (RP-HPLC, 15–35% B in A in 15 min): 5.16 min.

Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.carres.2016.03.005.

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