High-Yielding Microwave-Assisted Synthesis of Triazole-Linked Glycodendrimers by Copper-Catalyzed [3+2] Cycloaddition

John A. F. Joosten,[a] Niels T. H. Tholen,[a] Fatna Ait El Maate,[a] Arwin J. Brouwer,[a] G. Wilma van Esse,[a] Dirk T. S. Rijkers,[a] Rob M. J. Liskamp,*[a] and Roland J. Pieters*[a]

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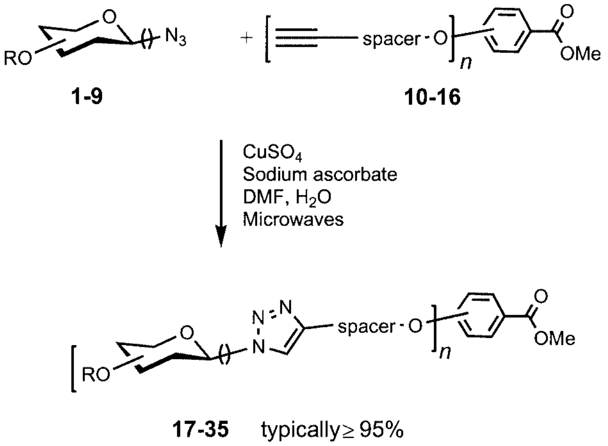
A facile and high-yielding synthesis of multivalent 1,4-disubstituted 1,2,3-triazole-linked glycodendrimers is described. Azido carbohydrates are linked by a Cu^I-catalyzed [3+2] cycloaddition reaction to dendritic acetylenes using microwave irradiation.

Introduction

Efficient carbohydrate conjugation to scaffold molecules is an important goal in bio-organic chemistry. Glycoconjugates[1] are playing increasingly important roles in the interference with protein–carbohydrate interactions,[2] the generation of carbohydrate vaccines,[3] functionalized glycochips and related surfaces,[4] and probes for (glyco)proteomics purposes.[5] Multivalent display of carbohydrates is more and more frequently used as a method to increase affinities[6] in various contexts such as the binding of bacteria,[7] bacterial toxins,[8] galectins[9] and other lectins.[10] In such systems the carbohydrates are mostly connected to the scaffold through amide[11] or thiourea linkages,[9a] by reductive amination[12] or thiol photoaddition onto allylic systems.[13] These methods vary in efficiency and their compatibility with functional groups, and all yield side-products, undesirable in couplings involving precious carbohydrate structures. With the recent improvement of the Huisgen cycloaddition[14] by copper catalysis,[15] this 1,4-disubstituted 1,2,3-triazole-forming “click”[16] reaction has all of the earmarks to become a premier conjugation method for protected and unprotected carbohydrates. Initial studies on carbohydrates using cycloaddition showed promise, yet thus far yields and procedures were not optimal.[17,18] Besides the synthetic promise, triazole moieties are also attractive connecting units since they are known to be relatively stable to metabolic degradation[19] and the triazole ring is also capable of hydrogen bonding, which can be favorable in the binding of biomolecular targets and for solubility.[20] We here report the straightforward synthesis of glycodendrimers using optimized microwave-assisted protocols for the synthesis of the azido carbohydrates and their subsequent Cu^I-catalyzed cycloaddition with acetylene-bearing dendrimers (Scheme 1). After optimization of the conditions, the reactions were highly selective and efficient.



Scheme 1. Copper-(I)-mediated synthesis of triazole glycodendrimers under microwave conditions.

Results and Discussion

A systematic study was undertaken with azido carbohydrates **1**–**9** of different character, functionalization pattern and reactivity (Scheme 2). These were subjected to the cycloaddition reaction with dendrimers **10**–**16** (Scheme 3) and 19 different triazole glycodendrimers were obtained in good yields. Furthermore, the use of unprotected carbohydrates was demonstrated as well as the straightforward incorporation of a fluorescent label, relevant for biological evaluation.

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[a] Department of Medicinal Chemistry, Utrecht Institute for Pharmaceutical Sciences, Utrecht University,
P. O. Box 80082, 3508 TB Utrecht, The Netherlands

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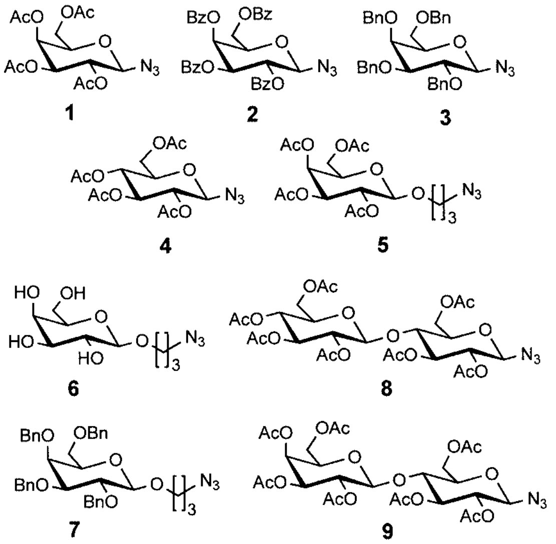
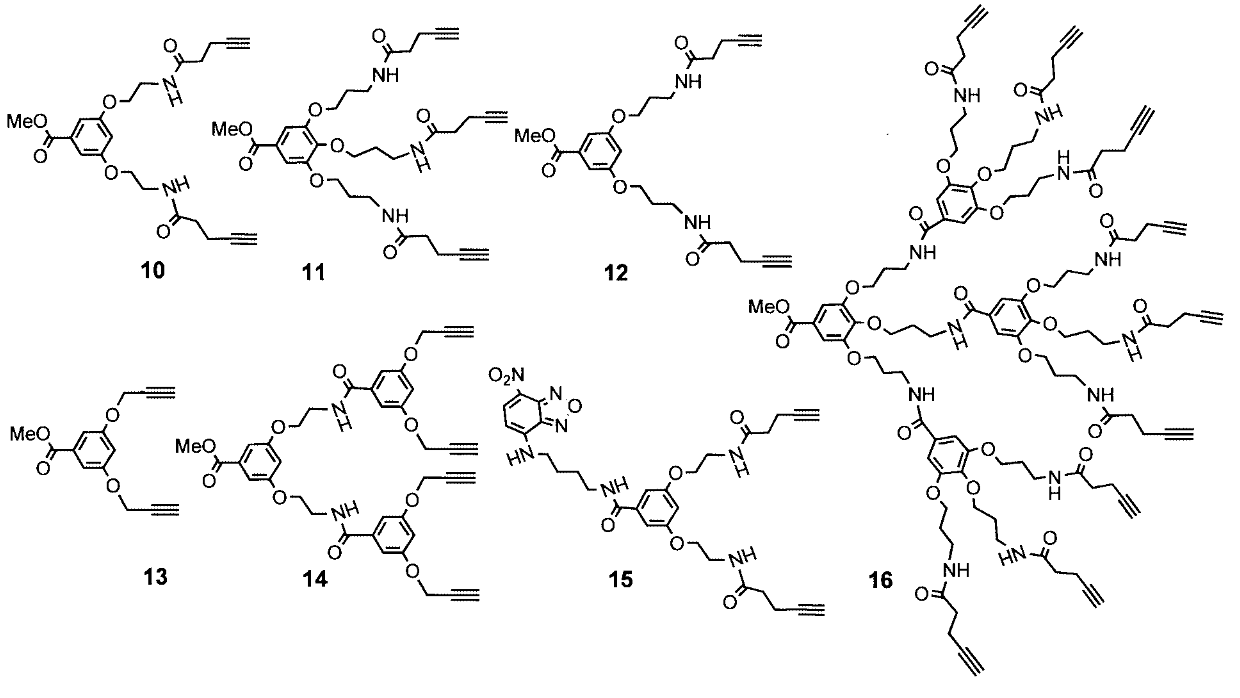


Scheme 2. Overview of the azido carbohydrates used in the cycloaddition reactions.

The carbohydrate derivatives included the monosaccharide galactose that was used without (**1**–**3**) or with (**5**–**7**) a spacer to the azido function. Its hydroxy protecting groups were also varied in order to evaluate this parameter. Besides galactose, the monosaccharide glucose (**4**), the disaccharides cellobiose (**8**) and lactose (**9**) were included. The anomeric glycosyl azides were prepared from their respective peracetylated counterparts by reaction with HBr in AcOH under microwave irradiation.[21] The resulting glycosyl bromides were treated with trimethylsilyl azide and tetrabutylammonium fluoride,[22] again using microwave irradiation, which gave the desired peracetylated β-azido sugars for the [3 + 2] cycloaddition reactions in high yields (≥ 78%). The procedure can be conveniently carried out on a gram scale. Interestingly, the microwave irradiation proved beneficial for both reactions as they were high-yielding and their rate was greatly improved. The spacer-containing azido carbohydrates **6** and **7** were prepared using a modified procedure described earlier by us,[7d] now employing microwave irradiation. The benzoylated (**2**) or benzylated compounds (**3** and **7**) were prepared from their acetylated precursors.

The azido carbohydrates were treated with di- and trivalent first generation dendrimers (**10**–**13**), one tetravalent second generation dendrimer (**14**), one nonavalent second generation dendrimer (**16**) as well as a divalent dendrimer with an NBD-fluorescent label (**15**). The dendritic main structures were derived from our previously reported systems,[23] and these amino acid dendrimers were readily synthesized in high yields. Their carbohydrate conjugates were shown to be effective multivalent scaffolds, increasing the binding potencies of their attached carbohydrates by several orders of magnitude.[7d,9a]

Initial efforts in the copper-catalyzed coupling of the azido carbohydrates to divalent alkyne-linked dendrimeric structures involved overnight stirring at room temperature. This only yielded a slow conversion to the monocoupled product. Microwave conditions[17b,24] gave faster reactions yielding only divalent products. Optimization of the reaction conditions using $CuSO_4$ and sodium ascorbate as the copper(I) source led to formation of the desired triazole glycodendrimers in high yields (Table 1). The use of an excess (1.5 equiv.) of the azido carbohydrate drove the reactions to completion, while the excess could be readily recovered by column chromatography. The relatively low product yields



Scheme 3. Multivalent alkyne-linked dendrimers used in the cycloaddition reactions.

in the reactions with unprotected glycosyl azide **6** or fluorescent-labeled **15** containing the first generation dendrimer reflects the product purification rather than the coupling efficiency. All coupling products were fully characterized by ^{1}H, ^{13}C, 2D-COSY NMR and MS.

Table 1. Microwave-assisted copper(I)-mediated synthesis of triazole glycodendrimers under microwave conditions.[a]

Entry	Azide	Dendrimer	Product	Valency	Yield[b]
1	**1**	**10**	**17**	2	93
2	**1**	**11**	**18**	3	95
3	**1**	**13**	**19**	2	97
4	**1**	**14**	**20**	4	98
5	**2**	**15**	**21**	2	68
6	**3**	**10**	**22**	2	95
7	**3**	**13**	**23**	2	97
8	**4**	**10**	**24**	2	97
9	**6**	**10**	**25**	2	73
10	**6**	**11**	**26**	3	80
11	**6**	**13**	**27**	2	94
12	**7**	**12**	**28**	2	95
13	**8**	**10**	**29**	2	95
14	**9**	**10**	**30**	2	92
15	**9**	**11**	**31**	3	91
16	**9**	**13**	**32**	4	98
17	**9**	**14**	**33**	4	91
18	**9**	**15**	**34**	2	86
19	**5**	**16**	**35**	9	97

[a] Reagents and conditions: alkyne-containing dendrimer (1 equiv.), azido carbohydrate (1.5 equiv. per alkyne), $CuSO_4$ (0.15 equiv. per alkyne), sodium ascorbate (0.3 equiv. per alkyne), DMF/H_2O, microwaves, 80 °C, 20 min. [b] Yield of isolated product.

Conclusions

In conclusion, the use of straightforward microwave-assisted procedures allowed the rapid preparation of azido carbohydrates. These were applied in a general microwave-enhanced regioselective Cu^I-catalyzed [3+2] cycloaddition reaction with different kinds of alkyne-bearing dendrimers, which led to triazole glycodendrimers, up to the nonavalent level, in high yields. Furthermore, two glycoconjugates containing a fluorescent label were prepared, important for the biological evaluation of these compounds.

Experimental Section

General Remarks: Reactions were carried out in a dedicated microwave oven, i.e. the Biotage Initiator. The microwave power was limited by the temperature control once the desired temperature was reached. Sealed vessels of 2 mL and 20 mL were used.

Synthesis of Anomeric Glycosyl Azides. Typical Procedure (Cellobiose Example): A solution of peracetylated cellobiose (500 mg), HBr (5 equiv.) and acetic anhydride (4 equiv.) in dry CH_2Cl_2 (10 mL) was exposed to microwave irradiation under sealed-vessel conditions at 80 °C for 20 min, after which it was concentrated. The residue was dissolved in dry THF (10 mL) and $TMSN_3$ (5 equiv.), and TBAF (5 equiv.) were added. The mixture was exposed to microwave irradiation at 80 °C for 15 min, then concentrated and purified on a silica column. Elution with toluene/EtOAc (30:1–5:1), gave **8** in 99% yield. Yield for the other anomeric glycosyl azides: **1** (78%), **4** (91%) and **9** (96%).

Copper-Catalyzed [2+3] Cycloadditions. General Procedure: The dendrimer (30–100 µmol) and the azido carbohydrate (1.5 equiv. per alkyne group), $CuSO_4$ (30 mol-%), and sodium ascorbate (60 mol-%), were dissolved in DMF (1–1.5 mL) containing several drops of H_2O. The solution was exposed to microwave irradiation at 80 °C for 20 min, then concentrated and purified on a silica column eluting first with CH_2Cl_2/EtOAc (6:1) to recover the excess of starting azido carbohydrate followed by elution with CH_2Cl_2/MeOH (6:1) to obtain the triazole-linked glycodendrimer in the indicated yields. For mass and NMR spectra of the produced compounds see Supporting Information.

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