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Sweet switches: azobenzene glycoconjugates synthesized by click chemistry†

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Azobenzene glycoconjugates can be switched between two isomeric states, *E* and *Z*, to change the spatial orientation of the conjugated carbohydrate ligands. Mono-, di- and trivalent azobenzene glycoconjugates were synthesized using click chemistry and their photochromic properties determined. Multivalency effects were observed in photoisomerisation.

Molecular recognition processes in biology are governed by the constitution and configuration of the interaction partners. Moreover, they are controlled and fine-tuned by conformational changes within the supramolecular environment of the biological system, such as a glycosylated cell surface.[1] In order to gain insight into conformational control of biological processes, photoswitchable bioprobes have become popular.[2] Typically, these are organic molecules that undergo a defined and reversible change between two sterically different states upon irradiation with light of an appropriate wavelength. Some of the best investigated molecular switches are azobenzenes that exist in a stable planar *E*-form and can be photoisomerised into a bent *Z*-isomer. Switching azobenzene conjugates between these two states effects a significant steric change within the molecule and alters the relative orientation of the conjugated bioprobes.

Azobenzene derivatives have been shown to be well-suited for biological applications[3] and their two isomeric forms (*E* and *Z*) can be independently addressed in biological testing. However, to date, only few examples have been published where carbohydrates have been combined with an azobenzene photoswitch.[4] It has been shown, though, that isomerisation of photoswitchable glycoconjugates changes their interactions with carbohydrate-recognizing proteins (lectins).[5,6]

As azobenzene glycoconjugates are important target molecules for glycobiological research it is necessary to improve their synthesis and facilitate their application in various contexts. The multiple assembly (clustering) of azobenzene glycoconjugates is of special relevance because it allows the investigation of conformational aspects of multivalency effects in carbohydrate recognition.[7] In the past small glycoclusters have proven to be very valuable tools for probing lectins.[8] Here, it has been our goal to utilize the concept of "click chemistry"[9] for the effective preparation of azobenzene glycosides and their di- and trivalent clustered analogues (*cf.* Fig. 1).

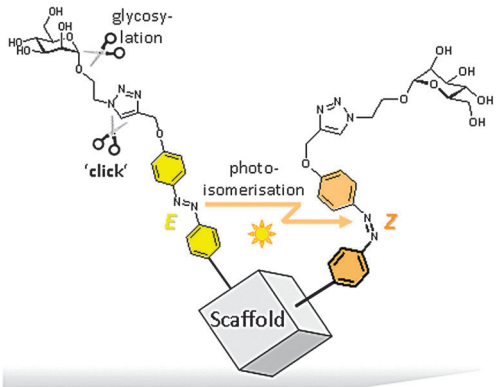

Fig. 1 The combination of glycosylation and click chemistry is a powerful method to create photoswitchable glycoconjugates of different valencies.

In order to employ click chemistry for the synthesis of photoswitchable glycoconjugates, the best approach is to use azobenzene propargyl ethers on the one hand and azido-functionalised carbohydrates on the other hand.

Firstly, ether-functionalised azobenzene derivatives are known for their favourable photochromic properties. Furthermore, the required azide-modified saccharides can be easily obtained in great variety. Owing to our long standing interest in mannose-specific lectins[10] we have selected mannoside **4** (Scheme 1) as the prototype azide for the current study.

The synthetic routes leading to mono- as well as bis-glycosylated azobenzene glycoconjugates were both started from 4,4′-dihydroxyazobenzene (**1**, Scheme 1) which was prepared according to the literature.[11] Regioselective conversion into the mono-propargyl ether **2** was achieved in 35% yield with recovery of ~40% starting material. The same reaction using 5 equivalents of both propargyl bromide and potassium carbonate leads to the bis-propargyl ether **3** as the only product in 91% yield.[12] Both azobenzene propargyl ethers, **2** and **3**, were subjected to 1,3-dipolar cycloaddition under "click conditions"[13] using the 2-azidoethyl mannoside **4**, which was easily obtained from D-mannose in three high-yield steps.[14] The desired triazole derivatives **5** and **7**, respectively, were obtained in high yield.

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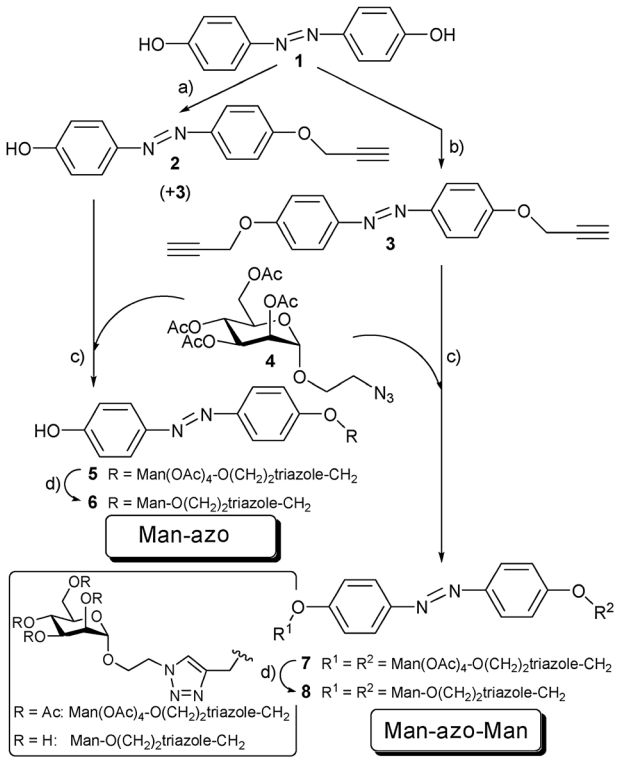
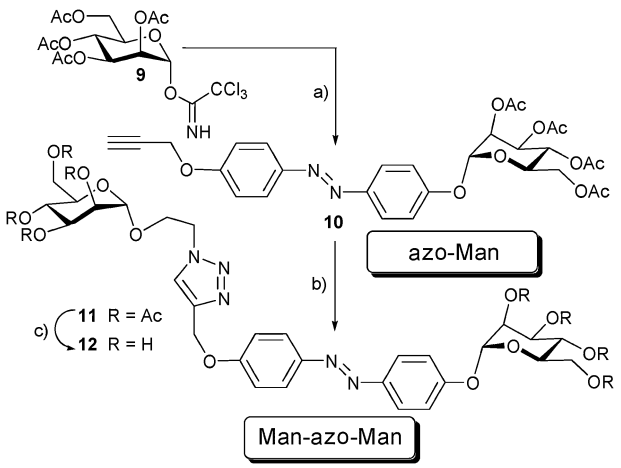


Scheme 1 Reaction conditions: (a) propargyl bromide (1.1 equiv.), K_2CO_3 (0.5 equiv.), 35% **2**, 7% **3**, 42% recovered **1**; (b) propargyl bromide (5 equiv.), K_2CO_3 (5 equiv.), 91%; (c) Cu/$CuSO_4$, *t*-BuOH/H_2O (1 : 1), rt, 30 h, 89% (**5**), 83% (**7**); (d) NaOMe, MeOH, rt, overnight: 92% (**6**), 96% (**8**).



Scheme 2 Reaction conditions: (a) **2**, BF_3–Et_2O, CH_2Cl_2, rt, 12 h, 88%; (b) **4**, Cu/$CuSO_4$, *t*-BuOH/THF/H_2O, 70 °C, 16 h, 82%; (c) NaOMe, MeOH, 93%.

De-*O*-acetylation under Zemplén conditions[15] afforded the unprotected mono- and bis-glycosylated azobenzene conjugates **6** (Man-azo) and **8** (Man-azo-Man) in nearly quantitative yields.

Alternatively, bis-glycosylated azobenzene derivatives can be prepared by combination of a glycosylation and a cycloaddition step (Scheme 2). Accordingly, Lewis acid-promoted mannosylation of **2** with the mannosyl donor **9**[16] gave the α-mannoside **10**, which upon reaction with the azide **4** under Cu(I) catalysis led to the triazole derivative **11** in 82% yield. Zemplén deprotection afforded the OH-free bis-glycosylated azobenzene glycoconjugate **12**.

The two prepared conjugates of the "Man-azo-Man" type, **8** and **12**, differ in that **8** is a symmetrical compound as reflected in the ^{1}H NMR spectrum, whereas **12** consists of two different fragments and displays two signal sets for the differently attached mannosidic moieties.

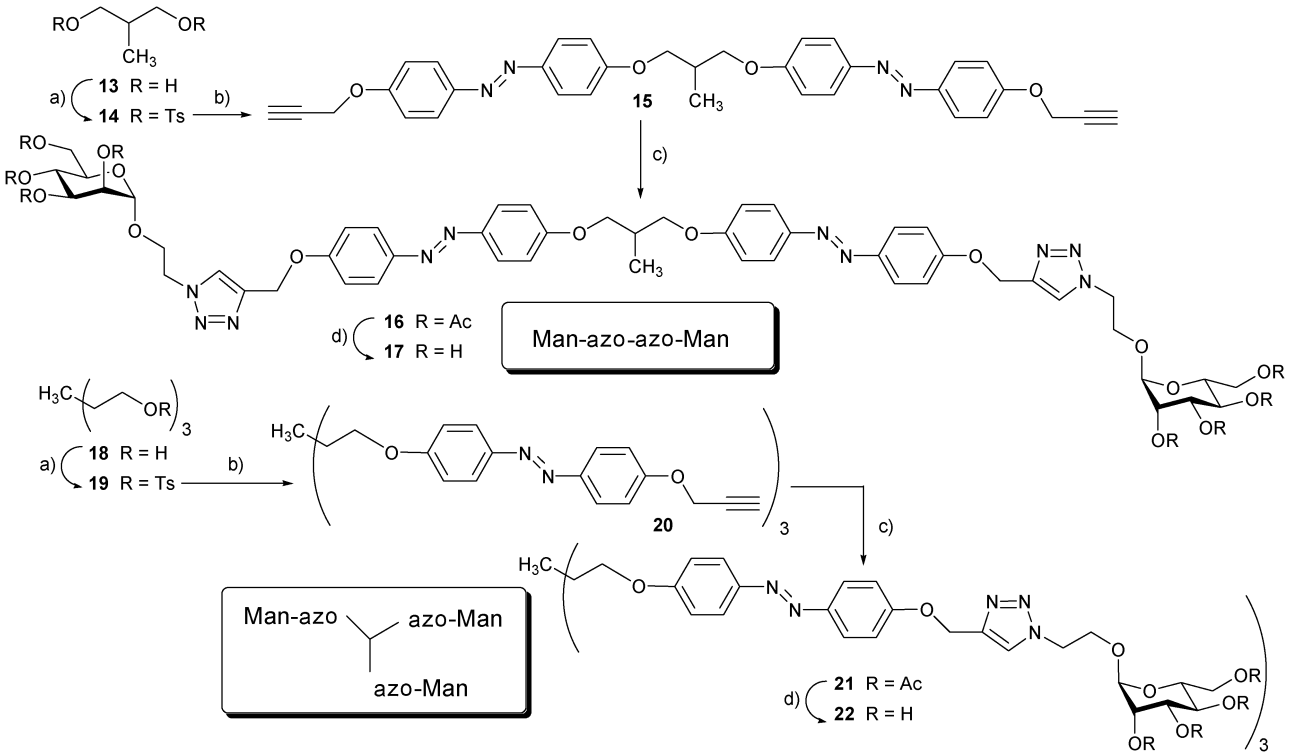
Next, clustering of glycoazobenzene moieties was investigated. As scaffolding core molecules, the diol **13** and the branched triol **14** were employed (Scheme 3). Our first attempt to use Mitsunobu conditions for the alkylation of these alcohols with the phenol **2** failed unexpectedly. Therefore, hydroxyl groups were activated by tosylation to subsequently undergo a classical nucleophilic substitution reaction. Reaction of the tosylates **14**[17] and **19**[18] using the azobenzene derivative **2** led to the desired di- and trivalent azobenzene alkynes **15** and **20**, respectively. They formed the starting materials for the subsequent click reaction with the azide **4** using a mixture of $CuSO_4$ and copper powder, leading to the triazole-linked glycoconjugates **16** and **21** in good yields. Again Zemplén deprotection occurred without any problem, furnishing the unprotected symmetric di- and trivalent azobenzene mannoside clusters **17** (Man-azo-azo-Man) and **22** ([Man-azo]$_3$).

The photochromic properties of mono-, di- and trivalent azobenzene glycoconjugates were tested next. In the ground state (GS) all azobenzene glycoconjugates, **6**, **8**, **12**, **17**, and **22**, existed in their *E*-configuration almost exclusively (Table 1).

Irradiation in DMSO solution for 30 min at 365 nm led to the respective photostationary states (PSS) with varying *E* : *Z* ratios, as determined by integration of the ^{1}H NMR signals. The PSS *E* : *Z* ratios of the monovalent bis-glycosylated azobenzene glycoconjugates **8** and **12** are excellent (3 : 97 and 1 : 99, respectively) with long half lives allowing independent biological evaluation of both isomeric forms. For the monoglycosylated azobenzene alcohol **6** on the other hand, the PSS could not be determined by UV-VIS spectroscopy. This is presumably due to the extremely fast kinetics of thermal relaxation of the *Z* isomer, as has been described earlier for *p*-hydroxy-substituted azobenzene derivatives.[19] The electron-donating effect of the OH-substituent lowers the barrier for thermal back isomerisation (Fig. 2) leading to relaxation times in the ms range. This feature makes such sweet switches interesting candidates for applications, where fast information processing is required.

Interestingly, photoisomerisation of the di- and trivalent azobenzenes was less effective than that in the case of **8** and **12**, leading to *E* : *Z* ratios in the PSS of 33 : 67 and 63 : 37, respectively. These ratios are averaged over an equilibrium mixture of *EE*, *EZ* and *ZZ* isomers for **17** having two azobenzene branches and a mixture of *EEE*, *EEZ*, *EZZ*, and *ZZZ* isoforms for **22** having three azobenzene branches (*cf.* ^{1}H NMR spectra in the ESI†).

The photoswitching in this case is most likely influenced by electronic effects including intramolecular quenching processes such as excitonic coupling, which was also shown for azobenzene SAMs.[20] The di- and trivalent azobenzene clusters may easily adopt a conformation in which the azobenzene branches are stacked, leading to decay of the excited state before isomerisation takes place. In addition, nonradiative decay could be responsible for less effective photoisomerisation.



Scheme 3 Reaction conditions: (a) tosyl chloride, pyridine, 83% (**14**), quant. (**19**); (b) **2**, K_2CO_3, DMF, 100 °C, 16 h, 74% (**15**), 65% (**20**); (c) **4**, $Cu/CuSO_4{\cdot}H_2O$, *t*-BuOH/THF/H_2O (1 : 1 : 1), 70 °C, 48 h, 81% (**16**), 79% (**21**); (d) NaOMe, MeOH, rt, overnight, 91% (**17**), quant. (**22**).

Table 1 Photochromic properties of azobenzene glycoconjugates

Azobenzene glycoconjugate		$E : Z^a$ GS	$E : Z^a$ PSS	$\tau_{1/2}$ (h)
6	Man-azo	99 : 1	—	—
8	Man-azo-Man	98 : 2	3 : 97	14
12	Man-azo-Man	99 : 1	1 : 99	21.3
17	Man-$(azo)_2$-Man	99 : 1	33 : 67[b]	15.5
22	$(Man\text{-}azo)_3$	99 : 1	63 : 37[b]	16

[a] *E* : *Z* ratios were determined by ^{1}H-NMR spectroscopy (10 mg in 0.5–0.6 mL). [b] Averaged over all branches. GS: ground state; PSS: photostationary state; $\tau_{1/2}$: half life, determined by UV-VIS spectroscopy (*cf.* ESI).

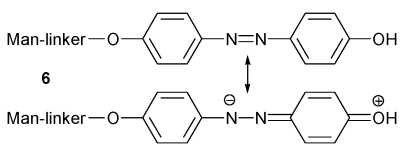


Fig. 2 Two resonance structures of **6** explaining fast $Z \rightarrow E$ relaxation.

The detailed kinetics of photoisomerisation of azobenzene clusters **17** and **22** will be determined in due course.

In conclusion, mono- and oligovalent azobenzene glycoconjugates with exciting photochromic properties have been made available employing click chemistry. Photoisomerisation of the di- and trivalent azobenzene clusters **17** and **22** is influenced by intramolecular effects leading to a mixture of isoforms. Multivalent glycoazobenzenes will be further investigated in advanced NMR studies and utilised for fabrication of photoswitchable multivalent glycoassemblies (arrays) for biological testing.

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