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Joining Hydroxyazobenzene and Mannose under Mitsunobu Conditions

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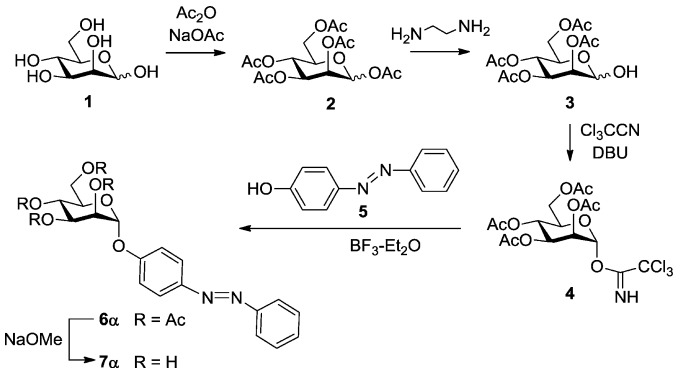
Abstract: The Mitsunobu reaction has been revisited to join hydroxyazobenzene and mannose derivatives to supply photoswitchable glycoconjugates. These are suited to modulating and controlling carbohydrate-lectin interactions, as well as to switching bacterial adhesion to surfaces. Employing hydroxyazobenzene in a Mitsunobu protocol, free mannose led to a mixture of azobenzene pyranosides and furanosides. Protected reducing mannose derivatives can give good yields of azobenzene β-D-mannopyranoside, and unprotected alkyl α-D-mannosides can be converted to 6-*O*-azobenzene-modified glycosides in a single step. Thus, valuable "sweet switches" can be obtained under Mitsunobu conditions, requiring a minimum (or no) protecting group chemistry.

Keywords: azobenzene glycosides · carbohydrates · glycoconjugates · Mitsunobu reaction · photoswitchable glycoconjugates

Azobenzene-functionalized carbohydrates have recently gained interest as photosensitive glycoconjugates. This is because photoisomerization of the azobenzene N=N double bond can effect a defined orientational change of the conjugated carbohydrate ligands, and thus, carbohydrate recognition can be modulated or, respectively, controlled. Back in the 1990s, Willner showed that a thiophenfulgide-based molecular switch can indeed be used to influence carbohydrate-lectin interactions.[1] Some time later, the group of Jayaraman synthesized amide-linked azobenzene glycoconjugates to photoswitch the interaction between α-D-mannosides and Concanavalin A.[2] Since 2012, our group has contributed to the synthesis and investigation of azobenzene-based "sweet switches" to study photoswitching of cellular adhesion.[3] In fact, we demonstrated very recently that photoisomerization of azobenzene α-D-mannoside ligands, ligated to a surface, allows us to reversibly switch the carbohydrate-specific adhesion of live *Escherichia coli* bacteria to such glycoarrays.[4]



Scheme 1. A five-step synthesis furnishes the azobenzene mannopyranoside **7α** in typically ~60% yield from mannose.[5]

As we will extend our studies on the specific photocontrol of cell adhesion to glycosylated surfaces, we are simultaneously concerned with the chemical synthesis of the required azobenzene glycosides. In Scheme 1, the typical synthesis of an azobenzene glycoside, the α-D-mannoside **7α**, is depicted, comprising five steps, starting from mannose.[5] When more complex azobenzene glycosides are targeted, even more protecting group chemistry, and thus, additional reaction steps are required.[6] Thus, we put further emphasis on making azobenzene glycosides, without the need for protecting group chemistry. This led us to the investigation of the Mitsunobu reaction,[7] in which an alcohol is substituted by an acid/nucleophile component, mediated by the redox system triarylphosphine/dialkyl azodicarboxylate.

A review of the literature shows that the Mitsunobu reaction has been employed regularly in carbohydrate chemistry, however, only occasionally in the synthesis of glycosides. Phenol was especially successfully reacted in Mitsunobu glycosylation, leading, for example, to phenyl α-D-mannoside in one step, and with yields greater than 80%.[8] A more complex phenol was employed *en route* to hygromycin A.[9] In this case, however, an otherwise

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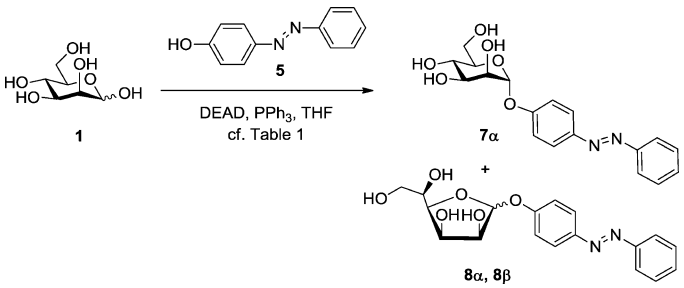
protected 1-OH-free carbohydrate had to be used, leading to an anomeric mixture of arabinosides.

The present study aims at testing *p*-hydroxyazobenzene (*p*-hydroxyazophenol, **5**) as the nucleophile in a Mitsunobu reaction[10] to explore an alternative means of access to azobenzene glycoconjugates. As D-mannose is of foremost importance in our biological studies, this sugar was used as the alcohol component. It was planned to vary the protecting group pattern of the employed mannose alcohol; however, in our first attempt, we took the most direct approach to azobenzene α-D-mannoside, reacting OH-free mannose and *p*-hydroxyazobenzene (Scheme 2).



Scheme 2. One-step synthesis of azobenzene mannosides based on a Mitsunobu protocol.

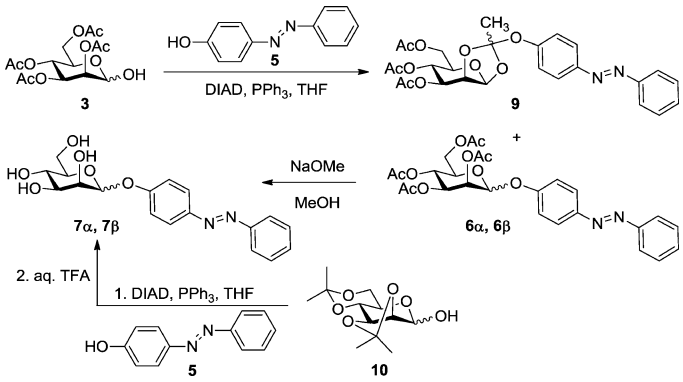
We were surprised that under Mitsunobu conditions, only the α-D-configured azobenzene mannopyranoside **7α** was isolated, whereas the β-mannopyranoside was not seen. On the other hand, both anomeric furanosides, **8α** and **8β**, were formed. Anomeric configurations of the obtained mannosides were determined according to C1-H1 hetero coupling constants and literature data.[11] Depending on the reaction conditions, varying pyranoside/furanoside ratios were isolated. As the Mitsunobu reaction is especially sensitive to the sequence of reagent addition, several modifications were attempted, of which two representative prototypes are listed in Table 1. When diethyl azodicarboxylate (DEAD) was added to a solution of **1**, **5**, and Ph_3P, pyranosidic and furanosidic products were formed in almost equal amounts. However, when a solution of *p*-phenylazophenol and DEAD was added to a mixture of **1** and Ph_3P, only minor amounts of furanosides were isolated. At the same time the anomeric ratios of formed furanosides **8α** and **8β** varied, whereas a β-configured mannopyranoside **7β** was not seen in any of our experiments. The scope of this straightforward synthesis of azobenzene glycosides should be further explored, as it provides a complementary option in the synthetic arsenal used for preparation of azobenzene glycoconjugates.

Table 1. Typical reaction conditions as applied to the reaction of Scheme 2. Reactions were performed in THF at room temperature.

Entry	Reaction mixture	Added reagents	Reaction time	Products[c] **7α**, **8** (α/β)[d]
1	**1**, **5**, PPh_3	DEAD[a]	1 d	15, 14 (20/80)
2	**1**, PPh_3	**5**, DEAD[b]	4 d	33, 5 (36/64)

[a] Added in THF over 10 min at 0 °C; [b] Added as a mixture in THF over 1 h; [c] Isolated yields in %; [d] Anomeric ratios according to ^1H NMR integration.

To test whether the yields of the Mitsunobu glycosylation with hydroxyazobenzene (**5**) can be improved when 1-OH-free, otherwise protected carbohydrates were employed as the alcohol components. The readily available reducing mannose derivative **3** was reacted under Mitsunobu conditions, this time adding diisopropyl azodicarboxylate (DIAD) to a mixture of the carbohydrate (**3**), the azobenzene **5**, and Ph_3P. This procedure led to an anomeric mixture of azobenzene mannopyranosides (**6α** and **β**), with minor amounts of the orthoester **9** formed besides (Scheme 3). The latter could be readily isolated



Scheme 3. Mitsunobu glycosidation of **5** employing the reducing mannose derivatives **3** and **10**.

after recrystallization, whereas the anomeric mixture of **6** could be purified best after de-*O*-acetylation, leading to **7** as an anomeric mixture with α:β approx. 1:1. Yields were around 60%. Notably, the NMR spectra of the obtained azobenzene mannosides (*cf.* supporting information) can be complicated by peaks of the *Z*-configured stereoisomers present in the ground photochemical state of the molecule.

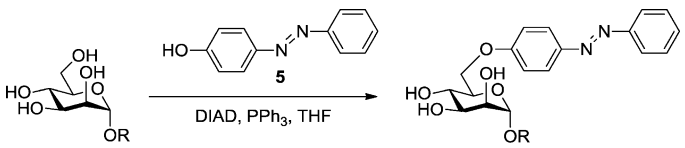
When the same procedure was applied to 2:3,4:6-diisopropylidene-protected mannose **10**, the β-mannopyranoside **7β** was formed almost exclusively in yields over 80%. Our results with diisopropylidene mannose **10** are related to literature reports, where **10** was reacted under similar Mitsunobu conditions with phenol[12] or with 4-methylumbelliferone,[13] mainly providing the respective β-D-mannosides in equally pleasant yields. Thus, the reducing mannose derivative **10** can be regarded as an excellent starting material for the Mitsunobu synthesis of β-D-mannosides. During the Mitsunobu reaction with **3**, on the other hand, S_N1 and S_N2 substitution processes are apparently competing, as was discussed previously.[14] We will employ this reaction to prepare the β-configured glycoarray analogues of those photoswitchable surfaces that

allowed photocontrol of bacterial adhesion.[4] Through the biological testing of a surface, analogous to that tested earlier, we will gain further insight into this novel biological phenomenon.

Finally, in a third part of this study, regioselective Mitsunobu etherification of allyl and propargyl α-D-mannosides **11**[15] and **12**[16] was attempted with hydroxyazobenzene (**5**). This approach is interesting, because the resulting azobenzene-modified glycosides have the potential of a photoswitchable hinge that can be installed onto any scaffold or surface, respectively, utilizing the allylic or propargylic aglycon function.

Regioselective Mitsunobu esterifications are rather common,[17] whereas regioselective etherifications with phenolic reaction partners are rare. A regioselective Mitsunobu 6-*O*-etherification of methylglucoside with phenol was reported with 30% yield.[18] Employing mannosides **11** and **12**, we were pleased to see that the Mitsunobu protocol with **5** led to the 6-*O*-monosubstituted azobenzene glycoconjugates **13** and **14** in ~60% yield (Scheme 4). Encouraged by these results, we will eventually submit additionally substituted azobenzene alcohols to the same reaction to achieve carbohydrate-scaffolded photosensitive molecular joints. Additionally, it is worth mentioning that the column chromatographic purification of the azobenzene-functionalized products is chromophore-supported.[19]

In conclusion, our study shows that *p*-phenylazophenol can be employed readily in Mitsunobu reactions to regioselectively obtain various photoswitchable glycoconjugates from free sugars (5×OH), and protected reducing sugars (1×OH, anomeric), as well as from glycosides (4×OH, 6-selective). Some interesting mechanistic aspects of our findings, as well as the photochromic properties of the synthesized azobenzene derivatives, will be investigated next and the scope of this reaction will be tested with substituted phenylazophenol derivatives. Yields and anomeric selectivity will be further optimized, employing, for example, ultrasound and trialkyl phosphines, such as Bu_3P.



11 R = allyl

12 R = propargyl

13 R = allyl

14 R = propargyl

Scheme 4. Regioselective Mitsunobu reaction of **5** with mannosides **11** and **12**.

Experimental

Mitsunobu reaction: General procedure employed for the conversion of 3, and of 10–12: The carbohydrate starting material (1.00 mmol), *p*-phenylazophenol (**5**, 1.10 mmol) and triphenyl phosphine (525 mg, 2.00 mmol) were dissolved in dry THF (15.0 mL) under a nitrogen atmosphere. The solution was cooled to 0 °C, and then a solution of diisopropyl azodicarboxylate (DIAD, 0.42 mL, 2.00 mmol) in dry THF (5.00 mL) was added dropwise over 30 min, and then the reaction mixture was stirred at rt until the TLC showed no further consumption of the carbohydrate starting material.

For NMR data of synthesized azobenzene glycoconjugates, see the supporting information.

***E*-*p*-(Phenylazo)phenyl α,β-D-mannopyranoside (7α, 7β) and 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-*E*-(*p*-(phenylazo)phenoxyethylidene)-β-D-mannopyranose (9) from 3:** According to the general procedure, the acetyl-protected carbohydrate **3** (348 mg, 1.00 mmol) was reacted at rt for 16 h. Then the mixture was co-evaporated with toluene *in vacuo* and the resulting crude product was recrystallized from CH_2Cl_2/MeOH (7:20) to give the orthoester **9** in the form of fine pale yellow crystals (47.6 mg, 0.09 mmol, 9%, mp: 198 °C, $[\alpha]_D^{21} = +28$ ($c = 0.08$, $CHCl_3$). The mother liquor was evaporated *in vacuo*, dissolved in dry methanol (20.0 mL) under a nitrogen atmosphere, and deprotected with freshly prepared 2 M sodium methoxide solution (200 μL). After neutralization with Amberlite IR 120 ion exchange resin, the crude product was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 99:1→9:1) to give an anomeric mixture (α/β = 52:48) of the title mannoside as a yellow solid (223 mg, 0.62 mmol, 62%; the product contains minor impurities, *cf.* NMR spectrum in the supporting information).

***E*-*p*-(Phenylazo)phenyl α,β-D-mannopyranoside (7α, 7β) from 10:** According to the general procedure, the isopropylidene-protected carbohydrate **10** (260 mg, 1.00 mmol) was reacted at rt for 3 d. Then water (5.00 mL) was added, followed by dropwise addition of trifluoroacetic acid (consecutively 2×1.70 mL) to effect deprotection. After the isopropylidene groups were completely removed, the mixture was co-evaporated with toluene *in vacuo* and the resulting crude product was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 99:1→9:1) to give an anomeric mixture (α/β = 5:95) of the title mannoside as a dark orange solid (298 mg, 0.83 mmol, 83%).

Allyl 6-*O*-*E*-[*p*-(phenylazo)phenyl] α-D-mannopyranoside (13): According to the general procedure, allyl α-D-mannopyranoside (**11**, 220 mg, 1.00 mmol) was reacted at rt for 5 d and subsequently evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/MeOH. 9:1) to give the title mannoside as a yellow syrup (253 mg, 0.63 mmol, 63%); R_f = 0.44 (CH_2Cl_2/MeOH, 9:1); $[\alpha]_D^{21} = +36$ ($c = 0.52$, MeOH).

Propargyl 6-*O*-*E*-[*p*-(phenylazo)phenyl] α-D-mannopyranoside (14): According to the general procedure, propargyl α-D-mannopyranoside (**12**, 218 mg, 1.00 mmol) was reacted at rt for 3 d and subsequently evaporated *in*

vacuo. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 99:1→9:1) to give the title mannoside as a yellow solid (217 mg, 0.55 mmol, 55%). R_f = 0.40 (CH_2Cl_2/MeOH, 9:1); mp: 138 °C; $[\alpha]_D^{21} = +99$ (c = 0.61, MeOH).

Acknowledgements

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