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Novel Efficient Routes to Indoxyl Glycosides for Monitoring Glycosidase Activities

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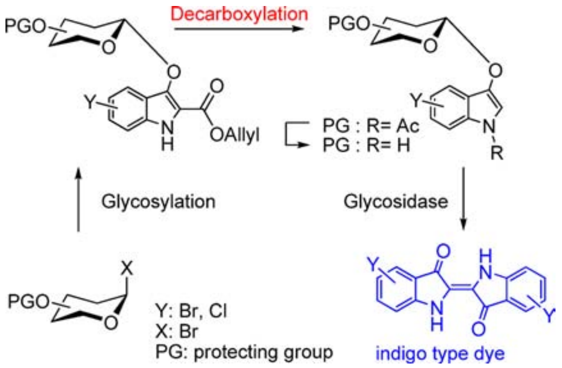
ABSTRACT



A new efficient synthesis for broad access to indoxyl glycosides was developed. Indoxylic acid allyl ester linked to a sugar structure served as the key intermediate in this route. Selective ester cleavage and mild decarboxylation led to the corresponding indoxyl glycosides in good yields. This synthesis was applied for preparation of indoxyl glycosides of fucose, sialic acid, and 6′-sialyl lactose.

Indoxyl glycosides are powerful tools for histochemical dectection of glycosidase activity.[1] Enzymatic hydrolysis of the glycosidic linkage releases free indoxyl which is rapidly oxidized to an indigo type dye (Figure 1). This method allows fast and easy *in vivo* screening without isolation or purification of enzymes as well as rapid tests of multiple biocatalysts at the same time, e.g. on microwells.

Unfortunately the synthesis of the corresponding glycosides proved to be difficult. Previously the most common synthetic pathway was by use of sodium hydroxide in acetone for glycosylation employing the respective indoxyl as an acceptor.[2−4]

Due to yield decreasing side reactions, such as dye formation and elimination, the isolation of the product is challenging. In particular glycosides containing a glucose unit or a glucose derivative in the reducing end only provided low yields so far. For example (*N*-acetyl-5-bromo-4-chloro-indol-3-yl)-2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside was obtained in 15% yield, and (*N*-acetyl-5-bromo-indol-3-yl)-2,3,2′,3′,4′-penta-*O*-acetyl-*β*-D-xylobioside, in 26% yield.[3,4] Obviously this protocol requires substantial improvements, especially for more complex and rare sugar structures.

As early as 1927, A. Robertson developed a synthesis for Indicane.[5] Indoxylic acid methyl ester was used as the acceptor, and after ester cleavage and decarboxylation, Indicane was obtained in approximately 50% yield.

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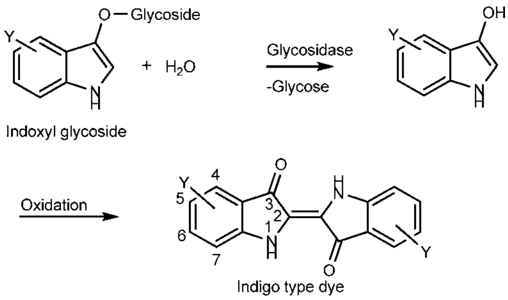


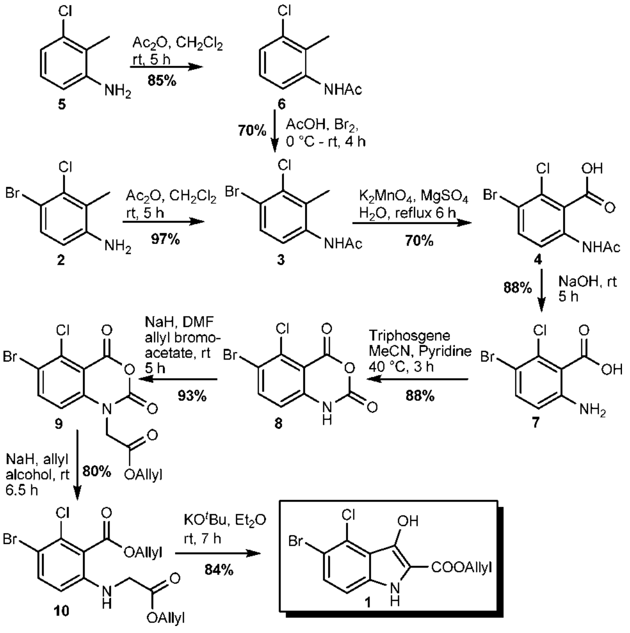
Figure 1. Monitoring glycosidase activity employing indoxyl glycosides.

Apparently this compares favorably with a yield of 15% for the above-mentioned indoxyl glucopyranoside. One potential problem in this approach seems to be the high temperature required (160 °C) for decarboxylation. Furthermore, the glycoside would need to be deprotected before decarboxylation. Both these steps are known to cause side reactions and decomposition, especially for sensitive structures such as di-, tri,- and oligosaccharides for which these conditions are not suitable. We here report a novel efficient synthetic route for the preparation of indoxyl glycosides, which was tested for monosaccharides as well as for more complex and sensitive sugar structures.

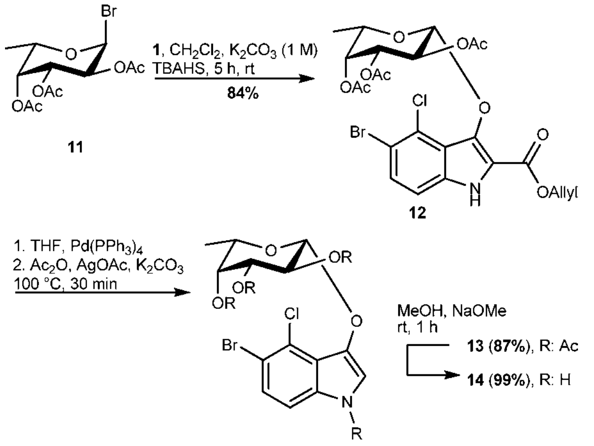
First glycosylation of the sugar donor with indoxylic acid allyl ester was carried out by phase transfer catalysis (PTC). The use of indoxylic acid allyl ester instead of indoxyl reduced the side reactions to a minimum and made selective cleavage of the allyl ester[6] possible. Subsequent mild silver mediated decarboxylation (90–100 °C) gave the protected X-glycosides in good overall yields (X: common abbreviation for 5-bromo-4-chloro-indoxyl). A modification of the recently published decarboxylation method could be favorably used in this step.[7] Instead of NMP or DMF, acetic anhydride as solvent with potassium carbonate in combination with silver acetate turned out to be the best conditions for high yields, as well as moderate temperatures (80–100 °C). Deacetylation under Zemplén conditions[8] and, if required ester hydrolysis, gave the unprotected compounds in good yields.

The indoxylic acid allyl ester **1** was prepared in seven steps in an overall yield of 33% (Scheme 1). 4-Bromo-3-chloro-2-methylaniline (**2**) was first acetylated (**3**, 97%) and then oxidized with KMnO$_4$ (**4**, 70%).[9] An alternative was to start from cheap 3-chloro-2-methylaniline (**5**), *N*-acetylate to obtain **6** (88%) and then treat this with bromine in acetic acid to give intermediate **3** in 70% yield.[10] 5-Bromo-6-chloro-anthranilic acid (**7**, 88%) was obtained after deprotection with aqueous sodium hydroxide solution.[11] Treatment with triphosgene/pyridine in MeCN[12] gave the isatoic anhydride (**8**, 88%) which was easily *N*-alkylated[13] using sodium hydride and allyl bromoacetate (**9**, 93%). Opening of the anhydride with allyl alcohol and a catalytic amount of sodium hydride gave 3-bromo-2-chloro-*N*-(methoxycarbonylallyl)-anthranilic acid allyl ester (**10**) in 80% yield.

Scheme 1. Synthesis of Indoxylic Acid Allyl Ester (**1**)



Scheme 2. Synthesis of Fucose Indoxyl Glycoside (X-Fuc, **14**)



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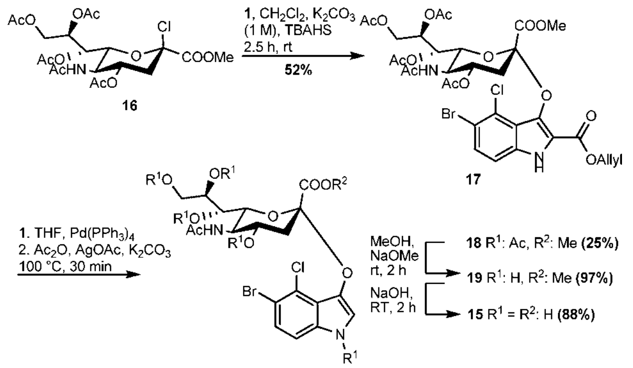
Finally 5-bromo-4-chloro-indoxylic acid allyl ester was obtained via Dieckmann condensation by use of KO^{t}Bu in Et$_2$O (**1**, 88%). Workup and purification of all steps was easy and carried out by precipitation/crystallization and aqueous workup. All reactions could be performed in scales up to 20 g and gave reproducibly high yields.

At first this route was tested with fucose (Scheme 2), a robust monosaccharide. Starting with the fucosyl bromide donor **11**, the first step was glycosylation with **1** by PTC which gave compound **12** (84%). Allyl ester deprotection[6] and subsequent decarboxylation proceeded well to give (*N*-acetyl-5-bromo-4-chloro-indol-3-yl)-2,3,4-tri-*O*-acetyl-*β*-D-fucopyranoside (**13**) in excellent yield (87%). Zemplén deacetylation[8] gave the free indoxyl glycoside **14** (X-Fuc) in 99% yield.

A corresponding synthetic route was also applied to the synthesis of sialic acid indoxyl glycoside (X-SA, **15**) (Scheme 3). Starting from the chloro donor (**16**), PTC glycosylation gave glycoside **17** in moderate yield (52%). By allyl ester deprotection[6] and subsequent decarboxylation, the sialic acid indoxyl glycoside **18** was obtained in 25% yield. Concomitant decompositions were the principal factors for the decreased yield. Zemplén deacetylation[8] (**19**, 97%) and ester hydrolysis gave 5-bromo-4-chloro-indol-3-yl-(5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulo-pyranosylonic acid) (**15**) in 88% yield. Even though the overall yield of 11% was slightly lower than reported in the literature, this new route is superior since it does not require purification by HPLC, as the intermediates could be crystallized.[14]

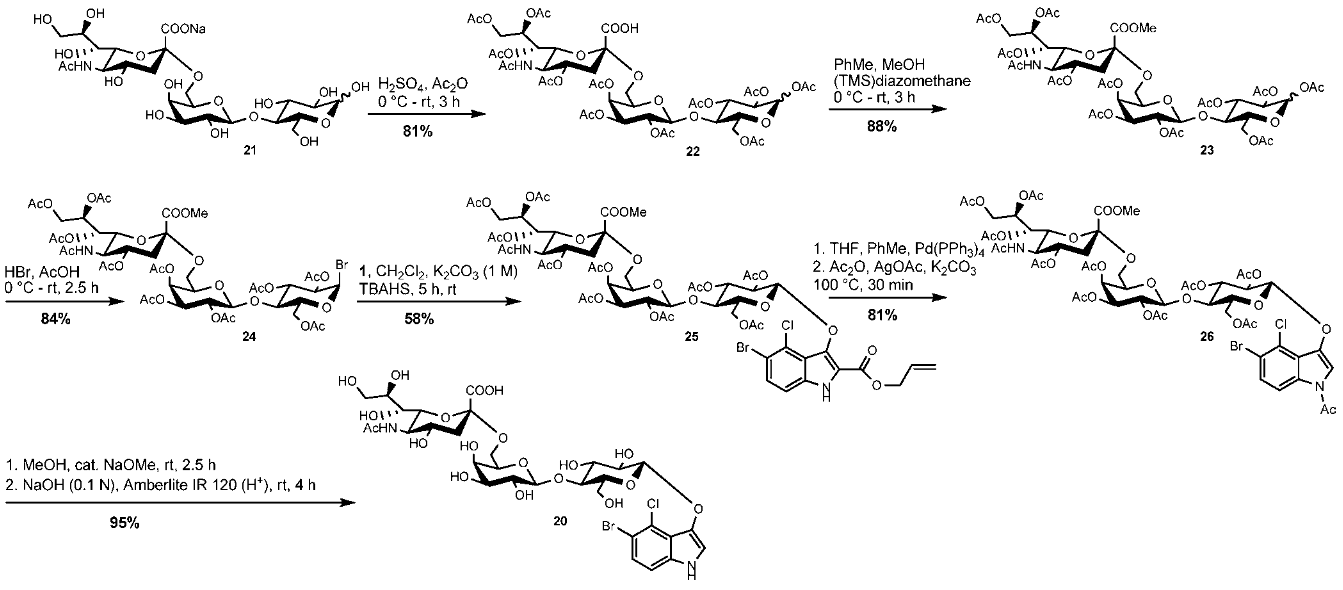
Scheme 3. Synthesis of Sialic Acid Indoxyl Glycoside (X-SA, **15**)



Finally this novel route was applied to the synthesis of 6′-sialyl lactose indoxyl glycoside (X-6′SL, **20**). This was realized in seven steps, starting from the unprotected sodium salt of 6′SL (**21**) (Scheme 4). The preparation of the donor started with peracetylation[15] of the unprotected saccharide (**22**, 81%). After esterification using TMS-diazomethane[16] the fully protected compound (**23**) was obtained in 88% yield. Careful treatment with HBr in AcOH gave the glycosyl bromide (**24**) in 84% yield. With rigid temperature control and a short reaction time, cleavage of the sialic acid was kept to a minimum. The PTC was carried out under common conditions, and compound **25** was obtained in 58% yield. Selective cleavage of the allyl ester by $Pd(PPh_3)_4$ and morpholine[6] followed by subsequent silver salt mediated decarboxylation gave the protected 6-′sialyl lactose indoxyl glycoside (**26**) in an excellent yield of 81%. Finally Zemplén deacetylation[8] and subsequent hydrolysis of the ester in an aqueous solution of sodium hydroxide gave (5-bromo-4-chloro-indol-3-yl)-(5-acetamido-3,5-dideoxy-α-D-galacto-D-glycero-2-nonulopyranosylonic acid)-(2−6)-(*β*-D-galactopyranosyl)-(1−4)-*β*-D-glucopyranoside (6′SL-X, **20**) in 95% yield.

In conclusion, a novel and advantageous synthetic access to indoxyl glycosides has been developed. The indoxylic acid acceptor was prepared in a scalable synthesis over seven

Scheme 4. Synthesis of 6′-Sialyl Lactose Indoxyl Glycoside (X-6′SL, **20**)



steps in 33% yield. Starting from peracetylated glycosyl halides, glycosylations to obtain protected indoxyl glycosides were performed by PTC. Selective allyl ester cleavage and silver salt mediated decarboxylation under mild conditions gave the protected indoxyl glycosides in moderate to excellent yields. Deprotection was carried out by Zemplén deacetylation,[8] and if required ester hydrolysis was employed with aqueous sodium hydroxide.

We are convinced that this approach will provide access to a variety of novel indoxyl glycoside compounds, as well as other indoxyl structures. Further indoxyl glycosides will be synthesized this way, with a focus on known structures for yield comparison, and this will be reported in due course. The synthesis of the indoxylic acid acceptors can be modified by using other starting materials or intermediates, having the requisite substitution pattern, and can be performed in larger scale.

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Supporting Information Available. Experimental procedures for preparation of all novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.