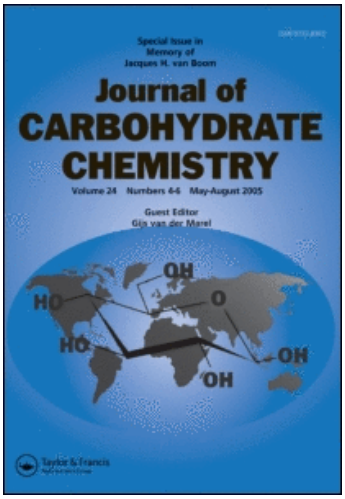


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# Synthesis and Evaluation of 2-Deoxy-2-amino-β-cellobiosides as Cellulase Inhibitors

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# Synthesis and Evaluation of 2-Deoxy-2-amino-$\beta$-cellobiosides as Cellulase Inhibitors

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The cellulase mixture of *Hypocrea jecorina* (formerly *Trichoderma reesei*) contains a variety of exo- and endoglucanases that belong to different structural families. As such, these enzymes form an interesting model system to study the enzyme-ligand interactions in glycoside hydrolases. The nucleophilic carboxylate of retaining $\beta$-glycosidases is believed to form a hydrogen bond with the 2-hydroxyl group of their substrate. Consequently, replacing this hydroxyl group with an amino group should result in a stronger electrostatic interaction and thus an increased affinity for the ligand. In this study, several modified cellobiosides were synthesized and evaluated as cellulase inhibitors. The introduction of an amino group was found to have an unpredictable effect on the inhibitory power of the ligands. However, the enzymes display a very high affinity for the corresponding 2-azido compounds, precursors in the synthetic route. The new ligand *m*-iodobenzyl 2-deoxy-2-azido-$\beta$-cellobioside even is the strongest inhibitor of cellobiohydrolase I known to date ($K_I = 1\ \mu M$).

**Keywords** Glycosidase; Cellulase; Inhibition; 2-Amino-2-deoxy-cellobioside

## INTRODUCTION

Glycoside hydrolases (GH, EC 3.2.1) are a diverse and ubiquitous group of enzymes that are responsible for the degradation of carbohydrate chains,

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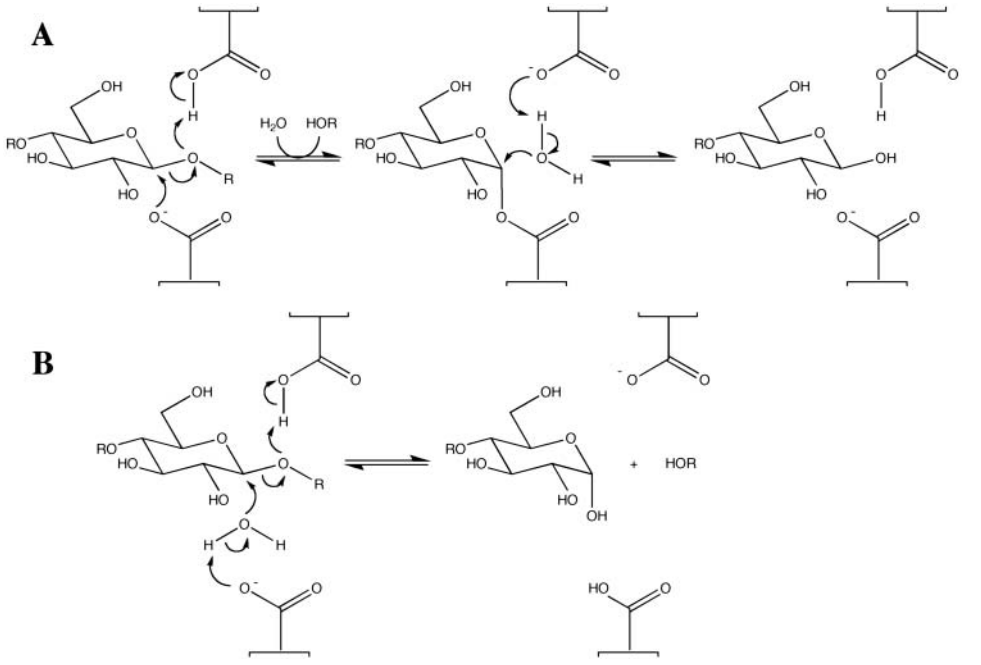


**Figure 1:** General reaction mechanisms of $\beta$-glycoside hydrolases. A: The double displacement mechanism of retaining glycosidases. B: The single displacement mechanism of inverting glycosidases. Conformational changes during hydrolysis are not shown.[28]



glycosides, and glycoconjugates. They are classified in more than 100 different families, reflecting their wide variation in sequence and structure.[1] Nevertheless, nearly all glycosidases follow one of two general reaction mechanisms that involve two carboxylic amino acids (Asp/Glu) as catalytic residues (Fig. 1).[2] A single displacement reaction results in inversion of the anomeric configuration, while a double displacement reaction results in net retention.

With a half-life in the order of millions of years, glycosidic bonds are known to be extremely stable.[3] Consequently, glycoside hydrolases rank among the most proficient biocatalysts, achieving rate enhancements up to $10^{17}$. This means that glycosidases have a very high affinity for the transition state (TS) and hence, are very sensitive to inhibition by TS mimics.[4] Such compounds can have numerous applications (e.g., in the treatment of diabetes or viral infections).[5, 6] Examples of commercially available therapeutic glycosidase inhibitors are acarbose (Glucobay) and oseltamivir (Tamiflu), which target $\alpha$-glucosidase and neuraminidase, respectively.

Inhibitor design requires a thorough knowledge of enzyme-ligand interactions, one of the most important being that between the catalytic nucleophile of retaining $\beta$-glycosidases and the 2-hydroxyl group of their substrate.[7] Indeed,

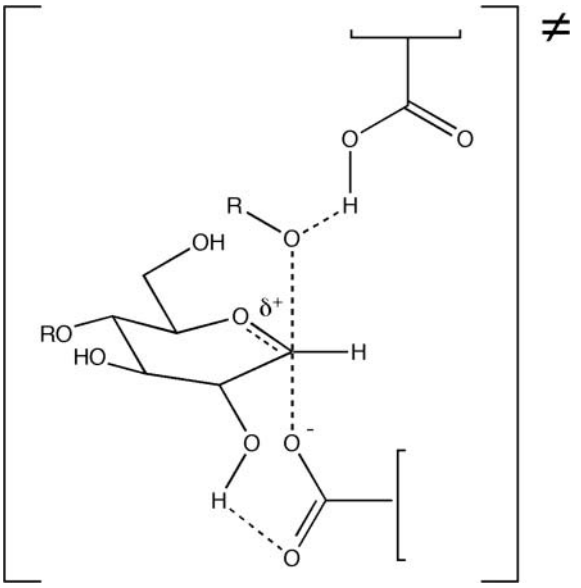


**Figure 2:** The interaction between the catalytic nucleophile of retaining $\beta$-glycosidases and the 2-hydroxyl group of their substrate. The interaction is believed to be optimized, both geometrically and electronically, at the level of the transition state.



this interaction is believed to be optimized, both electronically and geometrically, at the level of the transition state (Fig. 2) and contributes strongly (30 to 40 kJ/mol) to its stabilization.[8–10] Replacing the 2-hydroxyl group of a transition state mimic by an amino group should, therefore, strengthen the interaction with the negatively charged nucleophile, resulting in an increased affinity for the inhibitor. This has indeed been observed with glucosamine-based inhibitors of $\beta$-glucosidases.[11] On average, a 10-fold increase in affinity could be achieved, although the effect was dependent on the nature of the glycosidic heteroatom.

To determine whether this strategy can be expanded to other $\beta$-glycosidases, aromatic 2-deoxy-2-amino-$\beta$-cellobiosides have presently been synthesized and tested as inhibitors of cellulases from *Hypocrea jecorina* (formerly *Trichoderma reesei*). The coupling of a nonactivated aromatic aglycon to the (modified) cellobiosides should allow the ligands to span the active site in a distorted, transition state-like conformation without being hydrolyzed.[2] Cellulases consist of a complex mixture of different enzymes that comprise both endoglucanases (EG, EC 3.2.1.4) and cellobiohydrolases (CBH, EC 3.2.1.91).[12] These enzymes belong to different GH-families and, therefore, form an interesting model system to study the general features of enzyme-ligand interactions.

The four major cellulases of *H. jecorina* are CBH I (Cel7A), CBH II (Cel6A), EG I (Cel7B), and EG II (Cel5A), which together make up almost 99% of the cellulase content (nomenclature according to [13]). They have been extensively characterized and are well suited for the evaluation of novel ligands, as they display a variety of structures and mechanisms. All these enzymes hydrolyze their substrate with retention of the anomeric configuration, except for CBH II, which follows an inverting mechanism. Both cellobiohydrolases have an active site located in a tunnel, with CBH I and CBH II degrading cellulose from the reducing and nonreducing end, respectively. The endoglucanases, in contrast, have an active site located in a cleft and hydrolyze their substrate somewhere in the middle. Different binding modes of inhibitors are, therefore, often observed with the different enzymes.



## RESULTS

### Synthesis of Inhibitors

All compounds were synthesized starting from per-*O*-acetyl cellobiose (**1**) (Fig. 3). A nitrogen functionality was introduced at the 2-position by azidonitration[14, 15] of per-*O*-acetyl cellobial (**2**), which was prepared by converting **1** to acetobromo cellobiose followed by reductive elimination with $Zn/H_2PtCl_6$.[16] Treatment of **2** with ceric ammonium nitrate and sodium azide at –15°C yielded the 2-azido-2-deoxy-cellobiose derivatives **3** and **4**, both showing their 2-azido function in equatorial position. Although expected as a minor side product, the D-glucosyl-$\beta$(1′,4)-2-deoxy-2-azido-D-mannose derivate **5** with its 2-azido function in axial position appeared not to be present (TLC analysis), in contrast to what has been reported for the azidonitrations of D-galactal and D-glucal, where the corresponding axial 2-azido carbohydrate derivatives were formed in substantial amounts.[14, 15] Selective hydrolysis of the 1-nitrate ester of **3** yielded 2-deoxy-2-azido-3,6,2′,3′,4′,6′-hexa-*O*-acetyl cellobiose (**6**). In principle, the yield (36%) of the desired hemiacetal **6** may be further increased by converting the amide **4** (21% yield) to 2-deoxy-2-azido cellobiose (**7**) by means of acid hydrolysis, followed by peracetylation and selective anomeric deprotection.

Coupling of benzyl alcohol and *m*-iodobenzyl alcohol to the hemiacetal **6** and to 1-deprotected per-*O*-acetyl cellobiose[17] was with the trichloroacetimidate method.[18] Formation of the trichloroacetimidates **8** and **9**[19] was at rt to generate the thermodynamically more stable $\alpha$-anomer. The coupling reactions were at –15°C to avoid anomeric interconversion, respectively yielding the peracetates **10**, **11**, **12,** and **13**. Zemplen deacetylations to the final products **14**, **15**, **16,** and **17** were quantitative.

Attempts at catalytic reduction with $H_2$/Pd-C of the 2-azides **14** and **15** to the corresponding amines failed in our hands: the former underwent

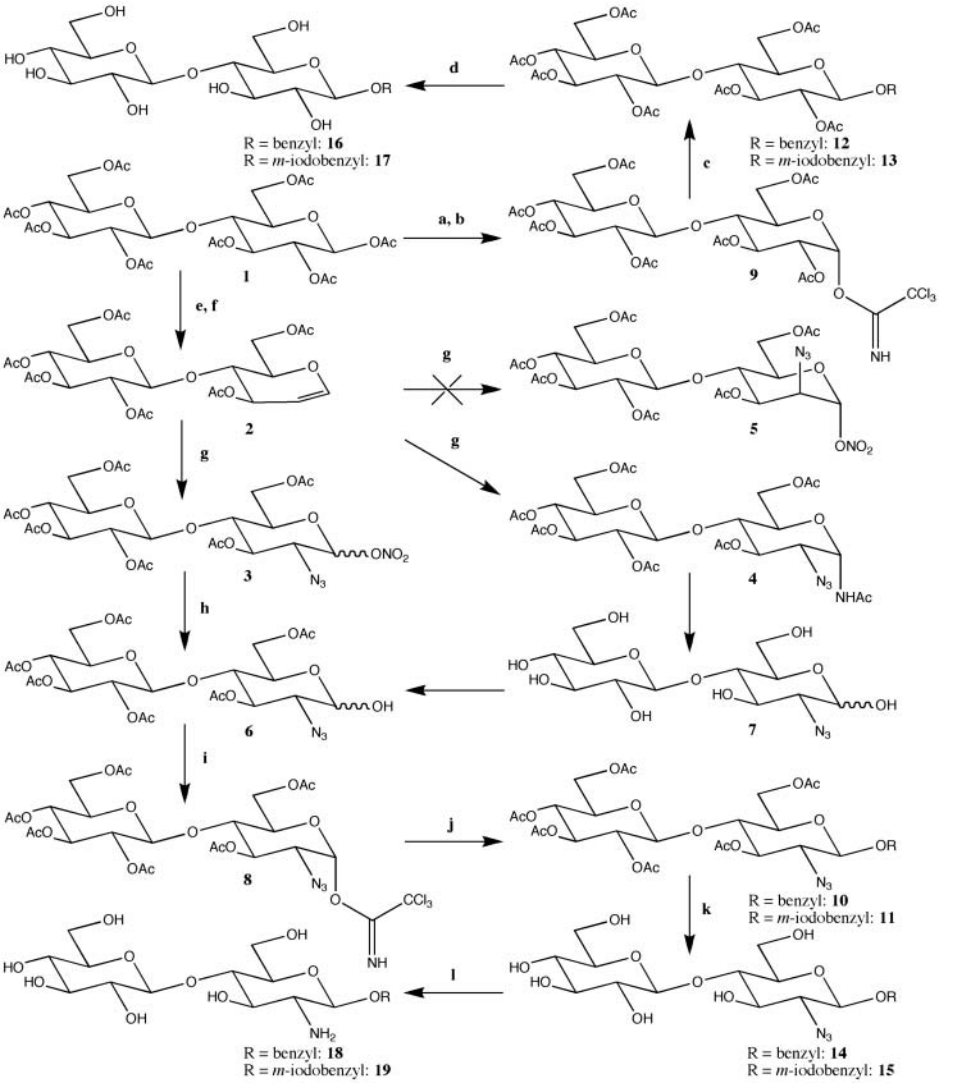


**Figure 3:** Synthetic routes for the different cellobiosides and their C2 analogues. a: hydrazine, DMF, 50°C (96%)[17]; b: $CCl_3CN$, DBU, $CH_2Cl_2$, rt, 1 h (90%)[19]; c: benzyl alcohol or *m*-iodobenzyl alcohol, $BF_3.OEt_2$, $CH_2Cl_2$, –15°C, 1 h (56% for **12**, 57% for **13**); d: NaOMe, MeOH, rt, 2 h (quant.); e: HBr/HOAc, $CHCl_3$, rt, 1 h (87%); f: Zn, $H_2PtCl_6$, HOAc, rt, 4 h (57%)[16]; g: $(NH_4^+)_2Ce^{IV}(NO_3^-)_6$, $NaN_3$, MeCN, 12 h, rt (21% for 4); h: $NaNO_2$, $H_2O$, dioxane, 8 h, 80°C (36% for **6** from **2**); i: $CCl_3CN$, DBU, $CH_2Cl_2$, rt, 2 h (58%), j: benzyl alcohol or *m*-iodobenzyl alcohol, $BF_3.OEt_2$, $CH_2Cl_2$, –15°C, 1h (79% for **10**; 72% for **11**); k: NaOMe, MeOH, rt, 2 h (quant.); l: triphenyl phosphine, MeOH/$H_2O$ (9/1), rt, 12 h (95% for **18**, 94% for **19**).

aglycon cleavage, whereas the latter did not react, presumably due to catalyst poisoning by the iodide function. They were successfully converted to the desired amines **18** and **19** by a Staudinger reduction[20] with triphenyl phosphine.

## Inhibition of Cellulases

Different aromatic cellobiosides (**14–19**) have been synthesized as potential inhibitors of cellulases (Fig. 3). Assuming that these compounds bind in subsites –2 to +1, distortion of the carbohydrate ring in subsite –1 to a transition state-like conformation should be possible. In that way, the replacement of the 2-hydroxyl group by an amino group could result in a stronger interaction with the catalytic nucleophile and hence, tighter binding of the inhibitor. Preliminary results have indicated that the corresponding 2-azido compounds, precursors in the synthetic route, incidentally display a high inhibitory power and they have therefore also been included in this study.

The use of a nonactivated aromatic aglycon is required to preclude enzymatic hydrolysis of the ligands. A benzyl group is known to accommodate efficiently in subsite +1 of most cellulases, because it resembles a carbohydrate ring in size and shape.[21] An iodobenzyl group was also evaluated as aglycon, as its increased polarity might result in improved interactions with the enzyme. Inhibition studies have been performed with four different cellulases, consisting of endo- and exo-acting enzymes from three different GH-families (Table 1). All inhibitions were found to be of the competitive type.

For CBH I (Cel7A), replacing the 2-OH by an amino group did not result in tighter binding but instead increased the $K_I$ of the ligands. However, introducing an azido group at this position had the opposite effect, resulting in $K_I$ values in the low $\mu$M range. When the aglycons are compared, a slight preference for an iodobenzyl over a benzyl is observed. Surprisingly, the cellobiosides **16** and **17** are hydrolyzed rather efficiently by EG I (Cel7B), excluding their use as inhibitors. All other ligands were only marginally hydrolyzed after 24 h incubation with the enzyme (not shown). The endoglucanase was also found to have a higher affinity for an azido than for an amino group at C2.

Unexpectedly, none of the synthesized ligands are able to inhibit EG II (Cel5A) to a significant extent. In contrast, inhibition was observed with the

**Table 1:** Inhibition constants for the various ligands synthesized in this study

| Inhibitor | | | $K_I$ ($\mu$M) | | |
|---|---|---|---|---|---|
| Nr. | aglycon | C2 | CBH I | EG I | CenA |
| 16 | benzyl | OH | 14 | — | 417 |
| 17 | iodobenzyl | | 7 | — | *n.d.* |
| 14 | benzyl | $N_3$ | 2 | 409 | 394 |
| 15 | iodobenzyl | | 1 | 248 | *n.d.* |
| 18 | benzyl | $NH_2$ | 72 | 623 | 661 |
| 19 | iodobenzyl | | 59 | 667 | *n.d.* |

Spectroscopic assays were performed continuously at pH 5.7 and 37°C. All measurements were performed in triplicate and had a CV of less than 10%. *n.d.*, not determined; —, hydrolysis.

endoglucanase CenA from family GH-6. This enzyme from *Cellulomonas fimi* was chosen for practical reasons because it is active on chromogenic substrates, while no such substrates have yet been identified for CBH II (Cel6A) from *H. jecorina*.[22] Only inhibitors with a benzyl group as aglycon have been tested, but this still allows an evaluation of the influence of the C2 substituent. An amino group has been found to decrease the ligands' affinity, while an azido group does not have a significant effect.

## DISCUSSION

As shown in this study, replacing the 2-OH group of cellobiosides with an amino functionality has a rather unpredictable effect on their inhibitory power toward cellulases. In fact, the proposed interaction with the catalytic nucleophile can only be realized if these ligands bind in subsites –2 to +1, which might not be the case for all cellulases. CBH I and EG I, for example, interact differently with cellobiose, although both cellulases belong to the same family GH-7. The former enzyme binds cellobiose very tightly ($K_I = 28\ \mu M$) in subsites +1/+2, while the latter binds it rather loosely ($K_I = 6$ mM) in subsites –2/–1.[23]

Considering its positioning of cellobiose, EG I might be expected to bind aromatic cellobiosides in subsites –2/+1. This is corroborated by the hydrolysis of ligands **16** and **17** at the heterosidic linkage. The strongest binding was achieved by the introduction of a 2-$N_3$ group, as compared to a 2-$NH_2$ group. Inspection of the crystal structure of the enzyme in complex with cellobiose (PDB 2OVW) reveals that subsite –1 of the active-site cleft is spacious enough to accommodate an azido group at C2 (Fig. 4). An azido functionality could potentially also interact with the catalytic nucleophile, as its central

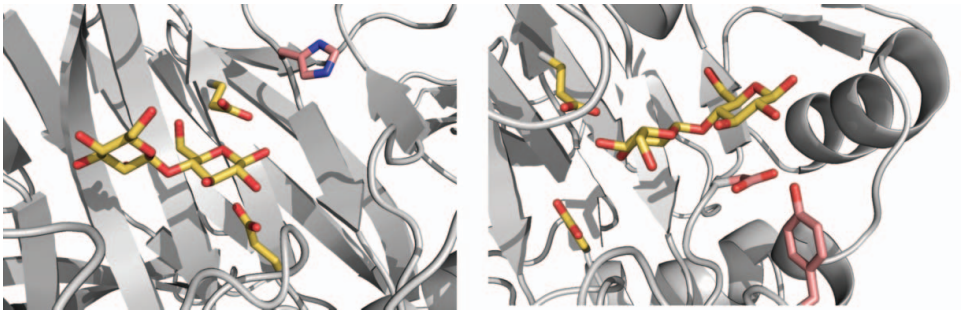

**Figure 4:** Binding of cellobiose in GH-7 cellulases. The ligand is positioned in subsites –2/–1 of EG I (left) and in subsites +1/+2 of CBH I (right). The figure was drawn using PyMol, with cellobiose and the catalytic amino acids in yellow and other important residues in pink. (Figure available in color online.)

nitrogen atom carries a partial positive charge. Surprisingly, different affinities for the aglycons are observed for the different types of inhibitors. The accommodation of an azido versus an amino group might, however, require a different conformation of the carbohydrate ring in subsite –1, resulting in different interactions with the aglycon in subsite +1. As the active site of EG I is composed of only four subsites (–2/+2), the only alternative positioning of cellobiosides would be in subsites –1/+2. In that case, the most likely interaction of the C2 substituent would be with a histidine residue (His209) in subsite +1, but it is uncertain how this would affect the binding strength of the inhibitors.

Of all *H. jecorina* cellulases, CBH I has the largest active site, containing at least 10 subsites (–7/+3). Consequently, many different binding modes could be envisaged for the ligands described here. Crystallographic and kinetic studies, however, have revealed that CBH I positions cellobiosides preferentially in subsites +1/+3.[24] This could explain why a 2-$NH_2$ group does not induce tight binding. Remarkably, the 2-$N_3$ compounds interact very strongly with the enzyme, resulting in $K_I$ values as low as 1 to 2 $\mu$M. The ligand *m*-iodobenzyl 2-deoxy-2-azido-$\beta$-cellobioside (**15**) is even the strongest CBH I inhibitor known to date. Its high affinity for CBH I has also been confirmed by mass spectrometry.[25] Inspection of the crystal structure of the enzyme in complex with cellobiose (PDB 3CEL) reveals that the C2 substituent in subsite +2 is pointing away from the active site, with relatively few interactions (Fig. 4). Only two residues (Tyr252 and Asp259) are in close vicinity (~4 Å) and could potentially form an interaction, especially with the longer azido functionality. For all inhibitor types, a similar preference for the aglycon groups is observed, implying that these are always positioned in the same subsite.

The cellulase CenA from family GH-6 has also been included in this study, although it operates by an inverting mechanism in which no catalytic nucleophile is present close to the 2-group of the substrate. It is, therefore, not surprising that modification of this group does not result in a significant improvement in binding strength. For EG II, the lack of inhibition by any of the reported ligands is probably due to their short chain length. Indeed, this enzyme is known to be active on chromogenic cellotriosides but not on the corresponding cellobiosides.[21]

In conclusion, the replacement of the 2-OH group of cellobiosides cannot be considered to be a generic strategy for increasing their inhibitory power toward cellulases. Nevertheless, a novel ligand for CBH I has been identified that ranks among its most efficient inhibitors known to date. As this enzyme is the major constituent of cellulase mixtures (up to 60% in *H. jecorina*), its inhibition has a dramatic effect on the total cellulolytic activity.[12] Consequently, compound **15** might be considered for practical applications, such as the treatment of termite infestations.[26]

## EXPERIMENTAL

### General

$^1$H NMR spectra (500 MHz) were recorded in $CDCl_3$ or $D_2O$ on a Bruker WP-500 or AM-500 spectrometer, with residual protons of the solvent as internal standard; chemical shifts are expressed in ppm relative to tetramethylsilane. IR spectra were recorded on a Beckman Acculab 4 spectrometer. MS data were recorded by LCMS with an Agilent 1100 series HPLC equipped with an Agilent G1946C ES-single quadrupole detector (as electrospray in positive or negative mode from 1:1 0.5 mM aqueous ammonium acetate-$CH_3CN$). Melting points were determined with a Reichert 269156 microscope and are uncorrected. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Thin-layer chromatography (TLC) was on Merck $60F_{254}$ silica gel plates, with detection by observation under UV (254 nm) and carbonization on a hot plate after dipping in a 10% soln of $H_2SO_4$ in isopropanol. Column chromatography was with Merck silica gel 100 (0.063–0.200 mm), which was dried in vacuo at 200°C when indicated. Toluene and EtOAc were distilled prior to use, $CH_2Cl_2$ was freshly distilled over $P_2O_5$, and $Et_2O$ and MeCN were kept over 4 Å molecular sieves that were dried in vacuo at 200°C. All reactions were run with magnetic stirring under nitrogen atmosphere.



### Azidonitration and Nitrate Hydrolysis

N-*acetyl-3,6,2′,3′,4′,6′-hexa*-O-*acetyl-2-deoxy-2-azido-cellobiosylamine (**4**) and 2-deoxy-2-azido-3,6,2′,3′,4′,6′-hexa*-O-*acetyl cellobiose (**6**)*

A soln of ceric ammonium nitrate (39.1 g, 71.4 mmol) in MeCN (150 mL) was dried over 4 Å molecular sieves (10 g) for 3 h at rt, and then cooled to –30°C (cooling bath heptanol/solid $CO_2$). Sodium azide (2.32 g, 35.7 mmol) and **2** (10 g, 17.86 mmol) were added under vigorous stirring. The mixture was allowed to reach rt and was further stirred overnight. After pouring in ice-cold water (100 mL) and stirring for 15 min, celite (20 g) was added and the mixture was filtered; the solids were washed with $CH_2Cl_2$ (3 × 100 mL). The combined organic layers were washed with water (2 × 100 mL) and concentrated in vacuo. The residue was dissolved in dioxane (100 mL) and a soln of sodium nitrite (10 g) in water (20 mL) was added. After stirring for 8 h at 80°C, the soln was cooled to rt and extracted with $CH_2Cl_2$ (3 × 100 mL). The combined organic layers were washed with water (100 mL) and dried over anhydrous $MgSO_4$. After concentration in vacuo, the residue was preliminary purified by column chromatography (eluent: toluene/EtOAc: 1/1). The first eluted compound was recrystallized from isopropanol (100 mL; the mother liquor was concentrated to 20 mL for a second crop), yielding **6** as white crystals (4.0 g, 36%). The

second eluted compound was further purified by column chromatography using the same eluent, yielding **4** as a white powder (2.48 g, 21%).

N-*acetyl-3,6,2′,3′,4′,6′-hexa*-O-*acetyl-2-deoxy-2-azido-cellobiosylamine (**4**)*

Brutoformula: $C_{24}H_{36}O_{17}N_4$ (MW = 660); mp 87°C; $[\alpha]_D^{23}$: +25 ($c$ = 0.1; $CHCl_3$); Rf (toluene/EtOAc: 4/6): 0.28; IR (KBr): 2100, 1750, 1700, 1530, 1370, 1230, 1040 $cm^{-1}$; $^1H$ NMR (500 MHz; $CDCl_3$): 6.55 (m, 1H), 5.72 (dd, 1H, $J$ = 5.6 and 6 Hz), 5.27 (dd, 1H, $J$ = 9 and 10 Hz), 5.14 (dd, 1H, $J$ = 9.4 and 9.4 Hz), 5.08 (dd, 1H, $J$ = 9.7 and 9.7 Hz), 4.93 (dd, 1H, $J$ = 8.1 and 9.1 Hz), 4.47 (d, 1H, $J$ = 7.8 Hz), 4.39 (dd, 1H, $J$ = 4.3 and 12.5 Hz), 4.36 (dd, 1H, $J$ = 1 and 12 Hz), 4.12 (dd, 1H, $J$ = 4.4 and 12 Hz), 4.09 (dd, 1H, $J$ = 1 and 12 Hz), 3.87 (dd, 1H, $J$ = 5.6 and 10.2 Hz), 3.75 (m, 1H), 3.70 (dd, 1H, $J$ = 8.6 and 8.6 Hz), 3.65 (m, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.09 (s + s, 6H), 2.03 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H) ppm; MS: $m/z$ (pos) 169 (8%), 331 [M/2+$H^+$] (32%), 661 [M+$H^+$] (100%), 683 [M+$Na^+$] (20%); $m/z$ (neg) 659 [M−$H^+$] (62%), 719 [M+$OAc^-$] (100%).

*2-Deoxy-2-azido-3,6,2′,3′,4′,6′-hexa-O-acetyl cellobiose (**6**)*

Brutoformula: $C_{24}H_{33}O_{16}N_3$ (MW = 619); mp 172°C; $[\alpha]_D^{25}$: +47 ($c$ = 2.0; $CHCl_3$); Rf (toluene/EtOAc: 4/6): 0.48; IR (KBr): 3600–3300, 2100, 1750, 1430, 1370, 1230, 1030, 900 $cm^{-1}$; $^1H$ NMR (500 MHz; $CDCl_3$): 5.49 (dd, 1H, $J$ = 9.3 and 10.4 Hz), 5.34 (m, 1H), 5.17–5.07 (m, 2H), 4.93 (dd, 1H, $J$ = 8 and 8 Hz), 4.54 (d, 1H, $J$ = 8.0 Hz), 4.50 (m, 1H), 4.39 (dd, 1H, $J$ = 4.1 and 8.4 Hz), 4.18 (m, 1H), 4.13 (dd, 1H, $J$ = 4.5 and 12.0 Hz), 4.07 (dd, 1H, $J$ = 2.0 and 12.2 Hz), 3.72 (dd, 1H, $J$ = 9.7 and 9.7 Hz), 3.67 (m, 1H), 3.38 (dd, 1H, $J$ = 8.3 and 10.2 Hz), 3.28 (dd, 1H, $J$ = 2.7 and 10.5 (Hz), 2.13 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H) ppm; MS: $m/z$ (pos) 331 (14%), 637 [M+$NH_4^+$] (100%); $m/z$ (neg) 410 (37%), 470 (84%), 530 (100%), 618 [M−$H^+$] (95%).

## Coupling of the Aglycons

*2-Deoxy-2-azido-1α-trichloroacetamido-hexa-O-acetyl cellobioside (**8**)*

To a soln of **6** (2 g, 3.23 mmol) and trichloroacetonitrile (1.3 mL, 12.9 mmol) in $CH_2Cl_2$ (40 mL) at rt, 1,8-diazabicyclo-[5,4,0]-undec-7-ene (0.323 mmol, 48 $\mu$L) was added. After stirring for 2 h, the solvent was removed in vacuo and the residue was purified by column chromatography with oven-dried silica gel (eluent: toluene/EtOAc: 7/3), yielding **8** as a slightly yellowish foam (1.44 g, 58%). This compound is unstable and must be used within hours.

Brutoformula: $C_{26}H_{33}O_{16}N_4Cl_3$ (MW = 763.5); mp ±100°C (dec); Rf (toluene/EtOAc: 4/6): 0.58; IR (KBr): 2100, 1750, 1680, 1370, 1220, 1030 $cm^{-1}$.

*2-Deoxy-2-azido-1β-benzyl-hexa*-O-*acetyl cellobioside (**10**)*

A soln of **8** (610 mg, 0.799 mmol) and benzyl alcohol (0.163 mL, 1.60 mmol) in $CH_2Cl_2$ (10 mL) with 4 Å molecular sieves (0.5 g) was stirred for 2 h at rt.

After cooling to –15°C (bath with benzyl alcohol/solid $CO_2$), a soln of $BF_3.OEt_2$ (9.8 $\mu$L, 0.080 mmol) in $CH_2Cl_2$ (100 $\mu$L) was added dropwise during 5 min; the reaction was monitored by TLC and was complete after 1 h. Solid $NaHCO_3$ (100 mg) was added and the mixture was brought to rt. After filtration, the solvent was removed in vacuo and the residue was purified by column chromatography (eluent: toluene/EtOAc: 7/3) yielding **10** as a white powder (450 mg, 79%).

Brutoformula: $C_{31}H_{39}O_{16}N_3$ (MW = 709); mp 133°C; $[\alpha]_D^{23}$: –20 ($c$ = 0.1; $CHCl_3$); Rf (toluene/EtOAc: 6/4): 0.63; IR (KBr): 2100, 1750, 1430, 1370, 1230, 1160, 1050, 900 $cm^{-1}$; $^1H$ NMR (500 MHz; $CDCl_3$): 7.37–7.30 (m, 5H), 5.13 (dd, 1H, $J$ = 9.4 and 9.4 Hz), 5.06 (dd, 1H, $J$ = 9.7 and 9.7 Hz), 4.93 (dd, 1H, $J$ = 9.4 and 10.4 Hz), 4.90 (d, 1H, $J$ = 11.8 Hz), 4.89 (dd, 1H, $J$ = 8.0 and 9.4 Hz), 4.67 (d, 1H, $J$ = 11.8 Hz), 4.53 (dd, 1H, $J$ = 1.8 and 12.0 Hz), 4.48 (d, 1H, $J$ = 8.0 Hz), 4.40 (d, 1H, $J$ = 8.1 Hz), 4.38 (dd, 1H, $J$ = 4.3 and 12.5 Hz), 4.11 (dd, 1H, $J$ = 5.1 and 12.0 Hz), 5.05 (dd, 1H, $J$ = 2.1 and 12.5 Hz), 3.69 (dd, 1H, $J$ = 9.5 and 9.5 Hz), 3.66 (ddd, 1H, $J$ = 2.2, 4.2 and 9.8 Hz), 3.52 (ddd, 1H, $J$ = 1.8, 4.9 and 9.9 Hz), 3.46 (dd, 1H, $J$ = 8.1 and 10.3 Hz), 2.15 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H) ppm; MS: $m/z$ (pos) 727 [M+$NH_4^+$] (100%); $m/z$ (neg) 769 [M+$OAc^-$] (100%).

*2-Deoxy-2-azido-1β-[m-iodo]benzyl-hexa*-O-*acetyl cellobioside (**11**)*

Recipe as for **10**. Starting with **8** (610 mg, 0.799 mmol) and *m*-iodobenzyl alcohol (0.209 mL, 1.60 mmol), **11** was obtained as a white powder (497 mg, 72%).

Brutoformula: $C_{31}H_{38}O_{16}N_3I$ (MW = 835); mp 134°C; $[\alpha]_D^{23}$: –18 ($c$ = 0.1; $CHCl_3$); Rf (toluene/EtOAc: 6/4): 0.66; IR (KBr): 2100, 1750, 1430, 1380, 1230, 1160, 1040, 900 $cm^{-1}$; $^1H$ NMR (500 MHz; $CDCl_3$): 7.71 (s, 1H), 7.65 (d, 1H, $J$ = 8 Hz), 7.32 (d, 1H, $J$ = 8 Hz), 7.09 (dd, 1H, $J$ = 8.0 and 8.0 Hz), 5.13 (dd, 1H, $J$ = 9.3 and 9.3 Hz), 5.06 (dd, 1H, $J$ = 9.8 and 9.8 Hz), 4.95 (dd, 1H, $J$ = 9.3 and 10.3 Hz), 4.90 (dd, 1H, $J$ = 8.0 and 9.3 Hz), 4.82 (d, 1H, $J$ = 12.1 Hz), 4.60 (d, 1H, $J$ = 12.1 Hz), 4.52 (dd, 1H, $J$ = 2.0 and 7.9 Hz), 4.48 (d, 1H, $J$ = 7.9 Hz), 4.38 (d, 1H, $J$ = 8.1 Hz), 4.37 (dd, 1H, $J$ = 4.4 and 12.5 Hz), 4.09 (dd, 1H, $J$ = 5.1 and 12.1 Hz), 4.04 (dd, 1H, $J$ = 2.2 and 12.4 Hz), 3.69 (dd, 1H, $J$ = 9.5 and 9.5 Hz), 3.65 (ddd, 1H, $J$ = 2.2, 4.4 and 9.9 Hz), 3.53 (ddd, 1H, $J$ = 2.0, 5.1 and 9.9 Hz), 3.46 (dd, 1H, $J$ = 8.1 and 10.3 Hz), 2.145 (s, 3H), 2.085 (s, 3H), 2.075 (s, 3H), 2.015 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H) ppm; MS: $m/z$ (pos) 853 [M+$NH_4^+$] (100%); $m/z$ (neg) 894 [M+$OAc^-$] (100%).

*1β-Benzyl-hepta*-O-*acetyl cellobioside (**12**)*

Recipe as for **10**. Starting with **9** (1.42 g, 2.133 mmol) and benzyl alcohol (0.441 mL, 4.266 mmol) in $CH_2Cl_2$ (30 mL), with 4 Å molecular sieves (1 g), a soln of $BF_3.OEt_2$ (27 $\mu$L, 0.22 mmol) in $CH_2Cl_2$ (0.5 mL), workup with $NaHCO_3$ (200 mg), and column chromatography with the same eluent, **12** was obtained as a white powder (870 mg, 56%).

Brutoformula: $C_{33}H_{42}O_{18}$ (MW = 726); mp 170°C; $[\alpha]_D^{23}$: +44 ($c$ = 0.1; $CHCl_3$); Rf (toluene/EtOAc: 4/6): 0.46; IR (KBr): 2940, 2860, 1750, 1430, 1370, 1230, 1040, 900 $cm^{-1}$; $^1H$ NMR (500 MHz; $CDCl_3$): 7.36–7.25 (m, 5H), 5.13 (2 × dd, 2H, $J$ obs. = 9.5 and 9.5 Hz), 5.06 (dd, 1H, $J$ = 9.8 and 9.8 Hz), 4.97 (dd, 1H, $J$ = 8.0 and 9.5 Hz), 4.92 (dd, 1H, $J$ = 8.6 and 8.7 Hz), 4.86 (d, 1H, $J$ = 12.2 Hz), 4.59 (d, 1H, $J$ = 12.2 Hz), 4.54 (dd, 1H, $J$ = 2 and 11.9 Hz), 4.51 (d, 1H, $J$ = 7.9 Hz), 4.49 (d, 1H, $J$ = 7.9 Hz), 4.37 (dd, 1H, $J$ = 4.1 and 12.5 Hz), 4.10 (dd, 1H, $J$ = 4.8 and 11.9 Hz), 4.03 (dd, 1H, $J$ = 2 and 12.5 Hz), 3.79 (dd, 1H, $J$ = 9.5 and 9.5 Hz), 3.65 (ddd, 1H, $J$ = 2, 4 and 9 Hz), 3.57 (ddd, 1H, $J$ = 2, 4 and 9 Hz), 2.15 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H) ppm; MS: $m/z$ (pos) 744 [M+$NH_4^+$] (100%); $m/z$ (neg) 785 [M+$OAc^-$] (100%).

*1β-[m-Iodo]benzyl-hepta-*O*-acetyl cellobioside (**13**)*

Recipe as for **10**. Starting with **9** (500 mg, 0.641 mmol) and *m*-iodobenzyl alcohol (0.163 mL, 1.28 mmol) in $CH_2Cl_2$ (10 mL), with 4 Å molecular sieves (0.5 g), a soln of $BF_3.OEt_2$ (8.0 μL, 0.065 mmol) in $CH_2Cl_2$ (100 μL), workup with $NaHCO_3$ (100 mg), and column chromatography with the same eluent, **13** was obtained as a white powder (266 mg, 57%).

Brutoformula: $C_{33}H_{41}O_{17}I$ (MW = 852); mp 202°C; $[\alpha]_D^{23}$: +40 ($c$ = 0.1; $CHCl_3$); Rf (toluene/EtOAc: 1/1): 0.59; IR (KBr): 1750, 1430, 1240–1210, 1160, 1130, 1050–1030, 900, 780, 730 $cm^{-1}$; $^1H$ NMR (500 MHz; $CDCl_3$): 7.64 (s, 1H), 7.63 (d, 1H, $J$ = 7 Hz), 7.21 (d, 1H, $J$ = 7.7 Hz), 7.07 (dd, 1H, $J$ = 7 and 7.7 Hz), 5.15 (dd, 1H, $J$ = 9.4 and 9.4 Hz), 5.13 (dd, 1H, $J$ = 9.5 and 9.3 Hz), 5.06 (dd, 1H, $J$ = 9.8 and 9.8 Hz), 4.97 (dd, 1H, $J$ = 8.0 and 9.6 Hz), 4.92 (dd, 1H, $J$ = 8.0 and 9.2 Hz), 4.79 (d, 1H, $J$ = 12.7 Hz), 4.54 (dd, 1H, $J$ = 2.0 and 12.1 Hz), 4.53 (d, 1H, $J$ = 12.7 Hz), 4.51 (d, 1H, H, $J$ = 8.0 Hz), 4.48 (d, 1H, $J$ = 8.0 Hz), 4.37 (dd, 1H, $J$ = 4.4 and 12.5 Hz), 4.10 (dd, 1H, $J$ = 4.9 and 12.1 Hz), 4.04 (dd, 1H, $J$ = 2.1 and 12.5 Hz), 3.80 (dd, 1H, $J$ = 9.6 and 9.6 Hz), 3.65 (ddd, 1H, $J$ = 2.2, 4.3 and 9.9 Hz), 3.37 (ddd, 1H, $J$ = 1.9, 4.8, and 9.9 Hz), 2.149 (s, 3H), 2.078 (s, 3H), 2.058 (s, 3H), 2.029 (s, 3H), 2.015 (s, 3H), 2.008 (s, 3H), 1.980 (s, 3H) ppm; MS: $m/z$ (pos) 870 [M+$NH_4^+$] (100%); $m/z$ (neg) 911 [M+$OAc^-$] (100%).

## Deprotection

*2-Deoxy-2-azido-1β-benzyl cellobioside (**14**)*

Compound **10** (200 mg, 282 μmol) was taken in a soln of sodium methanolate (0.1 eq., 28.2 μmol) in MeOH (3.2 mL of an 8.7 μM soln). Workup as for **16** gave **14** as a white powder (quant).

Brutoformula: $C_{19}H_{26}O_{10}N_3$ (MW = 457); mp 175°C (isopropanol); $[\alpha]_D^{23}$: –10 ($c$ = 0.1; MeOH); $^1H$ NMR (500 MHz; $D_2O$): 7.540–7.450 (m, 5H), 5.000 (d, 1H, $J$ = 11.7 Hz), 4.835 (d, 1H, $J$ = 11.7 Hz), 4.675 (d, 1H, $J$ = 8.2 Hz), 4.545 (d, 1H, $J$ = 7.8 Hz), 4.025 (dd, 1H, $J$ = 1.9 and 12.3 Hz), 3.940 (dd, 1H,

$J$ = 1.9 and 12.3 Hz), 3.865 (dd, 1H, $J$ = 5.3 and 12.3 Hz), 3.751 (dd, 1H, $J$ = 5.7 and 12.3 Hz), 3.735 (dd, 1H, $J$ = 9.5 and 9.5 Hz), 3.620 (dd, 1H, $J$ = 9.5 and 10.1 Hz), 3.610 (ddd, 1H, $J$ = 1.9, 5.3, and 9.5 Hz), 3.535 (dd, 1H, $J$ = 9.3 and 9.4 Hz), 3.515 (ddd, 1H, $J$ = 1.9, 7.7, and 9.3 Hz), 3.440 (dd, 1H, $J$ = 8.9 and 9.4 Hz), 3.400 (dd, 1H, $J$ = 8.5 and 10.1 Hz), 3.335 (dd, 1H, $J$ = 8.2 and 8.9 Hz) ppm; MS: $m/z$ (pos) 250 (80%), 296 (26%), 350 (27%), 475 [M+$NH_4^+$] (100%), 480 (85%); $m/z$ (neg) 456 [M−$H^+$] (100%), 457 (24%), 516 [M+$OAc^-$] (14%), 570 (17%), 913 [2M−$H^+$] (29%).

*2-Deoxy-2-azido-1β-[m-iodo]benzyl cellobioside (**15**)*

Compound **11** (200 mg, 239 μmol) was taken in a soln of sodium methanolate (0.1 eq., 23.9 μmol) in MeOH (2.7 mL of an 8.7 μM soln). Workup as for **16** gave **15** as a white powder (quant).

Brutoformula: $C_{19}H_{25}O_{10}N_3I$ (MW = 583); mp 197°C (isopropanol); $[\alpha]_D^{23}$: –12 ($c$ = 0.1; MeOH); $^1$H NMR (500 MHz; $D_2O$): 7.94 (s, 1H), 7.83 (d, 1H, $J$ = 8.2 Hz), 7.51 (d, 1H, $J$ = 7.6 Hz), 7.25 (dd, 1H, $J$ = 7.6 and 8.2 Hz), 4.94 (d, 1H, $J$ = 11.7 Hz), 4.77 (d, 1H, $J$ = 11.7 Hz), 4.65 (d, 1H, $J$ = 8.2 Hz), 4.54 (d, 1H, $J$ = 7.8 Hz), 4.02 (dd, 1H, $J$ = 1.9 and 12.3 Hz), 3.94 (dd, 1H, $J$ = 2.0 and 12.3 Hz), 3.86 (dd, 1H, $J$ = 5.0 and 12.3 Hz), 3.76 (dd, 1H, $J$ = 5.0 and 12.3 Hz), 3.73 (dd, 1H, $J$ = 9.1 and 9.7 Hz), 3.62 (dd, 1H, $J$ = 9.1 and 9.7 Hz), 3.60 (ddd, 1H, $J$ = 1.9, 5.0, and 9.1 Hz), 3.53 (dd, 1H, $J$ = 9.1 and 9.5 Hz), 3.51 (ddd, 1H, $J$ = 2.0, 5.0, and 9.5 Hz), 3.44 (dd, 1H, $J$ = 9.1 and 9.1 Hz), 3.39 (dd, 1H, $J$ = 7.8 and 9.1 Hz), 3.33 (dd, 1H, $J$ = 8.2 and 9.1 Hz) ppm; MS: $m/z$ (pos) 150 (68%), 172 (43%), 350 (32%), 388 (52%), 432 (55%), 476 (48%), 601 [M+$NH_4^+$] (100%), 606 (73%), 964 (75%); $m/z$ (neg) 582 [M−$H^+$] (100%), 617 (13%), 642 [M+$OAc^-$] (12%), 696 (29%).

*1β-Benzyl cellobioside (**16**)*

Compound **12** (200 mg, 275 μmol) was taken in a soln of sodium methanolate (0.1 eq., 27.5 μmol) in MeOH (3.2 mL of an 8.7 μM soln, freshly prepared from 10 mg Na in 50 mL MeOH). The reaction was complete after 2 h at rt. A spoontip of silica gel was added, and after 5 min the soln was filtered and concentrated in vacuo, yielding **16** as a white powder (quant).

Brutoformula: $C_{19}H_{28}O_{11}$ (MW = 432); mp 162°C (isopropanol); $[\alpha]_D^{23}$: –38 ($c$ = 0.1; MeOH); $^1$H NMR (500 MHz; $D_2O$): 7.52–7.43 (m, 5H), 4.970 (d, 1H, $J$ = 11.7 Hz), 4.785 (d, 1H, $J$ = 11.7 Hz), 4.585 (d, 1H, $J$ = 8.2 Hz), 4.540 (d, 1H, $J$ = 8.2 Hz), 4.020 (dd, 1H, $J$ = 1.6 and 12.3 Hz), 3.940 (dd, 1H, $J$ = 1.9 and 12.3 Hz), 3.850 (dd, 1H, $J$ = 5.0 and 12.3 Hz), 3.760 (dd, 1H, $J$ = 5.7 and 13.3 Hz), 3.685 (dd, 1H, $J$ = 9.2 and 9.2 Hz), 3.630 (dd, 1H, $J$ = 8.8 and 9.2 Hz), 3.610 (ddd, 1H, $J$ = 1.6, 5.0, and 9.2 Hz), 3.535 (dd, 1H, $J$ = 9.2 and 9.3 Hz), 3.515 (ddd, 1H, $J$ = 1.9, 5.7, and 9.2 Hz), 3.440 (dd, 1H, $J$ = 9.0 and 9.3 Hz), 3.380 (dd, 1H, $J$ = 8.2 and 8.7 Hz), 3.335 (dd, 1H, $J$ = 8.2 and 8.7 Hz) ppm; MS: $m/z$ (pos) 163 (32%), 253 (22%), 271 (40%), 325 (100%),

450 [M+$NH_4^+$] (79%), 455 [M+$Na^+$] (53%), 887 [2M+$Na^+$] (79%); *m/z* (neg) 431 [M−$H^+$] (100%), 863 [2M−$H^+$] (29%).

*1β-[m-Iodo]benzyl cellobioside (**17**)*

Compound **13** (500 mg, 587 μmol) was taken in a soln of sodium methanolate (0.1 eq., 58.7 μmol) in MeOH (6.7 mL of an 8.7 μM soln). Workup as for **16** gave **17** as a white powder (quant).

Brutoformula: $C_{19}H_{27}O_{11}I$ (MW = 558); mp 183°C (isopropanol); $[\alpha]_D^{23}$: –35 (*c* = 0.1; MeOH); $^1$H NMR (500 MHz; $D_2O$): 7.92 (s, 1H), 7.81 (d, 1H, *J* = 8.2 Hz), 7.49 (d, 1H, *J* = 7.9 Hz), 7.24 (dd, 1H, *J* = 7.9 and 8.2 Hz), 4.91 (d, 1H, *J* = 11.9 Hz), 4.75 (d, 1H, *J* = 11.9 Hz), 4.56 (d, 1H, *J* = 8.2 Hz), 4.54 (d, 1H, *J* = 8.2 Hz), 4.01 (dd, 1H, *J* = 1.9 and 12.3 Hz), 3.94 (dd, 1H, *J* = 1.9 and 12.3 Hz), 3.85 (dd, 1H, *J* = 5.1 and 12.3 Hz), 3.76 (dd, 1H, *J* = 5.6 and 12.3 Hz), 3.68 (dd, 1H, *J* = 8.6 and 8.6 Hz), 3.63 (dd, 1H, *J* = 8.6 and 8.8 Hz), 3.60 (ddd, 1H, *J* = 1.9, 5.1, and 8.6 Hz), 3.53 (dd, 1H, *J* = 9.3 and 9.3 Hz), 3.52 (ddd, 1H, *J* = 1.9, 5.6, and 9.2 Hz), 3.44 (dd, 1H, *J* = 9.1 and 9.3 Hz), 3.38 (dd, 1H, *J* = 8.2 and 6.8 Hz), 3.34 (dd, 1H, *J* = 8.2 and 9.1 Hz) ppm; ESIMS: *m/z* (pos) 271 (26%), 325 (100%), 576 [M+$NH_4^+$] (73%), 581 [M+$Na^+$], (49%) 1042 [2M+$Na^+$] (35%); *m/z* (neg) 557 [M−$H^+$] (100%), 1015 [2M−$H^+$] (15%).



## Reduction of the Azido Functionality

*2-Deoxy-2-amino-1β-benzyl cellobioside (**18**)*

To a soln of **14** (63 mg, 138 μmol) in MeOH/water: 9/1 (1 mL), triphenyl phosphine (72 mg, 276 μmol) was added. After stirring for 2 d at rt, the mixture was concentrated in vacuo and purified by column chromatography (eluent: isopropanol/water/saturated aq. ammonia: 18/2/1), yielding **18** as a white powder (56 mg, 95%).

Brutoformula: $C_{19}H_{29}O_{10}N$ (MW = 431); mp 105°C; $[\alpha]_D^{23}$: –36 (*c* = 0.05; MeOH); Rf (isopropanol/water/saturated aq. ammonia: 18/2/1): 0.35; $^1$H NMR (500 MHz; $D_2O$): 7.52–7.44 (m, 5H), 4.98 (d, 1H, *J* = 11.7 Hz), 4.77 (d, 1H, *J* = 11.7 Hz), 4.57 (d, 1H, *J* = 8.2 Hz), 4.54 (d, 1H, *J* = 8.2 Hz), 4.03 (dd, 1H, *J* = 1.9 and 12.3 Hz), 3.94 (dd, 1H, *J* = 2.2 and 12.3 Hz), 3.87 (dd, 1H, *J* = 5.0 and 12.3 Hz), 3.75 (dd, 1H, *J* = 6.0 and 12.3 Hz), 3.69 (dd, 1H, *J* = 9.5 and 9.8 Hz), 3.61 (ddd, 1H, *J* = 1.9, 5.0, and 9.8 Hz), 3.57 (dd, 1H, *J* = 9.5 and 10.0 Hz), 3.54 (dd, 1H, *J* = 9.5 and 9.5 Hz), 3.51 (ddd, 1H, *J* = 2.2, 6.0, and 9.5 Hz), 3.44 (dd, 1H, *J* = 9.5 and 9.5 Hz), 3.34 (dd, 1H, *J* = 8.2 and 9.5 Hz), 2.80 (dd, 1H, *J* = 8.2 and 10.0 Hz) ppm; MS: *m/z* (pos) 324 (29%), 432 [M+$H^+$] (100%), 454 [M+$Na^+$] (15%), 734 (26%), 863 [2M+$H^+$] (72%), 864 (33%), 885 [2M+$Na^+$] (18%); *m/z* (neg) 430 [M−$H^+$] (100%), 466 (27%), 490 [M+$OAc^-$] (37%), 439 (14%), 544 (14%), 861 [2M−$H^+$] (37%).

*2-Deoxy-2-amino-1β-[m-iodo]benzyl cellobioside (**19**)*

To a soln of **15** (100 mg, 171 μmol) in MeOH/water: 9/1 (2 mL), triphenyl phosphine (89 mg, 342 μmol) was added. After stirring for 2 d at rt, the mixture was concentrated in vacuo and purified by column chromatography (eluent: isopropanol/water/saturated aq. ammonia: 18/2/1), yielding **19** as a white powder (89 mg, 94%).

Brutoformula: $C_{19}H_{28}O_{10}NI$ (MW = 557); mp 130°C; $[\alpha]_D^{23}$: –32 ($c$ = 0.1; MeOH); Rf (isopropanol/water/saturated aq. ammonia: 18/2/1): 0.40; $^1$H NMR (500 MHz; $D_2O$): 7.93 (s, 1H), 7.82 (d, 1H, $J$ = 8.2 Hz), 7.50 (d, 1H, $J$ = 7.7 Hz), 7.24 (dd, 1H, $J$ = 7.7 and 8.2 Hz), 4.96 (d, 1H, $J$ = 11.7 Hz), 4.77 (d, 1H, $J$ = 11.7 Hz), 4.60 (d, 1H, $J$ = 8.2 Hz), 4.54 (d, 1H, $J$ = 8.0 Hz), 4.03 (dd, 1H, $J$ = 1.9 and 9.5 Hz), 3.94 (dd, 1H, $J$ = 2.0 and 9.5 Hz), 3.86 (dd, 1H, $J$ = 5.0 and 9.5 Hz), 3.75 (dd, 1H, $J$ = 5.0 and 9.5 Hz), 3.70 (dd, 1H, $J$ = 9.5 and 9.5 Hz), 3.60 (ddd, 1H, $J$ = 1.9, 5.0, and 9.5 Hz), 3.58 (dd, 1H, $J$ = 9.5 and 10.0 Hz), 3.54 (dd, 1H, $J$ = 9.5 and 9.5 Hz), 3.51 (ddd, 1H, $J$ = 2.0, 5.0, and 9.5 Hz), 3.44 (dd, 1H, $J$ = 9.5 and 9.5 Hz), 3.33 (dd, 1H, $J$ = 8.2 and 9.5 Hz), 2.82 (dd, 1H, $J$ = 8.0 and 10.0 Hz) ppm; MS: $m/z$ (pos) 324 (15%), 558 [M+H$^+$] (100%), 580 [M+Na$^+$] (23%), 1015 [2M+H$^+$] (46%), 1016 (13%), 1037 [2M+Na$^+$] (12%); $m/z$ (neg) 556 [M−H$^+$] (100%), 592 (17%), 616 [M+OAc$^-$] (31%), 770 (12%), 1113 [2M−H$^+$] (28%).



## Enzyme Assays

The cellulases CBH I, EG I, EG II, and CenA were kindly supplied by J. Ståhlberg (Uppsala University, Zweden), M. Siika-aho (VTT, Finland), R. Fagerström (Röhm Enzyme, Finland), and J.-P. Belaich (Université Marseille I, France), respectively. Their activity was determined using 2-chloro-4-nitrophenyl β-lactoside (CBH I and EG I), β-cellobioside (CenA), or β-cellotrioside (EG II) as substrate.[21] The release of the chromophore was measured continuously in a microplate reader (EL 808, Bio-Tek Instruments) at 405 nm. All experiments were performed in a 0.1 M sodium phosphate buffer at pH 5.7 and 37°C. The substrate and inhibitor concentrations were varied between 1/5 and 5× the value of $K_M$ and $K_I$, respectively. The inhibition constants were calculated from Dixon plots.[27] The measurements were performed in triplicate and had a CV of less than 10%. Hydrolysis of the inhibitors was checked by means of HPAEC-PAD, using a CarboPac PA-100 column, and the settings supplied by the manufacturer (Dionex, California).

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