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Circular Dichroic Studies of 2-Amino-2-deoxy-galactopyranosides - Conformations of the 2-(*N*-Acetyl-*p*-bromobenzamido) Group

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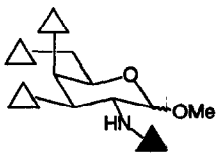
This paper is dedicated to the memory of Günther Snatzke.

Abstract : The CD spectra of methyl 2-amino-2-deoxy-D-galactopyranoside mono-*N*-acylates (acetyl or *p*-bromobenzoyl) resemble those of the corresponding *O*-acylates and can be accounted for by the additivity rule. However, the CD of pyranosides containing the *N*-acetyl-*p*-bromobenzamido imide group (NAcBz) are far more complex than those of mono-*N*-acylates and their *O*-counterparts, and furthermore, the solvent- and temperature-dependent changes differ for the α- and β-anomers. These differences can be accounted for by the different conformations of the 2-NAcBz group.

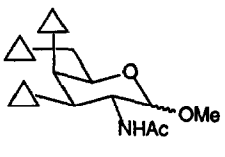
Introduction

The CD exciton chirality method offers a versatile approach for determining the absolute configuration and conformation of a variety of molecules in solution.[1] When the electric transition moments of two chromophores interact through space, they give rise to "split" CD curves, the signs of which depend on the chiral environment of the two chromophores. Furthermore, an additivity relation[2,3] exists in multiple chromophoric systems, in which the observed CD curve is simply the sum of all pairwise interactions, i.e., the observed CD of a system comprising three interacting chromophores ABC is the sum of the CDs arising from A/B, B/C and A/C interactions. This additivity relation is valid independent of whether the interacting chromophores are the same or not.

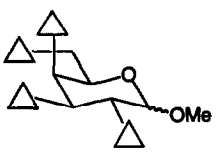
Ongoing studies with amino sugars to improve and develop the microscale CD method for structural determination of oligosaccharides[3] led to the preparation of the per-*p*-bromobenzoates of methyl 2-acetamido-2-deoxy-α- and β-D-galactopyranoside (α- and β-D-GalNAcBBBB),[4] compounds **7** and **8**, respectively. The two compounds exhibited unexpected CD curves which could not be rationalized by the additivity rule. CD measurements under various conditions and MM calculations of a series of methyl 2-amino-2-deoxy-D-galactopyranosides containing *N*-acetyl-*p*-bromobenzamido (NAcBz) group have led to the conclusion that the anomalies are due to the conformational differences of the 2-NAcBz group.



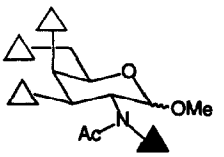
1, α-OMe
2, β-OMe



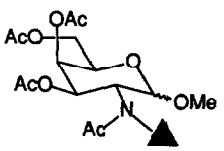
3, α-OMe
4, β-OMe



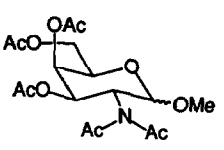
5, α-OMe
6, β-OMe



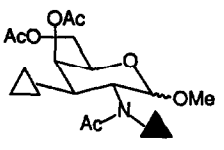
7, α-OMe
8, β-OMe



9, α-OMe
10, β-OMe



11, α-OMe
12, β-OMe



13, α-OMe
14, β-OMe

△ = *p*-Br-C_6H_4-CO-O-
▲ = *p*-Br-C_6H_4-CO-

Results and Discussion

Mono-*N*-acylates. Unless otherwise stated the UV and CD data are for MeCN, rt, wavelengths in nm, Δε in parentheses (clear extrema due to exciton coupling are denoted in bold).

α-D-GalNBBBB **1**: UV 243 CD **232 (-18.7), 250 (+54.8)**; A +74.
β-D-GalNBBBB **2**: UV 243 CD **234 (-20.0), 250 (+60.9)**; A +81.
α-D-GalNAcBBB **3**: UV 244 CD 227 (+4.0), **239 (-0.6), 254 (+13.9)**; A +15.
β-D-GalNAcBBB **4**: UV 244 CD 226 (+3.5), **242 (-0.5), 255 (+10.5)**; A +11.
α-D-GalBBBB **5**: (MeOH) UV 244 CD **237 (-29), 253 (+70)**; A +99.[5]
β-D-GalBBBB **6**: (MeOH) UV 244 CD **237 (-26), 252 (+74)**; A +100.[5]

As shown by **1 / 2**, and by **5 / 6**, the C-1 configuration of tetrachromophoric methyl pyranosides exert a relatively minor effect on the CD (Fig. 1).[3] The A values for **1** and **2** are similar, and smaller than those of oxygen analogs. Not surprisingly, the CD data of *p*-bromobenzamides **1 / 2** differ somewhat from those of benzoates **5 / 6**. Upon lowering the temperature to -80°C, the A value (*in MeOH*) of **1** increased from +77 → +91 and that of **2** from +78 → +96, thus becoming similar to those of **5** and **6** at room temperature. The first Cotton effects (CE) of α-D-GalNAcBBB **3** and β-D-GalNAcBBB **4** at 255 nm are red-shifted from the

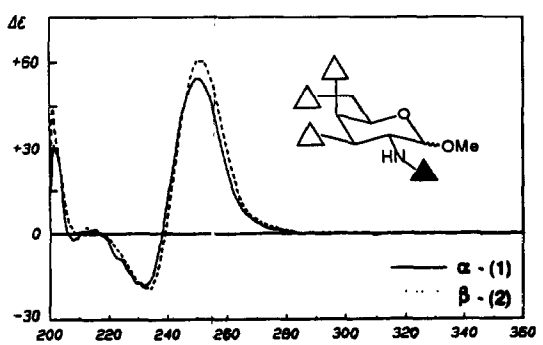


Fig. 1. CD of α - **1** and β - **2** in acetonitrile.

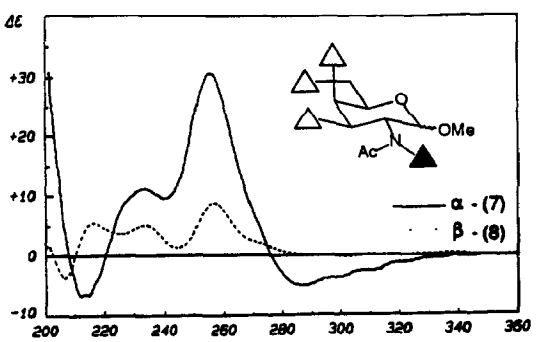


Fig. 2. CD of α - **7** and β - **8** in acetonitrile.

normal 250 nm,[1] and the second CEs are quite small. The reason why the first Cotton effect is bathochromically shifted and the second CE is buried could be the superimposition of the n→π* transition of the amide chromophore. The observed A values of +15 for **3** and +11 for **4** match the summation value of +8 estimated from the Gal pairwise interactions.[6]

N,N-Diacylates.

α-D-Gal**NAcBBBB 7**:	UV 245	CD 212 (-7.2), 233 (+11.5), 255 (+31.0), 286 (-5.3).
β-D-Gal**NAcBBBB 8**:	UV 245	CD 207 (-4.5), 216 (+5.8), 234 (+5.5), 257 (+8.9).
8: (MeOH)	UV 245	CD 216 (+9.7), 235 (+11.3), 257 (+6.4), 298 (-1.0).
8: (MeOH, -80°C)		CD 205 (-10.7), 216 (+10.3), 236 (+20.2), 253 (-2.3), 273 (-5.8).
8: (P5M1)	UV 245	CD 214 (+10.2), 233 (+18.4), 250 (-23.0).
8: (P5M1, -80°C)		CD 214 (+21.9), 236 (+35.3), 254 (-24.8), 295 (-2.4).
α-D-Gal**NAcBAAA 9**:	UV 258	CD 211 (-13.1), 242 (+17.5), 259sh (+11.6), 287 (-5.1).
β-D-Gal**NAcBAAA 10**:	UV 249	CD 238 (-14.2), 276 (+4.6), 294sh (+2.2).
α-D-Gal**NAcBBAA 13**:	UV 248	CD 210 (-16.4), 234 (+10.2), 257 (+23.0), 285 (-6.1), 311sh (-3.5), 322sh (-2.1).
β-D-Gal**NAcBBAA 14**:	UV 246	CD 208 (-7.3), 218 (-0.5), 233sh (-4.9), 240 (-5.7), 263 (+6.0), 289sh (+1.2).

Despite the similarity in UV spectra with absorption maxima at 245 nm and inflections at 285 nm, the CD curves of α- and β-D-Gal**NAcBBBB** (**7** and **8**, Fig. 2) are totally different. They are also different from the non-acetylated analogs, α-D-Gal**NBBBB 1** and β-D-Gal**NBBBB 2**. α-D-Gal**NAcBBBB 7** has a unique negative CE at 286 nm, which is also seen for other α-anomers (**9** and **13**). Whereas the CD of α-anomer **7** is not affected much by experimental conditions, the CD of β-anomer **8** revealed striking solvent- and temperature-dependence. The changes, however, are too complicated for clear-cut interpretations because of the involvement of three further aromatic chromophores in addition to the 2-NAcBz group. These aspects are discussed later with β-anomer **14** (Fig. 6).

^{1}H NMR spectrum of the β-anomer **8** showed split signals in a 2.5 : 1 ratio (in $CDCl_3$, methanol or acetonitrile) suggesting the presence of conformational equilibria. This was confirmed by heating the sample, upon which the signals collapsed to a single set. The same phenomenon has also been observed for the other β-anomers **10** and **14**. In contrast, the α-anomers **7**, **9** and **13** showed no splitting ($CDCl_3$). Very similar coupling constants of the ring protons between α- and the corresponding two sets of β-anomers ruled out the possibility of inverted ring conformations. Therefore, the orientation of the 2-NAcBz group must be responsible for the different NMR behavior of α- and β-anomers.

The simpler per-acetates Gal**NAcBAAA**, **9** and **10**, were therefore prepared in order to understand the chiroptical properties of the 2-NAcBz chromophore. Both compounds absorb in the red compared to the normal *p*-bromobenzamide chromophore (λ_{max} 240 nm); furthermore, the λ_{max} of the α-anomer is 9 nm red-shifted from the β-anomer. These trends reflect the different chromophoric nature of the imide group, NAcBz, and also indicate that the conjugation is stronger in the α-anomer **9** than in the β-anomer **10**. The two compounds exhibit CD curves of opposite signs (Fig. 3). Different conformations of the 2-NAcBz group are thus responsible for the CD differences. The CD of cyclic imides show strong substituent dependence,[7] supporting this conclusion. The CD spectra of compounds **9** and **10** represent several transitions, mainly, the

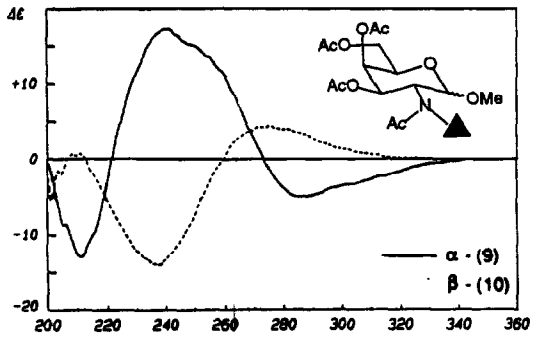


Fig. 3. CD of α - **9** and β - **10** in acetonitrile.

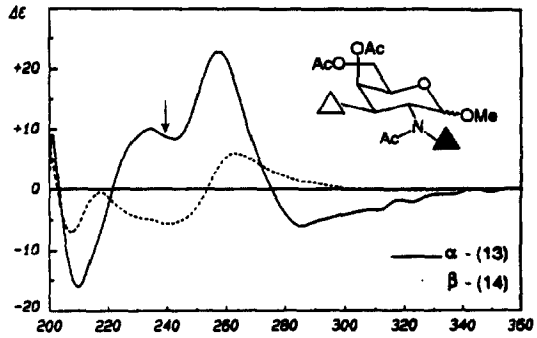
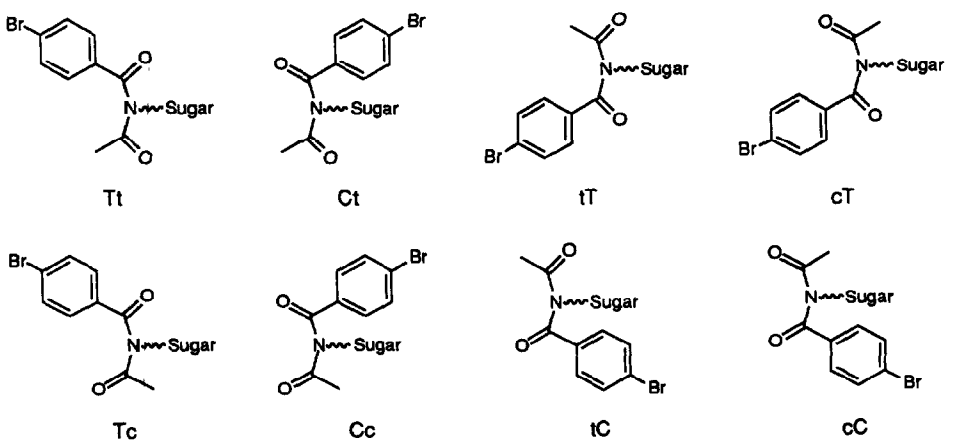


Fig. 4. CD of α - **13** and β - **14** in acetonitrile.

p-bromobenzamide ^1L_b (280 nm) and ^1L_a (240 nm) of opposite signs, as well as the n→π* transition displayed in α-anomer **9** as a shoulder around 260 nm. Similarly, a rigid PhCO-N-$COCH_3$ chromophore fused to a cholestane skeleton at C-2 and C-3 exhibited a ^1L_b band at 288 nm (Δε = -12), a ^1L_a band at 237 nm (Δε = +12) and a strong n→π* transition at 268 nm (Δε = -16).[8]

MM calculations[9] were performed for the simplest anomeric pair **9** and **10** and for α- and β-GalNAcAAAA (**11** and **12**) in order to better understand the conformational behavior of the 2-NAcBz group. Since the conformations of acetates are similar to those of benzoates, and calculations do not need the more complex π-minimization, acetates **9** and **10** were used instead of the more complicated per-*p*-bromobenzoates **7** and **8**. While two conformers (ct and tt)[10] were found for the model compound **11**, only one (ct) was found for the corresponding β-anomer **12**. As for compounds **9** and **10**, the various confomers of the NAcBz group are depicted in Scheme I. In all cases the 2-NAcBz group adopts the more stable perpendicular orientation with respect to the pyranoside ring.



Scheme I

The results from MM calculations show that the top four conformers (Tt, tT, Ct and cT) are favored over the bottom four (Tc, tC, Cc and cC). This is in agreement with the fact that the cis-cis conformation is

the least favored for the imide group.[11] Although a clear-cut correlation between various conformers and CD spectra cannot be expected, MM calculations seem to indicate that the four favored conformers (Tt, tT, Ct and cT) should be considered in analyzing the CD data.

α- and β-D-GalNAcBBAA (13 and 14). Molecular models of the four main conformers showed that the interaction of ^1L_a electric transition moments of the 2-NAcBz chromophore and the C-3 *p*-bromobenzoate group should lead to a negative exciton couplet only in conformer Ct, a positive exciton couplet in conformer Tt, while weak positive couplets as well as non-coupled contributions would be expected from the other two conformers (tT and cT). In order to experimentally check these points, we have prepared α- and β-D-GalNAcBBAA, **13** and **14**, and analyzed their CD spectra for possible exciton interactions between the 2-NAcBz and the C-3 *p*-bromobenzoate chromophores. The CD data of compounds **13** and **14** are shown in Fig. 4. α-D-GalNAcBBAA **13** exhibited a positive CE around 257 nm, assigned to the first CE of the exciton couplet (second CE at 240 nm, see arrow in Fig. 4), together with a positive CE around 235 nm and a broad negative CE at a longer wavelength. In consistence with other α-anomers, the CD of **13** was not affected much by either solvent or temperature. This positive couplet suggests that the NAcBz group favors conformers Tt / tT / cT.

Comparison of the α-anomers **7** and **13** with α-D-GalNAcBAAA **9** revealed that the broad negative Cotton effect at *ca.* 285 nm in these compounds is due to the weak ^1L_b band of the NAcBz group, and that the positive band around 235 nm arises from a non-coupled ^1L_a transition. This indicates that in these three compounds the 2-NAcBz group adopts a similar conformation. Thus, subtraction of the CD spectrum of **9** from that of compound **13** led to the expected positive CD couplet centered at 250 nm (CH_3CN: 259 nm, Δε +11.2; 241 nm, Δε -9.0; A value +20) (Fig. 5). The resulting positive exciton contribution is approximately one third that of methyl 2,3-di-*O*-(*p*-bromobenzoyl)-galactopyranoside derivative (A value +73). Similarly, based on the general validity of pairwise additivity in exciton-coupled systems,[2] subtraction of the CD of **13** from that of **7** led to a spectrum similar in intensity and shape (MeCN: 254 nm, Δε +9.3) to that of α-D-GalNAcBBB **3** (254 nm, Δε +13.9). Therefore, the total contribution of the NAcBz chromophore to the CD spectra of α-anomers **7** and **13** is the sum of its non-coupled CE and a small net positive exciton couplet.

The β-anomer **14**, with an equatorial 1-OMe, showed a much higher degree of solvent- and temperature-dependence. In polar sovents (MeCN, MeOH) it exhibited a positive CD couplet of a much smaller intensity than its corresponding α-anomer **13** (Fig. 4), while in the non-polar solvent system P5M1 (isopentane / methylcyclohexane = 5 : 1) the sign of the couplet changed to negative (Fig. 6). These experimental observations suggest the presence of tT, cT, Tt and Ct confomers in polar solvents and the dominance of this last one in the non-polar solvent system P5M1. Namely, when the solvent polarity changes from polar to non-polar, the conformational population of the Ct conformer increases at the expense of the Tt / tT / cT.

The important anisotropic shieldings observed in the ^1H NMR spectra (in $CDCl_3$) of compounds **7**, **8**, **9**, **10** , **13** and **14** are consistent with the previous results derived from the CD studies. Thus, the 1-methoxy groups of the α-anomers **7**, **9** and **13** (δ 3.09, 3.07 and 3.05 ppm) are shielded as compared with those of the corresponding major / minor components of β-anomers **8**, **10** and **14** (δ 3.59 / 3.48, 3.50 / 3.44 and 3.55 / 3.45 ppm) confirming that the NAcBz chromophore is adopting the tT or cT conformations in this solvent.

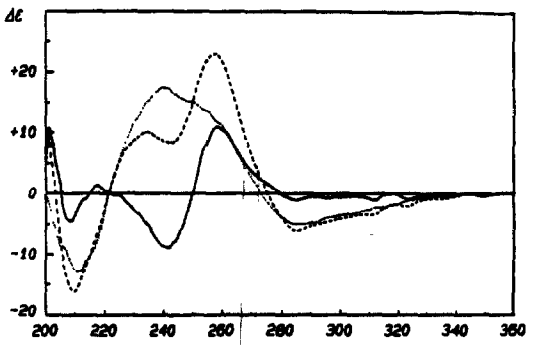


Fig. 5. CD of α - **9** (·····), α - **13** (----) and the difference spectrum (**13** - **9**) (——) in acetonitrile.

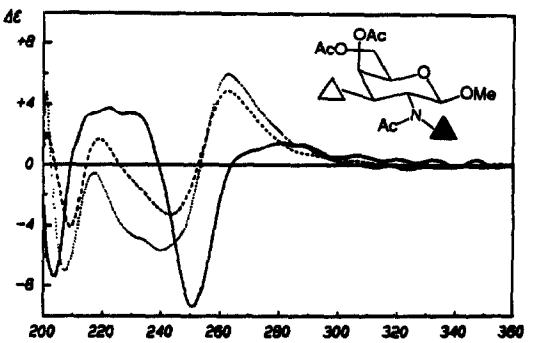


Fig. 6. CD of β - **14** in different solvents; CH_3CN (·····), CH_3OH (----) and isopentane / methylcyclohexane (5 : 1) (——).

However, in those of the β-anomers (**8**, **10** and **14**) the proton H-2 in the major component is shielded (δ 4.50, 4.20 and 4.45 ppm) with respect to the same proton in the minor component (δ 5.19, 4.63 and 5.02 ppm, respectively) and therefore, in agreement with a Ct conformer.

The following trends for the 2-NAcBz group in α- and β-anomers can be deduced based on the CD data measured under different conditions and the NMR studies. Namely, first for α-anomers, conformers Tt / tT / cT dominate over Ct in polar as well as in non-polar solvents. Second, for β-anomers, the conformational population of the Ct conformer dominates in both types of solvents. The small positive exciton couplet observed in polar solvents can be explained by a higher net positive contribution of Tt / tT / cT conformers versus the negative one from the Ct conformer.

Although slightly higher dipole moments have been reported for imides in the cis-trans conformation than in the less stable trans-trans conformation,[11] comparative studies of *N*-benzoyl with *N*-acetyl lactams revealed the importance of the benzene ring in altering the dipole moment. Thus, that conformer Ct is favored in non-polar solvents is consistent with the previous results.

Conclusion

These results demonstrate that in exciton-coupled systems, the effect of configurations of nearby substituents is rather small, except when non-bonded steric interactions with the chromophoric system are present. The uncommon and complex features of the CD of methyl 2-(*N*-acetyl-*p*-bromobenzamido)-2-deoxy-D-galactopyranosides can be accounted for by conformational analysis of the 2-NAcBz imide group, which leads to exciton-coupled as well as non-coupled Cotton effects. The conformation of the NAcBz group is affected by the configuration at the anomeric center, the conformationally locked α-anomers giving rise to CD spectra which are much less influenced by solvent and temperature as compared with those of the more flexible β-anomers.

Experimental Section

General. ^1H NMR spectra were obtained at 250 and 400 MHz. MMX force field was used for molecular mechanics calculations.[9] UV spectra were performed on a Perkin Elmer Model 550D. CD spectra were recorded on a JASCO J-600 spectropolarimeter. Low temperature CD measurements were obtained by incorporating a Jobin-Yvon Variocryostat system to the spectropolarimeter. High resolution mass spectroscopies (h.r.m.s.) were measured on a JEOL JMS-DX303HF Mass Spectrometer. Prior to

measurement of CD spectra, all compounds were purified by HPLC with a μ-Porasil column, 30 cm x 7.8 mm ID (EtOAc / n-Hexane solvent systems). The concentrations of the CD samples were ascertained from the UV spectra by using the standard ε values: 19500, 38200, 57200 and 76400 respectively for the mono-, bis-, tris- and tetrakis(*p*-bromobenzoate).[3] Density correction was realized in all CD low-temperature measurements.

Methyl 2-acetamido-3,4,6-tri-*O*-(*p*-bromobenzoyl)-2-deoxy-α-D-galactopyranoside (α-D-GalNAcBBB)[3] (3) : To a solution of methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (7.0 mg) in 250 μL of anhydrous pyridine was added *p*-bromobenzoyl chloride (14.6 mg), AgOTf (17.1 mg) and a catalytic amount of DMAP. The reaction mixture was stirred at room temperature for 12 h, then quenched with two drops of water, concentrated to dryness. The desired product was purified with silica gel preparative TLC (R_f 0.63, Benzene / Acetone = 2 / 1). ^{1}H NMR (250 MHz, $CDCl_3$): 1.86 (s, 3 H, OAc), 3.44 (s, 3 H, OCH_3), 4.33 (dd, 1 H, J = 10.1, 5.3 Hz, 6-H), 4.43 (dd, 1 H, J = 6.4, 5.3 Hz, 5-H), 4.52 (dd, 1 H, J = 10.1, 6.4 Hz, 6-H'), 4.81-4.93 (m, 2 H, 1-H and 2-H), 5.47 (dd, 1 H, J = 10.8, 3.1 Hz, 3-H), 5.75 (d, 1 H, J = 9.3 Hz, NH), 5.82 (d, 1 H, J = 2.6 Hz, 4-H), 7.43 (d, 2 H, J = 8.4 Hz, aromatic), 7.51-7.67 (m, 6 H), 7.82 (d, 2 H, J = 8.4 Hz), 7.90 (d, 2 H, J = 8.4 Hz). h.r.m.s. (FAB): $[M + H]^+$ 781.9219 (calcd. 781.9236 for $C_{30}H_{27}O_9NBr_3$).

Methyl 2-acetamido-3,4,6-tri-*O*-(*p*-bromobenzoyl)-2-deoxy-β-D-galactopyranoside (β-D-GalNAcBBB)[3] (4) : Same procedure was also used to prepared this compound, which shows R_f 0.63 (Benzene / Acetone = 2 / 1) on silica gel TLC plate. ^{1}H NMR (250 MHz, $CDCl_3$): 1.88 (s, 3 H, OAc), 3.57 (s, 3 H, OCH_3), 4.19-4.39 (m, 3 H, 2-H, 5-H and 6-H), 4.61 (dd, 1 H, J = 11.0, 6.3 Hz, 6-H'), 4.76 (d, 1 H, J = 8.3 Hz, 1-H), 5.48 (d, 1 H, J = 8.7 Hz, NH), 5.61 (dd, 1 H, J = 11.2, 3.3 Hz, 3-H), 5.81 (d, 1 H, J = 3.1 Hz, 4-H), 7.44 (d, 2 H, J = 8.5 Hz, aromatic), 7.54 (d, 2 H, J = 8.5 Hz), 7.59-7.66 (m, 4 H), 7.83 (d, 2 H, J = 8.5 Hz), 7.91 (d, 2 H, J = 8.4 Hz). h.r.m.s. (FAB): $[M + H]^+$ 781.9258 (calcd. 781.9236 for $C_{30}H_{27}O_9NBr_3$).

Methyl 2-(*N*-acetyl-*p*-bromobenzamido)-3,4,6-tri-*O*-(*p*-bromobenzoyl)-2-deoxy-α-D-galacto-pyranoside (α-D-GalNAcBBBB) (7) : Similar to the procedure for compound **3** except that more *p*-bromobenzoyl chloride was added and that the reaction time was longer (24 h). R_f = 0.61 (Hexane / EtOAc = 2 / 1). ^{1}H NMR (250 MHz, $CDCl_3$): 1.80 (s,3 H, OAc), 3.09 (s, 3 H, OCH_3), 4.31 (dd, 1 H, J = 9.7, 4.5 Hz, 6-H), 4.45-4.58 (m, 2 H, 5-H and 6-H'), 5.04 (d, 1 H, J = 3.2 Hz, 1-H), 5.64 (dd, 1 H, J = 11.8, 3.2 Hz, 2-H), 5.98 (d, 1 H, J = 3.7 Hz, 4-H), 6.07 (dd, 1 H, J = 11.8, 3.0 Hz, 3-H), 7.43 (d, 2 H, J = 8.4 Hz, aromatic), 7.51 (d, 2 H, J = 8.4 Hz), 7.56-7.59 (m, 6 H), 7.67 (d, 2 H, J = 8.5 Hz), 7.79 (d, 2 H, J = 8.5 Hz), 7.87 (d, 2 H, J = 8.5 Hz). h.r.m.s. (FAB): $[M + H]^+$ 963.8635 (calcd. 963.8603 for $C_{37}H_{30}O_{10}NBr_4$).

Methyl 2-(*N*-acetyl-*p*-bromobenzamido)-3,4,6-tri-*O*-(*p*-bromobenzoyl)-2-deoxy-β-D-galacto-pyranoside (β-D-GalNAcBBBB) (8) : Similar to the procedure for compound **4** except that more *p*-bromobenzoyl chloride was added and that the reaction time was longer (24 h). R_f = 0.57 (Hexane / EtOAc = 2 / 1). ^{1}H NMR (250 MHz, $CDCl_3$):[12] Major component, 2.16 (s, 3 H, OAc), 3.59 (s, 3 H, OCH_3), 5.47 (d, 1 H, J = 7.8 Hz, 1-H), 5.82 (d, 1 H, J = 3.0 Hz, 4-H), 6.16 (dd, 1 H, J = 10.9, 3.2 Hz, 3-H); Minor component, 1.78 (s, 3 H, OAc), 3.48 (s, 3 H, OCH_3), 5.17 (1 H, 1-H), 5.19 (1 H, 2-H), 5.90 (1 H, 4-H), 6.02 (1 H, 3-H). h.r.m.s. (FAB): $[M + H]^+$ 963.8588 (calcd. 963.8603 for $C_{37}H_{30}O_{10}NBr_4$).

Methyl 2-(*p*-bromobenzamido)-3,4,6-tri-*O*-(*p*-bromobenzoyl)-2-deoxy-α-D-galactopyranoside (α-D-GalNBBBB)[3] (1) : This compound was prepared by treating compound **7** (5.0 mg) with 4 M HCl / dioxane (1 mL) and MeOH (1 mL). The reaction mixture was stirred at room temperature for 48 h. The

product was purified with silica gel preparative TLC (R_f = 0.43, Hexane / EtOAc = 2 / 1). ^{1}H NMR (400 MHz, $CDCl_3$): 3.47 (s, 3 H, OCH_3), 4.37 (dd, 1 H, *J* = 10.6, 5.6 Hz, 6-H), 4.48 (t, 1 H, *J* = 6.3 Hz, 5-H), 4.56 (dd, 1 H, *J* = 10.6, 6.5 Hz, 6-H'), 5.03-5.06 (m, 2 H, 1-H and 2-H), 5.64 (dd, 1 H, *J* = 10.8, 3.3 Hz, 3-H), 5.88 (d, 1 H, *J* = 2.4 Hz, 4-H), 6.40 (d, 1 H, *J* = 9.6 Hz, NH), 7.40 (d, 2 H, *J* = 8.6 Hz, aromatic), 7.51-7.65 (m, 6 H), 7.84 (d, 2 H, *J* = 8.6 Hz), 7.96 (d, 2 H, *J* = 8.6 Hz). h.r.m.s. (FAB): $[M + H]^+$ 921.8445 (calcd. 921.8497 for $C_{35}H_{28}O_9NBr_4$).

Methyl 2-(*p*-bromobenzamido)-3,4,6-tri-*O*-(*p*-bromobenzoyl)-2-deoxy-β-D-galactopyranoside (β-D-GalNBBBB) (2) : This compound was prepared by treating galactosamine per-*p*-bromobenzoate with TMS-OTf and MeOH. D-Galactosamine hydrochloride was first per-*p*-bromobenzoylated with *p*-bromobenzoyl chloride, AgOTf and DMAP in pyridine, from which 2-(*p*-bromobenzamido)-1,3,4,6-tetra-*O*-(*p*-bromobenzoyl)-2-deoxy-galactopyranoside was obtained. The per-*p*-bromobenzoate (5.0 mg) was then treated with TMS-OTf (80 μL), CH_2Cl_2 (500 μL) and MeOH (20 μL) at room temperature for 3 h. The desired product was purified with silica gel preparative TLC (R_f 0.28, Hexane / EtOAc = 2 / 1). ^{1}H NMR (250 MHz, $CDCl_3$): 3.57 (s, 3 H, OCH_3), 4.25 (dd, 1 H, *J* = 6.7, 6.1 Hz, 5-H), 4.32 (dd, 1 H, *J* = 11.1, 6.7 Hz, 6-H), 4.51 (ddd, 1 H, *J* = 11.0, 8.7, 8.4 Hz, 2-H), 4.63 (dd, 1 H, *J* = 11.1, 6.1 Hz, 6-H'), 4.80 (d, 1 H, *J* = 8.4 Hz, 1-H), 5.69 (dd, 1 H, *J* = 11.0, 3.1 Hz, 3-H), 5.85 (d, 1 H, *J* = 3.1 Hz, 4-H), 6.12 (d, 1 H, *J* = 8.7 Hz, NH), 7.39 (d, 2 H, *J* = 8.6 Hz, aromatic), 7.49-7.63 (m, 10 H), 7.83 (d, 2 H, *J* = 8.5 Hz), 7.95 (d, 2 H, *J* = 8.4 Hz). h.r.m.s. (FAB): $[M + H]^+$ 921.8467 (calcd. 921.8497 for $C_{35}H_{28}O_9NBr_4$).

Methyl 2-(*N*-acetyl-*p*-bromobenzamido)-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside (α-D-GalNAcBAAA) (9) : Methyl 2-acetamido-2-deoxy-α-D-galactopyranoside was per-acetylated with acetic anhydride and DMAP in pyridine to give methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside. The per-acetate (11.4 mg) was then stirred with *p*-bromobenzoyl chloride (20.7 mg), AgOTf (24.3 mg) and a catalytic amount of DMAP in 500 μL of anhydrous pyridine. The product was purified with silica gel preparative TLC (R_f = 0.45, Hexane / EtOAc = 1 / 1). ^{1}H NMR (250 MHz, $CDCl_3$): 1.87, 1.94, 2.02, 2.15 (s, 3 H, OAc), 3.07 (s, 3 H, OCH_3), 4.09 (m, 2 H, 6-H and 6-H'), 4.13 (m, 1 H, 5-H), 4.91 (d, 1 H, *J* = 3.4 Hz, 1-H), 5.18 (dd, 1 H, *J* = 12.0, 3.4 Hz, 2-H), 5.52 (d, 1 H, *J* = 3.1 Hz, 4-H), 5.73 (dd, 1 H, *J* = 12.0, 3.1 Hz, 3-H), 7.60 (m, 4 H, aromatic). h.r.m.s. (FAB): $[M + H]^+$ 544.0825 (calcd. 544.0818 for $C_{22}H_{27}O_{10}NBr$).

Methyl 2-(*N*-acetyl-*p*-bromobenzamido)-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (β-D-GalNAcBAAA) (10) : Starting with methyl *N*-acetyl-2-amino-2-deoxy-β-D-galactopyranoside, the same procedure as that for compound **9** was applied to this compound. The product shows R_f = 0.52 (Hexane / EtOAc = 1 / 1) or 0.82 (Benzene / Acetone = 2 / 1). ^{1}H NMR (250 MHz, $CDCl_3$):[12] Major component, 3.50 (s, 3 H, OCH_3), 5.27 (d, 1 H, *J* = 8.0 Hz, 1-H), 5.35 (d, 1 H, *J* = 2.8 Hz, 4-H), 5.75 (dd, 1 H, *J* = 11.1, 3.0 Hz, 3-H); Minor component, 3.44 (s, 3 H, OCH_3), 4.63 (dd, 1 H, *J* = 11.1, 8.4 Hz, 2-H), 5.00 (d, 1 H, *J* = 8.4 Hz, 1-H), 5.45 (d, 1 H, *J* = 2.2 Hz, 4-H), 5.61 (dd, 1 H, *J* = 11.1 Hz, 2.6, 3-H). h.r.m.s. (FAB): $[M + H]^+$ 544.0818 (calcd. 544.0818 for $C_{22}H_{27}O_{10}NBr$).

Methyl 2-(*N*-acetyl-*p*-bromobenzamido)-4,6-di-*O*-acetyl-3-*O*-(*p*-bromobenzoyl)-2-deoxy-α-D-galactopyranoside (α-D-GalNAcBBAA) (13) : Methyl 2-acetamido-2-deoxy-α-galactopyranoside was partially *p*-bromobenzoylated. Among the many products, methyl 2-acetamido-3-*O*-(*p*-bromobenzoyl)-2-deoxy-α-D-galactopyranoside was isolated and then per-acetylated with acetic anhydride and DMAP to offer

methyl 2-acetamido-4,6-di-*O*-acetyl-3-*O*-(*p*-bromobenzoyl)-2-deoxy-α-D-galactopyranoside, followed by *p*-bromobenzoylation with *p*-bromobenzoyl chloride (10 mg), AgOTf (10 mg) and a catalytic amount of DMAP in 500 μL of anhydrous pyridine. Compound **13** was then purified with silica gel preparative TLC (R_f = 0.87, Benzene / Acetone = 2 / 1). ^{1}H NMR (400 MHz, $CDCl_3$): 1.82, 2.02, 2.08 (s, 3 H, OAc), 3.05 (s, 3 H, OCH_3), 4.11 (m, 2 H), 4.22 (t, 1 H), 4.93 (d, 1 H, J = 3.5 Hz, 1-H), 5.48 (dd, 1 H, J = 11.9, 3.5 Hz, 2-H), 5.67 (d, 1 H, J = 3.3 Hz, 4-H), 5.92 (dd, 1 H, J = 11.9, 3.3 Hz, 3-H), 7.53 (d, 2 H, J = 8.5 Hz, aromatic), 7.59 (s, 4 H), 7.78 (d, 2 H, J = 8.6 Hz). h.r.m.s. (FAB): $[M + H]^+$ 684.0051 (calcd. 684.0080 for $C_{27}H_{28}O_{10}NBr_2$).

Methyl 2-(*N*-acetyl-*p*-bromobenzamido)-4,6-di-*O*-acetyl-3-*O*-(*p*-bromobenzoyl)-2-deoxy-β-D-galactopyranoside (β-D-GalNAcBBAA) (14) : Starting with methyl 2-acetamido-2-deoxy-β-D-galactopyranoside, the same procedure as that for compound **13** was applied to this compound. The product shows R_f = 0.30 (Hexane / EtOAc = 2 / 1). ^{1}H NMR (400 MHz, $CDCl_3$):[12] Major component, 3.55 (s, 3 H, OCH_3), 4.45(dd, 1 H, J = 11.2, 8.0 Hz, 2-H), 5.37 (d, 1 H, J = 8.0 Hz, 1-H), 5.53 (d, 1 H, J = 3.0 Hz, 4-H), 6.01 (dd, 1 H, J = 11.2, 3.4 Hz, 3-H); Minor component, 3.45 (s, 3 H, OCH3), 5.02 (1 H, 2-H), 5.03 (1 H, 1-H), 5.59 (d, 1 H, J = 2.6 Hz, 4-H), 5.88 (dd, 1 H, J = 7.8, 2.4 Hz, 3-H). h.r.m.s. (FAB): $[M + H]^+$ 684.0048 (calcd. 684.0080 for $C_{27}H_{28}O_{10}NBr_2$).

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12. These compounds show splitting signals, therefore, only part of the spectra are assigned.