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Synthesis and Antiproliferative Evaluation of 2-Deoxy-*N*-glycosylbenzotriazoles/imidazoles

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Abstract: A series of 2-deoxy-2-iodo-α-D-mannopyranosylbenzotriazoles was synthesized using the benzyl, 4,6-benzylidene and acetyl protected D-glucal in the presence of *N*-iodosuccinimide (NIS). Subsequent removal of the iodine at the C-2 position using tributyltin hydride under free radical conditions afforded the 2-deoxy-α-D-glucopyranosylbenzotriazoles in moderate to high yields. This method was extended to the preparation of substituted 2-deoxy-β-D-glucopyranosylimidazoles as well. The stereoselectivity of the addition reaction and the effect of the protecting group and temperature on anomer distribution of the benzotriazole series were also investigated. The anticancer properties of the newly synthesized compounds were evaluated in a series of viability studies using HeLa (human cervical adenocarcinoma), human breast and lung cancer cell lines. The *N*-[3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-1*H*-benzotriazole and the *N*-[3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl]-2*H*-benzotriazole were found to be the most potent cancer cell inhibitors at 20 μM concentrations across all four cell lines.

Keywords: benzotriazole; D-glucal; cytotoxic activity; stereochemistry; heterocycles

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1. Introduction

Benzotriazoles are a versatile class of heterocycles with a wide range of applications and usages including antibacterial [1], antiviral [2] and antiparasitic [3], among others [4]. Compounds containing the benzotriazole moiety have been reported to possess powerful effects in inhibiting cell proliferation and arresting cancer development. For example, the [2-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-benzotriazole]-dichlorocopper (II) complex has been reported to have very potent superoxide dismutase (SOD) activity inhibiting and preventing cancer cell growth [5]. Benzotriazole-containing alkanoic acids have also been demonstrated to be agonists of peroxisome proliferator-activated receptors (PPARs) and, as such, are capable of inducing apoptosis in multiple cell lines and in vivo tumors [6]. Carbohydrate-based benzotriazoles have also been found to inhibit solid tumor growth in mice. This includes the acetyl protected glucose and *N*-acetylglucosamine benzotriazole derivatives [7]. Substituted rybofuranosyl benzotriazoles and benzoimidazoles have also been reported to be powerful protein kinase CK2 inhibitors [8]. While the mechanism of action of the ribose derivatives is well-documented [9], little is known about the mode of action of *N*-glycosyl benzotriazoles.

Interest in the generation of 2-deoxy analogs of compound **I** arose from the abundance of reports on the biological activity displayed by 2-deoxy-D-glucose. This compound has

been shown to significantly slow down cancer metabolism and induce cell death [10,11], yet cell viability studies using 2-deoxy-D-glucopyranosylbenzotriazoles cannot be found in the current literature. Various methods exist in the literature for the chemical synthesis of 2-deoxy sugars. Early work in this area focused on the chemical synthesis of 2-deoxy-β-glycosides [12,13]. Glycals have been used extensively as building blocks in the preparation of 2-deoxy sugars via their hydration or hydro-alkoxylation catalyzed by methanolic hydrogen halide [14–16]. Other methods include the use of methane sulfonic acid as catalyst [17], enzymes [16,18], alkoxymercuration of a glycal followed by reduction with sodium borohydride [19,20] and halohydration followed by dehydrogenation [21–23]. More closely related to this study is the method developed by Kashyap and co-workers via the reaction of various protected D-glucal with a mixture of tetra-*n*-butylammonium iodide with sodium periodate to afford 2-deoxyglycosides [24].

We have previously reported the synthesis of the benzyl protected *N*-glycosyl benzotriazole **I** (Figure 1) using glycosyl halides and silver triflate (AgOTf) as starting materials. Compound **I** effectively inhibited growth of HeLa (cervical adenocarcinoma) cells (86%, 100 μM) [25]. The goal of this study was to chemically synthesize a series of analogs of compound **I** and evaluate their antiproliferative and cytotoxic effects using four different established human cancer cell lines (A549, HeLa, HCC827 and MDA-MB-231). Our initial efforts went into the generation of the known *N*-glycosyl benzotriazoles **1–4** (Figure 1) and the study of the effects of the protecting groups of the sugar on their viability as cancer drugs. Our laboratory also became interested in the chemical synthesis of various substituted 2-deoxy-D-glucopyranosylbenzotriazole/imidazoles **5–14** (Figure 1) and their antiproliferative properties were studied as well.

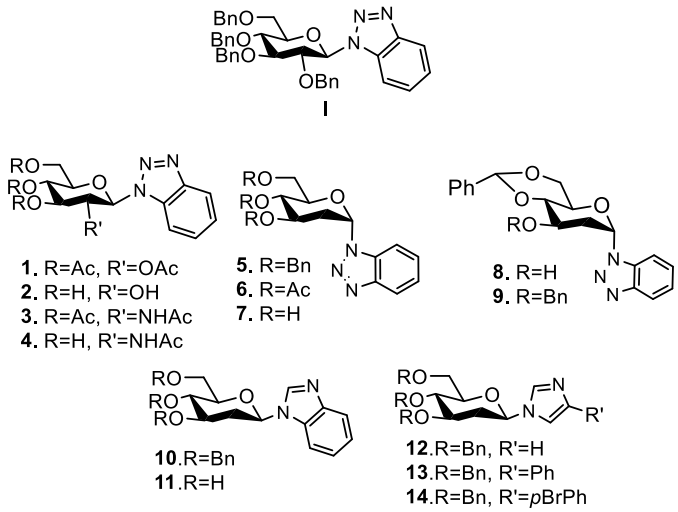
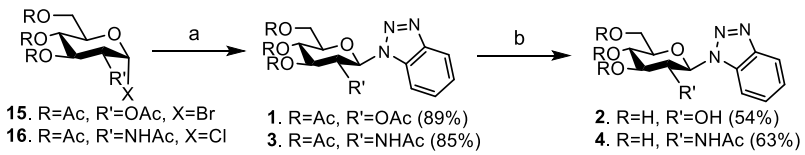


Figure 1. Targeted compounds.

2. Results and Discussion

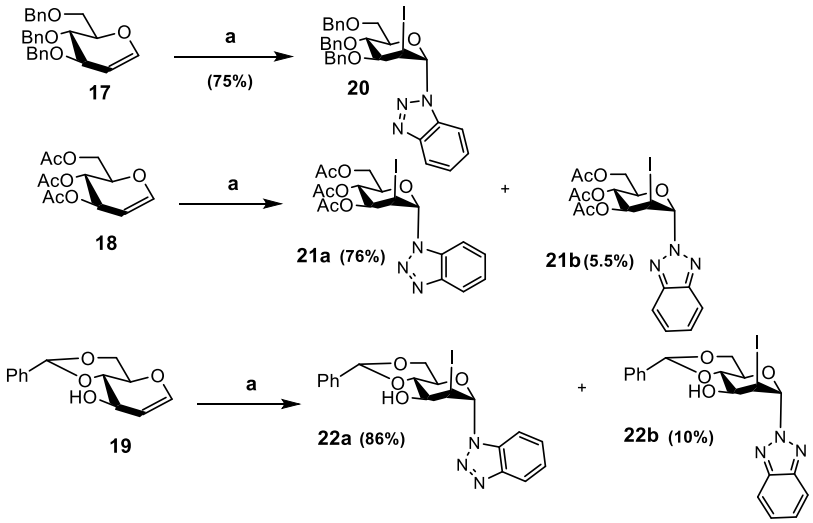
Our work began with the synthesis of compounds **1–4** using an established procedure employing commercially available acetyl protected glycosyl halides **15** and **16** (Scheme 1) as starting materials [7].



Scheme 1. Addition of benzotriazoles to glycosyl halides. Reagent and conditions: (a) benzotriazole/AgOTf/ACN/rt/2 h; (b) $NaOCH_3/CH_3OH$.

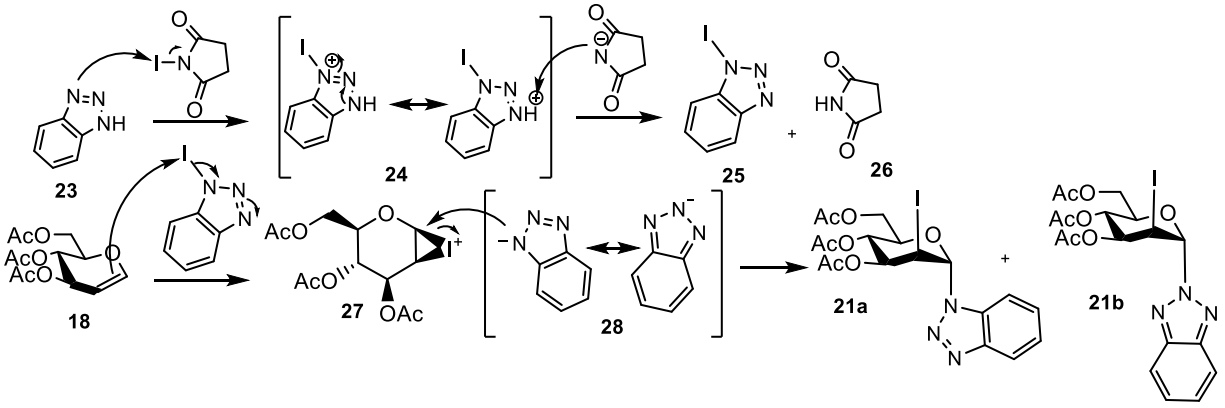
Compounds **1–4** were initially tested using a range of concentrations (data not shown) in a series of viability studies using HeLa cell line. None of these compounds were capable of inhibiting cell growth, even at a concentration of 100 μM. We hypothesized that perhaps a lack of lipophilicity, especially for analogs **2** and **4** when compared to compound **I** (Figure 1), may have played a role in the uptake and crossing of the cell membrane in our cell assay giving rise to the poor results observed. Interestingly, this pattern was observed throughout our study, where the benzyl protected sugar outperformed the acetyl, benzylidene and fully deprotected constructs in subsequent viability studies.

In the next phase of our study, we proceeded to synthesize our 2-deoxy sugar analog series starting with the benzyl protected compound **5** (Figure 1). Our laboratory has extensive experience preparing 2-deoxy-*N*-glycosides and has previously reported the synthesis of compound **5** [25]. Access to compounds **5–10** (Figure 1) was achieved using 2-deoxy-2-iodo-α-mannopyranosylbenzotriazoles as precursors (Scheme 2). The benzyl protected D-glucal gave the α-mannose 1*H*-*N*-glycosylbenzotriazole as the single isomer **20** ($^3J_{1\text{-}2}$ = 4.0 Hz) [25], while a 13:1 (1*H*:2*H*-benzotriazole) ratio of the 2-deoxy-2-iodo-α-mannose isomer **21a** and **21b** was obtained for the acetyl protected sugar. This type of benzotriazole isomerization has been previously described in the literature [26]. In the case of the 4,6-benzylidene protected sugar, the 2-deoxy-2-iodo-α-mannose 1*H*-*N*-glycosylbenzotriazole isomer **22a** was obtained as the major product, while less than 10% of the 2-deoxy-2-iodo-α-mannose 2*H* isomer **22b** was isolated after column chromatography (Scheme 2). The addition reaction was repeated at 0 °C and room temperature and very similar product ratios were obtained for the acetyl and benzylidene protected sugar. The best yields were obtained when the reaction was refluxed for 2 h for the acetyl and 4,6-benzylidene protected D-glucal. Product ratios were determined by measuring the integration values of the anomeric protons of the crude mixture using ^{1}H-NMR acetone-d_6.



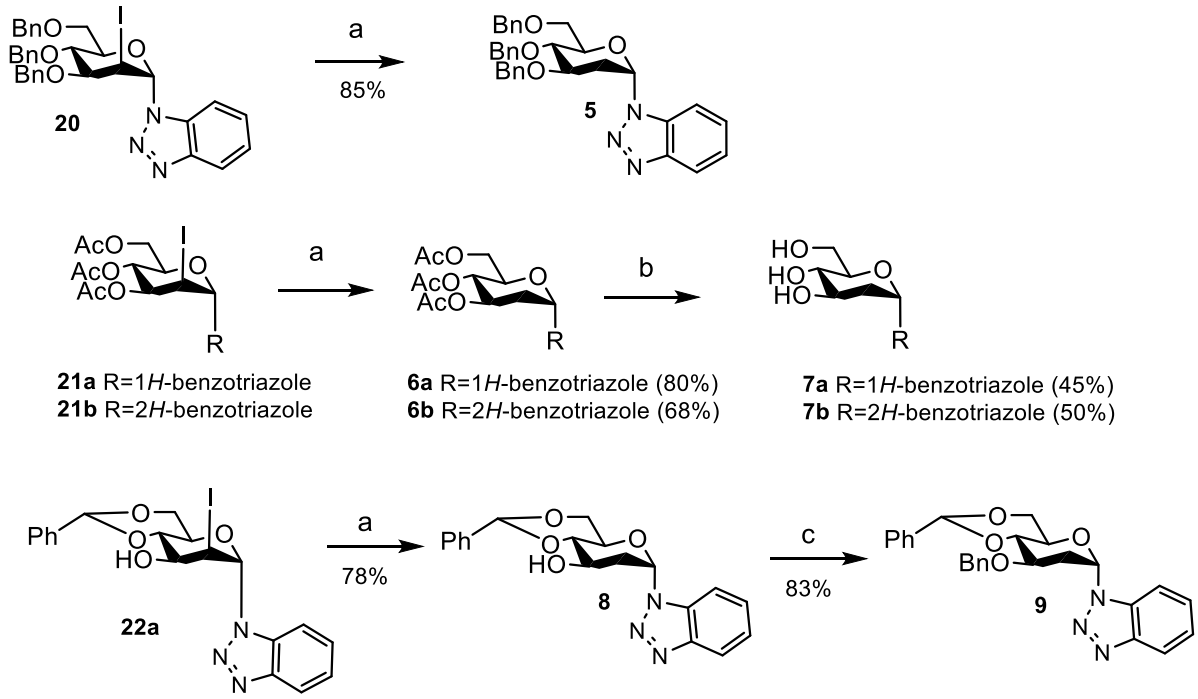
Scheme 2. Addition of benzotriazole to glycal. Reagent and conditions: (a) NIS/benzotriazole/propionitrile/reflux/2 h. Yields were calculated as isolated yields.

It is our hypothesis that the addition reaction of the benzotriazole to the acetyl and 4,6-benzylidene protected D-glucal may follow a mechanism very similar to the well documented addition of amides to glycals using NIS as the halogen source (Scheme 3) [27–29]. We envision the more nucleophilic benzotriazole **23** reacting with NIS generating intermediate **25**. The newly formed compound **25** delivers the iodine to the acetyl protected D-glucal **18**. Although the formation of compound **27** defies the majority rule [30,31], such an intermediate has been proposed in the isolation of the α-mannose isomer when alcohols are employed as the nucleophile in the presence of NIS [32–34]. Nucleophilic attacks by the benzotriazole anion from the bottom face of the three membered ring iodonium ion **27** give rise to the 2-deoxy-2-iodo-α-mannose isomers **21a** and **21b**.



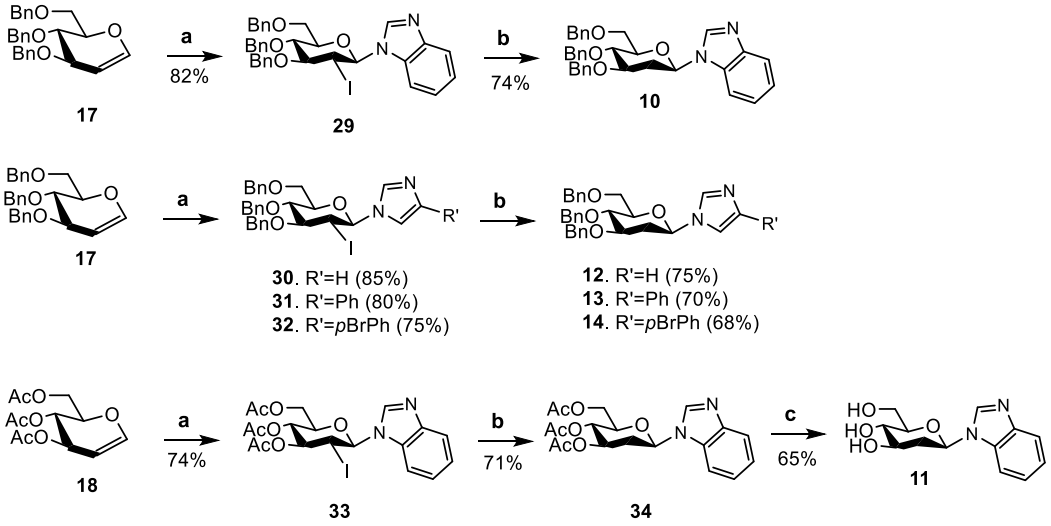
Scheme 3. Formation of the 1*H* and 2*H*-glycosylbenzotriazole.

Preparation of compounds **5–9** was accomplished via the removal of the iodine using tributyltin hydride (Bu_3SnH) and 2,2′-azobis(2-methylpropionitrile) (AIBN) in toluene under refluxing conditions (Scheme 4).



Scheme 4. Preparation of 2-deoxy-α-D-glycosylbenzotriazole. Reagent and conditions: (a) Bu_3SnH/AIBN/toluene/reflux/2 h; (b) $NaOCH_3/CH_3OH$; (c) NaH/BnBr/THF/rt.

Access to the benzyl protected 2-deoxy-β-D-glucopyranosylimidazoles **10–14** (Scheme 5) was achieved under free radical dehalogenation conditions using a procedure previously published by our laboratory [25]. We employed benzoimidazole and other commercially available substituted imidazoles. The addition reaction of the imidazole series resulted in the β-anomer **29–33** (Scheme 5) being the exclusive product in high to moderate yields. Such results can be explained based on the tendency of nitrogen glycosides to favor the equatorial and not axial position in virtue of the reverse anomeric effect [35].



Scheme 5. Preparation of 2-deoxy-β-D-glycosylimidazoles. Reagent and conditions: (a) NIS/imidazole/propionitrile/reflux/2 h; (b) Bu_3SnH/AIBN/toluene/reflux/2 h; (c) $NaOCH_3/CH_3OH$.

The newly synthesized compounds **5–14** were initially tested for antiproliferative activity in a series of viability studies using HeLa cells. Those that demonstrated cytotoxicity are included in Figure 2. Cells were treated with the synthesized compounds at 10 μM (grey bars) and 100 μM (black bars) concentrations to determine which compounds exhibit cytotoxic effects before further evaluation.

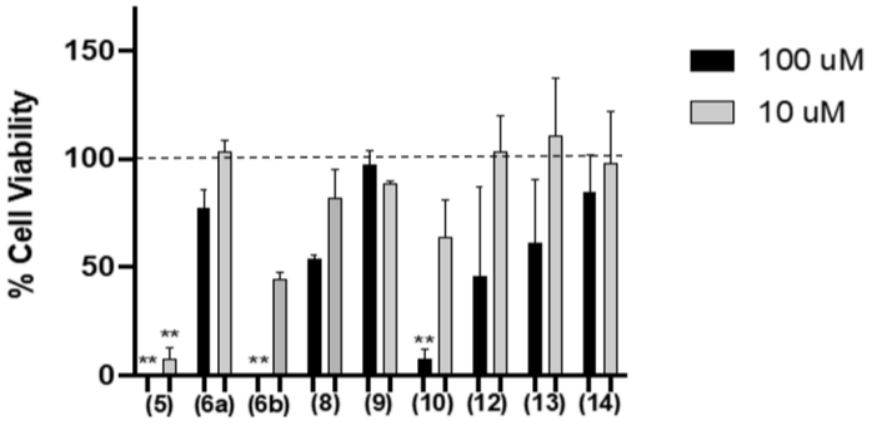
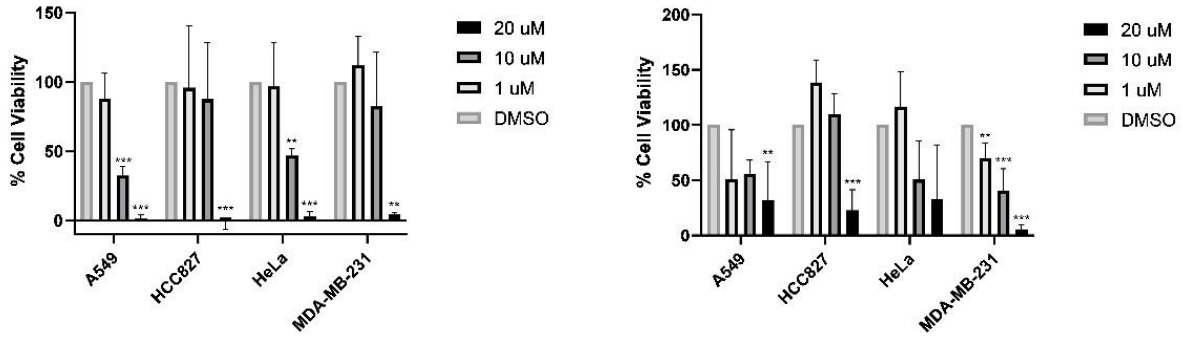


Figure 2. HeLa cell viability studies. Cells were treated with either a DMSO control (dashed line) or the synthesized compounds at a concentration of 100 μM (black bars) or 10 μM (grey bars).

Our results suggest that the benzyl protected 1*H*-2-deoxy-α-D-glucopyranosylbenzotriazole **5** was the most potent of all compounds and is capable of complete inhibition of cell viability at 100 μM and by 92% (8% survival) at 10 μM concentration. Our results also show a striking contrast in activity between the acetyl protected 1*H* (**6a**) and 2*H*-2-deoxy-α-D-glucopyranosylbenzotriazole **6b**. For compound **6b** (0%, 100μM) and (45%, 10 μM) cell survival was observed, while for compound **6a** (78%, 100 μM) and (100%, 10 μM), respectively. When comparing compound **5** and **6a** (acetyl protected analogue), we can observe a significant drop off in activity even at concentrations as high as 100 μM. As previously stated, we believe this may be attributed to the enhanced lipophilicity provided by the benzyl protecting group found in compound **5**, perhaps making it more effective at crossing cell membranes. More importantly, our results show that the isomer **6b** is as effective as compound **5** in killing our cancer cell lines at 100 μM, even with the acetyl protecting group present. We also observed moderate activity for the 4,6-benzylidene protected compound **8** (54%, 100 μM). Benzylation of the hydroxyl group at the C-3 position

rendered compound **9** completely ineffective. From the glycoimidazole series, compound **10** showed the most promising results in terms of (8%, 100 μM) survival rate. The remaining substituted imidazoles gave very poor results at our tested concentrations. It should be worth mentioning that all the fully deprotected compounds showed no activity at either concentration (results not shown).

Next, we set out to determine the cytotoxicity of compounds **5** and **6b** using established lung epithelial cancer cell lines A549 and HCC827 as well as the established metastatic breast cancer cell lines MDA-MB-231 and compared them to HeLa cells (Figure 3). Compounds **5** and **6b** proved to be excessively toxic at 100 μM concentrations, resulting with a zero percent survival rate. A dose–response analysis was performed to determine the appropriate concentration of compound required to inhibit the growth of cancer cells by 50% (IC_{50}) in HeLa cells before proceeding. The IC_{50} was determined to be 2.908 μM for compound **5** and 9.940 μM for compound **6b**. DMSO was used as a control and the cells were treated similarly as in Figure 2, this time using 20 μM, 10 μM and 1 μM to determine their potency at lesser concentrations in other established cancer cell lines. On average, there was less than 5% survival rate across all cell lines tested at 20 μM for compound **5** and less than 24% for compound **6b**. The effectiveness of both compounds **5** and **6b** decreased as concentrations were lowered to 10 μM and 1 μM.



(**a**) (**b**)

Figure 3. Viability studies using compound **5** (**a**) and compound **6b** (**b**).

3. Materials and Methods

3.1. Chemistry

All ^{1}H and ^{13}C NMR spectra were recorded on an ECS 400 MHz JEOL spectrometer. ^{1}H NMR spectra were recorded in acetone-d_6 and are referenced to residual $(CH_3)_2CO$ at δ = 2.04 ppm and the ^{13}C NMR spectra are referenced to the peak at δ = 205 ppm. ^{1}H NMR spectra recorded in $CDCl_3$ are referenced to residual $CHCl_3$ at δ = 7.24 ppm and ^{13}C NMR spectra are referenced to the central peak of $CDCl_3$ at δ = 77.0 ppm (See Supplementary Materials). Assignments were made by standard gCOSY and gHSQC experiments. HRMS data were obtained with a Bruker Ultraflex MALDI-TOF mass spectrometer. Tri-*O*-benzyl-D-glucal, tri-*O*-acetyl-D-glucal and *N*-iodosuccinimide (NIS) were purchased from Aldrich. Heterocycles were purchased from Aldrich Chemical Co. 4,6-*O*-Benzylidene-D-glucal was purchased from Carbosynth Ltd. All column chromatography was performed on silica gel 60 (EM Science, 70–230 mesh). Reactions were monitored by TLC on Kieselgel 60 F254 (EM Science) and the compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH_3CH_2CN, CH_2Cl_2 was distilled from CaH_2 and stored over molecular sieves (3 Å).

General Synthetic Procedures

Synthesis of compound **1–4** was performed as previously described [25]. Synthesis of the *N*-[2-deoxy-2-iodo-α-D-mannopyranosyl]-1*H*-benzotriazole series of compounds **20**, **21a**, **21b, 22a** and **22b** was performed as follows. D-glucal **17, 18** and **19** (1.83 mmol) was diluted in freshly distilled propionitrile (5 mL) followed by the addition of benzotriazole (3.67 mmol). NIS (2.74 mmol) was added to the reaction mixture and refluxed for 2 h. The reaction was cooled to room temperature and quenched with deionized water. The crude mixture was diluted in dichloromethane (50 mL) and washed with saturated $Na_2S_2O_3$ solution (100 mL) and (3 × 100 mL) deionized water. The organic layer was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude mixture was purified by flash column chromatography on silica gel (hexane/ethyl acetate eluent mixture).

Preparation of 2-deoxy-α-D-glycosylbenzotriazole series **5–9** was performed as follows. Compound **20**, **21a, 21b** and **22a** (1.38 mmol) was diluted in toluene (5 mL). Tributyltin hydride (2.76 mmol) was added dropwise followed by AIBN (1% by weight) and the resulting mixture was refluxed for 1 h. The reaction was allowed to cool down to room temperature and diluted in dichloromethane (50 mL). The organic layer was washed with deionized water (3 × 100 mL) and dried with $MgSO_4$, followed by the subsequent removal of the solvent under reduced pressure. The crude mixture was purified by flash column chromatography on silica gel (hexane/ethyl acetate eluent mixture).

N-[3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl]-1H-benzotriazole (**5**), The crude mixture (0.51 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 5:1, *v/v*) to give a clear oil (0.40 g, 85%). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.01 (d, 1H, J = 8.0 Hz, Ar), 7.88 (d, 1H, J = 8.4 Hz, Ar), 7.39–7.29 (m, 9H, Ar), 7.28–7.23 (m, 8H, Ar), 6.56 (d, 1H, $J_{1,2}$ = 5.6 Hz, H-1), 4.76 (dd, 2H, J = 12.8 Hz, J = 10.8 Hz, CH_2-Ph), 4.66 (d, 1H, J = 12.6 Hz, CH_2-Ph), 4.52 (d, 1H, J = 10.8 Hz, CH_2-Ph), 4.41–4.29 (m, 3H, H-3, CH_2-Ph), 3.63-3.58 (m, 2H, H-4, H-6′), 3.47 (d, 1H, $J_{5,4}$ = 10.8 Hz, H-5), 3.25 (dd, 1H, $J_{2,3}$ = 8.0 Hz, $J_{2,1}$ = 4.8 Hz, H-2), 3.12 (dd, 1H, $J_{6,5}$ = 5.2 Hz, $J_{6,6'}$ = 4.2 Hz, H-6), 2.21–2.16 (dd, 1H, $J_{2',3}$ = 8.5 Hz, $J_{2',1}$ = 4.4 Hz, H-2′) ppm. ^{13}C NMR (125 MHz, acetone-d_6) δ 146.27, 137.9, 137.6, 137.5, 133.4, 128.25, 127.82, 127.74, 127.8, 127.61, 126.2, 111.61, 82.11, 77.60, 77.47, 74.33, 70.45, 72.81, 71.33, 69.04, 32.28 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{33}H_{33}N_3NaO_4$ 558.2369; found 558.2365 $[M + Na]^+$.

N-[3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl]-1H-benzotriazole (**6a**), The crude mixture (0.59 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 3:1, *v/v*) to give a white solid (0.43 g, 80%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.95 (d, 1H, J = 8.0 Hz, Ar), 7.75 (d, 1H, J = 8.1 Hz, Ar), 7.50 (t, 1H, J = 8.1 Hz, J = 7.9 Hz, Ar), 7.35 (t, 1H, J = 8.2 Hz, J = 7.9 Hz, Ar), 6.58 (d, 1H, $J_{1,2}$ = 5.6 Hz, H-1), 5.81 (m, 1H, 3-H), 5.01 (dd, 1H, $J_{4,3}$ = 9.6 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 4.10 (dd, 1H, $J_{6,5}$ = 6.8 Hz, $J_{6,6'}$ = 5.6 Hz, H-6), 3.79 (dd, 1H, $J_{6',5}$ = 6.5 Hz, $J_{6',6}$ = 5.8 Hz, H-6′), 3.33 (m, 1H, H-5), 3.12 (dd, 1H, $J_{2,1}$ = 6.0 Hz, $J_{2,2'}$ = 2.5 Hz, H-2), 2.42–2.39 (ddd, 1H, $J_{2',3}$ = 7.6 Hz, $J_{2',1}$ = 5.6 Hz, $J_{2',2}$ = 2.5 Hz, H-2′), 1.91–1.06 (s, 9H, CH_3) ppm. ^{13}C NMR (125 MHz, acetone-d_6) δ 169.72, 169.45, 146.27, 132.96, 127.95, 124.55, 119.59, 111.20, 81.15, 70.41, 69.18, 68.97, 61.81, 32.04, 20.07, 19.85, 19.77 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{18}H_{21}N_3NaO_7$ 414.1277; found 414.1276 $[M + Na]^+$.

N-[3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl]-2H-benzotriazole (**6b**), The crude mixture (0.062 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 3:1, *v/v*) to give a white solid (0.028 g, 68%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.81 (d, 2H, J = 8.0 Hz, Ar), 7.36 (dd, 2H, J = 7.9 Hz, Ar), 6.56 (d, 1H, $J_{1,2}$ = 5.6 Hz, H-1), 5.97 (dd, 1H, $J_{3,4}$ = 8.9 Hz, $J_{3,2}$ = 7.0 Hz, H-3), 5.06 (dd, 1H, $J_{4,5}$ = 9.4 Hz, $J_{4,3}$ = 9.2 Hz, H-4), 4.11 (dd, 1H, $J_{6,5}$ = 9.4 Hz, $J_{6,6'}$ = 4.8 Hz, H-6), 3.84–3.80 (m, 2H, H-5, H-6′), 2.84 (dd, 1H, $J_{2,1}$ = 5.0 Hz, $J_{2,2'}$ = 2.4 Hz, H-2), 2.37 (ddd, 1H, $J_{2',3}$ = 7.0 Hz, $J_{2',1}$ = 6.0 Hz, $J_{2',2}$ = 3.0 Hz, H-2′), 1.89–1.79 (s, 9H, CH_3) ppm. ^{13}C NMR (125 MHz, acetone-d_6) δ 169.24, 169.45, 145.00, 132.96, 127.95, 124.55, 119.59, 111.20, 81.15, 70.41, 69.18, 68.97, 61.81, 32.04, 29.63, 29.04, 28.85, 28.47, 20.07, 19.85, 19.77 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{18}H_{21}N_3NaO_7$ 414.1277; found 414.1276 $[M + Na]^+$.

*N-[2-deoxy-α-*D*-glucopyranosyl]-2H-benzotriazole* (**7a**). The crude mixture (0.301 g) was purified by flash column chromatography on silica gel ($CHCl_3/CH_3OH$, 9:1, *v/v*) to give a white solid (0.13 g, 45%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.90 (d, 1H, J = 8.0 Hz, Ar), 7.83 (t, 1H, J = 10.4 Hz, J = 8.1 Hz, Ar), 7.47 (t, 1H, J = 7.6 Hz, J = 7.2 Hz, Ar), 7.36 (t, 1H, J = 7.6 Hz, J = 7.6 Hz, Ar), 6.43 (d,1H, $J_{1,2}$ = 5.2 Hz, H-1), 4.30–4.24 (m, 1H, H-3), 3.62–3.54 (m, 2H, H-6), 3.38 (dd, 1H, $J_{4,5}$ = 10.0 Hz, $J_{4,3}$ = 9.6 Hz, H-4), 3.05 (dd, 1H, $J_{2,1}$ = 5.2 Hz, $J_{2,2'}$ = 2.1 Hz, H-2), 2.82–2.79 (m, 1H, H-5), 2.19–2.11 (ddd, 1H, $J_{2',3}$ = 7.0 Hz, $J_{2',2}$ = 6.1 Hz, $J_{2',1}$ = 5.2 Hz, H-2′) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ 145.69, 133.01, 127.78, 124.74, 118.51, 111.64, 82.88, 75.23, 71.16, 68.94, 60.93, 34.51 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{12}H_{15}N_3NaO_4$ 288.0960; found 288.0957 $[M + Na]^+$.

*N-[2-deoxy-α-*D*-glucopyranosyl]-2H- benzotriazole* (**7b**). The crude mixture (0.041 g) was purified by flash column chromatography on silica gel ($CHCl_3/CH_3OH$, 9:1, *v/v*) to give a white solid (0.019 g, 50%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.61 (dd, 2H, J = 3.2 Hz, J = 3.2 Hz, Ar), 8.18 (dd, 2H, J = 2.8 Hz, J = 2.8 Hz, Ar), 7.26 (d, 1H, $J_{1,2}$ = 5.6 Hz, H-1), 5.28–5.24 (m, 1H, H-3), 4.49–4.44 (m, 2H, H-6), 4.24 (dd, $J_{4,3}$ = 9.6 Hz, $J_{4,5}$ = 9.2 Hz, H-4), 4.12–4.08 (m, 1H, H-5), 3.61 (dd, 1H, $J_{2,1}$ = 5.2 Hz, $J_{2,2'}$ = 2.8 Hz, H-2), 2.99–2.91 (ddd, 1H, $J_{2',3}$ = 7.6 Hz, $J_{2',2}$ = 6.0 Hz, $J_{2',1}$ = 5.1 Hz, H-2′) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ 144.08, 126.91, 118.00, 88.37, 76.21, 71.06, 68.38, 60.94, 35.16 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{12}H_{15}N_3O_4$ 265.1063; found 288.0960 $[M + Na]^+$.

*N-[4,6-O-benzylidene-3-hydroxyl-2-deoxy-α-*D*-glucopyranosyl]-1H-benzotriazole* (**8**). The crude mixture (0.41 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 2:1, *v/v*) to give a white solid (0.28 g, 78%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.93 (d, 1H, J = 7.6 Hz, Ar), 7.80 (d, 1H, J = 8.0 Hz, Ar), 7.40 (t, 1H, J = 8.2 Hz, J = 2.0 Hz, Ar) 7.27–7.25 (m, 6H, Ar), 6.54 (dd, 1H, $J_{1,2}$ = 5.6 Hz, 1-H), 5.54 (s, 1H, C*H*-Ph), 4.71 (d, 1H, J = 4.0 Hz, OH), 4.58 (dd, 1H, $J_{3,4}$ = 7.2 Hz, $J_{3,2}$ = 6.0 Hz, 3-H), 3.85 (d,1H, $J_{4,3}$ = 6.8 Hz, $J_{4,5}$ = 5.2 Hz, 4-H), 3.63 (m, 2H, 5-H, 6-H), 3.03 (dd,1H, $J_{2,1}$ = 5.6 Hz, $J_{2,2}$ = 3.8 Hz, 2-H), 2.29 (dd, 1H, $J_{2',1}$ = 5.5 Hz, $J_{2',2}$ = 4.0 Hz, H-2′) ppm. ^{13}C NMR (125 MHz, acetone-d_6) δ 146.23, 138.24, 133.03, 128.74, 127.44, 127.86, 126.45, 124.40, 119.54, 111.09, 101.58, 83.40, 82.37, 68.07, 65.88, 65.77, 65.28, 35.26 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{19}H_{19}N_3O_4$ 353.1376; found 354.1454 $[M + H]^+$.

*N-[4,6-O-benzylidene-3-O-benzyl-2-deoxy-α-*D*-glucopyranosyl]-1H-benzotriazole* (**9**). The crude mixture (0.81 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 2:1, *v/v*) to give a white solid (0.58g, 83%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.94 (d, 1H, J = 8.0 Hz, J = 5.2 Hz, Ar), 7.72 (d, 1H, J = 8.4 Hz, Ar), 7.49–7.45 (m, 1H, Ar), 7.36–7.31 (m, 5H, Ar), 7.29–7.14 (m, 5H, Ar), 7.16 (t, 1H, J = 8.2 Hz, J = 6.1 Hz, Ar), 6.58 (d, 1H, $J_{1,2}$ = 5.6 Hz, H-1), 5.62 (s, 1H, CHPh), 4.73 (dd, 2H, J = 12.6 Hz, J = 10.5 Hz, CH_2Ph), 4.55–4.50 (m, 1H, H-3), 3.91–3.84 (m, 2H, H-4, H-5), 3.71 (t, 1H, $J_{6,5}$ = 10.4 Hz, $J_{6,6'}$ = 10.4 Hz, H-6), 3.19–3.08 (m, 2H, H-2, H-6), 2.36–2.29 (m, 1H, H-2′) ppm. ^{13}C NMR (125 MHz, acetone-d_6) δ 146.20, 138.15, 128.71, 128.20, 128.02, 127.92, 127.55, 127.34, 126.24, 124.45, 119.57, 111.02, 101.25, 82.89, 82.18, 73.75, 72.27, 68.12, 65.25, 33.71 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{26}H_{25}N_3O_4$ 443.1845; found 444.1923 $[M + H]^+$.

*N-[3,4,6-tri-O-benzyl-2-deoxy-β-*D*-glucopyranosyl]-1H-benzoimidazole* (**10**). The crude mixture (0.62 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 2:1, *v/v*) to give a white solid (0.39g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H, NC*H*N), 7.71 (d, 1H, J = 5.2 Hz, Ar), 7.41 (d, 1H, J = 4.0 Hz, Ar), 7.26–7.16 (m, 18H, Ar), 5.44 (d, 1H, $J_{1,2}$ = 10.8 Hz, H-1), 4.87 (d, 1H, J = 10.8 Hz, CH_2Ph), 4.61 (dd, 2H, J = 11.6 Hz, J = 10.2 Hz, CH_2Ph), 4.49–4.39 (m, 3H, CH_2Ph), 3.81–3.75 (m, 1H, H-3), 3.73–3.60 (m, H-4, H-5, H-6), 2.47 (dd, 1H, $J_{2,1}$ = 9.6 Hz, $J_{2,2'}$ = 2.8 Hz, H-2), 2.25 (dd, 1H, $J_{2,1}$ = 11.6 Hz, $J_{2,2'}$ = 3.8 Hz, H-2′) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.00, 139.46, 137.21, 137.12, 137.03, 131.96, 127.61, 127.51, 126.84, 122.51, 121.93, 119.61, 110.26, 80.43, 78.60, 77.02, 74.39, 72.61, 71.09, 67.88 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{34}H_{34}N_2O_4$ 534.2519; found 535.2597 $[M + H]^+$.

*N-[2-deoxy-β-*D*-glucopyranosyl]-1H-benzoimidazole* (**11**). The crude mixture (0.75 g) was purified by flash column chromatography on silica gel ($CHCl_3/CH_3OH$, 9:1 *v/v*) to give a

white solid (0.31g, 65%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (d, 1H, J = 7.2 Hz, NC*H*N), 7.75 (d, 1H, J = 8.4 Hz, Ar), 7.26–7.19 (m, 2H, Ar), 6.10 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 4.00–3.91 (m, 1H, H-3), 3.67–3.59 (m, 2H, H-6), 3.38 (dd, 1H, $J_{4,3}$ = 8.8 Hz, $J_{4,5}$ = 8.8 Hz, H-4), 2.93–2.90 (m, 1H, H-5), 2.83 (dd, 1H, $J_{2,1}$ = 9.0 Hz, $J_{2,2'}$ 4.4 Hz, H-2), 2.18–2.13 (m, 1H, H-2′) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ 142.90, 141.41, 133.43, 123.42, 122.82, 118.63, 112.75, 80.59, 74.64, 70.85, 68.77, 60.73, 34.06 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{13}H_{16}N_2O_4$ 264.1110; found 265.1188 $[M + H]^+$.

*N-[3,4,6-tri-O-benzyl-2-deoxy-β-*D*-glucopyranosyl]-1H-4-phenylimidazole* (**13**). The crude mixture (0.51 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 3:1 *v/v*) to give a white oil (0.32 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, 1H, J = 7.6 Hz, Ar), 7.68 (s, 1H, NC*H*N), 7.37–7.31 (m, 20H, Ar), 5.26 (1H, $J_{1,2}$ = 10 Hz, H-1), 4.93 (d, 1H, J = 10.8 Hz, CH_2Ph), 4.74–4.50 (m, 5H, CH_2Ph), 3.81–3.75 (m, 3H, H-6, H-4, H-3), 3.68 (dd, 1H, $J_{5,4}$ = 9.2 Hz, $J_{5,6}$ = 8.8 Hz, H-5), 3.63–3.60 (m, 1H, H-6), 2.54 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{2',2}$ = 6.2 Hz, H-2′), 2.13 (dd, 1H, $J_{1,2}$ = 12.0 Hz, $J_{2,2'}$ = 11.6 Hz, H-2) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 141, 136.7, 134.76, 127.4, 127.36, 127.27, 127.23, 126.89, 126.74, 126.70, 126.64, 126.54, 126.51, 123.70, 111.52, 80.82, 78.11, 76.61, 75.86, 74.03, 72.35, 70.72, 67.54, 35.80 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{36}H_{36}N_2O_4$ 560.2675; found 561.2753 $[M + H]^+$.

*N-[3,4,6-tri-O-benzyl-2-deoxy-β-*D*-glucopyranosyl]-1H-5-(4-bromophenyl)-3H-imidazole* (**14**). The crude mixture (0.64 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 3:1 then 1:1 *v/v*) to give a yellowish oil (0.38 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, 1H, J = 10.4 Hz, Ar), 7.37–7.20 (m, 20H, Ar), 5.25 (dd, 1H, $J_{1,2}$ = 9.6 Hz, H-1) 4.93 (d, 1H, J = 10.0 Hz, CH_2Ph), 4.69 (dd, 2H, J = 10.0 Hz, J = 9.6 Hz, CH_2Ph), 4.62–4.5 (m, 3H, CH_2Ph), 3.81–3.77 (m, 3H, H-5, H-4, H-3) 3.63–3.60 (m, 2H, H-6), 2.56–2.52 (m, 1H, H-2), 2.15–2.07 (m, 1H, H-2) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 141, 137.98, 136.07, 128.71, 128.67, 128.57, 128.53, 128.20, 128.04, 128.01, 127.96, 127.84, 127.81, 125.01, 112.84, 82.11, 79.39, 75.34, 73.64, 72.01, 68.81, 37.08 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{36}H_{35}BrN_2O_4$ 638.1780; found 639.1858 $[M + H]^+$.

*N-[3,4,6-tri-O-acetyl-2-deoxy-2-iodo-α-*D*-mannopyranosyl]-1H-benzotriazole* (**21a**). The crude mixture (0.95 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 4:1, *v/v*) to give a clear oil (0.72 g, 76%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.97 (d, 1H, J = 8 Hz, Ar), 7.83 (d, 1H, J = 8.4 Hz, Ar), 7.53 (t, 1H, J = 8.3 Hz, J = 8.0 Hz, Ar), 7.37 (t, 1H, J = 8.1 Hz, J = 8.0 Hz, Ar), 6.73 (d, 1H, $J_{1,2}$ = 4.4 Hz, H-1), 5.60 (dd, 1H, $J_{2,1}$ = 4.4 Hz, $J_{2,3}$ = 4.2 Hz, H-2), 5.29 (dd, 1H, $J_{3,2}$ = 4.0 Hz, $J_{3,4}$ = 3.6 Hz, H-3), 5.23 (dd, 1H, $J_{4,5}$ = 7.0 Hz, $J_{4,3}$ = 6.8 Hz, H-4), 4.42 (dd, 1H, $J_{6,5}$ = 7.2 Hz, $J_{6,6}$ = 5.6 Hz, H-6), 4.0 (dd, 1H, $J_{6,5}$ = 10 Hz, $J_{6,6}$ = 6.4 Hz, H-6), 3.81–3.76 (m, 1H, H-5), 2.04–1.83 (m, 9H, CH_3) ppm. ^{13}C NMR (125 MHz, acetone-d_6) δ 169.91, 169.27, 168.86, 146.04, 132.70, 128.26, 124.74, 119.72, 111.04, 85.10, 73.40, 70.07, 67.01, 61.04, 25.86, 20.11, 19.92, 19.82 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{18}H_{20}IN_3O_7$ 517.0346; found 518.0424 $[M + H]^+$.

*N-[3,4,6-tri-O-acetyl-2-deoxy-2-iodo-α-*D*-mannopyranosyl]-2H- benzotriazole* (**21b**). The crude mixture (0.95 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 4:1, *v/v*) to give a clear oil (0.055 g, 5.5%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.82 (d, 2H, J = 8.8 Hz, Ar), 7.39 (d, 2H, J = 9.6 Hz, Ar), 6.67 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.37 (dd, 1H, $J_{2,3}$ = 4.0 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 5.31 (dd, 1H, $J_{3,2}$ = 4.0 Hz, $J_{3,4}$ = 2.0 Hz, H-3), 4.27 (dd, 1H, $J_{6,5}$ = 6.4 Hz, $J_{6,6}$ = 6.0 Hz, H-6), 4.18 (dd, 1H, $J_{4,5}$ = 2.4 Hz, $J_{4,3}$ = 2.0 Hz, H-4), 4.07 (dd, 1H, $J_{6,6}$ = 9.6Hz, $J_{6,5}$ = 2.8 Hz, H-6), 3.91 (dd, 1H, $J_{5,6}$ = 7.2 Hz, $J_{5,4}$ = 7.2 Hz, H-5), 2.02–1.86 (m, 9H, CH_3) ppm. ^{13}C NMR (125 MHz, acetone-d_6) δ 169.82, 169.21, 168.9, 144.28, 127.69, 118.58, 91.72, 73.58, 69.78, 67.17, 61.30, 25.35, 20.07, 19.94, 19.81 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{18}H_{20}IN_3O_7$ 517.0346; found 518.0424 $[M + H]^+$.

*N-[4,6-O-benzylidine-3-hydroxyl-2-deoxy-2-iodo-α-*D*-mannopyranosyl]-1H-benzotriazole* (**22a**) The crude mixture (1.12 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 8:1, *v/v*) to give a white solid (0.87 g, 86%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.92 (d, 1H, J = 8.4 Hz, Ar), 7.67 (d, 1H, J = 8.4 Hz, Ar), 7.48 (t, 1H, J = 8.1 Hz, J = 7.2 Hz, Ar), 7.36 (t, 1H, J = 7.6 Hz, J = 7.2 Hz, Ar), 7.28–7.26 (m, 2H, Ar), 7.17–7.11 (m,

3H, Ar), 5.50 (s, 1H, C*H*Ph), 5.48 (dd, 1H, $J_{1,2}$ = 4.8 Hz, H-1), 4.08 (dd, 1H, $J_{4,3}$ = 9.6 Hz, $J_{4,5}$ = 9.2 Hz, H-4), 3.98–3.92 (m, 1H, H-6), 3.88–3.85 (m, 2H, H-3, H-6), 3.78–3.73 (m, 2H, H-6′, H-2), 3.11–3.05 (m, 1H, H-5) ppm. ^{13}C NMR (125 MHz, acetone-d_6) δ 145.58, 137.43, 128.64, 128.40, 127.69, 126.15, 125.05, 119.03, 111.01, 101.97, 89.18, 80.31, 67.63, 67.04, 66.37, 60.23, 33.17 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{19}H_{18}IN_3O_4$ 479.0342; found 480.0420 $[M + H]^+$.

*N-[4,6-O-benzylidine-3-hydroxyl-2-deoxy-2-iodo-α-*D*-mannopyranosyl]-2H-benzotriazole* (**22b**). The crude mixture (1.12 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 3:1, *v/v*) to give a yellowish oil (0.10 g, 10%). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.95–7.93 (m, 2H, Ar), 7.50-7.44 (m, 2H, Ar), 7.43-7.32 (m, 5H, Ar), 5.72 (s, 1H, C*H*Ph), 5.41 (dd, 1H, $J_{1,2}$ = 4.8 Hz, H-1), 4.15 (dd, 1H, $J_{4,3}$ = 10.8 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 4.11–4.09 (m, 1H, H-6), 4.07–4.03 (m, 2H, H-6, H-2), 3.87 (dd, 1H, $J_{3,2}$ = 10.0 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.77–3.75 (m, 1H, H-5) ppm. ^{13}C NMR (125 MHz, acetone-d_6) δ 145.98, 127.96, 126.47, 124.74, 119.64, 111.28, 101.74, 88.96, 80.79, 67.16, 67.09, 66.50, 59.75, 20.06, 13.70 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{19}H_{18}IN_3O_4$ 479.0342; found 480.0420 $[M + H]^+$.

*N-[3,4,6-tri-O-benzyl-2-deoxy-2-iodo-β-*D*-glucopyranosyl]-1H-benzoimidazole* (**29**). The crude mixture (0.81 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 3:1, *v/v*) to give a white solid (0.65g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H, NC*H*N), 7.75 (d, 1H, *J* = 7.2 Hz, Ar), 7.46 (d, 1H, *J* = 7.2 Hz, Ar), 7.34–7.16 (m, 17H, Ar), 5.63 (d, 1H, $J_{1,2}$ = 10 Hz, H-1), 4.95–4.81 (m, 3H, CH_2Ph), 4.63–4.40 (m, 3H, CH_2Ph, H-2), 3.87 (dd, 1H, $J_{3,2}$ = 3.6 Hz, $J_{3,4}$ = 2.0 Hz, H-3), 3.77–3.65 (m, 4H, H-4, H-5, H-6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.15, 140.85, 136.74, 136.45, 136.26, 127.57, 127.48, 127.38, 127.09, 127.05, 126.89, 126.70, 126.55, 110.52, 86.05, 84.88, 77.89, 77.24, 74.85, 74.25, 72.50, 67.02, 32.0 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{34}H_{33}IN_2O_4$ 660.1485; found 661.1563 $[M + H]^+$.

*N-[3,4,6-tri-O-benzyl-2-deoxy-2-iodo-β-*D*-glucopyranosyl]-1H-4-phenylimidazole* (**31**). The crude mixture (0.70 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 8:1 *v/v*) to give a white solid (0.56 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H, NC*H*N), 7.75 (d, 1H, *J* = 7.2 Hz, Ar), 7.46 (d, 1H, *J* = 7.2 Hz, Ar), 7.34–7.16 (m, 17H, Ar), 5.63 (d, 1H, $J_{1,2}$ = 10 Hz, H-1), 4.95–4.81 (m, 3H, CH_2Ph), 4.63–4.40 (m, 3H, CH_2Ph, H-2), 3.87 (dd, 1H, $J_{3,2}$ = 3.6 Hz, $J_{3,4}$ = 2.0 Hz, H-3), 3.77–3.65 (m, 4H, H-4, H-5, H-6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.15, 140.85, 136.74, 136.45, 136.26, 127.57, 127.48, 127.38, 127.09, 127.05, 126.89, 126.70, 126.55, 110.52, 86.05, 84.88, 77.89, 77.24, 74.85, 74.25, 72.50, 67.02, 32.0 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{36}H_{35}IN_2O_4$ 686.1642; found 687.1720 $[M + H]^+$.

*N-[3,4,6-tri-O-benzyl-2-deoxy-2-iodo-β-*D*-glucopyranosyl]-1H-5-(4-bromophenyl)-3H-imidazole* (**32**). The crude mixture (1.01 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 6:1 then 3:1 *v/v*) to give a yellowish oil (0.68 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, 1H, *J* = 8.4 Hz, Ar), 7.42–7.12 (m, 20H, Ar), 5.30 (d, 1H, *J* = 10.4, H-1), 4.92 (d, 1H, *J* = 10.4 Hz, CH_2Ph), 4.84 (d, 1H, *J* = 10.0 Hz, CH_2Ph), 4.75 (d, 1H, *J* = 10.4 Hz, CH_2Ph), 4.54 (d, 1H, *J* = 9.6 Hz, CH_2Ph), 4.45 (dd, 2H, *J* = 10.4 Hz, *J* = 10.0 Hz, CH_2Ph), 4.17 (dd, 1H, $J_{1,2}$ = 10.4 Hz, $J_{2,2}$ = 10.0 Hz, H-2), 3.80–3.69 (m, 3H, H-3, H-4, H-5), 3.66–3.62 (m, 2H, H-6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.0, 137.20, 131.77, 128.70, 128.61, 128.57, 128.23, 128.11, 128.03, 127.97, 127.91, 126.67, 120.91, 112.15, 87.41, 85.89, 78.80, 78.27, 75.89, 75.32, 73.72, 68.17, 32.0 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{36}H_{34}BrIN_2O_4$ 764.0747; found 765.0825 $[M + H]^+$.

*N-[3,4,6-tri-O-acetyl-2-deoxy-2-iodo-β-*D*-glucopyranosyl]-1H-benzoimidazole* (**33**). The crude mixture (1.01 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 2:1 *v/v*) to give a clear oil (0.71 g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H, NC*H*N), 7.82 (t, 1H, *J* = 5.6 Hz, *J* = 3.2 Hz, Ar), 7.62 (t, 1H, *J* = 6.0 Hz, *J* = 4.1 Hz, Ar), 7.33-7.25 (m, 2H, Ar), 6.21 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 5.11 (d, 1H, $J_{2,1}$ = 6.6 Hz, H-2), 4.82 (t, 1H, $J_{3,2}$ = 8.4 Hz, $J_{3,4}$ = 3.6 Hz, H-3), 4.14–4.13 (m, 1H, H-6), 4.04 (dd, 1H, $J_{4,3}$ = 7.6 Hz, $J_{4,5}$ = 3.2 Hz, H-4), 3.68–3.66 (m, 1H, H-5), 2.25–2.00 (s, 12H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.84, 170.60, 169.61, 169.55, 169.28, 144.26, 144.18, 141.79, 141.63, 132.16, 123.84,

123.35, 120.90, 111.69, 111.14, 86.71, 70.83, 66.69,61.78, 60.51, 59.91, 32.0, 26.97, 24.67, 21.09, 20.83, 20.70, 14.29 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{19}H_{21}IN_2O_7$ 516.0393; found 517.0472 $[M + H]^+$.

*N-[3,4,6-tri-O-acetyl-2-deoxy-β-*D*-glucopyranosyl]-1H-benzoimidazole* (**34**). The crude mixture (0.81 g) was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 2:1 *v/v*) to give a white solid (0.69 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H, NC*H*N), 7.77 (t, 1H, *J* = 6.4 Hz, *J* = 2.4 Hz, Ar), 7.60 (t, 1H, *J* = 6.4 Hz, *J* = 3.0 Hz, Ar), 7.28–7.20 (m, 2H, Ar), 6.0 (d, 1H, $J_{1,2}$ = 4.8 Hz, H-1), 5.23 (m, 2H, H-3, H-5), 4.23 (dd, 1H, J_6 = 9.2 Hz, $J_{6,6'}$ = 5.2 Hz, H-6), 3.91 (dd, 1H, $J_{6,5}$ = 10.4 Hz, $J_{6,6'}$ = 2.0 Hz, H-6′), 3.44–3.45 (m, 1H, H-4), 3.03 (dd, 1H, $J_{2,1}$ = 4.0 Hz, $J_{2,2'}$ = 3.2 Hz, H-2), 2.34–2.28 (m, 1H, H-2′), 2.06–1.96 (s, 9H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.68, 170.63, 169.70, 144.26, 140.94, 133.35, 123.86, 123.31, 120.61, 112.11, 79.71, 70.12, 69.52, 67.66, 61.60, 31.75, 21.01, 20.82, 20.77 ppm. HR-MALDI-Tof/MS: *m/z* calculated for $C_{19}H_{22}N_2O_7$ 390.1427; found 391.1505 $[M + H]^+$.

3.2. Biology

HeLa cells were seeded in 96-well plates and left to grow overnight to approximately 80% confluence. The following day, cells were treated with either the DMSO control (represented as a black dashed line in Figure 2 or light grey bars in Figure 3) or the synthesized compounds at a concentration of 100 μM (black bars) or 10 μM (grey bars). Cell viability was determined relative to the DMSO control and is represented by the dashed black line (100% cell viability).

Cell Viability Assays were conducted as follows. All cells were seeded in 96-well plates and were grown to approximately 80% confluence before adding the compounds tested. Compounds were solubilized and compared to dimethyl sulfoxide (DMSO; Sigma Aldrich, St. Louis, MO). Viability assays were performed in duplicate wells using 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Thermo Fisher Scientific, Waltham, MA). A 5 mg/mL of MTT solution was made in phosphate buffered saline (PBS), filter sterilized and stored at −20 °C until used. On the day of assay, 20 μL of MTT stock solution was added to cells growing in 100 μL of pre-existing media and allowed to incubate for 2 h to allow for the uptake of the MTT dye. Following this, the media containing MTT was removed and the cells were gently washed once with 100 μL pre-warmed PBS. In order to release the internalized dye, 50 μL of DMSO was added to each well and incubated for 10 min on an orbital shaker for 10 min. Absorbance of the solubilized dye was measured and recorded at 540 nm using a multiplate reader.

All statistical analyses were performed using Graphpad Prism 9.1.1. The significance of difference (*p* value) of pooled results was determined by one-way analysis of variance (ANOVA) followed by the post hoc Dunnett's tests after three independent trials. Significance was defined according to the scale ** = $p < 0.05$ or *** = $p < 0.001$.

4. Conclusions

In summary, we report the synthesis of a series of 2-deoxy-2-iodo-α-D-mannopyranosylbenzotriazoles using the benzyl, 4,6-benzylidene and acetyl protected D-glucal in the presence of *N*-iodosuccinimide (NIS). The best yields were obtained when the reaction was refluxed for 2 h. During the addition reaction, the benzyl protected D-glucal produced the single 1*H*-benzotriazole isomer while a mixture of the 1*H* and 2*H*-benzotriazole isomers was obtained for the acetyl and 4,6-benzylidine protected D-glucal. Removal of the iodine at the C-2 position under free radical conditions afforded the 2-deoxy-D-glucopyranosylbenzotriazoles. The addition of various substituted imidazoles to the benzyl protected D-glucal in the presence of NIS was also accomplished, resulting in the chemical synthesis of a series of 2-deoxy-2-iodo-β-D-glucopyranosylimidazoles. Access to the 2-deoxy-D-glucopyranosylimidazoles was similarly achieved relative to the benzotriazole series. All prepared 2-deoxy sugars were screened for their anticancer activity using four established human cell lines. Our results strongly suggest that both 2-deoxy-α-D-glucopyranosylbenzotriazoles, **5** and **6b**, are cytotoxic to the cervical, lung and breast

cancer cell lines tested against in this study. Compound **5** showed an average of less than 5% survival rate at 20 μM concentrations, while the acetyl protected compound **6b** was less than 24% for all four cell lines. The IC_{50} was found to be 2.908 μM and 9.940 μM for compounds **5** and **6b**, respectively. When examining the nature of the different protecting groups for the 1*H*-2-deoxy-D-glucopyranosylbenzotriazole series, the more lipophilic benzyl ethers outperformed the acetyl and 4,6-benzylidene counterparts in cell viability studies. This pattern was also observed for the glucose derivatives **1–4** when compared to compound **I**. For the 2-deoxy-*N*-glycoimidazole series, only the benzimidazole containing compound **10** showed potency at 100 μM concentration in our cell viability studies. The cytotoxic screening of compounds **5** and **6b** using a broader cell panel is currently underway, as is their optimization and an investigation into their mode of action, with results forthcoming in future publications.

Supplementary Materials: The following are available online. Copies of ^{1}H and ^{13}C NMR spectra and HRMS spectra of all reported compounds.

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