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ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 675–682
Bioorganic & Medicinal Chemistry

Design, synthesis, and radiosensitizing activities of sugar-hybrid hypoxic cell radiosensitizers

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Received 21 September 2007; revised 9 October 2007; accepted 12 October 2007
Available online 17 October 2007

Abstract—We have designed sugar-hybrid TX-1877 derivatives conjugated with sugar moieties including β-glucose (β-Glc), β-galactose (β-Gal), α-mannose (α-Man) and *N*-acetyl-β-galactosamine (β-GalNAc). Compound **1** (TX-1877) was glycosylated with appropriate peracetylated sugars using BF_3-OEt_2 to give acetylated sugar-hybrids, **5** (TX-2244), **6** (TX-2245), **7** (TX-2246), and **10** (TX-2243). Removal of the acetyl groups afforded the sugar-hybrids having free hydroxyl groups, **11** (TX-2141), **12** (TX-2218), **13** (TX-2217) and **14** (TX-2068). We evaluated their radiosensitizing activities by an in vitro radiosensitization assay. All free hydroxyl hybrids have lower enhancement ratio (ER) values (ER $\leqslant$ 1.43) and lower *n*-octanol/water partition coefficient (P_{oct}) values ($P_{oct} < 1.00 \times 10^{-2}$) than does **1** (TX-1877, ER = 1.75, P_{oct}: 5.60×10^{-2}). All acetylated hybrids have similar P_{oct} values (3.55×10^{-2}–1.05×10^{-1}) to **1** (TX-1877) and have improved ER values (ER $\geqslant$ 1.47) compared to the hybrids having free hydroxyl groups. Among these, **5** (TX-2244) is the most active radiosensitizer (ER = 2.30). We found a good correlation (r = 0.866) between the magnitude of P_{oct} ($\log P_{oct}$) and the ER value of **5** (TX-2244), **6** (TX-2245), **7** (TX-2246), **10** (TX-2243) and **1** (TX-1877), suggesting that increasing the hydrophobicity is reflected in increased in vitro radiosensitizing activity. In the present study, we have succeeded in producing sugar-hybrid hypoxic cell radiosensitizers that have an increased radiosensitizing activity that does not depend on increased hydrophobicity.

1. Introduction

Hypoxic cells that exist in many human solid tumors are characterized by radioresistance.[1] The resistance to radiation treatment caused by tumor hypoxia is a major complicating factor in cancer therapy.[2–4] Thus, the development of compounds that can sensitize hypoxic tumor cells to radiation has been an important goal for medicinal chemists.[5]

Representative hypoxic cell radiosensitizers are listed in Figure 1. Etanidazole and misonidazole are well-known hypoxic cell radiosensitizers but have had limited thera-

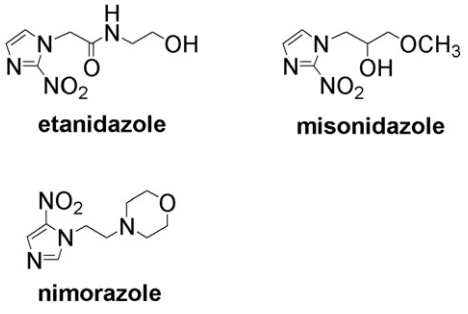

Figure 1. Representative hypoxic cell radiosensitizers.

Keywords: Hypoxic cell radiosensitizers; Sugar-hybrids; Electronic states; Hydrophobicity.
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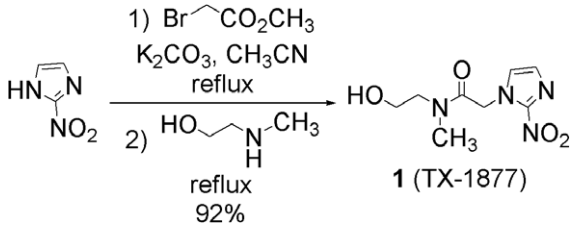
doi:10.1016/j.bmc.2007.10.035

peutic impact on radiotherapy due to clinical problems that include dose-limiting side effects such as neurotoxicity.[6] Nimorazole, used locally in Denmark for radiotherapy of head and neck cancer, is the only hypoxic cell radiosensitizer currently in clinical use.[7]

Our research has been directed toward exploring new strategies for the development of more effective radiosensitizers that will be useful in the clinic.

The sugar moiety has been used effectively in drug design to improve water solubility and for molecular recognition. An additional factor relevant to this study is the significant increases of glucose uptake into tumor tissue that have been ascribed to enhanced tumor glycolysis. Thus, one possible strategy to improve tumor affinity of drugs is to link a sugar to a drug or drug pharmacophoric moiety. Sugar-hybrid molecules designed to exploit this strategy have been prepared by other groups. Examples include glyco-conjugated porphyrins,[8,9] sugar-pendant diamines,[10] and other drug candidates (e.g., cytosine,[11] quinoxaline,[12] glycidol,[13] and β-carboline[14]). The above considerations led us to initiate research into the design of sugar-hybrid hypoxic cell radiosensitizers.

In this paper, we describe the design and synthesis of sugar-hybrid hypoxic cell radiosensitizers. We then discuss the correlations of the in vitro radiosensitizing activities and the physicochemical characteristics.



Scheme 1. One-pot synthesis of TX-1877.

2. Results

As the substrate to be linked to sugars, we chose TX-1877 (Scheme 1), previously reported by us to be more potent radiosensitizer than etanidazole[15] and have many additional biological activities such as antimetastatic[15] and immunopotentiation activity.[15–18] TX-1877 was conjugated with several sugar moieties, including β-glucose (β-Glc), β-galactose (β-Gal), α-mannose (α-Man), *N*-acetyl-β-galactosamine (β-GalNAc), tetra-*O*-acetyl β-Glc (β-Glc(OAc)$_4$), tetra-*O*-acetyl β-Gal (β-Gal(OAc)$_4$), tetra-*O*-acetyl α-Man (α-Man(OAc)$_4$), and tri-*O*-acetyl β-GalNAc (β-GalNAc(OAc)$_3$), to provide the sugar-conjugated molecules shown in Figure 2.

2.1. Molecular orbital calculation

We carried out molecular orbital calculations of the sugar-conjugated molecules in order to evaluate the utility of our sugar-hybrid drug design as a novel hypoxic cell radiosensitizer (Fig. 3). In all compounds the LUMO is characterized by a localized increased electron density on the 2-nitroimidazole ring as same as **1**.[15] In contrast to the LUMO, the HOMO is dependent on the structure of the sugar. Thus, the HOMO for **11**, **5**, **12**, **6**, **13**, and **7** are localized on the *N*-methylamide moiety, while for **14** and **10** increased electron densities are localized on the 2-acetamide moiety of the sugar. Energy values of LUMO (E_{LUMO}) and HOMO (E_{HOMO}) for the series are given in Table 1. The values ranged from −1.2 to −0.9 eV and were similar to **1**.

2.2. Chemistry

Reaction of 2-nitroimidazole with methyl bromoacetate in the presence of potassium carbonate in acetonitrile, followed by reaction with *N*-methylethanolamine (one pot), gave **1** in 92% yield (Scheme 1). Consistent with our previous report,[15] the ^{1}H NMR spectrum of **1** at 25 °C showed proton signals corresponding to the presence of two rotamers due to a C-N rotational barrier

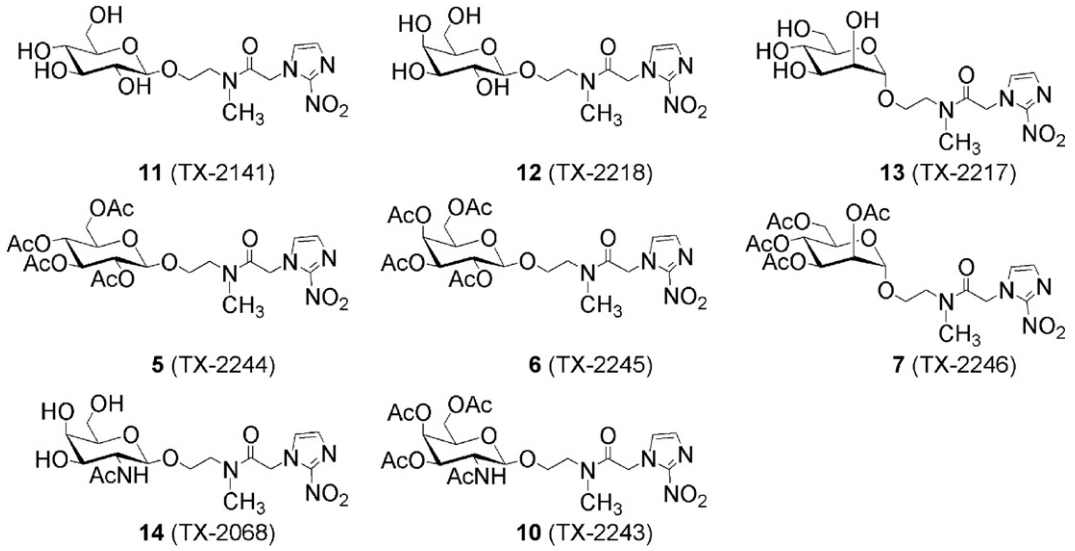


Figure 2. Structure of sugar-hybrid hypoxic cell radiosensitizers.

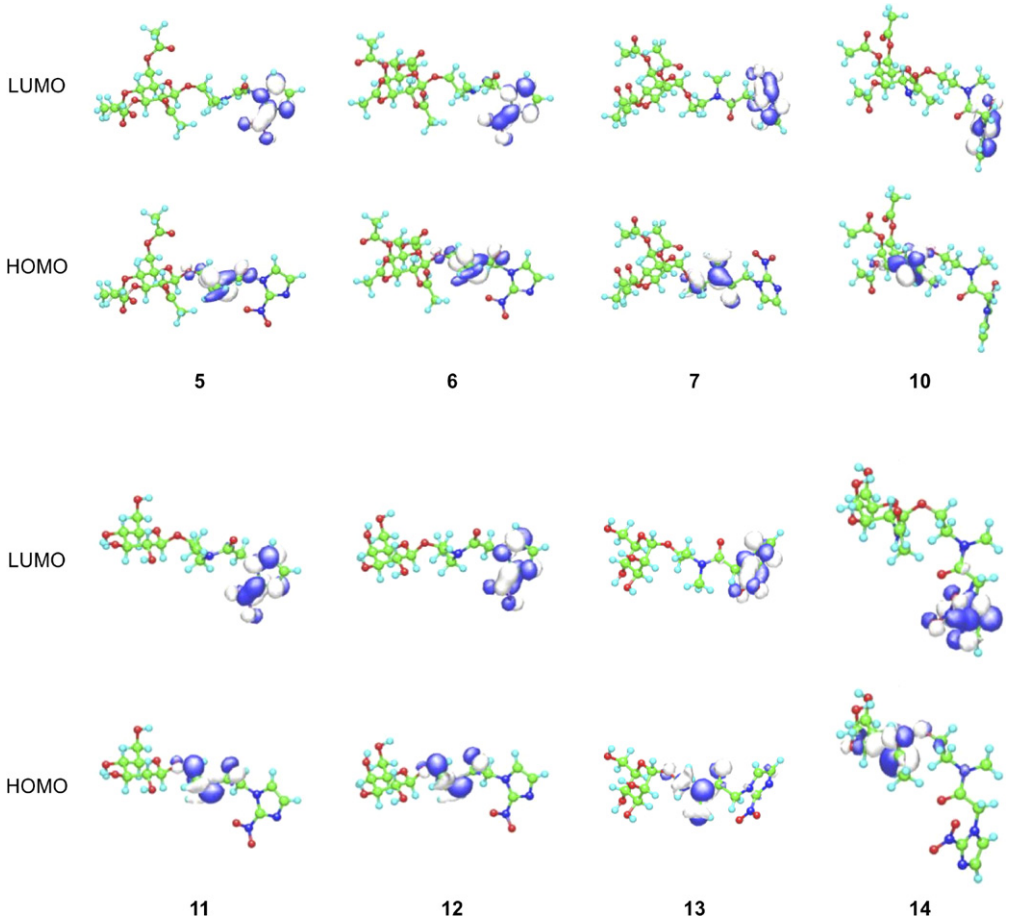


Figure 3. Distribution of the electron density (white and blue lobe) of LUMO and HOMO for sugar-hybrid hypoxic cell radiosensitizers.

Table 1. Energy of LUMO (E_{LUMO}) and HOMO (E_{HOMO}) for sugar-hybrid hypoxic cell radiosensitizers and **1**

Compound	E_{LUMO} (eV)	E_{HOMO} (eV)
1	−1.1095	−10.1018
5	−0.9610	−9.9749
6	−1.0060	−9.9804
7	−0.9731	−9.7424
10	−1.1428	−9.9510
11	−0.9912	−9.9044
12	−0.9670	−9.8643
13	−1.1479	−10.2101
14	−1.0418	−9.8371

(see Experimental section). Compound **1** was glycosylated with various peracetylated sugars in the presence of BF_3-OEt_2 in nitromethane (Table 2). The reaction of **2**, **3**, and **4** gave **5**, **6**, and **7** in poor yield (7–15%) along with aglycon acetate **8** (12–18% yield). Formation of aglycon acetate by using peracetylated sugars as glycosyl donor has been studied by some researchers.[19–22] In contrast, glycosylation of **9** with **1** under the same conditions produced **10** in 37% without formation of **8**. The reason for this difference may be that the *N*-acetyl group of **9** can stabilize an *N*,*O*-oxazoline intermediate. Removal of the *O*-acetyl group from **5**, **6**, **7**, and **10** was carried out with sodium methoxide in methanol to give **11**, **12**, **13**, and **14** in 45–99% yield (Scheme 2). The ^{1}H NMR spectra of all compounds also showed proton signals corresponding to rotamers, as described above.

2.3. Hydrophobic parameters

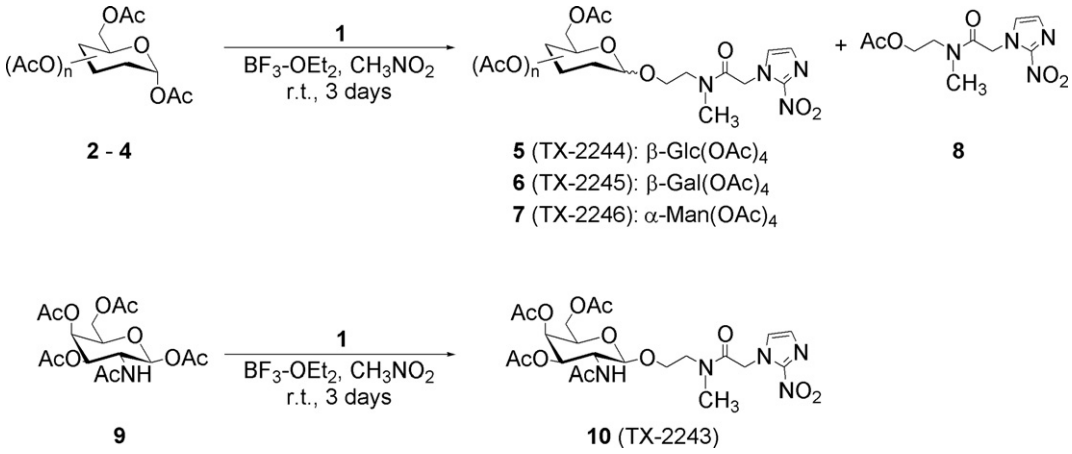
Two hydrophobic parameters-the calculated partition coefficients (calcd*P*) and *n*-octanol/water partition coefficients (P_{oct})-were determined for each compound in the series. As shown in Table 3, the calcd*P* values of **11** or **12** or **13**, and **14**, **5** or **6** or **7**, and **10** were 4.0×10^{-4}, 1.3×10^{-4}, 5.0×10^{-2}, and 5.1×10^{-3}, respectively. The P_{oct} values of **5**, **6**, **7**, and **10** were 1.05×10^{-1}, 3.55×10^{-2}, 6.97×10^{-2}, and 5.10×10^{-2}, respectively. The P_{oct} values of **11**, **12**, **13**, and **14** were undetectable ($<1.00\times10^{-2}$).

2.4. Radiosensitizing activity

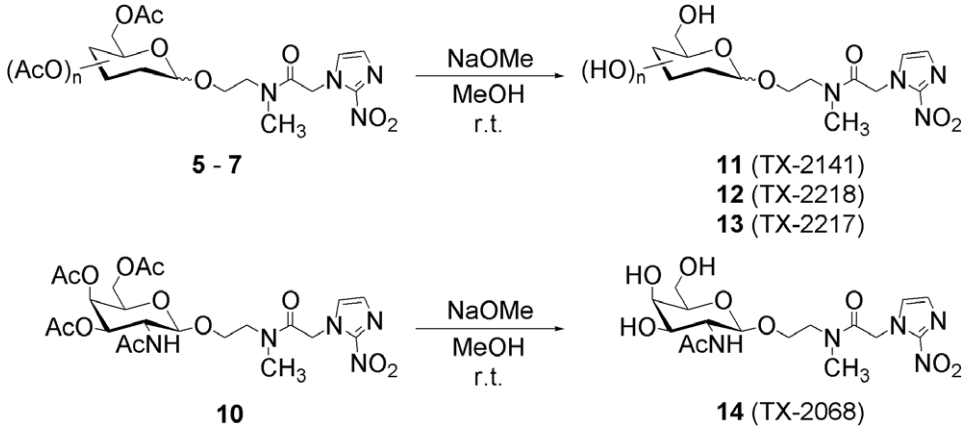
In vitro radiosensitizing activities of the sugar-hybrid hypoxic cell radiosensitizers were measured at a dose of 1 mM in EMT6/KU cells under hypoxic conditions.

The enhancement ratios (ER) are shown in Table 3. The order of radiosensitizing activity was **5** (β-Glc(OAc)$_4$) ≫ **7** (α-Man(OAc)$_4$) > **1** > **6** (β-Gal(OAc)$_4$) > **10** (β-GalNAc(OAc)$_3$) > **14** (β-GalNAc) ≈ **13** (α-Man) ≈ **12** (β-Gal) > **11** (β-Glc). All sugar-hybrids having free

Table 2. Glycosylation of **1** with various peracetylated sugars, **2**–**4** and **9**



Run		Sugar	BF_3-OEt_2		Glycoside (%)		**8** (%)
1	**2**	α-Glc$(OAc)_5$	2.0 equiv	**5**	(TX-2244)	8	12
2	**3**	α-Gal$(OAc)_5$	2.0 equiv	**6**	(TX-2245)	7	18
3	**4**	α-Man$(OAc)_5$	2.0 equiv	**7**	(TX-2246)	15	17
4	**9**	β-GalNAc$(OAc)_4$	2.0 equiv	**10**	(TX-2243)	37	—



Scheme 2. Deprotection of **5**–**7** and **10**.

Table 3. Hydrophobic parameter (calcd$P$ and P_{oct}) and radiosensitizing activities of sugar-hybrid hypoxic cell radiosensitizers and **1**

Compound	calcd$P$	P_{oct}	ER[a]
1	4.5×10^{-2}	5.60×10^{-2}	1.75[b]
5	5.0×10^{-2}	1.05×10^{-1}	2.30
6	5.0×10^{-2}	3.55×10^{-2}	1.63
7	5.0×10^{-2}	6.97×10^{-2}	1.88
10	5.1×10^{-3}	5.10×10^{-2}	1.47
11	4.0×10^{-4}	$<1.00\times10^{-2}$	1.33
12	4.0×10^{-4}	$<1.00\times10^{-2}$	1.40
13	4.0×10^{-4}	$<1.00\times10^{-2}$	1.41
14	1.3×10^{-4}	$<1.00\times10^{-2}$	1.43

[a] Based on ER value of etanidazole (ER = 1.72).[26]
[b] Data from Ref. 15.

hydroxyl groups including **11** (ER = 1.33), **12** (ER = 1.40), **13** (ER = 1.41), and **14** (ER = 1.43) are more hydrophilic radiosensitizers ($P_{oct} < 1.00\times10^{-2}$) than **1** ($P_{oct} = 5.60\times10^{-2}$). The ER values of these hybrids were smaller than that of **1** (ER = 1.75). On the other hand, the acetylated sugar-hybrids, **5** (ER = 2.30), **6** (ER = 1.63), **7** (ER = 1.88), and **10** (ER = 1.47), have similar hydrophobicities (P_{oct}: 3.55×10^{-2}–1.05×10^{-1}) to **1** and have improved ER values compared to the corresponding hydroxyl-free hybrids. Among these, **5** was the most active radiosensitizer in the series.

3. Discussion

Hypoxic cells present in tumors are a major complicating factor in cancer therapy, and are an important target for anticancer drug design. Previous work in drug candidate development has explored the introduction of a sugar moiety as a device to improve tumor affinity.[8–14] The focus of the present work is on the design, synthesis, and evaluation of a series of sugar-hybrid radiosensitizers as an approach to preparing more effective hypoxic cell radiosensitizers.

The effectiveness of the sugar-hybrid radiosensitizers was evaluated by an in vitro radiosensitizing assay under hypoxic condition. As shown in Table 3, **11** (TX-2141), **12** (TX-2218), **13** (TX-2217), and **14** (TX-2068) were less potent radiosensitizers having ER less than 1.6 (the minimum effective ER of radiosensitizers in the in vitro assay) compared to the ER of **1** (1.75). These hybrid radiosensitizers were more hydrophilic ($P_{oct} < 1.00 \times 10^{-2}$) than **1** (TX-1877, $P_{oct} = 5.60 \times 10^{-2}$). We found a good correlation ($r = 0.866$) between the magnitude of P_{oct} ($\log P_{oct}$) and the ER value of **5** (TX-2244), **6** (TX-2245), **7** (TX-2246), **10** (TX-2243), and **1** (TX-1877), suggesting that increasing the hydrophobicity is reflected in increased radiosensitizing activity. This result suggests that the hydrophobicity is one of the major factors affecting the in vitro radiosensitizing activity.

Electron affinity ($EA = -E_{LUMO}$) is one of the indicators of radiosensitizing activity.[23] Previously, Kasai et al. demonstrated that a decreased EA value or a shift of the localization of the LUMO electron-density to the amide moiety resulted in decreased radiosensitizing activity.[15] As shown in Figure 3 and Table 1, no significant difference in E_{LUMO} value and LUMO localization of the sugar-hybrids was observed from MO calculations, indicating that the electronic states of sugar-hybrid hypoxic cell radiosensitizers do not affect their radiosensitizing activities. Although it is unclear why differences in the radiosensitizing activity were observed depending on the nature of the sugar, the most active radiosensitizer is **5** (TX-2244) having an acetylated glucose moiety. Since the acetyl group is a hydrolytically labile functional group, it is possible that intracellular glucose metabolism could contribute to the radiosensitizing activity of this derivative.[24,25] To reduce toxicity, it is necessary for a drug candidate to have a P_{oct} value no greater than that of misonidazole.[26] In this regard, **5** (TX-2244) not only has lower hydrophobicity ($P_{oct} = 1.05 \times 10^{-1}$) but also higher radiosensitizing activity (ER = 2.30 at 1 mM) than misonidazole ($P_{oct} = 4.22 \times 10^{-1}$, ER = 1.72 at 1 mM).[27] We thus have achieved the design of a sugar-hybrid hypoxic cell radiosensitizer that has increased radiosensitizing activity along with controlled hydrophobicity.

In summary, we have presented herein the design and synthesis of sugar-hybrid hypoxic cell radiosensitizers. The synthesis was based on glycosylation of **1** (TX-1877), a radiosensitizer previously prepared in our laboratory, with appropriate peracetylated sugars in the presence of BF_3-OEt_2. Removal of the *O*-acetyl group afforded sugar-hybrids containing the free hydroxyl groups. In vitro radiosensitizing activity of the compounds in the series was related to their hydrophobicities. Among these, **5** (TX-2244) is the most active radiosensitizer having both higher radiosensitizing activity and lower hydrophobicity compared to misonidazole. We thus succeeded in the design of a hybrid molecule which has an increased radiosensitizing activity that does not depend on increased hydrophobicity.

4. Experimental

4.1. General procedures

All reactions were carried out under a nitrogen atmosphere and monitored by TLC using UV light and an ethanol solution of ammonium molybdate as a developing agent. Column chromatography was performed on Kanto Chemical silica gel 60 N (spherical, neutral, 40–50 or 230–400 mesh). ^{1}H NMR spectra were recorded in $CDCl_3$, CD_3OD or CD_3CN on a JOEL JNMEX400 spectrometer (400 MHz) with tetramethylsilane as the internal standard. Chemical shifts are reported in ppm and coupling constants are reported in Hz. HRMS were measured on a JOEL JMS-SX 102A mass spectrometer using a FAB method. IR spectra were measured on a Perkin Elmer spectrum RX 1. All melting points were determined with a Yanagimoto micro melting point apparatus (MP-S3 model) and are uncorrected. Calculated partition coefficients (calcd*P*) were determined by the PrologP modules in the Pallas 3.0 program of CompuDrug Chemistry Ltd. (South San Francisco, California). A Hitachi U-2000 spectrometer was used for measurement of P_{oct}. All chemicals and solvents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Kanto Chemical Co., Inc. (Tokyo, Japan), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and Sigma Aldrich Japan (Tokyo, Japan).

4.2. Synthesis

4.2.1. One-pot synthesis of 1 (TX-1877). Methyl bromoacetate (1.1 mL, 11.3 mmol, 1.1 equiv) was added to a mixture of 2-nitroimidazole (1.13 g, 10.0 mmol) and potassium carbonate (845 mg, 6.00 mmol, 0.6 equiv) in CH_3CN (40 mL), and the mixture was refluxed for 20 min. After cooling to room temperature, *N*-methylethanolamine (2.5 mL, 31.1 mmol, 3.1 equiv) was added and the mixture was refluxed for 17 h. After cooling to room temperature, the precipitate was filtered and washed with CH_3CN. The filtrate was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 20:1) to give TX-1877 (2.09 g, 92%) as a light yellow solid. ^{1}H NMR (400 MHz, CD_3OD): δ 7.40 (d, $J = 0.98$ Hz, 0.45H), 7.37 (d, $J = 0.98$ Hz, 0.55H), 7.16 (d, $J = 0.98$ Hz, 0.55H), 7.15 (d, $J = 1.2$ Hz, 0.45H), 5.54 (s, 1.1H), 5.44 (s, 0.9H), 3.79 (t, $J = 5.1$ Hz, 1.1H), 3.67 (t, $J = 5.6$ Hz, 0.9H), 3.55 (t, $J = 5.1$ Hz, 1.1H), 3.51 (t, $J = 5.6$ Hz, 0.9H), 3.21 (s, 1.35H), 2.98 (s, 1.65H). Other experimental data were consistent with previous work.[15]

4.2.2. General procedure for glycosylation. BF_3-OEt_2 (2.0 equiv) was added dropwise to a mixture of TX-1877 and peracetylated sugar (1.0 equiv) in CH_3NO_2 at 0 °C. After stirring for 30 min, the mixture was warmed to room temperature and then stirred for 3 days. The mixture was diluted with CH_2Cl_2 and saturated $NaHCO_3$ solution. The organic layer was washed with saturated NaCl solution and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatog-

raphy (EtOAc/MeOH or CH_2Cl_2/MeOH as the eluent) to give the product and/or **8**.

4.2.2.1. [2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-methyl-acetamido]ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (5, TX-2244). Light yellow solid (8%); mp 67–69 °C ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.44 (d, J = 0.98 Hz, 0.67H), 7.19 (d, J = 0.98 Hz, 0.33H), 7.18 (d, J = 0.98 Hz, 0.67H), 7.13 (d, J = 0.98 Hz, 0.33H), 5.40–4.98 (m, 5H), 4.52 (d, J = 8.1 Hz, 0.67H), 4.50 (d, J = 7.8 Hz, 0.33H), 4.27 (dd, J = 4.4, 12.4 Hz, 1H), 4.19 (dd, J = 2.4, 4.4 Hz, 0.67H), 4.16 (dd, J = 2.4, 4.4 Hz, 0.33H), 4.11–4.07 (m, 0.67H), 3.98–3.93 (m, 0.33H), 3.76–3.62 (m, 3H), 3.56–3.50 (m, 1H), 3.16 (s, 2H), 2.96 (s, 1H), 2.09 (s, 2H), 2.08 (s, 1H), 2.06 (s, 1H), 2.04 (2s, 3H), 2.03 (s, 2H), 2.01 (s, 3H); FAB-HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{22}H_{31}N_4O_{13}$, 559.1888; found, 559.1909.

4.2.2.2. [2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-methyl-acetamido]ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (6, TX-2245). Light yellow solid (7%); mp 74-76 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.48 (d, J = 0.98 Hz, 0.67H), 7.19 (d, J = 1.2 Hz, 0.33H), 7.17 (d, J = 1.2 Hz, 0.67H), 7.12 (d, J = 0.98 Hz, 0.33H), 5.44–5.40 (m, 2H), 5.30–5.18 (m, 1H), 5.13 (d, J = 10.0 Hz, 0.67H), 5.09 (d, J = 10.0 Hz, 0.33H), 5.06–5.01 (m, 1H), 4.49 (d, J = 7.8 Hz, 0.67H), 4.48 (d, J = 7.8 Hz, 0.33H), 4.22 (dd, J = 6.6, 8.5 Hz, 0.67H), 4.19 (dd, J = 6.6, 8.5 Hz, 0.33H), 4.15–4.10 (m, 2H), 4.00–3.90 (m, 1H), 3.78–3.63 (m, 2H), 3.56–3.47 (m, 1H), 3.17 (s, 1H), 2.97 (s, 2H), 2.19 (s, 2H), 2.17 (s, 1H), 2.07 (s, 1H), 2.06 (s, 2H), 2.05 (s, 1H), 2.02 (s, 2H), 2.01 (s, 2H), 1.99 (s, 1H); FAB-HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{22}H_{31}N_4O_{13}$, 559.1888; found, 559.1907.

4.2.2.3. [2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-methyl-acetamido]ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (7, TX-2246). Light yellow solid (15%); mp 60-62 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.46 (d, J = 0.98 Hz, 0.43H), 7.19 (2d, J = 1.2 Hz, 1H), 7.17 (d, J = 0.98 Hz, 0.57H), 5.36–5.28 (m, 3H), 5.26–5.18 (m, 2H), 4.89 (d, J = 1.7 Hz, 0.43H), 4.82 (d, J = 1.7 Hz, 0.57H), 4.30 (dd, J = 5.4, 12.4 Hz, 0.43H), 4.25 (dd, J = 5.7, 12.3 Hz, 0.57H), 4.16–4.10 (m, 1H), 4.02–3.97 (m, 1H), 3.93–3.86 (m, 1H), 3.74–3.68 (m, 1H), 3.67–3.44 (m, 2H), 3.27 (s, 1.71H), 3.04 (s, 1.29H), 2.18 (s, 1.29H), 2.17 (s, 1.71H), 2.07 (s, 3H), 2.06 (s, 1.71H), 2.05 (s, 1.29H), 2.01 (s, 3H); FAB-HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{22}H_{31}N_4O_{13}$, 559.1888; found, 559.1901.

4.2.2.4. [2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-methyl-acetamido]ethyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-galactopyranoside (10, TX-2243). Light yellow solid (37%); mp 90-92 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.56 (d, J = 0.98 Hz, 0.71H), 7.20 (d, J = 0.98 Hz, 0.29H), 7.17 (d, J = 0.98 Hz, 0.71H), 7.15 (d, J = 0.98 Hz, 0.29H), 5.84 (d, J = 9.0 Hz, 1H), 5.40–5.04 (m, 4H), 4.62 (d, J = 8.3 Hz, 0.71H), 4.49 (d, J = 8.5 Hz, 0.29H), 4.27–4.10 (m, 4H), 4.02–3.83 (m, 1.71H), 3.77–3.64 (m, 1.71H), 3.52–3.47 (m, 0.29H), 3.39–3.32 (m, 0.29H), 3.17 (s, 0.87H), 2.95 (s, 2.13H), 2.17 (2s, 3H), 2.06 (s, 2.13H), 2.04 (s, 0.87H), 2.02 (s, 2.13H), 2.01 (s, 0.87H), 1.96 (s, 0.87H), 1.90 (s, 2.13H); FAB-HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{22}H_{32}N_5O_{12}$, 558.2048; found, 558.2075.

4.2.3. General procedure for deprotection. The mixture of peracetylated glycoside and a small amount of sodium methoxide in MeOH was stirred at room temperature. After consumption of starting material, Dowex resin (50W × 4, H^+ form) was added and the precipitate was filtered. The filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 4:1) to give the product.

4.2.3.1. [2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-methyl-acetamido]ethyl β-D-glucopyranoside (11, TX-2141). White solid (64%); mp 89–91 °C; ^{1}H NMR (CD_3CN, 400 MHz): δ 7.25(d, J = 1.2 Hz, 0.33H), 7.21 (d, J = 1.2 Hz, 0.67H), 7.11 (d, J = 1.2 Hz, 0.33H), 7.10 (d, J = 0.98 Hz, 0.67H), 5.46 (d, J = 16.6 Hz, 0.67H), 5.40 (d, J = 16.6 Hz, 0.67H), 5.26 (s, 0.66H), 4.30 (d, J = 7.6 Hz, 0.67H), 4.24 (d, J = 7.8 Hz, 0.33H), 3.95–3.86 (m, 2H), 3.71–3.64 (m, 2H), 3.52–3.43 (m, 4.33H), 3.31–3.18 (m, 5.34H), 3.12 (s, 1H), 3.07–2.97 (m, 0.33H), 2.92 (s, 2H); FAB-HRMS: *m*/*z* $[M + H]^+$ calcd for $C_{14}H_{23}N_4O_9$, 391.1465; found, 391.1473; FT-IR (KBr): 3384, 2892, 1654, 1543, 1493, 1416, 1369, 1299, 1162, 1078, 1034 cm^{-1}.

4.2.3.2. [2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-methyl-acetamido]ethyl β-D-galactopyranoside (12, TX-2218). White solid (45%); mp 91–94 °C; ^{1}H MNR (CD_3OD, 400 MHz): δ 7.52 (d, J = 0.98 Hz, 0.67H), 7.42 (d, J = 0.98 Hz, 0.33H), 7.16 (d, J = 0.98 Hz, 0.33H), 7.13 (d, J = 0.98 Hz, 0.67H), 5.68 (d, J = 16.6 Hz, 0.67H), 5.58 (d, J = 16.6 Hz, 0.67H), 5.46 (d, J = 16.6 Hz, 0.33H), 5.42 (d, J = 16.6 Hz, 0.33H), 4.31 (d, J = 7.8 Hz, 0.67H), 4.22 (d, J = 7.1 Hz, 0.33H), 4.06–3.93 (m, 1.67H), 3.82 (d, J = 2.2 Hz, 1H), 3.79–3.63 (m, 4H), 3.59–3.44 (m, 3.33H), 3.23 (s, 0.99H), 2.98 (s, 2.01H); FAB-HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{14}H_{23}N_4O_9$, 391.1465; found, 391.1492; FT-IR (KBr): 3400, 2935, 1651, 1548, 1494, 1370, 1299, 1161, 1075 cm^{-1}.

4.2.3.3. [2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-methyl-acetamido]ethyl α-D-mannopyranoside (13, TX-2217). White solid (99%); mp 187–189 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.44 (d, J = 1.2 Hz, 0.5H), 7.40 (d, J = 1.2 Hz, 0.5H), 7.17 (d, J = 1.2 Hz, 1H), 5.56–5.45 (m, 2H), 4.83 (d, J = 1.7 Hz, 0.5H), 4.76 (d, J = 1.7 Hz, 0.5H), 4.02–3.98 (m, 0.5H), 3.93–3.86 (m. 2.5H), 3.85–3.47 (m, 7H), 3.23 (s, 1.5H), 3.00 (s, 1.5H); FAB-HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{14}H_{23}N_4O_9$, 391.1465; found, 391.1444; FT-IR (KBr): 3383, 2938, 1649, 1544, 1495, 1414, 1371, 1299, 1226, 1137, 1063, 975, 845, 809, 682, 656, 637, 609 cm^{-1}.

4.2.3.4. [2-(2-Nitro-1*H*-imidazol-1-yl)-*N*-methyl-acetamido]ethyl 2-acetamido-2-deoxy-β-D-galactopyranoside (14, TX-2068). White solid (79%); mp 121–123 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.72 (d, J = 1.2 Hz, 0.78H), 7.43 (d, J = 1.2 Hz, 0.22H), 7.17 (d, J = 1.2 Hz, 0.22H), 7.15 (d, J = 1.2 Hz, 0.78H), 5.57

(d, J = 16.6 Hz, 1H), 5.44 (d, J = 16.6 Hz, 1H), 4.34 (d, J = 8.3 Hz, 0.78H), 4.33 (d, J = 8.3 Hz, 0.22H), 4.11–4.05 (m, 1.67H), 4.00–3.95 (m. 0.33H), 3.83 (d, J = 3.2 Hz, 1H), 3.80–3.61 (m, 4.67H), 3.58–3.48 (m, 2.33H), 3.19 (s, 0.67H), 2.96 (s, 2.33H), 1.99 (s, 0.67H), 1.98 (s, 2.33H); FAB-HRMS: m/z $[M + Na]^+$ calcd for $C_{16}H_{25}N_5O_9Na$, 454.1544; found, 454.1571; FT-IR (KBr): 3397, 2945, 2894, 1650, 1600, 1549, 1508, 1495, 1413, 1371, 1300, 1227, 1161, 1146, 1123, 1082, 1058, 845, 789, 750, 650, 613 cm^{-1}.

4.3. Molecular orbital calculation

The semi-empirical molecular orbital calculations were carried out using the program MOPAC 2000 (Fujitsu WinMOPAC ver. 3.0. Fujitsu Ltd., Tokyo, Japan) with PM 3 Hamiltonian.

4.4. Measurement of *n*-octanol/water partition coefficient (P_{oct})

The measurement of partition coefficients (P_{oct}) was referred to Shake Flask Method. Stock solutions of each compound were prepared by dissolving 1 mg of each compound in 0.1 M phosphate buffer (pH 7.40) and the absorbance at 325 nm (ABS_{pre}) was measured. The stock solution (3 mL) and phosphate buffer-saturated *n*-octanol (20–300 mL) were combined and the mixture was vigorously stirred at room temperature for 2 h. Following separation of the two phases, the aqueous layer was collected and the absorbance was measured at 325 nm (ABS_{post}). The values of P_{oct} were calculated as shown in Eq. 1.

$$P_{oct} = \frac{ABS_{pre} - ABS_{post}}{ABS_{post}} \times \frac{\text{stock solution (ml)}}{n - \text{octanol (ml)}} \quad (1)$$

4.5. In vitro radiosensitizing assay

In vitro radiosensitization was measured in EMT6/KU single cells under hypoxic conditions. The hypoxic cell radiosensitizer was added to an EMT6/KU cell (2×10^6 cells/mL) suspension at a dose of 1 mM in test tubes and treated with 95% N_2–5% CO_2 gas for 1 hr. The cells were irradiated with a linear accelerator (MEVATRON KD2 PRIMUS) by 8–36 Gy at an X-ray dose rate of 2.0 Gy/min. After irradiation, colony formation assays were performed. Enhancement ratios (ERs) were determined from the ratio of radiation doses required to reduce the surviving fraction of EMT6/KU cells to 1%. Usually, each ER value of radiosensitizer was obtained from survival curves consisting of four or five points per curve and converted based on the ER value of etanidazole (ER = 1.72).[26]

Acknowledgments

This work was partially supported by Grant-in-Aid for Young Scientist (B) (No. 18790892) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors thank Dr. Hiromu Nishitani, Mr. Yasuo Nishimoto, Mr. Taro Kishi, and the staff of Department of Radiology, School of Medicine, The University of Tokushima, for generous help with the radiological experiments. The authors also thank Ms. Maki Nakamura, Ms. Kayoko Yamashita, and the staff of our Faculty for measurement of NMR, and Ms. K. Kirk acknowledges support from the NIDDK intramural research program.

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