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Chemical Probes

6-Azido-6-deoxy-L-idose as a Hetero-Bifunctional Spacer for the Synthesis of Azido-Containing Chemical Probes

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Abstract: The design of 6-azido-6-deoxy-L-idose for use as a hetero-bifunctional spacer is reported. The hemiacetal at one terminus is an equivalent of an aldehyde and can react with nucleophiles, such as amino groups and electron-rich aromatics. The azido group at the other terminus bio-orthogonally undergoes a Hüisgen [3+2] cycloaddition with an acetylene. The idose derivative exhibited a higher level of reactivity towards oxime formation than a corresponding glucose derivative. The ^{13}C NMR spectrum of the uniformly ^{13}C-labeled 6-azido-idose indicated that the acyclic forms of the sugar totaled 0.3% of all the isomers, whereas those of glucose totaled 0.01%. The larger population of the acyclic forms of the idose derivative would result in higher reactivity towards electrophilic addition in comparison with glucose derivatives. Finally, we prepared a *C*-idosyl epigallocatechin gallate (EGCG) that bears an azido group through *C*-glycosylation of EGCG with 6-azido-idose. This glycosyl form of the *C*-idosyl EGCG exhibited a cytotoxicity against U266 cells that was comparable to that of EGCG. These results suggested that the EGCG derivative could be used as an effective chemical probe for the elucidation of EGCG biological functions.

Introduction

Identifying the receptors of biologically active natural products that exhibit unique biological activities is an effective strategy for elucidating the roles of these biomolecules. Natural product derivatives possessing functional devices, such as a bio-orthogonal functional group, a fluorescent dye, a photoaffinity labeling unit, and affinity beads, are effective chemical probes for the elucidation of the mechanism of the biological action.[1] The synthesis of chemical probes that are based on natural products can be categorized into two approaches. The first is a de novo synthesis approach based on the preparation of chemical probes from simple building blocks.[2] This approach affords a high level of flexibility in the design of the chemical probes. However, the de novo synthesis of chemical probes frequently requires laborious efforts by well-trained synthetic chemists. A second version is referred to as semisynthesis, and it involves modifying natural products.[3] This approach omits the synthesis of the structure of natural products and minimizes the synthetic efforts required to synthesize the chemical probes. However, incorporating spacers that possess bio-orthogonal functional groups in natural products that have multiple reactive functional groups is difficult to accomplish without loss of their biological activities.

Hydrophilic hetero-bifunctional spacers that possess both a chemoselectively reactable functional group and a bio-orthogonal functional group are effective tools when preparing hybrid molecules by connecting two functional molecules.[4] The hydrophilicity of the spacers is important to minimize non-specific interactions with biomolecules. Reducing sugars that possess a bio-orthogonal functional group, such as an azido group or a terminal acetylene group, have served as useful hetero-bifunctional spacers in chemical glycobiology.[5] These sugars undergo enzymatic glycosidation in vivo to introduce a bio-orthogonal functional group into the targeted biomolecules. The azido group can be stained with a fluorescent dye possessing an alkyne moiety through a Hüisgen [3+2] cycloaddition.[6] On the other hand, reducing sugars chemically react with nucleophiles, such as amino groups in aqueous media.[7] Imine formation is often applied to the synthesis of sugar-containing materials and biochemical probes.[8] In addition, reducing sugars undergo electrophilic substitution of electrically rich aromatics in the presence of a Lewis acid.[9] Rauter and co-

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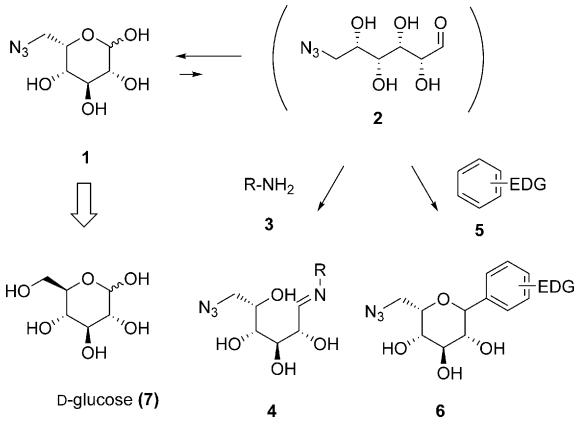
workers reported the synthesis of *C*-glycosyl flavanones through *C*-glycosylation of flavanones with various sugars catalyzed either by $Sc(OTf)_3$ or $Pr(OTf)_3$ in aqueous media.[10] One of the important factors determining the reactivity of reducing sugars towards electrophilic reactions is the population of the acyclic forms involving acetals and aldehydes.[7,11] Serianni and co-workers quantified the population of the acyclic forms of glucose by using ^{13}C NMR analysis and determined it to be 0.01%.[12] On the other hand, idose, a C5 epimer of glucose, involved the acyclic forms in 0.8%, which was the maximum among aldohexoses. These results suggested to us that idose would exhibit a higher reactivity towards electrophilic reactions than other aldohexoses. Herein, we report the use of 6-azido-6-deoxy-L-idose[13] (**1**) as a hetero-bifunctional spacer and its application to the synthesis of (−)-8-(6-azido-6-deoxy-β-L-idopyranosyl)-3-(3,4,5-trihydroxybenzoyl)epigallocatechin (**22**) as a chemical probe.

Results and Discussion

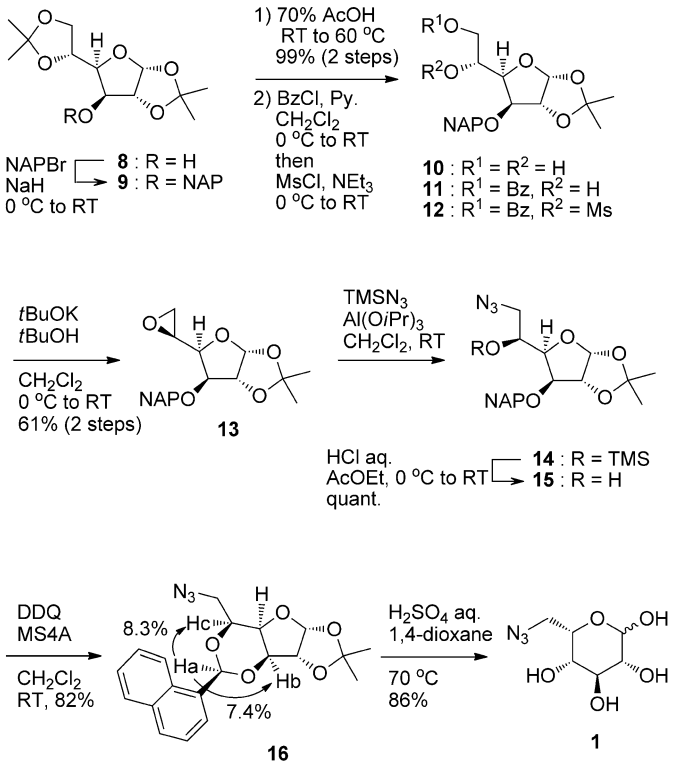
We designed the 6-azido-idose **1** as a hetero-bifunctional spacer (Scheme 1). The spacer **1** was chemically coupled not only with the electron-rich aromatic compounds **5** to provide the *C*-glycosyl-type compounds **6**, but also with the amines **3** to provide the imines **4**. An enriched population of the acyclic forms **2** of compound **1** resulted in a high reactivity of compound **1** towards electrophilic addition. The remaining azido group could be bio-orthogonally reacted with a terminal acetylene group through a Hüisgen [3+2] cycloaddition for the further conjugation of a functional device. The 6-azido-idose **1** could be prepared from D-glucose (**7**) through epimerization at the 5-position and incorporation of the azido group at the 6-position.

Scheme 2 shows the synthesis of the 6-azido-idose **1** from diacetone-D-glucose (**8**) by using the epoxide **13** as an intermediate. The preparation of compound **13** was achieved based on a reported method[14] for the synthesis of a related compound possessing a benzyl ether group instead of a (2-naphthyl)methyl ether. Etherification of the diacetonide **8** with 2-(bromomethyl)naphthalene under basic conditions to provide the ether **9**, was followed by hydrolysis of the acetal to provide the 1,2-diol **10** in 99% yield from compound **8**. Selective acylation of the primary alcohol function of compound **10**, followed by mesylation of the remaining secondary alcohol of compound **11** provided the mesylate **12**. Exposure of compound **12** under basic conditions resulted in the hydrolysis of the benzoate, followed by intramolecular etherification to provide the epoxide **13** in 61% total yield from compound **10**. We next examined the incorporation of an azido group through epoxide-opening of compound **13**. Treatment of the epoxide **13** with $TMSN_3$ (TMS = trimethylsilyl) in the presence of $Al(OiPr)_3$ at room temperature,[15] followed by hydrolysis of the resultant TMS ether **14** with 1 M HCl provided the azide **15** quantitatively. Oxidative acetalization of compound **15** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) under anhydrous conditions stereoselectively provided the acetal **16** in 82% yield. NOE experiments with compound **16** showed that the irradiation of the proton Ha enhanced the signals of both protons Hb (7.4%) and Hc (8.3%). These results revealed that the stereochemistry of the generated chiral center in acetal **16** is *S*. Removal of all the protecting groups of compound **16** under acidic conditions provided the 6-azido-idose **1** in 86% yield.

We first examined the oxime formation of the sugars **1**, **7**, and **18** with hydroxylamine (Scheme 3). *O*-Benzylhydoxylamine hydrochloride was treated with an equivalent of the sugars **1**, **7**, and **18** in a phosphate buffer (pH 7.0) at 0 °C for 22 h. The

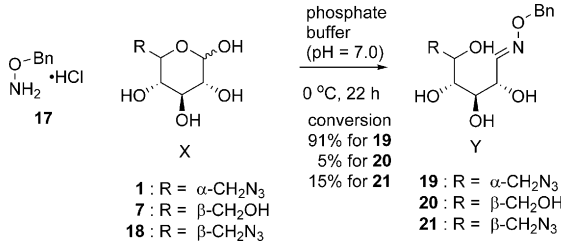


Scheme 1. Design of 6-azido-6-deoxy-L-idose (**1**) as a hetero-bifunctional spacer.



Scheme 2. Synthesis of the 6-azido-idose **1**. NAP = 2-naphthylmethyl, Ac = acetyl, Bz = benzoyl, Py = pyridine, Ms = mesyl.

reaction mixtures were analyzed by using HPLC based on UV absorption at $\lambda = 254$ nm. The transformation rates of hydroxylamine to the oximes **20** and **21** from D-glucose (**7**) and the 6-azido-glucose **18** were 5 and 15%, respectively. On the other hand, the transformation rate to the oxime **19** from the 6-azido-idose **1** was 91%. The yield of the isolated compound **19** was 85%. These results clearly indicated that the 6-azido-idose **1** exhibited a higher reactivity than the glucose derivatives **7** and **18**.



Scheme 3. Oxime formation. Bn = benzyl.

Next, we estimated the population of the acyclic forms of the 6-azido-idose **1** by using ^{13}C NMR measurement of the uniformly ^{13}C-labeled 6-azido-idose U-^{13}C-**1**. The U-^{13}C-6-azido-idose U-^{13}C-**1** was prepared from uniformly ^{13}C-labeled D-glucose through our established method. Figure 1 shows the ^{13}C NMR spectrum of the U-^{13}C-6-azido-idose U-^{13}C-**1**. The assignment of each signal was achieved based on a previous report[16] by comparing the chemical shifts and the scalar coupling constants. The populations of the six isomers of compound **1** involving the furanoses F-α and F-β, the pyranoses P-α and P-β, and the acyclic acetal and aldehyde Acetal and Alde were 12.7, 16.4, 36.1, 34.4, 0.2, and 0.1%, respectively, based on each ^{13}C peak intensity (Scheme 4). The acyclic forms totaled 0.3% of all the isomers. The population of the acyclic forms of compound **1** was more than ten times that of glucose.[12] The larger population of the acyclic forms may explain the higher level of reactivity of the 6-azido-idose **1**.

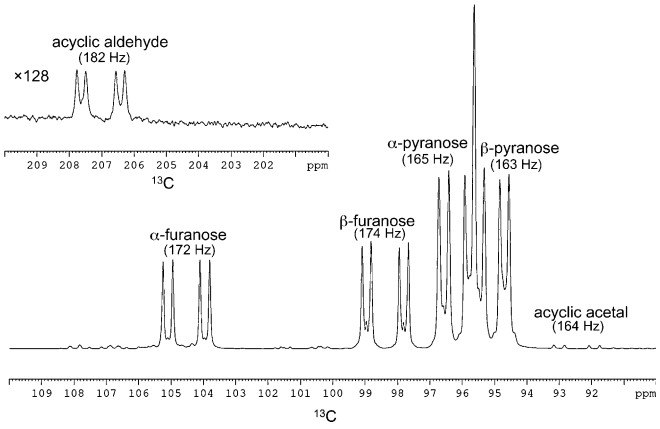
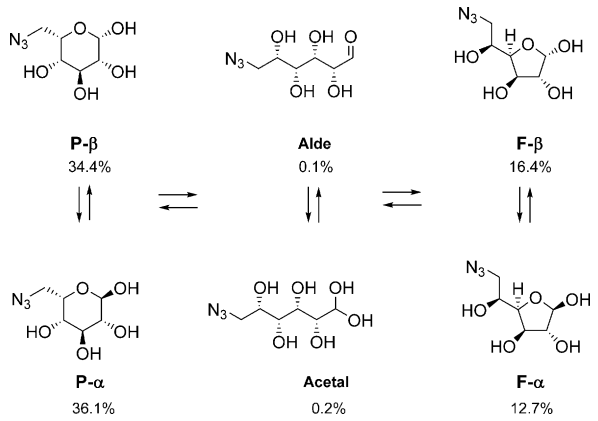


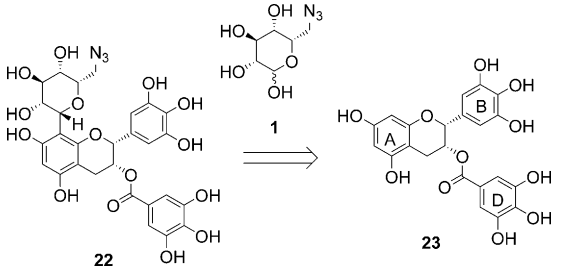
Figure 1. 150 MHz ^1H-coupled ^{13}C NMR spectrum of the U-^{13}C-6-azido-idose U-^{13}C-**1** (anomeric carbon region) dissolved in D_2O. The inset shows the vertical scale expansion of the aldehyde signal. Each C1 signal appears in a quartet, due to ^1J(C1,C2) (≈40 Hz) and ^1J(C1,H1) (160–180 Hz) scalar couplings. The ^1J(C1,H1) coupling constants are indicated for each peak.



Scheme 4. The six isomers of compound **1** involving the furanoses F-α and F-β, the pyranoses P-α and P-β, and the acyclic acetal and aldehyde Acetal and Alde.

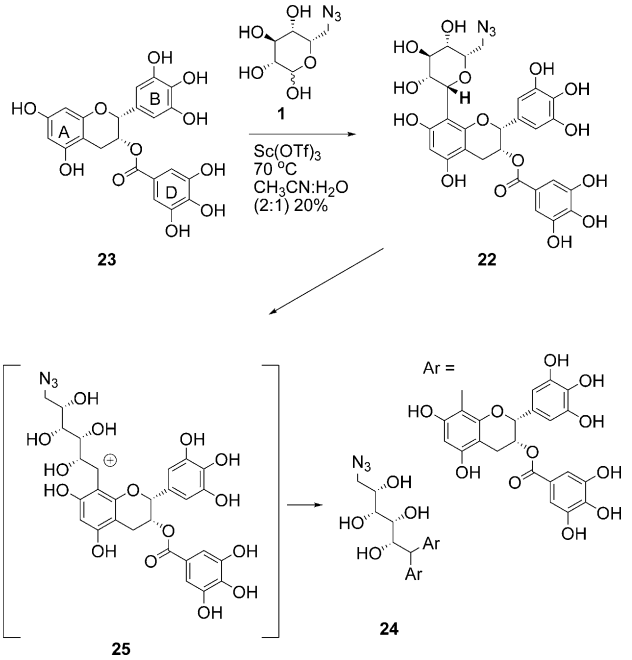
To demonstrate the utility of the 6-azido-idose **1**, we planned the synthesis of (−)-8-(6-azido-6-deoxy-β-L-idopyranosyl)-3-(3,4,5-trihydroxybenzoyl)epigallocatechin (**22**) through the direct *C*-glycosylation of epigallocatechin gallate (EGCG, **23**) with the 6-azido-idose **1**. The EGCG **23** is a phytochemical found in tea leaves that exhibits various biological activities (Scheme 5).[17] The gallate acts as a pharmacophore to bind a 67 kDa laminin receptor (67 LR), which mediates anti-cancer and anti-allergic actions.[18] In addition, the B-ring of the EGCG was easily oxidized under physiological conditions to an *ortho*-quinone moiety and was reacted with an amino group and a thiol group on proteins.[19] The group of Nakayama reported that the adduct of EGCG and proteins was stained with a functionalized boronic acid through esterification of the catechol moiety on the D-ring.[20] We expected the 6-azido-idose **1** to undergo *C*-glycosylation of the EGCG **23** at the C8- or C6-position under mildly acidic conditions. The resultant *C*-glycosyl EGCG **22** possessing an azido group was an effective chemical probe for exploring the mode of the biological action of the EGCG.

We first compared the reactivity of the 6-azido-idose **1** and the 6-azido-glucose **17** (Scheme 6). The EGCG **23** was exposed to either an equivalent of the 6-azido-idose **1** or the 6-azido-glucose **18** in the presence of $Sc(OTf)_3$ at 70 °C for 3 h.[21] The



Scheme 5. Synthesis of the *C*-idosyl EGCG **22** from the EGCG **23** and the 6-azido-idose **1**.

conversion of the reaction using compounds **1** and **18** was estimated to be 60 and 12%, respectively, through HPLC analysis of the remaining EGCG **1** based on UV absorption at $\lambda =$ 254 nm. The EGCG was stable under the acidic conditions without the sugars **1** and **18**. These results clearly indicated that the 6-azido-idose **1** would be more reactive towards electrophilic substitution of the aromatic rings than the 6-azido-glucose **18**. To identify the major product of the reaction, we treated the EGCG **23** with an equivalent of the 6-azido-idose **1** in the presence of $Sc(OTf)_3$ at 70 °C for 9 h. HPLC-MS analysis of the crude mixture indicated that the reaction mixture contained several mono-glycosyl products (molecular weight (MW) = 645), and the compound (MW = 1104). These results suggested that the reaction mixture from the 6-azido-idose **1** might contain the α- and β-anomeric stereoisomers and the pyran and furan derivatives as well as the regioisomers at C6- and C8-positions. In addition, in the reaction a further electrophilic addition of the benzylic cation **25** with the EGCG **23** might have occurred to provide the 1:2 complex **24** of the sugar and the EGCG. A major product was purified by preparative HPLC based on a MS-triggered detection to provide the *C*-glycosyl EGCG **22** in 20% yield as a single isomer. Although the yield was not high, the single-step transformation of the natural EGCG to the azido-containing EGCG **22** would be useful for the synthesis of biochemical probes for the elucidation of the mechanism of the function of the EGCG. It should be noted that when the isolated *C*-glycosyl EGCG was exposed to the reaction conditions, it provided several isomers possessing the same molecular weight (i.e., MW = 645). These results indicated that the idose unit would be easily isomerized under acidic conditions not only to an α-isomer but also to furan derivatives.



Scheme 6. Direct *C*-glycosylation of the EGCG **23** with the hetero-bifunctional spacer **1**.

Structure determination of the *C*-glycosyl product was achieved by ^1H and ^{13}C NMR spectroscopy as well as 2D NMR analyses (i.e., COSY, HMQC, and HMBC experiments) (Figure 2). In the ^1H NMR spectra of compound **22** the signal for protons on the A ring disappeared. A broad singlet at $\delta = 5.65$ ppm assigned to H-1‴, showed HMQC correlations with the ^{13}C signal at $\delta = 76.2$ ppm and HMBC correlations with the ^{13}C signals at $\delta = 157.8$, 153.0, and 103.2 ppm. These results indicated that *C*-glycosylation of the EGCG **23** with the idose **1** occurred at the C6- or C8-position. The stereochemistry of the glycosidic linkage was determined by NOE observation between H-1‴ and H-5‴ (6.4%) to be β. According to the report on the synthesis of *C*-glycosyl flavonoids,[10,22] HMBC correlation between H-2 and aromatic carbon atoms enabled assignment of a ^{13}C signal at the 8a-position, which was critical information to determine the position of the *C*-glycosylation. However, the requisite HMBC correlation was not observed. We finally determined the position of the *C*-glycosylation of the EGCG to be C8 based on NOE observation between H-1‴ and H-2′ (2.0%).

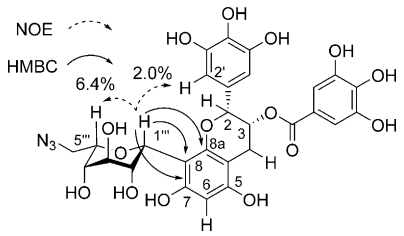


Figure 2. Key HMBC correlations and NOE observations for the structural assignment of compound **22**.

Next, we investigated the anti-cancer effect of the *C*-idosyl EGCG **22** in U266 cells expressing 67LR (a mouse melanoma cell line) (Figure 3). The anti-cancer effects against U266 cells served as an index for the affinity of EGCG-related compounds to 67LR. EGCG was used as a positive control. The U266 cells were incubated with compound **22** (0, 5.0, 10, or 25 μM) for 96 h at 37 °C. The relative number of viable cells was estimated by using an ATPlite assay. Our data suggested that the *C*-idosyl EGCG **22** exhibited a comparable cytotoxicity against U266 cells to that of EGCG. These results suggested that the azido-bearing EGCG derivative could be used not only as a chemical probe for the analysis of covalent adducts from EGCG, but also as a precursor for the synthesis of chemical probes analyzing EGCG-associated proteins involving 67LR.

Conclusion

In conclusion, 6-azido-6-deoxy-L-idose (**1**) was an effective hetero-bifunctional spacer for the synthesis of azido-containing chemical probes. The azido group can bio-orthogonally react with terminal acetylene through a Hüisgen [3+2] cycloaddition. The masked aldehyde **1** was more reactive towards imination than either D-glucose (**7**) or the 6-azido-glucose **18** due to its enhanced population of the acyclic forms involving the acetal and the aldehyde. The population of the acylic forms of

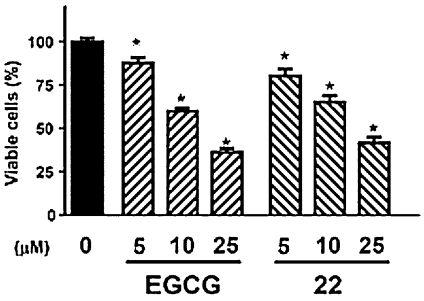


Figure 3. Anti-cancer effect of the *C*-idosyl EGCG **22** in human multiple myeloma cells. U266 cells were treated with either EGCG or the *C*-idosyl EGCG for 96 h and the relatively viable cell number was assessed through an AT-Plite assay ($n=3$) repeated three independent times. Error bars represent the standard deviation. (*, $P<0.05$ vs. control; corresponding to 0 μM). Statistical analysis was performed with ANOVA followed by a Dunnett's test (vs. control; corresponding to 0 μM)).

compound **1** was estimated to be 0.3% by using the ^{13}C NMR spectrum of the U-^{13}C-6-azido-idose U-^{13}C-**1**, which was more than ten times the reported data for glucose. We next applied the 6-azido-idose **1** to the synthesis of a *C*-glycosyl EGCG as a chemical probe. Treatment of EGCG with the 6-azido-idose **1** in the presence of $Sc(OTf)_3$ provided the *C*8-idosyl EGCG **22** in 20% yield. The azido-containing EGCG **22** exhibited cytotoxicity against U266 cells that was comparable to that of EGCG. These results suggested that the azido-containing EGCG **22** could be used for biochemical probes to elucidate the mode of the biological action. Biological research using compound **22** is now in progress.

Experimental Section

General information: NMR spectra were recorded on a JEOL Model ECP-400 spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C) and a Bruker instrument in the indicated solvent. Chemical shifts were reported in parts per million [ppm] relative to the signal ($\delta=$ 0.00 ppm) for internal tetramethylsilane solutions in $CDCl_3$. The ^1H NMR spectral data are reported as follows: $CDCl_3$ ($\delta=7.26$ ppm), CD_3OD ($\delta=3.31$ ppm). The ^{13}C NMR spectral data are reported as follows: $CDCl_3$ ($\delta=77.16$ ppm), CD_3OD ($\delta=49.0$ ppm). Multiplicities are reported by the following abbreviation: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad, J = coupling constants in Hertz. IR spectra were recorded on a Perkin–Elmer Spectrum One FTIR spectrometer. Only the strongest and/or structurally important peaks are reported as IR data given in [cm^{-1}]. ESI-TOF mass spectra were measured with a Waters LCT Premier™ XE. HRMS (ESI-TOF) were calibrated by using Leu-enkephalin. Optical rotations were measured on a JASCO model P-1020 polarimeter. All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plate (60F-254) with UV light ($\lambda=$ 254 nm), visualized by using a *p*-anisaldehyde solution. Column chromatography separations were performed by using silica gel (Merck, silica gel). Analytical HPLC was carried out on a SSC-3461 pump with a SSC-5410 UV detector by using a 4.6×250 mm^2, 3 μm, Inertsil ODS-3 column (GL Sciences Inc.). Preparative HPLC was carried out on a Waters AutoPurification™ System (a Waters 600 controller pump and a Waters 2767 sample manager) with a Waters 2767 Dual λ absorbance detector by using a $20\times$ 250 mm^2, 5 μm, Inertsil ODS-3 column (GL Sciences Inc.). THF and CH_2Cl_2 were dried by using a glass contour. *tert*-Butanol was distilled from CaH_2. For 0.1 M $BnONH_2$ solution with pH 7.0 phosphate buffer, $BnONH_2$·HCl (80 mg, 0.50 mmol) was dissolved in phosphate buffer solution (5.0 mL), which was prepared by dissolving Na_2HPO_4 (200 mg) in water. D-Glucose-$^{13}C_6$ (99 atom% ^{13}C) was purchased from SI Science Co., Ltd.

1,2-*O*-Isopropylidene-3-*O*-(2-naphthyl)methyl-α-D-glucofuranose (10): NaH (60% in mineral oil, 5.30 g) in a round bottom flask was washed with hexane to remove the mineral oil. After decanting the hexane, THF (134 mL) was added to the reaction vessel. Then, 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**8**) (17.4 g, 66.8 mmol), 2-(bromomethyl)naphthalene (15.5 g, 70.1 mmol), and tetrabutylammonium iodide (1.20 g, 3.25 mmol) were sequentially added to the reaction mixture at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was poured into water at 0 °C and extracted with two portions of ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was used in the next reaction without further purification. The residue was dissolved in 70% aqueous acetic acid (200 mL, 2450 mmol). After being stirred at 60 °C for 2 h, the reaction mixture was poured into an aqueous solution (200 mL) of NaOH (88.0 g, 2200 mmol) and $NaHCO_3$ (21.0 g, 250 mmol) at 0 °C and extracted with two portions of ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane = 2:1) to yield compound **10** (23.8 g, 66.0 mmol, 99% in two steps). $[\alpha]_D^{29}=-33.8$ ($c=0.755$, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=7.84$–7.78 (m, 4H; aromatic), 7.49–7.43 (m, 3H; aromatic), 5.94 (d, $J=3.4$ Hz 1H; H-1), 4.85 (d, $J=$ 12.2 Hz, 1H; benzyl), 4.71 (d, $J=12.2$ Hz, 1H; benzyl), 4.65 (d, $J=$ 3.9 Hz, 1H; H-2), 4.16–4.12 (m, 2H; H-3, H-4), 4.07–4.04 (m, 1H; H-5), 3.81 (dd, $J=11.7$, 3.4 Hz, 1H; H-6a), 3.70 (dd, $J=11.7$, 5.8 Hz, 1H; H-6b), 1.47 (s, 3H; acetal), 1.30 ppm (s, 3H; acetal); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=134.7$ (aromatic), 133.4 (aromatic), 133.3 (aromatic), 128.8 (aromatic), 128.1 (aromatic), 127.9 (aromatic), 127.0 (aromatic), 126.5 (aromatic), 126.4 (aromatic), 125.6 (aromatic), 112.0 (acetal), 105.3 (C-1), 82.3 (C-2), 82.1 (C-3), 80.2 (C-4), 72.4 (benzyl), 69.4 (C-5), 64.5 (C-6), 26.9 (CH_3), 26.4 ppm (CH_3); FTIR (neat): $\tilde{\nu}=436$, 2937, 1376, 1215, 1165, 1079, 1020, 752 cm^{-1}; HRMS (ESI-TOF): *m/z* calcd for $C_{20}H_{24}O_6$: 383.1471 $[M+Na]^+$; found: 383.1465.

5,6-*O*-Anhydro-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)methyl-β-L-idofuranose (13): Pyridine (57.0 mL, 708 mmol) and benzoyl chloride (8.25 mL, 71.1 mmol) were sequentially added to a solution of compound **10** (23.8 g, 66.0 mmol) in CH_2Cl_2 (200 mL) at 0 °C. After being stirred for 2 h at room temperature, methanesulfonyl chloride (8.25 mL, 107 mmol) and triethylamine (30 mL, 216 mmol) were sequentially added to the solution at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was poured into 3 M aqueous HCl solution, and the mixture was extracted with two portions of ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was used in the next reaction without further purification. Potassium *tert*-butoxide (15.0 g, 134 mmol) was added to a solution of the obtained residue in *tert*-butanol (200 mL) and CH_2Cl_2 (200 mL) at 0 °C. After being stirred at room temperature for 5 h, the reaction mixture was poured into a saturated aqueous NH_4Cl solution and the obtained mixture was extracted with two portions of ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on

silica gel (ethyl acetate/hexane = 1/3) and recrystallized to give compound **13** (13.8 g, 40.3 mmol, 61% in two steps). $[\alpha]_D^{26} = -64.9$ ($c = 0.385$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.84–7.76$ (m, 4H; aromatic), 7.49–7.42 (m, 3H; aromatic), 6.03 (d, $J = 3.4$ Hz, 1H; H-1), 4.89 (d, $J = 12.7$ Hz, 1H; benzyl), 4.68–4.64 (m, 2H; H-2, benzyl), 4.00 (d, $J = 3.9$ Hz, 1H; H-3), 3.81 (dd, $J = 5.9$, 3.4 Hz, 1H; H-4), 3.33–3.31 (m, 1H; H-5), 2.76 (t, $J = 5.0$ Hz, 1H; H-6a), 2.51 (dd, $J = 4.9$, 2.9 Hz, 1H; H-6b), 1.44 (s, 3H; acetal), 1.32 ppm (s, 3H; acetal); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 134.7$ (aromatic), 133.2 (aromatic), 133.2 (aromatic), 128.5 (aromatic), 127.9 (aromatic), 127.8 (aromatic), 126.7 (aromatic), 126.4 (aromatic), 126.3 (aromatic), 125.5 (aromatic), 112.0 (acetal), 105.6 (C-1), 82.6 (C-3), 82.5 (C-4), 82.2 (C-2), 72.0 (benzyl), 50.3 (C-5), 43.3 (C-6), 26.9 (CH_3), 26.4 ppm (CH_3); FTIR (neat): $\tilde{\nu} = 2989$, 1164, 1076, 1028, 856 cm^{-1}; HRMS (ESI-TOF): *m/z* calcd for $C_{20}H_{22}O_5$: 365.1365 $[M+Na]^+$; found: 365.1379.

6-Azido-6-deoxy-1,2-*O*-isopropylidene-3-*O*-(2-naphthyl)methyl-β-L-idofuranose (15): A mixture of $Al(OiPr)_3$ (0.358 g, 1.75 mmol) and Me_3SiN_3 (1.65 mL, 12.6 mmol) in CH_2Cl_2 (15.0 mL) was stirred at room temperature for 2 h. A solution of compound **13** (0.955 g, 2.79 mmol) in CH_2Cl_2 (4.2 mL) was added to the mixture at room temperature. After being stirred at room temperature for 29 h, the reaction mixture was poured into a biphasic solution composed of 1 M aqueous HCl solution and ethyl acetate. After being stirred at room temperature for 12 h, the mixture was extracted with two portions of ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/toluene = 1/4) to yield compound **15** (1.09 g, 2.83 mmol, 100%). $[\alpha]_D^{28} = -60.3$ ($c = 0.115$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.87–7.82$ (m, 3H; aromatic), 7.75 (s, 1H; aromatic), 7.52–7.48 (m, 2H; aromatic), 7.42 (dd, $J = 8.3$, 1.4 Hz, 1H; aromatic), 6.02 (d, $J = 3.9$ Hz, 1H; H-1), 4.89 (d, $J = 12.2$ Hz, 1H; benzyl), 4.71 (d, $J = 3.9$ Hz, 1H; H-2), 4.62 (d, $J = 12.2$ Hz, 1H; benzyl), 4.16–4.12 (m, 2H; H-4, H-5), 4.05 (d, $J = 3.4$ Hz, 1H; H-3), 3.36 (dd, $J = 12.7$, 6.3 Hz, 1H; H-6a), 3.28 (dd, $J = 13.2$, 4.4 Hz, 1H; H-6b), 1.49 (s, 3H; acetal), 1.34 ppm (s, 3H; acetal); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 133.9$ (aromatic), 133.3 (aromatic), 133.2 (aromatic), 128.9 (aromatic), 128.0 (aromatic), 127.9 (aromatic), 127.3 (aromatic), 126.6 (aromatic), 126.5 (aromatic), 125.6 (aromatic), 112.2 (acetal), 105.1 (C-1), 82.8 (C-3), 82.4 (C-2), 79.8 (C-4), 72.1 (benzyl), 69.8 (C-5), 53.2 (C-6), 26.9 (CH_3), 26.4 ppm (CH_3); FTIR (neat): $\tilde{\nu} = 2929$, 2103, 1164, 1075, 1028 cm^{-1}; HRMS (ESI-TOF): *m/z* calcd for $C_{20}H_{23}N_3O_5$: 386.1716 $[M+H]^+$; found: 386.1715.

(*S*)-6-Azido-6-deoxy-1,2-*O*-isopropylidene-3,5-*O*-(2-naphthyl)methylidene-β-L-idofuranose (16): Activated molecular sieve (4 Å) (0.540 g) and DDQ (0.670 g, 2.95 mmol) were sequentially added to a solution of compound **15** (1.03 g, 2.67 mmol) in CH_2Cl_2 (16.0 mL) at room temperature. After being stirred at room temperature for 12 h, the reaction mixture was poured into a saturated aqueous solution of $NaHCO_3$. After being stirred at room temperature for 11 h, the reaction mixture was extracted with two portions of dichloromethane. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane = 1/3) to yield compound **16** (0.840 g, 2.20 mmol, 82%). $[\alpha]_D^{26} = +2.8$ ($c = 0.310$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.99$ (s, 1H; aromatic), 7.87–7.82 (m, 3H; aromatic), 7.58 (d, $J = 8.3$ Hz, 1H; aromatic), 7.51–7.47 (m, 2H; aromatic), 6.05 (d, $J = 3.9$ Hz, 1H; H-1), 5.71 (s, 1H; benzyl), 4.69 (d, $J = 3.9$ Hz, 1H; H-2), 4.51 (d, $J = 2.0$ Hz, 1H; H-3), 4.28 (ddd, $J = 8.8$, 4.4, 2.0 Hz, 1H; H-5), 4.12 (t, $J = 2.4$ Hz, 1H; H-4), 3.83 (dd, $J = 13.2$, 8.3 Hz, 1H; H-6a), 3.46 (dd, $J = 13.2$, 4.4 Hz, 1H; H-6b), 1.53 (s, 3H; acetal), 1.35 ppm (s, 3H; acetal); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 134.3$ (aromatic), 133.7 (aromatic), 132.9 (aromatic), 128.4 (aromatic), 128.1 (aromatic), 127.7 (aromatic), 126.5 (aromatic), 126.2 (aromatic), 125.6 (aromatic), 123.5 (aromatic), 112.2 (acetal), 105.8 (C-1), 99.3 (benzyl), 83.2 (C-2), 79.6 (C-3), 75.7 (C-5), 72.3 (C-4), 51.7 (C-6), 26.7 (CH_3), 26.2 ppm (CH_3); FTIR (neat): $\tilde{\nu} = 2102$, 1165, 1141, 1096, 1073, 1015, 859, 823 cm^{-1}; HRMS (ESI-TOF): *m/z* calcd for $C_{20}H_{22}N_3O_5$: 384.1559 $[M+H]^+$; found: 384.1538.

6-Azido-6-deoxy-L-idose (1): A 1 M aqueous H_2SO_4 solution (2.80 mL) was added to a solution of compound **16** (0.353 g, 0.921 mmol) in 1,4-dioxane (2.80 mL) at room temperature. After being stirred for 11 h at 70 °C, Amberlite® IRA-67 free base was added to the solution at 0 °C. The mixture was filtered through a pad of Celite and concentrated in vacuo. The residue was purified by reverse-phase column chromatography on Bondesil-C18 (water) to yield compound **1** (0.163 g, 0.795 mmol, 86%). $[\alpha]_D^{30} = +12.7$ ($c = 0.565$, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 5.36$ (d, $J = 3.9$ Hz), 5.09 (s), 4.99 (d, $J = 2.9$ Hz), 4.98 (s), 4.32–4.28 (m), 4.16 (t, $J = 4.9$ Hz), 4.10–3.97 (m), 3.79 (t, $J = 4.9$ Hz), 3.61–3.52 (m), 3.46–3.45 (m), 3.41–3.35 ppm (m); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 104.1$, 97.5, 96.3, 94.0, 83.3, 82.9, 79.8, 78.4, 77.5, 77.3, 74.5, 72.1, 71.5, 71.5, 71.2, 71.1, 71.0, 70.0, 68.9, 54.9, 54.6, 52.6, 52.0 ppm; FTIR (neat): $\tilde{\nu} = 3436$, 2535, 2117, 1634 cm^{-1}; HRMS (ESI-TOF): *m/z* calcd for $C_6H_{11}N_3O_5$: 206.0777 $[M+H]^+$; found: 206.0815.

(−)-8-(6-Azido-6-deoxy-β-L-idopyranosyl)-3-(3,4,5-trihydroxybenzoyl)-epigallocatechin (22): Scandium trifluoromethanesulfonate (12.0 mg, 0.0244 mmol) was added to a mixture of (−)-epigallocatechin-3-*O*-gallate (**23**) (55.9 mg, 0.122 mmol) and compound **1** (25.0 mg, 0.122 mmol) in acetonitrile/water (2:1; 0.364 mL) at room temperature. After being stirred at 70 °C for 9 h, the reaction mixture was diluted with water and purified by reverse-phase column chromatography (VARIAN Bond ELUT® C18) to remove unreacted monosaccharide and scandium trifluoromethaesulfonate. The organic layer was concentrated in vacuo. The residue was subjected to chromatography by using a preparative 20 × 250 mm^2, 5 μm, Inertsil ODS-3 column (GL Sciences Inc.). Chromatography was performed by using 20% acetonitrile/water containing 0.1% formic acid as the eluent. After the solvent was removed in vacuo, the residue was suspended in water, and freeze-dried to afford compound **22** (15.7 mg, 20%). $[\alpha]_D^{29} = -46.9$ ($c = 0.55$, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 6.94$ (s, 2H; H-2″), 6.51 (s, 2H; H-2′), 5.96 (s, 1H; H-6), 5.65 (s, 1H; H-1‴), 5.55 (brs, 1H; H-3), 5.02 (s, 1H; H-2), 4.12 (t, $J = 5.8$ Hz, 1H; H-5‴), 3.99 (t, $J = 2.9$ Hz, 1H; H-3‴), 3.72–3.66 (m, 2H; H-2‴, H-6‴a), 3.60 (s, 1H; H-4‴), 3.52 (dd, $J = 13.2$, 5.4 Hz, 1H; H-6‴b), 3.00 (dd, $J = 17.6$, 4.9 Hz, 1H; H-4a), 2.86 ppm (dd, $J = 17.6$, 2.4 Hz, 1H; H-4b); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 167.7$ (carbonyl), 157.8 (C-7), 157.2 (C-5), 153.0 (C-8a), 146.8 (C-3′), 146.3 (C-3″), 139.8 (C-4″), 133.8 (C-4′), 130.6 (C-1′), 121.5 (C-1″), 110.3 (C-2″), 106.6 (C-2′), 103.2 (C-8), 99.3 (C-5a), 97.3 (C-6), 78.5 (C-2), 77.0 (C-5‴), 76.2 (C-1‴), 73.1 (C-2‴), 70.1 (C-4‴), 69.7 (C-3‴), 69.5 (C-3), 49.6 (C-6‴), 26.7 ppm (C-4); FTIR (neat): $\tilde{\nu} = 3271$, 2114, 1679, 1451, 1338, 1239, 1146, 1041 cm^{-1}; HRMS (ESI-TOF): *m/z* calcd for $C_{28}H_{27}N_3O_{15}$: 668.1340 $[M+Na]^+$; found: 668.1346.

NMR experiments: Quantitative ^{13}C NMR spectra were obtained by using an AVANCE 600 spectrometer equipped with a TXI probe (BrukerBioSpin). The probe temperature was set at 25 °C and the ^{13}C NMR spectra were obtained with 16000 scans at a recycle time of 11 s. To avoid ^{13}C–^{1}H heteronuclear NOEs, ^{1}H-decoupling was not applied throughout the measurement. ^{13}C chemical shifts (in [ppm]) were calibrated by using an exterior standard chemical shift (4,4-dimethyl-4-silapentane-1-sulfonic acid, set to $\delta = 0$ ppm). NMR data processing and analysis were performed by using TOPSPIN (version 2.1, BrukerBioSpin).

Cell culture and ATPlite assay: The anti-cancer effects of EGCG and the *C*-idosyl EGCG were assessed as described (Kumazoe et al., Scientific Reports 2015 srep09474).[23] Briefly, the human multiple myeloma cell line U266 was cultured in RPMI1640 supplemented with 10% fetal bovine serum in a humidified atmosphere with 5% CO_2 at 37 °C. U266 cells were plated into 24-well plates (50000 cells per well) and treated with the indicated concentrations of EGCG and the *C*-idosyl EGCG for 96 h in RPMI1640 medium supplemented with 1% fetal bovine serum, 200 units mL^{-1} catalase, and 5 units mL^{-1} superoxide dismutase. After 96 h, viable cells were assessed through an ATPlite One step™ assay (Perkin–Elmer, Montreal, Canada) according to the protocol of the manufacturer. Statistical analysis was performed with ANOVA followed by a Dunnett's test.

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