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SYNTHESIS AND RADIOIODINATION OF TEN ARYL-CARBOHYDRATE COMPOUNDS FOR LABELING MONOCLONAL ANTIBODIES.

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SUMMARY

Ten aryl-carbohydrate ligands have been synthesized that potentially increase the tumor retention and decrease the deiodination of iodinated monoclonal antibodies used in the imaging and treatment of cancer. The compounds were synthesized via reductive amination reactions, purified with cation exchange chromatography, and characterized using ^{1}H and ^{13}C NMR. These compounds were then radioiodinated to assess their iodination characteristics, for subsequent experiments involving attachment to monoclonal antibodies.

KEY WORDS: Aryl-Carbohydrate, Radioiodination, Monoclonal Antibodies.

INTRODUCTION

Radioiodinated monoclonal antibodies are being used in many centers for imaging and treatment of cancer. Iodine-131 is frequently used because of its energetic beta particle emission ($E\beta_{max}$ = 607 Kev) and principal γ-emission of 364 Kev. Together these properties permit γ-camera imaging for biodistribution studies of trace labeled and therapeutic doses of tumor associated monoclonal antibodies. *In vivo* use of radioiodinated antibodies can be limited by deiodination followed by rapid clearance of the radioiodine. Not only does deiodination, result in unwanted radiation to normal organs, but it also can result in rapid clearance from the tumor, with subsequent reduced radiation dose.

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Three processes can cause deiodination of the antibody. Small percentages of radioiodine can be lost by hydrolytic and enzymatic deiodination (1). Dehalogenases are present in cells to maintain iodine levels in thyroid metabolism, where iodinated tyrosine is especially vulnerable to deiodination enzymes. In addition, deiodination can occur through catabolic biochemical pathways (2). After antibody modulation intracellular protein catabolism can produce free iodine or single amino acids that may be more subject to transport mechanisms and/or enzymatic deiodination than the original labeled antibody. Once these labeled metabolic products are formed, they are rapidly cleared from the tumor site.

Radioiodinated tyramine-cellobiose (*I-TCB) adduct has been used to determine the sites of low density lipoprotein (LDL) catabolism in cultured fibroblasts and animal models. Both models showed increased cellular retention of the label at the site of metabolism (3, 4). We postulated that this same non-metabolizable sugar was applicable to the situation where radiolabel clearance was unacceptably fast after internalization and catabolism of antibodies. If modulation occurred after antibody-antigen association the *I-TCB-antibody would be trapped intracellularly. This is because the sugar is not readily metabolized, and because its polarity, in the absence of active cell transport, would inhibit its transport. Trapped compounds should have the desirable property of increasing the residence time of the label in the target cell. Tyramine cellobiose (TCB) was first synthesized by Pittman (3) by coupling tyramine to cellobiose (a ß-1-4 disaccharide of glucose) through reductive amination of the aldose. This provided a carrier that could be radioiodinated and that was not metabolizable by mammalian systems (5). We synthesized TCB and found that *I-TCB labeled antibody, when tested in a mouse lymphoma model, demonstrated increased tumor retention and similar normal organ pharmacokinetics when compared to chloramine-T iodinated antibody (6). Synthesis of the aryl-carbohydrate compounds was an attempt to provide a chemical solution to the deiodination and clearance problems encountered by the direct labeling of monoclonal antibodies.

Modification of the aryl substituent of these compounds could imporove on this solution rendering the iodine label less susceptible to deiodination by enzymatic and hydrolytic processes. The iodinated tyramine portion of TCB offers no advantages over conventional electrophilic iodination in this respect. However, we proposed that minimizing hydrolytic deiodination could be accomplished via a stronger aryl-iodine bond, and minimization of enzymatic deiodination could be

accomplished by eliminating the adjacent hydroxyl groups. With these considerations in mind we examined a series of aryl substituents. Phenethylamine has the advantages of a possible stronger para iodine bond (7,8) and a different molecular appearance. This compound, however, is not activated toward electrophilic substitution and is therefore more difficult to iodinate. Aniline has the same advantages as phenethylamine, however, it is more activated toward electrophilic iodination. The structure of 2,6-dimethylaniline blocks iodination at the ortho positions, while 3,5-dimethylaniline provides a very different molecular appearance for the minimization of enzymatic deiodination.

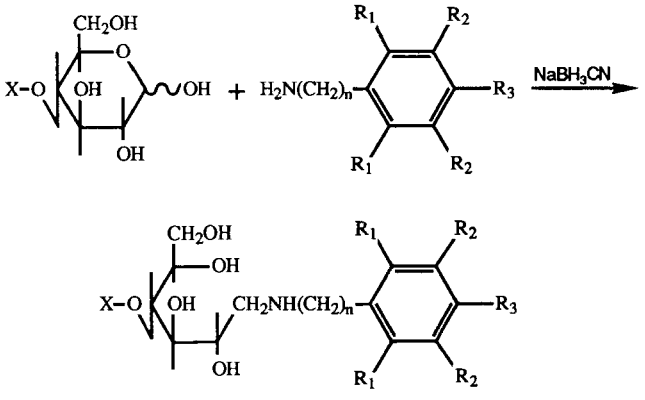
Glucose enters many cells by faciliated diffusion through the glucose transporter rather than by co-transport with sodium ion. The subsequent phosphorylation of glucose assures its retention by the cells. These mechanisms suggest that a more elementary and systematically elegant ligand might involve glucose as a carbohydrate portion of the molecule. A parallel series of compounds using glucose for the carbohydrate substituent was synthesized, with the possibility that it would have the same *in vivo* advantages as cellobiose. Synthesis and iodination of each of the aryl substituents with both carbohydrate substituents was attempted. This produced a set of ten compounds, each with its own unique set of advantages and disadvantages, as radioiodination schemes for monoclonal antibodies.

RESULTS and DISCUSSION

Linking of the aryl substituent to the carbohydrate molecule for all these aryl-carbohydrate compounds was accomplished through a reductive amination reaction (Scheme I). This reaction takes place between an amine and the ring open aldehyde of an aldose sugar in the presence of a reducing agent, sodium cyanoborohydride. The reaction is assumed to follow a Schiff base or imine mechanism (9).

The rate of the reductive amination may be critically dependent on several reaction conditions: pH, solvent, temperature, and molar ratios (Table 1). Imine formation is preceded by nucleophilic attack by an unprotonated amine, therefore basic conditions would increase the amount of reactable amine. On the other hand, the amines used in these reactions are more soluble at a low pH. In order to solvate all the starting materials a slightly acidic pH and/or a water/ethanol

Scheme I: Generic Reductive Amination



X = H or Glucose
n = 0 or 2
R_1 & R_2 = H or CH_3
R_3 = H or OH

solvent system was needed. The reactions were refluxed as opposed to stirring at room temperature (3) to increase the molecular collision frequency and the rate at which the aldohexose ring opened to expose a reactable aldehyde. A molar excess of amine is also important to maintain a high concentration of reactable amine. With these conditions, reaction times were between 3 and 96 hours for all the compounds except the sterically hindered 2,6-dimethylaniline compounds. The reductive amination could probably be optimized by extending the reaction times at higher pH.

Purification of the desired products was achieved by cation exchange chromatography under conditions where the amine was protonated. A linear ammonium hydroxide gradient was used to separate the product and reactants on the column. ^{1}H and ^{13}C nuclear magnetic resonance was used to identify and determine the structure of the compounds as well as a means of determining relative purity. NMR of the products showed a 1:1 relationship between the integral of the aromatic and carbohydrate portions, indicating complete purification was achieved.

There are at least three factors other than the reaction conditions contributing to the large variation of synthetic yields in Table I. A number of reactions were not allowed to go to completion. In these cases, if the TLC showed significant product formation the reaction was stopped. Steric hindrance is a likely reason for the lowered yields in the 2,6-dimethylaniline

Table I

Compound	pH	Solvent	A/C Ratio[1]	Yield (3 hrs)	Later Yield (at hrs)
Tyramine cellobiose	5.4	H_2O	2.5	27%	78% (96)
Tyramine glucose	6.4	H_2O	2.4	48%	48% (20)
Phenethylamine cellobiose	5.9	H_2O	2.5	25%	59% (96)
Phenethylamine glucose	6.4	H_2O	2.0	44%	44% (3)
Aniline cellobiose	6.4	H_2O	3.2	36%	36% (3)
Aniline glucose	6.4	H_2O	2.0	66%	66% (3)
3,5-Dimethylaniline cellobiose	5.4	H_2O	3.0	29%	50% (73)
3,5-Dimethylaniline glucose	6.4	EtOH/H_2O	2.5	56%	30% (7)
2,6-Dimethylaniline cellobiose	5.4	H_2O	3.0		6% (117)
2,6-Dimethylaniline glucose	5.4	EtOH/H_2O	2.0		30% (48)

Table I: lists the synthetic yields and conditions of the various aryl carbohydrate reactions.
[1] A/C = Amine/Carbohydrate reactant molar ratio.

reactions. The last factor affecting the product yield is the purification method. The elution profile of these compounds from the cation exchange column exhibits some tailing of the product that overlaps with elution of the free amine. Since only fractions that did not overlap with the amine were isolated, quantitative recovery of the products was not achieved. The losses due to the purification are relative to the particular ligand and its elution profile. Because of these factors the percent yield data reflect only semiquantitively the degree to which the reactions went to completion.

Once the ligands were synthesized, purified, and identified, we examined their iodination characteristics. Iodination was accomplished through electrophilic addition using chloramine-T (10). The reaction was quenched with sodium thiosulfate and excess sodium iodide. A single iodination protocol was used for all molecules; conditions were not individually optimized for these derivatives.

Table II

Compound	%Iodination[1]
Tyramine Cellobiose	94
Tyramine Glucose	90
Phenethylamine Cellobiose	25
Phenethylamine Glucose	22
Aniline Cellobiose	83
Aniline Glucose	75
3,5-Dimethylaniline Cellobiose	95
3,5-Dimethylaniline Glucose	93
2,6-Dimethylaniline Cellobiose	36
2,6-Dimethylaniline Glucose	32

Table II: [1]The iodination yields were based on the percent of the total radioiodine incorporated in the adduct and are the averages of at least three labeling.

The carbohydrate component of the product does not substantially affect the iodination yields, however, there is a trend for lower iodination yields with the glucose substituent compared to that of the cellobiose substituent. The iodination yield is largely dependent on whether or not the aryl substituent is activated toward electrophilic addition. The tyramine substituent is most activated because of the phenolic group ortho to the iodination site. Phenethylamine is not activated and showed much lower iodination yields. Aniline and its dimethyl derivatives are activated by the resonance donating amino nitrogen, principally in the para position and secondarily in the ortho positions, resulting in high iodination yields. The methyl groups of 3,5-dimethylaniline activate both ortho and para positions, resulting in high iodination yields. The methyl groups of 2,6-dimethylaniline block the ortho positions while at the same time deactivating the para position, resulting in lower iodination yields.

Regiochemistry of the ring iodination of the aryl glucose conjugates was determined by assignment of the aromatic protons in the NMR spectra of the reaction mixtures. Isomer ratios of the various products were determined by the ratio of the peak intergrals. Iodination of tyramine glucose gave 79% ortho and 21% meta to the -OH group. Iodination of 2,6-dimethylaniline gave the para isomer as expected. ^{1}H NMR spectra of iodinated phenethylamine glucose, aniline glucose and 3,5-dimethylaniline glucose showed only the para-isomer. However, these results do not exclude the presence of the ortho-isomer since starting material was present to a large extent in these preparations.

EXPERIMENTAL

All chemicals obtained from commercial sources were reagent grade or the highest grade available and were not further purified. Proton and carbon-13 nuclear magnetic resonance (^{1}H & ^{13}C NMR) spectra were obtained on a Varian VXR-300 nuclear magnetic resonance spectrometer in either D_2O or $CDCl_3$. Micro analyses were performed by Galbraith Laboratories Inc., Knoxville, TN.

General reaction conditions: The aryl amine was dissolved in water or an ethanol/water mixture with pH adjusted using glacial acetic acid. The carbohydrate was added such that there was a 2 - 3 molar excess of amine. Sodium cyanoborohydride was added in a 1:1 molar ratio with

the amine. The reaction was then refluxed with periodic pH adjustment until TLC indicated substantial product. The reaction mixture was then acidified with HCl and refluxed to destroy the hydride. The synthetic yields for all aryl carbohydrate compounds except the sterically hindered 2,6-dimethylaniline conjugates was determined at three hours and for some molecules at later time.

Chromatography procedures: Analytical separations used silica HPLC, 250mm x 4.6mm column (Econosil, Alltech Associates Inc., Deerfield, IL) with mobile phase of acetonitrile / 20% acetic acid = 60/40 and a flow rate of 1mL/min.

Preparative purification of the coupling products was accomplished by eluting the acidic reaction mixtures through a 2.5 x 20 cm cation exchange resin column (AG 50W x 8, ammonium form, BioRad, Richmond, CA). The column was equilibrated with approximately 500 mL of distilled water and was eluted with water until carbohydrate was no longer present in the eluate. A linear 24 hr gradient of water with either 0.2 M, 0.5M, or 1.0 M ammonium hydroxide was run to elute the product frist, followed by the free amine.

The preparative column elution profile was monitored using thin layer chromatography (TLC), (250 μm silica gel plates, Whatman, Hillsboro, OR) developed in butanol:glacial acetic acid:water (7:1:2). Unreacted cellobiose and glucose ran in the TLC system with R_f of 0.04 - 0.07 and 0.12 - 0.13, respectively. The different unreacted amines had different R_f values (0.50 - 0.85). The starting materials and product were visualized by doing duplicate TLC's and staining one for amine and one for carbohydrate. The amine was visualized by spraying the TLC plate with ninhydrin spray (2 mg ninhydrin/mL ethanol), yielding a purple color in 5 - 10 minutes at 100 °C. The carbohydrate was visualized by spraying the TLC plate with a reagent of equal volumes of 2 mg/mL naptholresorcinol in ethanol and 20% sulfuric acid; carbohydrates yielded a brown color in 5-10 minutes at 100 °C. Alternatively, spraying the TLC plate with 10% sulfuric acid, yielding a brown color in 5-10 minutes at 100 °C, or spraying the TLC plate with a 5% ammonium molybdate in 10% sulfuric acid stained glucose, yielding a blue color in 5-10 minutes at 100 °C. The TLC fractions were pooled, rotary evaporated to reduce the volume, and lyophilized.

Synthesis of tyramine cellobiose: Tyramine (6.85 g, 0.050 mol) was suspended in water (500 mL, distilled) with stirring. Sufficient glacial acetic acid was added to dissolve the tyramine.

D-Cellobiose (predominantly beta anomer) (6.85 g, 0.020 mol) and sodium cyanoborohydride (3.15 g, 0.050 mol) were added with magnetic stirring. The pH of the reaction mixture was then adjusted to 5.4 with glacial acetic acid and the reaction mixture was refluxed with magnetic stirring for 96 hrs., with readjustment to pH 5.4 at daily intervals. The mixture was then allowed to cool and the pH was adjusted to 3.5 with acetic acid, followed by 24 hr of refluxing to destroy any remaining sodium cyanoborohydride. The reaction mixture was rotary evaporated to approximately 50 mL and purified by chromatography to give tan crystals (3.85 g, 78%), TLC R_f 0.20 (Tyramine R_f 0.50). ^1H NMR (D_2O), δ 6.95 (d, 2H, J=8.2 Hz), 6.64 (d, 2H, J= 8.2 Hz), 4.36 (d, 1H, J= 7.7 Hz), 3.88 (m, 1H), 3.74 - 3.05 (m), 3.00 - 2.46 (m, 6H); ^{13}C NMR (D_2O), δ 155.4, 130.0 (2C), 128.6, 116.0 (2C), 102.1, 78.4 - 68.6 (8C), 61.9, 60.3, 50.0, 49.4, 31.9. Anal. Calcd for $C_{20}H_{34}O_{11}N$: C, 51.72; H, 7.33; N, 3.02. Found: C, 51.43; H, 7.41; N, 3.04.

Synthesis of tyramine glucose: Tyramine (7.32 g, 0.053 mol) was added to 250 mL of water with magnetic stirring. The pH was adjusted to 6.4 with glacial acetic acid. α-D-Glucose (4.80 g, 0.022 mol) was added to the reaction mixture, followed by sodium cyanoborohyride (3.35 g, 0.053 mol). The reaction mixture was refluxed with magnetic stirring for 20 hrs., followed by acidification to pH 3.5 and 2 more hours of refluxing to destry any remaining sodium cyanoborohydride. The mixture was rotary evaporated and purified by chromatography to give tan crystals (3.85 g, 48%), TLC R_f = 0.36. ^1H NMR (D_20), δ 6.90 (d, 2H, J=8.2 Hz), 6.58 (d, 2H, J=8.2 Hz), 3.72 (m, 1H), 3.66 - 3.35 (m, 5H), 2.70 - 2.65 (m, 6H); ^{13}C NMR (D_2O), δ 156.8, 129.9 (2C), 128.7, 116.4, 70.9 - 70.1 (4C), 62.8, 50.0, 49.7, 32.9. Anal. Calcd for $C_{14}H_{23}O_6N$: C, 55.81; H, 7.64; N, 4.65. Found: C, 56.1; H, 7.72; N, 4.67.

Synthesis of phenethylamine cellobiose: Phenethylamine (3.0 g, 0.025 mol) was added to 250 mL of water with magnetic stirring and enough glacial acetic acid was added to bring the phenethylamine into solution. D-Cellobiose (3.4 g, 0.010 mol) was added, followed by sodium cyanoborohydride (1.6 g, 0.025 mol). The pH was adjusted to 5.9, then refluxed with magnetic stirring and daily pH adjustment for 96 hr., after which time the mixture was acidified to pH 3.6, followed by 5 hrs. of refluxing. The reaction mixture was rotary evaporated to reduce the volume and purified. The phenethylamine cellobiose fractions were pooled and rotary evaporated to dryness to give white crystals (2.62 g, 59%), TLC R_f = 0.29 (Phenethylamine R_f 0.53). ^1H NMR (D_2O), δ 7.08 - 7.27 (m, 5H), 4.36 (d, 1H, J=7.7 Hz), 3.87 - 3.69 (m, 2H), 3.69 - 3.10 (m),

2.85 - 2.72 (m, 5H), 2.52 (dd, 1H, J=9.0, 12 Hz); ^{13}C NMR (D_2O), δ 139.2, 128.7 (4C), 126.5, 102.1, 78.4 - 69.2 (8C), 62.0, 60.2, 50.6, 49.9, 34.2. Anal. Calcd for $C_{20}H_{34}O_{10}N$: C, 53.57; H, 7.59; N, 3.13. Found: C, 53.05; H, 7.69; N, 3.11.

Synthesis of phenethylamine glucose: Phenethylamine (6.8 g, 0.056 mol) was dissolved in water (150 mL) with magnetic stirring. The pH was adjusted to 6.4 with glacial acetic acid. Glucose (5.07 g, 0.028 mol) was added to the reaction mixture followed by sodium cyanoborohydride (3.52 g, 0.056 mol). The mixture was refluxed with magnetic stirring for three hours followed by a pH adjustment to 3.5. The mixture was refluxed for another hour to destroy the hydride. The reaction mixture was rotary evaporated to reduce the volume and purified by chromatography to give white crystals (1.19 g, 15%), TLC R_f = 0.38. ^1H NMR (D_2O), δ 7.22 - 7.04 (m, 5H), 3.75 - 3.35 (m, 6H), 2.75 - 2.51 (m, 5H), 2.46 (dd, 1H, J=8.5, 12 Hz); ^{13}C NMR (D_2O), δ 140.3, 129.3 (2C), 129.2 (2C), 126.9, 71.5 - 71.2 (4C), 63.4 50.8, 50.4, 35.3. Anal. Calcd for $C_{14}H_{23}O_5N$: C, 58.95; H, 8.07; N, 4.91. Found: C, 58.94; H, 8.07; N, 4.84.

Synthesis of aniline cellobiose: Aniline (3.91 g, 0.042 mol) was added to 70 mL of water with magnetic stirring. The pH was adjusted to 6.4 with glacial acetic acid. Cellobiose (4.46 g, 0.013 mol) was added followed by sodium cyanoborohydride (2.64 g, 0.042 mol). The reaction mixture was refluxed with magnetic stirring for 3 hrs. followed by a pH adjustment to 3.5 and refluxing for another hour. The mixture was rotary evaporated to reduce the volume and purified by chromatography to give tan crystals (2.71 g, 36%), TLC R_f = 0.39 (Aniline R_f 0.84). ^1H NMR (D_2O), δ 7.13 (t, 2H, J=8.2 Hz), 6.70 (d, 3H, J=8.2 Hz), 4.39 (d, 1H, J=7.7 Hz), 3.95 (m, 1H), 3.82 - 3.12 (m), 2.97, (dd, 1H, J=8.5, 12 Hz); ^{13}C NMR (D_2O), δ 147.9, 129.5 (2C), 119.0, 114.7 (2C), 102.4, 79.2 - 69.2 (8C), 62.0, 60.0, 46.6. Anal. Calcd for $C_{18}H_{30}O_{10}N$: C, 51.43; H, 7.14; N, 3.33. Found: C, 50.95; H, 7.41; N, 3.30.

Synthesis of aniline glucose: Aniline (5.40 g, 0.058 mol) was solvated in 100 mL of water at pH 6.4. Glucose (5.22 g, 0.029 mol) was then dissolved in the reaction mixture with magnetic stirring followed by the addition of sodium cyanoborohydride (3.64 g, 0.058 mol). The mixture was refluxed with magnetic stirring for three hours followed by acidification to pH 3.5 and another hour of refluxing. The mixture was rotary evaporated to reduce the volume and purified by chromatography to give tan crystals (3.6 g, 66%), TLC R_f = 0.68. ^1H NMR (D_2O), δ 7.13 (t,

2H, J=8.2 Hz), 6.71 (t, 3H, J=8.2 Hz), 3.84 (m, 1H), 3.72 - 3.45 (m, 5H), 3.24 (dd, 1H, J=4.5, 13 Hz), 2.99 (dd, 1H, J=8.5, 14 Hz); ^{13}C NMR (D_2O), δ 147.7, 129.4 (2C), 118.9, 114.7 (2C), 71.4 - 70.6 (4C), 62.7, 46.3. Anal. Cacld for $C_{12}H_{19}O_5N$: C, 56.03; H, 7.39; N, 5.45. Found: C, 56.37; H, 7.36; N, 5.48.

Synthesis of 3,5-dimethylaniline cellobiose: 3,5-Dimethylaniline (6.5 g, 0.054 mol) was added to 300 mL of water with magnetic stirring. The pH was adjusted to 5.4 with glacial acetic acid. Not all the 3,5-dimethylaniline was solvated. Cellobiose (6.13 g, 0.018 mol) was added to the reaction mixture followed by sodium cyanoborohydride (3.37 g, 0.054 mol). Refluxing with magnetic stirring was commenced for 73 hrs. followed by acidification to pH 3.5 and refluxing for another hour. The mixture was extracted with CH_2Cl_2 (3 x 50 mL) to remove excess amine. The reaction mixture was rotary evaporated to reduce the volume and purified by chromatography to give tan crystals (3.98 g, 50%), TLC R_f = 0.46 (3,5 Dimethylaniline R_f 0.83). ^{1}H NMR (D_20), δ 6.35 (s, 1H), 6.33 (s, 2H), 4.35 (d, 1H, J=7.7 Hz), 3.89 (m, 1H), 3.80 - 3.07 (m), 2.91 (dd, 1H, J=8.5, 13), 2.05 (s, 6H); ^{13}C NMR (D_20), δ 148.0, 139.6 (2C), 120.5, 112.4 (2C), 102.4, 79.3 - 69.1 (8C), 62.0, 60.2, 46.7, 20.6 (2C). Anal. Calcd for $C_{20}H_{34}O_{10}N$: C, 53.57; H, 7.59; N, 3.13. Found: C, 53.38; H, 7.59; N, 3.12.

Synthesis of 3,5-dimethylaniline glucose: 3,5-Dimethylaniline (5.33 g, 0.044 mol) was solvated in approximately 10 mL of ethanol. Glucose (3.16 g, 0.0175 mol) was added to the mixture followed by enough water to dissolve the sugar (approximately 60 mL). The pH was adjusted to 6.4 and sodium cyanoborohydride (2.76 g, 0.044 mol) was added. The mixture was refluxed with magnetic stirring for approximately 7 hours followed by acidification to pH 3.5 and two more hours of refluxing followed by chromatographic purification to give tan crystals (1.5 g, 30%), TLC R_f = 0.62. ^{1}H NMR (D_2O), δ 6.37 (s, 1H), 6.34 (s, 2H), 3.80 (m, 1H), 3.68 - 3.40 (m, 5H), 3.18 (dd, 1H, J=4.5,13 Hz), 2.94 (dd, 1H, J=8.5, 13 Hz), 2.05 (s, 6H); ^{13}C NMR (D_2O), δ 148.0, 139.7 (2C), 120.4, 112.3 (2C), 71.3 - 70.6 (4C), 62.7, 46.3, 20.4 (2C). Anal. Calcd for $C_{14}H_{23}O_5N$: C, 58.95; H, 8.07; N, 4.91. Found: C, 58.43; H, 8.13; N, 4.88.

Synthesis of 2,6-dimethylaniline cellobiose: 2,6-Dimethylaniline (6.52 g, 0.054 mol) was added to 300 mL of water with magnetic stirring. The pH was adjusted to 5.4 with glacial acetic acid. Not all the aniline dissolved in the reaction mixture. Cellobiose (6.13 g, 0.018 mol)

was added followed by sodium cyanoborohydride (3.38 g, 0.054 mol). The mixture was refluxed for 117 hr with daily pH readjustment to 5.4. The reaction mixture was acidified to pH 3.5 and refluxed for one hour followed by extraction with CH_2Cl_2 (3 x 50 mL) to remove the excess aniline. The water layer was rotary evaporated to reduce the volume and purified by chromatography to give tan crystals (0.52 g, 6%), TLC R_f = 0.37 (2,6-Dimethylaniline R_f 0.85). ^1H NMR (D_2O), δ 6.94 (d, 2H, J=6.6 Hz), 6.78 (t, 1H, J=6.6 Hz), 4.36 (d, 1H, J=8.2 Hz), 3.88 - 3.10 (m), 2.97 (dd, J= 8.5, 14 Hz), 2.16 (s, 6H); ^{13}C NMR (D_2O), δ 144.3, 129.6, 129.0 (2C), 129.0, 122.8, 102.4, 79.6 - 69.2 (8C), 62.0, 60.5, 50.2, 17.8 (2C). Anal. Calcd for $C_{20}H_{34}O_5N$: C, 53.57; H, 7.59; N, 3.13. Found: C, 53.13; H, 7.70; N, 3.14.

Synthesis of 2,6-dimethylaniline glucose: 2,6-Dimethylaniline (6.30 g, 0.052 mol) was solvated in 300 mL of 33% ethanol in water (v/v). The pH was adjusted to 5.4 with glacial acetic acid. Glucose (4.68 g, 0.026 mol) was added to the reaction mixture followed by sodium cyanoborohydride (4.08 g, 0.065 mol). The mixture was refluxed for 48 hours followed by acidification to pH 3.5 and two hours of refluxing. The reaction mixture was cooled and extracted with dichloromethane (3 X 75 mL) to remove excess amine. The mixture was then rotary evaporated to reduce the volume and purified by chromatography to give tan crystals (2.21 g, 30%), TLC R_f = 0.55. ^1H NMR (D_2O), δ 6.92 (d, 2H, J=5.5Hz), 6.78 (t, 1H, J= 5.5 Hz), 3.78 (m, 1H), 3.74 - 3.45 (m, 6H), 3.11 (br d, 1H, J=12 Hz), 2.85 (dd, 1H, J=8.5, 12 Hz), 2.18 (s, 6H). ^{13}C NMR (D_2O), δ 144.2, 130.1 (2C), 129.1 (2C), 123.1, 71.7 - 71.0 (4C), 62.9, 50.0, 17.8 (2C). Anal. Calcd for $C_{14}H_{23}O_5N$: C, 58.95; H, 8.07; N, 4.91. Found: C, 57.80; H, 8.03; N, 4.87.

Radioiodination: The ligands were iodinated using the chloramine-T (CT) method (8). 0.25 mL of 0.5 M phosphate buffered saline (PBS) pH 7.2 or four times the volume of radioiodine was added to either I-125 or I-131 as NaI, approximately 0.46 and .0.98 nmoles per mCi, respectively (New England Nuclear, Boston, MA). 1 nmol of ligand was added to every 0.02 - 0.9 nmol of iodine. 0.45 - 0.50 nmol of CT was then added for every nmol of ligand. The reaction mixture was vortexed and incubated at room temperature for 5 minutes. The reaction was quenched by the addition with vortexing of 1.35 - 1.50 nmol of sodium thiosulfate per nmol of ligand and an excess of cold sodium iodide. The total reaction volume was less than 0.5 mL. The iodination yield was determined by radio-HPLC using a stationary phase consisting of a 250mm x 4.6mm weak

anionic exchange column (Lichrosorb-NH_2, Alltech Associates Inc., Deerfield, IL) , and a mobile phase of acetonitrile /water = 85/15, with a flow rate of 1mL/min. The radiolabelled peaks were detected by running the HPLC effluent line by a well-type NaI(Tl) crystal with associated electronics.

Regiochemistry of Aryl Glucose Compounds: Aryl glucose conjugates were iodinated using the chloramine-T method. Equivalent amounts of aryl glucose compounds and sodium iodide were used. ^1H NMR (D_2O, only the aromatic resonances are reported) resonances of iodinated tyramine glucose corresponding to ortho isomer to -OH group were d 7.65 (dd, 1H), 7.57 (dd, 1H), 7.30 (dd, 1H). The ^1H NMR resonances corresponding to the meta-isomer to the -OH group were d 7.50 (dd, 1H), 7.05 (dd, 1H), 6.78 (dd, 1 H). ^1H NMR (D_2O) resonances of iodinated phenethylamine were d 7.68 (d, 2H, J= 8.2 Hz) 7.67 (d, 2H, J= 8.2 Hz). ^1H NMR ($CDCl_3$) resonances of iodinated aniline glucose were d 7.30 (d, 2H, J= 7.5 Hz), 7.79 (d 2H, J= 7.5 Hz). ^1H NMR (D_2O) resonance of iodinated 3,5-dimethylaniline glucose was d 7.01 (s, 2H). ^1H NMR (D_2O) resonance of iodinated 2,6-dimethylaniline glucose was d 6.92 (s 2H).

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