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Chemical and Enzymatic Synthesis of Fructose Analogues as Probes for Import Studies by the Hexose Transporter in Parasites

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Abstract—Various D-fructose analogues modified at C-1 or C-6 positions were synthesized from D-glucose by taking advantage of the Amadori rearrangement or using the aldol condensation between dihydroxyacetone phosphate and appropriate aldehyde catalyzed by fructose 1,6-diphosphate aldolase from rabbit muscle. The affinities of the analogues for the glucose transporter expressed in the mammalian form of *Trypanosoma brucei* were determined by inhibition of radiolabelled 2-deoxy-D-glucose (2-DOG) transport using zero-trans kinetic analysis. Interestingly, the analogues bearing an aromatic group (i.e. a fluorescence marker) at C-1 or C-6 positions present comparable apparent affinities to D-fructose for the transporter. This result could find applications for hexose transport studies and also provides criteria for the design of glucose import inhibitors. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

The glycolytic metabolism of D-glucose is the unique source of energy for the parasite *Trypanosoma brucei* (mammalian form), the causative agent of African sleeping sickness.[1] The transport of the sugar, from the bloodstream of the host into the parasite, is ensured by the glucose transporter THT1 which consequently represents a potential target for anti-trypanosomal drugs.[2] Whereas the mammalian erythrocyte glucose transporter (GLUT1) only recognises D-glucose, interestingly, that of the parasite also accepts D-fructose as a furanose ring; there is some evidence that the affinity for this hexose comes from hydrogen bonds directed at the C-3, C-4 positions and the intra-cyclic oxygen atom (Fig. 1).[3] Therefore fructose analogues modified at C-1, C-2 or C-6 positions are of interest for the study of the parasite transporter either for blocking D-glucose uptake or for taking advantage of this transporter to internalize specifically into the parasite inhibitors directed against glycolytic enzymes.[4]

Among these fructose derivatives, those bearing a fluorescence marker are potentially useful probes for the study of complex recognition and transport phenomena in which THT1 (and also other sugar transporters) is involved. Biomolecule-fluorescent reporter or marker groups conjugates have shown their utilities in many areas of biology, i.e.: inhibitors of viral attachement,[5] antisense oligonucleotides,[6] macromolecular transport or diffusion,[7] intracellular enzyme substrats,[8] etc.

Taking the above considerations into account, we report the synthesis of D-fructose analogues modified at the C-1 or C-6 position. The basic route to obtain the expected conjugates was to synthesize, chemically or chemoenzymatically, the corresponding amino-deoxy-fructoses which were subsequently derivatized with a fluorescent marker. The dansyl group was used in this work owing to its relative weak hindrance compared to other markers and its fluorescence properties.[5,9] The resulting D-fructose analogues, that have been examined as inhibitors of hexose uptake before the future fluorescence assays for the binding to THT1, allow to glean additional information concerning trypanosome sugar transport system.

Results and Discussion

D-Fructose modified at C-1

The chemical synthesis of fructose derivatives **1**–**3**, starting from glucose, is shown in Scheme 1. First,

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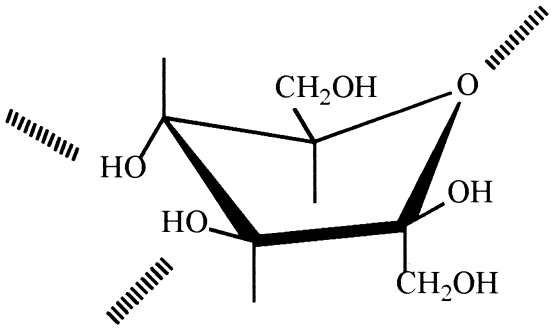


Figure 1. Proposal of the β-D-frutofuranose binding of the trypanosome transporter. This model is based on that from ref 3. Hatched lines represent hydrogen-bonding side chains from the transporter directed at C-3, C-4 and oxygen ring of the hexose.

1-amino-1deoxy-fructose acetate **2** was obtained quantitatively from D-glucose by taking advantage of the Amadori rearrangement through 1-deoxy-1-toluidino-fructose intermediate **1** followed by catalytic hydrogenation.[10] Then, the resulting ammonium salt **2** was subsequently sulfonylated with dansyl chloride after deprotonation. We observe that the best results were obtained when the reaction was carried out in dry methanol in presence of Dowex resin (OH^- form) rather than in aqueous solution with organic cosolvent and tertiary amine. The fluorescent fructose derivative **3** was isolated in 37% yield after preparative thin layer chromatography. ^{13}C NMR allowed to determine the percentages of the α/β furanose and pyranose forms at equilibrium in D_2O (see Table 1).[11]

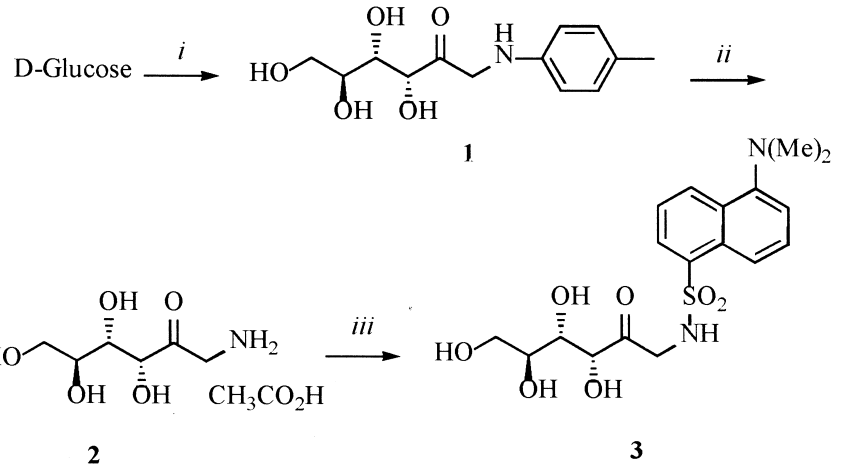
D-fructose modified at C-6

Rabbit muscle aldolase (EC 4.1.2.13) reversibly catalyzes the formation of fructose 1,6-diphosphate from two triose-phosphates: dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate. This enzyme which is rather selective towards the dihydroxyacetone phosphate structure, accepts a large variety of aldehydes as substrate and is frequently in use for synthetic purposes.[12] Thus, the expected fructofuranosides **5**–**8** were chemoenzymatically synthesized starting from (*R*) 3-azido-2-hydroxypropanal diethyl acetal **4** (Scheme 2).[4] Aldol condensation of 2-fold excess of aldehyde, resulting from deprotection of ketal **4**, with dihydroxyacetone phosphate (formed in situ from fructose 1,6-diphosphate) catalyzed by aldolase (1 mmol scale) gave the ketose-phosphate which was hydrolyzed in presence of acid phosphatase to yield 6-azido-6-deoxy-fructose **5** as unique reaction product. Subsequent ketalization of the azido ketose gave the corresponding methyl fructofuranoside **6** as a mixture of α- and β-forms.[13] This compound allowed to obtain 6-amino-6-deoxy-fructose derivative **7**, isolated after reductive amination, while direct reductive amination of compound **5** would have given the undesired aza sugar.[14]

Finally, sulfonylation of the ammonium salt with dansyl chloride, as described above for **2**, furnished the fluorescent α- and β-methylfructofuranosides, **8a** and **8b** respectively, which were separated by preparative thin layer chromatography. Attempts to obtain **8a,b**, through fructose derivative **10**, starting from aldol condensation between dansyled (*R*) 3-amino-2-hydroxy-propanal diethylacetal **9** (obtained from **4**) and dihydroxyacetone phosphate catalyzed by aldolase (Scheme 2) were unsuccessful. This is likely due to either the poor solubility of the aldehyde in aqueous solution or to the possible enzyme inactivation when high level of organic cosolvent was used to make the aldehyde soluble.

Interaction of D-fructose analogues with THT1

The affinities of the fructose derivatives for the glucose transporter expressed in the mammalian form of *T. brucei* were examined through the inhibition of the transport of radiolabelled 2-deoxy-D-[1-^{3}H]-glucose (2-DOG).[15] The kinetic parameters of the transport system were investigated using the zero-trans kinetic analysis (see Experimental). The resulting competitive inhibition constant values (K_i), reported in the table, were obtained using different inhibitor concentrations for a fixed (100 μM) 2-DOG concentration (see Experimental).



Scheme 1. Synthesis of the fructose derivatives **1**–**3**. (i) *p*-toluidine, H_2O, AcOH. (ii) H_2, $PdO/BaSO_4$, H_2O, AcOH. (iii) DOWEX 1X8 (OH^- form), dansyl chloride, MeOH, 37%.

Table 1. Inhibition constants for fructose derivative interactions with trypanosome glucose transporter. Percentages were determined from ^{13}C NMR spectra.[11] The inhibition constants for both substrates D-glucose and D-fructose are indicated for comparison

Inhibitor	Percentage of				K_i values with 100 μM 2-DOG (nM)
	Furanose		Pyranose		
	α	β	α	β	
D-Glucose	—[b]	—	36	46[a]	1.0
D-Fructose	5	18	—	77[a]	5.0
2-DOG	—	—	(100)		0.5
1	9	13	—	78	8.2
2	13	16	—	71	40
3	8	27	—	69	3.5
6	42	58	—	—	10
7	42	58	—	—	3.6
8a	100	—	—	—	4.7
8b	—	100	—	—	3.1

[a]From ref 3.
[b]n.d., not done.

The C-1 analogue (1-amino-1-deoxy-D-fructose **2**) binds to the transporter with 8-fold less affinity than D-fructose (Table 1). Addition of an aromatic group at C-1 position restaures recognition to an affinity close, with analogue **1**, to that of D-fructose, or even higher with the more hindered analogue **3**. These effects should not be assigned to equilibrium changes between furanose and pyranose forms since these latter are present in comparable proportions to that of D-fructose (Table 1). This suggests that the transporter accomodates a relative bulky hydrophobic group located at C-1 position of the hexose.

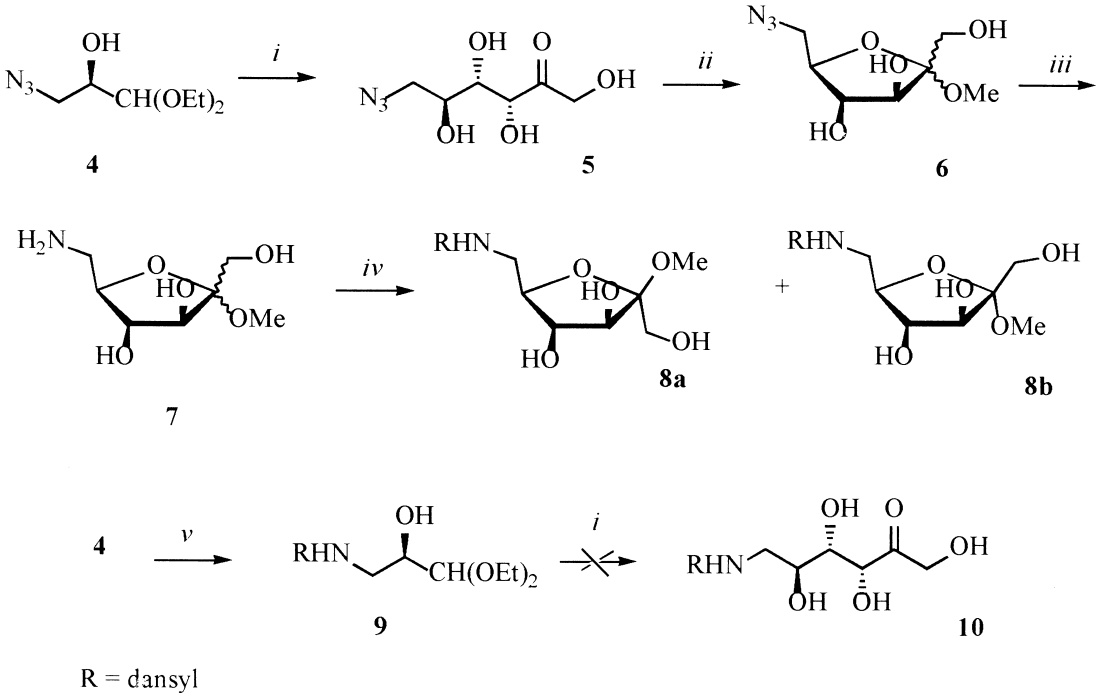
In contrast, the C-6 analogues (6-azido-6-deoxy-methyl-D-fructofuranose **6** and 6-amino-6-deoxy-methyl-D-fructofuranose **7**) inhibits 2-DOG uptake apparently to the same extent as D-fructose (Table 1). Nevertheless, these analogues exist exclusively in the furanose form, a situation a priori favourable for the recognition by the transporter,[3] while for D-fructose this form represents only 23% of the mixture at equilibrium (Table 1). Addition of an aromatic group at C-6 position does not alter the affinity for the transporter of the resulting analogues (compounds **8a,b**). Moreover, in a previous work,[3a,b] Eisenthal could not descriminate affinities between the α- and β-furanose forms towards the transporter; this can be done here, with compounds **8a** and **8b** which appear with similar affinities.

Beyond this complementary information concerning THT1, these could give criteria for further applied developments: (i) identification of the glucose binding site on THT1 by azido bearing aromatic group attached either at C-1 or C-6 position; (ii) design of glucose import inhibitors, resulting from a combinatorial approach of suitable substitued aromatic derivatives; (iii) results obtained with compounds **3** and **8a,b** (Table 1) indicate that they are convenient as fluorescent probes for hexose transport systems studies.

Experimental

Materials and methods

D-Fructose 1,6-diphosphate trisodium salt (>98%), NADH, glycerol-3-phosphate dehydrogenase (EC



Scheme 2. Synthesis of fructose derivatives **5**–**8**. (i) (a) H^+, (b) fructose 1,6-diphosphate, triose phosphate isomerase, aldolase, (c) acid phosphatase. (ii) H^+, MeOH. (iii) H_2, Pd/C, MeOH. (iv) DOWEX 1X8 (OH^- form), dansyl chloride, MeOH. (v) (a) H_2, Pd/C, (b) DOWEX 1X8 (OH^- form), dansyl chloride.

1.1.1.8), triose-phosphate isomerase (EC 5.3.1.1.), rabbit muscle aldolase and acid phosphatase (EC 3.1.3.2) were obtained from Boehringer-Mannheim. The NMR spectra were recorded on a Bruker AC80 (80 MHz ^{1}H NMR) or a Bruker AC200 (200 MHz ^{1}H NMR, 50 MHz ^{13}C NMR). All chemical shifts are reported in parts per million with respect to TMS. Chemicals obtained from commercial suppliers were used without further purification. 2-Deoxy-D-[1-^{3}H]glucose (2-DOG) was from Amersham. Optical rotations were measured with a Perkin–Elmer 241 polarimeter in a 1-dm cell. All liquid chromatography separations were performed using Merck silica gel 60 (230–400 mesh). Elemental analyses were performed by the Ecole Nationale de Chimie de Toulouse, France.

Determination of inhibition constant (K_i) for analogue interaction with trypanosome glucose transporter

Long-slender bloodstream forms of *T. brucei* strain 427 were grown in rats and isolated by DEAE ion exchange chromatography, as previously described.[16] The cells were washed three times in PBS (0.15 M NaCl, 5 mM K-phosphate pH 7.4) at 4 °C and resuspended in PBS at a concentration of 10^9 cells mL^{-1}. After incubation 2 min at 25 °C, 100 μL of cell suspension were incubated during 30 s with 2-deoxy-D-[1-^{3}H]glucose as substrate (100 μM, 0.1 μCi per point) and different fructose analogues as inhibitors (0.01 to 10 mM). The uptake was stopped by centrifugation, spinning the cells through an oil cushion of 1-bromododecane, as previously described.[15] The amount of radioactivity contained in the cell pellet was determined by liquid scintillation counting. Inhibition constant (K_i) values of inhibiting analogues were determined from the relationship $v_0/v = 1 + [I]/K_i$ where v_0 and v are the uninhibited and inhibited initial rates respectively and I is the concentration of inhibitor.[3a]

Chemical syntheses

1-(*N*-Toluidino)-amino-1-deoxy-D-fructose (1). (The synthesis was carried out through the procedure described previously.[10c]) A mixture of *p*-toluidin (80 g, 0.748 mol), D-glucose (100 g, 0.556 mol) and acetic acid 2 N (5 mL) in water (25 mL) was stirred for 30 min in a water bath. The mixture was diluted with absolute ethanol (100 mL) and kept at 4 °C for 24 h. The resulting precipitate was washed (absolute ethanol:diethyl ether, 2:3; 100 mL) and dried at 100 °C over P_2O_5 for 24 h to yield **1** as a white powder (82.5 g, 0.306 mol, 55%). ^{1}H NMR (CD_3OD, 200 MHz): δ 6.9 (d, $^3J_{HH}$ = 8.4 Hz, 2H), 6.6 (dd, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 2.1 Hz, 2H), 4.9 (s, 5H), 4.1–4.0 (m, 2H), 3.9–3.8 (m, 3H), 3.7–3.6 (m, 2H), 2.2 (s, 3H). ^{13}C NMR (CD_3OD, 50 MHz) **1** (β-pyranose form): δ 149.2, 130.5, 114.6, 99.4, 71.9, 71.3, 70.4, 64.6, 51.4, 20.5. **1** (β-furanose form): δ149.2, 130.5, 114.5, 102.4, 83.5, 79.1, 77.0, 64.3, 50.8, 20.5. **1** (α-furanose form): δ 149.2, 130.5, 115.1, 103.0, 84.2, 83.8, 78.7, 62.2, 49.9, 20.5. Mass spectrometry (DCI, NH_3) *m/z* 287 [$M + NH_4^+$], 270 [MH^+]. Anal. calcd. For $C_{13}H_{19}NO_5$: C, 57.98; H, 7.11; N, 5.20. Found: C, 57.63; H, 7.17; N, 5.27.

1-Amino-1-deoxy-D-fructose, acetate salt (2). (The synthesis was carried out through the procedure described previously.[10d]) To a suspension of PdO/BaSO4 (1.5 g) in water (37 mL) was added **1** (10.5 g, 37.1 mmol) in 37 mL of acetic acid 1 N. The mixture was hydrogenated for 24 h under stirring at room temperature. The catalyst was filtered off, the solution concentrated; the residue was diluted with absolute ethanol (100 mL). The resulting precipitate was isolated by filtration, washed with absolute ethanol and dried over P_2O_5 to give **2** as a white powder (9 g, 37 mmol, 96%). ^{1}H NMR (D_2O, 200 MHz): δ 3.92–3.85 (m, 2H), 3.81–3.74 (m, 1H), 3.89–3.58 (m, 2H), 3.11 (m, 2H), 1.79 (s, 3H). ^{13}C NMR (D_2O, 50 MHz) **2** (β-pyranose form): δ 184.1, 98.0, 72.2, 72.0, 71.6, 66.5, 47.7, 25.9. **2** (β-furanose form): δ 184.1, 101.3, 84.9, 80.1, 76.8, 64.6, 46.0, 25.9. **2** (α-furanose form): δ 184.1, 102.2, 84.9, 83.4, 78.6, 63.4, 47.2, 25.9. Mass spectrometry (electrospray <0) *m/z* 180.1 [M-acetate]$^-$. Anal. calcd. For $C_8H_{17}NO_7$: C, 40.17; H, 7.16; N, 5.86. Found: C, 39.84; H, 7.17; N, 5.86.

1-(*N*-(5-(Dimethylamino)naphth-1-yl)sulfonyl)amino-1-deoxy-D-fructose (3). Salt **2** (0.2 g, 0.837 mmol) was treated with 5 mL of Dowex 1×8 resin (OH^- form) in methanol (10 mL) for 30 min. Dansyl chloride (0.398 g, 0.837 mmol) was added by portion under stirring to the mixture cooled to 4 °C, then warmed to room temperature for 1 h. The resin was filtered off and the solvent evaporated under reduced pressure. The remaining residue was purified by thin layer chromatography (dichloromethane:methanol, 8:2) to yield **3** as a green oil (0.125 g, 0.31 mmol, 37%). ^{1}H NMR (CD_3OD, 200 MHz): δ 8.54 (dd, $^4J_{HH}$ = 0.9 Hz, $^3J_{HH}$ = 8.5 Hz, 1H), 8.37 (d, $^3J_{HH}$ = 8.7 Hz, 1H), 8.20 (dd, $^4J_{HH}$ = 1.2 Hz, $^3J_{HH}$ = 7.3 Hz, 1H), 7.63–7.53 (m, 2H), 7.26 (d, $^3J_{HH}$ = 7.6 Hz, 1H), 3.84–3.73 (m, 3H), 3.49–3.36 (m, 2H), 3.34 (s, 2H), 2.86 (s, 6H). ^{13}C NMR (CD_3OD, 50 MHz) **3** (β-pyranose form): δ 153.2, 136.5, 131.3, 131.2, 130.3, 129.3, 124.3, 120.4, 116.5, 98.5, 71.7, 71.1, 69.7, 64.6, 49.3, 45.8. **3** (β-furanose form): δ 153.2, 136.5, 131.3, 131.2, 130.3, 129.3, 124.3, 120.4, 116.5, 102.0, 83.1, 78.1, 76.4, 64.0, 48.2, 45.8. Mass spectrometry (DCI, NH_3) *m/z* 413 [MH^+], 430 [$M + NH_4^+$], 395 [$MH^+ - H_2O$], 323 [$MH^+ - 5H_2O$]. HRMS: calcd for $C_{18}H_{24}N_2O_7S$ 413.1384; found 413.1382.

(2*R*) 3-Azido-2-hydroxy-propanal diethyl acetal (4). Sodium azide (2.67 g, 41.1 mmol) and ammonium chloride (0.488 g, 6.09 mmol) were added to a mixture of D-glycidaldehyde-diethyl acetal (0.6 g, 4.11 mmol) in 40 mL of methanol and 5 mL of water. The mixture was stirred for 20 h at room temperature, then, the solvent was evaporated under reduced pressure and the residue diluted with absolute ethanol (50 mL). The formed precipitate was filtered off, the filtrate concentrated under reduced pressure and the resulting residue purified by flash chromatography (dichloromethane:methanol, 98:2) to give **4** as a colourless oil (0.703 g, 3.71 mmol, 90.5%). ^{1}H and ^{13}C spectra were consistent with those reported in the literature.[4a]

6-Azido-6-deoxy-D-fructose (5). To 10 mL of water, containing 150 μL of concentrated HCl 35%, was added

acetal **4** (0.23 g, 1.21 mmol). The solution was warmed to 45 °C and the progress of the reaction was monitored by TLC (dichloromethane:methanol, 95:5). After completion of hydrolysis, which afforded the free aldehyde, fructose-1,6-biphosphate (trisodium salt, 0.165 g, 0.3 mmol) was added and the pH adjusted at 6.5 by addition of NaOH. The solution was degassed with argon, and aldolase (EC 4.1.2.13, 300 U) and TIM (EC 5.3.1.1, 500 U) were added. After 48 h, the reaction was stopped by addition of $BaCl_2$, $2H_2O$ (0.488 g, 2 mmol), the pH was adjusted to 7.8 with NaOH and acetone (150 mL) was added. The precipitate was isolated by centrifugation (9000 rpm; 0 °C; 30 min) and washed with acetone. The white precipitate was suspended in distilled water (25 mL) and HCl added until pH = 1. A solution of Na_2SO_4 (0.284 g, 2 mmol) was then added and the pH adjusted to 6.5 with NaOH. The suspension was filtered through Celite 545, to remove $BaSO_4$. The pH of the filtrate was then adjusted to 4.9 with acetic acid, and acid phosphatase (EC 3.1.3.2, 100 U) was added. After complete reaction (12 h), the solution was neutralised and then freeze-dried, and the residue triturated with absolute ethanol and filtered. The filtrate was recovered and the solvant eliminated to afford **5** as an oil (0.2 g, 0.98 mmol, 80%). ^1H and ^{13}C spectra were consistent with those reported in the literature.[4a]

Methyl-6-azido-6-deoxy-D-fructofuranoside (6). A solution of methanol (3 mL), containing 8 μL of sulfuric acid 95% was added dropwise, at room temperature, to a stirred solution of azido fructose **5** (0.2 g; 0.97 mmol) in anhydrous methanol (10 mL). The progress of the reaction was monitored by TLC (eluent dichloromethane:methanol, 90:10). The mixture was neutralised with 5 mL of Amberlite IRA 410 resin (HCO_3^- form). The resin was filtered off and washed with methanol (3×5 mL). The solvent was removed under reduced pressure to afford **6** as a pale orange oil, mixture of α and β fructofuranoside forms (0.220 g, 0.97 mmol, 99%). ^1H NMR (D_2O, 200 MHz): δ 4.2–3.9 (m, 3H), 3.7–3.5 (m, 4H), 3.3 (s, 1.6H), 3.1 (s, 1.4H). ^{13}C NMR (D_2O, 50 MHz) **6** (β-furanose form): δ 106.7, 82.0, 79.2, 78.2, 62.3, 55.5, 51.7. **6** (α-furanose form): δ 111.2, 84.4, 82.8, 81.1, 60.2, 54.2, 50.9. Mass spectrometry (DCI, NH_3) *m/z* 237 [$M+NH_4^+$], 205 [$M+NH_4^+$–CH_3OH]. Anal. calcd. For $C_7H_{13}N_3O_5$, 0.6 EtOH: C, 39.90; H, 6.78; N, 17.02. Found: C, 40.26; H, 6.66; N, 17.37.

Methyl-6-amino-6-deoxy-D-fructofuranoside (7). A mixture of Pd/C (10%, 0.2 g, 0.14 mmol) and **6** (0.144 g, 0.66 mmol) in 10 mL of ethanol was degassed and hydrogenated overnight. The catalyst was filtered off and the solvent removed under reduced pressure to yield **7** as a colourless oil (0.128 g, 0.66 mmol, 99%) which was rapidly used in the next step without further purification. ^{13}C NMR (D_2O, 62 MHz) **7** (β-furanose form): δ 175.0, 107.1, 81.4, 79.6, 79.1, 62.0, 51.8, 45.5, 24.9. **7** (α-furanose form): δ 175.0, 111.1, 82.3, 81.9, 78.8, 60.3, 51.1, 43.9, 24.9.

Methyl-6-(*N*-(5-(dimethylamino)naphth-1-yl)sulfonyl)-amino-6-deoxy-D-fructofuranoside (8a–b). A mixture of 5 mL of Dowex 1×8 resin (OH^- form) and amino fructose **7** (0.128 g, 0.66 mmol) in 10 mL of methanol was stirred for 30 mn and cooled to 4 °C. Dansyl chloride (0.357 g, 0.75 mmol) was added by portion, then the mixture was warmed to room temperature for 1 h. The resin was filtered off and the mixture concentrated under reduced pressure and purified by preparative TLC (dichloromethane:methanol, 8:2) to yield α-furanoside **8a** (0.022 g, 0.052 mmol, 7.8%) and β-furanoside **8b** (0.044 g, 0.103 mmol, 15.6%). Compound **8a** (α-furanose form) $[\alpha]_D^{25} = -19.8$ ($c = 1$, EtOH). ^1H NMR (CD_3OD, 200 MHz): δ 8.7–8.1 (m, 3H), 7.7–7.2 (m, 3H), 3.6–3.3 (m, 5H), 3.0 (s, 3H), 2.9 (s, 6H). ^{13}C NMR (CD_3OD, 50 MHz): δ 153.2, 138.5, 131.2, 131.0, 130.2, 129.1, 124.3, 120.7, 116.6, 109.1, 82.5, 82.3, 80.1, 60.3, 49.0, 46.0, 45.8. HRMS: calcd for $C_{18}H_{24}N_2NaO_7S$ ($M+Na^+$) 449.136; found 449.138. Compound **8b** (β-furanose form) $[\alpha]_D^{25} = +15.0$ ($c = 1$, EtOH). ^1H NMR (CD_3OD, 200 MHz): δ 8.56 (m, 1H), 8.38 (m, 1H), 8.20 (m, 1H), 7.58 (m, 2H), 7.26 (m, 1H), 4.02 (m, 1H), 3.74–3.58 (m, 4H), 3.40 (s, 2H), 2.99 (s, 3H), 2.83 (s, 6H). ^{13}C NMR (CD_3OD, 50 MHz): δ 153.2, 149.1, 136.2, 131.0, 130.2, 129.1, 124.3, 120.7, 116.6, 105.4, 81.4, 78.6, 78.5, 61.2, 49.3, 47.8, 45.8. HRMS calcd for $C_{18}H_{25}N_2O_7S$ (MH^+) 427.154; found 427.155.

(2*R*) 3-(*N*-(5-(Dimethylamino)naphth-1-yl)sulfonyl)amino-2-hydroxy-propanal diethyl acetal (9). To a stirred mixture of (2*R*)-3-amino-2-hydroxy propanal diethyl acetal (0.423 g, 2.6 mmol), obtained from azide **4**,[4a] and *N,N*-diisopropylethylamine (0.67 g, 5.2 mmol) in 20 mL of anhydrous diethyl ether, dansyl chloride (0.681 g, 2.6 mmol) in anhydrous diethylether (10 mL) was added dropwise, at room temperature. The hydrochlorhydrate was filtered off, the solvent evaporated, the oil chromatographied (eluent dichloromethan) to afford **9** as a green oil (0.68 g, 1.716 mmol, 66%). ^1H NMR ($CDCl_3$, 200 MHz): δ 8.5–7.1 (m, 6H), 5.8 (t, 1H), 4.2 (d, 1H), 3.6–3.0 (m, 7H), 2.8 (s, 6H), 1.0 (t, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 151.9, 134.6, 130.4, 129.6, 128.4, 123.2, 118.9, 115.2, 103.1, 70.1, 63.7, 64.2, 45.4, 44.2, 15.3. Mass spectrometry (DCI, NH_3) *m/z* 397 [MH^+], 414 [$M+NH_4^+$].

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