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A simple and efficient method to label L-fucose

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Abstract—This letter reports a new labeling method of fucoidan and more precisely of its monomer, L-fucose. We studied the coupling processes of new aryliodide precursors to L-fucose in order to prepare the next step, that is, the fucoidan radiolabeling. The use of precursors containing hydrazide and hydroxylamines is an alternative procedure avoiding the use of toxic borohydride or organic borane used for reductive amination. These coupling reactions are efficient and stereoselective. They provide stable products.

Fucoidans, polysaccharides containing substantial percentages of L-fucose and sulfate ester groups are extracted from Phaeophycophyta (or brown algae) such as *Fucus vesiculosus* or *Ascophyllum nodosum*.[1–4] They have potent biological activities: anticoagulant/ antithrombotic,[5,6] anti-inflammatory,[7] antiviral,[8] anti-poison,[9] and anti-angiogenic.[10] The aim of this work was to develop a labeling technique for fucose containing polysaccharides using a simple chemical reaction in order to simplify their detection for biological applications. It is essential to label macromolecules without altering their biological properties. According to the literature, labeling polysaccharides with fluorescence or UV-detectable[11–15] groups is the most commonly used, but these techniques may not be appropriate for in vivo studies because of their low sensitivity.

The introduction of radioactive atoms associated to the macromolecule could solve this problem. The usefulness of radioactively labeled compounds in general stems from the ease of detecting and measuring extremely small amounts. Carbon-14 and tritium are extensively used as radiolabels. The isotopes of iodine[16] have two major advantages over ^{14}C and ^{3}H. All available iodine isotopes are γ emitters that can be detected directly without the use of a costly scintillation cocktail. The second advantage is the relative shortness of their half-lives (max 60 days for ^{125}I in comparison to 12.35 years for ^{3}H and 5730 years for ^{14}C).

A previous paper[17] described the radioiodination of a macromolecule, scleroglucan. The reaction consisted first in a hydroxyl group activation as a *p*-tosylate that was displaced by iodide in the second step. This method was useful and did not alter the polysaccharide. In our case, unfortunately, this method could not be employed because of a lack of reactive hydroxyl groups on the macromolecule.

The purpose of this work was thus to develop original precursors for fucoidan radioiodination on the reducing end. In order to achieve this goal, we designed several molecules containing an aromatic ring carrying two active functions: one used for iodination while the other forms a covalent link with the macromolecule. It should be then possible to radiolabel[16,18] the precursor prior to coupling it to the desired molecule in two successive reactions. The advantage of this method is to decrease the risk of degradation of the molecule by avoiding reductive conditions used during usual radiolabeling methods. This method could be then adapted to other non reduced polysaccharides.

According to the literature,[18] three coupling functions can be used (amine, hydrazide, and hydroxylamine). Amines[19,20] condense with aldehydes to form a Schiff

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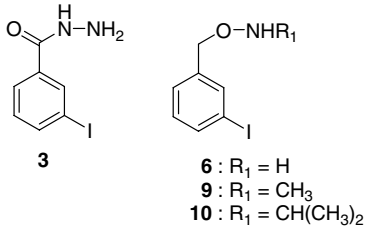
base linkage. This reaction is reversible and requires a second step, the imine reduction, to stabilize the linkage. Usually toxic borohydride or organic boranes are used for that purpose. Furthermore, the ring opening of the reducing end residue, which occurs during reductive amination, may have a detrimental effect on the biological activity or immunogenicity of the carbohydrate.[21]

In contrast to reductive amination, hydrazides,[22–25] and hydroxylamines[26–29] condense with aldehydes to form stable osazones and oximes, respectively.

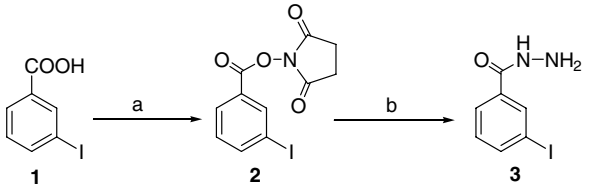
This letter describes the syntheses of two types of prosthetic groups (Scheme 1): 3-iodophenylhydrazide **3**, *O*-(3-iodobenzyl)-hydroxylamine **6**, *N*-methyl-*O*-(3-iodobenzyl)-hydroxylamine **9**, and *N*-isopropyl-*O*-(3-iodobenzyl)-hydroxylamine **10**.

To define appropriate conditions for the coupling reaction toward carbohydrate, a preliminary chemistry work was performed. This study permitted to define the coupling reaction conditions and their stereoselectivity. For that purpose, we substituted the polysaccharide with its L-fucose monomer during chemical reactions. This allowed us to work with non radioactive iodinated precursors in order to characterize the reaction products using conventional methods. Of course, the final goal is to substitute stable iodine by radioactive iodine.

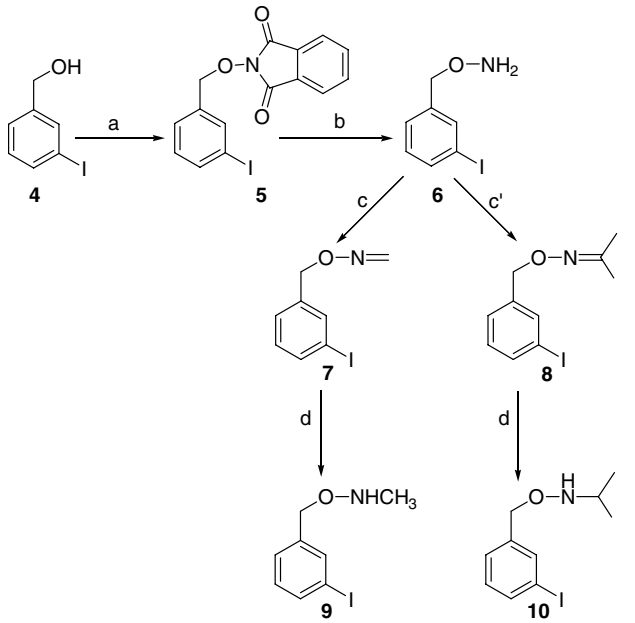
3-Iodophenylhydrazide **3** (Scheme 2) was prepared[30] in a two-step reaction with 95% global yield. This efficient reaction started with 3-iodobenzoic acid **1**. The starting material was activated as *N*-hydroxysuccinimidyl ester using TSTU (tetrafluoroborate *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(*N*-succinimidyl)-uronium) according to the Al-Jammaz method[31] to give compound **2** in a reproducible yield. Hydrazide **3** was then obtained quantitatively by reacting with hydrazine monohydrate.



Scheme 1. Precursors used for the iodination of carbohydrates.



Scheme 2. Synthesis of 3-iodophenylhydrazide **3**. Reagents and conditions: (a) TSTU (2.5 equiv), NEt_3 (2 equiv), anhydrous CH_3CN, rt, 95% and (b) NH_2–NH_2·H_2O (5 equiv), NEt_3 (1 equiv), anhydrous CH_3CN, rt, 100%.

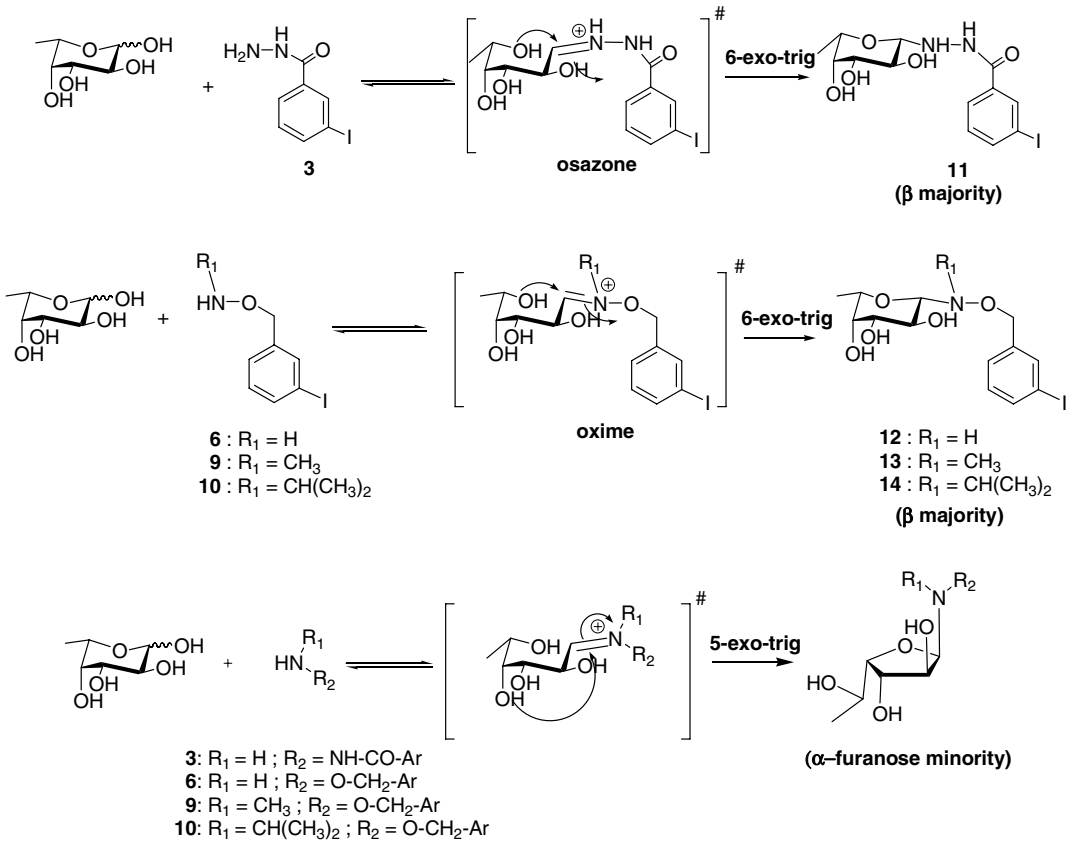


Scheme 3. Syntheses of *O*-(3-iodobenzyl)-hydroxylamines **6**, **9**, and **10**. Reagents and conditions: (a) *N*-hydroxyphthalimide (2 equiv), PPh_3 (2 equiv), diethylazodicarboxylate (2 equiv), anhydrous $CHCl_3$, rt, 15 h, 71%; (b) NH_2–NH_2·H_2O (10 equiv), EtOH abs, reflux, 3 h, 80%; (c) HCHO (1.5 equiv), anhydrous $CHCl_3$, reflux, 1 h, 95%; (c′) acetone, reflux, 1 h, 90%, and (d) $NaCNBH_3$ (3 equiv), EtOH, HCl (12 N) until pH 3, rt, 30 min, quantitative.

In the following synthesis (Scheme 3), we used a prosthetic group containing *O*-substituted-hydroxylamine function. It was also interesting to study the N-substitution effect regarding glycosylation reaction. O-Substituted hydroxylamines **6**, **9**, and **10** were obtained[32] according to a Mitsunobu method[33,34] starting with 3-iodobenzylalcohol **4**. This method permitted the introduction of a protected amine using a phthalimido protective group. *O*-(3-Iodobenzyl)-*N*-hydroxyphthalimide **5** was obtained in 71% yield. Amine deprotection was performed with hydrazine monohydrate to give *O*-(3-iodobenzyl)-hydroxylamine **6** with 80% yield. Aldehyde and ketone carbonyls were reacted with the highly nucleophilic aminooxy group to form oximes **7** and **8**. $NaCNBH_3$ reduction of these oximes was performed at pH 3 and gave quantitatively N-substituted hydroxylamines **9** and **10**.

Once the synthesis of these four precursors was performed, we studied the glycosylation reactions with L-fucose.[35] The reactions (Scheme 4) proceeded in polar organic solvent ($MeOH/CH_3COOH$ glacial (85/15)) at room temperature. The iodinated precursors were condensed on the anomeric position. Hydrazides and hydroxylamines condensed with the aldehyde to form, respectively, osazone or oxime. These acyclic intermediates were more or less stable and also existed in cyclic forms. The reactions were complete within 20 h and provided glycosylated compounds in good and reproducible yields (Table 1).

Regarding each glycosylation reaction, the resulting products consisted of a mixture of two cyclic forms



Scheme 4. Glycosylation of iodinated precursors.

Table 1. Yields and stereoselectivity of glycosylations on L-fucose

Product	Precursor	Yield (%)	% Cyclic form	% (β-Pyranose/ α-furanose)
11	**3**	96	100	95/5
12	**6**	90	50	95/5
13	**9**	98	100	92.5/7.5
14	**10**	85	100	95/5

(β-pyranose and α-furanose) and also of the acyclic form for hydroxylamine **12**. These compounds were not isolated but characterized by NMR spectroscopy. It is noteworthy that the analysis of the $^{3}J_{H1-H2}$ coupling constant values[36] indicated that the reactions proceeded with high stereoselectivity. We noticed a large excess of β-pyranose forms, that is, the cis-position of the precursor on C_1 toward CH_3-5.[37] The presence of the α-furanose form was ascertained on the basis of the typical deshielded chemical shift values[36] as well as the coupling constants observed by NMR spectroscopy.

Iodinated precursors gave the β-pyranose form in majority, except for *O*-(3-iodobenzyl)-hydroxylamine **6** where acyclic forms were detected in equal proportions. The β-pyranose major formation can be rationalized (Scheme 4) by the bulky OH-2 group which induces a preferential 6-*exo*-trig ring closure with OH-5 attacking the Si oxy-iminium face. The α-furanose forms arise from 5-*exo*-trig ring closure with OH-4. Addition of hydroxylamine **6** on L-fucose resulted in a stable oxime, which may explain the equilibrium observed between the cyclic and acyclic forms. N-Substitution of the hydroxylamines seems to destabilize the oxy-iminium intermediate inducing ring closure.

We have studied the feasibility of L-fucose glycosylation prior to working with the polysaccharide structure, fucoidan. We have demonstrated that these reactions proceeded chemoselectively on the aldehyde without activation of the anomeric center. Glycosylations were obtained in good and reproducible yields giving products stable in a fridge for several months. Addition of the precursors gave essentially the final cyclic forms, with β-pyranose prevailing, with the exception of *O*-(3-iodobenzyl)-hydroxylamine **6**, which gave 50% of acyclic form. We observed that addition of *N*-methyl and *N*-isopropyl substituted *O*-(3-iodobenzyl)-hydroxylamine induced cyclisation.

In conclusion, all precursors have shown good properties and could be potential candidates for polysaccharide radiolabeling. Regarding radiolabeling applications, the essential factor would be the resulting stability of the radiolabeled polysaccharides in vivo. In fact, this study was important to optimize the coupling reaction between precursors and polysaccharides prior to substituting cold iodine by radioactive iodine. We defined conditions smooth enough to be used for polysaccharide radiolabeling without denaturation.

Acknowledgements

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30. Synthesis of **3**: 3-Iodobenzoic acid **1** (1 equiv, 2 g, 8.06 mmol) was dissolved in 5 ml of anhydrous CH_3CN under inert atmosphere. NEt_3 (2 equiv, 2.25 ml, 16.12 mmol), and 2.5 equiv of TSTU (6.1 g, 20.16 mmol) were added in the round-bottom flask at room temperature. The reaction mixture was stirred at room temperature. A brown colour appeared immediately. After 15 min, the reaction was finished. The mixture was concentrated in vacuo. The product was purified by column chromatography in 95% yield (2.65 g, 7.68 mmol). Eluent: $CHCl_3$/AcOEt (95/5); R_f: 0.9; $[M+H^+]$: 346; ^1H NMR ($CDCl_3$): 2.9 (s, 4H, $2\times CH_2$); 7.27 (t, 1H, H_5, $^3J = 7.9$ Hz); 8 (d, 1H, H_4, $^3J = 7.9$ Hz); 8.1 (d, 1H, H_6, $^3J = 7.9$ Hz); 8.47 (s, 1H, H_2). ^{13}C NMR ($CDCl_3$): 27.7 ($2\times CH_2$); 96 (C_3); 129 (C_6); 131.7 (C_5); 132.5 (C_1); 141.2 (C_2); 145.8 (C_4); 162.6 (C=O); 170.8 ($2\times$ C=O).
O-(*N*-Succinimidyl)-3-iodobenzoate ester **2** (1 equiv, 0.5 g, 1.45 mmol) was dissolved with 5 ml of anhydrous CH_3CN under inert atmosphere. Monohydrate hydrazine (5 equiv, 203 μl, 7.25 mmol) and 1 equiv of NEt_3 (202 μl, 1.45 mmol) were dissolved in 5 ml of anhydrous CH_3CN and were added dropwise to the reaction mixture. A white suspension appeared after few minutes. The mixture was filtered on celite. The filtrate was concentrated. The final product was purified by flash chromatography on silica gel in a quantitative yield (0.38 g, 1.45 mmol). Eluent: $CHCl_3$/MeOH (9/1); R_f: 0.4; $[M+H^+]$: 263; ^1H NMR (DMSO-d_6): 4.56 (s, 2H, NH_2); 7.29 (t, 1H, H_5, $^3J = 7.9$ Hz); 7.86 (d, 1H, H_4, $^3J = 8.3$ Hz); 7.91 (d, 1H, H_6, $^3J = 7.9$ Hz); 8.18 (s, 1H, H_2); 9.9 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 94.7 (C_3); 127.4 (C_6); 131.4 (C_5); 136.3 (C_1); 137.3 (C_2); 141.6 (C_4); 167.9 (C=O).
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32. Synthesis of **6**: 3-iodobenzyl alcohol **4** (0.27 ml, 2.14 mmol) was dissolved into 30 ml of anhydrous $CHCl_3$ under nitrogen atmosphere. PPh_3 (1.12 g, 4.3 mmol) and *N*-hydroxyphthalimide (0.7 g, 4.3 mmol) were added to the reaction mixture. The reaction was stirred at room temperature to dissolve all components. Diethylazodicarboxylate (0.67 ml, 4.3 mmol) was added. The reaction mixture was stirred for 15 h at room temperature. The solvent was evaporated under reduced pressure. The product was purified by column chromatography in 71% yield (0.57 g, 1.52 mmol). Eluent: $CHCl_3$ (100); R_f: 0.6; $[M+H^+]$: 380; ^1H NMR ($CDCl_3$): 5.06 (s, 2H, CH_2); 7.05 (t, 1H, H_5, $^3J = 7.8$ Hz); 7.45 (d, 1H, H_4, $^3J = 7.7$ Hz), 7.7 (m, 5H, $H_6/H_{2',3',4',5'}$); 7.8 (s, 1H, H_2). ^{13}C NMR ($CDCl_3$): 78.8 (CH_2); 94 (C_3); 123.6 ($C_{2'}/C_{5'}$); 128.86 ($C_{3'}/C_{4'}$); 130.3 ($C_{1'}/C_{6'}/C_6$); 134.5 (C_5); 135.9 (C_1); 138.3 (C_2/C_4); 160.3 (C=O); 163.4 (C=O).
O-(3-Iodobenzyl)-*N*-hydroxyphthalimide **5** (0.57 g, 1.51 mmol) was dissolved in 50 ml of absolute ethanol. Hydrazine monohydrate (10 equiv, 727 μl, 15.1 mmol) was added. The reaction mixture was stirred and heated at reflux for 3 h. The mixture was cooled: a white precipitate appeared. The solid was filtered and rinsed with ethanol. The filtrate was concentrated. The product was obtained after purification by column chromatography in 80% yield (0.3 g, 1.21 mmol). Eluent: $CHCl_3$/MeOH (90/10); R_f: 0.3; $[M+H^+]$: 250; ^1H NMR ($CDCl_3$): 4.61 (s, 2H, CH_2); 5.43 (s, 2H, NH_2); 7.09 (t, 1H, H_5, $^3J = 7.7$ Hz); 7.31 (d, 1H, H_4, $^3J = 7.7$ Hz), 7.64 (d, 1H, H_6, $^3J = 8$ Hz); 7.72 (s, 1H, H_2). ^{13}C NMR ($CDCl_3$): 75.3 (CH_2); 97.5(C_3); 126.2 (C_6); 130.3 (C_5); 136.2 (C_2); 136.3 (C_4); 142.5 (C_1).

Synthesis of **9**: Paraformaldehyde (54 mg, 1.8 mmol) was dissolved in 10 ml of anhydrous $CHCl_3$ and was heated at 30 °C for 5 min for depolymerisation. *O*-(3-Iodobenzyl)-hydroxylamine **6** (0.3 g, 1.2 mmol) was added. The reaction mixture was heated at reflux for 1 h and then cooled. The solvent was evaporated. Product was obtained after purification by column chromatography in 95% yield (0.3 g, 1.14 mmol). Eluent: $CHCl_3$/MeOH (90/10); R_f: 0.3; $[M+H^+]$: 262; ^1H NMR ($CDCl_3$): 5.06 (s, 2H, CH_2); 6.49 (d, 1H, CH_2=N, $^3J = 8.2$ Hz); 7.1 (d, 1H, CH_2=N, $^3J = 7.9$ Hz); 7.1 (t, 1H, H_5, $^3J = 7.6$ Hz); 7.33 (d, 1H, H_4, $^3J = 7.6$ Hz), 7.65 (d, 1H, H_6, $^3J = 7.6$ Hz); 7.73 (s, 1H, H_2). ^{13}C NMR ($CDCl_3$): 75.6 (CH_2); 93 (C_3); 128.4 (C_6); 130 (C_5); 135.4 (C_2); 135.9 (C_4); 136.4 (CH_2=N); 141 (C_1). Oxime **7** (0.3 g, 1.14 mmol) was dissolved in 5 ml of absolute ethanol. Sodium cyanoborohydride (0.2 g, 3.4 mmol) was added. The pH was adjusted to 3 with HCl 12 N. The reaction was stirred for 30 min at room temperature under nitrogen atmosphere. The solvent was evaporated. A NaOH aqueous solution was used to obtain a pH 8. The aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and evaporated. Product **9** was obtained quantitatively (0.3 g, 1.14 mmol). $[M+H^+]$: 264; ^1H NMR ($CDCl_3$): 2.73 (s, 3H, CH_3); 4.64 (s, 2H, CH_2); 5.12 (s, 1H, NH); 7.08 (t, 1H, H_5, $^3J = 7.6$ Hz); 7.32 (d, 1H, H_4, $^3J = 7.3$ Hz), 7.63 (d, 1H, H_6, $^3J = 8$ Hz); 7.73 (s, 1H, H_2). ^{13}C NMR ($CDCl_3$): 33.9 (CH_3); 73.3 (CH_2); 94.5 (C_3); 127.2 (C_6); 130.3 (C_5); 135.9 (C_2); 136.3 (C_4); 141.5 (C_1).

Synthesis of **10**: *O*-(3-Iodobenzyl)-hydroxylamine **6** (0.1 g, 0.4 mmol) was dissolved in acetone. The mixture was heated at reflux for 1 h and was then cooled. The solvent was evaporated. The product was purified by column chromatography in 90% yield (104 mg, 0.36 mmol). Eluent: $CHCl_3$/MeOH (90/10); R_f: 0.3; $[M+H^+]$: 290; ^1H NMR ($CDCl_3$): 1.88 (s, 3H, CH_3); 1.9 (s, 3H, CH_3); 5 (s, 2H, CH_2); 7.08 (t, 1H, Hc, $^3J = 8$ Hz); 7.32 (d, 1H, Hb, $^3J = 7.6$ Hz), 7.62 (d, 1H, Hd, $^3J = 7.3$ Hz); 7.7 (s, 1H, Ha). ^{13}C NMR ($CDCl_3$): 15.8 (CH_3); 21.9 (CH_3); 74.19 (CH_2); 94.3 (C_3); 126.9 (C_6); 130 (C_5); 136.7 (C_2/C_4); 140.8 (C_1); 155.7 (N=CH_2).

Oxime **8** (104 mg, 0.36 mmol) was dissolved in 5 ml of absolute ethanol. Sodium cyanoborohydride (67.8 mg, 1.08 mmol) was added. The pH was adjusted to 3 with 12 N HCl. The reaction was stirred for 30 min at room temperature under nitrogen atmosphere. The solvent was evaporated. A 6 N NaOH aqueous solution was used to obtain a pH 8. The aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and evaporated. Product **10** was obtained quantitatively (104.7 mg, 0.36 mmol). $[M+H^+]$: 292; ^1H NMR ($CDCl_3$): 1.07 (d, 6H, $2\times CH_3$, $^3J = 6.1$ Hz); 3.17 (m, 1H, CH, $^3J = 6.4$ Hz); 4.65 (s, 2H, CH_2); 5.37 (s, 1H, NH); 7.08 (t, 1H, H_5, $^3J = 7.6$ Hz); 7.32 (d, 1H, H_4, $^3J = 7.6$ Hz), 7.63 (d, 1H, H_6, $^3J = 7.9$ Hz); 7.72 (s, 1H, H_2). ^{13}C NMR ($CDCl_3$): 21.7 ($2\times CH_3$); 53.3 (CH); 76.9 (CH_2); 95.9 (C_3); 128.9 (C_6); 131.6 (C_5); 138.3 (C_2); 138.7 (C_4); 142.1 (C_1).

Synthesis of **11**: L-fucose (12.5 mg, 0.076 mmol); 3-iodophenylhydrazide **3** (40 mg, 0.15 mmol). Eluent of chromatography: $CHCl_3$/MeOH (80/20). $R_f = 0.4$. Yield: 96% (29.9 mg, 0.073 mmol). $[M-H^+] = 407$. ^1H NMR (DMSO-d_6): 1.12 (d, CH_3 α form, $^3J = 6.4$ Hz); 1.14 (d, CH_3 β form, $^3J = 6.4$ Hz); 3.4 (m; 8H, $H_{2'}/H_{3'}/H_{4'}/H_{5'}$ β form + $H_2/H_3/H_4/H_5$ α form); 3.81 (d, 1H, $H_{1'}$β, $^3J = 8.4$ Hz); 4.39 (d, 1H, $H_{1'}$α, $^3J = 5.2$ Hz); 5 (s, 3H, OH); 5.9 (s, 1H, NH); 7.28 (t, 1H, H_5, $^3J = 8$ Hz); 7.87 (d, 1H, H_4, $^3J = 7.6$ Hz); 7.91 (d, 1H, H_6, $^3J = 8$ Hz); 8.2 (s, 1H, H_2); 10.2 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 18.6 (CH_3); 55.9 ($C_{5'}$(α)); 71.2 ($C_{2'}$);71.9 ($C_{3'}$(α)); 72.2 ($C_{5'}$(β) + $C_{3'}$(β)); 73.6 (CH_2 + $C_{4'}$); 87 ($C_{1'}$(α)); 91 ($C_{1'}$(β)); 95.2 (C_3); 126.7 (C_6); 130.5 (C_5); 134.9 (C_2); 135.5 (C_4); 139.7 (C_1); 163.9 (C=O).

Synthesis of **12**: L-Fucose (9.8 mg, 0.06 mmol); *O*-(3-iodobenzyl)-hydroxylamine **6** (30 mg, 0.12 mmol). Eluent of chromatography: $CHCl_3$/MeOH(90/10). $R_f = 0.2$. Yield: 90% (21.4 mg, 0.054 mmol). $[M-H^+] = 394$. ^1H NMR (DMSO-d_6): 1.05 (d, 3H, CH_3); 3.1–3.6 (m, 8H, $H_{2'}/H_{3'}$ cyclics + $H_{3'}/H_{4'}/H_{5'}$ acyclics + 3OH); 3.83 (quint, 1H, $H_{5'}$ cyclic, $^3J = 6.4$ Hz); 4.1 (d, 1H, $H_{4'}$ cyclic, $^3J = 6.4$ Hz); 4.3 (d, 1H, $H_{1'}$β, $^3J = 7.63$ Hz); 4.55 (d, 1H, $H_{1'}$α, $^3J = 5.17$ Hz); 4.6 (d, 1H, $H_{2'}$ acyclic, $^3J = 7$ Hz); 4.96 (s, 2H, CH_2); 7.17 (t, 1H, H_5, $^3J = 7.6$ Hz); 7.35 (d, 1H, H_4, $^3J = 7.6$ Hz); 7.49 (d, 1H, $H_{1'}$ acyclic, $^3J = 7.3$ Hz); 7.65 (d, 1H, H_6, $^3J = 7.6$ Hz); 7.69 (s, 1H, H_2). ^{13}C NMR (DMSO-d_6): 20 (CH_3); 64.9 ($C_{5'}$); 68 ($C_{2'}$); 72.2/72.7 ($C_{3'}$/CH_2); 73.6 ($C_{1'}$ cyclic/$C_{4'}$); 94.7 (C_3); 127.3 (C_6); 130.5 (C_5); 136.3 (C_2/C_4); 140.6 (C_1); 153.4 ($C_{1'}$ acyclic).

Synthesis of **13**: L-Fucose (9.9 mg, 0.06 mmol); *N*-methyl-*O*-(3-iodobenzyl)-hydroxylamine **9** (32 mg, 0.12 mmol). Eluent of chromatography: $CHCl_3$/MeOH(90/10). $R_f = 0.3$. Yield: 98% (24.4 mg, 0.059 mmol). $[M-H^+] = 408$. ^1H NMR ($(CD_3)_2CO$): 1.17 (d, CH_3 α form, $^3J = 6.4$ Hz); 1.18 (d, CH_3 β form, $^3J = 6.4$ Hz); 2.6 (s, 3H, CH_3–N); 3.6 (m, 8H, $H_{2'}/H_{3'}/H_{4'}/$ $H_{5'}$ β form+ $H_{2'}/$ $H_{3'}/H_{4'}/H_{5'}$ α form); 3.97 (d, 1H, $H_{1'}$(β), $^3J = 8.9$ Hz); 4.38 (d, $H_{1'}$(α), $^3J = 5.2$ Hz); 4.68 (s, 2H, CH_2); 7.13 (t, 1H, H_5, $^3J = 7.9$ Hz); 7.41 (d, 1H, H_4, $^3J = 7.6$ Hz); 7.63 (d, 1H, H_6,$^3J = 7.9$ Hz); 7.74 (s, 1H, H_2). ^{13}C NMR ($(CD_3)_2CO$): 16.9 (CH_3); 39 (CH_3–N); 68.9 ($C_{5'}$(α)); 72.5 ($C_{2'}$); 72.9 ($C_{5'}$(β)); 74.7 ($C_{3'}$(α)); 74.9 ($C_{3'}$(β)); 76 (CH_2); 88.2 ($C_{4'}$); 94.4 ($C_{1'}$(α)); 94.5 ($C_{1'}$(β)); 101.3 (C_3); 129 (C_6); 131.1 (C_5); 137.5 (C_2); 138.5 (C_4); 141.7 (C_1).

Synthesis of **14**: L-Fucose (8.5 mg, 0.052 mmol); *N*-isopropyl-*O*-(3-iodobenzyl)-hydroxylamine **10** (30 mg, 0.1 mmol). Eluent of chromatography: $CHCl_3$/MeOH (90/10). $R_f = 0.4$. Yield: 85% (19.2 mg, 0.044 mmol). $[M-H^+] = 436$. ^1H NMR (CD_3OD): 1.05 (d, 6H, CH_3–CH, $^3J = 6.1$ Hz); 1.18 (d, CH_3 α form, $^3J = 6.4$ Hz); 1.21 (d, CH_3 β form, $^3J = 6.4$ Hz); 2.97 (m, 1H, CH); 3.5 (m, 8H, $H_{2'}/H_{3'}/H_{4'}/H_{5'}$ β form + $H_{2'}/H_{3'}/H_{4'}/H_{5'}$ α form); 4.3 (d, 1H, $H_{1'}$ (β), $^3J = 8.9$ Hz); 4.5 (d, $H_{1'}$(α), $^3J = 4.8$ Hz); 4.7 (s, 2H, CH_2); 6.96 (t, 1H, H_5, $^3J = 7.6$ Hz); 7.18 (d, 1H, H_4, $^3J = 7.7$ Hz); 7.7 (d, 1H, H_6, $^3J = 7.8$ Hz); 7.8 (s, 1H, H_2). ^{13}C NMR (CD_3OD): 17.6 (CH_3); 19 ($2\times CH_3$); 47 (CH); 64.1 ($C_{5'}$(α)); 66.4 ($C_{2'}$); 67.1 ($C_{5'}$(β)); 71.1 ($C_{3'}$(α)); 74.1 ($C_{3'}$(β)); 75 ($C_{4'}$); 75.1 (CH_2); 82.1 ($C_{1'}$); 97.5 (C_3); 126.2 (C_6); 130.3 (C_5); 135.9 (C_2); 136 (C_4); 143 (C_1).

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