# β-Glucosyl and β-Galactosyl Transfer Catalysed by β-1,4-Galactosyltransferase in Preparation of Glycosylated Alkaloids

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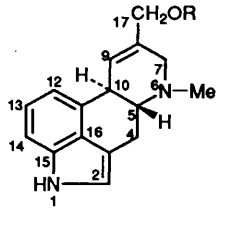
Bovine β-1,4-galactosyltransferase mediates the transfer of galactose from uridine 5'-diphosphogalactose and also transfer of glucose from uridine 5'-diphosphoglucose to elymoclavine 2-acetamido-2-deoxy-β-D-glucopyranoside **2a**, yielding the respective diglycosides **3** and **4**. Both reactions were optimised on a semipreparative scale. The products were characterised by NMR spectroscopy and mass spectrometry.

Different biological activities of various ergot alkaloids are widely employed, *e.g.*, for treatment of acromegaly, hypertension, hypergalactinaemia, migraine, parkinsonism, senile cerebral disturbances, and many other diseases.[1]

Most of the medicinally used representatives of this group are derivatives of natural compounds or semisynthetic products. Ergot alkaloid monoglycosides containing β-D-galactopyranosyl[2] (β-Gal), β-D-fructofuranosyl,[3] β-D-glucopyranosyl (β-Glc), 2-acetamido-2-deoxy-β-D-glucopyranosyl (β-GlcNAc), and 2-acetamido-2-deoxy-β-D-galactopyranosyl (β-GalNAc)[4] moieties have been prepared by biotransformation in our laboratory. Some of these glycosides are expected to have altered or new activities. Glycosides are more water-soluble than are the corresponding aglycones, thus improving their bioavailability. β-Glucosides are, *e.g.*, expected to pass more easily through the haematoencephalic (blood–brain) barrier. Selected alkaloid glycosides are currently being tested for their immunomodulation activity.

## Results and Discussion

Alkaloids bearing a β-Gal(1→4)β-GlcNAc (β-LacNAc) moiety were required for immunomodulation tests. An enzymic method for glycosylation was chosen because of the relative instability of the parent aglycone (*e.g.*, elymoclavine **1**). Galactosyltransferase was used instead of glycosidase for its regioselectivity and the higher yields attained in the glycosyl transfer. The starting material, *i.e.* alkaloids bearing a β-GlcNAc moiety, were recently prepared using hexosaminidase transglycosylation.[4]



**1** R = H
**2a** R = β-GlcNAc
**2b** R = β-Glc
**3** R = β-Gal(1→4)β-GlcNAc
**4** R = β-Glc(1→4)β-GlcNAc

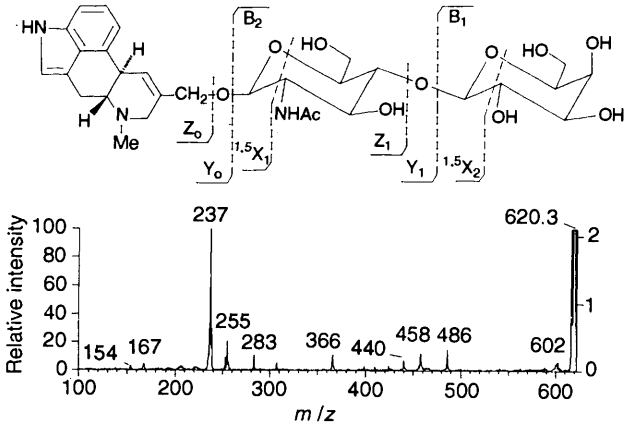
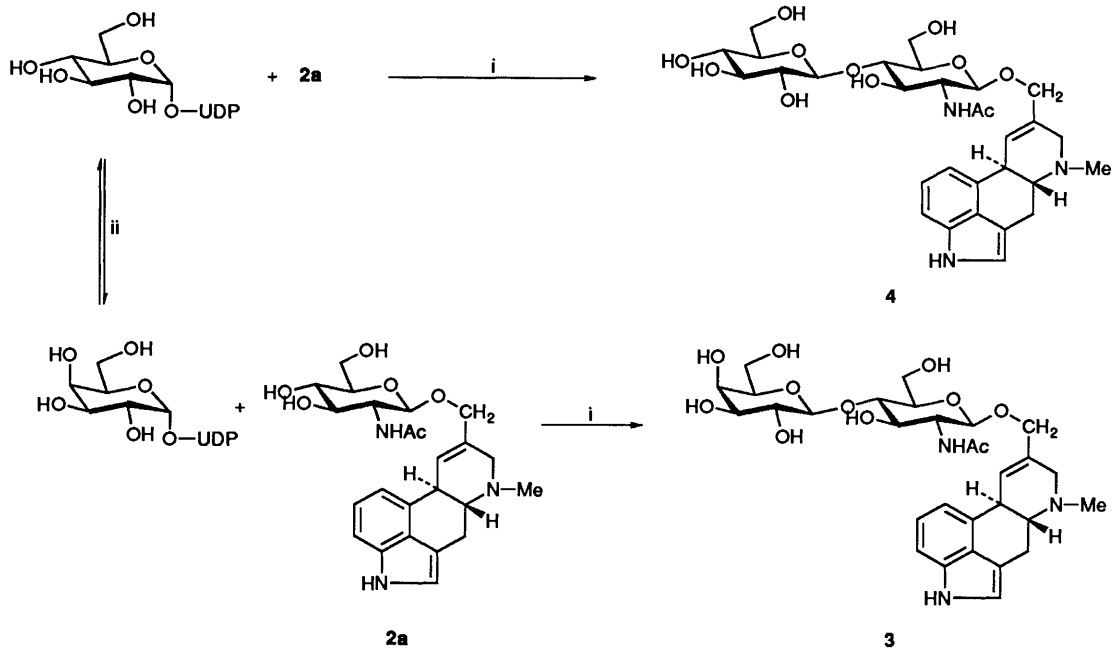



**Fig. 1** Daughter-ion linked-scan FAB mass spectrum of compound **3**. For data see Table 1.

In an analytical experiment, elymoclavine 17-*O*-(2-acetamido-2-deoxy-β-D-glucopyranoside) **2a** was subjected to galactosyl transfer catalysed by bovine β-1,4-galactosyltransferase (EC 2.4.1.22). Uridine 5'-diphosphogalactose (UDP-Gal) served as a substrate and alkaline phosphatase from calf intestine (EC 3.1.3.1) was used to remove feedback inhibition caused by the UDP produced. The reaction (monitored by TLC) was almost quantitatively completed within 24 h. The product **3** gave an $[M + H]^+$ ion *m/z* 620 ($C_{30}H_{42}N_3O_{11}$) in its FAB spectrum (Table 1). The ion with *m/z* 237, corresponding to dehydrated aglycone, dominates in the fragment-ion linked scan of the $[M + H]^+$ ion (Fig. 1). All fragment ions can be rationalised in terms of structure **3**. Besides the ergoline resonances, two contiguous spin systems were identified in the $^1H$ NMR spectra by a COSY experiment. Both are due to sugars of β-configuration ($J_{1',2'}$ 8.5 and $J_{1'',2''}$ 7.4 Hz), that one exhibiting large vicinal couplings only was assigned to the GlcNAc moiety. The other, with $J_{3'',4''}$ 3.2 and $J_{4'',5''}$ 1.0 Hz, belongs to a galactose moiety. A carbon atom, resonating in a characteristic downfield position ($\delta_C$ 81.82) and therefore participating in the glycosidic bond,[5–7] is directly coupled to




**Scheme 1** *Enzymes used:* i, Galactosyltransferase (bovine colostrum, EC 2.4.1.22); ii, UDP-galactose 4′-epimerase (EC 5.1.3.2)

**Table 1** High-resolution MS data obtained from FAB measurement and principal daughter ions [*m/z* (relative intensity %)] observed in *B/E* FAB mass spectra of $[M + H]^+$ ions of studied compounds (for nomenclature of listed ions see Fig. 1)

| | Compound | |
|---|---|---|
| Ion | **3** | **4** |
| $[M + H]^+_{meas.}$ | 620.2835 | 620.2813 |
| $[M + H]^+_{calc.}$ | 620.2819 | 620.2819 |
| $[M + H]^+$ elemental composition | $C_{30}H_{42}N_3O_{11}$ | $C_{30}H_{42}N_3O_{11}$ |
| $[M + H - H_2O]^+$ | 602(5) | 602(10) |
| $[^{1,5}X_2 + H]^+$ | 486(12) | 486(11) |
| $[Y_1 + 2H]^+$ | 458(9) | 458(10) |
| $[Z_1]^+$ | 440(5) | 440(5) |
| $[B_2]^+$ | 366(9) | 366(11) |
| $[^{1,5}X_1 + H]^+$ | 283(10) | 293(5) |
| $[Y_0 + 2H]^+$ | 255(19) | 255(32) |
| $[Z_0]^+$ | 237(100) | 237(100) |
| $[C_{12}H_9N]^+$ | 167(3) | 167(5) |
| $[C_{11}H_8N]^+$ | 154(1) | 154(0.5) |

4′-H of the GlcNAc part. This confirms a 1→4 attachment of galactose in elymoclavine *N*-acetyl-β-lactosaminide **3**.

In a semipreparative experiment using a larger amount of compound **2a** (70 mg, 0.15 mmol), the expensive UDP-Gal was replaced by the cheaper UDP-Glc and uridine 5′-diphosphoglucose 4′-epimerase (EC 5.1.3.2) was added. During this reaction (4 h), formation of another compound with $R_f$-value slightly higher than that of compound **3** was observed. Upon complete consumption of compound **2a** (23 h), the mixture was lyophilised and subjected to flash chromatography that afforded compound **3** (65 mg, 65%) and the side product **4** (19 mg, 20%). Concomitant formation of both products (**3** and **4**) was observed only in the presence of UDP-Gal-generating system (*in situ*), *e.g.*, of UDP-Glc and epimerase.

To confirm the suspected glucose transfer by galactosyl transferase, the experiment was repeated with compound **2a** (9 mg, 0.02 mmol—final conc$^n$. 0.1 mol dm$^{-3}$) and a higher concentration of UDP-Glc (final concn. H 0.2 mol dm$^{-3}$). First traces of the expected product appeared after 1 h; the reaction was complete after 100 h. Flash chromatography afforded compound **4** (7.3 mg, 57%), identical (MS, $^1$H and $^{13}$C NMR) with the above side product. The mass spectrum (Table 1) was similar to that of compound **3**. According to $^1$H NMR analysis, both sugar units have the β-configuration at their anomeric centres ($J_{1',2'}$ 8.4 and $J_{1'',2''}$ 7.9 Hz). Large vicinal coupling constants in both systems (axial–axial coupling) indicate a *gluco* configuration. The anomeric proton of the first residue was assigned on the basis of its nuclear Overhauser effect (NOE) to both 17-H protons of the aglycone. The diagnostic carbon atom ($\delta_C$ 81.82) represents C-4′ according to heteronuclear correlation, so that the linkage of two sugar moieties is again 1→4. Therefore, compound **4** is Glcβ-(1→4)GlcNAcβ-*O*-elymoclavine (Scheme 1).

We tested the possibility of an analogous transfer of glucose under similar conditions (however, in the presence of α-lactalbumin) with the Glcβ-*O*-elymoclavine **2b** as an acceptor. Nevertheless, after 100 h no trace of the expected cellobioside was formed. After unsuccessful incubation, the activity of the mixture (enzyme and donor integrity) was confirmed by addition of 4′-epimerase. Immediately after its addition a β-lactoside of compound **1** was produced.

For an efficient and fast Glc-1,4-transfer, the high concentration (0.2 mol dm$^{-3}$) of UDP-Glc is essential. This concentration of a relatively acidic substrate (two mole equivalents) completely unbalances the buffering capacity of 50 mmol dm$^{-3}$ cacodylate buffer (sodium dimethylarsinate) (pH 7.5) used. To maintain the pH optimum for the enzymes it is necessary to neutralise the acidity by treatment with 1 mol dm$^{-3}$ NaOH (approx. 50 mm$^3$ per 1 cm$^3$ of the mixture). The pH should be checked during the reaction and especially after the addition of new substrates. The concentration of $Mn^{2+}$ should be kept under 5 mmol dm$^{-3}$. Even though its higher concentration (optimum 13 mmol dm$^{-3}$) promotes the enzyme's activity, it accelerates the decomposition of UDP-Glc.

The possibility of Glc transfer by galactosyltransferase was excluded *ca.* 20 years ago by Ebner *et al.*[8] in their survey article based on the findings of Babad and Hassid.[9] However, this possibility was later demonstrated by Andree and Berliner,[10] who found the relative rate of reaction with UDP-Glc to be ~0.3% of that with UDP-Gal (when using GlcNAc as an acceptor in the absence of α-lactalbumin). They also found that galactosyltransferase in the presence of α-lactalbumin transfers Glc (from UDP-Glc) onto the free glucose, forming a disacch-

aride—most likely cellobiose. However, all these findings were based on paper chromatography and the tentative cellobiose produced by enzymic reaction had slightly different chromatographic behaviour from that of cellobiose standard. Glc transfer onto the GlcNAc moiety was recently confirmed by Palcic and Hindsgaul[11] using a model lipophilic acceptor β-GlcNAc-*O*-$[CH_2]_8CO_2Me$ on a 1 mg scale. In the reaction product only two anomeric β-protons were detected by $^1H$ NMR spectroscopy, showing that the second sugar has the β-configuration, but they were unable to confirm its nature and its site of attachment to the GlcNAc moiety.

The transfer rate of the glucose *vs* galactose by galactosyltransferase was estimated with our substrate **2a** (in the given concentrations) to be approx. in one order higher (3–5%) than the value reported (0.3%)[10] for GlcNAc as an acceptor. This estimation was based on the reaction times needed for completion of the reactions. This could be explained partly by the fact that functionalised GlcNAc (or Glc) has a different affinity towards galactosyltransferase[12] and partly by the nature of the aglycone, *i.e.*, ergot alkaloid **1** used. Amphiphilic properties of the ergoline skeleton (tertiary nitrogen, heteroaromatic nature of the indole moiety) common to all ergot alkaloids could interact with other sites on the enzyme surface in an unspecified manner. This activation effect of the ergot alkaloid moiety was observed with another glycosyltransferase.[13] Also, a high concentration (0.2 mol $dm^{-3}$) of the UDP-Glc is crucial for a reasonable reaction rate.

We report here an optimised semipreparative method for an application of this reaction to the preparation of glycoconjugates of pharmaceutically active compounds, with full spectral characterisation of the products.

## Experimental

$^1H$ and $^{13}C$ NMR spectra were measured on a Varian VXR-400 spectrometer (399.952 and 100.577 MHz, respectively) for samples in $CD_3OD$ at 25 °C. Residual solvent signal ($\delta_H$ 3.33, $\delta_C$ 49.3) served as an internal reference. Chemical shifts are given in the δ-scale; digital resolution was 0.0002 and 0.006 ppm, respectively. *J*-Values are given in Hz. Carbon-signal multiplicity was determined by an APT (Attached Proton Test) experiment. Manufacturer's software was used for 2D NMR spectroscopy (COSY, ROESY, HOM2DJ, HETCOR). Alkaloid numbering is given in structures **1**–**4**, letters a, e denote axial or equatorial protons, respectively. Primed numbers were used for the first sugar residue, double primed numbers for the second one. Letters d and u mean the downfield or upfield resonating protons in diastereotopic methylene groups, respectively.

All positive-ion fast atom bombardment (FAB) mass spectra were recorded on a double-focusing Finnigan MAT 90 instrument of *BE* geometry (magnetic sector preceding the electrostatic one) under conditions described previously.[14] The standard saddle-field FAB gun (Ion Tech Ltd., Teddington, GB) was operated at 2 mA current and 6 kV energy, using xenon as the bombarding gas ($1 \times 10^{-5}$ mBar).* *m*-Nitrobenzyl alcohol (Aldrich) was used as the matrix.

Products of collisionally activated decompositions in the first field-free region of the instrument were analysed by daughter-ion linked-scan (*B*/*E* constant) using manufacturer's software. The collision gas (He) pressure was adjusted for 50% attenuation of the primary ion beam, with collision-cell voltage maintained at the ground potential. The mass range was scanned at a rate of 40s/100 daltons and the conventional resolution was adjusted to 2000 (10% valley).

* 1 Bar = $10^5$ Pa.

*Preparation of* β-D-*Galactopyranosyl*-(1→4)-2-*acetamido*-2-*deoxy*-β-D-*glucopyranosyl*-(1→O)-*elymoclavine* **3**.—2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→*O*)-elymoclavine **2a** was prepared by enzymic transglycosylation according to the previously published procedure.[4] Elymoclavine **1** used for this preparation was kindly donated by Galena Ltd. (Opava, Czech Republic).

A solution of compound **2a** (70 mg, 0.15 mmol) and uridine 5′-diphosphoglucose (Sigma) (60 mg, 0.1 mmol) in 50 mmol $dm^{-3}$ sodium cacodylate buffer, pH 7.5 (2 $cm^3$) containing 5 mmol $dm^{-3}$ $MnCl_2$ was adjusted to pH 7.5 by 1 mol $dm^{-3}$ NaOH (~150 $mm^3$). Galactosyltransferase (Sigma) (0.8 U), UDP-Glc 4′-epimerase (Sigma) (4 U) and calf intestinal alkaline phosphatase (Boehringer) (40 U) were added and the mixture was incubated at 37 °C. After 8 h, UDP-Glc (60 mg, 0.1 mmol) was added (pH adjusted) and more enzymes, *i.e.*, galactosyltransferase (0.4 U) and UDP-Glc 4′-epimerase (1.8 U), were added. The reaction was monitored by TLC, alkaloid spots being detected by ergot alkaloid-specific Ehrlich reagent.[2,3] Depletion of UDP-Glc was followed by ion-exchange TLC on PEI cellulose (Merck) according to the described procedure.[15] The reaction was stopped after 23 h by quick freezing and lyophilisation. Flash chromatography on silica gel (6–35 μm) (SDS, France) with a mixture $CH_2Cl_2$–MeOH–EtOH–water–$NH_3$ (7:3.5:1:0.8:0.05) afforded compound **3** (65 mg, 65% isolated yield) and side product **4** (19 mg, ~20%).

*Preparation of* β-D-*Glucopyranosyl*-(1→4)-2-*acetamido*-2-*deoxy*-β-D-*glucopyranosyl*-(1→O)-*elymoclavine* **4**.—A solution of compound **2a** (9 mg, 0.02 mmol) and uridine 5′-diphosphoglucose (24 mg, 0.04 mmol) in 50 mmol $dm^{-3}$ sodium cacodylate buffer, pH 7.5 (~150 $mm^3$) containing 5 mmol $dm^{-3}$ $MnCl_2$ was adjusted to pH 7.5 by 1 mol $dm^{-3}$ NaOH and the solution was made up to 200 $mm^3$ by the buffer. Galactosyltransferase (0.2 U) was added and the mixture was incubated at 37 °C. After 24 h, when ~30% of the acceptor had been converted into compound **4**, another aliquot of transferase (0.06 U), UDP-Glc (6 mg) and phosphatase (20 U) were added. The same addition, except phosphatase, was made after 67 h of reaction. The pH of the mixture was continually adjusted to 7.5. The reaction was complete after 100 h. Flash chromatography afforded compound **4** (7.3 mg, 57%).

*Spectral Characterisation of Compounds* **3** *and* **4**.—Compound **3**, $\delta_H$ 2.000 (3 H, s, Ac), 2.558 (3 H, s, NMe), 2.624 (1 H, m, 5-H), 2.784 (1 H, ddd, *J* 13.8, 11.8, and 1.7, 4-$H^a$), 3.022 (1 H, m, 7-$H^e$), 3.368 (1 H, dd, *J* 13.8 and 4.4, 4-$H^e$), 3.406 (1 H, ddd, *J* 9.9, 5.5, and 2.3, 5′-H), 3.494 (1 H, dd, *J* 9.7 and 3.2, 3″-H), 3.540 (1 H, dd, *J* 9.7 and 7.4, 2″-H), 3.544 (1 H, dd, *J* 10.3 and 9.9, 4′-H), 3.558 (1 H, m, 7-$H^a$), 3.595 (1 H, ddd, *J* 7.8, 4.6 and 1.0, 5″-H), 3.625 (1 H, dd, *J* 10.3 and 8.5, 3′-H), 3.697 (1 H, dd, *J* 11.5 and 4.6, 6″-$H^u$), 3.774 (1 H, dd, *J* 11.5 and 7.8, 6″-$H^d$), 3.794 (1 H, dd, *J* 8.5 and 8.5, 2′-H), 3.798 (1 H, m, 10-H), 3.814 (1 H, dd, *J* 12.1 and 5.5, 6′-$H^u$), 3.823 (1 H, dd, *J* 3.2 and 1.0, 4″-H), 4.005 (1 H, dd, *J* 12.1 and 2.3, 6′-$H^d$), 4.222 (1 H, dm, *J* 12.0, 17-$H^u$), 4.304 (1 H, dm, *J* 12.0, 17-$H^d$), 4.353 (1 H, d, *J* 7.4, 1″-H), 4.458 (1 H, d, *J* 8.5, 1′-H), 6.529 (1 H, m, 9-H), 6.940 (1 H, ddd, *J* 7.1, 1.4 and 0.7, 12-H), 6.944 (1 H, d, *J* 1.7, 2-H), 7.082 (1 H, dd, *J* 8.2 and 7.1, 13-H) and 7.163 (1 H, ddd, *J* 8.2, 0.8 and 0.7, 14-H); $\delta_C$ 23.40 (q, Ac), 27.61 (t, C-4), 41.28 (q, NMe), 42.07 (d, C-10), 56.95 (d, C-2′), 58.15 (t, C-7), 62.80 (2 C, t, C-6′ and 6″), 66.09 (d, C-5), 70.61 (d, C-4″), 72.87 (d, C-2″), 73.20 (t, C-17), 74.57 (d, C-3′), 75.14 (d, C-3″), 77.23 (d, C-5′), 77.45 (d, C-5″), 81.73 (d, C-4′), 101.98 (d, C-1′), 105.50 (d, C-1″), 110.33 (d, C-14), 111.85 (s, C-3), 113.31 (d, C-12), 119.97 (d, C-2), 123.70 (d, C-13), 124.94 (d, C-9), 127.80 (s, C-16), 132.11 (s, C-8), 134.20 (s, C-11), 135.61 (s, C-15) and 173.73 (s, C=O).

Compound **4**, $\delta_H$ 1.998 (3 H, s, Ac), 2.649 (3 H, s, NMe), 2.780

(1 H, m, 5-H), 2.839 (1 H, ddd, *J* 14.4, 11.4, and 1.8, 4-H$^a$), 3.172 (1 H, dm, 7-H$^e$), 3.222 (1 H, dd, *J* 9.0 and 7.9, 2″-H), 3.288 (1 H, dd, *J* 9.6 and 8.7, 4″-H), 3.354 (1 H, m, 5″-H), 3.381 (1 H, dd, *J* 9.0 and 8.7, 3″-H), 3.405 (1 H, dd, *J* 14.4 and 4.8, 4-H$^e$), 3.408 (1 H, m, 5′-H), 3.533 (1 H, dd, *J* 9.6 and 8.5, 4′-H), 3.629 (1 H, dd, *J* 10.3 and 8.5, 3′-H), 3.638 (1 H, dm, 7-H$^a$), 3.649 (1 H, dd, *J* 11.9 and 5.8, 6″-H$^u$), 3.776 (1 H, dd, *J* 8.4 and 10.3, 2′-H), 3.823 (1 H, dd, *J* 12.1 and 5.6, 6′-H$^u$), 3.864 (1 H, m, 10-H), 3.890 (1 H, dd, *J* 11.9 and 2.2, 6″-H$^d$), 4.020 (1 H, dd, *J* 12.1 and 2.2, 6′-H$^d$), 4.238 (1 H, dm, *J* 12.3, 17-H$^u$), 4.321 (1 H, dm, *J* 12.3, 17-H$^d$), 4.401 (1 H, d, *J* 7.9, 1″-H), 4.480 (1 H, d, *J* 8.4, 1′-H), 6.567 (1 H, m, 9-H), 6.955 (1 H, ddd, *J* 7.1, 1.5, and 0.8, 12-H), 6.966 (1 H, d, *J* 1.8, 2-H), 7.092 (1 H, dd, *J* 8.2 and 7.1, 13-H) and 7.175 (1 H, ddd, *J* 8.2, 0.9, and 0.8, 14-H); $\delta_C$ 23.39 (q, Ac), 27.39 (t, C-4), 41.02 (l, NMe), 41.80 (d, C-10), 57.14 (d, C-2′), 57.92 (t, C-7), 62.79 (t, C-6″), 62.74 (t, C-6′), 66.08 (d, C-5), 71.73 (d, C-4″), 73.06 (t, C-17), 74.51 (d, C-3′), 75.29 (d, C-2″), 77.22 (d, C-3″), 78.24 (d, C-5′), 78.50 (d, C-5″), 81.82 (d, C-4′), 102.01 (d, C-1′), 105.01 (d, C-1″), 110.48 (d, C-14), 111.41 (s, C-3), 113.40 (d, C-12), 120.12 (d, C-2), 123.76 (d, C-13), 124.99 (d, C-9), 127.70 (s, C-16), 131.62 (s, C-8), 133.71 (s, C-11), 135.63 (s, C-15) and 173.79 (s, C=O).

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