

Synthesis and cytotoxicity evaluation of natural α -bisabolol β -D-fucopyranoside and analogues

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ABSTRACT

α -Bisabolol β -D-fucopyranoside, a cytotoxic naturally occurring compound, was efficiently synthesized along with five other α -bisabolol glycosides (β -D-glucoside, β -D-galactoside, α -D-mannoside, β -D-xyloside and α -L-rhamnoside). Glycosidation of α -bisabolol was performed using Schmidt's inverse procedure and provided excellent yields (83–95%). Cytotoxicity was evaluated against a broad panel of cancerous cell lines including human and rat glioma (U-87, U-251 and GL-261) since the anticancer activity of α -bisabolol was previously demonstrated against brain tumor cell lines. The addition of a sugar moiety markedly increased α -bisabolol cytotoxicity in most cases. Among the synthesized glycosides, α -bisabolol α -L-rhamnopyranoside exhibited the strongest cytotoxic activity with IC_{50} ranging from 40 to 64 μ M. According to ADME *in silico* predictions, this glycoside closely respects physicochemical parameters necessary to cross the blood–brain barrier passively.

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1. Introduction

α -(–)-Bisabolol (**1**) (Fig. 1), a nontoxic sesquiterpene alcohol, is widely used in fragrances and cosmetic preparations (Madhavan, 1999). This oily compound with a sweet floral odor is frequently found in essential oils of various plants such as chamomile (*Matricaria chamomilla*), which may contain up to 50% (Issac, 1979). α -Bisabolol (**1**) is well known for its anti-inflammatory properties (Thiele et al., 1969; Jakovlev et al., 1979) and for its ability to enhance transepidermal drug penetration (Kadir and Barry, 1991). Recently, Cavalieri et al. (2004) have shown that α -bisabolol (**1**) exhibits a cytotoxic apoptosis-inducing effect (Darra et al., 2008) against human glioblastoma and pancreatic carcinoma cell lines.

Like many natural sesquiterpene alcohols, α -bisabolol (**1**) exists in the plant kingdom as a glycosidically bound volatile compound (Stahl-Biskup et al., 1993). Indeed, α -bisabolol β -D-fucopyranoside (**5b**) (Fig. 1) was already isolated from the aerial parts of *Carthamus lanatus* (San Feliciano et al., 1982) and exhibited noticeable cytotoxic activity by the *Artemia salina* assay (Mikhova et al., 2004). α -Bisabolol (**1**) is a lipophilic compound and, consequently, it is poorly soluble in biological fluids. This physicochemical property limits its possible pharmacological applications. However, glycosidation of a molecule increases its hydrophilicity and thereby influences its physicochemical and pharmacokinetic properties (Křen and Martínková, 2001). Moreover, glycosidation can also improve

the permeability through biological barriers such as the blood–brain barrier (Poduslo and Curran, 1994; Egleton et al., 2001). Thus, we thought that it would be of interest to synthesize α -bisabolol glycosides and study their pharmacological potential especially as very few reports about glycosidation of volatile compounds are currently available.

In this work, we report the synthesis of *O*-glycoside derivatives of α -bisabolol (**1**). Cytotoxicity of pure compounds was evaluated *in vitro* against several tumor cell lines including human and rat glioma (U-87, U-251 and GL-261). Also, in order to estimate the potential of α -bisabolol glycosides to cross through the blood–brain barrier, we evaluated *in silico* some important pharmacokinetic parameters (log *P*, PSA and HBD) (Hitchcock and Pennington, 2006) using the ADME prediction program QikProp version 2.5 (Jorgensen and Duffy, 2002).

2. Results and discussion

2.1. Synthesis of α -bisabolol glycosides

Quite surprisingly, studies on the glycosidation of volatile compounds are scarce in the literature whereas many surveys have been published on the isolation of glycosidically bounded volatiles (Stahl-Biskup et al., 1993). Only a few reports about the synthesis of either monoterpenyl or phenylpropanoid glycosides by chemical methods (Mbaïrroua et al., 1994; Mastelić et al., 2004; Patov et al., 2006) or biotransformation (Vijayakumar and Divakar, 2007) are currently available and these approaches generally provide low

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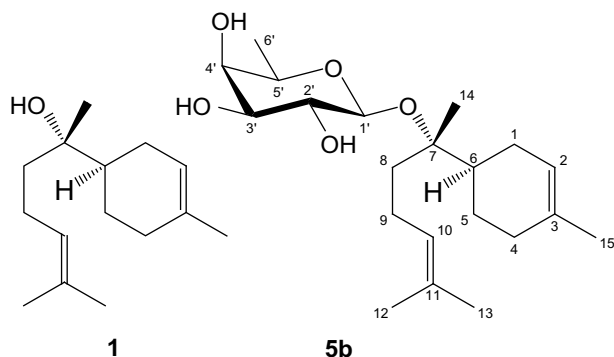
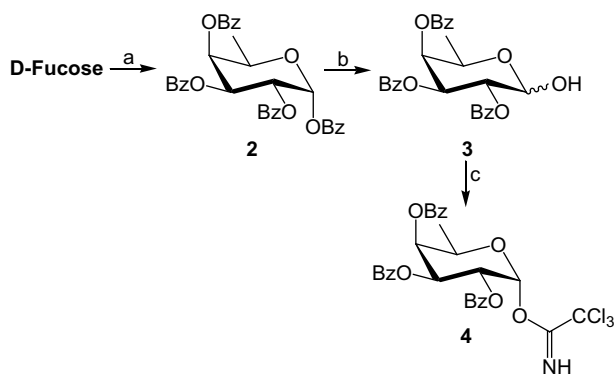


Fig. 1. Chemical structures of α -(-)-bisabolol (**1**) and α -bisabolol β -D-fucopyranoside (**5b**).

yields. For the synthesis of α -bisabolol glycosides, we chose to use the trichloroacetimidate (TCA) method of Schmidt (Schmidt and Kinzy, 1994). This method usually provides higher yields than the Koenigs–Knorr procedure and it has never been used previously for the glycosidation of volatile compounds.

TCA activated sugar donors were prepared from D-galactose (Rio et al., 1991), D-mannose (Ikeda et al., 1997), D-xylose (Schmidt and Junq, 2000), L-rhamnose (Gauthier et al., 2006), D-glucose (Gauthier et al., 2006) and D-fucose (Ross et al., 2001) following previously reported procedures. As a typical example (Scheme 1), D-fucose was first benzoylated using benzoyl chloride (BzCl) with 4-dimethylaminopyridine (DMAP) as the catalyst to afford the derivative **2** (94%). Selective deprotection of the anomeric position was achieved by bromination with HBr/HOAc followed by basic hydrolysis in the presence of silver carbonate (Ag_2CO_3) to provide **3** (85%, two steps). The TCA derivative **4** was then prepared in 71% yield according to Schmidt's procedure (Schmidt and Kinzy, 1994) using trichloroacetonitrile (CCl_3CN) and cesium carbonate (Cs_2CO_3) as catalyst (Urban et al., 1990).

The synthesis of α -bisabolol glycosides (**5b–10b**) was achieved according to the reaction sequence shown in Table 1. Lewis acid trimethylsilyl trifluoromethanesulfonate (TMSOTf) was used as the promoter of the reaction. Water is undesirable since it is a competitive acceptor, so the reaction was performed under rigorously anhydrous conditions. Glycosidation was first performed following the normal procedure (Deng et al., 1999) at room temperature in



Scheme 1. Synthesis of 2,3,4-tri-O-benzoyl- α -D-fucopyranosyl trichloroacetimidate (**4**). Reagents and conditions: (a) BzCl (6.0 equiv.), DMAP (0.1 equiv.), py, 0° to rt, overnight, 94%; (b) i-HBr/HOAc 33%, CH_2Cl_2 , rt, 2 h; ii- Ag_2CO_3 (1.35 equiv.), acetone/ H_2O 20:1, rt, 1 h, 85% (two steps); (c) CCl_3CN (6.0 equiv.); Cs_2CO_3 (0.2 equiv.); CH_2Cl_2 , rt, 4 h, 71%.

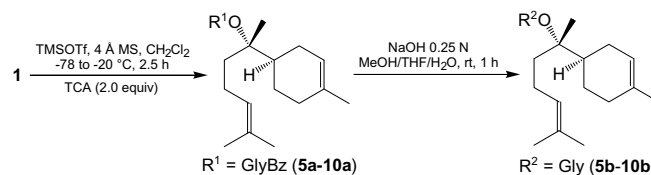
anhydrous CH_2Cl_2 using a catalytic amount of TMSOTf but dehydration of the tertiary alcohol was observed. Indeed, GC–MS analysis showed the formation of a complex mixture with α -bisabolene as a major compound (data not shown). To circumvent this problem, we performed the glycosidation reaction at -78°C using the inverse procedure (Schmidt and Toepfer, 1991) in which catalytic TMSOTf and α -bisabolol (**1**) are mixed before the dropwise TCA addition. Fully protected α -bisabolol glycosides (**5a–10a**) were obtained after 2.5 h at low temperatures (-78 to -20°C) in excellent yields (83–95%). Thereafter, deprotection of the benzoyl groups (NaOH, MeOH/THF/ H_2O 1:2:1) afforded α -bisabolol glycosides (**5b–10b**) in isolated yields ranging from 56% to 98%. The configuration of the glycosidic linkage of molecules **5b–10b** was determined by the chemical shifts and the vicinal coupling constants of the anomeric protons in ^1H NMR experiments (Agrawal, 1992). As expected, 1,2-*trans*-glycosides were exclusively obtained because of the presence of benzoyl protecting groups at the C-2' position of sugar TCA, which directed the anomeric selectivity of the glycosidation reaction (Flitsch, 2005). The structures of the sesquiterpene glycosides were confirmed by 1D and 2D NMR spectroscopic experiments (^1H , ^{13}C , DEPT-135, COSY, HSQC and HMBC) and HRMS. The physical and analytical data (^1H and ^{13}C NMR and $[\alpha]_D^{25}$) of α -bisabolol β -D-fucopyranoside (**5b**) were in agreement with those reported for the natural product isolated from *C. lanatus* (San Feliciano et al., 1982).

2.2. Cytotoxicity evaluation

α -Bisabolol (**1**) and synthesized α -bisabolol glycosides (**5b–10b**) were evaluated for their anticancer activity against a broad panel of tumor cell lines. The panel comprised human lung carcinoma (A549), colon adenocarcinoma (DLD-1), breast adenocarcinoma (MCF-7), melanoma (SK-MEL-2), ovary teratocarcinoma (PA-1), prostate adenocarcinoma (PC-3), pancreas adenocarcinoma (PANC 05.04), glioma (U-251), glioblastoma (U-87) and murine glioma (GL-261). Assessments were also carried out on normal human skin fibroblasts (WS1). Inhibition of growth cells was assessed by DNA quantification using Hoechst assay (Rago et al., 1990) and results are presented in Table 2. They are expressed as the concentration (μM) inhibiting 50% of the cell growth (IC_{50}). Etoposide and betulinic acid (BetA) were used as positive controls in this assay.

Recently, strong anticancer activity for α -bisabolol (**1**) against U-87 glioma cells (EC_{50} 2 μM) has been reported in the literature (Cavalieri et al., 2004). However, in our study, α -bisabolol (**1**) was found to be weakly cytotoxic against U-87 with an IC_{50} of 130 μM (29 $\mu\text{g}/\text{ml}$). This difference may be explained by the fact that Cavalieri et al. (2004) calculated the EC_{50} value estimating that only 2.5% of the initial amount of α -bisabolol (**1**) was dissolved in the culture medium used for biological assays.

On the whole, α -bisabolol glycosides exhibited stronger activity than the aglycone **1** except for α -bisabolol β -D-galactopyranoside (**9b**) ($\text{IC}_{50} > 100 \mu\text{M}$). As shown in Table 2, α -bisabolol α -L-rhamnopyranoside (**6b**) was the most potent glycoside: pronounced cytotoxic activity was observed for all tumor cell lines especially against A549 (IC_{50} 40 μM) and PA-1 (IC_{50} 40 μM). These results were significantly different to those obtained for **1** ($\text{IC}_{50} > 100 \mu\text{M}$, $P < 0.05$). With regard to brain tumor cell lines (U-251, U-87 and GL-261), cellular growth inhibitions were also observed for glycoside **6b** (IC_{50} 46–58 μM) with significant differences compared to the activity of α -bisabolol (**1**) ($P < 0.05$). Moreover, α -bisabolol α -D-mannopyranoside (**10b**) also provided moderate inhibitory effects particularly against MCF-7 (IC_{50} 45 μM) and GL-261 (IC_{50} 44 μM) cancer cell lines. Furthermore, the enhancement of α -bisabolol cytotoxicity seems not to be solely due to an increase in water solubility since more polar glycosides such as

Table 1Synthesis of α -bisabolol glycosides (**5b–10b**) by Schmidt's inverse procedure.

TCA ^a		R ¹	Yield ^b (%)		R ²	Yield ^b (%)	α/β ^c
	5a	Fuc-(OBz) ₃	88	5b	Fuc	98	0:1
	6a	Rha-(OBz) ₃	95	6b	Rha	95	1:0
	7a	Xyl-(OBz) ₃	95	7b	Xyl	87	0:1
	8a	Glc-(OBz) ₄	85	8b	Glc	95	0:1
	9a	Gal-(OBz) ₄	90	9b	Gal	89	0:1
	10a	Man-(OBz) ₄	83	10b	Man	56	1:0

Fuc, β -D-fucopyranose; Rha, α -L-rhamnopyranose; Xyl, β -D-xylopyranose; Glc, β -D-glucopyranose; Gal, β -D-galactopyranose; Man, α -D-mannopyranose; Gly, glycone.^a Trichloroacetimidate donors.^b Isolated yield.^c The configuration of the glycosidic linkage was determined by ¹H NMR analysis.

glucopyranoside **8b** and galactopyranoside **9b** (two hexoses) were significantly less active than the less polar rhamnopyranoside **6b** (a deoxy sugar). Thus, these preliminary cytotoxic results showed that adding a sugar moiety may improve the cytotoxic activity of α -bisabolol (**1**) depending on the nature of the glycone. Otherwise, as α -bisabolol (**1**) and sugar moieties are natural compounds devoid of toxicity (Madhavan, 1999), we may suppose that resulting glycosides should also be biocompatible. Further *in vivo* biological assays are needed to confirm this hypothesis.

2.3. *In silico* prediction of pharmacokinetic parameters

The major difficulty in the discovery of anti-glioma drugs is to find active compounds, which must be nontoxic, sufficiently soluble in blood to be bioavailable and sufficiently lipophilic to pass through the blood–brain barrier (BBB). Many promising anti-glioma drugs are discarded because they do not respect one of these conditions. Glycosidation is an interesting method to improve the water solubility of hydrophobic compounds (Křen and Martínková,

Table 2*In vitro* cytotoxicity of α -bisabolol glycosides (**5b–10b**) measured by Hoechst assay.

Compound	Panel cell lines/IC ₅₀ (μ M) ^{a,b}										
	A549	DLD-1	MCF-7	MEL-2	PA-1	PANC	PC-3	U-251	U-87	GL-261	WS1
1	80 $\pm$ 9	117 $\pm$ 7	106 $\pm$ 9	140 $\pm$ 12	93 $\pm$ 10	>150	>150	>150	130 $\pm$ 15	114 $\pm$ 6	>150
5b	61 $\pm$ 6	96 $\pm$ 8	75 $\pm$ 2	92 $\pm$ 8	64 $\pm$ 7	109 $\pm$ 6	98 $\pm$ 9	107 $\pm$ 6	84 $\pm$ 9	90 $\pm$ 7	87 $\pm$ 3
6b	40 $\pm$ 4	61 $\pm$ 4	45 $\pm$ 4	62 $\pm$ 4	40 $\pm$ 3	64 $\pm$ 5	58 $\pm$ 4	58 $\pm$ 4	46 $\pm$ 4	54 $\pm$ 3	64 $\pm$ 4
7b	63 $\pm$ 3	98 $\pm$ 8	61 $\pm$ 3	100 $\pm$ 2	52 $\pm$ 4	99 $\pm$ 6	89 $\pm$ 8	112 $\pm$ 5	83 $\pm$ 9	82 $\pm$ 6	93 $\pm$ 3
8b	94 $\pm$ 6	112 $\pm$ 5	88 $\pm$ 2	>150	81 $\pm$ 10	99 $\pm$ 4	128 $\pm$ 7	>150	96 $\pm$ 13	123 $\pm$ 9	109 $\pm$ 8
9b	111 $\pm$ 14	>150	>150	>150	118 $\pm$ 15	>150	>150	>150	>150	>150	>150
10b	53 $\pm$ 4	79 $\pm$ 7	45 $\pm$ 1	81 $\pm$ 5	49 $\pm$ 4	74 $\pm$ 3	72 $\pm$ 1	92 $\pm$ 7	68 $\pm$ 7	44 $\pm$ 1	88 $\pm$ 4
BetA ^c	10 $\pm$ 1	15 $\pm$ 1	41 $\pm$ 1	36 $\pm$ 5	23 $\pm$ 1	NT ^d	43 $\pm$ 1	33 $\pm$ 2	NT ^d	NT ^d	12 $\pm$ 1
Etoposide	<1.0	<1.0	<1.0	<1.0	<1.0	3 $\pm$ 2	<1.0	<1.0	<1.0	<1.0	<1.0

^a Data represent mean values $\pm$ standard deviations for two independent experiments made in triplicate.^b According to NCI criteria a pure compound is considered weakly active when IC₅₀ is between 100 and 50 μ M, moderately active when IC₅₀ is between 49 and 10 μ M, and strongly active when IC₅₀ is between 9 and 1 μ M (Boyd, 1997).^c Betulinic acid from Indofine Chemical Company Inc. was used as a positive control (Kessler et al., 2007).^d Not tested.

2001). However, it is less well known that the addition of a sugar moiety can also enhance the BBB permeability. This seems paradoxical but it has already been reported that glycosidation of neuropeptides can enhance their entry into the brain (Eggleston et al., 2001).

The *in silico* prediction of pharmacokinetic parameters is a useful tool, which allows estimating the ability of compounds to pass through the BBB based on its physicochemical properties. Hitchcock and Pennington (2006) have recently established a list of parameters involved in the BBB penetration and their optimal values based on the 25 top-selling central nervous system drugs in 2004. Suggested physicochemical property ranges for increasing the potential for BBB penetration are shown in Table 3. Important parameters are molecular mass (MW, g mol⁻¹), hydrogen bond donor (HBD), polar surface area (PSA, Å) and lipophilicity which is indicated by the calculated partition coefficient (clogP). The ADME prediction program QikProp version 2.5 (Jorgensen and Duffy, 2002) was used to evaluate these parameters *in silico*. As shown in Table 3, α -bisabolol (**1**) falls within the limits for passing through the BBB. However, the lipophilic character of **1** (clogP = 4.3) decrease its bioavailability and, consequently, may limit its chances to reach the BBB. If we compare the two most active glycosides against glioma cell lines (**6b** and **10b**), α -bisabolol α -L-rhamnopyranoside (**6b**) fits within the limits for passing through the BBB (clogP = 2.8; MW = 368.3 g/mol, PSA = 74.8 Å). In fact, for the glycoside **6b**, the HBD (=3) is the only parameter not satisfied since the suggested limit is less than 3. Furthermore, the additional alcohol at C-6 in the α -bisabolol glycosides derived from the three hexoses (**8b–10b**) apparently does not allow them to fulfill the permeability conditions (HBD >3; PSA >90 Å). Thus, this *in silico*

study suggests that the lipophilicity of α -bisabolol (**1**) has been decreased by the addition of a rhamnose moiety without dramatically altering its capacity to pass through biological barriers especially the BBB. It would be interesting to confirm this hypothesis with an *in vitro* model of the BBB which imitates the *in vivo* situation.

3. Conclusions

In summary, the synthesis of a series of α -bisabolol glycosides (**5b–10b**), including the natural α -bisabolol β -D-fucopyranoside (**5b**), was achieved. Glycosidation of the sesquiterpene alcohol α -bisabolol (**1**) was performed using Schmidt's inverse procedure and provided excellent yields. The biological screening showed that glycosidation may improve the cytotoxic activity of α -bisabolol (**1**) especially when adding α -L-rhamnopyranoside or α -D-mannopyranoside moieties. With regard to glioma cell lines, α -bisabolol α -L-rhamnopyranoside (**6b**) was much more active than α -bisabolol (**1**) itself, and according to *in silico* predictions, this glycoside closely fits with physicochemical parameters necessary to cross the BBB passively. Other functional groups could be attached to the α -bisabolol (**1**) structure. For example, some specific bioremovable moieties could be introduced with the aim of focusing on definite organs especially when brain delivery is targeted (Burger and Abraham, 2003). Such structural modifications of α -bisabolol (**1**) are currently in progress in our laboratory.

4. Experimental

4.1. General experimental procedures

Air and water sensitive reactions were performed in flame-dried glassware under Ar atmosphere. Moisture sensitive reagents were introduced via a dry syringe. CH₂Cl₂ was distilled from CaH₂ and THF was distilled from sodium with benzophenone ketyl. Flash column chromatography (FCC) was carried out using either 60–230 mesh silica gel or a high performance flash chromatography system (HPFC-Analogix F12-40) equipped with a silica gel column (F12-M, 8 g). Analytical thin layer chromatography (TLC) was performed with silica gel 60 F₂₅₄, 0.25 mm pre-coated TLC plates and visualized using UV₂₅₄ and cerium molybdate (2 g Ce(SO₄)₄(NH₄)₄, 5 g MoO₄(NH₄)₂, 200 ml H₂O, and 20 ml H₂SO₄) with charring. All of the chemical yields are not optimized and generally represent the result of the mean of two experiments. ¹H NMR spectra were recorded at 400 MHz and ¹³C NMR were recorded at 100 MHz on an Avance 400 Bruker spectrometer equipped with a 5 mm QNP probe. Elucidations of chemical structures were based on ¹H, ¹³C,

Table 3*In silico* prediction of α -bisabolol glycosides parameters involved in BBB penetration.

Compound	Sugar	MW (g/mol) ^a	HBD ^b	clogP ^c	PSA (Å) ^d
1	–	222.4	1	4.3	19.3
5b	Fuc	368.3	3	3.2	70.3
6b	Rha	368.3	3	2.8	74.8
7b	Xyl	354.5	3	2.7	74.6
8b	Glc	384.5	4	2.2	93.6
9b	Gal	384.5	4	2.1	93.4
10b	Man	384.5	4	2.1	96.6
Suggested limits ^e		<500	<3	2–5	<90

^a Molecular weight.^b Hydrogen bond donor.^c Calculated partition coefficient.^d Polar surface area.^e Hitchcock and Pennington (2006).

DEPT-135, COSY, HSQC and HMBC NMR experiments. Chemical shifts are reported in parts per million (ppm) relative to residual solvent peaks or trimethylsilane (TMS). Signals are reported as m (multiplet), s (singlet), d (doublet), dd (doublet of doublets), t (triplet), br s (broad singlet), br t (broad triplet), and coupling constants are reported in Hertz (Hz). The labile OH NMR signals appearing sometimes were not listed. Optical rotations were obtained using sodium D line at ambient temperature on a Rudolph Research Analytical Autopol IV automatic polarimeter. Melting points are uncorrected. High resolution electrospray ionization mass spectral data (HR-ESI-MS) were obtained at the department of Chemistry, Queen's University, Ontario, Canada.

4.2. Synthesis of 1,2,3,4-tetra-O-benzoyl- α -D-fucopyranose (**2**)

Benzoyl chloride (4 ml, 36.5 mmol) was slowly added to an ice-cold solution of D-fucose (1.0 g, 6.0 mmol) in anhydrous pyridine (13 ml) with DMAP (74 mg, 0.61 mmol) as catalyst. The reaction was stirred overnight at room temperature and then quenched with MeOH (15 ml). The mixture was diluted with CH₂Cl₂ and washed with cold 5 N H₂SO₄, saturated NaHCO₃ solution and brine. The solvents of the dried solution (MgSO₄) were evaporated under reduced pressure and the residue was purified by FCC (hexanes/EtOAc, 4:1–7:3) to give **2** as a white foam (3.33 g, 94%); *R*_f 0.50 (hexanes/EtOAc, 7:3); mp 73–75 °C; [α]_D²⁵ +239.8 (c 1.00, CH₂Cl₂); ¹H NMR spectroscopic data of **2** were in agreement with those published in the literature (Ross et al., 2001). ¹³C NMR (CDCl₃) δ : 16.3 (C-6), 67.7 (C-2), 68.0 (C-5), 69.9 (C-3), 71.5 (C-4), 90.9 (C-1), 128.8–133.9 (C-Ar), 164.8, 165.7, 165.9, 166.0 (4 $\times$ C=O); HR-ESI-MS *m/z* 603.1609 [M+Na]⁺ (calcd. for C₃₄H₂₈O₉Na, 603.1631).

4.3. Synthesis of 2,3,4-tri-O-benzoyl- α , β -D-fucopyranose (**3**)

HBr/HOAc (3 ml, 33%) was added under Ar to a solution of **2** (2.4 g, 4.2 mmol) in dry CH₂Cl₂ (13 ml). The reaction mixture was stirred at room temperature for 2.5 h, then the solution was washed with saturated NaHCO₃ solution and brine. The organic

Table 5

¹³C NMR spectroscopic data [100 MHz, δ (ppm)] for compounds **5b–10b**.

C no.	5b ^a	6b ^b	7b ^c	8b ^b	9b ^b	10b ^a
1	27.0	27.1	27.3	27.8	27.8	26.7
2	120.6	121.7	121.2	121.7	121.8	120.6
3	134.5	134.9	134.7	135.0	134.9	134.3
4	31.1	32.1	31.7	32.1	32.1	32.0
5	23.5	25.0	24.0	24.5	24.5	23.6
6	41.0	42.4	41.8	42.5	42.5	40.7
7	81.8	81.8	82.3	82.5	82.3	81.3
8	37.9	38.3	38.6	39.0	39.0	36.1
9	21.8	23.2	22.5	22.8	22.8	22.8
10	125.0	125.7	125.5	126.3	126.3	124.2
11	131.2	132.1	131.6	131.7	131.6	131.7
12	18.0	17.8	17.8	17.9	17.9	17.8
13	25.8	25.9	25.9	25.9	25.9	25.8
14	20.3	20.6	20.4	20.4	20.5	21.0
15	23.5	23.5	23.6	23.6	23.6	23.5
1'	97.1	95.6	98.4	98.6	99.1	94.0
2'	72.0	72.5	74.4	75.4	72.9	72.8
3'	74.4	74.1	77.3	78.4	75.3	72.0
4'	71.9	73.8	70.6	71.7	69.9	66.6
5'	70.4	70.1	66.0	77.4	76.0	72.5
6'	16.7	18.0		63.0	62.0	61.1

^a In CDCl₃.

^b In MeOD.

^c In CDCl₃/MeOD 1:1.

layer was dried (MgSO₄), filtered and the solvents were evaporated under reduced pressure. The residue was dissolved in acetone (22 ml), H₂O (0.9 ml) and CH₂Cl₂ (2 ml). Ag₂CO₃ (2.0 g, 7.3 mmol) was added in portions and the reaction was stirred for 1 h at room temperature, then the mixture was filtered through a bed of celite and anhydrous MgSO₄. The filtrate was concentrated under reduced pressure and the residue was purified by FCC (hexanes/EtOAc, 7:3–3:2) to give **3** (α : β = 5:1) as a white foam (1.74 g, 85%); *R*_f 0.22 and 0.35 (hexanes/EtOAc, 7:3); mp 75–77 °C; [α]_D²⁵ +220.7 (c 1.00, CH₂Cl₂); lit. (Ross et al., 2001) [α]_D²² +247.4 (c 1.00, CHCl₃); ¹H NMR spectroscopic data of **3** were in agreement with

Table 4

¹³C NMR spectroscopic data [100 MHz, δ (ppm)] for compounds **5a–10a** in CDCl₃.

C no.	5a	6a	7a	8a	9a	10a
1	26.7	26.8	26.5	26.6	26.6	26.9
2	120.2	120.7	120.4	120.2	120.3	120.5
3	134.4	134.3	134.2	134.2	134.3	134.5
4	30.8	31.3	30.9	30.8	30.8	31.2
5	22.8	23.9	23.1	23.1	23.0	23.8
6	40.5	40.9	40.7	40.1	40.8	40.9
7	82.3	82.6	82.4	82.7	82.7	82.9
8	38.3	37.4	38.2	38.3	38.4	36.4
9	21.7	22.4	22.4	21.7	21.8	22.2
10	124.9	124.5	124.7	124.6	124.7	124.2
11	131.3	131.7	131.5	131.4	131.4	132.0
12	18.0	17.9	17.8	17.8	17.9	17.8
13	25.9	25.5	25.9	25.7	25.8	25.8
14	19.9	20.4	20.1	19.7	19.7	21.1
15	23.5	23.5	23.5	23.3	23.4	23.5
1'	95.8	91.8	95.2	96.0	96.3	91.8
2'	70.1	72.4	71.4	72.3	70.1	72.4
3'	72.5	70.4	71.6	73.4	72.1	70.4
4'	71.4	72.2	70.0	70.2	68.5	67.3
5'	69.6	67.1	61.8	72.1	71.3	69.3
6'	16.6	17.8		63.8	62.6	63.5
C-Ar	128.4–130.2 133.1–133.5	128.4–130.0 133.2–133.5	128.4–130.0 133.2–133.5	128.4–129.9 133.1–133.5	128.4–130.2 133.2–133.6	128.4–130.1 133.1–133.5
C=O	165.4 165.9 166.3	165.8 165.9 166.0	165.2 165.7 165.8	165.1 165.4 166.0 166.2	165.3 165.7 165.8 166.2	165.7 165.7 165.8 166.4

those published in the literature (Ross et al., 2001). ^{13}C NMR (CDCl_3) (anomer α) δ : 16.3 (C-6), 65.0 (C-5), 68.4 (C-3), 69.5 (C-2), 72.0 (C-4), 91.0 (C-1), 128.4–133.8 (C-Ar), 165.8, 166.1, 166.2 ($3 \times \text{C=O}$); HR-ESI-MS m/z 477.1547 $[\text{M}+\text{H}]^+$ (calcd. for $\text{C}_{27}\text{H}_{25}\text{O}_8$, 477.1549).

4.4. Synthesis of 2,3,4-tri-*O*-benzoyl- α -*D*-fucopyranosyl trichloroacetimidate (**4**)

CCl_3CN (2 ml, 20.1 mmol) was added to a solution of **3** (1.6 g, 3.4 mmol) and Cs_2CO_3 (220 mg, 0.67 mmol) in CH_2Cl_2 (27 ml). The reaction mixture was stirred overnight at room temperature and then filtered off. The solvents of the filtrate were evaporated under reduced pressure and the residue was purified by FCC (hexanes/EtOAc, 7:3) to give **4** as a white foam (1.6 g, 71%); R_f 0.41 (hexanes/EtOAc, 7:3); mp 70–72 °C; $[\alpha]_D^{25} +183.2$ (c 1.00, CH_2Cl_2); lit. (Kitagawa et al., 1989) $[\alpha]_D^{24} +196.0$ (CHCl_3); ^1H and ^{13}C NMR spectroscopic data of **4** were in agreement with those published in the literature (Ross et al., 2001). HR-ESI-MS m/z 642.0465 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{29}\text{H}_{24}\text{NO}_8\text{NaCl}_3$, 642.0465).

4.5. General procedure for the synthesis of benzoylated α -bisabolol glycosides (**5a–10a**)

A solution of α -bisabolol (1 equiv., **1**) in dry CH_2Cl_2 (10 ml mmol^{-1}) was stirred for 15 min with 4 Å molecular sieves at –78 °C in an ice CO_2 /acetone bath. TMSOTf (0.1 equiv.) was added under Ar while keeping rigorous anhydrous conditions. Then, a solution of imidate (2 equiv.) in dry CH_2Cl_2 (3 ml mmol^{-1}) cooled at 0 °C was added dropwise over 5 min with continuous stirring. The reaction was allowed to warm to –20 °C over 2.5 h. When no α -bisabolol (**1**) could be detected by TLC, the reaction was quenched by addition of Et_3N (4 equiv.). The solvents were evaporated under reduced pressure and the resulting residue was purified by FCC.

4.6. α -Bisabolol 2,3,4-tri-*O*-benzoyl- β -*D*-fucopyranoside (**5a**)

This compound was prepared from α -bisabolol (55 μl , 0.23 mmol, **1**) and 2,3,4-tri-*O*-benzoyl- α -*D*-fucopyranosyl trichloroacetimidate (Kitagawa et al., 1989) (285 mg, 0.460 mmol, **4**). Purification by FCC (hexanes/EtOAc 19:1–9:1) afforded **5a** as a white foam (138 mg, 88%); R_f 0.59 (hexanes/EtOAc 7:3); $[\alpha]_D^{25} +95.0$ (c 1.00, CH_2Cl_2); mp 147–149 °C; ^1H NMR (CDCl_3) δ : 0.95 (m, 1H, H-5 α), 1.06 (s, 3H, H-14), 1.30 (d, 3H, $J = 6.2$ Hz, H'-6), 1.46 (m, 1H, H-8 α), 1.55 (s, 3H, H-15), 1.59 (m, 1H, H-8 β), 1.60 (s, 3H, H-12), 1.64 (m, 1H, H-4 α), 1.67 (s, 3H, H-13), 1.73 (m, 1H, H-6), 1.76 (m, 1H, H-5 β), 1.83 (m, 1H, H-4 β), 1.85 (m, 1H, H-1 α), 1.97 (m, 1H, H-9 α), 2.27 (m, 1H, H-9 β), 4.03 (dd, 1H, $J = 12.9$ Hz, $J = 6.5$ Hz, H'-5), 4.92 (d, 1H, $J = 7.8$ Hz, H'-1), 5.06 (br t, 1H, $J = 6.8$ Hz, H-10), 5.18 (br s, 1H, H-2), 5.56 (dd, 1H, $J = 10.5$ Hz, $J = 3.5$ Hz, H'-3), 5.69 (d, 1H, $J = 3.3$ Hz, H'-4), 5.78 (dd, 1H, $J = 10.5$ Hz, $J = 7.8$ Hz, H'-2), 7.19–7.27 (m, 2H, H-Ar), 7.31–7.44 (m, 3H, H-Ar), 7.45–7.53 (m, 3H, H-Ar), 7.56–7.65 (m, 1H, H-Ar), 7.73–7.81 (m, 2H, H-Ar), 7.88–8.00 (m, 2H, H-Ar), 8.09–8.20 (m, 2H, H-Ar); ^{13}C NMR (CDCl_3) see Table 4; HR-ESI-MS m/z 703.3281 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{42}\text{H}_{48}\text{O}_8\text{Na}$, 703.3246).

4.7. α -Bisabolol 2,3,4-tri-*O*-benzoyl- α -*L*-rhamnopyranoside (**6a**)

This compound was prepared from α -bisabolol (55 μl , 0.23 mmol, **1**) and 2,3,4-tri-*O*-benzoyl- α -*L*-rhamnopyranosyl trichloroacetimidate (Gauthier et al., 2006) (284 mg, 0.460 mmol). Purification by FCC (hexanes/EtOAc 9:1) afforded **6a** as a white foam (150 mg, 95%); R_f 0.44 (hexanes/EtOAc 4:1); $[\alpha]_D^{25} +89.3$ (c 1.00, CH_2Cl_2); mp 44–46 °C; ^1H NMR (CDCl_3) δ : 1.27 (s, 3H, H-14), 1.32 (d, 3H, $J = 6.2$ Hz, H'-6), 1.43 (m, 1H, H-5 α), 1.59 (m, 1H,

H-8 α), 1.62 (m, 1H, H-8 β), 1.65 (s, 3H, H-12), 1.69 (s, 6H, H-13, H-15), 1.84 (m, 1H, H-6), 1.94 (m, 1H, H-1 α), 1.98 (m, 1H, H-5 β), 2.01 (m, 2H, H-1 β , H-4 α), 2.06 (m, 1H, H-4 β), 2.08 (m, 1H, H-9 α), 2.10 (m, 1H, H-9 β), 4.36 (m, 1H, H'-5), 5.13 (br t, 1H, $J = 7$ Hz, H-10), 5.32 (br s, 1H, H'-1), 5.43 (br s, 1H, H-2), 5.48 (m, 1H, H'-2), 5.69 (t, 1H, $J = 9.7$ Hz, H'-4), 5.86 (dd, 1H, $J = 10.2$ Hz, $J = 3.2$ Hz, H'-3), 7.23–7.31 (m, 2H, H-Ar), 7.35–7.45 (m, 3H, H-Ar), 7.46–7.55 (m, 3H, H-Ar), 7.57–7.63 (m, 1H, H-Ar), 7.83–7.87 (m, 2H, H-Ar), 7.98–8.02 (m, 2H, H-Ar), 8.09–8.14 (m, 2H, H-Ar); ^{13}C NMR (CDCl_3) see Table 4; HR-ESI-MS m/z 703.3274 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{42}\text{H}_{48}\text{O}_8\text{Na}$, 703.3246).

4.8. α -Bisabolol 2,3,4-tri-*O*-benzoyl- β -*D*-xylopyranoside (**7a**)

This compound was prepared from α -bisabolol (55 μl , 0.23 mmol, **1**) and 2,3,4-tri-*O*-benzoyl- α -*D*-xylopyranosyl trichloroacetimidate (Schmidt and Junq, 2000) (278 mg, 0.460 mmol). Purification by FCC (hexanes/EtOAc 9:1) afforded **7a** as a white foam (145 mg, 95%); R_f 0.69 (hexanes/EtOAc 7:3); $[\alpha]_D^{25} -43.6$ (c 1.00, CH_2Cl_2); ^1H NMR (CDCl_3) δ : 0.97 (m, 1H, H-5 α), 1.10 (s, 3H, H-14), 1.51 (m, 1H, H-8 α), 1.56 (s, 3H, H-15), 1.59 (s, 3H, H-12), 1.61 (m, 1H, H-8 β), 1.67 (s, 3H, H-13), 1.68 (m, 1H, H-6), 1.69 (m, 1H, H-1 α), 1.72 (m, 1H, H-4 α), 1.74 (m, 1H, H-5 β), 1.80 (m, 1H, H-4 β), 1.83 (m, 1H, H-1 β), 1.94 (m, 1H, H-9 α), 2.16 (m, 1H, H-9 β), 3.63 (dd, 1H, $J = 11.9$ Hz, $J = 8.1$ Hz, H'-5 α), 4.43 (dd, 1H, $J = 11.9$ Hz, $J = 4.6$ Hz, H'-5 β), 5.00 (d, 1H, $J = 6.2$ Hz, H'-1), 5.10 (br t, 1H, $J = 7.1$ Hz, H-10), 5.18 (br s, 1H, H-2), 5.32 (m, 1H, H'-4), 5.42 (dd, 1H, $J = 8.1$ Hz, $J = 6.2$ Hz, H'-2), 5.79 (t, 1H, $J = 8.1$ Hz, H'-3), 7.29–7.43 (m, 6H, H-Ar), 7.44–7.59 (m, 3H, H-Ar), 7.88–8.04 (m, 6H, H-Ar); ^{13}C NMR (CDCl_3) see Table 4; HR-ESI-MS m/z 689.3083 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{41}\text{H}_{46}\text{O}_8\text{Na}$, 689.3090).

4.9. α -Bisabolol 2,3,4,6-tetra-*O*-benzoyl- β -*D*-glucopyranoside (**8a**)

This compound was prepared from α -bisabolol (55 μl , 0.23 mmol, **1**) and 2,3,4,6-tetra-*O*-benzoyl- α -*D*-glucopyranosyl trichloroacetimidate (Gauthier et al., 2006) (340 mg, 0.460 mmol). Purification by FCC (hexanes/EtOAc 9:1–4:1) afforded **8a** as a white foam (143 mg, 85%); R_f 0.26 (hexanes/EtOAc 4:1); $[\alpha]_D^{25} -11.5$ (c 1.00, CH_2Cl_2); mp 139–141 °C; ^1H NMR (CDCl_3) δ : 1.00 (m, 1H, H-5 α), 1.10 (s, 3H, H-14), 1.47 (m, 1H, H-8 α), 1.50 (s, 3H, H-15), 1.53 (s, 3H, H-12), 1.56 (s, 3H, H-13), 1.62 (m, 1H, H-1 α), 1.63 (m, 1H, H-8 β), 1.64 (m, 1H, H-4 α), 1.68 (m, 1H, H-6), 1.69 (m, 1H, H-5 β), 1.76 (m, 1H, H-4 β), 1.80 (m, 1H, H-1 β), 1.94 (m, 1H, H-9 α), 2.14 (m, 1H, H-9 β), 4.15 (m, 1H, H'-5), 4.45 (dd, 1H, $J = 12.1$ Hz, $J = 5.9$ Hz, H'-6 α), 4.58 (dd, 1H, $J = 12.1$ Hz, $J = 3.0$ Hz, H'-6 β), 4.98 (br t, 1H, $J = 6.7$ Hz, H-10), 5.04 (d, 1H, $J = 7.8$ Hz, H'-1), 5.11 (br s, 1H, H-2), 5.58 (m, 1H, H'-2), 5.64 (m, 1H, H'-4), 5.93 (m, 1H, H'-3), 7.22–7.30 (m, 2H, H-Ar), 7.30–7.43 (m, 7H, H-Ar), 7.44–7.57 (m, 3H, H-Ar), 7.79–7.86 (m, 2H, H-Ar), 7.85–7.97 (m, 4H, H-Ar), 7.98–8.05 (m, 2H, H-Ar); ^{13}C NMR (CDCl_3) see Table 4; HR-ESI-MS m/z 823.3429 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{41}\text{H}_{46}\text{O}_8\text{Na}$, 823.3458).

4.10. α -Bisabolol 2,3,4,6-tetra-*O*-benzoyl- β -*D*-galactopyranoside (**9a**)

This compound was prepared from α -bisabolol (55 μl , 0.23 mmol, **1**) and 2,3,4,6-tetra-*O*-benzoyl- α -*D*-galactopyranosyl trichloroacetimidate (Rio et al., 1991) (340 mg, 0.460 mmol). Purification by FCC (hexanes/EtOAc 19:1–9:1) afforded **9a** as a white foam (147 mg, 90%); R_f 0.67 (hexanes/EtOAc 7:3); $[\alpha]_D^{25} +226.1$ (c 0.5, CH_2Cl_2); mp 57–59 °C; ^1H NMR (CDCl_3) δ : 1.00 (m, 1H, H-5 α), 1.10 (s, 3H, H-14), 1.49 (m, 1H, H-8 α), 1.52 (s, 3H, H-15), 1.59 (s, 6H, H-12, H-13), 1.56 (s, 3H, H-13), 1.62 (m, 1H, H-1 α), 1.65 (m, 1H, H-8 β), 1.67 (m, 1H, H-4 α), 1.71 (m, 1H, H-6), 1.73 (m, 1H, H-5 β), 1.78 (m, 1H, H-4 β), 1.80 (m, 1H, H-1 β), 1.98 (m,

1H, H-9 α), 2.22 (m, 1H, H-9 β), 4.29 (t, 1H, J = 6.5 Hz, H'-5), 4.41 (dd, 1H, J = 11.3 Hz, J = 6.2 Hz, H'-6 α), 4.57 (dd, 1H, J = 11.1 Hz, J = 6.8 Hz, H'-6 β), 5.01 (m, 2H, H'-1, H-10), 5.14 (br s, 1H, H-2), 5.62 (dd, 1H, J = 10.5 Hz, J = 3.5 Hz, H'-3), 5.83 (dd, 1H, J = 10.5 Hz, J = 7.8 Hz, H'-2), 5.98 (d, 1H, J = 2.9 Hz, H'-4), 7.20–7.29 (m, 2H, H-Ar), 7.33–7.46 (m, 5H, H-Ar), 7.46–7.65 (m, 5H, H-Ar), 7.75–7.83 (m, 2H, H-Ar), 7.91–7.98 (m, 2H, H-Ar), 7.99–8.05 (m, 2H, H-Ar), 8.06–8.14 (m, 2H, H-Ar); ^{13}C NMR (CDCl_3) see Table 4; HR-ESI-MS m/z 823.3441 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{41}\text{H}_{46}\text{O}_8\text{Na}$, 823.3458).

4.11. α -Bisabolol 2,3,4,6-tetra-*O*-benzoyl- α -D-mannopyranoside (**10a**)

This compound was prepared from α -bisabolol (55 μL , 0.23 mmol, **1**) and 2,3,4,6-tetra-*O*-benzoyl- α -D-mannopyranosyl trichloroacetimidate (Ikeda et al., 1997) (340 mg, 0.460 mmol). Purification by FCC (hexanes/EtOAc 19:1–9:1) afforded **10a** as a white foam (152 mg, 83%); R_f 0.66 (hexanes/EtOAc 7:3); $[\alpha]_D^{25}$ –61.6 (c 0.5, CH_2Cl_2); mp 56–58 °C; ^1H NMR (CDCl_3) δ : 1.27 (s, 3H, H-14), 1.45 (m, 1H, H-5 α), 1.60 (m, 1H, H-8 α), 1.62 (s, 3H, H-12), 1.67 (s, 3H, H-13), 1.68 (s, 3H, H-15), 1.69 (m, 1H, H-8 β), 1.87 (m, 1H, H-6), 1.93 (m, 1H, H-1 α), 2.02 (m, 1H, H-1 β), 2.03 (m, 1H, H-5 β), 2.04 (m, 1H, H-4 α), 2.07 (m, 2H, H-9), 2.13 (m, 1H, H-4 β), 4.48 (m, 1H, H'-6 α), 4.60 (m, 1H, H'-5), 4.62 (m, 1H, H'-6 β), 5.13 (br t, 1H, J = 7.0 Hz, H-10), 5.41 (m, 2H, H-2, H'-1), 5.53 (m, 1H, H'-2), 5.94 (dd, 1H, J = 10.2 Hz, J = 3.2 Hz, H'-3), 6.10 (t, 1H, J = 10.0 Hz, H'-4), 7.23–7.31 (m, 2H, H-Ar), 7.33–7.46 (m, 7H, H-Ar), 7.47–7.64 (m, 3H, H-Ar), 7.81–7.89 (m, 2H, H-Ar), 7.94–8.03 (m, 2H, H-Ar), 8.04–8.13 (m, 4H, H-Ar); ^{13}C NMR (CDCl_3) see Table 4; HR-ESI-MS m/z 823.3447 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{41}\text{H}_{46}\text{O}_8\text{Na}$, 823.3458).

4.12. General procedure for the deprotection of benzoylated α -bisabolol glycosides (**5b**–**10b**)

The benzoylated α -bisabolol glycoside (1 equiv.) was dissolved in a 0.25 N NaOH (20 equiv.) solution of MeOH/THF/ H_2O 1:2:1 (15 ml). The reaction mixture was stirred at room temperature for 1 h. The solution was neutralized to pH $\approx$ 7 with a suspension of amberlite IR-120 activated with 1 M H_2SO_4 . After filtration and evaporation of the solvents under reduced pressure, the residue was purified by FCC.

4.13. α -Bisabolol β -D-fucopyranoside (**5b**)

5a (120 mg, 0.176 mmol) was treated according to the corresponding general procedure. The residue was purified by HPFC (hexanes/EtOAc 7:3–3:7) to afford **5b** as an oil (64 mg, 98%); R_f 0.38 (EtOAc); $[\alpha]_D^{25}$ –18.7 (c 1.00, CHCl_3), lit. (San Feliciano et al., 1982) $[\alpha]_D$ –20.6 (c 0.97, CHCl_3); ^1H NMR (CDCl_3) δ : 1.13 (s, 3H, H-14), 1.25 (m, 1H, H-5 α), 1.27 (d, 3H, J = 6.4 Hz, H'-6), 1.46 (m, 1H, H-8 α), 1.57 (m, 1H, H-8 β), 1.59 (s, 3H, H-12), 1.63 (s, 3H, H-15), 1.67 (s, 3H, H-13), 1.75 (m, 1H, H-6), 1.76 (m, 1H, H-1 α), 1.92 (m, 2H, H-4 α , β), 1.94 (m, 1H, H-1 β), 1.96 (m, 1H, H-5 β), 1.98 (m, 1H, H-9 α), 2.15 (m, 1H, H-9 β), 3.56 (m, 1H, H'-5), 3.57 (m, 1H, H'-3), 3.62 (m, 1H, H'-2), 3.70 (br s, 1H, H'-4), 4.37 (d, J = 7.1 Hz, H'-1), 5.04 (br t, 1H, J = 6.2 Hz, H-10), 5.34 (br s, 1H, H-2); ^{13}C NMR (CDCl_3) see Table 5; HR-ESI-MS m/z 391.2452 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{21}\text{H}_{36}\text{O}_5\text{Na}$, 391.2460).

4.14. α -Bisabolol α -L-rhamnopyranoside (**6b**)

6a (97 mg, 0.14 mmol) was treated according to the corresponding general procedure. The residue was purified by FCC (hexanes/EtOAc 4:1 to EtOAc 100%) to afford **6b** as a colorless gum (49 mg, 95%); R_f 0.38 (EtOAc); $[\alpha]_D^{25}$ –58.6 (c 0.50, CHCl_3); ^1H

NMR (MeOD) δ : 1.16 (s, 3H, H-14), 1.21 (d, 3H, J = 6.4 Hz, H'-6), 1.30 (m, 1H, H-5 α), 1.54 (m, 2H, H-8), 1.61 (s, 3H, H-12), 1.63 (s, 3H, H-15), 1.67 (s, 3H, H-13), 1.71 (m, 1H, H-6), 1.86 (m, 1H, H-5 β), 1.88 (m, 2H, H-1), 1.96 (m, 2H, H-4), 2.01 (m, 1H, H-9), 3.36 (m, 1H, H'-3), 3.68 (m, 1H, H'-2), 3.70 (m, 1H, H'-4), 3.76 (m, 1H, H'-5), 4.95 (s, 1H, H'-1), 5.09 (br t, 1H, J = 7.1 Hz, H-10), 5.36 (br s, 1H, H-2); ^{13}C NMR (MeOD) see Table 5; HR-ESI-MS m/z 391.2441 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{21}\text{H}_{36}\text{O}_5\text{Na}$, 391.2460).

4.15. α -Bisabolol β -D-xylopyranoside (**7b**)

7a (135 mg, 0.203 mmol) was treated according to the corresponding general procedure. The residue was purified by FCC (hexanes/EtOAc 7:3–3:7) to afford **7b** as a white powder (62 mg, 87%); R_f 0.38 (EtOAc); $[\alpha]_D^{25}$ –50.8 (c 1.00, CHCl_3); ^1H NMR (MeOD/ CDCl_3 1:1) δ : 1.17 (s, 3H, H-14), 1.28 (m, 1H, H-5 α), 1.48 (m, 1H, H-8 α), 1.60 (m, 1H, H-8 β), 1.61 (s, 3H, H-12), 1.64 (s, 3H, H-15), 1.67 (s, 3H, H-13), 1.76 (m, 1H, H-6), 1.81 (m, 1H, H-1 α), 1.91 (m, 1H, H-4 α), 1.97 (m, 1H, H-9 α), 1.98 (m, 1H, H-1 β), 1.99 (m, 1H, H-4 β), 2.01 (m, 1H, H-5 β), 2.15 (m, 1H, H-9 β), 3.18 (m, 1H, H'-5 β), 3.23 (m, 1H, H'-2), 3.35 (m, 1H, H'-3), 3.54 (m, 1H, H'-4), 3.85 (dd, 1H, J = 11.4 Hz, J = 5.2 Hz, H'-5 α), 4.44 (d, 1H, J = 7.3 Hz, H'-1), 5.06 (br t, 1H, J = 7.1 Hz, H-10), 5.36 (br s, 1H, H-2); ^{13}C NMR (MeOD/ CDCl_3 1:1) see Table 5; HR-ESI-MS m/z 355.2498 $[\text{M}+\text{H}]^+$ (calcd. for $\text{C}_{20}\text{H}_{35}\text{O}_5$, 355.2484).

4.16. α -Bisabolol β -D-glucopyranoside (**8b**)

8a (203 mg, 0.254 mmol) was treated according to the corresponding general procedure. The residue was purified by FCC (hexanes/EtOAc 1:1 to EtOAc 100%) to afford **8b** as a white powder (80 mg, 82%); R_f 0.22 (EtOAc); $[\alpha]_D^{25}$ –26.0 (c 1.00, MeOH); ^1H NMR (MeOD) δ : 1.18 (s, 3H, H-14), 1.27 (m, 1H, H-5 α), 1.47 (m, 1H, H-8 α), 1.60 (s, 3H, H-12), 1.62 (s, 3H, H-15), 1.64 (m, 1H, H-8 β), 1.66 (s, 3H, H-13), 1.74 (m, 1H, H-6), 1.82 (m, 1H, H-1 α), 1.90 (m, 1H, H-4 α), 1.93 (m, 1H, H-4 β), 2.01 (m, 1H, H-1 β), 2.02 (m, 1H, H-9 α), 2.06 (m, 1H, H-5 β), 2.16 (m, 1H, H-9 β), 3.17 (m, 1H, H'-2), 3.22 (m, 1H, H'-5), 3.31 (m, 1H, H'-4), 3.35 (m, 1H, H'-3), 3.65 (dd, 1H, J = 11.6 Hz, J = 5.2 Hz, H'-6 α), 3.80 (dd, 1H, J = 11.7 Hz, J = 2.1 Hz, H'-6 β), 4.47 (d, 1H, J = 7.8 Hz, H'-1), 5.10 (br t, 1H, J = 6.3 Hz, H-10), 5.35 (br s, 1H, H-2); ^{13}C NMR (MeOD) see Table 5; HR-ESI-MS m/z 407.2392 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{21}\text{H}_{36}\text{O}_6\text{Na}$, 407.2409).

4.17. α -Bisabolol β -D-galactopyranoside (**9b**)

9a (47 mg, 0.059 mmol) was treated according to the corresponding general procedure. The residue was purified by FCC (hexanes/EtOAc 1:1 to EtOAc 100%) to afford **9b** as a colorless gum (20 mg, 89%); R_f 0.49 (CH_2Cl_2 /MeOH 9:1); $[\alpha]_D^{25}$ –8.8 (c 0.30, pyridine); ^1H NMR (MeOD) δ : 1.17 (s, 3H, H-14), 1.27 (m, 1H, H-5 α), 1.48 (m, 1H, H-8 α), 1.60 (s, 3H, H-12), 1.62 (s, 3H, H-15), 1.63 (m, 1H, H-8 β), 1.66 (s, 3H, H-13), 1.74 (m, 1H, H-6), 1.83 (m, 1H, H-1 α), 1.96 (m, 2H, H-4), 2.00 (m, 1H, H-1 β), 2.01 (m, 1H, H-9 α), 2.06 (m, 1H, H-5 β), 2.16 (m, 1H, H-9 β), 3.45 (m, 1H, H'-5), 3.47 (m, 1H, H'-3), 3.49 (m, 1H, H'-2), 3.64 (dd, 1H, J = 11.0 Hz, J = 6.0 Hz, H'-6 α), 3.72 (dd, 1H, J = 11.0 Hz, J = 6.8 Hz, H'-6 β), 3.86 (m, 1H, H'-4), 4.43 (d, 1H, J = 7.0 Hz, H'-1), 5.07 (br t, 1H, J = 6.8 Hz, H-10), 5.35 (br s, 1H, H-2); ^{13}C NMR (MeOD) see Table 5; HR-ESI-MS m/z 407.2391 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{21}\text{H}_{36}\text{O}_6\text{Na}$, 407.2409).

4.18. α -Bisabolol α -D-mannopyranoside (**10b**)

10a (135 mg, 0.169 mmol) was treated according to the corresponding general procedure. The residue was purified by HPFC (hexanes/EtOAc 2:3 to EtOAc 100%) to afford **10b** as a white

powder (36 mg, 56%); R_f 0.41 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1); $[\alpha]_D^{25} +8.5$ (c 0.50, MeOH); ^1H NMR (CDCl_3) δ : 1.12 (s, 3H, H-14), 1.27 (m, 1H, H-5 α), 1.45 (m, 1H, H-8 α), 1.57 (m, 1H, H-8 β), 1.58 (s, 3H, H-12), 1.63 (s, 3H, H-15), 1.66 (s, 3H, H-13), 1.70 (m, 1H, H-6), 1.78 (m, 1H, H-1 α), 1.85 (m, 1H, H-5 β), 1.88 (m, 2H, H-9), 1.90 (m, 1H, H-1 β), 1.93 (m, 2H, H-4), 3.70 (m, 1H, H'-6 α), 3.72 (m, 1H, H'-5), 3.81 (m, 1H, H'-2), 3.91 (m, 1H, H'-3), 3.98 (m, 1H, H'-6 β), 3.99 (d, 1H, $J = 10.0$ Hz, H'-4), 5.05 (br t, 1H, $J = 6.4$ Hz, H-10), 5.12 (s, 1H, H'-1), 5.34 (br s, 1H, H-2); ^{13}C NMR (CDCl_3) see Table 5; HR-ESI-MS m/z 407.2392 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{21}\text{H}_{36}\text{O}_6\text{Na}$, 407.2409).

4.19. Cell lines and culture conditions

All human (A549, DLD-1, U-251, U-87, PANC 04.05, PC-3, PA-1, MEL-2, MCF-7 and WS1) and rat (GL-261) cells lines were obtained from the American Type Culture Collection (ATCC). All cell lines were cultured in minimum essential medium containing Earle's salts and L-glutamine (Mediatech Cellgro, VA) to which were added 10% foetal bovine serum (Hyclone) and vitamins (1 $\times$) (Mediatech Cellgro, VA). Cells were kept at 37 °C in a humidified environment containing 5% CO_2 .

4.20. Cytotoxicity assay

Exponentially growing cells were plated in 96-well microplates (Costar, Corning Inc.) at the density of 5×10^3 cells per well in 100 μl of culture medium and were allowed to adhere for 16 h before treatment. Increasing concentrations of each compound in DMSO (Sigma–Aldrich) were then added (100 μl per well) and the cells were incubated for 48 h. The final concentration of DMSO in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Cytotoxicity was assessed with cellular DNA assay using Hoechst dye 33342 (Rago et al., 1990). Cell lysis was performed by adding a solution of SDS (0.01%) at -80 °C before the Hoechst assay was carried out. Measurements were performed on an automated 96-well fluoroskan Ascent F1™ plate reader (Lab-systems) using excitation and emission wavelengths of 365 and 460 nm. Fluorescence was proportional to the cellular metabolic activity in each well. Survival percentage was defined as the fluorescence in experimental wells compared to that in control wells after subtraction of blank values. Etoposide was used as positive control. Each experiment was carried out twice in triplicate. IC_{50} results were expressed as means $\pm$ standard deviation.

4.21. Statistical analysis

Data were subjected to analysis of variance (two-way ANOVA) followed by Student–Newman Keuls post-hoc test and were considered significantly different when $P < 0.05$. All computations were done using statistical software Sigma-Stat version 3.5.

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