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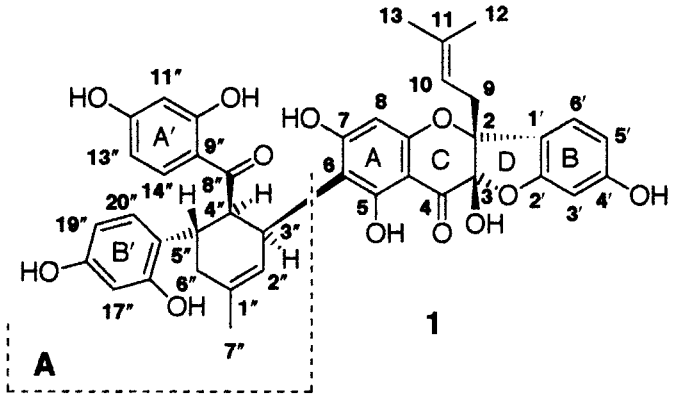
ABSOLUTE STRUCTURES OF 3-HYDROXY-2-PRENYLFLAVANONES WITH AN ETHER LINKAGE BETWEEN THE 2′- AND 3-POSITIONS FROM MORACEOUS PLANTS[1]

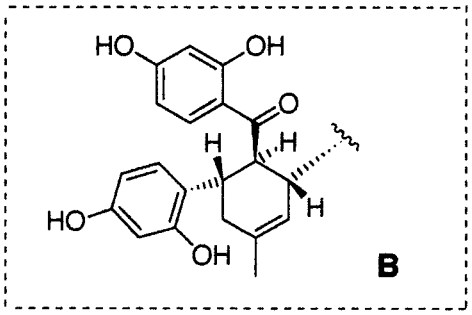
Ya-Qin Shi, Toshio Fukai, Masaru Ochiai, and Taro Nomura*

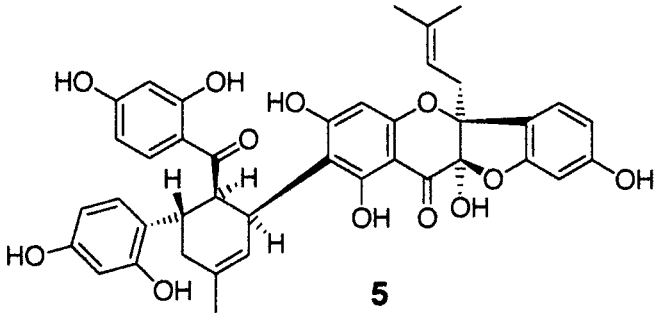
Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan, E-mail: nomura@phar.toho-u.ac.jp

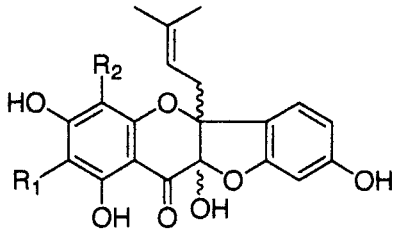
Abstract - Absolute configurations at C-2 and C-3 of eight sanggenon-type flavanones (3-hydroxy-2-prenylflavanones with an ether linkage between the C-2′ and C-3 positions, i.e., sanggenons A, C, L, and M, sanggenols F, and G, and soroceins D and F.) were determined as (2*R*,3*S*)-configurations by chemical and spectroscopic methods.

Many 3-hydroxy-2-prenylflavanones with an ether linkage between the 2′- and 3-positions have been isolated from moraceous plants, *e.g.*, *Morus cathayana*, *Sorocea bonplandii*, *S. ilicifolia*, and the traditional Chinese herbal medicine, "Sang-bai-pi".[2-16] Among these compounds, sanggenons C (**1**) and D (**2**) were isolated as hypotensive constituents of sang-bai-pi.[5, 6] In addition, sanggenon D (**2**) inhibited the specific ^{3}H-12-O-tetradecanoylphorbol-13-acetate binding to mouse skin particulate, the activation of protein kinase C with teleocidin, and the induction of ornithin decarboxylase activity by teleocidin in mouse skin.[14] These agents (**1** and **2**) are Diels-Alder type adducts derived from a chalcone (A′-ring–C-8″–C-4″–C-5″–B′-ring of **1**) and a sanggenon-type flavanone[17] with a 6-dehydroprenyl group by an enzymatic reaction. Recently, we reported that these sanggenon-type flavanones showed higher cytotoxic action against human oral tumor cell lines (HSC-2 and HSG) than against normal human gingival fibroblast.[18] However, absolute structures of these sanggenon-type flavanones have not yet been determined. We report here the elucidation of the absolute configurations at C-2 and C-3 of eight of these 2-prenyl-3-hydroxyflavanones. Previously, Nomura *et al.* reported that the relationship between the 2-prenyl and 3-hydroxyl groups of sanggenon A (**3**) was a *cis*-configuration in relative.[7, 16] Alkaline treatment of the compound (**3**, $[\alpha]_D$ + 176°) produced an angular type flavanone, sanggenon M [(±)-**4**],

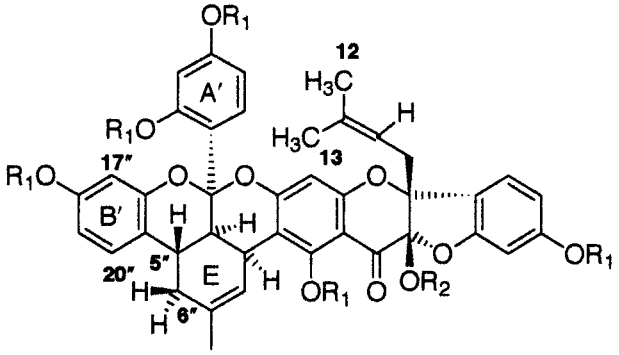




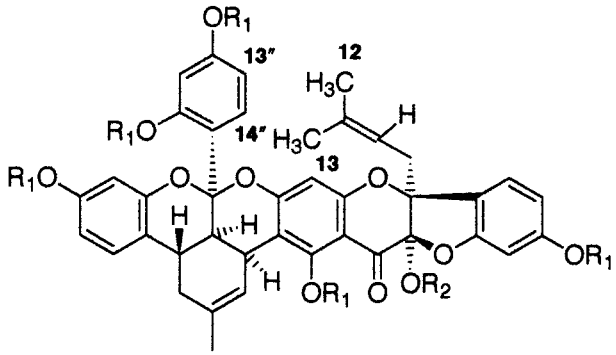




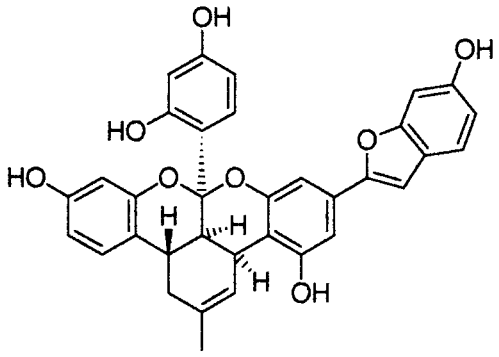
2 R_1 = **B**, R_2 = H
13 R_1 = H, R_2 = **A**



1a $R_1 = R_2$ = H
1b R_1 = Ac, R_2 = H
1c $R_1 = R_2$ = Ac



5a $R_1 = R_2$ = H
5b R_1 = Ac, R_2 = H
5c $R_1 = R_2$ = Ac



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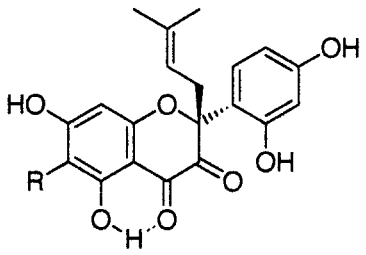
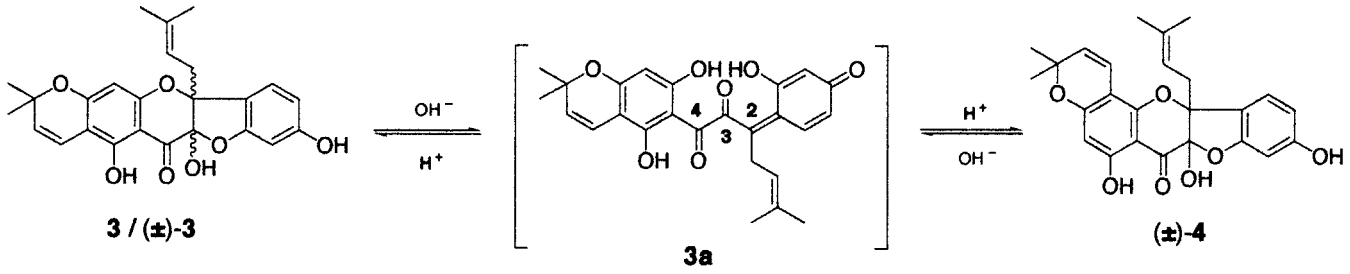


1e R = **A**
7 R = H

Figure 1. Structures of sanggenons C (**1**), D (**2**), O (**13**) , mulberrofuran G (**6**), and their derivatives.

as a racemate, and the starting material was also recovered as a racemic mixture [(±)-**3**, $[\alpha]_D \pm 0°$] as shown in Scheme 1.[9, 16]



Scheme 1. Mechanism of simultaneous epimerization of sanggenon A (**3**) at the C-2 and C-3 positions.

In the reaction, no diastereoisomer, (2*R*,3*R*)-**3**, (2*R*,3*R*)-**4**, (2*S*,3*S*)-**3**, or (2*S*,3*S*)-**4**, was obtained.[9, 16] Hano *et al.* reported that the absolute configurations at C-4″ and C-5″ of the methylcyclohexene ring of the Diels-Alder type adducts (**1** and **2**) were the same (4″*R*,5″*S*), but not at C-3″ (3″*S* for **1**, 3″*R* for **2**) when considering their CD spectra and sign of optical rotation.[19] However, the enantiomeric structures [(3″*R*,4″*S*,5″*R*)-**1** and (3″*S*,4″*S*,5″*R*)-**2**] for these compounds could not be ignored because the contributions of the additional asymmetric centers (C-2 and C-3) to their CD spectra and specific rotations are unknown.

Our strategy for determining the absolute configurations at C-2 and C-3 of the sanggenon-type flavanones was as follows: Sanggenon C (**1**) should be treated with the basic conditions that are similar to those of Scheme 1, to obtain the diastereomeric mixture of (2*R*,3*S*)-**1** and (2*S*,3*R*)-**1** or (2*R*,3*R*)-**1** and (2*S*,3*S*)-**1**. Flexible methylcyclohexene rings of the diastereoisomers would be anchored to the A ring by means of intramolecular ketalization.[11, 20] This would confirm the absolute configuration of the chiral centers of the methylcyclohexene moiety of these diastereomers by applying the property that the contribution of the flavanone moieties would disappear from the CD spectrum when the ratio of (2*R*,3*S*)-**1** and (2*S*,3*R*)-**1** is 1:1. Then, the relative configuration of the 2-prenyl group (or B ring) with the methylcyclohexene ring and B′ ring (or A′ ring) would be determined by NOE measurements of each isomer.

Sanggenon C (**1**) was dissolved in 0.5% sodium hydroxide solution and allowed to stand for 15 min at room temperature. The solution was then neutralized with 0.5% hydrochloric acid to yield a diastereoisomer (**5**, 14%)[21] and to recover **1** (55%). Under this condition, no constitutional isomer was obtained (HPLC and ^{1}H NMR analyses). The structure (**5**) was confirmed with COSY, NOESY [correlation between H-8 (δ 5.72, in acetone-d_6) and H_3-12 (δ 1.52)], and HMBC (correlation between 5-

OH and C-6) spectra, and resistance to an aluminum chloride-induced UV shift.[5, 22] The low yield of **5** can be explained with the slow rotation between the C-2 and C-3 bond of an intermediate of the reaction because of a steric hindrance of the methylcyclohexene ring, benzoyl group, or A′ ring. This rotation was presumably slower than that of sanggenon A (**3a** in Scheme 1).[23]

Cyclized compounds (**1a**)[11] and (**5a**), each *m/z* 691 $[M + H]^+$,[24] were derived by ketalization of **1** and **5**, respectively, under an acidic condition (in ethanol containing 1.5% H_2SO_4), as described previously.[11,19,20] These structures (**1a** and **5a**) were confirmed by the same spectroscopic methods as used for **5**. The relative configurations of the 2-prenyl group with the 3-hydroxy group in **1a** and **5a** were elucidated by comparisons of the ^{1}H NMR spectra of their pentaacetates (**1b** and **5b**) and hexaacetates (**1c** and **5c**),[24] respectively. The shifts of H_2-9 that were caused by acetylation of 3-OH (Table 1) indicated that the relationships between the 2-prenyl and 3-hydroxyl groups of both **1a** and **5a** were *cis*-configurations in relative as reported in **3**.[7, 16] The CD spectrum of 1:1 mixture of **1a** and **5a** [positive Cotton effects at 220, 237, 295 (sh), 309, 325 (infl.) and negative Cotton effects at 260 (sh), 276 nm] was similar to that of mulberrofuran G (**6**), positive Cotton effects at 220, 235, 290, 305 (sh), 334 nm, and a valley at 260 nm. The absolute structure of the compound (**6**) was determined as (3″*S*,4″*R*,5″*S*)-**6** by X-Ray crystallographic analysis of a bromide of its derivative.[19] Therefore, the absolute configurations at C-3″, C-4″, and C-5″ of **1**, **1a**, and **5a** were elucidated to (3″*S*,4″*R*,5″*S*)-configurations.

Table 1. ^{1}H NMR chemical shifts of H_2-9 of **1b**, **1c**, **5b**, and **5c** (in $CDCl_3$, 500 MHz)[a]

	1b	**1c**	Δδ (**1b** – **1c**)	**5b**	**5c**	Δδ (**5b** – **5c**)
H_A-9	3.06	2.82	+ 0.24	3.09	2.70	+ 0.39
H_B-9	2.76	2.88	– 0.12	2.77	2.78	– 0.01

[a] These signals were assigned with COSY spectra.

In the comparison of ^{1}H NMR spectra between **1a** and **5a** (Table 2), except for H-3″, protons of the methylcyclohexene ring (E ring) and B′-ring of **5a** appeared at the upper fields than those of **1a**. In contrast, the A′ ring protons of **1a** were observed at the upper field than those of **5a**. These upfield shifts, in comparison between **1a** and **5a** (in Table 2), were caused by the anisotropic effect of their B rings. This data indicates that the B ring of **5a** is located above the E and B′ rings, and the B ring of **1a** is above the A′ ring. Therefore, the absolute configurations at C-2 and C-3 of **1a** were determined as (2*R*,3*S*)-configurations, and those of **5a** as (2*S*,3*R*)-configurations.

Table 2. ^{1}H NMR data of **1a** and **5a** (in acetone-d_6, 400 MHz).

	1a	**5a**	Δδ (**1a** – **5a**)		**1a**	**5a**	Δδ (**1a** – **5a**)	
(E ring)				(B′ ring)				
H-2″	6.32	6.29	+ 0.03	H-17″	6.34	6.33	+ 0.01	
H-3″	3.28	3.29	– 0.01	H-19″	6.50	6.48	+ 0.02	
H-4″	3.39	3.37	+ 0.02	H-20″	7.13	7.08	+ 0.05	
H-5″	2.86	2.78	+ 0.08	(A' ring)				[Δδ (**5a** – **1a**)]
H-6″β	2.71	2.67	+ 0.04	H-11″	6.40	6.44	– 0.04	[+ 0.04]
H-6″α	2.01	1.98	+ 0.03	H-13″	6.19	6.28	– 0.09	[+ 0.09]
H_3-7″	1.76	1.73	+ 0.03	H-14″	6.89	7.07	– 0.08	[+ 0.08]

These configurations of **1a** and **5a** were further confirmed by their NOESY spectra (in acetone-d_6) as follows: The cross peaks with NOEs were observed between H-5″ of the E ring and H_3-13 (δ 1.45) of the prenyl group and between H-6″β (E ring) and H_3-12 (δ 1.56) (prenyl group) in the spectrum of **1a**. In addition, NOEs were observed between H-14″ (A′ ring) and H_3-13 (δ 1.49) (prenyl group), between H-14″ and H_3-12 (δ 1.70), and between H-14″ and H_3-13 in the spectra of **5a**.

The differential CD spectrum between **1a** and a 1:1 mixture of **1a** and **5a** (i.e., the spectrum of **1a** minus that of the mixture is that of flavanone moiety of **1a**) showed positive Cotton effects at 222, 250, 306, and 347 nm and negative Cotton effects at 240 and 279 nm (Table 3). The differential spectrum corresponds to the CD spectrum of (2*R*,3*S*)-sanggenon-type flavanones. In contrast, the differential CD spectrum between **5a** and the mixture exhibited positive Cotton effects at 240 and 279 nm and negative Cotton effects at 221, 250, 306, and 344 nm [corresponding to (2*S*,3*R*)-sanggenon-type flavanones].

The determinations of the relative configurations between 2-prenyl and 3-hydroxy groups of sanggenon C (**1**) and the stereoisomer (**5**) failed because of the broad ^{1}H NMR spectra of their octaacetates caused by the slow changes in the conformation of the methylcyclohexene ring or slow rotation between the C-5″ and C-15″ (or the C-6–C-3″) bond. Nevertheless, considering the configurations at C-2 and C-3 of **1a** and **5a** which were derived from **1** and **5** with the weak acid condition, the absolute configurations at C-2 and C-3 of **1** are (2*R*,3*S*) or (2*R*,3*R*), and those of **5** are (2*S*,3*R*) or (2*S*,3*S*). In the condition, the ring-open reaction at the C ring (rupture between C-2 and O-1 bond or O-1 and C-8a bond) could not occur. If isomerization at C-3 of **1** occurred in the acidic condition, the intermediate was presumably a dicarbonyl compound (**1e**). From consideration of a molecular model (**7**) calculated with Mopac 97 (PM3 method) and molecular dynamics (MM2), production of hemiketal (**1**) from **1e** occurred with the attack of 2′-OH

at the α-site of C-3, preventing 2′-OH from approaching the β-site of C-3. Therefore, the absolute configurations of C-2 and C-3 of **1** and **5** were (2*R*,3*S*)- and (2*S*,3*R*)-configurations, respectively. These configurations could be confirmed with the differential CD spectrum between **1** (or **5**) and a 1:1 mixture of **1** and **5** as described in **1a** and **5a** (Table 3). The differential spectrum between **1** and the mixture (**1** – mix) resembled that between **1a** and the mixture (**1a** – mix) that corresponds to (2*R*,3*S*)-sanggenon-type flavanones. Conversely, the differential spectrum between **5** and the mixture (**5** – mix) resembled that between **5a** and the mixture (**5a** – mix) that corresponds to (2*S*,3*R*)-sanggenon-type flavanones.

Table 3. Data of differential CD spectra[a] and CD spectra of sanggenon-type flavanones with a pyran ring (**3** and **4**), with a pyran ring and a prenyl group (**8** and **11**) and with one or two prenyl (geranyl) groups (**9**, **10**, and **12**)

	nm (molecular ellipicity)					
(**1a** – mix)[b]	222 (+ 59,700)	240 (– 3,000)	250 (+ 23,300)	279 (– 15,200)	307 (+ 8,900)	347 (+ 9,500)
(**5a** – mix)[b]	221 (– 55,700)	240 (+ 9,200)	250 (– 21,100)	279 (+ 15,300)	306 (– 7,000)	344 (– 9,100)
(**1** – mix)[b]	230 (+ 24,100)[c]		249 (+ 11,200)[c]	276 (– 3,200)	290 (+ 4,700)	330 (+ 15,000) 360 (+ 13,000)[c]
(**5** – mix)[b]	235 (– 13,200)	242 (– 7,900)	249 (– 10,200)	275 (+ 7,600)	291 (– 8,400)	334 (– 8,400) 360 (– 4,400)[c]
3	244 (+ 11,600)[c]		265 (+ 23,000)	285 (– 28,100)	312 (+ 4,600)	336 (+ 2,700)[e]
4	227 (+ 3,300)	241 (+ 1,200)[d]	261 (+ 3,600)	285 (– 860)	304 (+ 1,800)	330 (+ 1,000)[c]
8	227 (+ 8,900) 234 (+ 5,700)	243 (+ 11,800)[c]	267 (+ 20,900)	287 (– 35,500)	315 (+ 4,500)	336 (+ 2,700)[e]
9	226 (+ 15,300)	255 (+ 3,900)[d]	264 (+ 4,200)	296 (– 4,00)	329 (+ 3,400)	
10	235 (– 14,700)[d]		254 (+ 4,200)	274 (– 8,100)	293 (+ 9,200)	335 (+ 6,300)
11	224 (+ 2,200)	239 (– 10,500)	252 (+ 4,300)	282 (– 13,600)	311 (+ 6,100)	339 (+ 8,600)
12	230 (0)	242 (– 12,000)	252 (+ 11,900)	276 (– 14,300)	295 (+ 17,600)	338 (+ 11,900)

[a] Differential spectrum calculated by subtracting of the CD spectrum of the 1:1 mixture (**1a** and **5a**, or **1** and **5**) from that of the pure compound (**1a**, **5a**, **1**, and **5**). [b] Measured in MeCN. The other compounds were measured in MeOH. [c] Observed as a shoulder peak. [d] Observed as a valley. [e] Inflated range.

The relative configurations of the 2-prenyl groups with the 3-hydroxyl group of sanggenons L (**8**) and M (**4**),[9] sanggenols F (**9**) and G (**10**),[2b] and soroceins D (**11**)[3] and F (**12**)[4] could be assigned as *cis*-configurations because of similarities of chemical shifts and coupling constants of H_2-9 (2-prenyl group) among these compounds and sanggenon A (**3**, δ 2.77, br dd, *J* = 6 and 15 Hz, 3.16, br dd, *J* = 9 and 15 Hz, 400 MHz, acetone-d_6). The CD spectra of these compounds (Table 3)[25] were similar to the differential CD spectrum between **1a** and the mixture of **1a** and **5a** [corresponding to (2*R*,3*S*)-sanggenon-type

flavanones]. Therefore, the absolute configurations at C-2 and C-3 of these compounds were established as (2*R*,3*S*)-configurations.

8 R_1 = prenyl, R_2 = H
11 R_1 = H, R_2 = prenyl

9 R_1 = prenyl, R_2 = H
10 R_1 = H, R_2 = geranyl
12 R_1 = R_2 = prenyl

Figure 2. Structures of sanggenons L (**8**), sanggenols F (**9**), G (**10**), soroceins D (**11**), and F (**12**).

A study of the absolute configurations at C-2 and C-3 of the other class of sanggenon-type flavanones with a methylcyclohexene ring [Diels-Alder type adducts, i.e., sanggenons B, D (**2**), E, O (**13**), P (sorocein H), and S, and sanggenol J][2b, 6, 10–14] is in progress.

ACKNOWLEGMENT

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21. Compound **5**: MALDI-TOF-MS *m/z* 709 $[M + H]^+$, $[\alpha]^{22}_D$ – 159° (*c* 0.1, MeCN), ^{13}C NMR (125 MHz, acetone-d_6) δ 18.0 (C-12), 23.7 (C-7″), 25.5 (C-13), 31.9 (C-9), 32.7 (C-3″), 33.6 (C-6″), 35.6 (C-5″), 48.0 (C-4″), 91.8 (C-2), 96.5 (C-8), 99.3 (C-3′), 99.9 (C-4a), 102.2 (C-3), 103.4 (C-11″), 103.6 (C-17″), 107.5 (C-19″), 108.6 (C-13″), 108.8 (C-6), 109.5 (C-5′), 113.9 (C-9″), 118.4 (C-10), 121.2 (C-1′), 122.0 (C-15″), 122.6 (C-2″), 125.4 (C-6′), 129.0 (C-20″), 134.5 (C-14″), 135.2 (C-1″), 136.7 (C-11), 156.2 (C-16″), 157.6 (C-18″), 161.0 (C-2′, C-4′), 161.7 (C-8a), 163.6 (C-5), 165.7 (C-12″), 166.7 (C-10″), 167.6 (C-7), 188.1 (C-4), 208.5 (C-8″).
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23. A similar solution that was allowed to stand for 45 min at room temperature yielded 27% of compound **5**. **5**, $[\alpha]^{25}_D$ – 24° (*c* 0.1, MeOH), recovered **1**, $[\alpha]^{25}_D$ + 310° (*c* 0.1, MeOH), the starting material (**1**), $[\alpha]^{25}_D$ + 303° (*c* 0.06, MeOH).
24. All the MS data were obtained with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). **1b**, *m/z* 901 $[M + H]^+$, 923 $[M + Na]^+$, **1c**, *m/z* 965 $[M + Na]^+$, **5b**, *m/z* 923 $[M + Na]^+$, **5c**, *m/z* 965 $[M + Na]^+$.
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