Annonaceous Acetogenins from the Seeds of *Annona squamosa*. Non-adjacent Bis-tetrahydrofuranic Acetogenins

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Four non-adjacent bis-tetrahydrofuranic acetogenins, named squamostatins-B (2), -C (3), -D (4) and -E (5), have been isolated from the petroleum ether extract of *Annona squamosa* seeds. The structures of these acetogenins have been established on the basis of spectral evidence. C-15/C-16-*threo*, C-19/C-20-*threo*, C-23/C-24-*erythro* stereochemistry was assigned for squamostatins-B and -D, whereas C-15/C-16-*threo*, C-19/C-20-*threo*, C-23/C-24-*threo* stereochemistry was assigned for squamostatins-C and -E. All of these acetogenins, including squamostatin-A, have been established to have C-12/C-15-*trans*, C-20/C-23-*trans* stereochemistry by ^{13}C-NMR comparison with synthetic model mono-tetrahydrofuranic compounds. An improved ^{13}C-NMR assignment of squamostatin-A is presented.

Keywords annonaceous acetogenin; *Annona squamosa*; bis-tetrahydrofuran; squamostatin; Annonaceae

Annonaceous acetogenins have attracted much interest because of their wide range of biological activities and unique structures.[1] About 70 annonaceous acetogenins have been isolated so far from several genera of Annonaceae plants, which grow in tropical and subtropical climates. Their structures are characterized by one or two tetrahydrofuran (THF) rings, γ-lactone and unbranched aliphatic chain that are variously hydroxylated, acetylated, or ketonized. These acetogenins are classified into three groups, mono-tetrahydrofuran, adjacent bis-tetrahydrofuran and non-adjacent bis-tetrahydrofuran according to the number of THF rings and their connection pattern.[1]

In the preceding paper,[2] we described the isolation and structure elucidation of thirteen adjacent bis-tetrahydrofuranic acetogenins from the petroleum ether extract of the seeds of *Annona squamosa* L. (Annonaceae). In addition to these acetogenins, four non-adjacent bis-tetrahydrofuranic acetogenins were also isolated. In this paper we describe the structure elucidation of these four acetogenins, named squamostatins-B (**2**), -C (**3**), -D (**4**) and -E (**5**).[3] In connection with the stereochemical assignment of these compounds, stereochemically defined model mono-tetrahydrofurans **6**—**13** have been synthesized and a more accurate ^{13}C-NMR assignment of squamostatin-A (**1**) has been achieved.

Results and Discussion

In our earlier study, a waxy residue which precipitated from the petroleum ether extract of the ground seeds of *A. squamosa* afforded the major two acetogenins, squamocin[4] and squamostatin-A[5] (**1**). After the removal of the two acetogenins, the residual mixture was further separated by reversed-phase octadecyl silica (ODS) HPLC, using MeOH–H_2O (8:1) as an eluent, affording squamostatin-B (**2**) and -C (**3**). On the other hand, processing of the supernatant of the petroleum ether extract gave the polar fraction.[2] ODS-HPLC separation of this fraction as described above furnished squamostatins-D (**4**) and -E (**5**). The mobilities of **2**—**5** in HPLC and TLC are summarized in Table I.

Nearly ten non-adjacent bis-tetrahydrofuranic acetogenins have been reported from Annonaceae plants.[5-10] However, *trans* stereochemistry of the two substituents of the two THF rings has been proposed without any apparent evidence.[11] We have now firmly established that **1** has C-12/C-15-*trans*, C-20/C-23-*trans* stereochemistry. Our assignment has been made in connection with a study to obtain a better ^{13}C-NMR assignment of squamostatin-A (**1**). We will deal with this first.

Stereochemically defined model mono-tetrahydrofuranic compounds have been synthesized for the ^{13}C-NMR study. These were *threo*/*trans*- (**6**), *threo*/*cis*- (**7**), *erythro*/*trans*- (**8**) and *erythro*/*cis*- (**9**) isomers of 2-heptyl-5-(1-hydroxyheptyl)tetrahydrofurans, and *threo*/*trans*/*threo*- (**10**), *threo*/*cis*/*threo*- (**11**), *erythro*/*trans*/*threo*- (**12**) and *erythro*/*cis*/*threo*- (**13**) isomers of 2,5-di-(1-hydroxyheptyl)tetrahydrofuran. The ^{13}C assignments of these mono-tetrahydrofurans are listed in Table II.

We previously reported that the molecule of **1** contains

TABLE I. HPLC Mobility (ODS Column) and TLC Behavior

Compound	Retention time		*Rf* value	
	A	B	C	D
Squamostatin-A (**1**)	7.8	14.0		0.19
2	9.4	16.8		0.25
3	10.8	21.5		0.24
Squamocin	11.6	25.5	0.44	0.33
4	13.6		0.60	
5	15.7		0.57	

A Conditions: solvent MeOH–H_2O (13:1), flow rate 0.6 ml/min. B Conditions: solvent MeOH–H_2O (10:1), flow rate 1.0 ml/min. C $CHCl_3$:AcOEt:MeOH = 10:5:1. D $CHCl_3$:AcOEt:MeOH = 10:4:1.

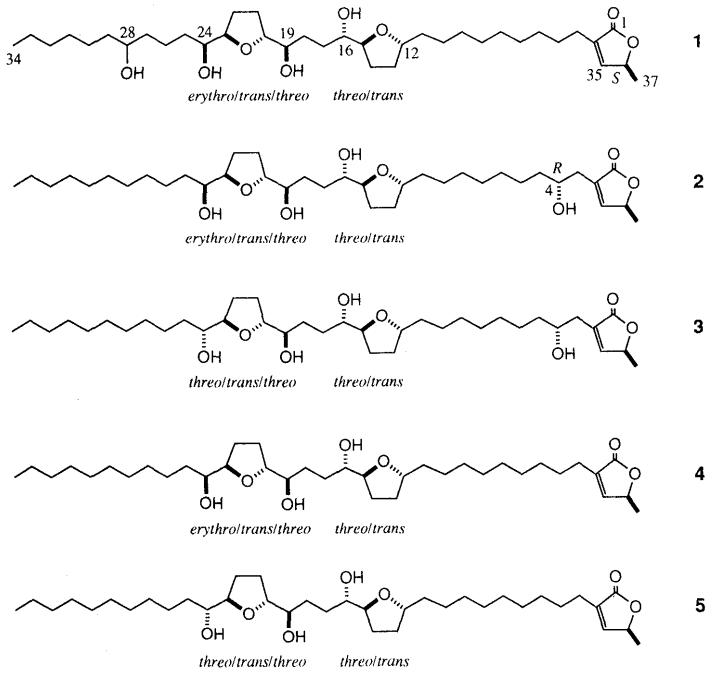

Chart 1. Structures of Non-adjacent Bis-tetrahydrofuranic Acetogenins (**1**—**5**)

The structures imply relative stereochemistry regarding to each THF ring, whereas C-4 and C-36 show absolute stereochemistry.

TABLE II. ^{13}C-NMR Data for Model Tetrahydrofuranic Compounds (**6**—**13**)[a] (125 MHz, $CDCl_3$)

Carbon	6	7	8	9	10	11	12	13
2	79.3	79.9	80.2	79.6	82.7	82.8	83.3	82.3
3	32.4	31.4	32.3	31.4	28.8	28.1	28.6	28.4
4	28.4	27.8	25.0	23.9	28.8	28.1	25.2	24.1
5	81.9	82.2	81.5	82.1	82.7	82.8	82.2	82.8
1′	35.7	36.1	36.1	35.8	74.0	74.3	74.3	74.2
2′	26.2	26.2	26.1	26.2	33.4	34.0	33.2	34.2
3′	29.7	29.7	29.7	29.7	25.5	25.6	25.5[f]	25.7[h]
4′	29.3[b]	29.3[c]	29.2[d]	29.2[e]	29.4	29.3	29.4[g]	29.3
1″	74.2	74.5	72.0	71.6	74.0	74.3	71.6	72.1
2″	33.4	34.0	32.6	32.6	33.4	34.0	32.5	33.1
3″	25.6	25.7	26.0	25.9	25.5	25.6	25.9[f]	25.9[h]
4″	29.4[b]	29.4[c]	29.4[d]	29.3[e]	29.4	29.3	29.3[g]	29.3

a) The chemical shifts of 5′-C (5″-C), 6′-C (6″-C) and 7′-C (7″-C) of **6**—**13** are δ 31.8, 22.6 and 14.1, respectively. *b*—*h*) Assignments may be interchanged within the column.

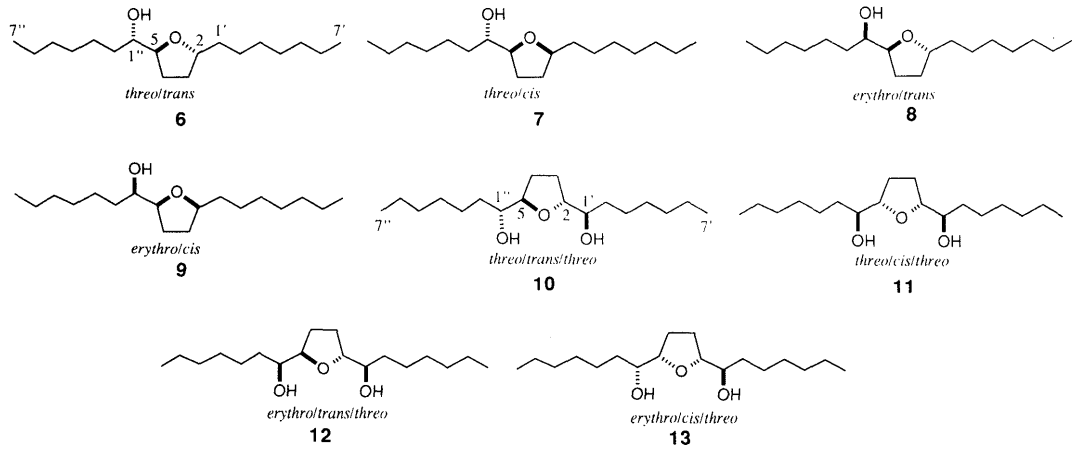

Chart 2. Structures of Model Tetrahydrofurans (**6**—**13**)

The structures imply relative stereochemistry. The numbering systems depicted here are used in the text.

three –CH(OH)–CH(OR)– units (C-15/C-16, C-19/C-20, and C-23/C-24)[5] and subsequently the stereochemistry of these three linkages was found to be two *threo* and one *erythro* by the application of Born's rule[12] (oxymethine proton and carbon alpha to the THF ring resonate at δ_H *ca.* 3.8 and δ_C *ca.* 71—72 for *erythro* compounds and at δ_H *ca.* 3.4 and δ_C *ca.* 74 for *threo* compounds). Evaluation of the ^{13}C assignment of **1** was started with the signals of A-THF ring,[13] more specifically C-11 to C-14. Each set (C-2, C-3, C-4, C-1′) of ^{13}C data for **6**—**9** was compared with the ^{13}C data for **1** (C-11 to C-14). The comparative study revealed that only the ^{13}C data for the *threo*/*trans*-isomer **6** are in good agreement with those for **1**, and consequently the signals at δ 35.6, 79.3, 32.4, 28.4 were assigned to C-11, C-12, C-13 and C-14, respectively. It is thus established that **1** has C-12/C-15-*trans*, C-15/C-16-*threo*-structure.

Since *threo*-structure was assigned to C-15/C-16, one of the C-19/C-20 and C-23/C-24 linkages must be *threo*- and the other must be *erythro*. We compared the ^{13}C-NMR data for the *erythro*/*trans*/*threo*- (**12**) or *erythro*/*cis*/*threo*- (**13**) isomers with those for **1** with respect to the chemical shifts of C-20 to C-24. The most diagnostic signal was found in one of the methylene signals of the THF rings (δ 25.2 *vs.* 24.1). *threo*/*trans*/*threo*- or *threo*/*cis*/*threo*-Structure for the B-THF ring was ruled out by the spectral comparison with those of **10** and **11**. C-20/C-23 *trans* stereochemistry was thus firmly established.

With these established configurations regarding the two THF rings of **1**, there are still two options in the assignment of *threo* and *erythro*-structures, *i.e.*, which one of C-19/C-20 and C-23/C-24 is *threo*. We favored C-19/C-20-*threo*, C-23/C-24-*erythro* stereochemistry on the basis of the similarity in the ^{13}C chemical shifts of C-25 (δ 32.5) and C-26 (δ 22.0) of **1** and squamocin (δ 32.5, 22.0, respectively). If the reverse (C-19/C-20-*erythro* and C-23/C-24-*threo*) is the case, these two chemical shifts might be altered. Another line of evidence supporting this assignment will be presented later in the text. Based on the ^{13}C-NMR data for **12**, the signals at δ 83.4, 28.6, 25.4, 82.2 and 71.6 of **1** were assigned to C-20, C-21, C-22, C-23 and C-24, respectively.

After the completion of the ^{13}C assignment described above, the remaining oxymethine signal at δ 82.0 was assigned to C-15. The C-16 and C-19 (δ 74.6 and 74.5) signals are still interchangeable. C-17 and C-18, previously erroneously assigned to δ 32.4 and 35.6, turned out to be buried in the methylene overlap region (*ca.* 29.5). We believe the present ^{13}C assignment (Table III) of squamostatin-A is the most reliable among a number of tentative assignments reported for non-adjacent type bis-tetrahydrofuranic acetogenins.

We will next describe the structure elucidation of squamostatins-B, -C, -D and -E, in the order of elution in ODS-HPLC.

Squamostatin-B (**2**) was isolated as white crystals, mp 98—101 °C, and the molecular formula was established as $C_{37}H_{66}O_8$ by HR-FAB-MS [(FAB-MS *m/z* 639 (MH^+)]. The spectral data (UV, IR and NMR) for **2** were characteristic of annonaceous acetogenins and indicated the presence of α,β-unsaturated-γ-lactone, two THF rings

TABLE III. ^{13}C-NMR Spectral Data for Non-adjacent Bis-tetrahydrofuranic Acetogenins (125 MHz, $CDCl_3$)

Carbon	1	2	3	4	5
1	173.9	174.6	174.6	173.9	173.9
2	134.3	131.2	131.2	134.3	134.3
3	25.2	33.4	33.4	25.2	25.1
4	27.4	70.0	70.0	27.4	27.4
5	a)	37.4	37.4	a)	a)
6	a)	25.5	25.5	a)	a)
7—9	a)	a)	a)	a)	a)
10	26.1	26.2	26.1	26.2	26.2
11	35.6	35.6	35.6	35.6	35.6
12	79.3	79.3	79.3	79.3	79.3
13	32.4	32.4	32.4	32.4	32.4
14	28.4	28.4	28.4	28.4	28.4
15	82.0	82.0	82.0	82.0	82.0
16	74.5b)	74.5c)	74.4d)	74.5e)	74.4f)
17	a)	a)	a)	a)	a)
18	a)	a)	a)	a)	a)
19	74.6b)	74.6c)	74.3d)	74.6e)	74.2f)
20	83.4	83.3	82.7	83.3	82.7
21	28.6	28.6	28.7	28.6	28.7
22	25.4	25.2	28.7	25.2	28.7
23	82.2	82.2	82.7	82.2	82.7
24	71.6	71.6	74.0	71.5	74.1
25	32.5	32.6	33.5	32.5	33.4
26	22.0	26.0	25.6	26.0	25.6
27	37.3	a)	a)	a)	a)
28	71.8	a)	a)	a)	a)
29	37.5	a)	a)	a)	a)
30	25.7	a)	a)	a)	a)
31	29.7	a)	a)	a)	a)
32	31.8	31.9	31.9	31.9	31.9
33	22.6	22.7	22.7	22.7	22.6
34	14.1	14.1	14.1	14.1	14.1
35	148.9	151.8	151.7	148.8	148.8
36	77.4	78.0	77.9	77.4	77.4
37	19.2	19.1	19.1	19.2	19.2

a) The signals were overlapped in the region of δ 29—39. *b*—*f*) Assignments may be interchanged within the column.

(δ 82.2, 82.0, 83.3 and 79.3) and four hydroxyl groups (δ 70.0, 74.6, 74.5 and 71.6). The signal at δ 79.3 is typical of C-12 of non-adjacent bis-tetrahydrofuranic acetogenins. The presence of four hydroxyl groups was supported by the formation of a tetra-acetate derivative. The chemical shifts and coupling pattern of the C-3 methylene proton signals (δ 2.40 and 2.53) and the chemical shifts of carbon signals due to the lactone moiety, including C-4 (δ 70.0), indicated that **2** is a C-4 hydroxylated acetogenin.[14] The remaining three oxymethine carbons could be assigned as those adjacent to the THF ring (C-16, -19, -24). This was verified by the ^1H-NMR data for the (*R*)-α-trifluoromethyl-α-methoxyphenylacetic acid (MTPA) ester of **2** (*vide infra*).

The positions of the two THF rings as well as the hydroxyl groups were established by mass spectral study. The electron impact (EI)-MS of **2** showed ion peaks at *m/z* 620, 602 and 584 arising from successive losses of water from the molecular ion. Also observed are a series of fragment ions starting with *m/z* 309, 379 and 449, which can be formed by cleavage at C-15/C-16, C-19/C-20$-H_2O$ and C-23/C-24$-H_2O$ in that order (Fig. 1). These ions were shifted up by 16 mass units from the corresponding ions of **1**.

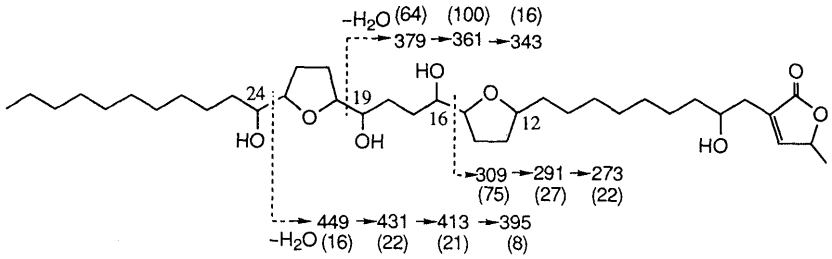

Fig. 1. Mass Fragmentation of Squamostatin-B (**2**)
The values in parentheses indicate relative intensity.

TABLE IV. ^{1}H-NMR Spectral Data for the Oxymethine Proton Signals in the (*R*)-MTPA Esters of **1**—**5** (500 MHz, $CDCl_3$)

Compd.	H-24/H-23	H-20/H-19	H-16/H-15	H-12	H-4	H-36
1	5.16/3.88	3.69/4.91	4.91/3.88	3.74	—	4.99
2	5.26/3.97	3.69/4.92[a]	4.90[a]/3.88	3.74	5.37	4.91
3	5.02/4.02	3.93/4.99	4.90/3.87	3.74	5.37	4.91
4	5.26/3.97	3.69/4.91	4.91/3.88	3.75	—	4.99
5	5.02[a]/4.03	3.93/4.99[a]	4.90/3.87	3.74	—	5.00[a]

a) The chemical shifts of the overlapped signals were estimated from the H–H COSY spectra.

The linkages at C-15/C-16, C-19/C-20, and C-23/C-24 were determined to be two *threo* and one *erythro*, based on the chemical shifts of the proton and carbon signals of these positions [δ_H 3.41/δ_C74.6, δ_H 3.41/δ_C 74.5 and δ_H *ca.* 3.8/δ_C 71.6 (the C–H connectivities were confirmed by C–H correlation spectroscopy (COSY) experiments on **2**)].[12] The *trans*-structures of the A and B rings were apparent because the ^{13}C-NMR data for **2** were closely similar to those for **1**. It is reasonable to assume the C-23/C-24-*erythro* structure for **2** by analogy with squamostatin-A.

The ^{1}H-NMR data for the tetra-(*R*)-MTPA ester of **2** (Table IV) supported the structure depicted in the formula **2**. The H–H COSY spectrum of the ester showed the presence of three –C<u>H</u>(OMTPA)–C<u>H</u>(OR)– units (C-15/C-16, C-19/C-20, C-23/C-24), one $-CH_2-$C<u>H</u>(OR)$-CH_2-$ unit (C-12), and one $-CH_2-$C<u>H</u>(OMTPA)$-CH_2-$lactone unit.

The NMR data further established 4*S* configuration, because the H-4, H-35, H-36 and H-37 signals were observed at the chemical shifts expected for natural 4*R*-acetogenins.[2,15] The negative Cotton effect at 242 nm of **2** established 36*S* configuration.[2] The structure of squamostatin-B is thus established to be as shown in the formula (**2**).

The ^{1}H-NMR (in benzene-d_6) data for **2** were in excellent agreement with those published for bullatalicin, isolated from *A. bullata*.[6] Identity of **2** with bullatalicin (reported mp 120—121 °C) has been confirmed by direct HPLC and TLC comparison with an authentic sample. Cherimoline (mp 116—117 °C),[7,8] isolated from *A. cherimolia* also seems to be identical with **2** on the basis of spectral comparison (^{1}H- and ^{13}C-NMR and MS). This is the first report of the isolation of **2** from *A. squamosa*.

Squamostatin-C (**3**) $C_{37}H_{66}O_8$ [FAB-MS *m/z* 639 (MH^+)], was isolated as white crystals, mp 95—97 °C. This compound seemed to be a stereoisomer of **2**, since the mass fragmentation pattern of **3** was similar to that of **2**. Comparison of the NMR data for **2** and **3** revealed that the chemical shifts attributable to H-24 and C-24 were significantly different from each other (Table II). The chemical shifts of δ_H 3.41/δ_C 74.0 (H-24/C-24) of **3**, together with those of H-16/C-16 and H-19/C-19, clearly indicated that C-23/C-24, C-15/C-16 and C-19/C-20 are all *threo* in **3**. The ^{13}C-NMR data for **3**, particularly C-12 to C-15, are compatible with those of *threo*/*trans*- **6**, but not *threo*/*cis*- **7**. This established that the A-THF ring of **3** has C-12/C-15-*trans* structure as well. Similar comparison of the ^{13}C-NMR data of **3** with those of the model compounds **10** and **11**, established that the B-THF ring must have C-20/C-23-*trans* structure.

The ^{1}H-NMR data for the tetra-(*R*)-MTPA ester of **3** are listed in Table IV. It can be seen from Table IV that the chemical shifts of H-12 as well as H-15/H-16 of **3** are closely similar to those of **1** and **2**. It is reasonable to assume that the structural alternation at C-24 or C-28 might not significantly affect the chemical shifts of H-12, H-15 and H-16. Thus, the signals at δ 4.90 and 3.87 are assignable to H-16 and H-15. In contrast, if the *erythro*–*threo* modification occurs at C-19/C-20, the chemical shifts of H-23/H-24 as well as H-15/H-16 might be changed. This constitutes another reason why we prefer C-23/C-24-*erythro* structure for **1** and **2** (and also **4**). This assumption is also supported by the observation that both C-23/C-24-*erythro* and -*threo* isomers exist in *A. squamosa* seeds, as demonstrated in the preceding[2] and present papers. The stereochemistry at C-4 and C-36 was determined as *R* and *S*, respectively, as described for **2**. The structure of squamostatin-C was thus established to be as shown in the formula (**3**).

German researchers have recently reported the isolation of a bis-tetrahydrofuranic acetogenin (mp 107—109 °C), named annonin-IV,[10] from *A. squamosa* seeds and they proposed a unique structure having a hydroxylated tetrahydrofuran ring. However, this structure needs to be revised since the spectral data for annonin-IV are essentially identical with those for **3**. Annonin-IV is most likely to be identical with squamostatin-C.

Squamostatin-D (**4**), $C_{37}H_{66}O_7$ [FAB-MS *m/z* 623 (MH^+)], was isolated as white crystals, mp 112—113.5 °C. This non-adjacent bis-tetrahydrofuranic acetogenin [δ_C 79.3 (C-12)] possesses three, not four, hydroxyl groups, as evidenced by the oxymethine signals (δ 71.5, 74.5 and 74.6) in the ^{13}C-NMR spectrum as well as the formation of a tri-(*R*)-MTPA ester. The ^{1}H-

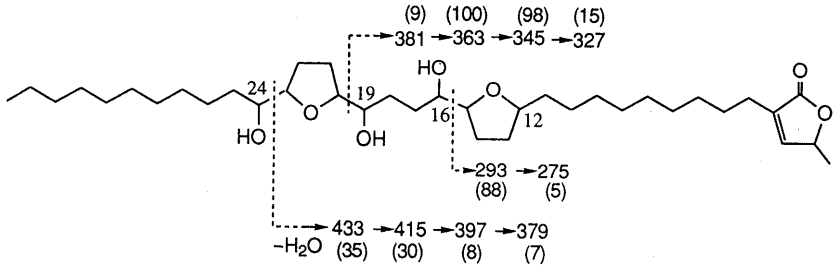

Fig. 2. Mass Fragmentation of Squamostatin-D (**4**)
The values in parentheses indicate relative intensity.

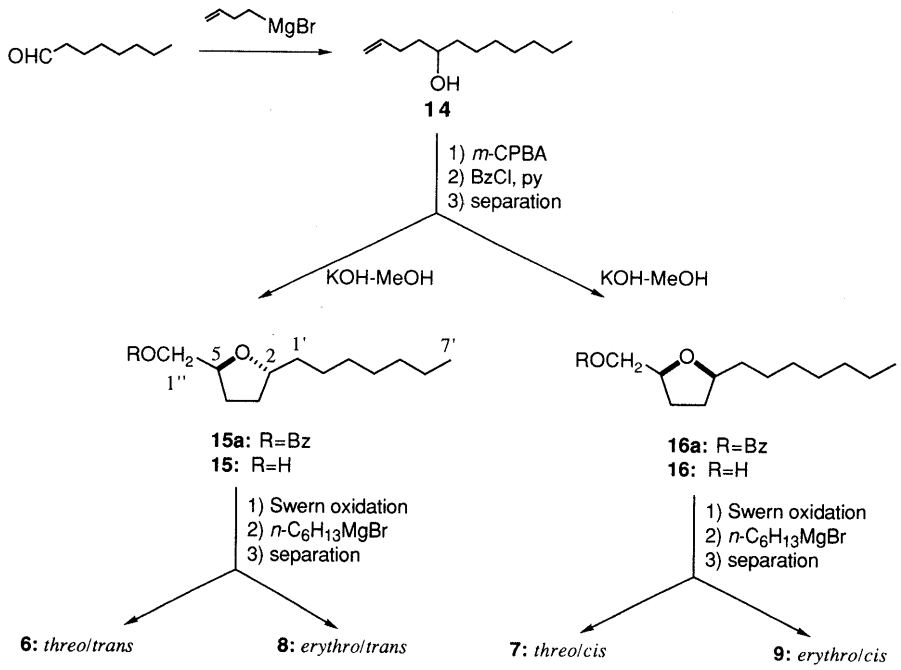

Chart 3

and ^{13}C-NMR data for **4** were in good agreement with the structure depicted in the formula **4**. The structure was also supported by the EI-MS data. The mass fragmentation pattern of **4** is shown in Fig. 2, which exhibits series of ion peaks arising from the fission of C-15/C-16, C-19/C-20 and C-23/C-24.

As can be seen in Table IV, the ^{1}H-NMR data for the non-adjacent bis-THF moiety of the (*R*)-MTPA ester of **4** were closely similar to those for **2**. Thus, the relative as well as absolute configuration of the bis-THF moiety was identical with that of **2**, as depicted in **4**. The C-36 configuration was depicted as *S*, as in the majority of *A. squamosa* acetogenins. Squamostatin-D can be referred to as 28-deoxysquamostatin-A or 4-deoxysquamostatin-B. Compound **4** is a new non-adjacent bis-tetrahydrofuranic acetogenin.

Squamostatin-E (**5**), $C_{37}H_{66}O_7$ [FAB-MS *m/z* 623 (MH^+)], was isolated as white crystals, mp 105—106 °C. The mass fragmentation pattern of **5** was essentially identical with that of **4**, thus suggesting the same plane structure for **4** and **5**. Comparison of the ^{13}C-NMR data for **3** and **5** revealed that **5** has all *threo* relationships at C-15/C-16, C-19/C-20 and C-23/C-24. The close similarity in the ^{1}H-NMR data (Table IV) for the tri-(*R*)-MTPA ester of **5** and tetra-(*R*)-MTPA ester of **3** indicated the identity of the absolute stereochemistry of the non-adjacent THF moiety. On the basis of these data, the structure of squamostatin-E was established to be as shown in **5**. The 36*S* configuration was assigned by analogy with the majority of *A. squamosa* acetogenins. This is a new acetogenin and can be referred to as 4-deoxysquamostatin-C.

Synthesis of Model Tetrahydrofurans The model (±)-2-heptyl-5-(1-hydroxyheptyl)tetrahydrofurans **6**—**9** have been synthesized according to Chart 3. Reaction of octanal with 3-butenylmagnesium bromide gave the alcohol **14**. Treatment of **14** with *m*-chloroperbenzoic acid gave a 1 : 1 mixture of *trans*- and *cis*-tetrahydrofurans (**15**/**16**). The separated *trans*-benzoate **15a** and the *cis*-isomer **16a** were hydrolyzed to give the *trans*- (**15**) and *cis*- (**16**) tetrahydrofurans. The *trans*- and *cis*-stereochemistries were determined at a later stage. Swern oxidation of **15** gave the corresponding aldehyde, which was reacted with *n*-hexylmagnesium bromide to give a mixture of *threo*/*trans*- (**6**) and *erythro*/*trans*- (**8**) alcohols in the ratio of 3 : 2, respectively. The *cis*-tetrahydrofuran **16** was similarly converted into the *threo*/*cis*- (**7**) and *erythro*/*cis*- (**9**) tetrahydrofurans in the ratio of 2 : 3, respectively.

Orientation of the two substituents on the THF ring was determined at this stage. Prior to the experiments, the three oxymethine protons and carbons were firmly assigned based on the H–H COSY and C–H COSY data for **6**—**9**. For example, the NMR data for **7** were analyzed as follows. The proton signal at δ 3.86 was readily assigned to H-2 (attached to the carbon at δ 79.9), because the other two oxymethine protons were coupled to each other. The two coupled protons at δ 3.36 and δ 3.70 were attached to the carbons at δ 74.5 and 82.2, respectively. By comparison of the two carbon chemical shifts, the signals at δ_H 3.70 and δ_C 82.2 were assigned to H-5 and C-5. These assignments were supported by the fact that the proton at δ 3.70 was coupled not only to the proton at 3.36 but to magnetically non-equivalent methylene protons (δ 1.89 and 1.66) on the carbon at δ 27.8, whereas the proton at δ 3.36 was further coupled to methylene protons at δ 1.42 attached to the carbon at δ 34.0. Similar analysis allowed us to assign almost all the carbon signals (Table II). Further, the chemical shifts of H-1″ (δ_H 3.36) and C-1″ (δ_C 74.5) clearly indicated that **7** has *threo* stereochemistry in the light of Born's rule.[12]

In the nuclear Overhauser effect (NOE) experiments on **7**, irradiation of the H-5 proton enhanced the signal intensity of H-2. In similar NOE experiments on **6**, the signal intensity of H-2 (δ 3.88) was not changed on

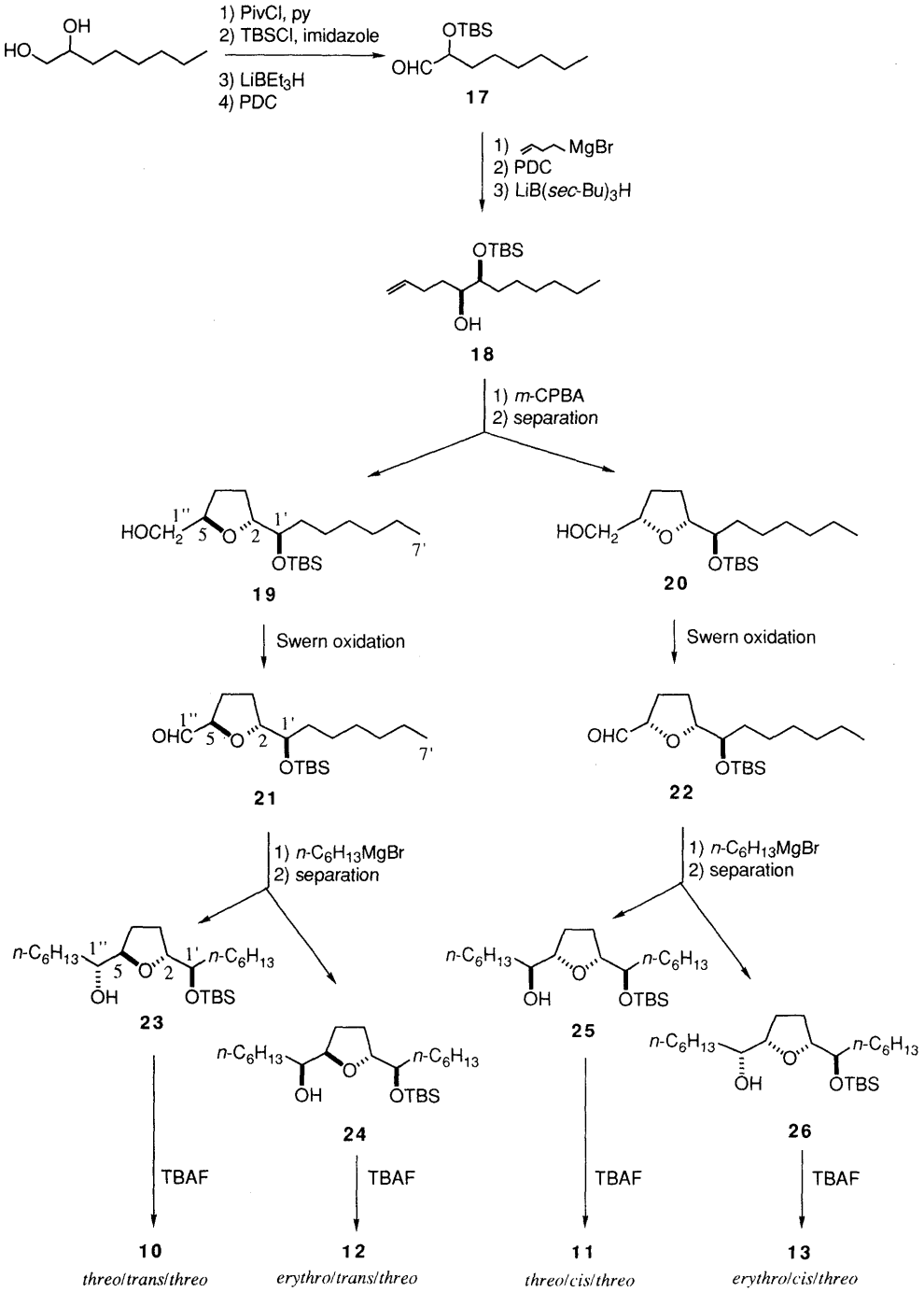

Chart 4

irradiation of H-5 (δ 3.78). Thus, it was firmly established that **15**, **15a**, **6** and **8** belong to the *trans*-series, whereas **16**, **16a**, **7** and **9** belong to the *cis*-series. It was also established that **6** has *threo*-stereochemistry and **8** and **9** have *erythro*-stereochemistry, as described for **7**.

The model (±)-2,5-di-(1-hydroxyheptyl)tetrahydrofurans **10**—**13** were prepared according to Chart 4. The requisite aldehyde **17** was prepared from 1,2-octanediol in four steps, *i.e.*, esterification with pivaloyl chloride, etherification with *tert*-butyldimethylsilyl (TBS) chloride, deprotection of the pivaloyl group with $LiBEt_3H$, and oxidation with pyridinium dichromate (PDC). Reaction of the aldehyde **17** with 3-butenylmagnesium bromide gave a 1 : 2 mixture of *threo*- and *erythro*-alcohols. The minor alcohol **18** was shown to be the *threo*-isomer since it was eventually converted into *threo*/*threo*-tetrahydrofurans **10** and **11** (*vide infra*). A two-step sequence, *i.e.*, oxidation with PDC and reduction with $LiB(sec\text{-}Bu)_3H$, transformed the alcohol mixture into the practically pure *threo*-alcohol **18** (*threo* : *erythro* = 20 : 1). Treatment of the *threo*-alcohol **18** with *m*-chloroperbenzoic acid gave a 1 : 1 mixture of the *trans*- (**19**) and *cis*- (**20**) tetrahydrofurans. The *trans*- and *cis*-stereochemistry was determined on the basis of NOE experiments. In **20**, irradiation of H-5 (δ 4.07, assigned from the H–H and C–H COSY spectra) increased the signal intensity of H-2 (δ 3.96). By contrast, in the NOE experiments on **19**, irradiation of H-5 (δ 4.08) did not cause such an enhancement of the signal intensity of H-2 (δ 3.92). Thus, it was established that compounds **19**, **21**, **23**, **24**, **10** and **12** have *trans*-stereochemistry, whereas compounds **20**, **22**, **25**, **26**, **11** and **13** have *cis*-stereochemistry.

Swern oxidation of **19** gave the *trans*-aldehyde **21**, which was allowed to react with *n*-hexylmagnesium bromide to give a mixture of the *threo*/*trans*/*threo*-alcohol **23** and the *erythro*/*trans*/*threo*-isomer **24** in the ratio of 4 : 3, respectively. The alcohols **23** and **24** were separately treated with tetrabutylammonium fluoride (TBAF) to give the *threo*/*trans*/*threo*-alcohol **10** and *erythro*/*trans*/*threo*-isomer **12**, respectively. Similar transformation of the *cis*-isomer **20** gave, *via* the aldehyde **22**, a mixture of the *threo*/*cis*/*threo*- (**25**) and *erythro*/*cis*/*threo*- (**26**) alcohols in the ratio of 1 : 4, respectively. Deprotection of **25** and **26** gave the *threo*/*cis*/*threo*- (**11**) and *erythro*/*cis*/*threo*- (**13**) tetrahydrofurans, respectively.

The *threo* and *erythro* stereochemistry of **10**—**13** was determined without difficulty by the application of Born's rule. The synthetic model tetrahydrofurans **6**—**13** with the known relative stereochemistry were successfully utilized in the structure elucidation of the non-adjacent bis-tetrahydrofuranic acetogenins **1**—**5**. Optical resolution of these model tetrahydrofurans is in progress in our laboratory, and should provide further information on the absolute stereochemistry of these acetogenins.

In conclusion, we have performed detailed structural analysis on the four non-adjacent bis-tetrahydrofuranic acetogenins isolated from the petroleum ether extract of *A. squamosa* seeds. The structures of **2**—**5** established in the present work were further supported by the results of precursor-ion scanning mass spectrometry combined with derivatization with *N*,*N*-dimethylethylenediamine.[16] Squamostatins-B and -D have C-15/C-16-*threo*, C-19/C-20-*threo*, C-23/C-24-*erythro* structure, whereas squamostatins-C and -E have C-15/C-16-*threo*, C-19/C-20-*threo*, C-23/C-24-*threo* structure. Squamostatins-B and -C have a C-4 hydroxy group. Squamostatin-C(**3**), -D(**4**) and -E (**5**) are new acetogenins. The stereochemistry at the non-adjacent THF moiety of these acetogenins was established to be C-12/C-15-*trans* and C-20/C-23-*trans* on the basis of ^{13}C-NMR spectral comparison with synthetic model tetrahydrofurans. Determination of the absolute stereochemistry of the non-adjacent THF moiety of acetogenins **1**—**5** remains to be achieved. The data for the (*R*)-MTPA esters of acetogenins **1**—**5** should be useful not only in the determination of the absolute stereochemistry, but also as a basis set of data for comparison and identification of acetogenins of this type.

Experimental

General procedures are described in the preceding paper.[2] Compounds **2** and **3** were isolated from the acetogenin mixture, after removal of squamocin and **1**,[4,5] by ODS-HPLC (column, STR Prep-ODS 25 cm × 20 mm; solvent, MeOH–water 11 : 1). The isolation of **4** and **5** from the polar fractions was performed as previously described.[2] Preparation of (*R*)-MTPA esters was carried out as described in the preceding paper.

Squamostatin-B (2) White crystals, mp 98—101 °C (from AcOEt). $[\alpha]_D^{25}$ +10.5° (*c* = 0.10, MeOH). IR ($CHCl_3$, cm^{-1}): 3590, 3450, 1745. UV λ_{max} nm (ε): 210 (7000). CD (MeOH) $\Delta\varepsilon$ (nm): −0.50 (238). HR-FAB-MS Calcd for $C_{37}H_{67}O_8$ (MH^+; *m*/*z*): 639.4836. Found: 639.4890. ^{1}H-NMR δ: 0.88 (3H, t, *J* = 6.8 Hz, H-34), 1.43 (3H, d, *J* = 6.8 Hz, H-37), 2.40 (1H, ddt, *J* = 15.0, 8.2, 1.5 Hz, H-3a), 2.53 (1H, ddt, *J* = 15.0, 3.0, 1.5 Hz, H-3b), 3.41 (2H, m, H-16, -19), 3.76—3.91 (6H, m, H-4, -12, -15, -20, -23, -24), 5.06 (1H, q, *J* = 6.8 Hz, H-36), 7.19 (1H, s, H-35).

Tetra-(*R*)-MTPA ester, ^{1}H-NMR δ: 0.88 (3H, t, *J* = 7.0 Hz, H-34), 1.31 (3H, d, *J* = 6.6 Hz, H-37), 2.59 (1H, ddt, *J* = 15.6, 2.0, 2.0 Hz, H-3a), 2.67 (1H, dd, *J* = 15.6, 7.8 Hz, H-3b), 3.470, 3.495, 3.523, 3.580 (3H each, s, OMe), 3.69 (1H, q, *J* = 7.5 Hz, H-20), 3.74 (1H, m, H-12), 3.88 (1H, q, *J* = 6.9 Hz, H-15), 3.97 (1H, m, H-23), 4.88—4.94 (3H, m, H-16, -19, -36), 5.26 (1H, m, H-24), 5.37 (1H, m, H-4), 6.96 (1H, s, H-35), 7.30—7.65 (20H, m, aromatic).

Squamostatin-C (3) White crystals, mp 95—97 °C (from AcOEt). $[\alpha]_D^{25}$ +12.0° (*c* = 0.20, MeOH). IR ($CHCl_3$, cm^{-1}): 3685, 3585, 3540, 1755. UV λ_{max} nm (ε): 210 (7000). CD (MeOH) $\Delta\varepsilon$ (nm): −0.50 (238). HR-FAB-MS Calcd for $C_{37}H_{67}O_8$ (MH^+; *m*/*z*): 639.4836. Found: 639.4890. ^{1}H-NMR δ: 0.88 (3H, t, *J* = 7.1 Hz, H-34), 1.43 (3H, d, *J* = 6.8 Hz, H-37), 2.40 (1H, ddt, *J* = 15.0, 8.2, 1.6 Hz, H-3a), 2.53 (1H, ddt, *J* = 15.0, 4.0, 2.0 Hz, H-3b), 3.41 (3H, m, H-16, -19, -24), 3.77—3.90 (5H, m, H-4, -12, -15, -20, -23), 5.06 (1H, q, *J* = 6.8 Hz, H-36), 7.19 (1H, s, H-35).

Tetra-(*R*)-MTPA ester, ^{1}H-NMR δ: 0.89 (3H, t, *J* = 7.0 Hz, H-34), 1.31 (3H, d, *J* = 6.8 Hz, H-37), 2.59 (1H, ddt, *J* = 15.6, 2.0, 2.0 Hz, H-3a), 2.67 (1H, dd, *J* = 15.6, 7.8 Hz, H-3b), 3.466, 3.495, 3.507, 3.530 (3H each, s, OMe), 3.74 (1H, m, H-12), 3.87 (1H, m, H-15), 3.93 (1H, m, H-20), 4.02 (1H, m, H-23), 4.90 (2H, m, H-16, -36), 4.99 (1H, m, H-19), 5.02 (1H, m, H-24), 5.37 (1H, m, H-4), 6.96 (1H, s, H-35), 7.30—7.64 (20H, m, aromatic).

Squamostatin-D (4) White crystals, mp 112—113.5 °C (from MeOH–H_2O). $[\alpha]_D^{25}$ +7.9° (*c* = 0.51, MeOH). IR ($CHCl_3$, cm^{-1}): 3560, 3450, 1750. HR-FAB-MS Calcd for $C_{37}H_{67}O_8$ (MH^+; *m*/*z*): 623.4887. Found: 639.4882. ^{1}H-NMR δ: 0.89 (3H, t, *J* = 5.9 Hz, H-34), 1.41 (3H, d, *J* = 6.7 Hz, H-37), 2.26 (2H, tt, *J* = 7.7, 7.1 Hz, H-3), 3.41 (2H, m, H-16, -19), 3.77—3.90 (5H, m, H-12, -15, -20, -23, -24), 4.99 (1H, qq, *J* = 6.8, 1.4 Hz, H-36), 6.98 (1H, br s, H-37).

Tri-(*R*)-MTPA ester, ^{1}H-NMR δ: 0.88 (3H, t, *J* = 7.0 Hz, H-34), 1.40 (3H, d, *J* = 7.0 Hz, H-37), 2.26 (2H, tt, *J* = 7.7, 7.1 Hz, H-3), 3.474, 3.525, 3.582 (3H each, s, MeO), 3.69 (1H, q, *J* = 7.5 Hz, H-20), 3.75 (1H, m, H-12), 3.89 (1H, q, *J* = 7.0 Hz, H-15), 3.97 (1H, m, H-23), 4.91 (2H, m, H-16, -19), 4.99 (1H, qq, *J* = 6.8, 1.4 Hz, H-36), 5.26 (1H, m, H-24), 6.97 (1H, d, *J* = 1.5 Hz, H-35), 7.33—7.63 (15H, m, aromatic).

Squamostatin-E (5) White crystals, mp 105—106 °C (from MeOH–

H_2O). $[\alpha]_D^{25}$ +14.7° (c=0.51, MeOH). IR ($CHCl_3$, cm^{-1}): 3560, 3450, 1750. *Anal.* Calcd for $C_{37}H_{66}O_7$: C, 71.34; H, 10.68. Found: C, 71.64; H, 10.98. ^{1}H-NMR δ: 0.88 (3H, t, J=6.7 Hz, H-34), 1.42 (3H, d, J=6.7 Hz, H-37), 2.26 (2H, t, J=7.7 Hz, H-3), 3.38—3.57 (3H, m, H-16, -19, -24), 3.77—3.92 (4H, m, H-12, -15, -20, -23), 4.98 (1H, qq, J=6.8, 1.4 Hz, H-36), 6.98 (1H, br s, H-35).

Tri-(*R*)-MTPA ester, ^{1}H-NMR δ: 0.88 (3H, t, J=7.0 Hz, H-34), 1.40 (3H, d, J=6.7 Hz, H-37), 2.26 (2H, t, J=7.7 Hz, H-3), 3.469, 3.508, 3.531 (3H each, s, MeO), 3.74 (1H, m, H-12), 3.87 (1H, q, J=7.0 Hz, H-15), 3.93 (1H, q, J=7.0 Hz, H-20), 4.03 (1H, m, H-23), 4.91 (1H, q, J=6.5 Hz, H-16), 4.96—5.05 (3H, m, H-19, -24, -36), 6.97 (1H, d, J=1.4 Hz, H-35), 7.33—7.62 (15H, m, aromatic).

1-Decen-5-ol (14) Octanal (9.01 ml, 57.7 mmol) in THF (10 ml) was added to a solution of Grignard reagent prepared from 4-bromo-1-butene (5.00 ml, 49.3 mmol) and magnesium (1.05 g, 43.4 mmol) in THF (20 ml) under nitrogen at room temperature. The reaction mixture was stirred for 5 min, and then diluted with saturated aqueous NH_4Cl and ether. Extractive (ether) work-up gave a crude product, which was chromatographed on silica gel with hexane–AcOEt (10 : 1) to give **14** (6.80 g, 85%) as a colorless oil. IR (neat, cm^{-1}): 3600, 2930, 2850, 1640, 1465, 1380, 1000, 915. ^{1}H-NMR δ: 0.88 (3H, t, J=7.3 Hz), 2.16 (2H, m), 3.61 (1H, m), 4.95 (1H, dq, J=10.5, 1.7 Hz), 5.05 (1H, dq, J=18.0, 1.7 Hz), 5.84 (1H, ddt, J=18.0, 10.5, 7.5 Hz). ^{13}C-NMR δ: 14.1, 22.6, 25.6, 29.3, 29.6, 30.1, 31.8, 36.5, 37.5, 71.5, 114.7, 138.7. *Anal.* Calcd for $C_{12}H_{24}O$: C, 78.20; H, 13.12. Found: C, 78.10; H, 13.24.

***trans*- (15) and *cis*-2-Heptyl-5-(hydroxymethyl)tetrahydrofurans (16)** A mixture of *m*-chloroperbenzoic acid (8.59 g, 49.8 mmol) and **14** (6.80 g, 36.9 mmol) in CH_2Cl_2 (90 ml) was stirred at room temperature for 24 h. Extractive (ether) work-up gave a crude product, which was chromatographed on silica gel with hexane–AcOEt (5 : 1) to give a mixture of **15/16** (5.76 g, 78%). Benzoylation of the mixture (5.72 g, 28.6 mmol) with benzoyl chloride (5.31 ml, 45.6 mmol) and pyridine (7 ml) gave **15a/16a**. The mixture was separated by medium-pressure liquid chromatography (a silica gel Lobar column) with hexane–ether (9 : 1) to give the more mobile **15a** (2.52 g, 29%) and the less mobile **16a** (2.96 g, 34%). **15a**: colorless oil. IR (neat, cm^{-1}): 2930, 2850, 1720, 1600, 1450, 1270. ^{1}H-NMR δ: 0.88 (3H, t, J=7.1 Hz), 1.75 (1H, m), 2.09 (2H, m), 4.02 (1H, m), 4.34 (3H, m), 7.43 (2H, m), 7.56 (1H, m), 8.06 (2H, dd, J=9.0, 1.2 Hz). ^{13}C-NMR δ: 14.1, 22.6, 26.1, 28.4, 29.2, 29.6, 31.8, 35.7, 67.1, 76.1, 79.8, 128.3, 129.7, 130.2, 132.9, 166.6. FAB-MS m/z: 305 (MH^+), 303. *Anal.* Calcd for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.82; H, 9.42. **16a**: colorless oil. ^{1}H-NMR δ: 0.88 (3H, t, J=7.0 Hz), 1.80 (1H, m), 2.01 (2H, m), 3.91 (1H, qui, J=6.9 Hz), 4.31 (3H, m), 7.43 (2H, m), 7.55 (1H, m), 8.06 (2H, dd, J=9.0, 1.2 Hz). ^{13}C-NMR δ: 14.1, 22.6, 26.1, 28.0, 29.2, 29.6, 30.9, 31.8, 35.9, 67.1, 76.5, 80.4, 128.3, 129.7, 130.2, 132.9, 166.6. *Anal.* Calcd for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.83; H, 9.34.

A mixture of **15a** (2.52 g, 8.28 mmol) in methanol (4 ml) and 5% KOH/methanol (2 ml) was stirred for 10 min at room temperature. Extractive (ether) work-up gave a crude product which was chromatographed on silica gel with hexane–AcOEt (5 : 1) to give **15** (1.50 g, 90%) as a colorless oil. IR (neat, cm^{-1}): 3430, 2920, 2850, 1460, 1375, 1095, 1045. ^{1}H-NMR δ: 0.89 (3H, t, J=7.0 Hz, Me), 1.87—2.20 (4H, m, H-3, -4), 3.48 (1H, dd, J=11.9, 6.4 Hz, H_a-1″), 3.63 (1H, dd, J=11.9, 3.7 Hz, H_b-1″), 3.91 (1H, m, H-2), 4.09 (1H, m, H-5). ^{13}C-NMR δ: 14.06, 22.63, 26.19, 27.54, 29.25, 29.68, 31.79, 32.03, 35.74, 65.07, 78.83, 79.52. FAB-MS m/z: 201 (MH^+), 199. *Anal.* Calcd for $C_{12}H_{24}O_2$: C, 79.15; H, 12.08. Found: C, 72.03; H, 12.38.

The *cis* isomer **16a** (2.96 g, 9.72 mmol) was hydrolyzed in the same way as described for **15a** to give **16** (1.80 g, 92%) as a colorless oil. ^{1}H-NMR δ: 0.89 (3H, t, J=6.9 Hz, Me), 1.82—2.05 (4H, m, H-3, -4), 3.48 (1H, dd, J=11.0, 6.4 Hz, H_a-1″), 3.69 (1H, dd, J=11.9, 3.7 Hz, H_b-1″), 3.86 (1H, m, H-2), 4.00 (1H, m, H-5). ^{13}C-NMR δ: 14.06, 22.64, 26.24, 27.04, 29.23, 29.66, 31.39, 31.80, 35.91, 65.28, 79.12, 80.21. *Anal.* Calcd for $C_{12}H_{24}O_2$: C, 79.15; H, 12.08. Found: C, 72.09; H, 12.02.

***threo/trans*- (6) and *erythro/trans*-2-Heptyl-5-(1-hydroxyheptyl)tetrahydrofurans (8)** Dimethyl sulfoxide (2.13 ml, 30.0 mmol) was added dropwise to a solution of oxalyl dichloride (1.31 ml, 15.0 mmol) in CH_2Cl_2 (50 ml) under nitrogen at −78 °C. After 5 min, a solution of **15** (1.50 g, 7.49 mmol) in CH_2Cl_2 (8 ml) was added at −78 °C and the mixture was stirred for 15 min at −40 °C. Triethylamine (5.22 ml, 37.5 mmol) was then added at the same temperature and the mixture was stirred for a further 10 min. Extractive (AcOEt) work-up gave a crude aldehyde (1.52 g) as a yellow oil. IR (neat, cm^{-1}): 2925, 2850, 1465, 1380, 1070. ^{1}H-NMR δ: 0.88 (3H, t, J=7.6 Hz, Me), 2.00 (2H, m), 2.19 (1H, m), 4.00 (1H, m, H-2), 4.32 (1H, ddd, J=9.7, 6.8, 2.0 Hz, H-5), 9.66 (1H, d, J=2.0 Hz, CHO). ^{13}C-NMR δ: 14.0, 22.6, 26.1, 27.2, 29.2, 29.6, 31.1, 31.8, 35.3, 81.2, 82.4, 203.2.

A THF (4 ml) solution of the aldehyde (1.52 g) was added to a solution of *n*-hexylmagnesium bromide prepared from *n*-hexyl bromide (2.70 ml, 19.2 mmol), magnesium (370 mg, 15.2 mmol), and THF (8 ml) under nitrogen at room temperature. The mixture was stirred for 5 min and saturated aqueous NH_4Cl and ether were added. Extractive (ether) work-up gave a crude product (2.33 g). The mixture was separated on a silica gel Lobar column with hexane–AcOEt (15 : 1) to give **6** (723 mg, 34% from **15**) and **8** (436 mg, 20% from **15**). **6**: a colorless oil. IR ($CHCl_3$, cm^{-1}): 3570, 2940, 2860, 1460, 1380, 1065. ^{1}H-NMR δ: 0.88 (6H, t, J=7.1 Hz, Me), 1.53—1.64 (2H, m, H_a-4, H_a-1′), 1.95 (1H, m, H_b-4), 2.03 (1H, m, H_a-3), 2.43 (1H, s, OH), 3.37 (1H, m, H-1″), 3.78 (1H, q, J=8.3 Hz, H-5), 3.88 (1H, m, H-2). ^{13}C-NMR data are shown in Table II. FAB-MS m/z: 285 (MH^+), 283. *Anal.* Calcd for $C_{18}H_{36}O_2$: C, 75.99: H, 12.76. Found: C, 76.23; H, 12.90. **8**: a white solid, mp 25—27 °C. IR ($CHCl_3$, cm^{-1}): 3560, 2940, 2860, 1460, 1375, 1065. ^{1}H-NMR δ: 0.88 (6H, t, Me), 1.57 (1H, m, H_a-1′), 1.77—1.91 (2H, m, H-4), 2.03 (1H, m, H_a-3), 3.78 (1H, m, H-1″), 3.88 (1H, m, H-5), 3.95 (1H, m, H-2). ^{13}C-NMR data are shown in Table II. *Anal.* Calcd for $C_{18}H_{36}O_2$: C, 75.99; H, 12.76. Found: C, 75.70; H, 12.46.

***threo/cis*- (7) and *erythro/cis*-2-Heptyl-5-(1-hydroxyheptyl)tetrahydrofurans (9)** The isomeric mono-tetrahydrofurans **7** and **9** were prepared from **16** (1.80 g, 8.99 mmol) in the same way as described for **15** *via* the aldehyde as a yellow oil. ^{1}H-NMR δ: 0.88 (3H, t, J=7.5 Hz, Me), 1.86—2.19 (3H, m), 4.03 (1H, m, H-2), 4.23 (1H, ddd, J=8.8, 5.4, 2.0 Hz, H-5), 9.68 (1H, d, J=2.0, H-1″). ^{13}C-NMR δ: 14.1, 22.6, 26.2, 27.9, 29.2, 29.6, 31.0, 31.8, 35.7, 81.5 ,82.9, 203.4. The isomers were separated on a silica Lobar column with hexane–AcOEt (15 : 1) to give **7** (743 mg, 29% from **16**) and **9** (1.16 g, 45% from **16**). **7**: a colorless oil. IR ($CHCl_3$, cm^{-1}): 3570, 2930, 2850, 1465, 1380, 1060. ^{1}H-NMR δ: 0.88 (6H, t, J=7.1 Hz, Me), 1.66 (1H, m, H_b-4), 1.89 (1H, m, H_a-4), 1.96 (1H, m, H_a-3), 3.36 (1H, q, J=5.5 Hz, H-1″), 3.70 (1H, q, J=6.4 Hz, H-5), 3.86 (1H, qui, J=6.4 Hz, H-2). ^{13}C-NMR data are shown in Table II. *Anal.* Calcd for $C_{18}H_{36}O_2$: C, 75.99; H, 12.76. Found: C, 75.77; H, 12.93. **9**: a colorles soil. IR ($CHCl_3$, cm^{-1}): 3560, 2930, 2850, 1465, 1385, 1060. ^{1}H-NMR δ: 0.87 (6H, t, J=6.8 Hz, Me), 1.58 (1H, m, H_a-1′), 1.73 (1H, m, H_a-4), 1.85 (1H, m, H_b-4), 1.95 (1H, m, H_a-3), 2.04 (1H, s, OH), 3.79—3.90 (3H, m, H-1″, -2, -5). ^{13}C-NMR data are shown in Table II. *Anal.* Calcd for $C_{18}H_{36}O_2$: C, 75.99; H, 12.76. Found: C, 76.22; H, 12.93.

2-(*tert*-Butyldimethylsilyloxy)octanal Pivaloyl chloride (9.4 ml, 77.2 mmol) was added to a solution of 1,2-octanediol (10.0 g, 68.4 mmol) in pyridine (50 ml) at 0 °C and the mixture was stirred at room temperature for 1 h. Extractive (ether) work-up gave a crude ester (17.3 g). A mixture of the ester, imidazole (9.3 g, 137 mmol) and TBS chloride (14.7 g, 95.0 mmol) in *N*,*N*-dimethylformamide (150 ml) was stirred at room temperature overnight. Extractive (ether) work-up gave a crude product, which was chromatographed on silica gel with hexane–AcOEt (15 : 1) to give the TBS ether (18.9 g, 80% from 1,2-octanediol) as a colorless oil. ^{1}H-NMR δ: 0.07 (3H, s), 0.08 (3H, s), 0.89 (9H,s), 0.87 (3H, t, J=7.0 Hz), 1.21 (9H, s), 1.48 (2H, m), 3.84 (1H, m), 3.96 (2H, d, J= 5.9 Hz).

Lithium triethylborohydride (1 M solution in THF, 200 ml) was added to a stirred solution of the ether (17.3 g, 50.2 mmol) in dry THF (200 ml) with ice-salt cooling under nitrogen and the mixture was stirred for 10 min. Extractive (ether) work-up gave a crude product, which was chromatographed on silica gel with hexane–AcOEt (8 : 1) to give the TBS ether (12.5 g, 95%) as a colorless oil. IR ($CHCl_3$, cm^{-1}): 3570, 3450, 2930, 2860, 1465, 1380, 1255, 1095, 835. ^{1}H-NMR δ: 0.09 (6H, s), 0.88 (3H, t, J=6.8 Hz), 0.91 (9H, s), 1.48 (2H, m), 1.89 (1H, t, J=7.0 Hz, OH), 3.45 (1H, m), 3.56 (1H, m), 3.73 (1H, m). *Anal.* Calcd for $C_{14}H_{32}O_2Si$: C, 64.55; H, 12.38. Found: C, 64.74; H, 12.66.

PDC (37.1 g, 98.6 mmol) was added to a mixture of the ether (10.6 g, 40.7 mmol) and molecular sieves 4A (40 g) in CH_2Cl_2 (280 ml) under nitrogen at room temperature. The mixture was stirred at reflux for 6 h. The mixture was cooled to room temperature, diluted with dry ether, and filtered through a Florisil column. Concentration of the filtrate gave a crude product, which was chromatographed on silica gel with hexane–AcOEt (10 : 1) to give the aldehyde **17** (5.9 g, 57%). IR ($CHCl_3$, cm^{-1}): 1720. ^{1}H-NMR δ: 0.07 (3H, s), 0.08 (3H, s), 0.88 (3H, t, J=6.8 Hz), 0.92 (9H, s), 1.61 (2H, m), 3.96 (1H, t, J=6.2, 2.0 Hz), 9.59 (1H, d, J=2.0 Hz). HR-FAB-MS Calcd for $C_{14}H_{31}O_2Si$ (MH^+; m/z): 259.2093. Found: 259.2060.

6-*tert*-Butyldimethylsilyloxy-5-hydroxy-1-dodecene (18) The aldehyde **17** (5.7 g, 22.1 mmol) was reacted with 3-butenylmagnesium bromide in the same way as described for the preparation of **14** to give a crude product. This was chromatographed on silica gel with hexane–AcOEt (8 : 1) to give a mixture (5.5 g, 79%) of *threo* and *erythro*-alcohols. Oxidation of this mixture (5.4 g, 17.2 mmol) with PDC in the same way as described above afforded the corresponding ketone (3.9 g, 73%) after chromatography on silica gel with hexane–benzene (1 : 1). L-Selectride (1 M solution in THF, 14 ml) was added to a stirred solution of the ketone (3.6 g, 11.5 mmol) in dry THF (13 ml) at −78 °C under nitrogen and the mixture was stirred for 10 min. Extractive (ether) work-up gave a crude product which was chromatographed on silica gel with benzene–AcOEt (10 : 1) with an eluent to give the TBS ether (3.3 g, 91%) as a colorless oil. IR ($CHCl_3$, cm^{-1}): 3550, 2925, 2840, 1640, 1460, 1255, 1075, 835. ^{1}H-NMR δ: 0.08 (3H, s), 0.09 (3H, s), 0.88 (3H, t, J=6.8 Hz), 0.90 (9H, s), 3.49 (2H, m), 4.97 (1H, dq, J=10.5, 1.7 Hz), 5.04 (1H, dq, J=18.0, 1.7 Hz), 5.84 (1H, ddt, J=18.0, 10.5, 7.5 Hz). ^{13}C-NMR δ: −4.6, −4.1, 14.0, 18.1, 22.6, 25.0, 25.9 (×3), 29.6, 30.2, 31.8, 33.4, 33.9, 72.0, 75.2, 114.6, 138.6. *Anal.* Calcd for $C_{18}H_{38}O_2Si$: C, 68.72; H, 12.18. Found: C, 68.95; H, 12.37.

In a preliminary experiment the mixture of *threo* and *erythro*-alcohols was converted into the pivaloyl ester. Separation of the esters on a silica gel column afforded the more mobile *threo*-ester (26%) and the less mobile *erythro*-ester (65%). Reduction of the separated *erythro*-ester with $LiAlH_4$ in ether at −40 °C gave the pure *erythro*-alcohol as a colorless oil. IR ($CHCl_3$, cm^{-1}): 3550, 2925, 2840, 1640, 1460, 1255, 1075, 835. ^{1}H-NMR δ: 0.07 (6H, s), 0.88 (3H, t, J=6.8 Hz), 0.90 (9H, s), 3.60 (2H, m), 4.98 (1H, dq, J=10.5, 1.7 Hz), 5.05 (1H, dq, J=18.0, 1.7 Hz), 5.84 (1H, ddt, J=18.0, 10.5, 7.5 Hz). ^{13}C-NMR δ: −4.4 (×2), 14.1, 18.1, 22.6, 25.7, 25.9 (×3), 29.5, 30.4, 30.7, 30.8, 31.8, 73.9, 75.3, 114.7, 138.5. *Anal.* Calcd for $C_{18}H_{38}O_2Si$: C, 68.72; H, 12.18. Found: C, 68.90; H, 12.32.

***threo/trans*- (19) and *threo/cis*-2-(Hydroxymethyl)-5-(1-*tert*-butyldimethylsilyloxyheptyl)tetrahydrofurans (20)** A mixture of *m*-chloroperbenzoic acid (2.20 g, 12.7 mmol) and **18** (3.30 g, 10.5 mmol) in CH_2Cl_2 (30 ml) was stirred at room temperature for 14 h. Extractive (ether) work-up gave a crude product, which was chromatographed on silica gel with hexane–AcOEt (5 : 1) to give a mixture of **19**/**20** (2.7 g, 79%). The mixture was separated on a silica gel Lobar column with hexane–AcOEt (5 : 1) to give the less mobile **19** (990 mg, 29%) and the more mobile **20** (916 mg, 26%). **19**: a colorless oil. IR ($CHCl_3$, cm^{-1}): 3600, 3460, 2940, 2850, 1460, 1255, 1075, 835. ^{1}H-NMR δ: 0.06, 0.07 (3H each, s, SiMe), 0.88 (3H, t, J=7.3 Hz, Me), 0.89 (9H, s, Me_3C), 3.48 (1H, m, H_a-1″), 3.57 (1H, m, H-1′), 3.64 (1H, m, H_b-1″), 3.92 (1H, m, H-2), 4.08 (1H, m, H-5). ^{13}C-NMR δ: −4.6, −4.1, 14.1, 18.3, 22.6, 25.6, 26.0 (×3), 27.7, 27.8, 29.5, 31.8, 33.0, 65.0, 75.1, 79.4, 81.1. FAB-MS m/z: 331 (MH^+), 329. *Anal.* Calcd for $C_{18}H_{38}O_3Si$: C, 65.40; H, 11.59. Found: C, 65.31; H, 11.89. **20**: a colorless oil. ^{1}H-NMR δ: 0.079, 0.084 (3H each, s, SiMe), 0.88 (3H, t, J=7.3 Hz, Me), 0.90 (9H, s, Me_3C), 3.47 (1H, m, H_a-1″), 3.60 (1H, m, H-1′), 3.75 (1H, br d, J=6.0 Hz, H_b-1″), 3.96 (1H, m, H-2), 4.07 (1H, m, H-5). ^{13}C-NMR δ: −4.5, −4.3, 14.1, 18.3, 22.6, 25.6, 25.9 (×3), 27.3, 27.7, 29.5, 31.8, 34.0, 65.3, 74.7, 79.4, 81.3. FAB-MS m/z: 331 (MH^+), 329. *Anal.* Calcd for $C_{18}H_{38}O_3Si$: C, 65.40; H, 11.59. Found: C, 65.36; H, 11.77.

***threo/trans*-(5-Formyl)-2-(1-*tert*-butyldimethylsilyloxyheptyl)tetrahydrofuran (21)** Swern oxidation of **19** (125 mg, 0.41 mmol) in the same way as described for **15** afforded a crude aldehyde (140 mg) as a colorless oil. IR ($CHCl_3$, cm^{-1}): 2940, 2860, 1730, 1465, 1255, 1075, 840. ^{1}H-NMR δ: 0.07, 0.08 (3H each, s, SiMe), 0.88 (3H, t, J=7.0 Hz, Me), 0.89 (9H, s, Me_3C), 3.61 (1H, m, H-1′), 4.07 (1H, m, H-2), 4.29 (1H, m, H-5), 9.66 (1H, d, J=2.0 Hz, CHO). ^{13}C-NMR δ: −4.5, −4.3, 14.1, 18.2, 22.6, 25.6, 25.9 (×3), 27.0, 27.5, 29.5, 31.8, 33.1, 74.5, 83.1, 85.0, 203.1. HR-FAB-MS Calcd for $C_{18}H_{37}O_3Si$ (MH^+; m/z): 329.2512. Found: 329.2475

***threo/cis*-(5-Formyl)-2-(1-*tert*-butyldimethylsilyloxyheptyl)tetrahydrofuran (22)** Oxidation of **20** (356 mg, 1.08 mmol) in the same manner as described above gave a crude aldehyde **22** (388 mg) as a colorless oil. IR ($CHCl_3$, cm^{-1}): 2940, 2860, 1730, 1465, 1255, 1075, 840. ^{1}H-NMR δ: 0.07 (6H, s, Me_2Si), 0.88 (3H, t, J=7.0 Hz, Me), 0.89 (9H, s, Me_3C), 3.62 (1H, m, H-1′), 4.07 (1H, m, H-2), 4.24 (1H, td, J=7.0, 2.0 Hz, H-5), 9.72 (1H, d, J=2.0 Hz, CHO). ^{13}C-NMR δ: −4.5, −4.3, 14.1, 18.2, 22.6, 25.5, 26.0 (×3), 27.2, 28.2, 29.5, 31.8, 33.7, 74.7, 83.3 (×2), 204.1. HR-FAB-MS Calcd for $C_{18}H_{37}O_3Si$ (MH^+; m/z): 329.2512. Found: 329.2475.

***threo/trans/threo*- (10) and *erythro/trans/threo*-2,5-Di-(1-hydroxyheptyl)tetrahydrofurans (12)** The crude aldehyde **21** (125 mg) was reacted with *n*-hexylmagnesium bromide in the same way as described for the preparation of **6** and **8** to give a crude product (104 mg). This was separated on a silica gel Lobar column with hexane–ether (7 : 1) to give the more mobile **23** (40 mg, 26% from **19**) and the less mobile **24** (28 mg, 18% from **19**). **23**: a colorless oil. ^{1}H-NMR δ: 0.06, 0.08 (3H each, s), 0.88 (3H, t, J=7.0 Hz), 0.89 (9H, s), 1.63 (2H, m), 1.92 (2H, m), 2.39 (1H, d, J=3.7 Hz, OH), 3.33 (1H, m), 3.55 (1H, m), 3.76 (1H, m), 3.86 (1H, m). HR-FAB-MS Calcd for $C_{24}H_{51}O_3Si$ (MH^+; m/z): 415.3607. Found: 415.3642. **24**: a colorless oil. ^{1}H-NMR δ: 0.05 (3H, s), 0.07 (3H, s), 0.88 (6H, t, J=7.0 Hz), 0.89 (9H, s), 3.54 (1H, m), 3.78 (1H, m), 3.84 (1H, m), 3.92 (1H, m). HR-FAB-MS Calcd for $C_{24}H_{51}O_3Si$ (MH^+; m/z): 415.3607. Found: 415.3652. A solution of **23** (40 mg, 0.097 mmol) and 0.2 ml of TBAF (1.0 M solution in THF) in THF (0.5 ml) was stirred at reflux for 12 h. Extractive (ether) work-up gave a crude product, which was chromatographed on silica gel with hexane–AcOEt (2 : 1) to give **10** (26 mg, 90%) as a white solid, mp 46—49 °C. IR ($CHCl_3$, cm^{-1}): 3450, 2920, 1465, 1065. ^{1}H-NMR δ: 0.88 (6H, t, J=7.1 Hz, Me), 1.66 (2H, m, H_a-3, H_a-4), 1.98 (2H, m, H_b-3, H_b-4), 3.41 (2H, m, H-2, -5), 3.79 (2H, q, J=8.3 Hz, H-1′, -1″). ^{13}C-NMR data are shown in Table II. FAB-MS m/z: 301 (MH^+), 299. HR-FAB-MS Calcd for $C_{18}H_{37}O_3$ (MH^+; m/z): 301.2743. Found: 301.2717.

Desilylation of **24** (28 mg, 0.068 mmol) in the same way as described for **21** afforded **12** (18 mg, 85%) as white crystals, mp 63—64 °C. IR ($CHCl_3$, cm^{-1}): 3570, 3450, 2930, 2850, 1465, 1065. ^{1}H-NMR δ: 0.88 (6H, t, J=7.3 Hz, Me), 1.64 (1H, m, H_a-3), 1.86 (1H, m, H_a-4), 1.91 (1H, m, H_b-4), 2.00 (1H, m, H_b-3), 3.40 (1H, m, H-1′), 3.82 (2H, m, H-2, -1″), 3.88 (1H, m, H-5). ^{13}C-NMR data are shown in Table II. *Anal.* Calcd for $C_{18}H_{36}O_3$: C, 71.95; H, 12.08. Found: C, 72.10; H, 11.80.

***threo/cis/threo*- (11) and *erythro/cis/threo*-2,5-Di-(1-hydroxyheptyl)tetrahydrofurans (13)** Grignard addition of the crude aldehyde **22** (356 mg, 1.08 mmol) in the same way as described for **21** afforded a mixture of **25** and **26**, which was separated into the less mobile **25** (42 mg, 9% from **20**) and the more mobile **26** (164 mg, 37% from **20**) on a silica gel Lobar column with hexane–ether (5 : 1). **25**: a colorless oil. ^{1}H-NMR δ: 0.07 (3H, s), 0.08 (3H, s), 0.88 (6H, t, J=7.0 Hz), 0.90 (9H, s), 2.66 (1H, d, J=6.0 Hz, OH), 3.37 (1H, m), 3.58 (1H, m), 3.78 (1H, m), 3.92 (1H, m). *Anal.* Calcd for $C_{24}H_{50}O_3Si$: C, 69.50; H, 12.15. Found: C, 69.65; H, 12.22. FAB-MS m/z: 415 (MH^+), 413. **26**: a colorless oil. IR ($CHCl_3$, cm^{-1}): 3480, 2930, 2860, 1465, 1255, 1065, 840. ^{1}H-NMR δ: 0.07 (3H, s), 0.08 (3H, s), 0.88 (6H, t, J=7.0 Hz), 0.90 (9H, s), 2.63 (1H, br s), 3.59 (1H, m), 3.80—3.97 (3H, m). FAB-MS m/z: 415 (MH^+), 413. *Anal.* Calcd for $C_{24}H_{50}O_3Si$: C, 69.50; H, 12.15. Found: C, 69.75; H, 12.32.

Desilylation of **25** (42 mg, 0.10 mmol) in the same way as described for **23** (the reaction was carried out at room temperature instead of at reflux) afforded **11** (26 mg, 85%) as a white solid, mp 32—35 °C. IR ($CHCl_3$, cm^{-1}): 3590, 3450, 2940, 2860, 1465, 1070, 840. ^{1}H-NMR δ: 0.88 (6H, t, J=7.3 Hz, Me),1.74 (2H, m, H_a-3, H_a-4), 1.93 (2H, m, H_b-3, H_b-4), 3.42 (2H, q, J=5.5 Hz, H-1′, -1″), 3.82 (2H, q, J=5.5 Hz, H-2, -5). ^{13}C-NMR data are shown in Table II. *Anal.* Calcd for $C_{18}H_{36}O_3$: C, 71.95; H, 12.08. Found: C, 72.18; H, 11.94. FAB-MS m/z: 301 (MH^+), 301, 283, 265.

Desilylation of **26** (154 mg, 0.37 mmol) in the same way as described for **25** gave **13** (106 mg, 95%) as a white solid, mp 50—52 °C. IR ($CHCl_3$, cm^{-1}): 3590, 3440, 2930, 2860, 1465, 1070. ^{1}H-NMR δ: 0.88 (6H, t, J=7.3 Hz, Me), 1.76 (1H, m, H_a-3), 1.80 (1H, m, H_a-4), 1.92 (1H, m, H_b-3), 1.96 (1H, m, H_b-4), 3.44 (1H, m, H-1′), 3.83 (2H, m, H-2, -1″), 3.90 (1H, m, H-5). ^{13}C-NMR data are shown in Table II. ^{13}C-NMR data are shown in Table II. *Anal.* Calcd for $C_{18}H_{36}O_3$: C, 71.95; H, 12.08. Found: C, 71.89; H, 11.81.

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