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4,5-*Seco*-probotryenols A-C, a new type of sesquiterpenoids from *Stachybotrys bisbyi*

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4,5-*Seco*-probotryenols A–C (1–3), a new type of sesquiterpenoids named *seco*-probotryane-type sesquiterpenoid, have been obtained from *Stachybotrys bisbyi* (PYH05-7), along with five new botryane skeleton sesquiterpenoids (4, 5, and 8–10), and two known ones (6 and 7). Their structures were determined by NMR analyses, chemical derivatization, and X-ray crystallography.

Introduction

Probotryane (or presilphiperfolane)-type sesquiterpenoids possess a 5/5/6 tricyclic system skeleton (Fig. 1),[1] and they were first isolated from the flowering plants *Eriophyllum staechadifolium* and *Flourensia heterolepis*.[2] Due to its special molecular structure, this carbon skeleton serves as the important branch point for the biosynthesis of many sesquiterpene natural products.[3] The classification of the downstream sesquiterpenoids could depend on ring rearrangement or ring cleavage of the probotryane skeleton. Polycyclic sesquiterpenoids, such as silphiperfolane, cameroonane, nopsane, prenopsane, isocomane, silphinane, terrecyclane, and modhephane (Fig. 1), are the downstream products from the rearrangement of the probotryane skeleton. Botryane sesquiterpenoids are generated from the cleavage of the probotryane skeleton C-10/C-15.[4] Botryane sesquiterpenoids possessing a bicyclic skeleton (6/5), are usually identified from the fungi of *Botrytis cinerea*[5-11] *Boletus edulis*,[12] *B. squamosa*,[13] *Daldinia concentrica*,[14] *Geniculosporium* sp.,[15] and *Hypocrea* sp.[16] and they exhibit phytotoxicity,[9, 11, 17] cytotoxicity,[14, 15] and antibiotic activity.[12]

In the course of our search for bioactive secondary metabolites from wetland fungi,[18] the chemical investigation of metabolites from *Stachybotrys bisbyi* (PYH05-7) was carried out, which led to the isolation of three unusual sesquiterpenoids [4,5-*seco*-probotryenols A–C (**1**–**3**)] and five new botryane sesquiterpenoids [dehydrobotrylactone (**4**), 12-acetoxydehydrobotrydienol (**5**), and dehydrobotryosides A–C (**8**–**10**)] along with two known ones [12-hydroxydehydrobotrydienol (**6**) and dehydrobotrydienol (**7**)]. Notably, 4,5-*seco*-probotryenols A–C (**1**–**3**) are a new type of sesquiterpenoid and are derived from cleavage of the probotryane skeleton at C-4/C-5. This new type is named *seco*-probotryane-type sesquiterpenoid. In addition, dehydrobotryosides A–C (**8**–**10**) are the first glycosylation derivatives of botryane sesquiterpenoids. Details of the isolation, elucidation of the structural and absolute configurations, antibiotic activity, and cytotoxicity of all isolated compounds are reported herein.

Results and discussion

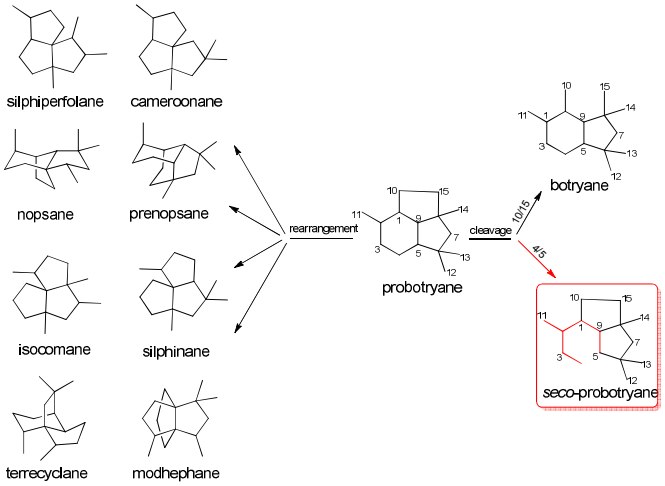

Fig. 1 Natural sesquiterpenoids derived from the probotryane skeleton.

4,5-*Seco*-probotryenol A (**1**) was obtained as a colorless crystal. Its molecular formula was determined to be $C_{15}H_{26}O_2$ by HR-ESI-MS, with 3 degrees of unsaturation. The ^{13}C NMR and DEPT-135 spectra showed 15 carbon signals ascribable to one sp^2 methine, one sp^2 quaternary carbon, two sp^3 quaternary carbons, three sp^3 methines [including an oxygenated one (δ_C 80.9)], four sp^3 methylenes [including an oxygenated one (δ_C 61.3)], and four sp^3 methyls (δ_C 31.3, 30.5, 19.9, and 17.9, respectively). An analysis of the ^1H-^1H COSY data revealed the presence of one isolated spin-system corresponding to a C-4−C-3−C-2(C-11)−C-1−C-10−C-15 subunit (Fig. 3). Combined with the ^1H-^1H COSY analysis and the degrees of unsaturation, the HMBC correlations from H_3-12 to C-5/C-6/C-7/C-13, from H_3-13 to C-5/C-6/C-7/C-12, from H-5 to C-1/C-6/C-7/C-8/C-9, from H_3-14 to C-7/C-8/C-9/C-15, from Ha-10/Hb-10 to C-1/C-8/C-9/C-15, and from H_3-11 to C-1/C-2/C-3 deduced the planar structure of **1** as shown in Fig. 3, and the assignments of all proton and carbon resonances are provided in Table 1. In the NOESY experiment, the observed correlations between H_3-14 and Ha-10/H-2, and between H-15 and H-1, signified that H_3-14, Ha-10 and H-2 were located on the same face of the cyclopentane ring (C-1–C-9–C-8–C-15–C-10), while H-15 and H-1 were on the other face. Furthermore, the NOESY correlations

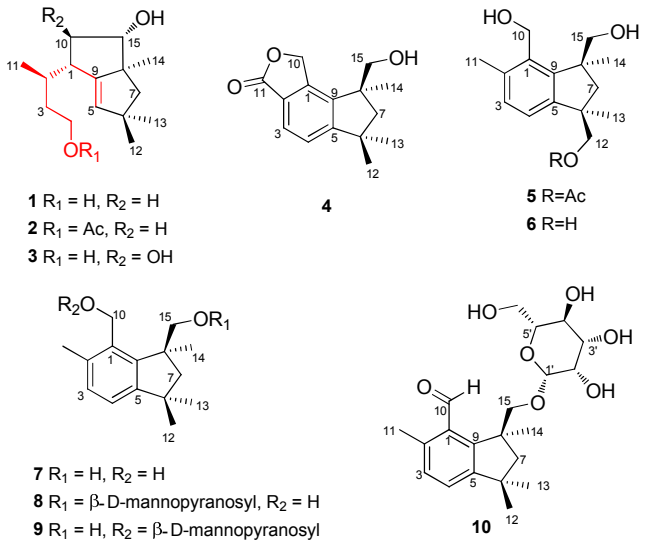

Fig. **2** Chemical structures of **1** – **10**

between Hb-7 and H_3-13/H_3-14, and between Ha-7 and H_3-12/H-15, signified that H_3-13, Hb-7 and H_3-14 were located on the same face of the cyclopentene ring (C-5–C-6–C-7–C-8–C-9), while Ha-7, H_3-12, and H-15 were on the other face. On the basis of the above analyses, the relative configurations of C-1, C-8, and C-15 in **1** were established as 1*S**, 8*S**, and 15*S**, respectively. The NOESY correlations between H_3-11 and Ha-10/Hb-10/H-1, and between Ha-3/Hb-3 and H-1/H-5 (Fig. 4), indicated that the relative configurations of C-1, C-2 were 1*S**, 2*R**. A single-crystal X-ray diffraction experiment unambiguously confirmed the above deduction. Furthermore, the Flack parameter of 0.02 (18) allowed the assignment of the absolute configuration of **1** was 1*S*, 2*R*, 8*S*, 15*S* (Fig. 5).

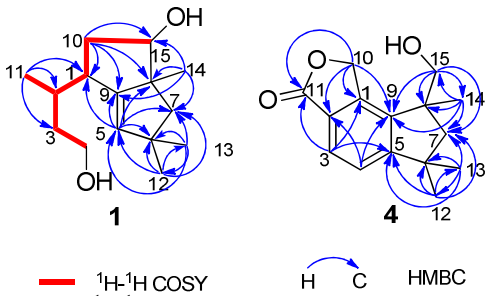

Fig. 3 Key ^{1}H–^{1}H COSY and HMBC correlations of **1** and **4**

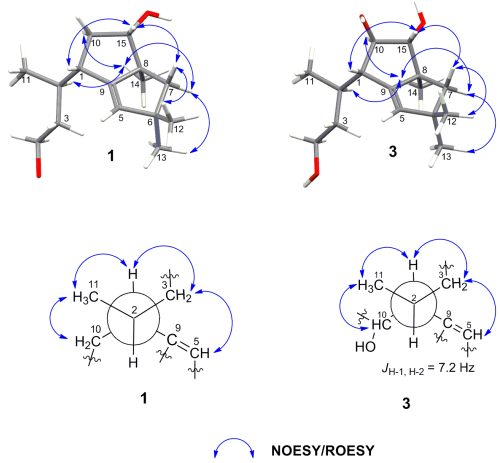

Fig. 4 Key NOESY/ROESY correlations of **1** and **3**

4,5-*Seco*-probotryenol B (**2**) was obtained as a colorless oil. Its molecular formula was determined to be $C_{17}H_{28}O_3$ by HR-ESI-MS. The molecular weight of **2** was 42 atomic mass units more than **1**, which indicated that **2** may be an acetylated derivative of **1**. Except for the signals for one ester carbonyl (δ_C 171.2) and one methyl (δ_C 21.0/δ_H 2.04), the NMR data (in $CDCl_3$) of **2** were similar to **1**. The key HMBC correlations from Ha-4/Hb-4 to the additional ester carbonyl (δ_C 171.2), and from the additional methyl protons (δ_H 2.04) to the ester carbonyl (δ_C 171.2) indicated the existence of the acetyl group and the acetylation at C-4. The planar structure of **2** was further established by the 2D NMR analyses (see the supporting information Table S2), and the assignments of all proton and carbon resonances are provided in Table 1. The similar observed NOESY correlations (see the supporting information Table S2) of **2** and **1** suggested that their relative configurations were identical. Since **2** and **1** coexist in the same strain, the absolute configurations of C-1, C-2, C-8, and C-15 in **2** should be the same as those in **1**. To confirm the above deduction, the derivatization of **2** was carried out. After hydrolysis in 2% sodium methanolate-methanol solution, compound **2** was transformed into **1**. Thus, the absolute configuration of **2** was assigned as 1*S*, 2*R*, 8*S*, 15*S*, which was the same as that of **1**.

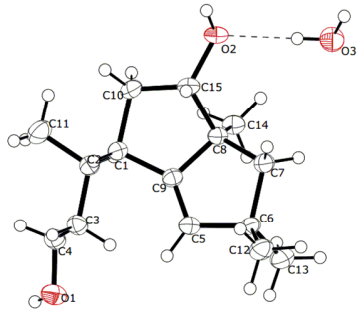

Fig. **5** X-ray structure of **1**

4,5-*Seco*-probotryenol C (**3**) was obtained as a colorless oil. Its molecular formula was determined to be $C_{15}H_{26}O_3$ by HR-ESI-MS (*m/z* 277.1781, $[M + Na]^+$), indicating that **3** has one more oxygen atom than **1**. Except for an oxygenated methine carbon at δ_C 83.1 and the loss of a methylene carbon at δ_C 40.9, the NMR spectroscopic data of **3** (Table 1) were similar to those of **1**, indicating **3** was a hydroxylated derivative of **1**. The key ^{1}H–^{1}H COSY correlation between H-10 (δ_H 4.00, t, J = 7.6 Hz) and H-15 (δ_H 3.48, d, J = 7.6 Hz), and the HMBC cross-peaks from H-10 to C-1/C-8/C-9/C-15, from H-1 to C-5/C-9/C-10/C-11, and from H-15 to C-7/C-8/C-10/C-14 indicated that the hydroxylation occurred at the C-10 position. The key ROESY correlations between H_3-14 and H-10/H-2, between H-15 and H-1, signified that H_3-14, H-10, and H-2 were located on the same face of the cyclopentane ring (C-1–C-9–C-8–C-15–C-10), while H-15 and H-1 were on the other face. Furthermore, the ROESY correlations between Hb-7 and H_3-13/H_3-14, and between Ha-7 and H_3-12/H-15, signified that H_3-13, Hb-7, and H_3-14 were located on the same face of the cyclopentene ring (C-5–C-6–C-7–C-8–C-9), while H_3-12, Ha-7, and H-15 were on the other face. On the basis of the above analyses, the relative configurations of C-1, C-8, C-10, and C-15 in **3** were established as 1*S**, 8*S**, 10*R**, and 15*R**, respectively (Fig. 4). The coupling constant between H-1 and H-2 ($^3J_{H-1, H-2}$ = 7.2 Hz), the ROESY correlations between H_3-11 and H-10/H-1, and the ROESY correlations between Ha-3/Hb-3 and H-1/H-5 (Fig. 4) suggested that the relative configurations of C-1, C-2 were 1*S**, 2*R**. Considering the co-occurrence of **1**–**3** in the fungus *S. bisbyi* (PYH05-7) the absolute configuration of **3** was assigned as 1*S*, 2*R*, 8*S*, 10*R*, 15*R*.

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Table 1 ^{1}H (400 Hz) NMR and ^{13}C NMR (100 Hz) data for **1**–**4** ($CDCl_3$)

No.	**1**		**2**		**3**		**4**	
	δ_C	$^{*}\delta_H$ (J in Hz)	δ_C	$^{*}\delta_H$ (J in Hz)	δ_C	$^{*}\delta_H$ (J in Hz)	δ_C	$^{*}\delta_H$ (J in Hz)
1	42.0	2.10	41.7	2.10	49.6	2.14, t (7.2)	143.4	
2	35.7	1.46	35.9	1.45	33.4	1.69	125.2	
3	38.1	1.95, a 1.29, b	33.7	1.99, a 1.34, b	37.2	1.86, a 1.36, b	124.9	7.76, d (8.0)
4	61.3	3.73, a 3.65, br dt (10.4, 7.5), b	63.0	4.15, ddd (10.8, 8.0, 5.3), a 4.05, dt (10.8, 7.6), b	61.0	3.73, a 3.65, br dt (10.3, 7.4), b	123.9	7.27, d (8.0)
5	134.8	5.13, s	134.9	5.13, s	135.2	5.18, s	159.5	
6	50.0		50.0		49.1		43.4	
7	53.7	1.65, d (12.8), a (β) 1.61, d (12.8), b (α)	53.6	1.65, d (12.8), a (β) 1.61, d (12.8), b (α)	54.3	1.68, d (12.4), a (β) 1.58, d (12.4), b (α)	51.6	2.11, d (13.6), a (β) 1.87, d (13.6), b (α)
8	57.7		57.6		56.0		48.8	
9	151.4		151.2		147.7		141.3	
10	40.9	2.36, ddd (12.8, 8.1, 7.4), a (α) 1.60, b (β)	40.9	2.37,ddd (12.8, 8.0, 7.6), a (α) 1.59, b (β)	83.1	4.00, t (7.6)	69.5	5.51, d (15.6), a 5.34, d (15.6), b
11	17.9	0.88, d (6.4)	17.7	0.89, d (6.4)	17.9	1.00, d (6.8)	171.5	
12	31.3	1.05, s	31.3	1.05, s	31.4	1.06, s	31.2	1.36, s
13	30.5	1.18, s	30.4	1.18, s	30.3	1.15, s	31.9	1.36, s
14	19.9	1.14, s	19.9	1.14, s	21.4	1.12, s	23.8	1.39, s
15	80.9	3.73, dd (10.4, 7.2)	80.8	3.74, dd (10.4, 7.2)	86.8	3.48, d (7.6)	70.7	3.71, s
CO			171.2					
CH_3			21.0	2.04, s				

* The indiscernible signals due to overlap or having complex multiplicity are reported without designating their multiplicity

Dehydrobotrylactone (**4**) was obtained as a colorless oil. Its molecular formula was determined to be $C_{15}H_{18}O_3$ by HR-ESI-MS, with 7 degrees of unsaturation. In the ^{1}H NMR data we observed characteristic protons for two aromatic protons [(δ_H 7.76, d, J = 8.0 Hz), (δ_H 7.27, d, J = 8.0 Hz)] and three methyl groups [δ_H 1.39 (s), 1.36 (s), and 1.36 (s)]. The ^{13}C NMR and DEPT-135 spectra (Table 1) showed 15 carbon signals including one carbonyl carbon (δ_C 171.5), six aromatic carbons (δ_C 159.5, 143.4, 141.3, 125.2, 124.9, and 123.9), three sp^3 methylene carbons, two sp^3 quaternary carbons, and three methyls (δ_C 31.9, 31.2, and 23.8). The above information indicated that **4** possessed a botryane skeleton. Combined with the molecular formula and chemical shifts, the HMBC correlations (MeOD) from H-3 to C-1/C-5/C-11, from H-4 to C-2/C-9, from H_3-12 to C-5/C-6/C-7/C-13, from H_3-13 to C-5/C-6/C-7/C-12, from H_3-14 to C-7/C-8/C-9/C-15, from Ha-15/Hb-15 to C-7/C-8/C-9/C-14, and from Ha-10/Hb-10 to C-1/C-2/C-9/C-11 deduced the planar structure of **4** as shown in Fig. 3. Since **4** and dehydrobotrydienol (**7**) coexist in the same fungus, the absolute configurations of C-8 in **4** should be the same as **7**. The X-ray crystallography analysis (Fig. 6) of **7** suggested the absolute configuration of C-8 is *S*, which was consistent with Collado's deduction from chemical derivatization of dehydrobotrydienal.[9] Thus, the absolute configuration of **4** was identified as 8*S*.

12-Acetoxydehydrobotrydienol (**5**) was obtained as a colorless oil. Its molecular formula was determined to be $C_{17}H_{24}O_4$ by HR-ESI-MS (*m/z* 293.1777, $[M + H]^+$). The ^{1}H NMR data exhibited characteristic protons for two aromatic protons at δ_H 7.12 (d, J = 8.0 Hz) and 7.05 (d, J = 8.0 Hz) and four methyl groups at δ_H 2.43 (s), 2.04 (s), 1.39 (s), and 1.34 (s). The ^{13}C NMR and DEPT-135 spectra showed 17 carbon signals including one ester carbonyl carbon, six aromatic carbons, four sp^3 methylene carbons, two sp^3 quaternary carbons, and four methyl carbons. Except for the presence of an acetyl group (δ_H 2.43, 3H, s; δ_C 171.3, and 18.9), the NMR data of **5** were similar to those of 12-hydroxydehydrobotrydienol (**6**)[9] suggesting that **5** might be an acetylated derivative of **6**. The HMBC correlation from H_2-12 to $-OCOCH_3$, suggested that the acetoxyl group was at C-12. The planar structure of **5** was further established by the analyses of 2D NMR (see the supporting information Table S6), and the assignments of all proton and carbon resonances are provided in Table 2. In the ROESY experiment, the observed correlations between Ha-7 and H_2-12/Ha-15/Hb-15, and between Hb-7 and H_3-13/H_3-14 indicated that C-12 and C-15 on the same face of the cyclopentene ring (C-5–C-6–C-7–C-8–C-9), while C-13 and C-14 were on the other face. On the basis of the above analyses, the relative configurations of C-6 and C-8 in **5** were established as 6*R** and 8*S**. Since **5** and **6** coexist in the same strain, the absolute

configurations of C-6 and C-8 in **5** should be the same as those in **6**. To confirm the deduction, the derivatization of **5** was carried out. After hydrolysis in 2% sodium methanolate-methanol solution, compound **5** was transformed into **6** whose absolute configuration was determined as 6*R*, 8*S* by X-ray crystallographic analysis (Fig. 6). Thus, the absolute configuration of **5** was identified as 6*R*, 8*S*.

The molecular formula of **8** was in accordance with $C_{21}H_{32}O_7$ as determined by HR-ESI-MS data. The ^{1}H NMR data exhibited characteristic protons for two aromatic protons at δ_H 7.07 (d, J = 7.6 Hz) and 6.97 (d, J = 7.6 Hz), one anomeric proton signal at 4.42 (1H, br s), and four methyls at δ_H 2.41 (s), 1.49 (s), 1.27 (s), and 1.25 (s). The ^{13}C NMR spectrum (Table 2) of **8** showed 21 carbon signals including six aromatic carbons (δ_C 151.1, 145.0, 137.5, 135.9, 130.4, and 122.4), two sp^3 quaternary carbons, three sp^3 methylenes [including two oxygenated ones (δ_C 58.7, 78.4)], four methyls (δ_C 32.1, 31.7, 26.9, and 19.3), and a set of sugar moiety carbons (δ_C 100.9, 71.0, 73.8, 66.1, 76.1, and 60.5). The

Table 2 ^{1}H NMR and ^{13}C NMR data for **5** and **8**–**10** ($CDCl_3$).

No.	**5**[a]		**8**[b]		**9**[a]		**10**[a]	
	δ_C	*δ_H (J in Hz)	δ_C	*δ_H (J in Hz)	δ_C	*δ_H (J in Hz)	δ_C	*δ_H (J in Hz)
1	134.8		135.9		130.7		132.8	
2	137.5		137.5		137.3		138.5	
3	130.4	7.12, d (8.0)	130.4	7.07, d (7.6)	130.0	7.06, d (7.6)	131.6	7.10, d (7.6)
4	123.5	7.05, d (8.0)	122.4	6.97, d (7.6)	123.3	7.02, d (7.6)	127.4	7.18, d (7.6)
5	147.3		151.1		152.4		152.0	
6	44.4		40.6		40.8		41.3	
7	49.3	2.37, d (13.2), a (β) 1.72, d (13.2), b (α)	55.0	1.92, d (13.6), a (β) 1.84, d (13.6), b (α)	53.1	2.28, d (13.2), a (β) 1.66, d (13.2), b (α)	53.8	2.19, d (13.6), a (β) 1.84, d (13.6), b (α)
8	50.4		48.4		50.2		49.1	
9	145.1		145.0		145.5		148.3	
10	58.3	4.77, d (11.6), a 4.71, d (11.6), b	58.7	5.02, d (11.6), a 4.57, d (11.6), b	65.2	5.08, d (10.0), a 4.46, d (10.0), b	195.8	10.61, s
11	20.9	2.04, s	19.3	2.41, s	19.2	2.33, s	21.2	2.49, s
12	72.4	4.09, s	32.1	1.25, s	32.3	1.27, s	31.3	1.27, s
13	27.1	1.34, s	31.7	1.27, s	31.1	1.27, s	32.0	1.29, s
14	26.9	1.39, s	26.9	1.49, s	26.4	1.30, s	26.9	1.51, s
15	70.9	3.92, d (11.6), a 3.64, d (11.6), b	78.4	4.06, d (9.4), a 3.45, d (9.4), b	69.8	3.99, d (11.2), a 3.52, d (11.2), b	77.5	4.08, d (9.6), a 3.60, d (9.6), b
CO	171.3							
CH_3	18.9	2.43, s						
1′			100.9	4.42, br s	100.1	4.49, br s	100.7	4.39, br s
2′			71.0	4.01, br s	71.1	3.80, br s	70.9	3.93, br s
3′			73.8	3.37, br d (7.4)	73.7	3.30	73.9	3.47, br d (8.4)
4′			66.1	3.74, t (8.3)	65.7	3.82	66.6	3.80
5′			76.1	2.94, br d (8.4)	76.2	3.07	76.0	3.09, br d (8.4)
6′			60.5	3.65, br d (11.4), a 3.49, d (11.4), b	60.7	3.82, a 3.72, d (12.0), b	61.1	3.80, a 3.72, d (11.2), b

[a] The data were recorded with a Bruker AV 400 spectrometer (^{1}H for 400 MHz, ^{13}C for 100 MHz).
[b] The data were recorded with a Bruker AV 600 spectrometer (^{1}H for 600 MHz, ^{13}C for 150 MHz).
* The indiscernible signals due to overlap or having complex multiplicity are reported without designating their multiplicity

structure of aglycone was established from the 1D and 2D NMR data (see the supporting information Table S8), and this was the same as that of dehydrobotrydienol (**7**).[8] In the sugar moiety of **8**, the *trans*-axial arrangement for H-3′, H-4′, and H-5′ was deduced from the large coupling constants ($^3J_{H\text{-}3',\ H\text{-}4'}$ = 8.3 Hz and $^3J_{H\text{-}4',\ H\text{-}5'}$= 8.4 Hz), whereas the *cis* relationship for H-2′ and H-3′ was evident on the basis of a small coupling constant. From the analysis of coupling constants and comparison with the previously reported ^{13}C NMR data of mannopyranose,[19] the sugar moiety of **8** was identified as mannopyranose (Man). After acid hydrolysis and derivatization of **8**, HPLC analysis revealed the presence of D-mannose as compared with derivatives obtained by the same method with standard monosaccharides[20] The Man unit in **8** was attached to the aglycone via a β-linkage on the basis of the mutual ROESY correlations between H-1′ and H-3′/H-5′. The connection position of the sugar to aglycone was established at C-15 on the basis of the HMBC correlation from H-1′ to C-15. Therefore, the structure of **8** was elucidated as 15-*O*-*β*-D-mannopyranosyl-dehydrobotrydienol, named dehydrobotryoside A.

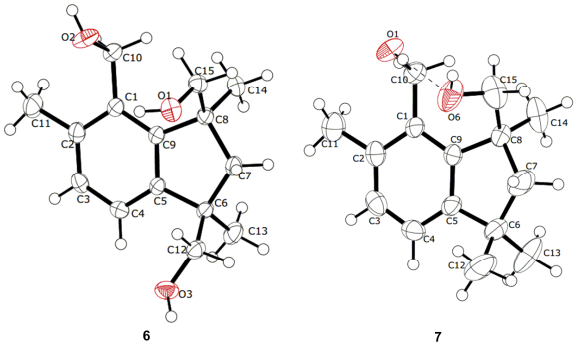

Fig. **6** X-ray structures of **6** and **7**.

Dehydrobotryoside B (**9**) was afforded as a colorless oil. The molecular formula was determined to be $C_{21}H_{32}O_7$ by HR-ESI-MS. The NMR data of **9** also showed the presence of a mannopyranose moiety and a dehydrobotrydienol aglycone, which was the same as **8**. The linkage of the mannopyranose moiety with the aglycone was established at C-10 on the basis of the HMBC correlation from H-1′ to C-10. After acid hydrolysis and derivatization of **9**, HPLC analysis revealed the presence of D-mannose as compared with derivatives obtained by the same method with standard monosaccharides.[20] The Man unit in **9** was attached to the aglycone via a β-linkage on the basis of the mutual NOESY correlations between H-1′ and H-3′/H-5′. Therefore, the structure of **9** was elucidated as 10-*O*-*β*-D-mannopyranosyl-dehydrobotrydienol, named dehydrobotryoside B.

Dehydrobotryoside C (**10**) was obtained as a colorless oil. Its molecular formula was determined to be $C_{21}H_{30}O_7$ by HR-ESI-MS data. The ^{1}H NMR data exhibited characteristic protons for one aldehyde hydrogen proton at δ_H 10.61 (s), two aromatic protons at δ_H 7.18 (d, J = 7.6 Hz) and 7.10 (d, J = 7.6 Hz), one anomeric proton signal at 4.39 (1H, br s), and four methyl groups at δ_H 2.49 (s), 1.51 (s), 1.29 (s), and 1.27 (s). The ^{13}C NMR and DEPT-135 spectra showed 21 carbon signals, including one aldehyde carbonyl carbon (δ_C 195.8), six aromatic carbons (δ_C 152.0, 148.3, 138.5, 132.8, 131.6, and 127.4), two sp^3 methylene carbons [including an oxygenated one (δ_C 77.5)], two sp^3 quaternary carbons, four methyls (δ_C 32.0, 31.3, 26.9, and 21.2), and a set of sugar moiety carbons (δ_C 100.7, 70.9, 73.9, 66.6, 76.0, and 61.1). The ^{1}H and ^{13}C NMR spectra of the aglycone were similar to those of dehydrobotrydienol (**7**), except that an oxygenated methylene carbon in dehydrobotrydienol was replaced by an aldehyde carbon (δ_C 195.8) in **10**. This assumption was confirmed by 2D NMR spectra analyses. The HMBC correlations from H-10 to C-1/C-2 determined the aldehyde group was located at the C-1 position. The sugar moiety was determined to be mannopyranose on the basis of its ^{13}C NMR chemical shifts and ^{1}H-^{1}H coupling constants compared with the previously reported NMR data.[19] The connection of the mannopyranose with the aglycone was established at C-15 on the basis of the HMBC correlation from H-1′ to C-15. After acid hydrolysis and derivatization of **10**, HPLC analysis revealed the presence of D-mannose as compared with derivatives obtained by the same method with standard monosaccharides.[20] The Man unit in **10** was attached to the aglycone via a β-linkage on the basis of the mutual ROESY correlations between H-1′ and H-3′/H-5′. Therefore, the structure of **10** was elucidated as 15-*O*-*β*-D-mannopyranosyl-dehydrobotryenalol, named dehydrobotryoside C.

All isolated compounds were subjected to biological assays for antimicrobial activity against two bacteria (*Staphylococcus aureus* 209P and *Escherichia coli* ATCC0111) and two fungi (*Candida albicans* FIM709 and *Aspergillus niger* R330). In addition, all isolated compounds were evaluated by the MTT method for their cytotoxicity against five tumor cell lines, including HL-60, SMMC-7721, A-549, MCF-7, and SW480, with cisplatin and paclitaxel as the positive controls. However, no compounds showed activity.

Conclusions

In summary, ten sesquiterpenoids were isolated from *S. bisbyi*, including eight new ones. Among them, 4,5-*seco*-probotryenols A–C (**1**–**3**) are a new type of sesquiterpenoids named *seco*-probotryane sesquiterpenoid, which possess a bicyclic skeleton (5/5) derived from probotryane skeleton cleaved at C-4/C-5. In the downstream products of probotryane sesquiterpenoids, most members result from ring rearrangement. For now, only botryane sesquiterpenoids are derived from ring cleavage at C-10/C-15. The identification of 4,5-*seco*-probotryenols A–C (**1**–**3**) signifies a new type of sesquiterpenoids. In addition, no glycosylation derivative of botryane sesquiterpenoid has been identified. Dehydrobotryosides A–C (**8**–**10**) are the first botryane glycosides. Furthermore, this is the first report about botryane skeleton sesquiterpenoids from *Stachybotrys* sp..

Experimental

General experimental procedures

The optical rotations were measured on a JASCO P-1020 polarimeter with a 1 cm cell at room temperature. The UV spectra were recorded on a JASCO V-550 UV/Vis spectrometer. The IR spectra were obtained using a JASCO FT/IR-480 plus spectrometer. The HR-ESI-MS spectra were acquired using a Waters Synapt G2 mass spectrometer. The NMR spectra were measured with Bruker AV-400 and Bruker AV 600 spectrometers using solvent signals ($CDCl_3$: δ_H7.26/δ_C77.0; CD_3OD: δ_H3.30/δ_C49.0) as internal standard. The analytical HPLC was performed on a Dionex HPLC system equipped with an Ultimate 3000 pump, an Ultimate 3000 diode array detector (DAD), an Ultimate 3000 Column Compartment, an Ultimate 3000 autosampler (Dionex, USA) and an Alltech (Grace) 2000 ES evaporative light scattering detector (ELSD) (Alltech, USA) using a reversed-phase C18 column (5 μm, 4.6 × 250 mm; Phenomenex, USA). The preparative HPLC was performed on a Shimadzu LC-6-AD Liquid Chromatography system with an SPD-20A Detector (Shimadzu, Japan) using a reversed-phase C18 column (5 μm, 21.2 × 250 mm; Phenomenex, USA). The semi-preparative HPLC was carried out on a Shimadzu LC-6AD Liquid Chromatography system with a SPD-20A Detector (Shimadzu, Japan) using a reversed-phase C18 column (5 μm, 10 × 250 mm; YMC, Japan). Column chromatography (CC) was carried out on Sephadex LH-20 (Pharmacia, USA) and ODS (50 μm, YMC, Japan), respectively. TLC was performed on precoated silica gel plates (SGF254, 0.2 mm, Yantai Chemical Indus-try Research Institute, China).

Fungus Material.

The strain PYH05-7 was isolated from the mud, collected at Poyang Lake, Jiangxi Province, China. The strain was identified as *Stachybotrys bisbyi* based on morphological characteristics, its ribosomal internal transcribed spacer (ITS), and the 5.8S rRNA gene (ITS1-5.8S-ITS2) sequence data (GenBank accession no. KP256004). The fungus was cultured on slants of potato dextrose agar at 25°C for 5 days. Agar plugs were used to inoculate four Erlenmeyer flasks (250 mL), each containing 100 mL of potato dextrose broth. Four flasks of the inoculated media were incubated at 25°C on a rotary shaker at 200 rpm for 5 days to prepare the seed culture. Fermentation was carried out in 22 Erlenmeyer flasks (500 mL), each containing 70 g of rice.

Distilled H_2O (105 mL) was added to each flask, and the rice was soaked overnight before autoclaving at 120°C for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at room temperature for 51 days.

Extraction and Isolation.
The culture was extracted three times with EtOAc, and the organic solvent was evaporated under vacuum to afford the dry crude extract (45.8 g). The crude extract was dissolved in 90% v/v aqueous MeOH (500 mL) and partitioned against the same volume of cyclohexane to afford a cyclohexane fraction (C, 15.3 g) and an aqueous MeOH fraction (W, 27.4 g). The aqueous MeOH fraction (W, 27.4 g) was separated by ODS CC eluting with MeOH−H_2O (30:70, 50:50, 70:30, and 100:0, v/v) to afford four fractions (W1 to W4). The fraction W2 (5.9 g) was further separated by ODS CC with a gradient of MeOH−H_2O (40%−65%, v/v) to yield eight fractions (W2a to W2h). Fraction W2c (319.2 mg) was subjected to Sephadex LH-20 CC using MeOH and purified with semi-preparative HPLC using CH_3CN−H_2O (27:73, v/v) at flow rate of 4 mL/min to yield **6** (40.0 mg). Fraction W2d (1.34 g) was subjected to Sephadex LH-20 CC with MeOH yield three fractions (W2d1 to W2d3). Fraction W2d2 (751.7 mg) was purified by semi-preparative HPLC with CH_3CN−H_2O (30:70, v/v) at flow rate of 4 mL/min to yield **3** (38.3 mg). Fraction W2e (352.2 mg) was subjected to Sephadex LH-20 CC with MeOH and purified with preparative HPLC using CH_3CN−H_2O (30:70, v/v) at flow rate of 8 mL/min to yield **1** (5.3 mg), **4** (8.3 mg), **5** (15.1 mg), **8** (20.3 mg), **9** (26.7 mg), and **10** (29.8 mg). Fraction W2f (1.35 g) was separated by Sephadex LH-20 CC with MeOH to afford four fractions (W2f1 to W2f4). Fraction W2f4 (168.7 mg) was subjected to semi-preparative HPLC with CH_3CN−H_2O (32:68, v/v) at a flow rate of 4 mL/min to yield **7** (15.3 mg). The fraction W3 (5.5 g) was further separated by ODS CC using a MeOH−H_2O gradient elution (55%−75%, v/v) to yield six fractions (W3a to W3f). Fraction W3b (1.12 g) was subjected to Sephadex LH-20 CC using MeOH and purified with semi-preparative HPLC using CH_3CN−H_2O (50:50, v/v) at flow rate of 3 mL/min to yield **2** (9.1 mg).

4, 5-*Seco*-probotryenol A (1). colorless crystals; $[\alpha]_D^{27}$ +19.5 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 208 (4.68) nm; IR (KBr) ν_{max} 3270, 2956, 2874, 1457, 1359, 1060 cm^{-1}; HR-ESI-MS (positive): *m/z* 261.1833 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1830); ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1.

4, 5-*Seco*-probotryenol B (2). colorless oil; $[\alpha]_D^{27}$ +10.9 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 209 (3.61) nm; IR (KBr) ν_{max} 3299, 2965, 1457, 1367, 1247, 1076 cm^{-1}; HR-ESI-MS (positive): *m/z* 303.1934 $[M + Na]^+$ (calcd for $C_{17}H_{28}O_3Na$, 303.1936); ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1.

4, 5-*Seco*-probotryenol C (3). colorless oil; $[\alpha]_D^{27}$ -11.6 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 209 (3.25), 327(2.58) nm; IR (KBr) ν_{max} 3275, 2963, 1727, 1471, 1256, 1067 cm^{-1}; HR-ESI-MS (positive): *m/z* 277.1781 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1780); ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1.

Dehydrobotrylactone (4). colorless oil; $[\alpha]_D^{27}$ -13.2 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 208 (4.69) nm; IR (KBr) ν_{max} 3420, 2842, 1686, 1579, 1243, 1070 cm^{-1} HR-ESI-MS (positive): *m/z* 247.1358 $[M + H]^+$ (calcd. for $C_{15}H_{19}O_3$, 247.1334); ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1.

12-Acetoxydehydrobotrydienol (5). colorless oil; $[\alpha]_D^{27}$ +11.7 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 209 (4.75), 253 (4.16) nm; IR (KBr) ν_{max} 3455, 2955, 1748, 1603, 1471, 1078 cm^{-1}; HR-ESI-MS (positive): *m/z* 293.1777 $[M + H]^+$ (calcd for $C_{17}H_{25}O_4$, 293.1753); ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 2.

12-Hydroxydehydrobotrydienol (6): colorless crystals; $[\alpha]_D^{27}$ +12.0 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 209 (4.30) nm; IR (KBr) ν_{max} 3455, 2955, 1655, 1471, 1078 cm^{-1}; ^1H (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) data, see the supporting information Table S7.

Dehydrobotrydienol (7): colorless crystals; $[\alpha]_D^{27}$ -13.0 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 209 (3.91) nm; IR (KBr) ν_{max} 3356, 2957, 1655, 1460, 1042 cm^{-1}.

Dehydrobotryoside A (8): colorless oil; $[\alpha]_D^{27}$ -26.6 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 209(4.67) nm; IR (KBr) ν_{max} 3398, 2962, 1457, 1367, 1247, 1076 cm^{-1}; HR-ESI-MS (positive): *m/z* 397.2224 $[M + H]^+$ (calcd. for $C_{21}H_{33}O_7$, 397.2226); ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2.

Dehydrobotryoside B (9): colorless oil; $[\alpha]_D^{27}$ -23.5 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 209 (4.69) nm; IR (KBr) ν_{max} 3317, 2882, 1446, 1388, 1236, 1041 cm^{-1}; HR-ESI-MS (positive): *m/z* 793.4368 $[2M + H]^+$ (calcd. for $C_{42}H_{65}O_{14}$, 793.4374); ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 2.

Dehydrobotryoside C (10): colorless oil; $[\alpha]_D^{27}$ -13.7 (c 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 209 (4.69) nm; IR (KBr) ν_{max} 3317, 2882, 1446, 1388, 1236, 1041cm^{-1}; HR-ESI-MS (positive): *m/z* 395.2119 $[M + H]^+$ (calcd. for $C_{21}H_{31}O_7$, 395.2070); ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 2.

Alkaline hydrolysis of 2 and 5
A sample of **2** (1 mg) dissolved in 2% sodium methanolate–methanol (3 mL) was stirred at 50 °C for 12 h. After neutralizing with 10% HCOOH and extracting with EtOAc, the EtOAc layer was evaporated to dryness and dissolved in MeOH. Then the mixture was purified by semi-preparative RP-HPLC with MeOH-H_2O (55:45, v/v) at flow rate of 4 mL/min to yield **2a** (0.9 mg), whose ^1H NMR spectrum, retention time and optical rotation ($[\alpha]_D^{27}$ +24.6, $CHCl_3$) were identical with those of **1** (see the supporting information S2).

A sample of **5** (1 mg) dissolved in 2% sodium methanolate–methanol (3 mL) was stirred at 50 °C for 12 h. After neutralizing with 10% HCOOH and extracting with EtOAc, the EtOAc layer was evaporated to dryness and dissolved in MeOH. Then the mixture was purified by semi-preparative RP-HPLC with MeOH-H_2O (46:54, v/v) at flow rate of 4 mL/min to yield **5a** (0.7 mg), whose ^1H NMR spectrum, retention time, and optical rotation ($[\alpha]_D^{27}$ +12.6, $CHCl_3$) were identical with those of **6** (see the supporting information S2).

Cytotoxicity assay.
Five human cancer cell lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480, were used in the cytotoxicity assay. All the cells were cultured in DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA), in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates[21, 22] with cisplatin and paclitaxel (Sigma, USA) as the positive controls. Cell viability after 48 h treatment was detected and the cell growth curve was graphed. The IC_{50} values were calculated by Reed and Muench's method.[23, 24]

Antimicrobial Assay.
Compounds were tested for antimicrobial activity using a disk-diffusion assay.[25, 26] Seed cultures of two bacteria (*Staphylococcus aureus* 209P and *Escherichia coli* ATCC0111) and two fungi (*Candida albicans* FIM709 and *Aspergillus niger* R330) were prepared by incubating the organism for 12 hours at 32 °C (fungi) or 37 °C (bacteria). Aliquots of the overnight cultures (80 µL) were spread onto the surfaces of nutrient agar (bacteria) or yeast extract agar with 2% (w/v) glucose (fungi). Sterile filter disks (6 mm diameter) infused with 6 µL of test solution (in DMSO) and positive control, vehicle only (DMSO) were added to the plates. The plates were left upright for 30 min at room temperature before being placed in an incubator for 12 hours at 32 °C (fungi) or 37 °C (bacteria). Then the diameter of the zone of growth inhibition around each disk was recorded. The continuous 2-fold dilution method was used to evaluate the minimal inhibitory concentrations. The MICs were defined as the lowest concentration at which no microbial growth could be observed.

Determination of the absolute configuration of mannose unit
Authentic samples of D/L-Man and L-cysteine methyl ester hydrochloride (1.0 mg each) were dissolved in pyridine (1.0 mL) and heated at 60 °C, and then *o*-tolylisothiocyanate (0.2 mL) was added to the mixture and heated at 60 °C for 1 h.
A sample of **8/9/10** (0.5 mg) was added to 2 mL of 2 mol/L HCl in a hydrolysis tube, and the mixture was heated at 100 °C for 2 h. The reaction was quenched with 3.0 mL of H_2O and extracted twice with 2.0 mL of EtOAc to remove the aglycone. After evaporation of the aqueous layer, 1.0 mg of L-cysteine methyl ester hydrochloride and 100 μL of pyridine were added, and the mixture was stirred at 60 °C for 1 h. *o*-Tolylisothiocyanate (50 μL) was added, and the solution was stirred at 60 °C for another 1 h. The reaction mixture was directly analyzed by RP HPLC (Phenomenex C18 column; 250 × 4.6 mm, 5 μm; 25% CH_3CN in H_2O with 0.1% HCOOH; 0.8 mL/min; 35 °C) detected at 250 nm. The resulting Man derivative (t_R 14. 253 min) coeluted with a derivatized D-Man standard (t_R 14. 253 min), but not with a derivatized L-Man standard (t_R 22.985 min).

X-ray crystallographic analysis of 1, 6, and 7.
Crystal data for 4, 5-*seco*-probotryenol A (**1**): Data were collected using a Sapphire CCD with graphite monochromated Cu Kα radiation, λ = 1.54184 Å at 173.0 K. Crystal data: $C_{15}H_{26}O_2 \cdot H_2O$, M = 256.37, space group orthorhombic, $P2_12_12_1$; unit cell dimensions were determined to be a = 6.30805(13) Å, b = 14.8331(3) Å, c = 16.7092(3) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 1563.44(5) Å^3, Z = 4, Dx = 1.089 mg/m^3, F (000) = 568, μ (Cu Kα) = 0.583 mm^{-1}. 12848 reflections were collected to θ_{max} = 62.77°, in which 2417 reflections were observed [$F^2 > 4\sigma(F^2)$]. The final refinement gave R = 0.0281, R_W = 0.0708, S = 1.083, Flack = 0.02(18), and Hooft = 0.04(8). Crystal data of **1** was deposited in the Cambridge Crystallographic Data Centre (CCDC 1060373).

Crystal data for 12-hydroxydehydrobotrydienol (**6**): Data were collected using a Sapphire CCD with graphite monochromated Cu Kα radiation, λ = 1.54184 Å at 173.0 K. Crystal data: $C_{15}H_{22}O_3$, M = 250.33, space group orthorhombic, $P2_12_12_1$; unit cell dimensions were determined to be a = 9.13361(13) Å, b = 10.68806(3) Å, c = 13.75024(19) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 1342.31(3) Å^3, Z = 4, Dx = 1.239 mg/m^3, F (000) = 544, μ (Cu Kα) = 0.678 mm^{-1}. 21683 reflections were collected to θ_{max} = 62.73°, in which 2078 reflections were observed [$F^2 > 4\sigma(F^2)$]. The final refinement gave R = 0.0286, R_W = 0.0726, S = 1.105, Flack = 0.07(19), and Hooft = 0.11(7). Crystal data of **6** was deposited in the Cambridge Crystallographic Data Centre (CCDC 1060374).

Crystal data for dehydrobotrydienol (**7**): Data were collected using a Sapphire CCD with graphite monochromated Cu Kα radiation, λ = 1.54184 Å at 173.0K. Crystal data: $C_{15}H_{26}O_2$, M = 234.33, space group orthorhombic, $P2_12_12_1$; unit cell dimensions were determined to be a = 8.8587(3) Å, b = 10.9481(4) Å, c = 13.7817(5) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 1336.62(9) Å^3, Z = 4, Dx = 1.164 mg/m^3, F (000) = 512, μ (Cu Kα) = 0.590 mm^{-1}. 12262 reflections were collected to θ_{max} = 62.86°, in which 1869 reflections were observed [$F^2 > 4\sigma(F^2)$]. The final refinement gave R = 0.0728, R_W = 0.2002, S = 1.032, Flack = -0.1(7), and Hooft = -0.1(2). Crystal data of **7** was deposited in the Cambridge Crystallographic Data Centre (CCDC 1060375).

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†Electronic Supplementary Information (ESI) available: NMR data of **1–10**, the HPLC analyses of the reaction products (**2a** and **5a**), and the 1D and 2D NMR spectra of **1–10**. See DOI: 10.1039/c000000x/

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