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Meroditerpenoids and Derivatives from the Brown Alga *Cystoseira baccata* and Their Antifouling Properties

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The brown alga *Cystoseira baccata* harvested along the Atlantic coasts of Morocco yielded seven new meroditerpenoids (**1**−**4**) and derivatives (**5**−**7**), whose chemical structures were elucidated mainly by 2D NMR and mass spectrometry. Surprisingly, for all these compounds, which possess a bicyclo[4.3.0]nonane ring system, a *trans* fusion of the bicyclic system was deduced by stereochemical studies even though such compounds isolated from *Cystoseira* species are known to have a typical *cis* orientation for the bridgehead methyls. The antifouling and antibacterial activities of compounds **1**−**5** and **7** were evaluated, as well as their toxicity toward nontarget species. Compounds **4**, **5**, and **7** showed antifouling activities against growth of microalgae, macroalgal settlement, and mussel phenoloxidase activity, while being nontoxic to larvae of sea urchins and oysters.

Marine brown algae of the genus *Cystoseira* (family Sargassaceae, order Fucales) are widely distributed through the subtropics with a prevalent center of speciation in the Mediterranean Sea.[1] Previous chemical investigations have demonstrated that species of this genus are prolific producers of diterpenoids, meroditerpenoids, and phlorotannins.[2]

As a part of our project focused on searching for new antifouling compounds from seaweeds, we have investigated specimens of *Cystoseira baccata* collected off the Atlantic coast of Morocco. This species is commonly found along the NE Atlantic shores from Ireland to Mauritania, and to date only a few studies have been published on the chemical composition of *C. baccata*.[3−6]

In this paper, further investigation of this species led to the isolation of compound **1**, which has the same planar structure as cyclo-1′-demethylcystalgerone (**8a**), a meroditerpenoid with a bicyclic diterpenoid side chain first isolated from a related Mediterranean species (*Cystoseira algeriensis*),[7] but also described from *C. baccata*.[6] However, further exploration of the stereostructure of **1** revealed a *trans* orientation of the two bridgehead methyls of the hydrindane system instead of a *cis* orientation in the case of **8a** and all the cyclic meroditerpenes previously described from species of the *Cystoseira* genus.[2,8,9] In addition to **1**, we report on the isolation and structural characterization by spectroscopic analysis (HRMS, UV, IR, 1D and 2D NMR) of six other novel *C. baccata* metabolites (**2**−**7**) that possess the same bicyclic system with a characteristic *trans* fusion. Relative configurations were determined by 2D NOE experiments, while absolute configurations were attributed with the help of CD measurements or using the modified Mosher ester method.[10,11] Compounds **1**−**5** and **7**, together with the crude extract of *C. baccata*, were screened for their potential antifouling activities toward model species representative of microfouling (marine and terrestrial bacteria; microalgae) and macrofouling species (macroalgae and invertebrates). Because the goal was to find new environmentally friendly compounds for formulation of antifouling paints, toxicity tests were also run toward two crucial indicator species (sea urchins and oysters).

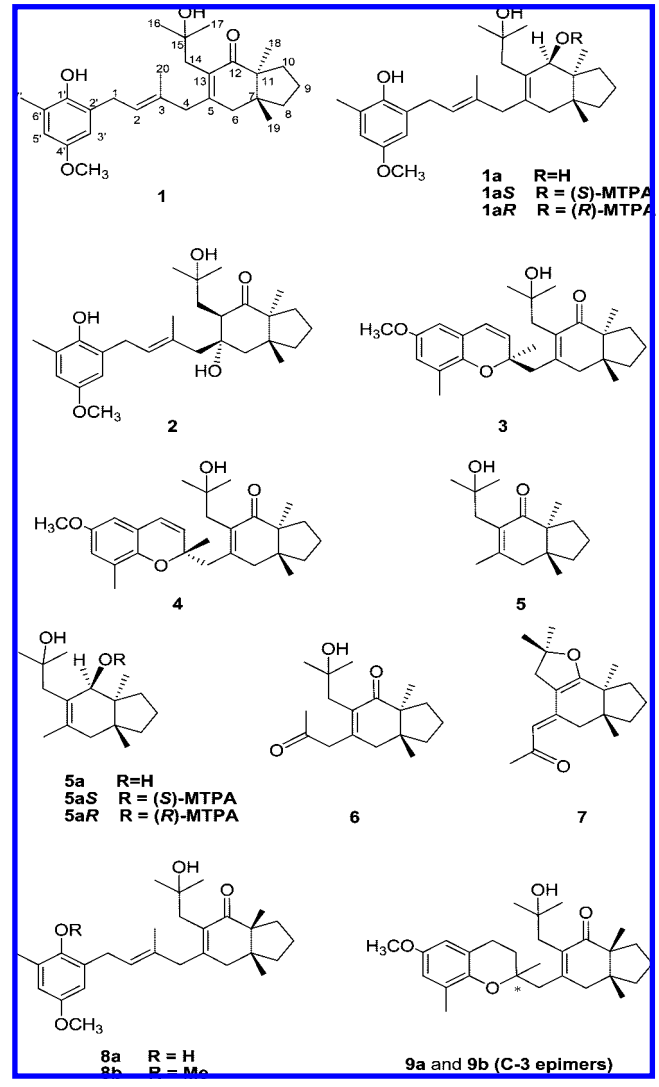

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Table 1. ^{13}C NMR Data for Compounds **1**−**7** (100 MHz, $CDCl_3$)

	1	**2**	**3**	**4**	**5**	**6**	**7**
position	δ_C, mult.[a]	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	29.7 CH_2	30.2 CH_2	123.6 CH	123.3 CH			
2	125.2 CH	127.7 CH	129.4 CH	130.2 CH		30.7 CH_3	31.7 CH_3
3	134.0 qC	135.2 qC	78.9 qC	78.6 qC		205.5 qC	197.6 qC
4	45.1 CH_2	47.5 CH_2	46.1 CH_2	46.2 CH_2	22.3 CH_3	50.2 CH_2	114.9 CH
5	154.2 qC	80.2 qC	153.4 qC	153.3 qC	153.6 qC	149.0 qC	107.3 qC
6	41.4 CH_2	42.3 CH_2	43.4 CH_2	44.3 CH_2	44.4 CH_2	43.1 CH_2	36.7 CH_2
7	44.5 qC	45.7 qC	44.8 qC	44.8 qC	44.5 qC	44.8 qC	46.7 qC
8	34.9 CH_2	35.5 CH_2	35.0 CH_2	35.0 CH_2	34.8 CH_2	35.0 CH_2	33.5 CH_2
9	18.8 CH_2	19.3 CH_2	18.8 CH_2	18.8 CH_2	18.8 CH_2	18.8 CH_2	19.9 CH_2
10	29.3 CH_2	28.7 CH_2	29.3 CH_2	29.4 CH_2	29.2 CH_2	29.2 CH_2	28.9 CH_2
11	54.9 qC	58.2 qC	54.8 qC	54.9 qC	54.8 qC	55.0 qC	45.9 qC
12	208.3 qC	213.2 qC	208.5 qC	208.7 qC	208.4 qC	206.5 qC	173.1 qC
13	132.1 qC	54.4 CH	133.0 qC	133.2 qC	130.6 qC	133.2 qC	153.5 qC
14	39.0 CH_2	34.7 CH_2	39.4 CH_2	39.6 CH_2	39.3 CH_2	38.7 CH_2	41.7 CH_2
15	71.2 qC	70.9 qC	71.1 qC	70.8 qC	71.2 qC	71.3 qC	88.8 qC
16	29.6 CH_3	28.9 CH_3	29.5 CH_3	28.8 CH_3	29.2 CH_3	29.5 CH_3	28.7 CH_3
17	30.3 CH_3	30.9 CH_3	30.7 CH_3	31.4 CH_3	30.7 CH_3	30.5 CH_3	28.3 CH_3
18	21.0 CH_3	22.4 CH_3	20.9 CH_3	20.9 CH_3	21.1 CH_3	21.1 CH_3	23.1 CH_3
19	22.5 CH_3	23.2 CH_3	22.4 CH_3	22.5 CH_3	22.6 CH_3	22.5 CH_3	23.6 CH_3
20	16.3 CH_3	18.3 CH_3	27.6 CH_3	27.0 CH_3			
1′	146.1 qC	146.3 qC	144.2 qC	144.1 qC			
2′	127.1 qC	127.2 qC	120.4 qC	120.5 qC			
3′	112.9 CH	113.0 CH	109.0 CH	109.1 CH			
4′	153.1 qC	153.1 qC	153.2 qC	152.9 qC			
5′	113.9 CH	114.0 CH	116.5 CH	116.6 CH			
6′	124.7 qC	125.0 qC	126.2 qC	126.0 qC			
7′	16.3 CH_3	16.3 CH_3	15.8 CH_3	16.0 CH_3			
4′-OMe	55.6 CH_3	55.6 CH_3	55.7 CH_3	55.7 CH_3			

[a] Multiplicities inferred from DEPT and HSQC experiments.

Results and Discussion

The lipophilic fraction derived from the crude extract of dried and powdered *C. baccata* was purified by a combination of Si gel column chromatography and reversed-phase semipreparative HPLC to yield compounds **1**−**7**.

Compound **1** was obtained as an optically active pale yellow oil ($[\alpha]^{25}_D$ +83) and displayed absorption bands for hydroxyl (3415 cm^{-1}) and conjugated carbonyl functionalities (1658 and 1604 cm^{-1}) in the IR spectrum. It gave an $[M + Na]^+$ ion by HRESIMS at *m/z* 463.2812, which suggested a molecular formula of $C_{28}H_{40}O_4$ and, therefore, possessed nine degrees of unsaturation. The ^1H NMR data (recorded in $CDCl_3$) indicated the presence of two *meta*-coupled aromatic protons (δ_H 6.56 and 6.51), an olefinic triplet (δ_H 5.37, J = 7.0 Hz), two D_2O exchangeable singlets (δ_H 4.71 and 3.63), one methoxy group (δ_H 3.73), and six singlet methyls (δ_H 2.22, 1.69, 1.21, 1.12, 1.03, and 0.80). The ^{13}C NMR and DEPT spectra revealed the occurrence of 11 quaternary carbons (δ_C 208.3, 154.2, 153.1, 146.1, 134.0, 132.1, 127.1, 124.7, 71.2, 54.9, and 44.5), three methines (δ_C 125.2, 113.9, and 112.9), seven methylenes (δ_C 45.1, 41.4, 39.0, 34.9, 29.7, 29.3, and 18.8), and, again, seven methyl groups (δ_C 55.6, 30.3, 29.6, 22.5, 21.0, 16.3, and 16.3). With nine degrees of unsaturation, **1** apparently contained two rings besides the carbonyl, the two double-bond groups (one trisubstituted and one tetrasubstituted), and the aromatic ring. The proton and protonated carbon signals in the NMR spectra of **1** were unequivocally assigned by the HSQC experiments (Tables 1 and 2), and $^1H-^1H$ COSY was then used to deduce extensive coupling systems. Homonuclear coupling correlations from H_3-7′ through H-5′, H-3′, H_2-1, and H-2 to H_3-20 and $H_{a,b}$-4 allowed formulation of fragment **a** (Figure 1), commonly found in *Cystoseira*-type metabolites. Fragment **b** was delineated by sequential couplings between H_b-6/H_3-19/H_a-8 and H_2-9/$H_{a,b}$-10/H_3-18 as well as by HMBC correlations from C-8 to H-9, C-9 to H_b-8, and C-10 to H_b-8 and H_2-9. HMBC correlations between H_3-19 and C-7, C-8, and C-11, on one hand, and between H_3-18 and C-7, C-10, and C-11, on the other, revealed the presence of a cyclopentane moiety in fragment **b**. Further heteronuclear couplings between H_3-18 and C-12 and between $H_{a,b}$-6 and C-5, C-7, C-11, C-13, and C-19 allowed not only the localization of the ketone and the second trisusbtituted double bond but also the completion of the carbon skeleton of substructure **b** as a bicyclo[4.3.0]nonane ring. Fragment **c** was deduced by HMBC cross-peaks between $H_{a,b}$-14 and C-15, C-16, and C-17, H_3-16 and C-14, C-15, and C-17, and H_3-17 and C-13, C-15, and C-16. The planar structure of **1** was completed by the linkage of these three fragments using additional HMBC cross-peaks between $H_{a,b}$-14 and C-5, C-12, and C-13, $H_{a,b}$-4 and C-5, C-6, and C-13, and $H_{a,b}$-6 and C-4. A detailed comparison of the NMR data of **1** with those reported for cyclo-1′-demethylcystalgerone (**8a**)[6,7] revealed the similarity of these molecules. However, the NOESY spectrum distinguished these two compounds based on spatial correlations of the bridgehead methyls (Figure 2), which were found to have a *trans* orientation in **1** instead of *cis* in the case of **8a**. To our knowledge this is the first report of such a structural feature in the Sargassaceae family. Finally, the geometry of the double bond at C-2 was assigned to be *E* on the basis of (i) the upfield ^{13}C NMR chemical shift of the vinyl methyl carbon at δ_C 16.3 (C-20)[12,13] and (ii) the NOE correlations observed between H-2 and H_2-4 as well as between H_2-1 and H_3-20.

Compound **2** was isolated as an optically active, yellowish oil, $[\alpha]^{25}_D$ +28, and had a molecular formula of $C_{28}H_{42}O_5$ (*m/z* 458, ESIMS), which was suggested by the HRESIMS of the M + Na adduct. Its IR spectrum showed absorption bands at 3432 and 1694 cm^{-1}, indicating the presence of both hydroxyl and nonconjugated carbonyl groups. Overall, the ^{13}C NMR features of **2** showed similarity to those of **1** except the replacement of two quaternary sp^2-hybridized carbons by two sp^3 carbons (a methine at δ_C 54.4 and a hydroxyl-bearing quaternary carbon at δ_C 80.2). On the basis of the assignments of the NMR data (Tables 1 and 2) by the $^1H-^1H$ COSY, HSQC, and HMBC experiments, the ^1H NMR spectrum of **2** showed, by comparison with those of **1**, that the chemical shift values of $H_{a,b}$-4, $H_{a,b}$-6, and $H_{a,b}$-14 were shifted significantly upfield and an additional methine triplet signal (δ_H 3.32, J = 5.5 Hz, H-13) was observed. These data suggested that the $\Delta^{5,13}$ double bond of **1** was replaced, in the case of **2**, by its hydrated counterpart. This

Table 2. ^{1}H NMR Data of Compounds **1**−**7** (400 MHz, $CDCl_3$, *J* in Hz)

position	1	2	3	4	5	6	7
1a	3.35 (d, 7.0)	3.39 (dd, 16.0, 7.5)	6.28 (d, 10.0)	6.32 (d, 10.0)			
1b		3.31 (dd, 16.0, 7.5)					
2	5.37 (t, 7.0)	5.32 (t, 7.5)	5.53 (d, 10.0)	5.61 (d, 10.0)		2.22 (s)	2.16 (s)
4a	3.05 (d, 14.5)	2.52 (d, 14.5)	2.78 (d, 14.0)	2.97 (d, 14.0)	1.92 (s)	3.62 (d, 17.0)	5.62 (br s)
4b	2.98 (d, 14.5)	2.14 (d, 14.5)	2.71 (d, 14.0)	2.53 (d, 14.0)		3.42 (d, 17.0)	
6a	2.44 (d, 18.5)	2.12 (d, 14.0)	2.63 (d, 18.5)	2.94 (d, 18.5)	2.57 (d, 18.0)	2.65 (d, 18.5)	3.40 (d, 18.0)
6b	2.21 (d, 18.5)	2.06 (d, 14.0)	2.51 (d, 18.5)	2.24 (d, 18.5)	2.21 (d, 18.0)	2.10 (d, 18.5)	2.57 (d, 18.0)
8a	1.73 (m)	1.62 (m)	1.67 (m)	1.73 (m)	1.74 (m)	1.76 (m)	1.70 (m)
8b	1.50 (m)	1.43 (m)	1.47 (m)	1.50 (m)	1.53 (m)	1.53 (m)	1.52 (m)
9	1.74 (m)	1.75 (m)	1.70 (m)	1.72 (m)	1.75 (m)	1.76 (m)	1.81 (m)
10a	1.97 (m)	1.98 (m)	1.95 (m)	1.96 (m)	1.97 (m)	2.00 (m)	1.81 (m)
10b	1.46 (m)	1.27 (m)	1.43 (m)	1.44 (m)	1.46 (m)	1.48 (m)	1.50 (m)
13		3.32 (t, 5.5)					
14a	2.73 (d, 14.0)	2.20 (dd, 15.0, 5.5)	2.67 (d, 14.5)	2.71 (d, 14.0)	2.60 (d, 14.0)	2.72 (d, 14.5)	2.50 (br d, 2.0)
14b	2.46 (d, 14.0)	1.75 (dd, 15.0, 5.5)	2.53 (d, 14.5)	2.49 (d, 14.0)	2.43 (d, 14.0)	2.33 (d, 14.5)	
16	1.12 (s	1.17 (s)	1.13 (s)	1.08 (s)	1.12 (s)	1.10 (s)	1.44 (s)
17	1.21 (s)	1.27 (s)	1.23 (s)	1.29 (s)	1.24 (s)	1.22 (s)	1.41 (s)
18	1.03 (s)	1.29 (s)	0.97 (s)	0.99 (s)	1.05 (s)	1.13 (s)	1.04 (s)
19	0.80 (s)	0.68 (s)	0.82 (s)	0.81 (s)	0.86 (s)	0.87 (s)	0.82 (s)
20	1.69 (s)	1.88 (s)	1.42 (s)	1.40 (s)			
3′	6.51 (d, 3.0)	6.52 (d, 3.0)	6.38 (d, 3.0)	6.39 (d, 3.0)			
5′	6.56 (d, 3.0)	6.56 (d, 3.0)	6.58 (d, 3.0)	6.57 (d, 3.0)			
7′	2.22 (s)	2.21 (s)	2.15 (s)	2.15 (s)			
4′-OMe	3.73 (s)	3.73 (s)	3.73 (s)	3.74 (s)			
OH	4.71 (s) 3.63 (s)	4.76 (s)	3.58 (s)	4.02 (s)	3.85 (br s)		

hypothesis was confirmed in particular by the HMBC correlations from H-13 to C-4, C-5, C-6, C-12, C-14, and C-15. The MS data (an extra H_2O and eight degrees of unsaturation) together with the IR and the NMR spectra were consistent with the occurrence of fragment **d** in the chemical structure of **2** instead of **b** in the case of **1**. The relative stereochemistry of **2** was determined from NOE enhancements observed in a NOESY experiment. NOE correlations from H_3-18 to H_b-6, H_a-8, H_a-10, and H-13 together with correlations from H_3-19 to H_b-4b, H_a-6, H_b-8, and H_b-10 suggested not only the *trans*-fused nature of the hydrindane moiety but also the relative stereochemistry at C-5 and C-13 by the fact that H-13, H_3-18, and the hydroxyl group at C-5 were all cofacial.

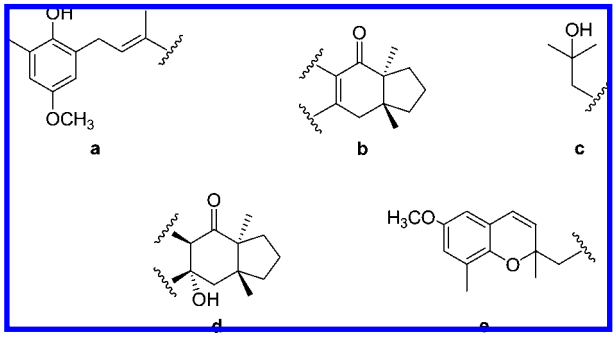

Figure 1. Partial structures **a**−**e** for compounds **1**−**5** and **7** as deduced by 2D NMR data.

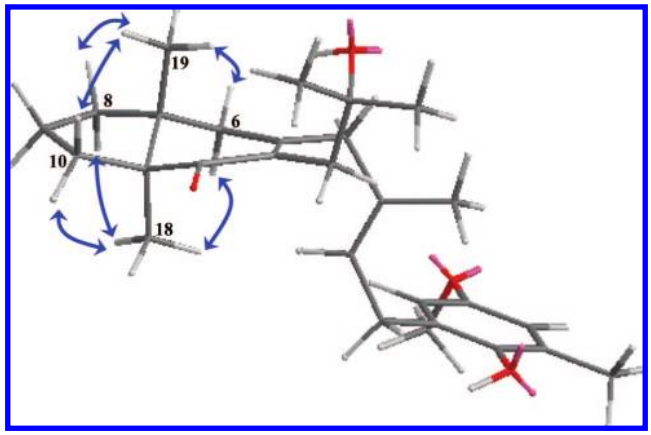

Figure 2. Computer-generated model for compound **1** using MM2 force field calculations and key NOE correlations.

Compound **3** was obtained as a clear yellow oil, with $[\alpha]^{25}_D$ −6, and showed IR absorption bands for hydroxyl (3421 cm^{-1}) and conjugated carbonyl (1657 and 1601 cm^{-1}) groups. It showed an HRESIMS $[M + Na]^+$ ion at *m/z* 479.2767, suggesting a molecular formula of $C_{28}H_{38}O_4$ (10 degrees of unsaturation). ^{13}C NMR analysis revealed the presence of 10 olefinic carbons in addition to the carbonyl, and hence, **3** possessed four rings. The 2D NMR experiments including ^{1}H−^{1}H COSY, HSQC, and HMBC displayed, by comparison of their ^{1}H and ^{13}C NMR data (Tables 1 and 2), that **3** and **1** shared the same fragments **b** and **c**. Moreover, as typical NMR signals were observed for the *O*-methyltoluquinol moiety, features differing from **1** were easily localized in the first isoprenoid unit using the HMBC spectrum. The main differences were, in the ^{1}H NMR data, the two signals of a pair of *cis*-coupled olefinic protons at δ_H 6.28 (d, J = 10 Hz, H-1) and 5.53 (d, J = 10 Hz, H-2) and, in the ^{13}C NMR spectrum, the occurrence of two sp^2 methine carbons at δ_C 123.6 (C-1) and 129.4 (C-2) and the quaternary oxygenated carbon at δ_C 78.9 (C-3). Thus, the planar structure of **3** was completed by the chromenol fragment **e**. Furthermore, the NOESY spectrum showed characteristic cross-peaks indicating, as for **1** and **2**, a *trans* fusion of the bicyclic system in fragment **b**. On the basis of the above observations, the structure of compound **3** was established and assigned as the chromene derivative of **1**. Moreover, the CD data (negative Cotton effect around 260−290 nm) established, by application of Crabbé's rule,[14,15] that the absolute configuration at C-3 was *R*.

Compound **4** (clear yellow oil, $[\alpha]^{25}_D$ +65) showed IR and NMR spectral features very similar to those of **3**, indicating that **4** was an isomer of **3**. This was further confirmed by HRESIMS (*m/z* 479.2735 $[M + Na]^+$ for $C_{28}H_{38}O_4$). A complete analysis of the 2D NMR data revealed that **4** possessed a planar structure identical with those of **3**. From a biosynthetic point of view, it seemed more likely that the stereochemical variation occurred for the oxygen-bearing quaternary carbon (C-3) rather than for asymmetric carbons (C-7 and C-11) of the bicyclo[4.3.0]nonane ring system. This was supported by NOE correlations, identical to those of **1**−**3** for the bridgehead methyls of the hydrindane part, implying a *trans* junction for **4**. Finally, these data allowed the description of compound **4**

as the C-3 epimer of **3**. The *S* configuration of the chromene moiety was corroborated by CD measurement (positive Cotton effect around 260−290 nm), which was opposite that of **3**. These two compounds are probably artifacts formed from **1** during the extraction or the isolation procedures.

The molecular formula $C_{16}H_{26}O_2$ of compound **5**, which was deduced by HRESIMS on the sodiated molecular ion at *m*/*z* 273, suggested a smaller chemical structure than the four compounds previously described. The IR spectrum indicated the presence of hydroxyl (3428 cm^{-1}) and conjugated carbonyl (1652 and 1612 cm^{-1}) groups. From the point of view of NMR data, the absence of aromatic and olefinic signals in the ^1H spectrum, together with the occurrence of the signals at δ_C 208.4, 153.6, and 130.6, allowed the attribution of the four degrees of unsaturation to one carbonyl, one tetrasubstituted double bond, and two rings in the molecule. A comparison of overall ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) established that differences between **5** and **1**, **3**, and **4** lie only in the lack of signals corresponding to the *O*-methyltoluquinol moiety and the first isoprenoid unit (fragment **a**). Thus, the chemical structure of **5** was constructed of fragments **b** and **c** and completed by a methyl group (δ_C 22.3, C-4; δ_H 1.92, s, H_3-4). This hypothesis was supported by the long-range heteronuclear correlations of both H_3-4 with C-5, C-6, and C-13 and $H_{a,b}$-6 with C-4 in the HMBC spectrum, thus establishing the structure of compound **5**. Analysis of the 2D NOESY data for **5** and similar optical rotation values gave the relative configuration of the bridgehead quaternary carbons as being consistent with those of **1**.

Compound **6** was obtained as an optically active, clear yellow oil ($[\alpha]^{25}_D$ +7). The molecular formula was determined to be $C_{18}H_{28}O_3$ by high-resolution ESIMS (*m*/*z* 315.1919, $[M + Na]^+$), suggesting five degrees of unsaturation. Comparison of the NMR data of **6** with those of **1**, **3**, **4**, and **5** showed the occurrence, for all of these compounds, of the same bicyclo[4.3.0]nonane ring system (fragment **b**) and the same fragment **c**. Additional signals at δ_C 30.7 (CH_3, C-2), 50.2 (CH_2, C-4), and 205.5 (qC, C-3) in the ^{13}C NMR spectrum of **6**, together with appropriate HMBC correlations between H_3-2 and C-3 and C-4 and between $H_{a,b}$-4 and C-3, were consistent with the presence of a 2-ketopropyl group. This structural feature was corroborated by an absorption band for a nonconjugated carbonyl (1718 cm^{-1}) in the IR spectrum of **6**. The connectivity between this moiety and the rest of the structure was fully established by HMBC correlations of $H_{a,b}$-4 with C-5, C-6, and C-13 and $H_{a,b}$-6 with C-4, and thus, all of these data supported the proposed planar structure for **6**. In fact, compound **6** was probably a product obtained during the treatment of the algal material, or the extraction procedure, by oxidation of **1** at the level of the Δ^2 double bond. This hypothesis was in agreement with that reported by Amico et al.,[16] who obtained an equivalent byproduct after ozonolysis of cystalgerone (**8b**).

Compound **7** showed an HRESIMS $[M + Na]^+$ ion at *m*/*z* 297.1807 for a molecular formula of $C_{18}H_{26}O_2$ (six degrees of unsaturation). With one ketone carbonyl (δ_C 197.6) and four other sp^2 carbons (δ_C 173.1, 153.5, 114.9, and 107.3), the ^{13}C NMR data accounted for three of the six double-bond equivalents, leaving a structure that contained three rings. In comparison with the NMR data of **6**, the main difference in the ^1H NMR spectrum of **7** was the replacement of a pair of doublets of nonequivalent methylene protons (δ_H 3.62 and 3.42, $H_{a,b}$-4) by an olefinic singlet signal at δ_H 5.62. This information was corroborated, in the ^{13}C and the HSQC spectra of **7**, by the occurrence of the corresponding signal for a sp^2 methine at δ_C 114.9, and it implied a Δ^4 double bond instead of a Δ^5 double bond in the case of **6**. In the NOESY spectrum, the occurrence of a correlation between H-4 and H_2-14, together with the absence of cross-peaks between H-4 and $H_{a,b}$-6, defined the absolute configuration of this double bond as *E*. Finally, the molecular formula (loss of 18 amu with respect to **6**) suggested the presence of an additional ring. The loss of a ketone resonance in the ^{13}C NMR spectrum taken along with all observed ^1H and ^{13}C chemical shifts allowed for the assignment of **7** as a highly conjugated derivative of **6** resulting from an intramolecular cyclization between C-15 and C-12 with loss of water.

Table 3. Results of the Antimacroalgal and Anti-invertebrate Assays for Compounds **4**, **5**, and **7**

	IC_{50} (μg/mL)		
compound	*Sargassum muticum*	*Ulva intestinalis*	phenoloxydase
4	2.5	>100	1
5	1	1	1
7	>100	2.5	2.5

Finally, in order to determine the absolute configuration of the bridgehead carbons (C-7 and C-11) of the hydrindane moiety, compound **1** was reduced with $NaBH_4$ to give the alcohol **1a** in 42% yield. The effectiveness of the reduction was checked by NMR. The ^1H NMR spectrum showed an additional signal at δ_H 3.81 (s, H-12) due to the oxymethine proton at C-12, while the ketone signal at δ_C 208.3 was replaced in the ^{13}C NMR spectrum by an oxygen-bearing methine carbon at δ_C 78.0. The absolute configuration of this newly appearing asymmetric center at C-12 was assigned as *R* by application of modified Mosher's method[10,11] (see experimental data on $\delta_S - \delta_R$). The *S* configuration was then suggested for the neighboring C-11 on the basis of NOESY cross-peaks between H-12 and H-18, while the presence of a *trans* fusion of the bicyclic system allowed us to attribute an *R* configuration to C-7. The same methodology was employed with **5**, and its absolute configuration was assumed to be 7 *R*, 11 *S*. The absolute stereochemistry of compounds **2**−**4**, **6**, and **7** was not determined due to a limited quantity of the isolated natural products. However, in consideration of the similar biogenetic origin of **1**−**7**, the absolute configurations at C-7 and C-11 of **2**−**4**, **6**, and **7** are proposed as *R* and *S*, respectively. In the case of **2**, NOE correlations suggested that the two other stereocenters at C-5 and C-13 are *S* and *R*, respectively.

To date, only two papers have been published describing chemical investigations of terpenoids from *C. baccata*, leading to isolation of a total of three metabolites: cyclo-1′-demethylcystalgerone (**8a**)[6] and its two chromanol derivatives (**9a** and **9b**).[3] For these compounds, primarily described by spectroscopic comparison with cystalgerone (**8b**) and similar metabolites, the hypothesis of a *cis*-fused bicyclic moiety has not been fully demonstrated. It seems more likely that compounds **8a**, **9a**, and **9b** possess the same absolute configurations at C-7 and C-11 as found in **1**−**7**. Comparison of $[\alpha]_D$ and NMR data of **1** with those of **8a** (isolated from *C. baccata*)[6] had identical values, thus supporting this hypothesis.

As regards the novelty of this *trans*-fused hydrindane carbon skeleton in the Sargassaceae family, actual studies held in our laboratory, on Mediterranean species of *Cystoseira*, are inclined to favor the existence of several biosynthetic pathways. Indeed, both *cis* and *trans* junctions of the cyclopentane ring have been recently observed in cyclized meroditerpenoids isolated from a same sample (M. El Hattab, A. Ortalo-Magné, and G. Culioli, unpublished data).

Compounds **1**−**5** and **7** and the crude extract of *C. baccata* were assayed for antifouling and antibacterial activities and in toxicity tests. No activity, at the concentrations tested (MICs > 100 μg/mL), were recorded on marine (*Pseudoalteromonas elyakovii*, *Vibrio aestuarianus*, and *Polaribacter irgensii*) or terrestrial bacteria (*Salmonella typhimurium, Escherichia coli*, and *Bacillus subtilis*). Among the three microalgae studied (*Exanthemachrysis gayraliae, Cylindrotheca closterium*, and *Pleurochrysis roscoffensis*), only *E. gayraliae* growth was inhibited by **5** with a MIC value of 1 μg/mL. Results on antimacroalgal and anti-invertebrate activities were promising for **4**, **5**, and **7** (see Table 3). None of the compounds tested showed significant toxicity toward larvae of sea urchin (*Echinus esculentus*) or oysters (*Crassostrea gigas*) (LC_{50} > 100 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 343 polarimeter, using a 10 cm microcell. CD spectra were obtained on a Jasco J-810 spectrophotometer. IR spectra were recorded with a Jasco model J-410 FT-IR spectrometer as KBr plates (films). 1D and 2D NMR spectra were obtained at 400 and 100 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Avance 400 MHz NMR spectrometer in $CDCl_3$. All chemical shifts were referenced to the residual solvent peak observed at δ_H 7.26 and δ_C 77.0. High-resolution mass spectra were carried out on an electrospray QTOF spectrometer (Applied Biosystems, Model QStar). HPLC isolations were performed using a Varian model Prostar 210 pump with RI monitoring (Varian RI-4).

Plant Material. The marine brown alga *Cystoseira baccata* was collected by hand at Sidi Bouzid near El Jadida on the Moroccan Atlantic coast in December 2005 and was identified by two of the authors (M.B.M. and M.D.). A voucher specimen is available from G.C. as collection number CB-EJ-12/05.

Extraction and Purification. The shade-dried material (268 g dry wt) was ground and extracted with mixtures of $CHCl_3$/MeOH (2:1 then 1:1 and finally 1:2, v/v) at room temperature. The concentrated extracts were combined and partitioned in a mixture of MeOH/$CHCl_3$/H2O (4:3:1, v/v/v) to yield, after solvent removal, 6.7 g of a dark brown lipophilic extract. This extract was subjected to CC on silica gel (Si60, 40−63 μm, Merck) with a solvent gradient from *n*-hexane to EtOAc and then from EtOAc to MeOH. The fraction eluting with 50% EtOAc in *n*-hexane was further purified by repeated semipreparative HPLC (Merck Purospher Star RP-18e 5 μm; 10 × 250 mm; 3 mL/min) using H_2O/MeCN (15:85, v/v) to afford five new compounds, **1** (9.8 mg), **3** (3.1 mg), **4** (3.7 mg), **5** (9.1 mg), and **7** (3.4 mg). From the fraction eluted with EtOAC/*n*-hexane (60:40, v/v), two new metabolites, **2** (4.6 mg) and **6** (1.2 mg), were also purified by reversed-phase HPLC with H_2O/MeCN (20:80, v/v) as eluent.

Compound 1: clear yellow oil; $[\alpha]^{25}_D$ +83 (*c* 0.1, MeOH); UV (EtOH) λ_{max} 300 nm (ε 2030), 330 nm (ε 520); IR (film) ν_{max} 3415, 2968, 2931, 2881, 1658, 1604, 1484, 1460, 1379, 1354, 1199, 1144, 1060, 1010 cm^{-1}; ^{13}C and ^{1}H NMR (400 MHz, $CDCl_3$), see Tables 1·2; HRESIMS *m/z* 463.2812 [M + Na]$^+$ (calcd for $C_{28}H_{40}O_4Na$, 463.2824).

Compound 2: clear yellow oil; $[\alpha]^{25}_D$ +28 (*c* 0.1, MeOH); UV (EtOH) λ_{max} 300 nm (ε 2290); IR (Film) ν_{max} 3432, 3203, 2950, 2877, 1694, 1482, 1453, 1380, 1331, 1224, 1186, 1139, 1054 cm^{-1}; ^{13}C and ^{1}H NMR (400 MHz, $CDCl_3$), see Tables 1 and 2; HRESIMS *m/z* 481.2904 [M + Na]$^+$ (calcd for $C_{28}H_{42}O_5Na$, 481.2930).

Compound 3: clear yellow oil; $[\alpha]^{25}_D$ −6 (*c* 0.1, MeOH); CD (MeOH) λ_{max} $\Delta\varepsilon_{274}$ −34.55; UV (EtOH) λ_{max} 290 nm (ε 3070), 330 nm (ε 2240); IR (film) ν_{max} 3421, 2966, 2927, 1657, 1601, 1469, 1377, 1193, 1143, 1059 cm^{-1}; ^{13}C and ^{1}H NMR (400 MHz, $CDCl_3$), see Tables 1 and 2; HRESIMS *m/z* 479.2767 [M + Na]$^+$ (calcd for $C_{28}H_{40}O_5Na$, 479.2773).

Compound 4: clear yellow oil; $[\alpha]^{25}_D$ +65 (*c* 0.1, MeOH); CD (MeOH) λ_{max} $\Delta\varepsilon_{274}$ +2.50; UV (EtOH) λ_{max} 275 nm (ε 1740), 330 nm (ε 970); IR (film) ν_{max} 3439, 2965, 2926, 1656, 1602, 1468, 1376, 1196, 1146, 1060 cm^{-1}; ^{13}C and ^{1}H NMR (400 MHz, $CDCl_3$), see Tables 1 and 2; HRESIMS *m/z* 479.2735 [M + Na]$^+$ (calcd for $C_{28}H_{40}O_5Na$, 479.2773).

Compound 5: clear yellow oil; $[\alpha]^{25}_D$ +53 (*c* 0.1, MeOH); UV (EtOH) λ_{max} 280 nm (ε 1070); IR (film) ν_{max} 3428, 2966, 2930, 2879, 1652, 1612, 1450, 1378, 1150 cm^{-1}; ^{13}C and ^{1}H NMR (400 MHz, $CDCl_3$), see Tables 1 and 2; HRESIMS *m/z* 273.1822 [M + Na]$^+$ (calcd for $C_{16}H_{26}O_2Na$, 273.1830).

Compound 6: clear yellow oil; $[\alpha]^{25}_D$ +7 (*c* 0.1, MeOH); IR (film) ν_{max} 3402, 2970, 2923, 1718, 1680, 1608, 1456, 1378, 1314, 1126, 1038 cm^{-1}; ^{13}C and ^{1}H NMR (400 MHz, $CDCl_3$), see Tables 1 and 2; HRESIMS *m/z* 315.1919 [M + Na]$^+$ (calcd for $C_{18}H_{28}O_3Na$, 315.1936).

Compound 7: clear yellow oil; $[\alpha]^{25}_D$ −11 (*c* 0.1, MeOH); UV (EtOH) λ_{max} 350 nm (ε 920), 270 nm (ε 350); IR (film) ν_{max} 3433, 2966, 2931, 2879, 1715, 1684, 1607, 1547, 1449, 1376, 1332, 1277, 1258, 1181, 1130 cm^{-1}; ^{13}C and ^{1}H NMR (400 MHz, $CDCl_3$), see Tables 1 and 2; HRESIMS *m/z* 297.1807 [M + Na]$^+$ (calcd for $C_{18}H_{26}O_2Na$, 297.1830).

Reduction of Compound 1 into the Corresponding Alcohol 1a. Reduction of **1** (5 mg) was conducted in MeOH (10 mL) with an excess of $NaBH_4$ (10 mg), and the solution was then allowed to stand overnight at room temperature. The reaction was quenched with H_2O, and then the mixture was extracted with Et_2O (3 × 25 mL). The combined ether extracts were dried over anhydrous $MgSO_4$. The products were purified using reversed-phase HPLC (Merck Purospher Star RP-18e 5 μm; 4.6 × 250 mm; 1 mL/min), with H_2O/MeCN (20:80, v/v) as eluent, to yield the pure alcohol **1a** (2.1 mg).

Alcohol 1a: colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 6.56 (1H, d, *J* = 3.0 Hz, H-5′), 6.52 (1H, d, *J* = 3.0 Hz, H-3′), 5.33 (1H, t, *J* = 7.0 Hz, H-2), 3.81 (1H, s, H-12), 3.73 (3H, s, H-4′OMe), 3.33 (2H, brd, *J* = 7.0 Hz, H-1), 3.12 (1H, d, *J* = 14.5 Hz, H-4a), 2.92 (1H, d, *J* = 14.5 Hz, H-14a), 2.54 (1H, d, *J* = 14.5 Hz, H-4b), 2.22 (3H, s, H-6′Me), 2.12 (1H, d, *J* = 14.5 Hz, H-14b), 2.05 (1H, m, H-10a), 1.95 (1H, d, *J* = 17.0 Hz, H-6a), 1.83 (1H, d, *J* = 17.0 Hz, H-6b), 1.74 (2H, m, H-9), 1.64 (3H, s, H-20), 1.54 (1H, m, H-8a), 1.39 (1H, m, H 8b), 1.30 (3H, s, H-17), 1.24 (1H, m, H-10b), 1.19 (3H, s, H-16), 0.97 (3H, s, H-19), 0.72 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 153.1 (C, C-4′), 146.3 (C, C-1′), 135.9 (C, C-3), 135.4 (C, C-5), 129.3 (C, C-13), 127.3 (C, C-2′), 125.0 (C, C-6′), 123.6 (CH, C-2), 114.0 (CH, C-5′), 112.8 (CH, C-3′), 78.0 (CH, C-12), 71.5 (C, C-15), 55.6 (CH_3, C-OMe), 47.1 (CH_2, C-14), 46.7 (C, C-11), 44.9 (CH_2, C-4), 41.1 (CH_2, C-6), 40.8 (C, C-7), 36.7 (CH_2, C-8), 31.5 (CH_3, C-17), 30.0 (CH_2, C-10), 29.9 (CH_2, C-1), 28.7 (CH_3, C-16), 24.8 (CH_3, C-19), 22.4 (CH_3, C-18), 19.1 (CH_2, C-9), 16.2 (CH_3, C-6'Me), 15.9 (CH_3, C-20).

Preparation of *S*- and *R*-MTPA Ester Derivatives of Compound 1a. Both (*S*)- and (*R*)-MTPA esters of **1a** (**1a*S***, **1a*R***) were obtained by treatment of **1a** (1.0 mg) with (*R*)- and (*S*)-MTPA chlorides (10 μL) in dry pyridine (0.5 mL) catalyzed with DMAP and stirred at room temperature overnight. The MTPA esters were purified using analytical HPLC (Merck Purospher Star RP-18e 5 μm; 4.6 × 250 mm; 1 mL/min), with H_2O/MeCN (10:90, v/v) as eluent.

Selected signals of **1a*S***: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.591 (H-17), 1.373 (H-16), 0.614 (H-19), 0.462 (H-18). Selected signals of **1a*R***: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.510 (H-17), 1.340 (H-16), 0.720 (H-19), 0.628 (H-18). $\Delta\delta_H$(**1a*S*** − **1a*R***): H-17, +0.081 ppm; H-16, +0.033 ppm; H-19, −0.106 ppm; H-18, −0.166 ppm.

Reduction of Compound 5 into the Corresponding Alcohol 5a. A sample of **5** (5 mg) was treated as described above for compound **1**. Usual workup followed by reversed-phase HPLC afforded **5a** (2.3 mg).

Alcohol 5a: colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 3.77 (1H, brs, H-12), 2.83 (1H, d, *J* = 14.5 Hz, H-14a), 2.08 (1H, d, *J* = 14.5 Hz, H-14b), 2.05 (1H, d, *J* = 17.5 Hz, H-6a), 2.04 (1H, m, H-10a), 1.91 (1H, d, *J* = 17.5 Hz, H-6b), 1.76 (2H, m, H-9), 1.57 (1H, m, H-8a), 1.42 (1H, m, H 8b), 1.66 (3H, s, H-4), 1.32 (3H, s, H-17), 1.27 (1H, m, H-10b), 1.20 (3H, s, H-16), 1.00 (3H, s, H-19), 0.75 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 134.5 (C, C-5), 126.9 (C, C-13), 78.5 (CH, C-12), 71.7 (C, C-15), 47.6 (CH_2, C-14), 47.0 (C, C-11), 43.9 (CH_2, C-6), 41.2 (C, C-7), 36.8 (CH_2, C-8), 31.8 (CH_3, C-17), 30.1 (CH_2, C-10), 28.7 (CH_3, C-16), 25.0 (CH_3, C-19), 22.5 (CH_3, C-18), 21.4 (CH_3, C-4), 19.3 (CH_2, C-9).

Preparation of *S*- and *R*-MTPA Ester Derivatives of Compound 5a. A solution of pure compound (2.3 mg) was divided into two parts, then transferred into clean NMR tubes and dried under the stream of N_2 gas. Deuterated pyridine (0.6 mL) and (*S*)-(−)-MTPACl (4 μL) or (*R*)-(+)-MTPACl (4 μL) were added successively to the NMR tube under a N_2 gas stream, and the tube was carefully shaken to mix the sample and MTPA chloride evenly. The reaction in the NMR tubes was monitored by ^{1}H NMR and was completed in approximately 6 h for the (*S*)-MTPA ester **5a*S*** and 48 h for the (*R*)-MTPA ester **5a*R***.

Selected signals of **5a*S***: ^{1}H NMR (C_5D_5N, 400 MHz) δ_H 1.711 (H-4), 1.694 (H-17), 1.670 (H-16), 1.206 (H-19), 0.715 (H-18). Selected signals of **5a*R***: ^{1}H NMR (C_5D_5N, 400 MHz) δ_H 1.706 (H-2), 1.496 (H-17), 1.423 (H-16), 1.279 (H-19), 0.812 (H-18). $\Delta\delta_H$(**5a*S*** − **5a*R***): H-4, +0.005 ppm; H-17, +0.198 ppm; H-16, +0.247 ppm; H-19, −0.073 ppm; H-18, −0.097 ppm.

Biological Assays. Biological assays of the compounds were performed toward bacteria (marine and terrestrial), microalgae, macroalgae, and marine invertebrates. Toxicity was checked against larvae of sea urchins and oysters. All the compounds were tested on a range of concentrations from 0.5 to 100 μg/mL (0.5, 1, 2.5, 5, 10, 25, 50, and 100 μg/mL). Each experiment and controls were run in six replicates and using two batches of organisms.

Antibacterial Assays. The compounds were tested for inhibitory activities against three strains of marine bacteria [*Pseudoalteromonas*

elyakovii (ATCC 700519), *Vibrio aestuarianus* (ATCC 35048), and *Polaribacter irgensii* (ATCC 700398)] and three strains of terrestrial bacteria [*Salmonella typhimurium* (ATCC 35986), *Escherichia coli* (ATCC 35049), and *Bacillus subtilis* (ATCC 55572)]. The experiments were performed as previously described by Maréchal et al.[17] Compounds were incubated at 25 °C for 48 h with the bacteria (2×10^8 cells/mL) in 96-well plates (Merck) in MHB medium (Mueller Hinton broth, Sigma), supplemented with NaCl (15 g/L) for marine bacteria and in TSM medium (trypsic soy broth, Sigma) for terrestrial bacteria. Minimum inhibitory concentrations (MICs), compared to the control (culture media), were determined by the microtiter broth dilution method.[18]

Antimicroalgal Assays. The compounds were screened for antimicroalgal activities against three microalgae: *Exanthemachrysis gayraliae* (AC 15), *Cylindrotheca closterium* (AC 170), and *Pleurochrysis roscoffensis* (AC 238). Compounds were incubated (at 18 °C, for 48 h under a light regime of 15:9 light:dark using 54 μmol photons/m^2•s cool-white fluorescent lamp) with the algae (0.4 μg/mL of chlorophyll A) in F/2 medium[19] as outlined by Plouguerne et al.[20] After 48 h, the relative optical density of the sample suspension was determined at 600 nm. Minimum inhibitory concentrations (MICs) compared to the seawater control were determined by the method of Tsoukatou et al.[21]

Antimacroalgal Assays. The compounds were screened for antimacroalgal activities against two species: *Sargassum muticum* and *Ulva intestinalis*. Compounds were incubated (at 18 °C, for 48 h under a light regime of 15:9 light:dark using 54 μmol photons/m^2•s cool-white fluorescent lamp) with the algae (3000 spores/mL) in F/2 medium[19] as outlined by Hellio et al.[22] After 48 h, the germination and attachment rates were determined as stated by Hellio et al.[22]

Anti-invertebrate Assays. Compounds were tested for their inhibitory activity of the phenoloxidase (PO) purified from *Mytilus edulis* following the method of Hellio et al.[23] Aliquots of pure enzyme were incubated for 2 h with the test compounds. Then, the PO activity was followed by monitoring the increase of absorbance at 475 nm using L-Dopa (10 mM) as substrate. In comparison with the control, percentages of inhibition of PO activity were determined for the test compounds.[24]

Toxicity Tests. In order to determine the toxicity, larvae of sea urchins (*Echinus esculentus*) and oysters (*Crassostrea gigas*) were incubated with the compounds according to the procedures of Hellio et al.[25] Ten to fifteen larvae were added to 2 mL of solution in the wells of a 24-well (Iwaki) plate. After 48 h exposure, the number of viable larvae was recorded. The data are expressed as a 24 h LC_{50} (= concentration of extract that produces 50% mortality in comparison with the control), which was determined using Sigma Plot 8.0.

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Supporting Information Available: Tables T1−T7 with complete NMR data together with all 1D and 2D NMR spectra of compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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