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Iriomoteolide-3a, a Cytotoxic 15-Membered Macrolide from a Marine Dinoflagellate *Amphidinium* Species

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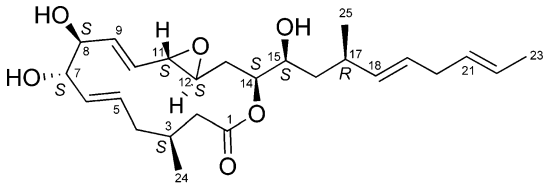
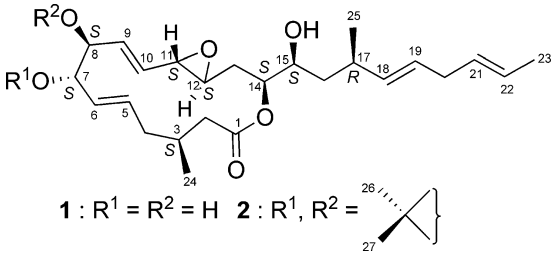


Iriomoteolide-3a (**1**)

A 15-membered macrolide, iriomoteolide-3a (**1**), with an allyl epoxide has been isolated from a marine benthic dinoflagellate *Amphidinium* sp. (strain HYA024), and the structure was assigned by detailed analyses of 2D NMR data. Relative and absolute configurations were elucidated on the basis of conformational studies of **1** and its acetonide (**2**) and modified Mosher's method of **1**, respectively. Iriomoteolide-3a (**1**) and the acetonide (**2**) exhibited potently cytotoxic activity against antitumor cells.

Marine dinoflagellates are known to produce bioactive secondary metabolites.[1] Members of *Amphidinium* are among the most abundant and diverse sand-dwelling benthic dinoflagellates worldwide,[2] and have been proven to be important sources of structurally unique polyketides.[3,4] Macrolides such as amphidinolides,[3,5] caribenolide-I,[6] and amphidinolactones,[7] isolated from symbiotic or free-swimming dinoflagellates *Amphidinium* sp., have various carbon chains as well as irregularly introduced C_1 branches and oxygen substituents. More than half of amphidinolides possess odd-numbered lactone rings such as 15-, 17-, 19-, 25-, 27-, and 29-membered macrolides.[3a]

Recently, we have screened numerous *Amphidinium* strains by using genetic analyses,[8] cytotoxic screening, and metabolomics analyses, and found an *Amphidinium* strain, named HYA024, that produced unknown cytotoxic macrolides. Three new cytotoxic 20-membered macrolides, iriomoteolides-1a, -1b, and -1c, have been isolated from the strain.[9] Further examination of the extract led to the isolation of a cytotoxic 15-membered macrolide, iriomoteolide-3a (**1**), with a novel carbon skeleton associated with an allyl epoxide moiety. Herein we describe the isolation and structure elucidation of **1**.



1 : $R^1 = R^2 = H$ **2** : $R^1, R^2 =$

The *Amphidinium* strain, HYA024, was monoclonally separated from sea sand collected off Iriomote Island, Japan. The cultured algal cells (15.3 g, dry weight) obtained from 400 L of the medium were extracted with the MeOH/toluene solvent system. The toluene-soluble materials of the extract were

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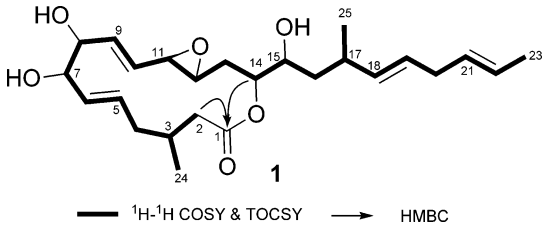

FIGURE 1. Selected 2D NMR correlations for iriomoteolide-3a (**1**).

subjected to SiO_2 gel, C_{18}, and NH_2-SiO_2 columns followed by C_{18} HPLC to afford iriomoteolide-3a (**1**, 0.015%), together with a known macrolide, iriomoteolide-1b.[9b] Iriomoteolides-1a[9a] and -1c[9b] were obtained from a less-polar fraction of the SiO_2 gel column.

Iriomoteolide-3a {**1**, $[\alpha]^{22}_D$ +24 (*c* 0.18, $CHCl_3$)} showed pseudomolecular ion peaks at *m/z* 457 $(M + Na)^+$ and 469 $(M + {}^{35}Cl)^-$ in the positive- and negative-mode ESIMS spectra, respectively. The molecular formula, $C_{25}H_{38}O_6$, of **1** was established by HRESIMS data [*m/z* 457.2566 $(M + Na)^+$, Δ +0.0 mmu]. ^{1}H and ^{13}C NMR data (Table S1, Supporting Information) in $CDCl_3$ assigned by using the HMQC spectrum disclosed the presence of a total of 25 carbon signals due to an ester carbonyl, eight sp^2 methines, eight sp^3 methines including six oxygenated ones, five sp^3 methylenes, and three methyls. Because five out of seven unsaturation degrees were accounted for, **1** was inferred to possess two rings in the molecule.

Detailed analyses of ^{1}H−^{1}H COSY and TOCSY spectra in $CDCl_3$ revealed a spin system from H_2-2 to H_3-23, H_3-24, and H_3-25 (Figure 1). Three disubstituted double bonds at C-5, C-9, and C-18 were indicated to possess *E*-geometries from *J*(H-5/H-6) (16.3 Hz), *J*(H-9/H-10) (15.5 Hz), and *J*(H-18/H-19) values (15.5 Hz), while *E*-geometry for the double bond at C-21 was deduced from the ^{13}C chemical shift for C-23 (δ_C 17.8)[10] as well as NOESY correlations for H_2-20/H-22 and H-21/H_3-23. The presence of a trans epoxide at C-11 was suggested by *J*(C-11/H-11) and *J*(H-11/H-12) values (180 and 2.3 Hz, respectively). The phase-sensitive HMBC[11] spectrum showed correlations from H_2-2 and H-14 to the ester carbonyl carbon (C-1), suggesting that C-14 was involved in an ester linkage with C-1. Thus, the planar structure of iriomoteolide-3a was concluded to be **1** possessing a 15-membered macrolactone ring.

The relative configuration of **1** was deduced from bond-rotation analyses based on ^{1}H−^{1}H coupling constants and NOESY data in $CDCl_3$. For the C-1−C-6 portion (Figure 2), ^{1}H−^{1}H coupling constants suggested anti for H-2b−H-3 (7.8 Hz), H-3−H-4b (8.9 Hz), and H-4a−H-5 (10.0 Hz) and gauche relationships for H-2a−H-3 (2.4 Hz), H-3−H-4a (4.0 Hz), and H-4b−H-5 (4.0 Hz).[12] Since NOESY correlations were observed for H-2a/H-5, H-3/H-6, H-4a/H-6, and H_2-4/H_3-24, the conformation for the C-1−C-6 portion was assigned as shown in Figure 2.

For the C-9−C-19 portion (Figure 3a), NOESY correlations for H-9/H-11 and H-10/H-12 and the *J*(H-10/H-11) value (9.8 Hz) indicated an anti relationship for H-10−H-11. The relative

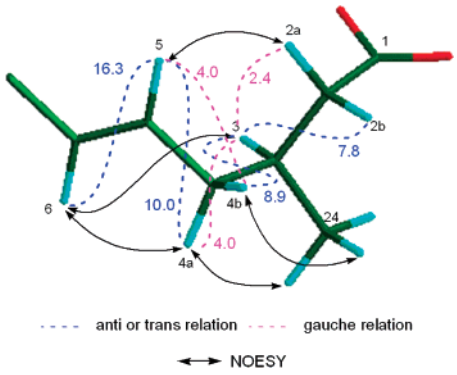

FIGURE 2. Relative stereochemistry for the C-1−C-6 portion in iriomoteolide-3a (**1**).

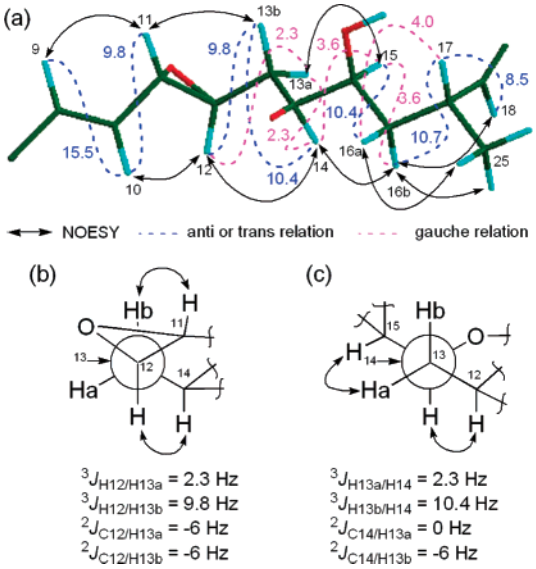

FIGURE 3. (a) Relative stereochemistry for the C-9−C-19 portion and rotations for (b) C-12−C-13 and (c) C-13−C-14 bonds in iriomoteolide-3a (**1**).

configuration for C-12−C-14 as well as orientation of the 11-(12)-epoxide oxygen atom were elucidated on the basis of the *J*-based configuration analysis[13] as follows. For the C-12−C-13 and C-13−C-14 bonds (Figures 3b and 3c), anti for H-12−H-13b and H-13b−H-14 and gauche relationships for H-12−H-13a and H-13a−H-14 were inferred by *J*(H-12/H-13a) (2.3 Hz), *J*(H-12/H-13b) (9.8 Hz), *J*(H-13a/H-14) (2.3 Hz), and *J*(H-13b/H-14) values (10.4 Hz) and NOESY correlation for H-12/H-14. Both gauche relationships for H-13a−11(12)-O and H-13b−11(12)-O were deduced from relatively large negative values for ^2J(C-12/H-13a) and ^2J(C-12/H-13b) (both −6 Hz), which were estimated from the intensities[14] of H-13a/C-11 and H-13b/C-11 cross-peaks in the phase-sensitive HMBC spectrum. The ^2J(C-14/H-13a) (0 Hz) and ^2J(C-14/H-13b) (−6 Hz) values were attributed to the anti and gauche relationships for H-13a−14-O and H-13b−14-O, respectively. Considering NOESY

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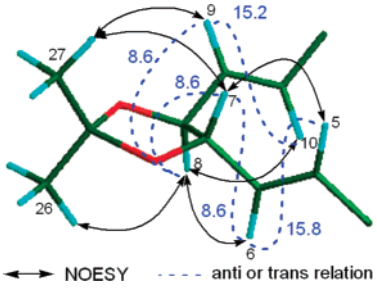

FIGURE 4. Relative stereochemistry for the C-4−C-11 portion in the 7,8-*O*-isopropylidene derivative (**2**) of iriomoteolide-3a (**1**).

correlations for H-11/H-13b, H-12/H-14, and H-13a/H-15, it was indicated that the epoxide oxygen atom was oriented to the outside of the macrolactone ring. NOESY correlations for H-13a/H-15 and H-14/H-16b and the *J*(H-14,H-15) value (3.4 Hz) were suggestive of the threo configuration for C-14−C-15. The 1,3-syn relation for C-15−C-17 was elucidated by *J*(H-15/H-16a), *J*(H-15/H-16b), *J*(H-16a/H-17), and *J*(H-16b/H-17) values (10.0, 3.6, 4.0, and 10.7 Hz, respectively) and NOESY correlations for H-14//H-16b, H-16b/H-18, and H_2-16/H_3-25.

The relative configuration for the C-6−C-9 portion for **1** was not determined, because H-7 (δ_H 3.965) and H-8 (δ_H 3.955) overlapped. Iriomoteolide-3a (**1**) was converted into the 7,8-*O*-isopropylidene derivative (**2**) by treatment with 2,2-dimethoxypropane and pyridinium *p*-toluenesulfonate. Two acetonide methyl signals at δ_H 1.44 (H_3-27) and 1.42 (H_3-26) showed NOESY correlations to H-7 (δ_H 4.02) and H-8 (δ_H 3.93), respectively, thus suggesting the 7,8-trans configuration (Figure 4). The relatively large *J*(H-6/H-7), *J*(H-7/H-8), and *J*(H-8/H-9) values (all 8.6 Hz) of **2** were indicative of anti relations for H-6−H-7, H-7−H-8, and H-8−H-9. The signal patterns for H-7 and H-8 of **1** agreed with those simulated as 8.6 Hz for *J*(H-6/H-7), *J*(H-7/H-8), and *J*(H-8/H-9) values using the NMR-PEAK.exe program by Nakamura[15] (see Figure S13, Supporting Information), indicating anti relationships for H-6−H-7, H-7−H-8, and H-8−H-9 in **1**. Considering the conformations shown in Figures 2−4, the relative configurations of the eight chiral centers in **1** were proposed.

Elucidation of the absolute configuration for **1** was examined by application of modified Mosher's method.[16] Treatment of **1** with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoro-2-phenylacetyl chloride (MTPACl) gave 7,8,15-tris-(*S*)- and (*R*)-MTPA esters (**3a** and **3b**, respectively) of **1**. Each of the ^{1}H NMR data for **3a** and **3b** were assigned by analyses of the ^{1}H−^{1}H COSY and TOCSY spectra, and chemical shifts differences ($\Delta\delta = \delta_S - \delta_R$) were shown in Figure 5. $\Delta\delta$ Values for H_2-16, H-17, H-18, and H_3-25 showed negative signs, while positive signs were observed for H-12, H_2-13, and H-14, thus suggesting that C-15 possessed *S*-configuration. Positive $\Delta\delta$ values for H-7 (+0.01) and H-8 (+0.03) corresponded to a typical $\Delta\delta$ pattern for diesters of *S*,*S*-1,2-diol with chiral anisotropic reagents reported by Riguera and co-workers.[17] Therefore, the absolute configurations of **1** were assigned as 3*S*, 7*S*, 8*S*, 12*S*, 13*S*, 14*S*, 15*S*, and 17*R*.

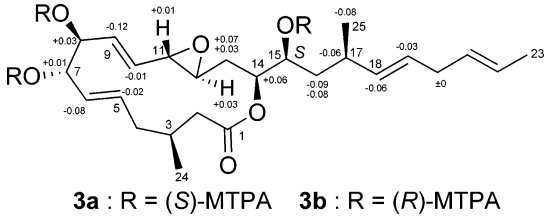

FIGURE 5. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained from 7,8,-15-tris-(*S*)- and (*R*)-MTPA esters (**3a** and **3b**, respectively) of iriomoteolide-3a (**1**).

Iriomoteolide-3a (**1**) is a new 15-membered macrolide[18] having an allyl epoxide, three hydroxyl groups, and two methyl branches. Although two classes of 15-membered macrolides such as amphidinolides J(S)[19] and O(P)[20] had been isolated from the symbiotic dinoflagellate *Amphidinium* species, the carbon chain length and C_1- and oxygen-substituted positions for **1** are quite different from those of these known 15-membered macrolides. Naturally occurring macrolides generally possess an even-numbered lactone ring, since these macrolides may be generated through lactonization of a successive polyketide chain, and the oxygenated carbons derived from the C-1 carbonyl of acetates or propionates are involved in an ester linkage. In the previous biosynthetic studies of amphidinolides,[21] however, the incorporation patterns revealed that they may be generated through non-successive polyketide including isolated C_1 units derived from C-2 of acetates, and the oxygenated carbons involved in an ester linkage are derived not only from the C-1 carbonyl but also the C-2 methyl of acetates. These biosynthetic features of *Amphidinium* macrolides may explain the generation of the odd-numbered lactone ring for **1**.

Our preliminary in vitro screening on antitumor and antiviral activities showed that iriomoteolide-3a (**1**) and its 7,8-*O*-isopropylidene derivative (**2**) exhibited potent cytotoxicity against human B lymphocyte DG-75 (IC_{50}: 0.08 and 0.02 μg/mL, respectively) and Raji cells (IC_{50}: 0.05 and 0.02 μg/mL, respectively), the latter of which was infected with Epstein−Barr virus (EBV). Further investigations on their biological activities are now in progress.

Experimental Section

Isolation. Cultivation and extraction were described previously.[9] The toluene-soluble fractions (2 g) obtained from the harvested HYA024 cells (15.3 g, from 400 L of culture) were subjected to SiO_2 column chromatography (40 × 200 mm), using a stepwise

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elution of $CHCl_3$ (200 mL) and $CHCl_3$/MeOH (98:2, 200 mL and then 95:5, 200 mL). The fraction eluted with $CHCl_3$/MeOH (95:5) was chromatographed successively by using a C_{18} (CH_3CN/H_2O, 7:3) and then NH_2-SiO_2 columns (*n*-hexane/EtOAc, 2:1). A macrolide-containing fraction was separated by C_{18} HPLC [YMC-Pack Pro C_{18}, 5 μm, YMC Co., Ltd., 10 × 250 mm; eluent, CH_3CN/H_2O (60:40); flow rate, 2 mL/min; UV detection at 210 nm] to afford iriomoteolide-3a (**1**, 2.3 mg, 0.015%).

Iriomoteolide-3a (1): colorless amorphous; $[\alpha]^{22}_D$ +24 (*c* 0.18, $CHCl_3$); IR (neat) ν_{max} 3438 (broad), 2920 1707, and 1215 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS (positive) *m*/*z* 457 $(M + Na)^+$; ESIMS (negative) *m*/*z* 469 and 471 [ca. 3:1, $(M + Cl)^-$]; HRESIMS *m*/*z* 457.2566 [calcd for $C_{25}H_{38}O_6Na$, $(M + Na)^+$ 457.2566].

7,8-*O*-Isopropylidene Derivative (2) of Iriomoteolide-3a (1). To a solution of iromoteolide-3a (**1**, 0.2 mg) in CH_2Cl_2 (20 μL) were added 2,2-dimethoxypropane (10 μL) and pyridinium *p*-toluenesulfonate (2 μg), and the mixture was stirred at 4 °C for 1 h. After evaporation of the solvent, the residue was subjected to a silica gel column (hexane/EtOAc, 8:1) to afford compound **2** (0.2 mg): ^1H NMR ($CDCl_3$) δ 1.01 (3H, d, J = 6.6 Hz, H_3-25), 1.05 (3H, d, J = 6.6 Hz, H_3-24), 1.28 (1H, m, H-16), 1.41 (1H, m, H-16), 1.42 (3H, s, H_3-26), 1.44 (3H, s, H_3-27), 1.57 (1H, m, H-13), 1.66 (3H, d, J = 6.6 Hz, H_3-23), 1.71 (1H, m, H-4), 1.86 (1H, m, H-3), 1.95 (1H, dd, J = 8.2 and 15.8 Hz, H-2), 2.22 (1H, br d, J = 14.0 Hz, H-13), 2.23 (1H, m, H-4), 2.37 (1H, m, H-17), 2.49 (1H, dd, J = 2.4 and 13.8 Hz, H-2), 2.67 (2H, m, H_2-20), 2.87 (1H, br d, J = 9.8 Hz, H-12), 3.06 (1H, dd, 2.3 and 9.8 Hz, H-11), 3.60 (1H, m, H-15), 3.93 (1H, t, J = 8.6 Hz, H-8), 4.02 (1H, t, J = 8.6 Hz, H-7), 5.17 (1H, m, H-14), 5.20 (1H, dd, 8.9 and 15.2 Hz, H-18), 5.32 (1H, dd, J = 9.8 and 15.2 Hz, H-10), 5.39−5.46 (3H, m, H-21, H-19, and H-22), 5.46 (1H, dd, J = 8.6 and 15.8 Hz, H-6), 5.82 (1H, ddd, J = 4.0, 10.0, and 15.8 Hz, H-5), and 5.60 (1H, dd, J = 8.6 and 15.2 Hz, H-9); ESIMS *m*/*z* 497.3 $(M + Na)^+$; HRESIMS *m*/*z* 497.2883 [calcd for $C_{28}H_{42}O_6Na$ $(M + Na)^+$ 497.2879].

7,8,15-Tris-(*S*)-MTPA Ester (3a) of Iriomoteolide-3a (1). To a solution of iriomoteolide-3a (**1**, 0.2 mg) in 1% 4-dimethylaminopyridine (DMAP) solution in CH_2Cl_2 (20 μL) were added Et_3N (1 μL) and (*R*)-(−)-MTPACl (0.8 μL), and the mixture was stirred at 4 °C for 15 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (2 μL), the solvent was evaporated in vacuo. The residue was passed through a silica gel column (hexane/acetone, 8:1) to afford the 7,8,-15-tris-(*S*)-MTPA ester (**3a**, 0.05 mg) of **1**: ^1H NMR ($CDCl_3$) δ 0.88 (3H, d, J = 6.6 Hz, H_3-25), 0.98 (3H, d, J = 6.6 Hz, H_3-24), 1.13 (1H, m, H-13b), 1.36 (1H, m, H-16b), 1.46 (1H, m, H-16a), 1.66 (3H, d, J = 6.6 Hz, H_3-23), 1.92 (1H, m, H-4b), 1.95 (1H, m, H-2b), 1.97 (1H, m, H-17), 2.20 (2H, m, H-2a and H-3), 2.22 (1H, m, H-13a), 2.39 (1H, m, H-4a), 2.62 (2H, s, H_2-20), 2.81 (1H, m, H-12), 2.83 (1H, m, H-11), 3.42 (3H, s), 3.45 (3H, s), 3.62 (3H, s), 5.09 (1H, dd, J = 8.5 and 15.5 Hz, H-18), 5.15 (1H, m, H-15), 5.25 (1H, m, H-6), 5.31 (1H, m, H-14), 5.34 (1H, m, H-19), 5.37−5.43 (2H, m, H-21 and H-22), 5.51 (2H, m, H-9 and H-10), 5.64 (1H, m, H-7), 5.70 (1H, m, H-8), 6.02 (1H, s, H-5), 7.35−7.42 (9H, m), and 7.50−7.58 (6H, m); ESIMS (positive) *m*/*z* 1105.4 $(M + Na)^+$; HRESIMS *m*/*z* 1105.3728 [calcd for $C_{55}H_{59}O_{12}F_9Na$ $(M + Na)^+$ 1105.3761].

7,8,15-Tris-(*R*)-MTPA Ester (3b) of Iriomoteolide-3a (1). Iriomoteolide-3a (**1**, 0.2 mg) was treated with DMAP (20 μg), Et_3N (1 μL), and (*S*)-(+)-MTPACl (0.8 μL) by the same procedure as described above to afford the 7,8,15-tris-(*R*)-MTPA ester (**3b**, 0.12 mg) of **1**: ^1H NMR ($CDCl_3$) δ 0.96 (3H, d, J = 6.6 Hz, H_3-25), 0.98 (3H, d, J = 6.6 Hz, H_3-24), 1.10 (1H, m, H-13b), 1.44 (1H, m, H-16b), 1.55 (1H, m, H-16a), 1.66 (3H, d, J = 6.6 Hz, H_3-23), 1.92 (1H, m, H-4b), 1.95 (1H, m, H-2b), 2.03 (1H, m, H-17), 2.15 (1H, m, H-13a), 2.19 (1H, m, H-2a), 2.20 (1H, m, H-3), 2.44 (1H, m, H-4a), 2.62 (2H, s, H_2-20), 2.78 (1H, m, H-12), 2.82 (1H, m, H-11), 3.35 (3H, s), 3.40 (3H, s), 3.53 (3H, s), 5.15 (1H, dd, J = 8.5 and 15.5 Hz, H-18), 5.17 (1H, m, H-15), 5.25 (1H, m, H-14), 5.33 (1H, m, H-6), 5.37 (1H, m, H-19), 5.37−5.43 (2H, m, H-21 and H-22), 5.52 (1H, m, H-10), 5.67 (1H, m, H-8), 5.63 (2H, m, H-7 and H-9), 6.04 (1H, s, H-5), 7.35−7.42 (9H, m), and 7.50−7.58 (6H, m); ESIMS (positive) *m*/*z* 1105.4 $(M + Na)^+$; HRESIMS *m*/*z* 1105.3772 [calcd for $C_{55}H_{59}O_{12}F_9Na$ $(M + Na)^+$ 1105.3761].

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Supporting Information Available: Spectral data for **1**, **2**, **3a**, and **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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