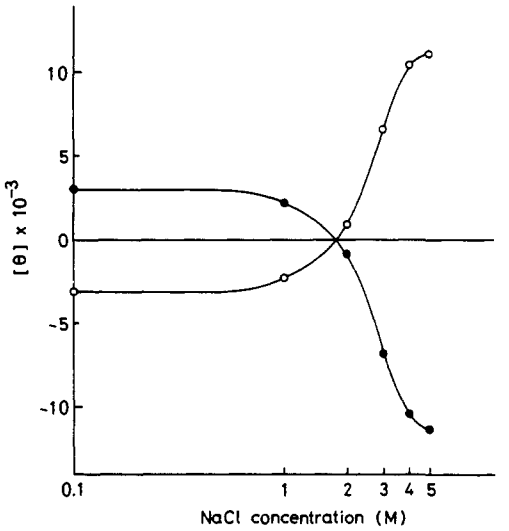

Figure 2. Salt concentration dependence of the B to Z transition for D-d(CGCGCG) (closed circles) and L-d(CGCGCG) (open circles) at 0 °C. [θ] values at 295 nm were plotted for each salt concentration. Experimental conditions were the same as those described in the caption below Figure 1 except for NaCl concentration.

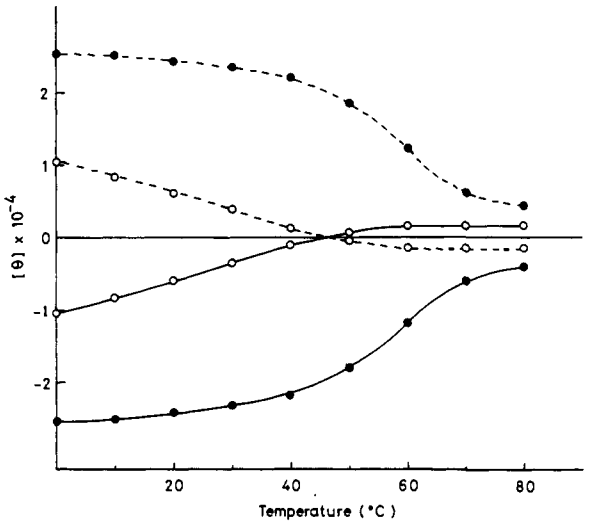

Figure 3. Temperature dependence of CD strength of D-d(CGCGCG) (solid line) and L-d(CGCGCG) (dashed line) under low salt conditions (0.1 M NaCl, closed circles) at 254 nm and under high salt conditions (4 M NaCl, open circles) at 295 nm.

nuclease P1 afforded the expected nucleotides and 5'-end deoxycytidine, but the L-isomer was resistant to digestion by this enzyme under the same conditions, as expected. This result indicates that the optical isomer of natural DNA cannot be recognized by natural enzymes consisting of L-amino acids.

The conformational properties of DNA oligomers or polymers containing the alternating C–G sequence have been well documented by circular dichroism (CD) spectra.[9] In 0.1 M NaCl solution, the CD spectrum of the D-hexamer showed a profile of the standard B form[10] (Figure 1a, solid line). The same magnitude but opposite sign was observed for the CD spectrum of L-d(CGCGCG) (Figure 1a, dashed line). The L-oligonucleotide thus adopts a mirror-image B form with left-handed double-helical conformation. The inversion of the CD band at 295 nm of the alternating C–G sequences under high salt conditions is known to be due to the conformational transition from the right-handed B form to the left-handed Z form.[11] The CD spectrum of D-d(CGCGCG) showed the characteristic negative band of the Z form at 295 nm in 4 M NaCl solution (Figure 1b, solid line), and that of the L-isomer showed an inversion profile (Figure 1b, dashed line). Both spectra were the same in magnitude at each wavelength. The L-hexamer thus adopts a mirror-image Z form with right-handed double-helical conformation.

The dynamic properties of both hexanucleotides were also compared. Both were noted to have the same salt concentration dependence on the B to Z conformational transition, whose midpoint was at 2.6 M NaCl (Figure 2). The same dependency on temperature, with opposite signs, was also noted for both isomers within experimental error under both low and high salt conditions (Figure 3). D- and L-DNA thus have the same type and strength of hydrogen bonding and base–base stacking interactions.

The present data clearly show both D- and L-DNA to possess the same conformation and dynamic properties except for chirality. The higher order structures of L-DNA are also the exact mirror images of that of natural DNA.

(9) (a) Pohl, F. M.; Jovin, T. M. *J. Mol. Biol.* **1972**, *67*, 375–396. (b) Pohl, F. M. *Nature* **1976**, *260*, 365–366. (c) Zacharias, W.; Larson, J. E.; Klysik, J.; Stirdivant, S. M.; Wells, R. D. *J. Biol. Chem.* **1982**, *257*, 2775–2782. (d) Uesugi, S.; Shida, T.; Ikehara, M. *Chem. Pharm. Bull.* **1981**, *29*, 3573–3585.

(10) Saenger, W. In *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 253–282.

(11) (a) Wang, A. H.-J.; Quigley, G. J.; Kolpak, F. J.; Crawford, J. L.; van Boom, J. H.; van der Marel, G.; Rich, A. *Nature* **1979**, *282*, 680–686. (b) Patel, D. J.; Kozlowski, S. A.; Nordheim, A.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 1413–1417.

(12) Fasman, G. D. *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; CRC Press: Boca Raton, FL, 1975; Vol. 1, p 589.

Nearest-Neighbor Recognition in Phospholipid Bilayers. Probing Lateral Organization at the Molecular Level[1]

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Developing a detailed understanding of supramolecular structure–function relationships within biological membranes represents one of the most important challenges presently facing chemists and biologists.[3] Although the lipid bilayer provides the basic structural element for all biological membranes, its precise lateral organization remains poorly defined. In particular, the question of whether or not lipids organize themselves into nonrandom clusters (i.e., domains) remains enigmatic.[4] The fact that natural phospholipids are rich in structural diversity could mean that a hierarchy of domains exists and that certain of these domains have functional importance, e.g., membrane fusion, transport, recognition, and catalysis.[5-7]

In this report we describe an experimental method that probes the thermodynamic preference for one phospholipid unit to become a *covalently attached nearest neighbor* of another in the bilayer state. We define such a preference as "nearest-neighbor recognition" (NNR). If the packing forces that govern NNR are the same as those that govern domain formation, then a systematic analysis of NNR should provide molecular-level insight into how the structure and composition of phospholipids influence their tendency to segregate within the lamellar phase. Our approach

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(2) Unilever Graduate Fellow, 1989–1991.

(3) Gennis, R. B. *Biomembranes: Molecular Structure and Function*; Springer-Verlag: New York, 1989; Chapter 4.

(4) Melchoir, D. L. *Science* **1986**, *234*, 1577; **1987**, *238*, 550.

(5) Jain, M. K. *Nonrandom Lateral Organization in Bilayers and Biomembranes. Membrane Fluidity in Biology*; Aloia, R. C., Ed.; Academic Press: New York, 1983; Vol. 1, p 1.

(6) Nagle, J. F.; Dilley, R. A. *J. Bioenerg. Biomembr.* **1986**, *18*, 55.

(7) Caruthers, M. A.; Melchoir, D. L. *Trends Biol. Sci.* **1986**, *11*, 331.

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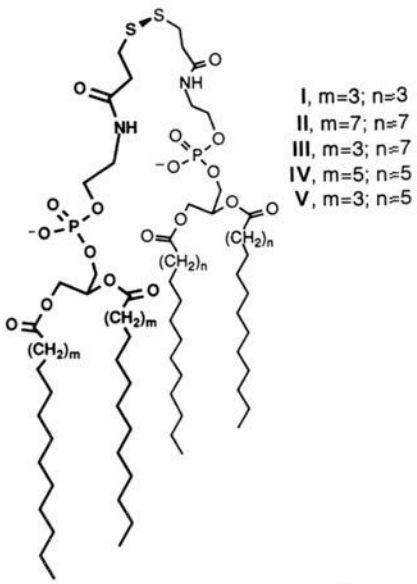
involves the equilibration of disulfide-based dimers via thiolate–disulfide interchange. Specifically, a 1:1 molar mixture of two different symmetrical phospholipid dimers (i.e., homodimers A and B) is equilibrated via thiolate–disulfide interchange.[8] In order to ensure that equilibrium has been reached, a similar exchange is performed starting with heterodimer C. An equilibrium mixture that departs from a statistical homodimer/heterodimer/"homodimer" ratio of 1:2:1 is then taken as a direct measure of NNR.[9]

Phospholipid dimers I–V, which may be regarded as cardiolipin analogues, were specifically chosen for the present study in order to demonstrate the feasibility of our approach and to examine whether or not a difference of four and/or eight methylene groups per lipid monomer is sufficient to establish a nearest-neighbor preference. Treatment of 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine with *N*-succinimidyl 3-(2-pyridyldithio)propionate afforded 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanol(2-pyridyldithio)propionamide (**1**); similar conversion of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine produced 1,2-distearoyl-*sn*-glycero-3-phosphoethanol(2-pyridyldithio)propionamide (**2**). Deprotection of **1** and **2** gave the corresponding thiol derivatives **3** and **4**, respectively. Reaction of **3** with **1**, **4** with **2**, and **3** with **2** generated the requisite dimers I, II, and III, in overall yields of 79, 81, and 84%, respectively. Phospholipids IV and V were prepared by similar means.[10]

Vortex mixing of a dried thin film of I in the presence of 10 mM borate buffer (140 mM NaCl, 2 mM NaN_3, pH 7.4) produced a multilamellar dispersion that exhibited a gel to liquid-crystalline phase transition temperature (T_m) of 22.7 °C; similar dispersions made from II and III showed T_m values of 55.4 and 33.9 °C, respectively. A 1/1 molar mixture of I/II (prepared from a chloroform solution) produced one endotherm at 22.5 °C and one at 50.2 °C.



Large unilamellar vesicles (LUVs, 1000-Å diameter) were prepared from multilamellar dispersions of III and also I/II (1/1 molar mixture prepared from chloroform) via standard extrusion procedures.[11] Each LUV dispersion (0.6 mg of lipid/mL) was then deoxygenated with argon, raised to pH 8.5, and incubated with 1 equiv of dithiothreitol in the fluid phase at 60 ± 1 °C. Aliquots were quenched by lowering their pH to 5.0, and the lipids were analyzed by HPLC, after being freeze-dried.[12,13] Similar

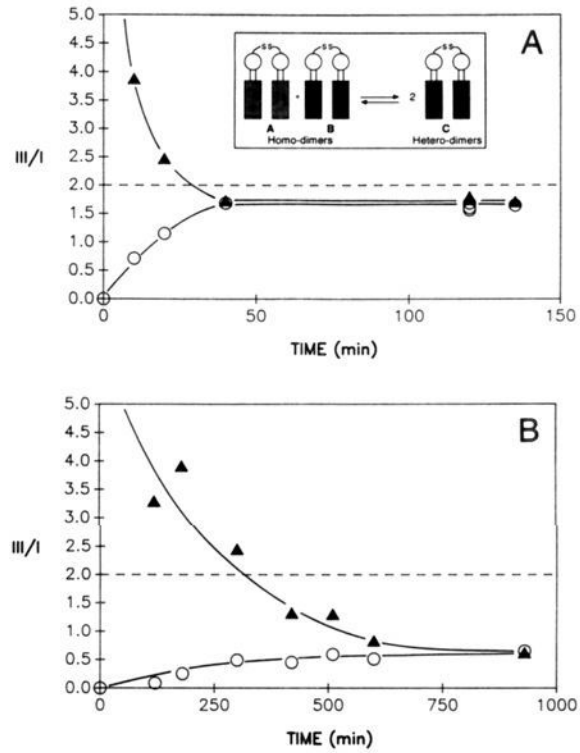


Figure 1. (A) Plot of molar ratio of III/I as a function of incubation time for vesicles prepared from pure III (▲) and a 1/1 mixture of I/II (○) that have been incubated at 60 ± 1 °C; six points are shown at 120 min. Insert: stylized illustration of thiolate–disulfide interchange of lipid dimers. (B) Same as A, except equilibration temperature was 33 ± 1 °C. In all cases, equal molar ratios of symmetrical dimers were produced (±5%).

dimer-equilibration experiments were also performed in the gel–fluid coexistence region at 33 ± 1 °C.

Those dimer distributions that were generated within *fluid* membranes made from pure III, and from the 1/1 molar mixture of I/II, are presented in Figure 1A as a function of time. From these data it is clear that equilibrium has been reached within 1 h and that the monomeric units of I and those of II show a very modest ability to "recognize" themselves; i.e., the 1/(1.68 ± 0.06)/1 molar ratio of I/III/II corresponds to a thermodynamic preference for forming homodimers of 0.12 ± 0.02 kcal/mol.[14] In the gel–fluid coexistence region, NNR is significantly enhanced. Here, the observed ratio of 1/(0.59 ± 0.05)/1 corresponds to ΔG = 0.74 ± 0.05 kcal/mol (Figure 1B).[15] Examination of a nonequilibrating 1/0.59/1 mixture of I/III/II by differential scanning calorimetry (DSC) further establishes that the gel–fluid coexistence is retained at 33 °C; i.e., two distinct endotherms persist.

In sharp contrast, analogous experiments carried out with I/V/IV reveal completely random mixing in both the fluid and gel–fluid states; i.e., 1/(2.00 ± 0.04)/1 equilibrium mixtures are observed. This result, together with the fact that a nonexchangeable 1/2/1 mixture of I/V/IV exhibits a *single* endotherm, provides compelling evidence that these monomeric components are randomly distributed not only at the molecular level but also at the supramolecular level. If one assumes that those factors that are responsible for NNR are also of primary importance for analogous intermolecular recognition, then our results with

(8) Singh, R.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 6304 and references cited therein.

(9) For a related approach, see: Roth, M. R.; Welti, R. *Biochim. Biophys. Acta* **1991**, *1063*, 242.

(10) Lipids I–V showed the expected ^1H NMR (500 MHz) spectra and HRMS.

(11) Hope, M. J.; Bally, M. B.; Webb, G.; Cullis, P. R. *Biochim. Biophys. Acta* **1985**, *812*, 55.

(12) Attempted equilibration of a 1/1 mixture of I/II for 4.5 h at pH 5 (1 equiv of DTT), and at pH 8.5 (in the absence of DTT), resulted in no detectable exchange.

(13) During these equilibration experiments, ca. 20% of the dimers were converted into equal molar amounts of **3** and **4**.

(14) Eight independent experiments; errors represent two standard deviations of the mean.

(15) Five independent experiments.

membranes made from I-III imply that a difference of eight methylene groups between two phospholipids is sufficient to produce domains in the fluid phase. The striking dichotomy between I/III/II and I/V/IV further reveals that NNR can depend on *both* molecular structure and the physical state of the membrane.

Studies that are now in progress are aimed at examining the influence of (i) differences in chain length, (ii) unsaturation within the *sn*-1 and/or *sn*-2 chains, (iii) cholesterol, (iv) membrane proteins, (v) temperature, and (vi) head-group structure on nearest-neighbor recognition within phospholipid membranes. Detailed kinetic analyses are also being carried out in order to probe the supramolecular organization of the *initial* state of these bilayers.

Tetraphenyldistibine: A Most Useful Reagent for Discriminating Radical Reactions

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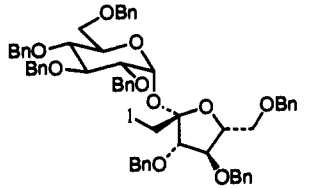
Recently, during our studies on the synthesis of sucrose,[1,2] we encountered a serious problem in effecting the S_N2 displacement of iodide **1** by oxygen-centered nucleophiles. Since all attempted bimolecular displacement reactions were unsuccessful, we sought solace in radical chemistry. Irradiation of **1** gave the corresponding alkyl radical, which was trapped with TEMPO, and the resultant adduct was subsequently converted into sucrose. These tribulations sensitized us to examine alternative, radical methods for the conversion of alkyl iodides into alcohols. Herein we report preliminary observations on the use of tetraphenyldistibine (**2**)[3,4] as a reagent for such substitution chemistry. Much to our surprise, **2** showed a remarkable chemoselectivity on photolysis in the presence of various alkyl iodides.



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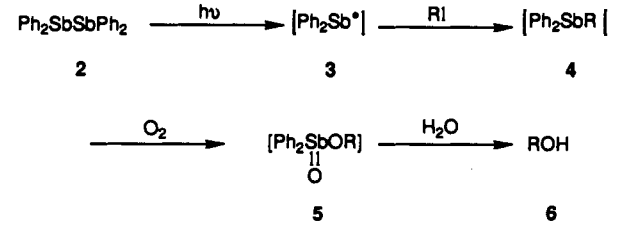
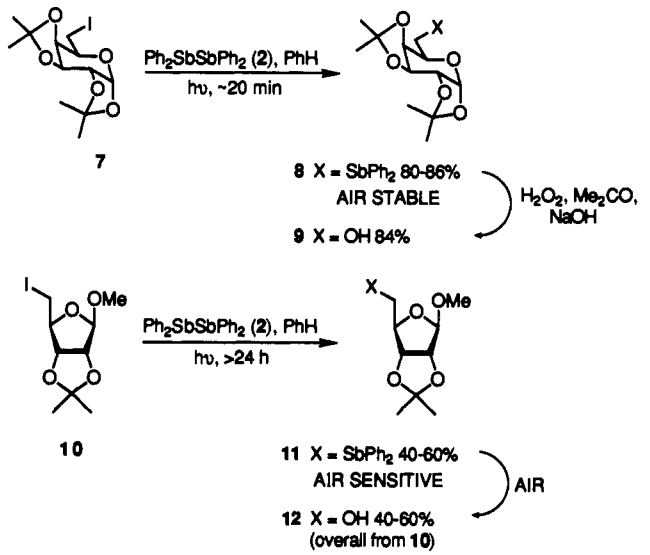
We considered that tetraphenyldistibine (**2**) should undergo homolysis, on irradiation, to produce the diphenylantimony(II) radical (**3**).[5] In turn, **3** should react with alkyl iodides, via a chain radical process,[6] to produce the air-sensitive[7] alkyl(diphenyl)stibine (**4**). In situ air oxidation and hydrolysis of the resultant compound **5** should provide the alcohol **6** (Scheme I). These expectations were reasonable since the conversion of **4** into **6** has precedent.[7]

Scheme I



Scheme II



Irradiation of **2** in the presence of iodide **7**[8] rapidly gave the stibine **8**[9] (80–88%) (Scheme II). Much to our surprise and in contrast to precedent,[4,5,7] this substance was not air sensitive although it was oxidized to produce **9** using basic H_2O_2. In contrast, the iodide **10**[8] reacted at a much slower rate with **2** on irradiation under identical conditions. Even after prolonged reaction, only 40–60% conversion into **11** was observed. The properties of **11** were also curious since aerial oxidation gave the alcohol **12** (40–60% overall).

(1) Barrett, A. G. M.; Bezuidenhoudt, B. C. B.; Melcher, L. M. *J. Org. Chem.* **1990**, *55*, 5196.

(2) Barrett, A. G. M.; Bezuidenhoudt, B. C. B.; Howell, A. R.; Lee, A. C.; Russell, M. A. *J. Org. Chem.* **1989**, *54*, 2275. Barrett, A. G. M.; Bezuidenhoudt, B. C. B.; Gasiecki, A. F.; Howell, A. R.; Russell, M. A. *J. Am. Chem. Soc.* **1989**, *111*, 1392. Barrett, A. G. M.; Bezuidenhoudt, B. C. B. *Heterocycles* **1989**, *28*, 209.

(3) Ashe, A. J., III. *Adv. Organomet. Chem.* **1990**, *30*, 77.

(4) Freedman, L. D.; Doak, G. O. *J. Organomet. Chem.* **1991**, *404*, 49. Houben-Weyl, *Methoden der Organische Chemie*; Thieme: Stuttgart, 1978; Band XIII-8. Doak, G. O.; Freedman, L. D. *Organometallic Compounds of Arsenic, Antimony and Bismuth*; Wiley-Interscience: New York, 1970. Dub, M. *Organometallic Compounds*, 2nd ed.; Springer: New York, 1968; Vol. 3. Poller, R. C. Organic Compounds of Antimony and Bismuth. In *Comprehensive Organic Chemistry*; Barton, D. H. R., Ollis, W. D., Eds.; Pergamon Press: New York, 1979; Vol. 3, pp 1111-1125.

(5) Cook, W. T.; Vincent, J. S. *J. Chem. Phys.* **1977**, *67*, 5766. See also: Davies, A. G.; Hook, S. C. W. *J. Chem. Soc. B* **1970**, 735. Ates, M.; Breunig, H. J.; Guelec, S. *Polyhedron* **1988**, *7*, 2601. Ates, M.; Breunig, H. J.; Soltani-Neshan, A.; Tegeler, M. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1986**, *41B*, 321. Breunig, H. J.; Jawad, H. *Ibid.* **1982**, *37B*, 1104. Ashe, A. J., III; Ludwig, E. J., Jr. *J. Organomet. Chem.* **1986**, *303*, 197; **1986**, *308*, 289. Breunig, H. J.; Jawad, H. *Ibid.* **1984**, *277*, 257; **1983**, *243*, 417. Nesmeyanov, A. N.; Borisov, A. F.; Novikova, N. V. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1967**, 815. Ashe, A. J., III; Ludwig, E. J., Jr.; Oleksyszyn, J. *Organometallics* **1983**, *2*, 1859. Baimbridge, C. L.; Mickey, C. D.; Zingaro, R. A. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1395.

(6) Neumann, W. P. *Synthesis* **1987**, 665. Curran, D. P. *Ibid.* **1988**, 417, 489. Crich, D.; Quintero, L. *Chem. Rev.* **1989**, *89*, 1413.

(7) Barton, D. H. R.; Bridon, D.; Zard, S. Z. *Tetrahedron* **1989**, *45*, 2615. Barton, D. H. R.; Ozbalik, N.; Schmitt, M. *Tetrahedron Lett.* **1989**, *30*, 3263. Barton, D. H. R.; Bridon, D.; Zard, S. Z. *J. Chem. Soc., Chem. Commun.* **1985**, 1066.

(8) Garegg, P. J.; Samuelson, B. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2866.

(9) The preparation of **8** and **9** is representative. A solution of **7** (275 mg) and **2** (500 mg) in dry PhH (10 mL) under N_2 was irradiated with a sun lamp for 20 min, cooled to 25 °C, and centrifuged, the supernatant layer was evaporated in vacuo, and the residue was chromatographed (silica; Et_2O/hexanes, 1:9) to afford **8** (341 mg, 88%) as a colorless oil: $[\alpha]_D$ −51° (*c* 3, $CHCl_3$); IR ($CHCl_3$) 3430, 2916, 1637, 1430, 1382, 1256, 1212, 1171, 1068, 1019, 896, 732 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.55–7.50 (m, 4 H), 7.29–7.25 (m, 6 H), 5.53 (d, 1 H, *J* = 5.0 Hz), 4.57 (dd, 1 H, *J* = 1.9, 7.9 Hz), 4.26 (dd, 1 H, *J* = 2.4, 5.1 Hz), 4.14 (dd, 1 H, *J* = 1.9, 7.9 Hz), 3.98 (m, 1 H), 2.34 (dd, 1 H, *J* = 8.8, 12.3 Hz), 2.09 (dd, 1 H, *J* = 6.1, 12.3 Hz), 1.48 (s, 3 H), 1.31 (s, 3 H), 1.27 (s, 3 H), 1.22 (s, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 138.3, 136.1, 135.7, 128.5, 128.4, 128.3, 128.1, 109.1, 108.3, 96.7, 73.8, 71.2, 70.3, 65.9, 26.0, 25.6, 24.8, 24.3, 22.6; MS (EI) *m/e* 518 ($M^{\bullet+}$) 441, 325, 275, 243; HRMS calcd for $C_{24}H_{29}O_5Sb$ ($M^{\bullet+}$) 518.1053, found ($M^{\bullet+}$) 518.1038. Anal. Calcd for $C_{24}H_{29}O_5Sb$: C, 55.63; H, 5.64. Found: C, 55.66; H, 5.83. H_2O_2 (30%, 2 mL) and Me_2CO (5 mL) were added to **8** (100 mg), THF (3 mL), and NaOH (30 mg) at 0 °C, and the mixture was warmed up to 25 °C. After 2 h, saturated $Na_2S_2O_3$ (15 mL) was added at 0 °C and the solution neutralized (1 M HCl) and evaporated in vacuo. The residue was extracted with EtOAc (4 × 40 mL), and the extract was dried ($MgSO_4$) and evaporated. Chromatography (silica; Et_2O/hexanes, 2:3) gave **9** (42 mg, 84%).

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