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Cholesterol Surrogates Incorporating a Benzophenone as Part of the Sterol Tetracycle

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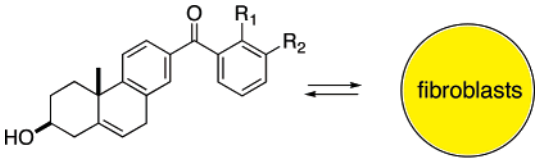

4, $R_1 = R_2 = H$ **5**, $R_1 = H$, $R_2 = CH_2CH(CH_3)_2$,
6, $R_1 = CH_2CH_2CH(CH_3)_2$, $R_2 = H$

Photoactivatable analogues **4**−**6** of cholesterol (**1**), having their cross-linking site in the ring D sterol region, have been synthesized starting from bromotetralone **14** via enantioselective Robinson annulation to enone **13** and Suzuki carbonylative coupling to the appropriate phenylboronic acid. Each of **4**−**6** was shown to substitute successfully for **1** in an assay of apo A−I-induced cellular cholesterol efflux, indicating that these analogues equilibrated with **1** in all major cellular pools.

Introduction

For use in studies of cellular cholesterol efflux and HDL formation,[1−3] we have been synthesizing photoactivatable analogues of cholesterol (**1**) having benzophenone groups at different defined positions relative to the sterol structure. Preparation of eight analogues with the potentially cross-linking benzophenone carbonyl group carbon atom at either end of the cholesterol structure in the positions indicated in Figure 1 has previously been described.[4] All of these analogues substituted successfully for cholesterol in an isotope dilution assay measuring apolipoprotein A−I-induced cholesterol efflux,[4] suggesting an unexpected tolerance of biological membranes regarding the incorporation of surrogates of **1** having differing structure. In the preceding paper,[5] the synthesis and preliminary biochemical evaluation of additional analogues lacking the steroidal tetracyclic nucleus is reported. Compounds **2** and **3**, exemplifying this type of analogue with the photo-cross-linking site in the ring B region (Figure 1), have also been shown to substitute successfully for **1** in the isotope dilution assay of cholesterol efflux.[5] These results again suggest that rather large structural variations can be tolerated in the major cellular cholesterol pools.

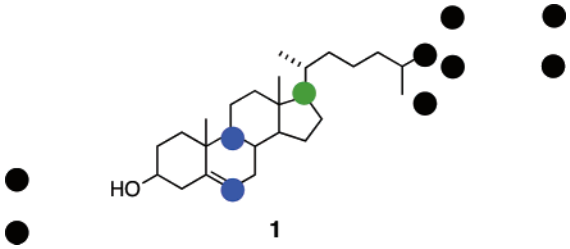

FIGURE 1. Structure of cholesterol (**1**) showing the approximate location of the photo-cross-linking carbonyl carbon atom when each of the analogues prepared to date is superimposed upon it. The black circles refer to compounds described in ref 4, the blue circles to compounds in ref 5, and the green circle to compounds described in this paper.

In this paper are described the preparation and preliminary biochemical evaluation of a third type of benzophenone-containing analogue of **1**, having the photophore located in the ring D region as shown in Figure 1, specifically compounds **4**, **5**, and **6**. Examination of Dreiding molecular models and use of the Spartan molecular modeling program indicated that both **5** and **6**, although having the surrogate ring D alkyl side chain in different positions on the benzophenone, can approximate the size and shape of cholesterol. The Spartan molecular

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(1) Fielding, P. E.; Russel, J. S.; Spencer, T. A.; Hakamata, H.; Nagao, K.; Fielding, C. J. *Biochemistry* **2002**, *41*, 4929−4937.

(2) Fielding, P. E.; Chau, P.; Liu, D.; Spencer, T. A.; Fielding, C. J. *Biochemistry* **2004**, *43*, 2578−2586.

(3) Nakamura, Y.; Kotite, L.; Gan, Y.; Spencer, T. A.; Fielding, C. J.; Fielding, P. E. *Biochemistry* **2004**, *43*, 14811−14820.

(4) Spencer, T. A.; Wang, P.; Li, D.; Russel, J. S.; Blank, D. H.; Huuskonen, J.; Fielding, P. E.; Fielding, C. J. *J. Lipid Res.* **2004**, *45*, 1510−1518.

(5) Gan, Y.; Blank, D. H.; Ney, J. E.; Spencer, T. A. *J. Org. Chem.* **2006**, *8*, 5864−5869.

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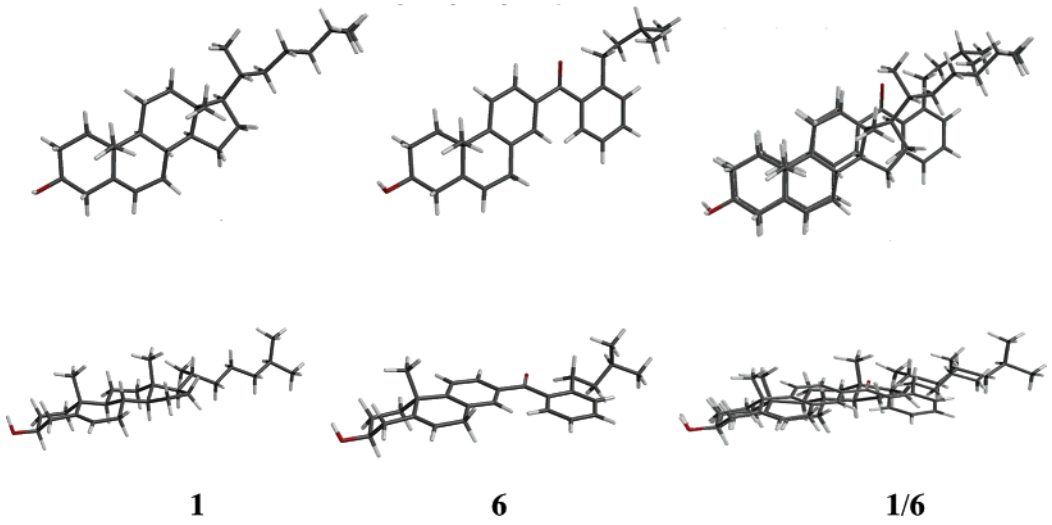
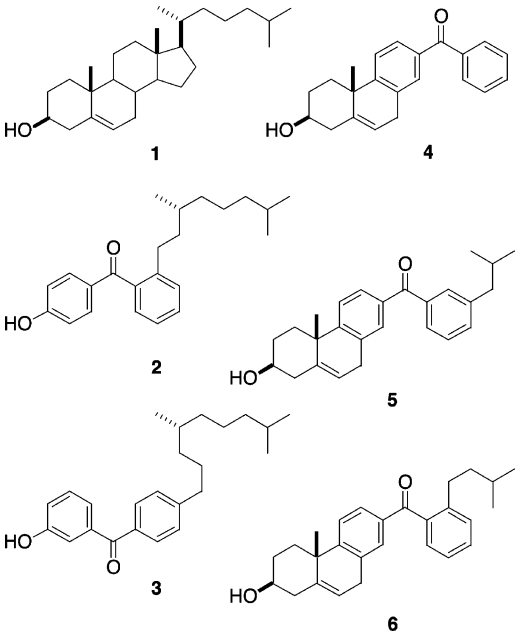


FIGURE 2. Spartan molecular modeling structures of cholesterol (**1**), analogue **6**, and **1** superimposed on **6** in top and side views. The structure of **1** was energy minimized and that of **6** was conformationally manipulated for most effective overlap using Dreiding models as a guide.

modeling structures of **1** and **6** shown in Figure 2 demonstrate that **6** can overlap **1** very well, with the sole exception of a protruding portion of the distal benzene ring of **6**.

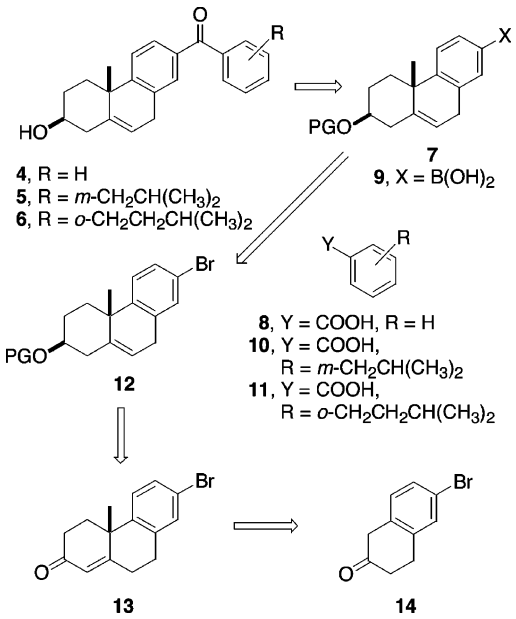


Results and Discussion

Synthesis. The strategy adopted for preparation of **4**−**6** envisioned coupling of a chiral tricyclic intermediate **7** with an appropriately substituted benzene to forge the benzophenone moiety near the end of the synthesis (Scheme 1). The first approach undertaken experimentally toward realization of this strategy involved Suzuki coupling of boronic acid **9** (**7**, X = $B(OH)_2$) with anhydride derivatives of benzoic acid (**8**) or substituted benzoic acids **10** or **11**. The key tricyclic intermediate **9** would be secured via protected allylic alcohol **12**, obtained by standard procedures from chiral enone **13**, in turn prepared from known bromotetralone **14**[6] by methylation and enantioselective Robinson annulation.

The requisite substituted benzoic acids **10**[7] and **11**[8,9] have each been prepared before by routes requiring at least five steps.

SCHEME 1

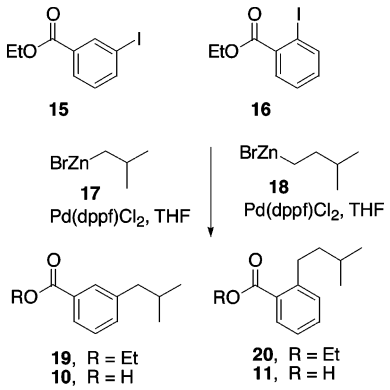


In the present work, these compounds were prepared much more expeditiously, as shown in Scheme 2, by Negishi coupling[10,11] of iodoesters **15** and **16** with the commercially available organozinc reagents **17** and **18** to obtain esters **19** and **20**, which were saponified to afford **10** and **11** in 89 and 90% overall yield, respectively.

The starting material for synthesis of the tricyclic boronic acid **9**, to be coupled with suitable derivatives of benzoic acid or substituted benzoic acids **10** or **11**, was bromotetralone **14**,[6] which was prepared in 71% yield from *p*-bromophenylacetic

(6) Tschaen, D. M.; Abramson, L.; Cai, D.; Desmond, R.; Dolling, U.-H.; Frey, L.; Karady, S.; Shi, Y.-J.; Verhoeven, T. R. *J. Org. Chem.* **1995**, *60*, 4324−4330.
(7) Agoulnik, S.; Akasaka, K.; Fang, F.; Harmange, J.-C.; Hawkins, L.; Jiang, Y.; Johannes, C.; Li, X.-Y.; Mcguinness, P.; Murphy, E.; Schiller, S.; Vermeulen, M.; Wu, J. Patent Appl. WO 2003-US390 20030108, 2003.
(8) Harvey, W. E. *J. Chem. Soc.* **1958**, 2060−2062.
(9) Andrews, E. D.; Harvey, W. E. *J. Chem. Soc.* **1961**, 4687−4689.
(10) Baba, S.; Negishi, E. *J. Am. Chem. Soc.* **1976**, *98*, 6729−6731.
(11) Negishi, E. *Acc. Chem. Res.* **1982**, *15*, 340−348.

SCHEME 2



acid by the interesting Friedel−Crafts method of Burckhalter and Campbell[12] (Scheme 3). Methylation of **14** by the Stork enamine method[13] gave 74% of **21**, which, it was hoped, could be converted to chiral enone **13** with high enantioselectivity by use of an appropriate chiral amine catalyst to effect Robinson annulation. The simple enantiomeric phenylethylamines, used by d'Angelo and co-workers[14] to effect enantioselective Michael additions, seemed an excellent choice.

When **21** was treated with *R*-(+)-1-phenylethylamine, the enantiomer predicted to give **13** selectively, then with methyl vinyl ketone, the major product obtained was crystalline bridged ketol **22** in 57% yield, analogous to results of d'Angelo[15,16] and of Morgan et al.[17] The stereochemistry at the hydroxyl-bearing carbon atom of **22** was assigned by NOE experiments, particularly observation of an interaction between the methyl group at that site and the benzylic proton shown in **22**. Isolation of the readily purified **22** ensured that, after conversion to **13** in 94% yield by treatment with acid,[15−17] we had essentially enantiopure tricyclic enone. The positive optical rotation of **13** ($[\alpha]_D^{25}$ = +117°) is consistent with those of similar tricyclic enones having angular methyl groups.[15,16,18]

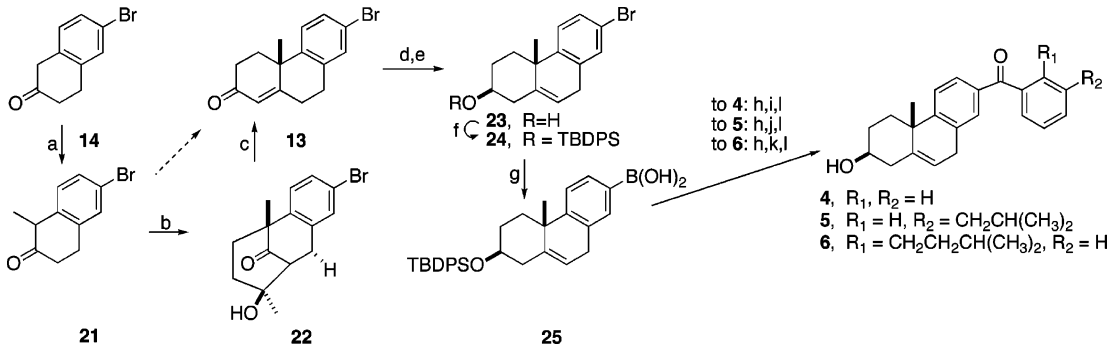
Completion of the synthesis of a tricyclic boronic acid **9** ready for formation of the benzophenone moiety (Scheme 3) began with a standard deconjugation−reduction sequence consisting in conversion of **13** to its dienol acetate derivative, followed by treatment with ethanolic $NaBH_4$ to give **23** in 88% overall yield, essentially according to the procedures of Joannou and Reeder.[19] After protection of **23** as its *tert*-butyldiphenylsilyl ether **24** in 82% yield, successive lithiation and treatment with triisopropoxyborane by the procedure of Brown and Cole[20] afforded the desired **25**. Suzuki coupling[21] of **25** with benzoic anhydride, followed by deprotection, afforded target analogue **4** in a modest but acceptable 41% yield. However, when **25** was coupled with *m*-isobutylbenzoic acid (**10**) by the mixed anhydride method employing pivaloyl anhydride,[21] only 18% of **5** could be obtained after deprotection. With *o*-isopentylbenzoic acid (**11**), only a trace of **6** could be obtained by this approach, presumably as a result of increased steric hindrance to coupling across the series benzoic acid, **10**, and **11**.

To overcome this problem, attention was turned to reversing the functional groups of the Suzuki coupling partners by using boronic acids **26**[22] and **27** (Scheme 4) to attach the second aromatic ring bearing an alkyl side chain. Compounds **26**[22] and **27** were synthesized from 3-bromo- and 2-bromobenzaldehyde, respectively, via Wittig reactions to afford alkenes **28**[23] (78% yield) and **29**[24] (90% yield of a 3:4 *E*:*Z* mixture), which, after nearly quantitative diimide reduction using tosylhydrazide[25] to afford **30**[22,26] and **31**,[27] were converted[20] to **26** and **27** in 82 and 53% yield, respectively. For coupling with **26** and **27**, tricyclic bromide was converted in 92% yield by the procedure of Klapars and Buchwald[28] to the more reactive iodide **32**, which was combined with **26** and **27** by the carbonylative Suzuki coupling procedure[29,30] to give 31% of **5** and 57% of **6**, respectively, after fluoride ion deprotection. Compound **4** was also prepared from **32** and phenylboronic acid by this procedure in the same 41% yield obtained via **25**. Although these yields of the target analogues remain relatively modest, more than ample material is readily available through this approach.

Preliminary Biochemical Evaluation. The synthesized analogues **4**, **5**, and **6** were evaluated as cholesterol surrogates in the same isotope dilution assay used previously[4] and in the preceding paper.[5] The results shown in Figure 3 indicate that this third type of analogue also can replace **1** in all major cellular pools. The tolerance for structural variation in successful cholesterol surrogates discussed in the preceding paper[5] thus extends to compounds **4**−**6** with the photo-cross-linking site in the ring D region. The ability of **4** to replace **1** is perhaps unexpected in view of reports[31,32] that sterols with severely truncated side chains do not promote lipid raft formation in model membranes. Analogues of all the types represented in Figure 1, possessing photophoric sites within and beyond each end of the cholesterol structure, have now been prepared and have met a demanding initial criterion as cholesterol surrogates. In future studies, we hope to be able to utilize the range of

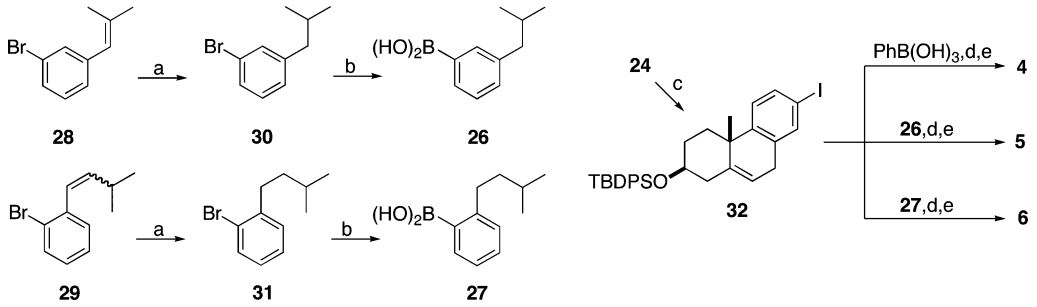
(12) Burckhalter, J. H.; Campbell, J. R. *J. Org. Chem.* **1961**, *26*, 4232−4235.
(13) Stork, G.; Brrizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrell, R. *J. Am. Chem. Soc.* **1963**, *85*, 207−222.
(14) Pfau, M.; Revial, G.; Guingant, A.; d'Angelo, J. *J. Am. Chem. Soc.* **1985**, *107*, 273−274.
(15) Volpe, T.; Revial, G.; Pfau, M.; d'Angelo, J. *Tetrahedron Lett.* **1987**, *28*, 2367−2370.
(16) d'Angelo, J.; Revial, G.; Volpe, T.; Pfau, M. *Tetrahedron Lett.* **1988**, *29*, 4427−4430.
(17) Morgan, B. P.; Swick, A. G.; Hargrove, D. M.; LaFlamme, J. A.; Moynihan, M. S.; Carroll, R. S.; Martin, K. A.; Lee, E.; Decosta, D.; Bordner, J. *J. Med. Chem.* **2002**, *45*, 2417−2424.
(18) Nerinckx, W.; Vandewalle, M. *Tetrahedron: Asymmetry* **1990**, *1*, 265−274.
(19) Joannou, G. E.; Reeder, A. Y. *Steroids* **1996**, *61*, 18−21.
(20) Brown, H. C.; Cole, T. E. *Organometallics* **1983**, *2*, 1316−1319.
(21) Goossen, L. J.; Ghosh, K. *Angew. Chem., Int. Ed.* **2001**, *40*, 3458−3460.
(22) Previously prepared by a different route: Murugesan, N.; Hunt, J. T. Eur. Pat. Appl. EP 569193 A1 19931110, 1993; Murugesan, N.; Hunt, J. T.; Stein, P. D. U.S. Patent 5514696 A 19960507, 1996.
(23) Biller, S. A.; Magnin, D. R. U.S. Patent 5157027 A 19921020, 1992.
(24) What appeaers to be the *E* isomer of **29** has been previously prepared by a different route: Atmaram, S.; Forrester, A. R.; Gill, M.; Napier, R. J.; Thomson, R. H. *Acta Chem. Scand. B* **1982**, *36*, 641−647.
(25) Harrowven, D. C.; Sutton, B. J.; Coulton, S. *Tetrahedron* **2002**, *58*, 3387−3400.
(26) Previously prepared by a different route: Okada, S.; Sawada, K.; Kayakiri, N.; Saitoh, Y.; Tanaka, H.; Hashimoto, M. Eur. Pat. Appl. EP 458207 A2 19911127, 1991.
(27) Previously prepared by a different route: Reich, H. J.; Goldenberg, W. S.; Gudmundsson, B. O.; Sanders, A. W.; Kulicke, K. J.; Simon, K.; Guzei, I. A. *J. Am. Chem. Soc.* **2001**, *123*, 8067−8079.
(28) Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 14844−14845.
(29) Ishiyama, T.; Kizaki, H.; Hayashi, T.; Suzuki, A.; Miyaura, N. *J. Org. Chem.* **1998**, *63*, 4726−4731.
(30) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457−2483.
(31) Xu, X.; Bittman, R.; Duportail, G.; Heissler, D.; Vilcheze, C.; London, E. *J. Biol. Chem.* **2001**, *276*, 33540−33546.
(32) Wenz, J. J.; Barrantes, F. J. *Biochemistry* **2003**, *42*, 14267−14276.

SCHEME 3 [a]



[a] Reagents and conditions: (a) pyrrolidine, PhH, Δ; CH_3I, dioxane, Δ; (b) *R*-(+)-1-phenylethylamine, $PhCH_3$, Δ; $H_2CCHCOCH_3$, THF, rt, 2 days; (c) *p*-TsOH, $PhCH_3$, Δ, 4 h; (d) Ac_2O, EtOAc, 70% $HClO_4$, rt, 10 min; (e) $NaBH_4$, EtOH, 0 °C, overnight; (f) TBDPSCl, imidazole, DMF, 80 °C, 4 h; (g) *n*-BuLi, THF, −78 °C, 30 min; B(O*i*Pr)$_3$, to rt, 3 h; (h) $Pd(OAc)_2$, PPh_3, THF; (i) $(PhCO)_2$, ∼2 equiv of H_2O, Δ, overnight; (j) **10**, $[(CH_3)_3CO]_2O$, ∼2 equiv of H_2O, Δ, overnight; (k) **11**, $[(CH_3)_3CO]_2O$, ∼2 equiv of H_2O, Δ, overnight; (l) TBAF, THF, rt, 4 h.

SCHEME 4 [a]



[a] Reagents and conditions: (a) $TsNHNH_2$, NaOAc, THF, Δ, overnight; (b) *n*-BuLi, THF, −78 °C, 40 min; B(O*i*Pr)$_3$, −78 °C, 2 h, rt, overnight; (c) CuI, NaI, D,L-dimethylcyclohexane-1,2-diamine, dioxane, 100 °C, 24 h; (d) $Pd(dppf)Cl_2$, CO, K_2CO_3, 80 °C, overnight, anisole; (e) TBAF, THF, rt, 4 h.

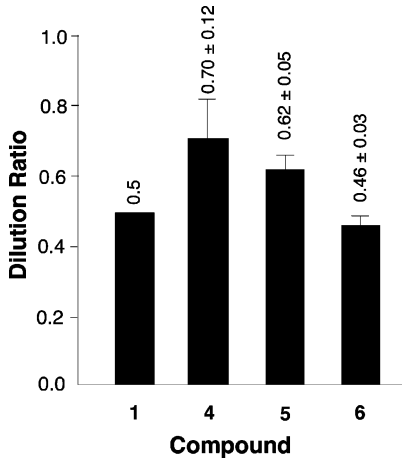


FIGURE 3. The dilution of [^{3}H]cholesterol ([^{3}H]**1**) label in fibroblast monolayers by **4**, **5**, or **6**. The dilution ratio is the reduction in [^{3}H]**1** efflux, induced by apolipoprotein A−I, to the cellular medium after fibroblast monolayers were equilibrated (48 h, 37 °C) with 10 μCi [^{3}H]**1** plus unlabeled **1** or analogue equal to the total sterol content of cells and medium compared with cells labeled with the same level of tracer [^{3}H]**1** only. Complete equilibration between sterol pools is indicated by a dilution ratio of 0.5, which is shown as the calculated dilution ratio for **1**. Values shown represent means ±1 standard deviation of three independent experiments, each including triplicate dishes of fibroblasts incubated as described in detail in ref 4 and in the Supporting Information for ref 5.

cross-linking locations that these analogues present to elucidate lipid−lipid and lipid−protein interactions in membranes of living cells.

Experimental Section

3-Isobutylbenzoic Acid (10). To a mixture of 1.50 g (5.43 mmol) of ethyl 3-iodobenzoate (**15**), prepared by Fischer esterification of 3-iodobenzoic acid, and 130 mg (0.16 mmol) of $Pd(dppf)Cl_2$ in 5 mL of THF was added 25 mL of 0.5 M isobutylzinc bromide (**17**) in THF via syringe. This mixture was stirred at rt for 6 h, diluted with 5 mL of NH_4Cl solution, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, filtered, and evaporated to give 1.21 g of crude **19** as a brown oil which was dissolved in 5 mL of ethanol and treated with a solution of 2 g of NaOH in 5 mL of water. The resulting mixture was stirred overnight, acidified by concentrated HCl to pH = 1, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, filtered, and evaporated to give 1.09 g of brown oil which was chromatographed with 30:1 hexane:ethyl acetate to give 857 mg (89%) of colorless oily **10**: ^{1}H NMR (300 MHz) δ 8.02−7.96 (m, 2H), 7.45−7.42 (m, 2H), 2.59 (d, J = 7.2 Hz, 2H), 2.02−1.88 (m, 1H), 0.96 (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz) δ 173.0, 142.4, 135.0, 131.0, 129.4, 128.5, 128.0, 45.4, 30.5, 22.5. Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.28; H, 8.08.

2-Isopentylbenzoic Acid (11). As in the preparation of **10**, 1.46 g (5.29 mmol) of ethyl 2-iodobenzoate (**16**) prepared by Fischer esterification of 2-iodobenzoic acid was treated with 3-methylbutylzinc bromide (**18**) to give 1.49 g of crude **20** which in turn gave 943 mg (93%) of **11**: mp 47−48 °C (lit.[8] 45.5−46 °C); ^{1}H NMR (300 MHz) δ 8.09−8.06 (m, 1H), 7.53−7.48 (m, 1H), 7.34−7.28 (m, 2H), 3.09−3.03 (m, 2H), 1.77−1.64 (m, 1H), 1.58−1.50 (m, 2H), 1.00 (d, J = 6.3 Hz, 6H); ^{13}C NMR (75 MHz) δ 174.2, 146.7, 133.2, 132.1, 131.6, 128.4, 126.1, 41.6, 33.1, 28.7, 22.8. Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39. Found: C, 74.83; H, 8.43.

10-Hydroxy-5-bromo-1,10-dimethyltricyclo[7.3.1.0.^{2,7}]trideca-2(7),3,5-trien-13-one (22). To a solution of 11.02 g (46.3 mmol)

of **21** in 100 mL of toluene was added 5.60 g (5.8 mL, 46.3 mmol) of (*R*)-(+)-phenylethylamine. The mixture was attached to a Dean−Stark trap and heated at reflux until no further separation of H_2O was detected. The solvent was evaporated, and the residue was dissolved in 100 mL of THF and treated with 3.53 g (4.2 mL, 50.4 mmol) of freshly distilled methyl vinyl ketone. The resulting mixture was stirred at rt for 2 days, diluted with 30 mL of H_2O and 5 mL of HOAc, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, filtered, and evaporated to give 12.83 g of brown oil which was chromatographed with 10:1 to 2:1 hexane:ethyl acetate to give 8.13 g (57%) of **22**: mp 171−173 °C. In another preparation, the crude product was washed with 3:1:1 hexane:ether:CH_2Cl_2 to give 50% of **22** without chromatography. Recrystallization from ethyl acetate gave **22**: mp 179−181 °C; ^{1}H NMR δ 7.40−7.38 (m, 1H), 7.28 (br s, 1H), 7.16−7.15 (m, 1H), 3.32 (dd, J = 18.0, 7.5 Hz, 1H), 3.18 (d, J = 18.0 Hz, 1H), 2.59 (d, J = 7.5 Hz, 1H), 2.20−2.14 (m, 1H), 1.85 (br s, 1H), 1.64−1.60 (m, 1H), 1.51−1.48 (m, 2H), 1.47 (s, 3H), 1.39 (s, 3H); ^{13}C NMR δ 213.7, 141.9, 136.3, 130.6, 130.3, 127.4, 120.8, 78.9, 57.7, 48.7, 40.7, 34.7, 32.5, 28.3, 19.9. Anal. Calcd for $C_{15}H_{17}BrO_2$: C, 58.27; H, 5.54. Found: C, 58.27; H, 5.45.

7-Bromo-4-methyl-4,4,9,10-tetrahydro-3*H*-phenanthren-2-one (13). To a solution of 113 mg (0.37 mol) of **22** in 5 mL of toluene was added 4 mg of *p*-toluenesulfonic acid. The mixture was heated at reflux for 4 h, diluted with 3 mL of saturated $NaHCO_3$ solution, and extracted with ether. The combined organic layers were washed with brine, dried, filtered, and evaporated to give 121 mg of yellow oil which was chromatographed with 4:1 hexane:ether to give 101 mg (94%) of **13** as a colorless oil: ^{1}H NMR δ 7.37−7.35 (m, 1H), 7.28−7.27 (m, 1H), 7.19−7.17 (m, 1H), 5.91 (br s, 1H), 3.02−2.97 (m, 1H), 2.91−2.85 (m, 1H), 2.76−2.65 (m, 2H), 2.57−2.50 (m, 2H), 2.38−2.33 (m, 1H), 2.09−2.02 (m, 1H), 1.56 (s, 3H); ^{13}C NMR δ 198.8, 168.8, 143.0, 137.3, 131.5, 130.3, 128.2, 124.7, 120.1, 39.2, 37.0, 34.9, 31.0, 31.0, 27.8; $[\alpha]_D^{25}$ = 117° (*c* 2.95, $CHCl_3$). Anal. Calcd for $C_{15}H_{15}BrO$: C, 61.87; H, 5.19. Found: C, 62.09; H, 5.37.

7-Bromo-4a-methyl-1,2,3,4,4a,9-hexahydrophenanthren-2-ol (23). To a solution of 6.01 g (20.7 mmol) of **13** in 300 mL of EtOAc and 40 mL of Ac_2O was added a solution of 0.2 mL of 70% $HClO_4$ in 250 mL of EtOAc. The mixture was stirred at rt for 10 min and diluted with 200 mL of saturated $NaHCO_3$ water solution. The organic layer was washed repeatedly with $NaHCO_3$ solution and then once with brine, dried, filtered, and evaporated to give crude yellow oily dienol acetate which was dissolved in 100 mL of ethanol, treated with 6.24 g (160 mmol) of $NaBH_4$, stirred at 0 °C overnight, and diluted with 10 mL of HOAc. The ethanol was evaporated, and the residue was partitioned between 50 mL of H_2O and 50 mL of ethyl acetate. The organic layers were washed with brine, dried, filtered, and evaporated to give 7.6 g of yellow oil which was chromatographed with 3:1 hexane:ethyl acetate to give 5.3 g (88%) of colorless oily **23**: ^{1}H NMR δ 7.33−7.19 (m, 3H), 5.61−5.60 (m, 1H), 3.65−3.58 (m, 1H), 3.44−3.30 (m, 2H), 2.52−2.48 (m, 1H), 2.43−2.38 (m, 1H), 2.18−2.14 (m, 1H), 2.03−1.97 (m, 2H), 1.81−1.73 (m, 1H), 1.59−1.53 (m, 1H), 1.38 (s, 3H); ^{13}C NMR δ 143.2, 138.2, 135.0, 131.0, 129.6, 128.2, 119.5, 118.8, 71.2, 42.1, 38.1, 37.3, 32.2, 29.8, 26.9. Anal. Calcd for $C_{15}H_{17}BrO$: C, 61.45; H, 5.84. Found: C, 61.19; H, 5.98.

((4b*S*,7*S*)-7-Hydroxy-4b-methyl-4b,5,6,7,8,10-hexahydrophenanthren-2-yl)(phenyl)methanone) (4) via Boronic Acid 25. To a solution of 1.17 g (2.2 mmol) of compound **24** in 10 mL of THF was added 2.7 mL of 1.0 M *n*-BuLi in hexane at −78 °C. After 30 min, 413 mg (2.2 mmol) of B(O-*i*-Pr)$_3$ was added dropwise. The resulting mixture was stirred for 1 h at −78 °C, stirred at rt for another 2 h, hydrolyzed by adding 25 mL of H_2O containing 0.1 mL of concentrated H_2SO_4, and quickly extracted twice with ether. The combined organic layers were washed with brine, dried, filtered, and evaporated to give 1.00 g of crude **25** as a colorless oil which was used without purification. According to the method of Goossen and Ghosh,[21] to a solution of 484 mg (0.98 mmol) of this crude **25** in 25 mL of THF were added 7 mg (0.03 mmol) of $Pd(OAc)_2$, 18 mg (0.068 mmol) of PPh_3, 44 μL (2.4 mmol) of H_2O, and 443 mg (1.17 mmol) of benzoic anhydride. This mixture was heated at reflux overnight, then passed through a pad of Celite, and washed with ether. The filtrate was collected and concentrated to give 313 mg of crude product, which was dissolved in 10 mL of THF. Then 2.5 mL of 1.0 M TBAF in THF was added, and the resulting mixture was stirred at rt for 4 h, quenched by adding 20 mL of saturated NH_4Cl solution, and extracted with ether. The combined organic layers were dried, filtered, and evaporated to give 312 mg crude product which was chromatographed with 1:1 hexane:ether to give 125 mg (41%) of **4** as a colorless oil: ^{1}H NMR δ 7.83−7.81 (m, 2H), 7.65−7.58 (m, 3H), 7.51−7.43 (m, 3H), 5.65 (br s, 1H), 3.66−3.60 (m, 1H), 3.51−3.38 (m, 2H), 2.54−2.50 (m, 1H), 2.46−2.41 (m, 1H), 2.26−2.22 (m, 1H), 2.18 (br s, 1H), 2.06−2.02 (m, 1H), 1.85−1.76 (m, 1H), 1.65−1.59 (m, 1H), 1.44 (s, 3H); ^{13}C NMR δ 196.9, 149.2, 138.1, 138.1, 135.2, 132.9, 132.6, 130.5, 130.3, 128.5, 128.4, 126.4, 119.2, 71.2, 42.3, 38.1, 37.9, 32.3, 30.1, 27.0. Anal. Calcd for $C_{22}H_{22}O_2$: C, 82.99; H, 6.96. Found: C, 82.88, H, 6.90.

((4b*S*,7*S*)-7-Hydroxy-4b-methyl-4b,5,6,7,8,10-hexahydrophenanthren-2-yl)(3-isobutylphenyl)methanone (5) via 25. As in the preparation of **4** via **25**, 447 mg (0.84 mmol) of **24** was converted to 501 mg of crude **25**. According to the method of Goossen and Ghosh,[21] to this crude **25** were added a solution of 150 mg (0.84 mmol) of **10** in 3 mL of THF, 10 mg (0.04 mmol) of $Pd(OAc)_2$, 25 mg (0.09 mmol) of PPh_3, 234 mg (1.26 mmol) of trimethyl acetic anhydride, and 37 μL (2.1 mmol) of H_2O. This mixture was heated at reflux overnight, then passed through a pad of Celite, and washed with ether. The filtrate was collected and concentrated to give 251 mg of crude product, which was dissolved in 10 mL of THF and deprotected as in the preparation of **4** from **25** to give 224 mg of crude product which was purified by preparative TLC on a 20 cm × 20 cm Analtech TLC plate with 1:1 hexane:ether to give 57 mg (18%) of colorless oily **5**: ^{1}H NMR δ 7.66−7.62 (m, 3H), 7.60 (br s, 1H), 7.57−7.38 (m, 3H), 5.66 (br s, 1H), 3.67−3.61 (m, 1H), 3.50−3.38 (m, 2H), 2.57−2.51 (m, 3H), 2.46−2.41 (m, 1H), 2.27−2.23 (m, 1H), 2.06−2.02 (m, 1H), 1.95−1.87 (m, 2H), 1.85−1.77 (m, 1H), 1.67−1.60 (m, 1H), 1.45 (s, 3H), 0.94 (d, J = 6.5 Hz, 6H); ^{13}C NMR δ 197.1, 149.0, 142.0, 138.1, 137.9, 135.4, 133.4, 132.8, 130.8, 130.5, 128.3, 128.2, 127.8, 126.3, 119.1, 71.2, 45.4, 42.2, 38.0, 37.8, 32.3, 30.5, 30.1, 26.9, 22.5. HRMS *m*/*z*: calcd for $C_{26}H_{30}O_2$, 374.2246; found 374.2239.

(*E*,*Z*)-1-Bromo-2-(3-methyl-1-butenyl)benzene (29). To a mixture of 14.5 g (36.7 mmol) of isobutyltriphenylphosphonium bromide in 50 mL of THF was added 25 mL of 1.6 M *n*-BuLi in hexane. This mixture was stirred at rt for 2 h, and a solution of 5.66 g (30.6 mmol) of 2-bromobenzaldehyde in 20 mL of THF was added dropwise via syringe. The resulting mixture was stirred overnight, quenched with 50 mL of saturated NH_4Cl solution and extracted with hexane. The combined organic layers were washed with brine, dried, filtered, and evaporated to a volume of ca. 5 mL. The precipitate of Ph_3PO was removed by filtration and washed with hexane. The filtrates were evaporated, and the 7.14 g of crude product was chromatographed with hexane to give 6.14 g (90%) of colorless oily **29** as a 3:4 mixture of *E* and *Z* isomers: ^{1}H NMR δ 7.63−7.61 (m, 1H), 7.58−7.53 (m, 2H), 7.33−7.26 (m, 3H), 7.16−7.08 (m, 2H), 6.74 (d, J = 16.5 Hz, 1H, *E*), 6.37 (d, J = 11.0 Hz, 1H, *Z*), 6.21−6.17 (m, 1H, *E*), 5.65−5.60 (m, 1H, *Z*), 2.74−2.67 (m, 1H, *Z*), 2.61−2.54 (m, 1H, *E*), 1.17 (d, J = 6.5 Hz, 6H, *E*), 1.07 (d, J = 7.5 Hz, 6H, *Z*); (lit.[24] ^{1}H NMR δ 7.5 (dd, J = 2 Hz, 2H), 7.23 (t, J = 2 Hz, 1H), 7.04 (t, J = 2 Hz, 1H), 6.68 (d, J = 5 Hz, 1H), 6.12 (dd, J = 2, 5 Hz, 1H), 2.5 (oct, J = 2 Hz, 1H), 1.08 (d, J = 2 Hz, 6H)); ^{13}C NMR δ 141.4, 141.2, 138.3, 138.0, 133.1, 132.8, 130.7, 128.4, 128.4, 127.6, 127.2, 127.1, 126.4, 126.2, 124.3, 123.6, 32.0, 27.5, 23.3, 22.6. Anal. Calcd for $C_{11}H_{13}Br$: C, 58.69; H, 5.82. Found: C, 58.40; H, 5.82.

***t*-Butyl((2*S*,4a*S*)-7-iodo-4a-methyl-1,2,3,4,4a,9-hexahydrophenanthren-2-yloxy)diphenylsilane (32).** According to a procedure of Klapars and Buchwald,[28] to a solution of 480 mg (0.90

mmol) of **24** in 1.5 mL of dioxane were added 85 mg (0.45 mmol) of CuI, 337 mg (2.25 mmol) of NaI, and 0.14 mL (0.9 mmol) of D,L-dimethylcyclohexane-1,2-diamine. The flask was purged with N_2, sealed with a septum, and heated at 100 °C for 24 h. The cooled reaction mixture was treated with 4 mL of concentrated NH_4OH solution and extracted with ether. The organic layer was washed with brine, dried, filtered, and evaporated to give 843 mg of a brown oil which was chromatographed to give 482 mg (92%) of colorless solid **32**. An analytical sample was obtained by recrystallization from hexane: mp 131−133.5 °C; ^{1}H NMR δ 7.72−7.69 (m, 4H), 7.47−7.37 (m, 8H), 7.02−7.00 (m, 1H), 5.34 (br s, 1H), 3.62−3.56 (m, 1H), 3.34−3.17 (m, 2H), 2.50−2.45 (m, 1H), 2.32−2.28 (m, 1H), 2.04−2.00 (m, 1H), 1.87−1.83 (m, 2H), 1.36 (m, 1H), 1.36 (s, 3H), 1.10 (m, 9H); ^{13}C NMR δ 144.2, 138.6, 137.1, 136.0, 136.0, 135.4, 135.4, 134.8, 134.8, 129.8, 129.8, 128.4, 127.8, 127.8, 118.3, 91.0, 72.8, 42.5, 38.2, 37.4, 32.6, 29.6, 27.2, 27.0, 19.4. Anal. Calcd for $C_{31}H_{35}ISiO$: C, 64.35; H, 6.1 Found: C, 64.31; H, 6.11.

Compound 4 via 32. According to the procedure of Miyaura et al.,[29] to a solution of 603 mg (1.13 mmol) of **32** in 6 mL of anisole were added 55 mg (0.07 mmol) of $Pd(dppf)Cl_2$, 152 mg (1.24 mmol) of phenylboronic acid, 467 mg (3.39 mmol) of K_2CO_3, and 850 mg (5.65 mmol) of NaI. The mixture was heated under CO at 80 °C overnight, then passed through a pad of Celite, which was washed with 50 mL of ether. The filtrate was evaporated to give 6.02 g of brown oil, which was dissolved in 10 mL of THF, and 2.5 mL of TBAF 1.0 M solution in THF was added. The resulting mixture was stirred at rt for 4 h, quenched with 20 mL of saturated NH_4Cl solution, and extracted with ether. The combined organic layers were washed with brine, dried, filtered, and evaporated to give 312 mg of oil which was chromatographed with 1:1 hexane:ether to give 125 mg (41%) of **4** as a colorless oil with ^{1}H and ^{13}C NMR spectra identical to those of **4** prepared via **25**.

Compound 5 via 32. As in the preparation of **4** via **32**, 454 mg (0.85 mmol) of **32** and 152 mg (0.85 mmol) of freshly prepared **26** gave 312 mg of oil which was chromatographed with 1:1 hexane:ether to give 91 mg (31%) of **5** as a colorless oil with ^{1}H and ^{13}C NMR spectra identical to those of **5** prepared via **25**.

((4b*S*,7*S*)-7-Hydroxy-4b-methyl-4b,5,6,7,8,10-hexahydrophenanthren-2-yl)(2-isopentylphenyl)methanone (6). 2-Isopentylphenylboronic acid (**27**) was prepared by adding 3 mL of 1.6 M *n*-BuLi to a solution of 934 mg (4.13 mol) of **31** in 5 mL of THF at −78 °C and, after stirring for 40 min at −78 °C, adding 178 mg (4.13 mmol) of B(O-*i*Pr)$_3$ dropwise via syringe. The resulting mixture was stirred at −78 °C for 2 h, allowed to stand at rt overnight, quenched with 20 mL of 1 N HCl, and extracted with ether. The combined organic layers were washed twice with brine, dried, filtered, and evaporated to give 901 mg of yellow oil which was chromatographed with 3:2 hexane:ether to give 420 mg (53%) of white solid **27**: ^{1}H NMR δ 8.20−8.19 (m, 1H), 7.51−7.48 (m, 1H), 7.32−7.28 (m, 2H), 3.22−3.18 (t, J = 8.0 Hz, 2H), 1.71−1.65 (m, 1H), 1.62−1.57 (m, 2H), 0.95 (d, J = 7.0 Hz, 6H); ^{13}C NMR δ 151.8, 137.5, 132.3, 129.9, 125.3, 42.8, 33.9, 28.4, 23.0. Upon prolonged standing or drying in vacuo, **27** underwent transformation to 2,4,6-tri-2-isopentylphenylboroxin: mp 87.5−89 °C. HRMS *m*/*z*: calcd for $C_{33}H_{45}O_3B_3$, 522.3648; found, 522.3641. As in the preparation of **4** via **32**, 208 mg (0.36 mmol) of **32** and 76 mg (0.40 mol) of freshly prepared **27** gave 231 mg of dark oil which was chromatographed with 1:1 hexane:ether to give 81 mg (57%) of **6** as a colorless oil: ^{1}H NMR δ 7.63−7.61 (m, 1H), 7.55 (m, 1H), 7.44−7.40 (m, 2H), 7.34−7.23 (m, 3H), 5.65 (br s, 1H), 3.67−3.60 (m, 1H), 3.48−3.36 (m, 2H), 2.66−2.63 (m, 2H), 2.54−2.50 (m, 1H), 2.46−2.40 (m, 1H), 2.25−2.21 (m, 1H), 2.05−2.00 (m, 1H), 1.84−1.76 (m, 1H), 1.68 (br s, 1H), 1.65−1.59 (m, 1H), 1.53−1.40 (m, 3H), 1.43 (s, 3H), 0.82 (d, J = 6.5 Hz, 6H); ^{13}C NMR δ 198.8, 149.8, 142.1, 138.9, 137.9, 135.7, 133.0, 130.5, 130.3, 130.2, 128.6, 128.3, 126.5, 125.3, 119.2, 71.2, 42.2, 41.2, 38.0, 37.9, 32.3, 31.4, 30.1, 28.2, 26.9, 22.6, 22.6. Anal. Calcd for $C_{27}H_{32}O_2$: C, 83.46; H, 8.3 Found: C, 83.38; H, 8.37.

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Supporting Information Available: General experimental methods, preparations of compounds **14**, **21**, **24**, **28**, **30**, **26**, and **31**, ^{1}H and ^{13}C NMR spectra for all compounds, NOE experiments on compound **22**, atom coordinates for molecular modeling of compounds **1**, **6**, and **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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