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Synthesis and antiproliferative properties of a photoactivatable analogue of ET-18-OCH$_3$

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Abstract—A photoreactive analogue of the antitumor ether lipid 1-*O*-octadecyl-2-*O*-methyl-glycero-3-phosphocholine (ET-18-OCH$_3$, **1**) was synthesized for the first time. The 3-(trifluoromethyl)-3-(*m*-iodophenyl)diazirine (TID) moiety was used as the photolabel in ether lipid **2a**. The TID group was tethered through an *O*-undecyloxy linkage to the *sn*-1 position of the ether lipid. Compound **2a** was found to qualitatively mimic the antiproliferative effects of **1** in the dark (i.e. in the absence of carbene generation), suggesting that this photoactivatable probe may be suitable for identification of the proteins that mediate the biological activities of **1**. Analogue **2a** was converted by Stille reaction to stannane intermediate **2b**, which was subjected to *ipso* [^{125}I]-iododestannylation to afford ^{125}I-labeled ether lipid **2c**. Photolysis (long wavelength UV light, 30 min) of **2c** in the presence of cytosolic proteins obtained from a breast cancer cell line (MCF-7), followed by NaDodSO$_4$/PAGE and autoradiography, showed radioiodination of primarily two polypeptides (with molecular masses of about 47- and 170-kDa), both of which were subject to competition by prior addition of excess **2a**. This study indicates that analogue **2c** may have utility in the characterization of the putative proteins that interact specifically with antitumor ether lipid **1**.

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1. Introduction

The antitumor ether lipid (AEL) 1-*O*-octadecyl-2-*O*-methyl-glycero-3-phosphocholine (**1**), also known as ET-18-OCH$_3$ and edelfosine, inhibits tumor cell growth by a multitude of processes.[1–3] Like other alkyl-lysophospholipids and unlike many other anticancer agents, **1** does not directly target cellular DNA. Although the principal mechanism of action of ET-18-OCH$_3$ has not yet been fully established, it is clear that the drug perturbs intracellular signaling transduction events after it is taken up spontaneously into cells.[4] We showed that ET-18-OCH$_3$ inhibits the translocation of Raf-1 to the plasma membrane of the mammary epithelial cancer cell line MCF-7, thereby directly blocking Ras-dependent activation of Raf-1 and subsequently blocking mitogen-activated protein (MAP) kinase activity and the Raf-1/MEK/ERK cascade.[5,6] ET-18-OCH$_3$ is also implicated in the c-Jun kinase (JNK) signaling pathway, since it activated JNK in leukemic cells.[7] These findings raise the possibility that the antiproliferative effects of ET-18-OCH$_3$ may arise, at least in part, from its ability to interfere with the activities of key proteins in various mitogenic signaling pathways of cells that are sensitive to ET-18-OCH$_3$. To examine protein-ET-18-OCH$_3$ interactions and thereby enhance our understanding of the drug's mechanism(s) of action, we seek to identify the intracellular signaling proteins that are inhibited by ET-18-OCH$_3$.

Photoactivatable analogues of biologically active compounds are an attractive means for determining the macromolecular targets of the parent compounds.[8] Photoreactive analogues generate highly reactive intermediates on activation with UV light. Carbene precursors have been used extensively to identify ligand-binding domains. The 3-trifluoromethyl-3-*m*-iodophenyl-3*H*-diazirine (TID) moiety is readily incorporated into the hydrophobic region of membranes,[9] and is thus a useful probe for the study of lipid–protein interactions.[8,10] In this paper, we report the first synthesis of a photoactivatable probe of ET-18-OCH$_3$ (**2a**), which bears a trifluoromethyl[11] iodophenoxydiazirine (TID) group at the terminus of the *sn*-1 *O*-alkyl moiety. To test whether the TID photolabel in **2a** perturbs its biological activities as an antitumor agent, we compared the antiproliferative activity of **2a** with that of the parent compound (**1**). Thus, we incubated a variety of epithelial cancer cell lines with **2a** in the dark, i.e. under conditions in which photoactivation of **2a** to produce the reactive carbene functionality is avoided. Our findings indicate that probe **2a** mimics the antiproliferative properties of **1** in the epithelial cancer cells we tested. To illustrate the utility of the photoactivatable probe, **2a** was converted in two steps into **2c**, the radioiodinated form of **2a**. **2c** was activated with UV light in the presence of cytosolic proteins derived from MCF-7

Keywords: antitumor compounds; diaziridenes/diazirines; lipids; phospholipids.

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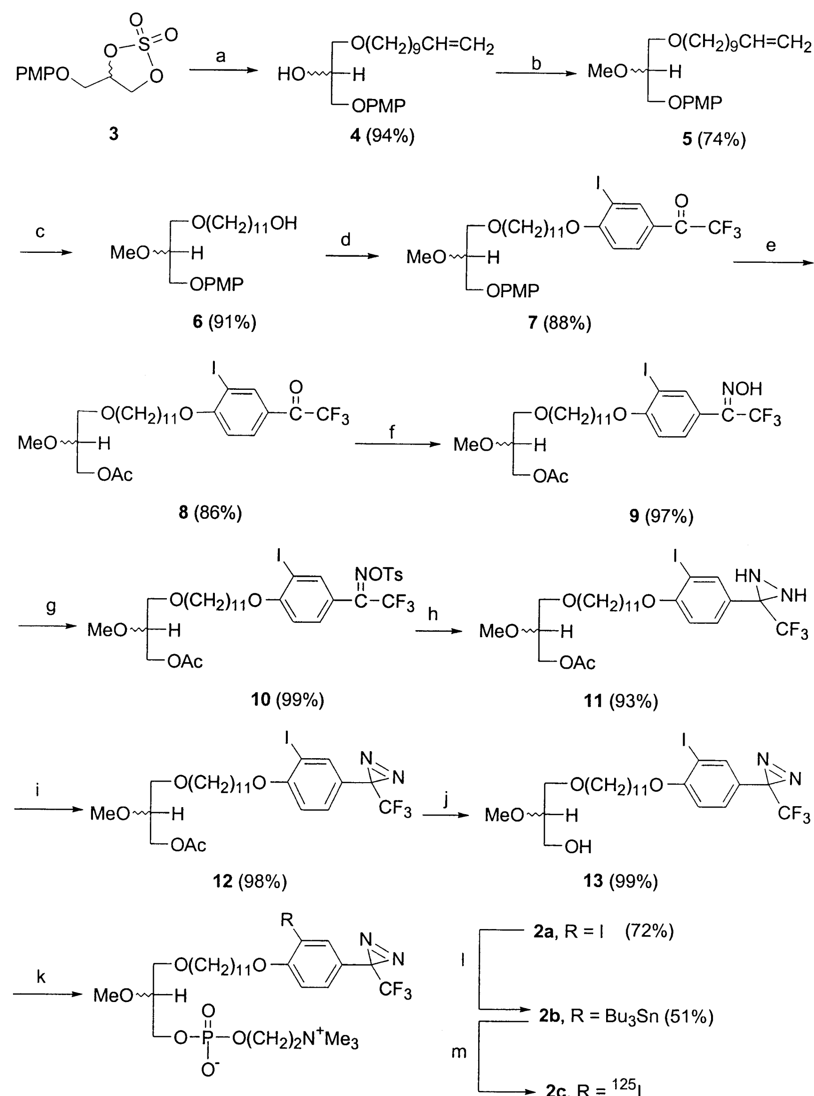
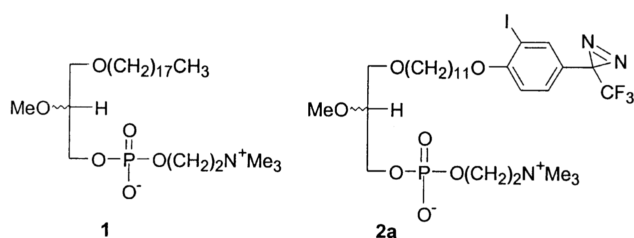


Scheme 1. Synthesis of photoaffinity labeled ET-18-OCH$_3$ analogue **2**. *Reagents and conditions*: a. CH$_2$=CH(CH$_2$)$_9$OH, *n*-BuLi, THF, 0°C; b. NaH, CH$_3$I, Bu$_4$NBr, THF; c. (i) 9-BBN, (ii) NaOH, H$_2$O$_2$; d. **17** (see Scheme 2), Ph$_3$P, DIAD, THF, rt, 18 h; e. (i) CAN, CH$_3$CN–H$_2$O (2:1), 0°C–rt, (ii) Ac$_2$O, DMAP, Et$_3$N, Et$_2$O; f. NH$_2$OH·HCl, Py-EtOH (1:6), 50–60°C, 30 h; g. *p*-TsCl, Et$_3$N, DMAP, CH$_2$Cl$_2$; h. liq. NH$_3$, CH$_2$Cl$_2$; i. I$_2$, Et$_3$N, MeOH, rt; j. NaOMe, MeOH, rt; k. (i) POCl$_3$, Et$_3$N, −10°C; (ii) choline tosylate, Py, CHCl$_3$, rt; l. Bu$_6$Sn$_2$, cat. Pd(MeCN)$_2$Cl$_2$, HMPA, 18 h, rt; m. Na^{125}I, chloramine-T, NaH$_2$PO$_4$, EtOH.

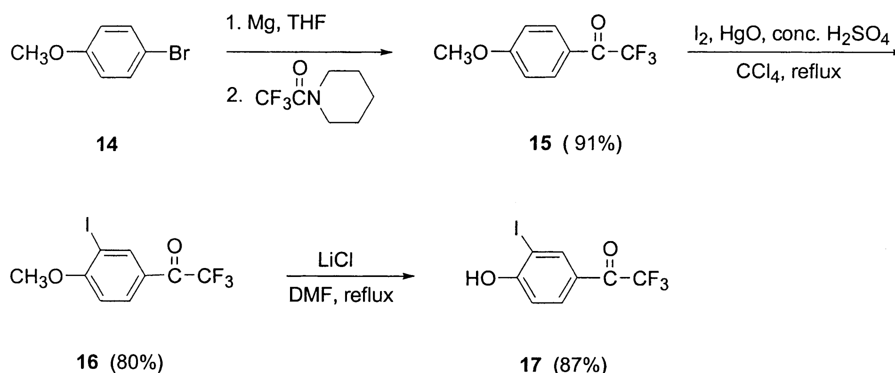
cells. Two proteins were prominently photolabeled; moreover, the extent of labeling was reduced markedly in competition experiments carried out with an excess of **2a**, indicative of a specific interaction with cellular proteins.



2. Results and discussion

2.1. Syntheses of probes 2a and 2c

Ring-opening reactions of (*S*)-1-(4′-methoxyphenyl)glycerol 2,3-cyclic sulfate (**3**)[11] with long-chain nucleophiles were recently shown to provide a convenient entry to protected glycerol derivatives.[12,13] The reaction of lithium 11-undecenoxide (2 equiv.) with cyclic sulfate **3** proceeded smoothly in THF at 0°C to give the *p*-methoxyphenyl (PMP)-protected glycerol derivative **4** in 94% yield. After the unreacted 11-undecenol was removed by converting it into 11-undecenyl methyl ether, alcohol **4** was *O*-methylated under phase-transfer conditions (excess NaH, MeI, cat. Bu$_4$NBr). Hydroboration of methoxy derivative **5** using 9-borabicyclo[3.3.1]nonane (9-BBN) afforded ω-alcohol **6**. Mitsunobu reaction[14] (DIAD/Ph$_3$P) of phenol derivative **15** (prepared as outlined in Scheme 2) with primary alcohol **6** afforded the coupling product **7** in 88% yield. The PMP ether group in **7** was removed with CAN in aqueous acetonitrile to give the free C-3 alcohol, which was acetylated to give **8** in 86% overall yield. The trifluoroacetyl group of acetate **8** was converted into the trifluoromethyldiazirinyl group in **12** by using conventional procedures.[15] Base-catalyzed hydrolysis of the acetate afforded alcohol **13** in virtually quantitative yield. Insertion of the phosphocholine



Scheme 2. Synthesis of compound **17**.

moiety by using phosphorus oxychloride followed by choline tosylate[16] gave product **2a**. Aryl iodide **2a** was subjected to Stille coupling[17] with hexa-*n*-butylditin (bis(tributyltin)) and catalytic $Pd(MeCN)_2Cl_2$ at room temperature, affording aryltin intermediate **2b** in 51% yield.[18] The latter is stable for months at −20°C. To illustrate the utility of **2a** as a photoaffinity label, radioiodine was incorporated by *ipso* destannylation of **2b** using $Na^{125}I$ with chloramine-T in buffered aqueous ethanol solution. Fractionation on solid-phase extraction cartridges (aminopropyl and reversed phase silica) provided product **2c**.

2.2. Synthesis of aryl iodide 17

As shown in Scheme 2, trifluoroacetylation of the Grignard reagent of **14** with *N*-(trifluoroacetyl)piperidine provided trifluoroacetophenone analogue **15** in high yield. Electrophilic aromatic iodination of **15** took place with very high regioselectivity; the methoxy and trifluoroacetyl groups both direct the iodo group to the desired position. *O*-Demethylation of anisole **16** with lithium chloride in DMF at reflux provided precursor **17** in high yield. Phenol **17** was coupled to alcohol **6** via Mitsunobu reaction (Scheme 1); it can also be coupled to primary alkyl halides via its phenolate group.

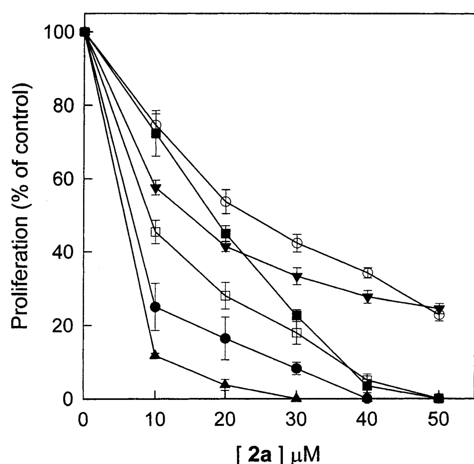


Figure 1. Effect of **2a** on the proliferation of epithelial cancer cell lines. Proliferating MCF-7 (●), MDA-MB-468 (■), MDA-MB-231 (▲), BT-549 (▼), A549 (○), and DU 145 (□) cells were incubated in 24-well plates with medium containing **2a** (0–50 μM). Cells in representative wells were counted on day 0 prior to the addition of **2a**. After 48 h, the medium was replaced with freshly prepared drug-containing media for another 48 h. The cell numbers were determined in each well, and the increase over day 0 for each concentration was expressed as a percentage of that in control wells with no drug. The results are the means of two different experiments with quadruplicate wells per experiment.

2.3. Review of the synthetic procedure

We chose an ether linkage as the means of coupling the aryl trifluoromethyldiazirine moiety to the *sn*-1 *O*-alkyl chain of the probe. An advantage of using an ether linkage to tether the carbene precursor to the lipid is that this linkage affords resistance to chemical and enzymatic hydrolysis. However, this linkage has the disadvantage of introducing a polar atom into the hydrophobic region of the molecule, which is accommodated in the interior region of the membrane bilayer.

Our synthesis of the new photoactivatable analogue of **1** features several noteworthy steps: (a) regioselective introduction of an iodine atom into the aromatic ring of compound **15** (Scheme 2) in the presence of excess HgO, affording aryl iodide **16** in high yield;[19] (b) *O*-demethylation of **16**, followed by Mitsunobu coupling of phenol **17** with ω-alcohol **6** (Scheme 1); (c) Stille coupling to replace the nonradiolabeled iodide in **2a** with a tin-containing group, affording the stable intermediate **2b**; and (d) chloramine-T mediated *ipso* iododestannylation of **2b**, giving radioiodinated **2c**.

The synthetic sequence used to prepare **2a** can be readily modified to prepare a range of photoactivatable compounds carrying linkers with different chain lengths at the *sn*-1 position coupled to the aryldiazirine moiety.

2.4. Biological

Incubation of epithelial cancer cells derived from breast (MCF-7, MDA-MB-231, MDA-MB-468, BT549), lung (A549), and prostate (DU145) with **2a** for 48 h resulted in a concentration-dependent decrease in the proliferation of the cells; at 50 μM of **2a**, the decrease ranged from 40 to 90% (data not shown). The cytotoxic properties of **2a** were manifested by incubating the cells with 30–50 μM of the compound for 96 h (Fig. 1). The order of decreasing susceptibility to the cytotoxic effects was: MDA-MB-231>MCF-7>DU145=MDA-MB-468. Under the same experimental conditions, **2a** was not cytotoxic toward

Table 1. Growth inhibitory properties of ET-18-OCH_3 (**1**) and **2a** on epithelial tumor cells

Cell line	IC_{50} (μM)		
	ET-18-OCH_3 (incubation period, 48 h)	**2a** (incubation period, 48 h)	**2a** (incubation period, 96 h)
MCF-7	2	17.5	7
MDA-MB-231	3	9	6
BT549	3.5	37	15
A549	8	37	23
MDA-MB-468	19	48	18
DU145	15	>50	9

Cells were incubated with **1** or **2a** for 48 or 96 h, as indicated. For details of the in vitro assay, see Section 4 and Ref. 23.

BT549 and A549, but the proliferation of the cells was reduced to approximately 20% of the controls. One can infer from these results that incubating BT549 or A549 cells for periods longer than 96 h would ultimately result in the death of the cells. Table 1 presents the IC_{50} values (drug concentrations required to inhibit growth by 50%) found for incubation of the cells with **2a** for 48 and 96 h. Also shown are the IC_{50} values obtained when the cells were incubated with ET-18-OCH_3 (**1**) for 48 h. An incubation period of 96 h with **2a** was needed to obtain IC_{50} values approaching those of the parent compound (**1**). While the reason for this is unclear, we speculate that the presence of polar atoms in the hydrophobic region of **2a** and/or the shorter *sn*-1 chain length in **2a** than in **1** may reduce the rate of uptake of the compound and its accumulation in the cells. Nevertheless, the above results indicate that **2a** is an effective probe since it retains the antiproliferative and cytotoxic activity of the parent compound (**1**).[20]

Fig. 2 shows the results of radioiodination of MCF-7 cytosolic proteins in the presence of **2c** (lane 1). Analysis by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed a prominent band of labeled protein with a molecular mass (Mr) of about 47 kDa. Another protein with a Mr of about 170 kDa was also present. Both of these bands were markedly reduced in intensity when an excess of **2a** was present (lanes 2 and 3), indicating that the interaction of **2c** with these proteins is specific. A band having Mr of about 62-kDa is also apparent in the presence of excess **2a**.

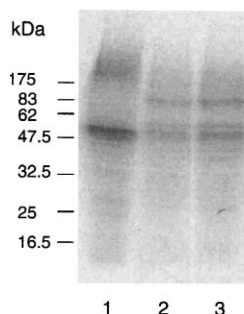


Figure 2. Radioiodination of MCF-7 cytosolic proteins by **2c**. Cytosolic proteins were photolyzed with **2c** (lane 1) and with **2c** in the presence of a 200-fold excess (lane 2) and a 100-fold excess (lane 3) of **2a** as described in Section 4.1. Proteins were separated on 10% SDS gels and subjected to autoradiography.

3. Conclusion

The synthesis of **2a**, the first photoactivatable probe of ET-18-OCH_3 (**1**), in 30% overall yield has been described here. This model photoactivatable probe was found to qualitatively mimic the antiproliferative properties of the parent compound (**1**) against a variety of epithelial cancer cell lines in the dark. The ability of **2c** to label cytosolic proteins obtained from MCF-7 cells was also demonstrated. This study suggests that intracellular proteins that interact with AELs can be identified by photoactivatable labeling.

4. Experimental

4.1. General methods

^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at 400 and 100 MHz on a Bruker spectrometer, respectively. The reference chemical shifts for $CDCl_3$ were δ 7.24 (^1H) and 77.0 ppm (^{13}C). Melting points are uncorrected. Flash chromatography was carried out with Merck 60 (230–400 mesh) silica gel. TLC was carried out on EM 0.25-mm thick silica gel 60 F_{254} aluminum sheets. Visualization was with 10% sulfuric acid solution in ethanol. Solid-phase 'Bond-Elut' extraction silica gel cartridges (aminopropyl, 500 mg, and C-18, 2 g) were purchased from Alltech Associates (Deerfield, IL). The solvents were dried by distillation from the following reagents before use: THF (sodium/benzophenone); chloroform (P_2O_5); CH_2Cl_2 (CaH_2); HMPA (redistilled). $Na^{125}I$ (17 Ci/mg) in 10^{-5} M NaOH (pH 8–11) was purchased from New England Nuclear Life Sciences. High-resolution MS data were recorded at the University of California, Riverside, Mass Spectrometry Facility. Aluminum foil was used to exclude light from the reaction flasks containing the TID probe. Exponentially growing MCF-7 cells were harvested, lysed by sonication, and the cytosol was obtained by centrifugation.[5] The cytosolic proteins were irradiated for 30 min at room temperature in plastic trays (Accutran purchased from Schleicher and Schuell) with long-wavelength (365 nm) UV light (Mineralight lamp model UVGL-58, UVP, Inc.) at a distance of 1 cm from the lamp.

4.1.1. (*S*)-1-(4′-Methoxyphenyl)glycerol 2,3-cyclic sulfate (3). The titled compound was synthesized from (*R*)-1-(4′-methoxyphenyl)glycerol as previously reported.[12]

4.1.2. 1-*O*-(11′-Undecenyl)-3-*O*-(4′-methoxyphenyl)-*sn*-glycerol (4). To an ice-cooled solution of 11-undecenol

(10.2 g, 60.0 mmol) in 250 mL of THF, 24 mL (60 mmol) of *n*-butyllithium (a 2.5 M solution in hexane) was added by a syringe under argon. The mixture was stirred for about 30 min until no more bubbles formed. A solution of 7.81 g (30 mmol) of cyclic sulfate **3** in 50 mL of THF was added dropwise at 0°C. The mixture was stirred overnight at room temperature. To facilitate the workup of the product (product **4** and 11-undecenol have very similar R_f values), methyl iodide (8.5 g, 60 mmol) was added to convert the unreacted 11-undecenol into the corresponding methyl ether. After the reaction mixture was stirred for 10 h, a solution of 80 mL of concentrated H_2SO_4 in 400 mL of water was added. After the solution was stirred overnight, the product was extracted with Et_2O. The ether layer was washed with water, saturated aqueous sodium bicarbonate solution, saturated aqueous sodium thiosulfate solution, brine, and then dried ($MgSO_4$). The residue obtained after concentration was purified by chromatography (elution with hexane/EtOAc 6:1) to provide 9.8 g (94%) of **4** as a clear oil; R_f 0.35 (hexane/EtOAc 6:1). ^{1}H NMR: δ 6.85–6.79 (m, 4H), 5.82–5.76 (m, 1H), 5.00–5.89 (m, 2H), 4.13–4.10 (m, 1H), 3.96–3.94 (m, 2H), 3.75 (s, 3H), 3.57–3.44 (m, 4H), 2.04–1.99 (m, 2H), 1.61–1.54 (m, 2H), 1.35–1.25 (m, 12H). ^{13}C NMR: δ 154.0, 152.7, 139.2, 115.5, 114.6, 114.1, 71.7, 71.4, 69.7, 69.1, 55.7, 33.8, 29.6, 29.5, 29.4, 29.1, 28.9, 26.1. DEI-MS 350 $(M)^+$; HRMS calcd for $(C_{21}H_{34}O_4)^+$ 350.2457, found 350.2461.

4.1.3. 1-*O*-(11′-Undecenyl)-2-*O*-methyl-3-*O*-(4′-methoxyphenyl)-*sn*-glycerol (5). To an ice-cooled solution of 7.65 g (21.8 mmol) of alcohol **4** in 200 mL of THF, 1.05 g (43.8 mmol) of sodium hydride was added. After about 40 min (when no bubbles were still formed), a solution of 9.29 g (66 mmol) of methyl iodide in 10 mL of THF and 0.5 g (1.6 mmol) of tetra-*n*-butylammonium bromide were added. The mixture was stirred for 20 h at room temperature, and then concentrated. After Et_2O (200 mL) and water (100 mL) were added, the aqueous layer was extracted with Et_2O (150 mL). The combined ether layer was washed with water and brine, and dried ($MgSO_4$). The product (5.92 g, 74%) was purified by chromatography (elution with hexane/EtOAc 20:1, then 10:1); R_f 0.65 (hexane/EtOAc 3:1). ^{1}H NMR: δ 6.86–6.79 (m, 4H), 5.82–5.75 (m, 1H), 4.99–4.89 (m, 2H), 4.03–3.07 (m, 2H), 3.74 (s, 3H), 3.74–3.67 (m, 1H), 3.60–3.56 (m, 2H), 3.49 (s, 3H), 3.45–3.42 (m, 2H), 2.04–1.98 (m, 2H), 1.56–1.51 (m, 2H), 1.36–1.24 (m, 12H). ^{13}C NMR: δ 153.8, 153.0, 139.2, 115.5, 114.5, 114.1, 78.8, 71.8, 69.9, 68.2, 58.2, 55.7, 33.8, 29.6, 29.5, 29.4, 29.1, 28.9, 26.1. DEI-MS 364 $(M)^+$; HRMS calcd for $(C_{22}H_{36}O_4)^+$ 364.2614, found 364.2612.

4.1.4. 1-*O*-(11′-Hydroxyundecyl)-2-*O*-methyl-3-*O*-(4′-methoxyphenyl)-*sn*-glycerol (6). To a solution of 1.82 g (5.0 mmol) of alkene **5** in 40 mL of THF, 15 mL (7.5 mmol) of a 0.5 M solution of 9-BBN in THF was added dropwise under argon. After the solution was stirred for 8 h at room temperature, the reaction was quenched with 1 mL of MeOH. A solution of 0.45 g of NaOH in 4 mL of water was added, and the mixture was cooled in an ice bath; then 2 mL of 30% H_2O_2 (18 mmol) was added dropwise. After the mixture was stirred overnight, THF was removed under reduced pressure, and the residue was extracted with Et_2O twice. The ether layer was washed with brine twice, dried ($MgSO_4$), and concentrated. Product **6** (1.84 g, 91%) was obtained by chromatography (elution with hexane/EtOAc 2:1) as a clear oil; R_f 0.10 (hexane/EtOAc 3:1). ^{1}H NMR: δ 6.90–6.83 (m, 4H), 4.07–4.17 (m, 2H), 3.79 (s, 3H), 3.79–3.61 (m, 5H), 3.54 (s, 3H), 3.50–3.46 (m, 2H), 1.61–1.55 (m, 4H), 1.47 (br s, 1H), 1.35–1.28 (m, 12H). ^{13}C NMR: δ 153.9, 153.0, 115.5, 114.6, 78.8, 71.8, 69.9, 68.3, 63.0, 58.2, 55.7, 32.8, 29.6, 29.5, 29.40, 29.38, 26.1, 25.7. DEI-MS 382 $(M)^+$; HRMS calcd for $(C_{22}H_{38}O_5)^+$ 382.2719, found 382.2707.

4.1.5. 4-Methoxytrifluoroacetophenone (15). The titled compound (Scheme 2) was synthesized as previously reported[19b] in 91% yield.

4.1.6. 3-Iodo-4-methoxy-trifluoroacetophenone (16). A mixture of 1.03 g (50 mmol) of **15**, 2.16 g (100 mmol) of HgO, 0.5 mL of concentrated H_2SO_4, and 1.27 g (50 mmol) of iodine in 25 mL of CCl_4 was heated to reflux with vigorous stirring for 5 h. The reaction mixture was cooled to room temperature, and then filtered through a pad of Celite. The filtrate was washed with 2N aqueous sodium thiosulfate solution and brine, and then dried (Na_2SO_4). The residue obtained after evaporation was purified by column chromatography (elution with hexane/EtOAc 9:1), affording 1.32 g (80%) of the product as a white solid; mp 52–54°C; R_f 0.22 (hexane/EtOAc 9:1). ^{1}H NMR: δ 8.47 (s, 1H), 8.04 (d, J=8.8 Hz, 1H), 6.89 (d, J=8.8 Hz, 1H), 3.98 (s, 1H). ^{13}C NMR: δ 177.9 (q, J_{C-F}=35 Hz), 163.6, 141.7, 132.7 (d, J=2.1 Hz), 124.3, 116.6 (q, J=145 Hz), 110.3, 86.5, 56.9.

4.1.7. 4-Hydroxy-3-iodo-trifluoroacetophenone (17). *O*-Demethylation of **16** was carried out as follows. A mixture of 3.35 g (10.0 mmol) of **16** and 1.3 g (31 mmol) of LiCl in 25 mL of DMF was heated at reflux under argon for 2 h. The reaction mixture was cooled to room temperature, poured into water (100 mL), and acidified with 10% HCl. The product was extracted with Et_2O (2×75 mL). The ethereal layer was washed with brine twice, dried ($MgSO_4$), and concentrated. The residue was dried under high vacuum, affording 2.8 g (87%) of **17** (R_f 0.45, CH_2Cl_2), which was used directly in the next step (see Scheme 1, step d). A small amount of the product was purified by chromatography (elution with CH_2Cl_2) to give a white solid; mp 87–88°C. ^{1}H NMR: δ 8.40 (d, J=0.4 Hz, 1H), 7.98 (d, J=8.6 Hz, 1H), 7.09 (d, J=8.8 Hz, 1H), 6.13 (s, 1H). ^{13}C NMR: δ 178.4 (q, J=35 Hz), 160.8, 141.2 (d, J=1.6 Hz), 132.9 (d, J=1.0 Hz), 125.9, 124.6, 118.0 (q, J=290 Hz), 115.3, 86.3. DEI-MS 316 $(M)^+$; HRMS calcd for $(C_8H_4F_3IO_2)^+$ 315.9208, found 315.9208.

4.1.8. 1-*O*-[11′-(2-(Iodo-4-(trifluoroacetyl)phenoxy)undecyl)]-2-*O*-methyl-3-*O*-(4′-methoxyphenyl)-*sn*-glycerol (7). To a mixture of 627 mg (1.64 mmol) of **6** was added a solution of 588 mg (1.84 mmol) of 4-hydroxy-3-iodo-trifluoroacetophenone (**17**) and 504 mg (1.92 mmol) of Ph_3P in 12 mL of THF, followed by a solution of 400 mg (1.88 mmol, 95% purity) of diisopropyl azodicarboxylate (DIAD) in 3 mL of THF. The mixture was stirred for 18 h at room temperature. After concentration, the residue was purified by chromatography (elution with hexane/EtOAc 6:1) to afford 0.99 g (88%) of **7** as an oil; R_f 0.40 (hexane/

EtOAc 3:1). ^{1}H NMR: δ 8.49 (d, J=1.7 Hz, 1H), 8.03 (dd, J=7.6 Hz, 0.8 Hz, 1H), 6.88–6.80 (m, 5H), 4.12 (t, J=6.4 Hz, 2H), 4.05–4.00 (m, 2H), 3.76 (s, 3H), 3.69–3.59 (m, 3H), 3.51 (s, 3H), 3.48–3.44 (m, 2H), 1.89–1.86 (m, 2H), 1.61–1.51 (m, 4H), 1.38–1.26 (br m, 12H). ^{13}C NMR: δ 177.9 (q, J=35 Hz), 163.2, 153.9, 153.0, 141.7, 132.7,124.0, 115.5, 114.6, 111.0, 87.0, 78.8, 71.8, 69.91, 69.89, 68.2, 58.2, 55.7, 29.7, 29.6, 29.53, 29.45, 29.43, 29.2, 28.7, 26.1, 25.9. FAB-MS 680 $(M)^+$; HRMS calcd for $(C_{30}H_{40}F_3IO_6)^+$ 680.1822, found 680.1808.

4.1.9. 1-*O*-[11′-(2-Iodo-4-(trifluoroacetyl)phenoxy)undecyl)]-2-*O*-methyl-3-*O*-acetyl-*sn*-glycerol (8). To an ice-cooled mixture of 1.89 g (2.76 mmol) of PMP ether **7** in 40 mL of MeCN and 20 mL of water was added 4.76 g (8.69 mmol) of ammonium ceric(IV) nitrate (CAN). The mixture was stirred for 2.5 h at room temperature, and then stirred with aqueous 1N $Na_2S_2O_3$ for 30 min. The mixture was extracted with Et_2O (2×150 mL), washed with brine, and dried ($MgSO_4$). After concentration, the residue was dissolved in 50 mL of anhydrous Et_2O. To the solution of the crude alcohol were added 1.5 g (14.9 mmol) of Et_3N, 25 mg (0.20 mmol) of DMAP, and 1.0 g (9.8 mmol) of acetic anhydride. The mixture was stirred overnight and then acidified with 2N HCl. After the mixture was extracted with Et_2O (twice), the combined organic layers were washed with saturated aqueous sodium bicarbonate solution and brine, and dried ($MgSO_4$). The product (1.46 g, 86% for two steps) was obtained as an oil by chromatography (elution with hexane/EtOAc 6:1, then 2:1); R_f 0.30 (hexane/EtOAc 3:1). ^{1}H NMR: δ 8.49 (d, J=1.6 Hz, 1H), 8.03 (d, J=8.6 Hz, 1H), 6.86 (d, J=8.9 Hz, 1H), 4.25 (dd, J=11.7, 3.8 Hz, 1H), 4.14–4.09 (m, 3H), 3.53–3.50 (m, 1H), 3.48–3.46 (m, 2H), 3.43 (s, 3H), 3.43–3.39 (m, 2H), 2.05 (s, 3H), 1.87–1.82 (m, 2H), 1.55–1.48 (m, 4H), 1.36–1.23 (m, 12H). ^{13}C NMR: δ 177.9 (q, J=35 Hz), 170.9, 163.2, 141.6, 132.6, 124.0, 116.6 (q, J=290 Hz), 111.0, 87.0, 78.0, 71.8, 69.9, 69.7, 63.7, 58.0, 29.52, 29.49, 29.43, 29.41, 29.22, 29.14, 28.7, 26.0, 25.9, 20.9.

4.1.10. 1-*O*-[11′-(2-Iodo-4-(trifluoroacetyloxime)phenoxy)undecyl)]-2-*O*-methyl-3-*O*-acetyl-*sn*-glycerol (9). A mixture of 240 mg (0.39 mmol) of **8** and 32 mg (0.47 mmol) of hydroxylamine hydrochloride in 3 mL of EtOH and 0.5 mL of pyridine was heated at 50–60°C for 30 h. The mixture was concentrated and partitioned between Et_2O and water. The ether layer was washed with 2N HCl, saturated aqueous sodium bicarbonate solution, brine, and dried ($MgSO_4$). On concentration and drying under vacuum, 239 mg (97%) of **9** was obtained as an oil, which was pure enough to be used in the next step. ^{1}H NMR: (as a mixture of the anti and syn stereoisomers) δ 8.96–8.67 (two broad peaks, 1H), 7.95–7.89 (2d, 1H), 7.47–7.40 (2dd, 1H), 6.83–6.76 (2d, 1H), 4.22–4.01 (m, 4H), 3.71–3.40 (m, 8H), 2.06 (s, 3H), 1.81–1.80 (m, 2H), 1.59–1.49 (m, 4H), 1.33–1.17 (m, 12H).

4.1.11. 1-*O*-[11′-(2-Iodo-4-(trifluoroacetyl)phenoxy)-undecyl)]-2-*O*-methyl-3-*O*-acetyl-*sn*-glycerol *O*-tosyl oxime (10). To an ice-cooled solution of 1.31 g (2.07 mmol) of *syn* and *anti* oximes **9**, 530 mg (5.3 mmol) of Et_3N, and 20 mg (0.16 mmol) of DMAP in 40 mL of dry CH_2Cl_2, *p*-TsCl (434 mg, 2.3 mmol) was added slowly. The mixture was stirred at room temperature for about 1 h. After CH_2Cl_2 was added, the mixture was washed with saturated aqueous sodium bicarbonate solution, brine, and dried ($MgSO_4$). After concentration, 1.61 g (99%) of *O*-tosyl oxime **10** was obtained; R_f 0.80 (CH_2Cl_2/MeOH 10:1). ^{1}H NMR ($CDCl_3$) (two stereoisomers): δ 7.98–7.83 (m, 2H), 7.77–7.74 (m, 1H), 7.41–7.29 (m, 3H), 6.81–6.55 (2d, 1H), 4.24–4.20 (m, 1H), 4.10–4.00 (m, 3H), 3.51–3.38 (m, 8H), 2.44–2.43 (2s, 3H), 2.04 (s, 3H), 1.81–1.79 (m, 2H), 1.52–1.48 (m, 4H), 1.36–1.21 (m, 12H). FAB-MS 786 $(MH)^+$; HRMS calcd for $(C_{32}H_{43}F_3INO_8S+H)^+$ 786.1786, found 786.1738.

4.1.12. 1-*O*-[11′-(2′-Iodo-4′-(3″-trifluoromethyldiazirinyl)phenoxy)undecyl]-2-*O*-methyl-3-*O*-acetyl-*sn*-glycerol (11). To a solution of 1.50 g (1.91 mmol) of **10** in 35 mL of CH_2Cl_2 in a pressure tube was added liquid ammonia (30 mL) at −78°C. After the mixture was stirred at room temperature for 2.5 h, the excess ammonia was evaporated, and the product was extracted with CH_2Cl_2. The organic layer was washed with water and brine, and dried ($MgSO_4$). The residue was purified by chromatography (elution with CH_2Cl_2/MeOH 20:1), providing 1.13 g (93%) of **11** as a viscous oil; R_f 0.60 (CH_2Cl_2/MeOH 10:1). ^{1}H NMR: δ 7.97 (d, J=2.0 Hz, 1H), 7.51 (dd, J=8.5 Hz, 1.8 Hz, 1H), 6.76 (d, J=8.6 Hz, 1H), 4.25–4.21 (m, 1H), 4.10–4.06 (m, 1H), 4.00 (t, J=6.3 Hz, 2H), 3.53–3.51 (m, 1H), 3.48–3.46 (m, 2H), 3.43 (s, 3H), 3.42–3.39 (m, 2H), 2.73 (d, J=8.7 Hz, 1H), 2.16 (d, J=8.7 Hz, 1H), 2.05 (s, 3H), 1.82–1.79 (m, 2H), 1.55–1.46 (m, 4H), 1.25 (br m, 12H). ^{13}C NMR: δ 171.0, 158.9, 138.9, 129.5, 125.2, 123.4 (q, J=277 Hz), 111.4, 86.5, 78.0, 71.8, 69.7, 69.4, 63.7, 58.0, 56.9 (q, J=145 Hz), 29.53, 29.51, 29.47, 29.46, 29.42, 29.19, 28.9, 26.02, 25.97, 20.9.

4.1.13. 1-*O*-[11′-(2′-Iodo-4′-(3″-trifluoromethyldiazirinyl)-phenoxy)undecyl]-2-*O*-methyl-3-*O*-acetyl-*sn*-glycerol (12). A mixture of 1.03 g (1.63 mmol) of **11**, 457 mg (1.79 mmol) of iodine, and 412 mg (4.08 mmol) of Et_3N in 15 mL of MeOH was stirred at room temperature for 0.5 h. After 150 mL of Et_2O was added, the mixture was poured into 1N $Na_2S_2O_3$ solution. The organic layer was washed with brine and dried ($MgSO_4$). The product (1.0 g, 98%) was obtained by chromatography (elution with CH_2Cl_2); R_f 0.70 (CH_2Cl_2/MeOH 50:1). ^{1}H NMR: δ 7.55 (d, J=2.2 Hz, 1H), 7.16 (dd, J=2.0, 8.6 Hz, 1H), 6.74 (d, J=8.7 Hz, 1H), 4.25–4.21 (m, 1H), 4.10–4.07 (m, 1H), 3.98 (t, J=6.4 Hz, 2H), 3.52–3.40 (m, 8H), 2.06 (s, 3H), 1.81–1.76 (m, 2H), 1.55–1.46 (m, 4H), 1.30–1.26 (m, 12H). ^{13}C NMR: δ 170.9, 158.8, 137.6, 128.2, 122.4, 22.0 (q, J=273 Hz), 111.5, 86.9, 78.1, 71.8, 69.8, 69.4, 63.7, 58.0, 29.55, 29.52, 29.46, 29.43, 29.2, 28.9, 27.4 (q, J=40.5 Hz), 26.03, 25.96, 20.90.

4.1.14. 1-*O*-[11′-(2′-Iodo-4′-(3″-trifluoromethyldiazirinyl)-phenoxy)undecyl]-2-*O*-methyl-*sn*-glycerol (13). To a solution of 1.0 g (1.59 mmol) of **12** in 25 mL of MeOH was added ~8 mg (0.33 mmol) of NaH. The mixture was stirred at room temperature for 2 h. After three drops of 10% HCl were added, the mixture was concentrated and the residue was extracted with Et_2O (2×75 mL). The ether layer

was washed with brine and dried ($MgSO_4$). The product (0.92 g, 99%) was purified by chromatography (elution with CH_2Cl_2/MeOH 100:2.5); R_f 0.40 (CH_2Cl_2/MeOH 50:1). ^{1}H NMR: δ 7.54 (d, J=2.3 Hz, 1H), 7.16 (dd, J=2.2 Hz, J=8.8 Hz, 1H), 6.74 (d, J=8.6 Hz, 1H), 3.98 (t, J=6.4 Hz, 2H), 3.73–3.63 (m, 2H), 3.53–3.50 (m, 2H), 3.44 (s, 3H), 3.437–3.39 (m, 3H), 1.83–1.76 (m, 2H), 1.56–1.44 (m, 4H), 1.44–1.23 (m, 12H). ^{13}C NMR: δ 158.8, 137.6, 128.3, 122.4, 122.0 (q, J=273 Hz), 111.5, 86.9, 79.8, 71.9, 70.6, 69.9, 69.4, 62.7, 57.7, 29.56, 29.51, 29.47, 29.46, 29.42, 29.2, 28.9, 27.5 (q, J=40.5 Hz), 26.1, 26.0.

4.1.15. 1-*O*-[11′-(2′-Iodo-4′-(3″-trifluoromethyldiazirinyl)-phenoxy)undecyl]-2-*O*-methyl-*sn*-glycero-3-phosphocholine (2a). A 50-mL flask was flame-dried and flushed with argon. A solution of 117 mg (0.76 mmol) of $POCl_3$ and 77 mg (0.76 mmol) of Et_3N in 0.5 mL of alcohol-free $CHCl_3$ was cooled to −10°C. A solution of 360 mg (0.61 mmol) of **13** in 3 mL of $CHCl_3$ was added. After the mixture was stirred for 1 h, choline tosylate (250 mg, 0.91 mmol) and 0.5 mL of pyridine were added. The reaction mixture, which became orange, was stirred overnight at room temperature. After water (0.2 mL) was added to quench the reaction, the mixture was stirred for 30 min. The residue obtained after concentration was passed through a TMD-8 resin (elution with THF/H_2O 9:1). The fractions containing the product were combined, concentrated, and purified by chromatography (elution with $CHCl_3$/MeOH 4:1, then with $CHCl_3$/MeOH/H_2O 65:25:4). The product (330 mg, 72%) was obtained after lyophilization from benzene and filtration of a chloroform solution of the product through a 0.2-μm Millipore filter to remove suspended silica gel; R_f 0.18 ($CHCl_3$/MeOH/H_2O 65:25:4). ^{1}H NMR: δ 7.53 (d, J=2.1 Hz, 1H), 7.16 (dd, J=8.5 Hz, 2.2 Hz, 1H), 6.72 (d, J=8.6 Hz, 1H), 4.23–3.74 (m, 9H), 3.45–3.30 (m, 16H), 1.78–1.73 (m, 2H), 1.46–1.40 (m, 4H), 1.27–1.20 (m, 12H). ^{13}C NMR: δ 158.8, 137.5, 128.2, 122.4, 122.0 (q, J=273 Hz), 111.5, 86.8, 79.6, 71.7, 70.2, 69.4, 66.3, 64.9, 59.2, 57.7, 54.3, 29.7, 29.6, 29.5, 29.2, 29.0, 28.9, 28.7, 27.5 (q, J=40.5 Hz), 26.1, 26.0. FAB-MS 752 $(MH)^+$, 724 $(MH-N_2)^+$; HRMS calcd for $(C_{32}H_{46}F_3IN_3PO_7+H)^+$ 752.2150, found 752.2189; UV (MeOH) λ_{max} (ϵ, M^{-1} cm^{-1}) 295 nm (1975), 375 nm (329).

4.1.16. 1-*O*-[11′-(2′-(Tri-*n*-butylstannyl)-4′-(3″-trifluoromethyldiazirinyl)phenoxy)undecyl]-2-*O*-methyl-*sn*-glycero-3-phosphocholine (2b). A mixture of 48 mg (0.064 mmol) of **2a**, 371 mg (0.64 mmol) of hexa-*n*-butylditin, and 8.0 mg (0.031 mmol) of $Pd(CH_3CN)_2Cl_2$ in 3 mL of HMPA was stirred for 18 h at room temperature under argon. The reaction mixture was diluted with 5 mL of $CHCl_3$, and then passed through an aminopropyl silica gel solid-phase extraction cartridge (500 mg) (elution with $CHCl_3$ to remove the solvent and unreacted hexabutylditin, then with $CHCl_3$/MeOH 4:1). The filtrate was passed through another aminopropyl cartridge (elution with $CHCl_3$/MeOH 4:1). After concentration, the residue was dissolved in a small volume of MeOH and loaded onto a C-18 cartridge (2 g) (elution with MeOH). The first part of the eluant consisted of starting material **2a** (9 mg), whereas the last part afforded 24.2 mg (51%) of the desired product. ^{1}H NMR: δ 7.14 (s, 1H), 7.07 (dd, J=2.0 Hz, 8.7 Hz, 1H), 6.73 (d, J=8.6 Hz, 1H), 4.28 (m, 2H), 3.89–3.77 (m, 6H), 3.50–3.34 (m, 17H), 1.73 (m, 2H), 1.51–1.25 (m, 28H), 1.03–0.87 (m, 6H), 0.85 (t, J=7.3 Hz, 3H). ^{13}C NMR: δ 164.3, 135.0, 131.5, 128.5, 122.4 (q, J=275 Hz), 120.8, 109.3, 79.7, 71.8, 70.4, 67.9, 66.4, 64.9, 59.3, 57.8, 54.4, 29.7, 29.63, 29.56, 29.46, 29.3, 29.2, 29.1, 29.0, 27.3, 26.14, 26.10, 13.6, 9.9.

4.1.17. 1-*O*-[11′-(2′-[^{125}I]-4′-(3″trifluoromethyldiazirinyl)-phenoxy)undecyl]-2-*O*-methyl-*sn*-glycero-3-phosphocholine (2c). The following iododestannylation procedure was used to incorporate radioiodine. To a 1.5-mL cone-shaped vial were added 100 μL (0.093 μmol) of a solution of arylstannane **2b** (1.7 mg of **2b** in 2 mL of absolute EtOH), 50 μL (10 μmol) of a solution of NaH_2PO_4 (276 mg of $NaH_2PO_4{\cdot}H_2O$ in 10 mL of H_2O), 40 μL (1.3 μmol) of unlabeled NaI, and 10 μL of $Na^{125}I$ (1 mCi). Then 50 μL (0.23 μmol) of chloramine-T (from a freshly prepared stock solution of 13 mg (0.046 mmol) of chloramine-T trihydrate in 10 mL of absolute EtOH) was added with stirring. The reaction mixture, which became pale yellow, was stirred for 30 min. The reaction was quenched with 50 μL of 30% aqueous $NaHSO_3$ solution. After 200 μL of chloroform was added, the mixture was shaken and the bottom layer was collected and concentrated to dryness by using a stream of nitrogen. The residue was dissolved in 100 μL of $CHCl_3$ and loaded onto an aminopropyl solid-phase extraction cartridge (500 mg). The vial was washed with an additional 100 μL of $CHCl_3$. The latter solution was also loaded onto the cartridge. Elution with 4 mL of $CHCl_3$ removed the byproducts (presumably tributyltin iodide and *p*-toluenesulfonamide); then elution with 4 mL of 1:1 $CHCl_3$/MeOH afforded product **2c** in a single fraction.

4.2. Effects of compounds 1 and 2a on cell proliferation

A 50 mM stock concentration of **2a** was prepared in absolute EtOH and stored at −80°C in the dark. The cells were maintained under subdued light to avoid carbene generation. The effects of compounds **1** and **2a** on the increase in cell numbers were assessed on the following panel of epithelial cancer cell lines: breast (MCF-7, MDA-MB-468, MDA-MB-231, BT549), prostate (DU145), and lung (A549). The in vitro assays have been described previously.[21–24] Briefly, cells were subcultured into 24-well plates and allowed to grow to the exponential phase. The medium was changed and replaced with one containing **2a** (0–50 μM) or **1** (0–20 μM) in 10% fetal bovine serum supplemented medium. The drug-containing media were prepared shortly before use. The cell numbers in representative wells were determined at the time of addition of the compounds. The cells were incubated in an atmosphere of 5% CO_2, and the plates were shielded from light with aluminum foil. At the end of the incubation period (48 or 96 h), the cells were detached with trypsin and the cell numbers were determined with a Coulter counter. The increase in cell numbers was expressed as a percentage relative to those in wells without any drug. When the cells were incubated with **2a** for 96 h, the medium in all wells was replaced after 48 h with fresh medium. All manipulations were carried out in a partially darkened room.

4.3. Gel electrophoresis and autoradiography

For radioiodination of MCF-7 cytosolic proteins, 2 μL of a solution of **2c** in EtOH (10 nmol, 2.2×10^6 cpm) was added to 500 μL of cytosolic proteins (1.2 mg of protein) in a 1.5-mL microfuge tube. Competition experiments were carried out in the presence of a 100- and a 200-fold excess of **2a** in 4 μL of EtOH. The tubes were rotated in the dark at room temperature prior to photolysis. Photolysis was carried out as described under Section 4.1. After photolysis, the samples were boiled at 100°C for 5 min in 300 μL of Laemmli SDS sample buffer.[5] The proteins were separated by SDS-PAGE on 10% gels, which were dried and exposed to film (Kodak X-OMAT AR) for 3 days.

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