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A novel photoaffinity probe for the LTD_4 receptor

Michel Gallant*, Nicole Sawyer, Kathleen M. Metters, Robert J. Zamboni

Merck Frosst Centre for Therapeutic Research, P.O.Box 1005, Pointe Claire-Dorval, Québec, Canada, H9R 4P8

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Abstract

A novel photoaffinity probe for the leukotriene D_4 receptor (LTD_4) is described. L-745310, which is structurally related to the potent LTD_4 antagonist MK-0476 (Singulair®), was found to selectively label a 43-kDa protein in guinea-pig lung membrane previously identified as the LTD_4 receptor.

Key words: Photoaffinity probe, leukotriene D_4 receptor, L-745310.

1. Introduction

The cysteinyl leukotrienes are derived from the biotransformation of an unstable epoxide intermediate, leukotriene A_4 (LTA_4). LTA_4 is produced from arachidonic acid in a two step oxidative process catalyzed by the 5-lipoxygenase in the presence of the activating protein FLAP [1]. LTA_4 is further biotransformed by two separate metabolic pathways to produce either the proinflammatory chemotatic agent LTB_4 or the cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4 [2]. They are produced by human eosinophils, macrophages, and mast cells [3]. The leukotrienes account collectively for the biological activity known as slow reacting substance of anaphylaxis thus exhibiting potent contractile actions on respiratory smooth muscle [4]. The leukotrienes have been shown to be key mediators in pathogenic events such as prolonged bronchoconstriction, increased mucus production, vascular permeability, and inflammatory processes [5]. Consequently, they have been associated with disease states such as human bronchial asthma and bronchial hyperreactivity. The leukotrienes produce their physiological effects through interaction with specific cell surface receptors [6]. The existence of a LTD_4 receptor has been demonstrated using radioligand binding assays along with a number of tissue- and cell-based assays including smooth muscle contraction assays [6,7]. In addition the clinical success of the LTD_4 receptor antagonist MK-0476 (**1**, Fig. 1) [8], in blocking agonist- and exercise-induced bronchoconstriction in in vivo pharmacological models and in asthmatic subjects, strongly support the importance of LTD_4 receptor mediated events in human bronchial asthma [9,10].

The LTD_4 receptor is a member of the G-protein coupled family of receptors [11], which are known to share a common motif of a single polypeptide chain with seven transmembrane domains. However, neither the amino-acid nor the nucleotide sequence have yet been determined for the LTD_4 receptor. In order to characterize this receptor, we reported in 1993 the preparation and reactivity of a radioiodinated photoactivatable analog of LTD_4 (^{125}I-azido-LTD_4, **2**, Fig. 1) [12]. Radiolabeling experiments with ^{125}I-azido-LTD_4 showed that the probe selectively labeled a 43-kDa protein in guinea-pig lung membrane preparations. Most convincingly, the photolabeling of this protein was inhibited by LTD_4, LTE_4, LTC_4, and the LTD_4 receptor antagonist MK-0571 (**3**, Fig. 1) [13] with a rank order and potencies identical to their respective IC_{50} values determined in equilibrium competition binding assays. This paper demonstrates that an analog of MK-0476, the antagonist L-745310 (**4**, Fig. 1), can equally be used as a photoaffinity probe, to selectively label and consequently isolate and characterize the LTD_4 receptor. L-745310 is synthetically more accessible than ^{125}I-azido-LTD_4 and includes a photoactivatable group suited for G-protein coupled receptors.

*Corresponding author.

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2. Results

L-745310 (**4**) contains a trifluoromethyl diazirine [14] unit as the photoactivatable group. It offers many advantages over the use of the traditional azido group. Under proper photolysis conditions (350 nM), which are not detrimental to biological systems, diazirine generates highly reactive carbene within minutes [15]. These unstable carbenes can efficiently undergo intermolecular insertion of carbon–hydrogen bonds with minimum side reactions. Though the trifluoromethyl diazirine unit forms, upon photolysis, an electrophilic linear diazo isomer as the main side product, the latter was shown to be stable and therefore less susceptible to induce non-specific labeling by nucleophilic attack [15,16]. In contrast to the azido group, diazirine moiety are hydrophobic and therefore potentially more accessible to membrane bound receptors [16].

2.1 Synthesis

The preparation of L-745310 was accomplished in a convergent manner by preparing the synthon containing the photoactivatable moiety independently from the backbone of the probe. The diazirine synthon **11** was prepared in 8 steps starting from methyl 3,5-diiodobenzoate (**5**, Scheme 1). Reduction of **5** with DIBAH at −40°C led to the desired benzyl alcohol which was converted directly to the corresponding silyl ether **6** in 60% overall yield. The next 6 steps have been reported in literature for different substrate [15,16]. The ether **6** was monoacylated by lithium halogene exchange at low temperature (−100°C) using *n*-BuLi, followed by addition of ethyl trifluoroacetate. The resulting trifluoromethylacetophenone **7** (80% yield) was converted to a stereoisomeric (*E*/*Z*) mixture of oximes by addition of $HONH_2$·HCl. The crude mixture of oximes was tosylated to give a 1.4 to 1 mixture of stable tosyl-oximes **8**, which could be separated on silica-gel. Since both tosyl-oximes could lead to the desired diaziridine **9** upon addition of ammonia, although the minor stereoisomer reacted at a slower rate, they were processed as a mixture. The diaziridine **9**, obtained in 97% yield, was oxidized to the corresponding diazirine using freshly prepared Ag_2O and was processed without purification. Commercially available Ag_2O resulted in an incomplete

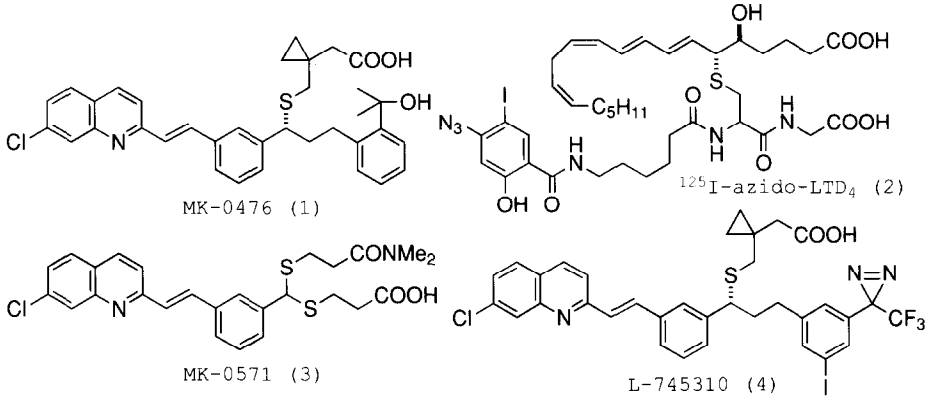
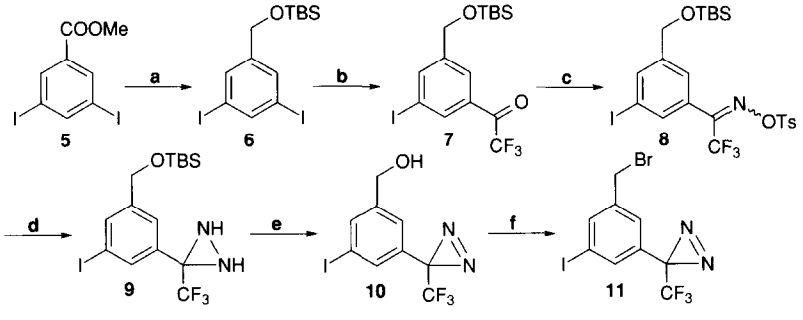
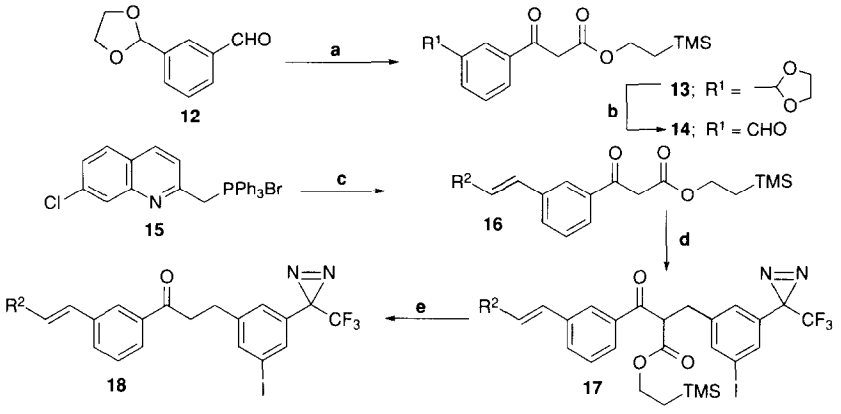


Fig. 1.



Scheme 1.' Reagents and conditions: (a) i. DIBAH, THF, −40°C; ii. TBSCl, imidazole, DMF, 60%. (b) i. *n*-BuLi, THF, −100°C; ii. CF_3COOEt, −100°C, 80%. (c) i. $HONH_2$·HCl, pyr, EtOH, 60°C; ii. TsCl, NEt_3, DMAP, 79%. (d) NH_3, Et_2O, 97%. (e) i. Ag_2O, Et_2O; ii. TBAF, THF, 0°C, 78%. (f) CBr_4, DIPHOS, CH_2Cl_2, 0°C, 81%.

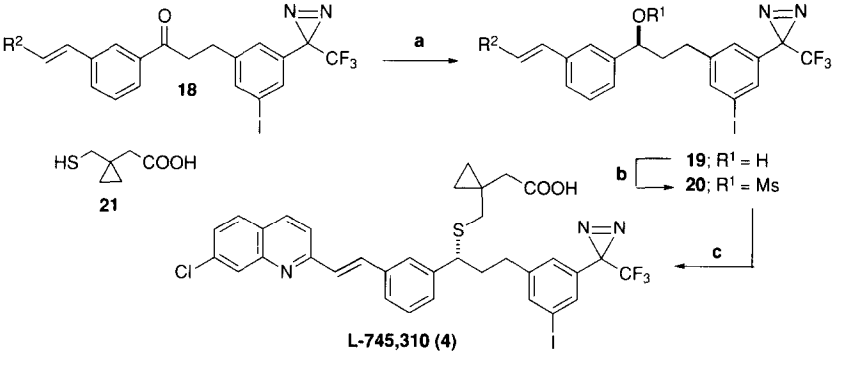


Scheme 2. Reagents and conditions: R^2 = 7-chloro-quinoline (a) i. 2-TMS-ethyl acetate, LDA, THF, −78°C; ii. **12**, 0°C, iii. MnO_2, 70%. (b) PPTS, Acetone, H_2O, 95%. (c) i. *t*-BuOK, THF, −78°C; ii. **14**, 0°C, 86%. (d) i. NaH, DMF, HMPA, 0°C; ii. **11**, 84%. (e) TBAF, AcOH, 84%.

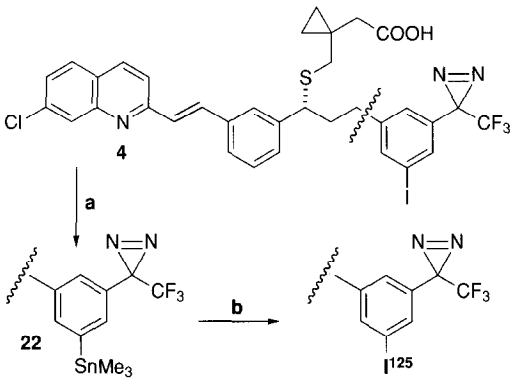
reaction. Deprotection of the silyl ether using TBAF afforded the benzylic alcohol **10** in an overall yield of 78% for the two steps. Finally the alcohol **10**, upon reaction with CBr_4 and DIPHOS, provided the desired diazirine–benzyl bromide **11** in 81% yield.

The backbone of the photoaffinity probe was prepared in 4 steps (Scheme 2) starting with the aldol condensation of the mono protected dialdehyde **12** [17] and the lithium enolate of trimethylsilylethyl acetate. The resulting hydroxy-ester was oxidized to the keto-ester **13** in an overall yield of 70%. Deprotection of **13** under acidic conditions provided the aldehyde **14**. Next, a Wittig reaction using the ylide generated by the deprotonation of the quinoline phosphonium salt **15** [13] with potassium *t*-butoxide produced the desired quinolinylethenylphenyl **16** in 86% yield. The photoactivatable diazirine was introduced by addition of the benzyl bromide **11** to the sodium enolate of **16** providing the benzylated keto-ester **17** in 84% yield. The keto-ester **17** was found to be inert to commercially available TBAF solution (1 M, THF) at reflux. The basicity of TBAF probably induces the formation of the enolate of keto-ester **17**, thus prohibiting the decarboxylation. On the other hand, the reaction proceeded by addition of AcOH (5 equiv.) to a solution of keto-ester **17** in 1 M TBAF (THF, 10 equiv.) yielding the ketone **18** in 84%.

The enantioselective reduction of ketone **18** using (−)-B-chlorodiisopinocampheylboron (DIPCl) developed by Brown and coworkers [18] gave the desired *β*-alcohol **19** in 92% yield with an 95% *ee* as determined by Mosher's ester (Scheme 3). The thiol-acid side chain was introduced with inversion of configuration according to an established procedure [8] by a SN_2 displacement of the corresponding benzylic mezylate **20**. Addition of the dianion of the thiol-acid **21** generated in THF using two equiv. of *n*-BuLi to the mezylate **20** afforded the desired photoaffinity probe L-745,310 (**4**) in 66% yield.



Scheme 3. Reagents and conditions: R^2 = 7-chloro-quinoline (a) DIPCl, Hunig's base, CH_2Cl_2, −40°C to 0°C, 92%, 95% *ee*. (b) MsCl, NEt_3, CH_2Cl_2, 0°C. (c) i. **21**, *n*-BuLi, THF, −22°C to 0°C; ii. **20**, 0°C, 66%.



Scheme 4. Reagents and conditions: (a) $Pd(PPh_3)_4$, $Me_3SnSnMe_3$, dioxane, 50°C, 80%. (b) NaI^{125}, chloramine-T, DMF, phosphate buffer, pH 7.

2.2 ^{125}I-Iodination

Radiolabeling of L-745310 was efficiently achieved in a two step process. L-745310 (**4**) was converted to the nucleophilic arylstannane intermediate **22** (Scheme 4) by a palladium catalyze coupling. The trimethylstannyl **22** was iodinated using NaI^{125} in presence of Chloramine-T® which generated in situ the highly reactive ^{125}ICl species [19]. The photoaffinity probe ^{125}I-L-745310 was finally purified by HPLC and used immediately. ^{125}I-L-745310 was found to be stable at −78°C though it would slowly decompose after a long period of time, leading to an increase in non-specific labeling.

2.3 Radioligand binding

The leukotriene D_4 receptor binding assay used in these studies has been previously described in detail [7,9]. The binding affinity of L-745310 for the LTD_4 receptor was found to be 100-fold less than MK-0476 (Table 1). The addition of 0.05% of human serum albumin (HSA) to the guinea-pig lung membrane preparation reduced this discrepancy by tenfold. This negative protein shift effect may be indicative of the increased hydrophobicity of L-745310 compared to MK-476. The presence of HSA could enhance the solubility of the probe by acting as a detergent or by preventing the probe from adhering to the surface of the incubation tubes and other membrane components.

2.4 Photoaffinity labeling

It was previously shown that ^{125}I-azido-LTD_4 (**2**) identified a single polypeptide (43-kDa) in photoaffinity labeling experiments on guinea-pig lung membrane preparations [12]. These labeling experiments could be inhibited in a concentration-dependent manner using agonist (LTD_4) or antagonist (MK-0571). In addition, the labeling was modulated by cations (Ca^{2+}) and by nucleotide analogs (GTPγS). These data, along with others described in the cited paper, confirmed that the labeled 43-kDa protein was the LTD_4 receptor. The incubation of ^{125}I-L-745310 with guinea-pig lung membrane preparation under similar conditions was found to label the same 43-kDa protein. In agreement, the labeling could be inhibited by LTD_4. However, a high level of nonspecific labeling was observed, which was rendered more intense upon addition of LTD_4 presumably because the displacement of the probe by LTD_4 increased its concentration. Consequently, optimization of the experimental conditions were undertaken.

Addition of detergent, such as taurocholate, increased specific labeling of the 43-kDa protein with respect to the nonspecific labeling as shown in Fig. 2. Optimum labeling was obtained when the concentration of taurocholate

Table 1

	Guinea-pig[a] IC_{50} (nM)	Guinea-pig HSA[b] IC_{50} (nM)
MK-476 (**1**)	0.41 ± 0.20 (n = 10)	0.53 ± 0.23 (n = 3)
Azido LTD_4 (**2**)	1.7 (n = 2)	
L-745310 (**4**)	27, 53	3.7, 7.8

[a]Inhibition of specific binding of $[^3H]LTD_4$ to guinea-pig lung membrane. Values are mean ± S.E.M. or individual determination.

[b]Binding assay performed as in [a] but the incubation is supplemented with 0.05% HSA.

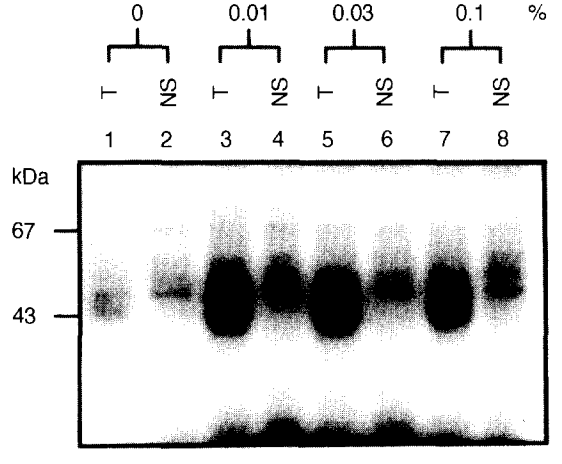


Fig. 2. Modulation of the photolabeling of the 43-kDa protein using ^{125}I-L-745310 by addition of detergent. Total (T) and nonspecific (NS; in presence of 1 μM LTD_4) photolabeling were performed in the presence of taurocholate (0–0.1%). Radiolabeled proteins were visualized by SDS–PAGE followed by autoradiography.

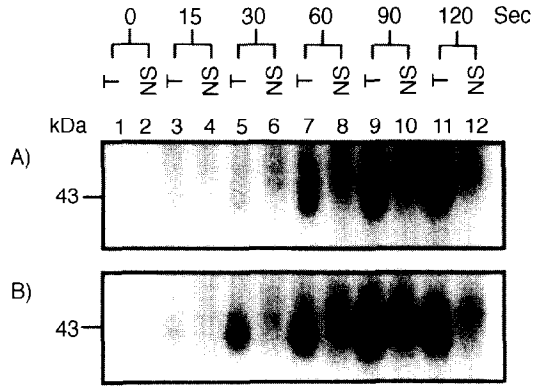


Fig. 3. Modulation of the photolabeling of the 43-kDa protein using ^{125}I-L-745310 by irradiation time and temperature. Total (T) and nonspecific (NS; in presence of 1 μM LTD_4) photolabeling were performed at different irradiation (350 nm) times from 0–120 s. Photolabeling experiments were conducted at rt (panel A) and ≈ -80°C (panel B). Radiolabeled proteins were visualized by SDS–PAGE followed by autoradiography.

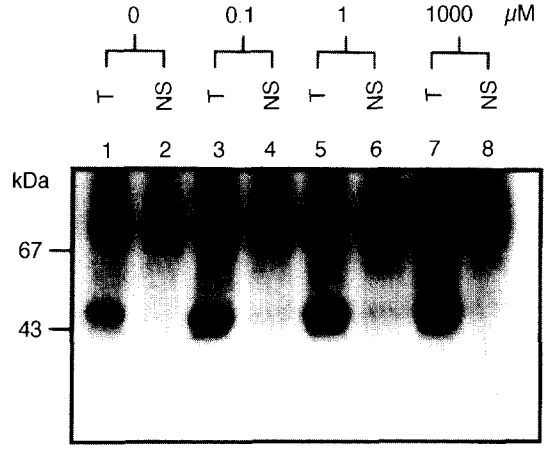


Fig. 5. Effect of GTPgS on the photolabeling of the 43-kDa protein by ^{125}I-L-745310. Total (T) and nonspecific (NS; in presence of 1 μM LTD_4) photolabeling were performed in the presence of GTPγS (0–1000 μM). Radiolabeled proteins were visualized by SDS–PAGE followed by autoradiography.

was set between 0.01 and 0.03% (lanes 3 and 5). The degree of labeling could also be modulated by irradiation time. Optimum photolabeling was achieved when the irradiation (350 nm) was maintained for 90 s or more, as exemplified by panels A and B (Fig. 3). Furthermore, the temperature at which the irradiation was performed was found to be critical. Panel B (Fig. 3), when compared to panel A, clearly shows that a higher degree of specific labeling can be attained when the photolabeling was performed at low temperature by allowing the protein-probe mixture to cool prior to irradiation on an aluminum block immersed in liquid nitrogen. Specific radiolabeling of the 43-kDa protein also increased with increasing guinea-pig lung membrane protein as expected for specific identification of a receptor (Fig. 4).

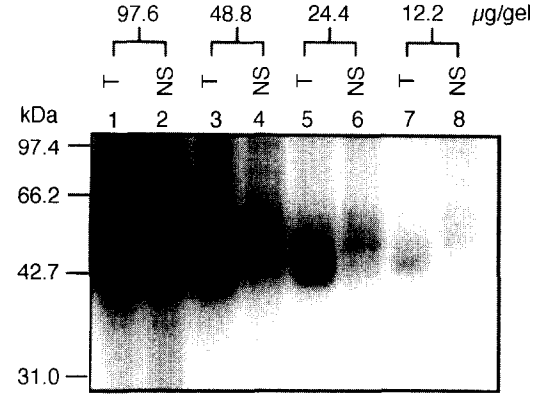


Fig. 4. Protein curve of the photolabeling of the 43-kDa protein by ^{125}I-L-745310. Total (T) and nonspecific (NS; in presence of 1 μM LTD_4) photolabeling were performed using different quantity of guinea-pig lung membrane protein (97.6–12.2 μg). Radiolabeled proteins were visualized by SDS–PAGE followed by autoradiography.

Having optimized the photolabeling experimental conditions, the labeling characteristics of ^{125}I-L-745310 were determined. The presence of GTPγS, a non-hydrolysable GTP analog, results in dissociation of the receptor-G-protein complex thereby converting the receptor to a low affinity state for agonist without affecting the binding of antagonist [20]. The presence of GTPγS did not modify the affinity of L-745310 for the guinea-pig lung LTD_4 receptor (Fig. 5), therefore confirming that this probe behaves as an antagonist toward the labeled receptor. This is in contrast to previous results using ^{125}I-azido-LTD_4 where the specific radiolabeling of the receptor was strongly inhibited by GTPγS. This suggests that ^{125}I-L-745310 will have greater utility in radiolabeling the receptor for purification since binding to the receptor will be independent to coupling to G-proteins.

3. Conclusion

The photoaffinity probe ^{125}I-L-745310 selectively radiolabeled the LTD_4 receptor (43-kDa) in guinea-pig lung membrane preparation. The labeling was successfully inhibited by both agonist (LTD_4) and antagonist (MK-0476). The optimization of the experimental conditions resulted in highly specific labeling of the targeted receptor. The probe was found to behave as an antagonist toward the LTD_4 receptor. The use of a trifluoromethyl diazirine as the photoactivatable moiety demonstrated an improvement over the previously

reported use of azido group for these type of labeling experiments. The antagonist ^{125}I-L-745310 exhibited superior labeling specificity over ^{125}I-azido-LTD_4. Preliminary experiments on the photolabeling of the human LTD_4 receptor have revealed that the latter is expressed in very small amount in lung membrane preparation thus making it a challenging target. To overcome this problem a new generation of photoaffinity probe with increased binding affinity for the human LTD_4 receptor is needed.

4. Experimental

4.1 General methods

All reagents and dry solvents were obtained from commercial sources and used without further purification. All reactions were carried out under an inert atmosphere and protected from light. Flash chromatography was performed on silica-gel (Merck, 230–400 mesh). ^{1}H and ^{13}C NMR were recorded on a Bruker ARX-400 or AMX-300 instrument. Infrared spectra were recorded on a Perkin–Elmer 681 spectrometer. Optical rotation were measured on a Perkin–Elmer 241 polarimeter. Melting points were taken on a Mettler FP61 apparatus and are uncorrected. High resolution mass spectra (HRMS) and elemental analysis (EA) were obtained from Oneida Research Services. The laser densitometer Molecular Dynamics, ImageQuant. was used for gel analysis.

*4.1.1 O-(t-Butyldimethylsilyl)-3,5-diiodo-benzylalcohol (**6**)*

To a solution of methyl-3,5-diiodo-benzoate (**5**) (19.89 g, 51.3 mmol) in dry toluene (200 ml) at −40°C was added over 40 min DIBAH (1.5 M in Tol, 75 ml, 113 mmol). The resulting mixture was stirred for 1 h at −40°C followed by addition of water (100 ml), warmed to rt and finally filtered on celite. The organic phase was separated and the aqueous phase was backwashed with toluene (100 ml). The combined organic extracts were washed with brine (100 ml), dried over $MgSO_4$, filtered, and concentrated. The residual solid, imidazole (7.98 g, 117 mmol), *t*-butyldimethylsilyl chloride (8.53 g, 56.6 mmol) were combined in dry DMF (150 ml). The resulting solution was stirred overnight at rt then diluted with ether (1 l), washed with water (3×600 ml), brine (500 ml), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (Hex) afforded the desired material as a light-yellow solid (14.68 g, 60%); mp 38.5–39.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.62 (s, 1H), 7.61 (s, 1H), 4.62 (s, 2H), 0.94 (s, 9H), 0.10 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.5, 143.4, 134.2, 94.7, 63.2, 25.9, 18.3, -5.3; IR (melted) 2950–2650, 1250, 1115, 840 cm^{-1}; LRMS (CI) *m/z* 475 (M + 1).

*4.1.2 5-(t-Butyldimethylsilyloxy)methyl-3-iodo-2,2,2-trifluoromethylacetophenone (**7**)*

To a solution of diiodobenzyl **6** (14.60 g, 30.8 mmol) in dry THF (150 ml) at −100°C was added dropwise over 30 min *n*-BuLi (2.5 M in Hex, 12.9 ml, 32.3 mmol). The solution was stirred for 15 min then ethyl trifluoroacetate (4.40 ml, 36.9 mmol) was added over 15 min. The resulting mixture was stirred at −100°C for 30 min, quenched at −100°C using saturated $NaHCO_3$ (200 ml) then extracted with ether (3×300 ml). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residual yellow oil was flash chromatographed (Tol:Hex, 2:1) to yield the desired material as a colorless oil (10.84 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.80 (s, 2H), 4.76 (s, 2H), 0.96 (s, 9H), 0.12 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.2 (q, J = 35 Hz), 144.9, 141.5, 137.0, 131.4, 126.5, 126.4, 116.3 (q, J = 292 Hz), 94.3, 63.2, 25.8, 18.2, −5.5; IR (neat) 2950–2850, 1720, 1200, 1150 cm^{-1}; LRMS (CI) *m/z* 445 (M + 1).

*4.1.3 5'-[(t-Butyldimethylsilyloxy)methyl]-3'-iodo-2,2,2-trifluoromethylacetophenone O-tosyl-oxime (**8**)*

To a solution of the trifluoromethylacetophenone **7** (10.84 g, 24.4 mmol) in pyridine (50 ml) and ethanol (50 ml) was added hydroxylamine hydrochloride (2.47 g, 35.5 mmol). The resulting mixture was stirred at 60°C overnight, cooled to rt and concentrated. The residual oil was dissolved in ether (500 ml), washed with water (2×250 ml), brine, dried over $MgSO_4$, filtered, and concentrated. The residual oil was dissolved in CH_2Cl_2 (100 ml), cooled to 0°C then NEt_3 (10 ml, 71.7 mmol), a catalytic amount of DMAP and tosyl chloride (6.95 g, 36.5 mmol) were added. The final mixture was allowed to reach rt overnight. The volatiles were evaporated and the residue was dissolved in ether (500 ml) washed with water (2×250 ml), brine, dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (Hex:CH_2Cl_2, 3:1) yielded the desired oximes (two stereoisomers) as a colorless oil (11.85 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88–7.84 (m, 2H), 7.79 (s, 1H), 7.56–7.49 (2s, 1H), 7.38–7.28 (m, 3H), 4.72–4.67 (m, 2H), 2.47 (s, major stereoisomer (1.4/1)), 2.45 (s, minor stereoisomer), 0.92 (m, 9H), 0.09 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.7 (q, J = 34 Hz, major), 152.5 (q, J = 33 Hz, minor), 146.3, 146.1, 144.6, 144.3, 138.4, 138.0, 137.9, 135.7, 134.9, 131.2, 131.0, 129.2, 129.1, 126.2, 125.4, 124.3, 119.3 (q, J = 278 Hz, major), 117.0 (q, J = 284 Hz, minor), 93.9, 93.7, 63.3, 25.8, 21.7, 18.3, −5.4; IR (neat) 2950–2850, 1390, 1200–1140 cm^{-1}; LRMS (CI) *m/z* 614 (M + 1).

*4.1.4 3-(5-((t-Butyldimethylsilyloxy)methyl)-3-iodo-phenyl)-3-trifluoromethyl diaziridine (**9**)*

To a degassed solution of oxime **8** (11.85 g, 19.3 mmol) in dry ether (150 ml), cooled in liquid N_2 was

condensed ammonia (≈10 ml). The tube was sealed and the resulting solution was gently shaken at rt for 2 days. After cooling the mixture in liquid N_2, the tube was opened and the excess ammonia was allowed to escape slowly. The mixture was filtered and concentrated to give the desired diaziridine **9** as a colorless oil (8.62 g, 97%), which was used without any purification. An analytical sample was obtained by flash chromatography (Tol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.74 (s, 1H), 7.56 (s, 1H), 4.71 (s, 2H), 2.79 (d, J=8.8 Hz, 1H), 2.20 (d, J=8.8 Hz, 1H), 0.94 (s, 9H), 0.10 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.4, 136.7, 135.3, 133.5, 124.9, 123.2 (q, J=278 Hz), 94.1, 63.6, 57.3 (q, J=49 Hz), 25.8, 18.3, -5.4; IR (neat) 3240, 2950–2850, 1180–1130, 835 cm^{-1}; LRMS (CI) m/z 459 (M+1).

*4.1.5 3-Iodo-5-(3-(trifluoromethyl)-3H-diazirine-3-yl)benzyl alcohol (**10**)*

To a solution of diaziridine **9** (8.62 g, 18.8 mmol) in ether (100 ml) was added freshly prepared Ag_2O (20.1 g, 87.0 mmol). The suspension was stirred 1 h at rt. The solid was filtered on celite, washed with ether, and the combined ether fractions were evaporated in vacuo. The residual oil was dissolved in dry THF (100 ml) cooled to 0°C then TBAF (1 M, 25 ml, 25 mmol) was added. The resulting mixture was stirred 5 h at rt, poured in ether (200 ml), washed with water (3×500 ml), brine (100 ml), dried over $MgSO_4$, filtered, and concentrated. The residual yellow solid was flash chromatographed (Tol: EtOAc, 9:1) to yield the desired diazirine **10** as a white solid (5.00 g, 78%); mp 70–72°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.42 (s, 1H), 7.16 (s, 1H), 4.66 (d, J=5.8 Hz, 1H), 1.87 (t, J=5.9 Hz, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.5, 136.9, 134.3, 131.2, 123.8, 121.7 (q, J=275 Hz), 94.5, 63.4, 27.7 (q, J=41 Hz); IR (KBr) 3400–3200, 2950–2850, 1615, 1600, 1565, 1450, 1345, 1200–1110 cm^{-1}; LRMS (CI) m/z 343 (M+1).

*4.1.6 3-(5-(Bromomethyl)-3-iodo-phenyl)-3-trifluoromethyl-3H-diazirine (**11**)*

To a solution of diazirine **10** (5.00 g, 14.6 mmol) in dry CH_2Cl_2 (50 ml) at 0°C was added CBr_4 (7.28 g, 22.0 mmol) and DIPHOS (3.51 g, 8.80 mmol). The resulting mixture was stirred at 0°C for 6 h, concentrated and the residual solid was suspended in 75 ml ether, filtered, and washed with ether (3×50 ml). The combined ether fractions were concentrated. Flash chromatography (Hex) afforded the desired diazirine **11** as a white solid (4.80 g, 81%); mp 41–41.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.43 (s, 1H), 7.17 (s, 1H), 4.36 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.5, 139.3, 135.2, 131.6, 126.4, 121.6 (q, J=275 Hz), 94.6, 30.7, 27.6 (q, J=40 Hz); IR (KBr) 3100–2850, 1560, 1340, 1200–1140 cm^{-1}; Anal. calcd for $C_9H_5BrF_3IN_2$: C, 26.69; H, 1.24; N, 6.92; F, 13.68; found: C, 27.19; H, 1.36; N, 7.01; F, 14.07.

*4.1.7 2-(Trimethylsilyl)ethyl-3-(3-(1,3-dioxolan-2-yl)-phenyl)-3-oxopropanoate (**13**)*

To a solution of LDA (0.95 M, 90 ml; 85.6 mmol) in dry THF at −78°C was added over 20 min a solution of 2-(trimethylsilyl)ethyl acetate (13.78 g, 85.9 mmol) in dry THF (10 ml). After stirring for 30 min at −78°C the resulting enolate solution was cannulated in a solution of aldehyde **12** (11.81 g, 66.3 mmol) in toluene (200 ml) at −78°C. The resulting mixture was allowed to reached 0°C slowly (3 h). The reaction was quenched with acetic acid (14 ml), then poured in a NaH_2PO_4-NaOH buffer (100 ml, 0.5 M, pH 7). The organic extract was washed with brine, dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (Tol:EtOAc, 9:1) afforded the desired alcohol (17.47 g, 78%). The resulting alcohol and MnO_2 (37.2 g, 428 mmol) in ethyl acetate (200 ml), were stirred overnight at rt. The mixture was filtered on celite, MnO2, washed with THF:EtOAc (1:1, 3×100 ml). The combined organic fractions were concentrated and flash chromatographed to yield the desired ester **13** (15.95 g, 72%) as a colorless oil. Both the keto and the enol form (2.5:1) were observed by NMR. Keto form; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J=1.7 Hz, 1H), 7.92 (dt, J=8.0, 1.5 Hz, 1H), 7.68 (d, J=7.7 Hz, 1H), 7.47 (t, J=7.7 Hz, 1H), 5.82 (s, 1H), 4.23-4.19 (m, 2H), 4.12–4.00 (m, 4H), 3.95 (s, 2H), 0.98-0.94 (m, 2H), 0.05–0.00 (m, 9H); enol form; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.75 (d, J=8.0 Hz, 1H), 7.54 (d, J=7.7 Hz, 1H), 7.40 (t, J=7.7 Hz, 1H), 5.81 (s, 1H), 5.64 (s, 1H), 4.30–4.26 (m, 2H), 4.12–4.00 (m, 4H), 1.06–1.01 (m, 2H), 0.57–0.00 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.2, 173.3, 170.9, 167.5, 139.0, 138.6, 136.2, 133.7, 131.8, 129.3, 129.2, 128.9, 128.6, 126.7, 124.2, 103.3, 103.0, 87.8, 65.4, 63.9, 62.6, 46.2, 17.4, 17.2, 1.5, 1.6; IR (neat) 2950, 2895, 1735, 1685, 1625 cm^{-1}; LRMS (CI) m/z 337 (M+1).

*4.1.8 2-(Trimethylsilyl)ethyl-3-(3-formyl-phenyl)-3-oxopropanoate (**14**)*

To a solution of ketoester **13** (2.75 g, 8.18 mmol) in 10 ml acetone and 2 ml water was added PPTS (411 mg, 1.64 mmol). The mixture was refluxed for 7 h, cooled to rt and concentrated. The residue was dissolved in ether (200 ml), washed with saturated $NaHCO_3$ (100 ml), brine, dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (Tol:EtOAc, 95:5) yielded the desired aldehyde **14** (2.275 g, 95%) as a colorless oil. Both the keto and the enol form (2.5:1) were observed by NMR. Keto form; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (CHO), 8.39 (t, J=1.7 Hz, 1H), 8.19 (dt, J=7.8, 1.7, 1.3 Hz, 1H), 8.08 (dt, J=7.6, 1.7, 1.3 Hz, 1H), 7.65 (t, J=7.7 Hz, 1H), 4.24–4.20 (m, 2H), 4.00 (s, 2H), 0.99–0.94 (m, 2H), −0.01 (s, 9H); enol form;

^{1}H NMR (400 MHz, $CDCl_3$) 10.03 (CHO), 8.24 (t, J=1.7 Hz, 1H), 8.00 (dt, J=7.8, 1.7, 1.3 Hz, 1H), 7.94 (dt, J=7.6, 1.7, 1.3 Hz, 1H), 7.57 (t, J=7.7 Hz, 1H), 5.70 (s, 1H), 4.31–4.27 (m, 2H), 1.17–1.02 (m, 2H), 0.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.6, 191.5, 191.1, 173.1, 169.5, 167.2, 136.9, 136.8, 136.7, 134.6, 134.0, 133.9, 131.7, 131.6, 129.8, 129.7, 129.4, 127.5, 88.6, 64.1, 62.9, 46.1, 17.4, 17.3, −1.5, −1.6; IR (neat) 3500, 2950, 1745, 1700, 1630, 1600 cm^{-1}; HRMS (FAB+) m/z calcd for $C_{15}H_{20}O_4Si$ ($M+NH_4^+$): 310.1473, found 310.1475.

*4.1.9 2-(Trimethylsilyl)ethyl-3-(3-(2-(7-chloroquinolin-2-yl)ethenyl)phenyl)-3-oxopropanoate (**16**)*

To a suspension of phosphonium salt **15** (3.08 g, 5.93 mmol) in dry THF (30 ml) at −78°C was added dropwise over 15 min a solution of potassium *t*-butoxide (1 M in THF, 6.5 ml, 6.5 mmol)). The resulting light yellow solution was stirred 15 min at −78°C then 30 min at 0°C. A solution of aldehyde **14** (1.45 g, 4.94 mmol) in dry THF (6 ml) was added via cannulation to the solution of the ylide at −78°C. The reaction mixture was stirred 10 min at −78°C followed by 3 h at 0°C then poured in phosphate buffer (100 ml, NaH_2PO_4 + NaOH, 0.2 M, pH 7), extracted (3×100 ml) with EtOAc. The combined organic fractions were washed with brine, dried over Na_2SO_4, filtered, then concentrated. Flash chromatography (Tol:EtOAc, 95:5) afforded the desired keto-ester **16** (1.84 g, 83%) as a light yellow solid. Both the keto and the enol form (5:4) were observed by NMR but could not be differentiated; mp 64–66°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (t, J=1.7 Hz), 8.06–8.03 (m, J=8.6, 7.9, 2.3 Hz), 8.00 (t, J=1.7 Hz), 7.85 (dt, J=8.0, 1.3 Hz), 7.80 (d, J=7.8 Hz), 7.73–7.64 (m, J=16.3, 16.2, 8.6, 8.3 Hz), 7.56 (d, J=8.5 Hz), 7.56 (d, J=8.5 Hz), 7.47 (t, J=7.7 Hz), 7.42–7.32 (m, J=16.3, 8.9, 8.6, 2.0 Hz), 5.67 (s), 4.32–4.28 (m), 4.26–4.22 (m), 3.99 (s), 1.08–1.04 (m), 1.01–0.97 (m), 0.06 (s), 0.00 (s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.3, 167.5, 156.2, 148.6, 137.1, 136.6, 136.2, 136.1, 135.6, 134.1, 133.5, 132.0, 129.9, 129.8, 129.4, 129.2, 123.0, 128.7, 128.5, 128.2, 127.2, 127.2, 127.1, 126.2, 125.7, 124.8, 119.8, 119.7, 87.9, 63.9, 62.7, 46.2, 17.4, 17.3, −1.4, −1.5; IR (melted) 3050, 2950, 2890, 1730, 1685, 1640–1590 cm^{-1}; HRMS (FAB$^+$) m/z calcd for $C_{25}H_{26}ClNO_3Si$ ($M+H^+$): 452.1447, found 452.1449.

*4.1.10 2-(Trimethylsilyl)ethyl-2-(3-iodo-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl-methyl)-3-(3-(2-(7-chloroquinolin-2-yl)ethenyl)phenyl)-3-oxopropanoate (**17**)*

To a solution of the keto-ester **16** (1.12 g, 2.48 mmol) in dry DMF (8 ml) and HMPA (0.65 ml, 3.74 mmol) at 0°C was added NaH (80%w, 89.0 mg, 2.97 mmol). The mixture was stirred 15 min at 0°C then 30 min at rt. A solution of **11** (1.10 g, 2.72 mmol) in DMF (2 ml) was added via cannulation to the sodium enolate solution at 0°C. The resulting mixture was stirred at rt overnight, diluted with ether (100 ml), washed with water (3×50 ml), brine, dried over $MgSO_4$, filtered, and concentrated. Flash chromatography yielded the title compound (1.703 g, 84%) as a light-yellow foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.06 (d, J=8.7 Hz, 1H), 8.04 (d, J=2.0 Hz, 1H), 7.88 (d, J=8.0 Hz, 1H), 7.80 (d, J=7.8 Hz, 1H), 7.72 (d, J=16.3 Hz, 1H), 7.67 (d, J=8.7 Hz, 1H), 7.66 (s, 1H), 7.58 (d, J=8.5 Hz, 1H), 7.47 (t, J=7.8 Hz, 1H), 7.41 (dt, J=8.7, 2.0 Hz, 1H), 7.37 (d, J=16.3 Hz, 1H), 7.34 (s, 1H), 7.02 (s, 1H), 4.54 (t, J=7.6, 7.2 Hz, 1H), 4.17–4.09 (m, 2H), 3.30 (dd, J=14.2, 7.7 Hz, 1H), 3.24 (dd, J=14.2, 7.2 Hz, 1H), 0.88–0.80 (m, 2H), −0.01 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.3, 168.7, 156.2, 148.7, 141.5, 139.6, 137.2, 136.5, 136.3, 135.7, 133.8, 133.6, 132.2, 131.3, 130.0, 129.3, 128.7, 128.6, 128.3, 127.4, 127.3, 126.6, 125.8, 121.8 (q, J=275 Hz), 119.9, 94.6, 64.5, 56.0, 34.1, 27.8 (q, J=45 Hz), 17.3, 1.6; IR (melted) 2970, 2860, 1730, 1690, 1605, 1495, 1155 cm^{-1}; HRMS (FAB$^+$) m/e calcd for $C_{34}H_{30}ClF_3IN_3O_3Si$ ($M+H^+$): 776.0817, found 776.0820.

*4.1.11 1-(3-(2-(7-Chloroquinolin-2-yl)ethenyl)phenyl)-3-(3-iodo-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl)-propan-1-one (**18**)*

To ester **17** (703 g, 0.91 mmol) was added a solution of TBAF (1.0 M in THF, 9.10 ml, 9.10 mmol) followed by acetic acid (260 μl, 4.54 mmol). The light brown solution was stirred for 6 h at rt, poured in ether (100 ml), washed with water (3×50 ml), brine, dried over $MgSO_4$, filtered, and concentrated. Flash chromatography afforded the desired ketone **18** (530 mg, 93%) as a light-yellow solid; mp 151–152.5°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.09 (d, J=8.6 Hz, 1H), 8.07 (d, J=1.8 Hz, 1H), 7.87 (d, J=7.8 Hz, 1H), 7.80 (d, J=7.8 Hz, 1H), 7.73 (d, J=16.3 Hz, 1H), 7.69 (d, J=8.9 Hz, 1H), 7.68 (s, 1H), 7.60 (d, J=8.6 Hz, 1H), 7.48 (t, J=7.7 Hz, 1H), 7.43 (dd, J=8.7, 2.0 Hz, 1H), 7.39 (d, J=16.4 Hz, 1H), 7.35 (s, 1H), 7.04 (s, 1H), 3.29 (t, J=7.3 Hz, 1H), 3.03 (t, J=7.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.8, 156.3, 144.3, 139.1, 137.1, 137.0, 136.4, 135.8, 133.9, 133.3, 131.8, 131.3, 129.7, 129.2, 128.7, 128.2, 128.1, 127.4, 126.7, 126.2, 125.8, 121.8 (q, J=275 Hz), 119.7, 94.6, 39.9, 29.4, 27.7 (q, J=45 Hz); IR (KBr) 3040, 1675, 1595, 1665, 1490, 1155 cm^{-1}; HRMS (FAB$^+$) m/z calcd for $C_{28}H_{18}ClF_3IN_3O$ ($M+H^+$): 632.0213, found 632.0214.

*4.1.12 1-(3-(2-(7-Chloroquinolin-2-yl)ethenyl)phenyl)-3-(3-iodo-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl)-S-propan-1-ole (**19**)*

To a solution of ketone **18** (528 mg, 0.84 mmol) in dry CH_2Cl_2 (5 ml) and diisopropylethylamine (30 μl, 0.17 mmol) at −40°C was cannulated dropwise a solution of (−)-B-chlorodiisopinocampheylboron (434 mg,

1.35 mmol) in dry CH_2Cl_2 (2 ml). The reaction mixture was allowed to reach 0°C slowly and stirred overnight, poured in 10% aqueous ethanolamine (50 ml), stirred for 1 h, extracted (3×50 ml) with ethyl acetate. The combined organic fractions were washed with brine, dried over $MgSO_4$, filtered, then concentrated. Flash chromatography (Tol:EtOAc, 95:5) afforded 571 mg of a pinene contaminated sample of the desired alcohol. Further purification was achieved by dissolving it in 10 ml ether then 85 μl of concentrated HCl was added. The yellow precipitate was filtered, washed with ether (3×20 ml), neutralized with 10% NEt_3 in CH_2Cl_2. The organic phase was washed with saturated $NaHCO_3$ (50 ml), brine, dried over $MgSO_4$, filtered, and concentrated to give the desired pure alcohol **19** (486 mg, 92%) as a light-yellow foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J=8.6 Hz, 1H), 8.06 (d, J=2.0 Hz, 1H), 7.71 (d, J=16.3 Hz, 1H), 7.70 (d, J=8.7 Hz, 1H), 7.62 (d, J=8.6 Hz, 1H), 7.60 (s, 1H), 7.59 (s, 1H), 7.55 (d, J=7.7 Hz, 1H), 7.43 (dd, J=8.6, 2.0 Hz, 1H), 7.41 (t, J=7.6 Hz, 1H), 7.37 (d, J=16.1 Hz, 1H), 7.32 (s, 1H), 7.28 (d, J=7.6 Hz, 1H), 6.96 (s, 1H), 4.70 (t, J=5.9 Hz, 1H), 2.77–2.61 (m, 2H), 2.14–1.97 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.8, 148.5, 145.0, 144.9, 139.0, 136.5, 136.2, 135.7, 135.0, 133.0, 131.1, 129.1, 128.7, 128.6, 128.0, 127.2, 126.7, 126.4, 126.0, 125.6, 124.8, 121.8 (q, J=275 Hz), 119.5, 94.6, 73.2, 40.0, 31.6, 27.8 (q, J=41 Hz); IR (KBr) 3350, 2920, 1600, 1560, 1495, 1150 cm^{-1}; HRMS (FAB$^+$) m/e calcd for $C_{28}H_{20}ClF_3IN_3O$ ($M+H^+$): 634.0369, found 634.0370.

*4.1.13 1-(((1-(3-(2-(7-Chloroquinolin-2-yl)ethenyl)-phenyl)-3-(3-iodo-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl)propyl)thio)methyl)cyclopropaneacetic acid (L-745310, **4**)*

To a solution of alcohol **19** (253 mg, 0.399 mmol) in dry CH_2Cl_2 (3 ml) at −22°C was added NEt_3 (85 μl, 0.61 mmol) then MsCl (37 μl, 0.48 mmol). The mixture was stirred for 1 h at 0°C then poured in saturated aqueous $NaHCO_3$ (50 ml) and extrated with EtOAc (3×50 ml). The combined organics extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to give the desired mesylate **20** in quantitative yield, which was used without further purification. In a separate flask, a solution of thiol-acid **21** (61.6 mg, 0.421 mmol) in dry THF (1 ml) was cooled to −22°C. A solution of *n*-BuLi (1.6 M in Hex, 550 μl, 0.88 mmol) was added dropwise. The resulting suspension was stirred for 15 min at 0°C then cooled back to −22°C. A solution of the mesylate **20** in dry THF (1.5 ml) was cannulated dropwise into the thiolate suspension. The resulting mixture was stirred at 0°C for 2 h, poured in 25% aqueous NH_4Cl (25 ml) and extracted with EtOAc (3×50 ml). The combined organics extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated. Flash chromatography (Tol:EtOAc:AcOH 94.9:5:0.1) afforded the desired acid (201 mg) in 66% yield. An analytical sample was obtained by HPLC using a Prep Nova-Pak® HR C18 column (MeOH 89.9%/H_2O 10%/AcOH 0.1%); ^{1}H NMR (400 MHz, acetone-d_6) δ 8.29 (d, J=8.6 Hz, 1H), 8.01 (d, J=2.1 Hz, 1H), 7.91 (d, J=8.7 Hz, 1H), 7.86 (d, J=16.3 Hz, 1H), 7.82 (d, J=8.6 Hz, 1H), 7.74 (t, J=1.1 Hz, 1H), 7.70 (s, 1H), 7.58 (dd, J=7.3, 1.4 Hz, 1H), 7.50 (dd, J=8.7, 1.2 Hz, 1H), 7.48 (d, J=16.3 Hz, 1H), 7.44 (s, 1H), 7.39 (t, J=7.5 Hz, 1H), 7.35 (dt, J=7.7, 1.5 Hz, 1H), 3.94 (t, J=7.5 Hz, 1H), 2.79–2.63 (m, 2H), 2.57 (s, 2H), 2.45 (d, J=16.0 Hz, 1H), 2.39 (d, J=16.0 Hz, 1H), 2.26–2.20 (m, 2H), 0.55–0.33 (m, 4H); ^{13}C NMR (100 MHz, acetone-d_6) δ 173.4, 157.8, 149.4, 146.2, 144.3, 140.3, 137.6, 137.2, 135.9, 135.7, 133.7, 131.3, 130.3, 129.8, 129.3, 129.2, 128.4, 127.8, 127.5, 127.0, 126.9, 126.7, 122.8 (q, J=274 Hz), 121.0, 95.2, 49.8, 40.0, 39.6, 38.5, 33.9, 28.4 (q, J=41 Hz), 17.5, 12.9, 12.6; IR (KBr) 1710, 1600, 1495, 1150 cm^{-1}; HRMS m/e calcd for $C_{34}H_{28}ClF_3IN_3O_2S.H^+$: 762.0669, found 762.0666; $[\alpha]_D^{20}$ + 57.5° (c = 1, $CHCl_3$); Anal. calcd for $C_{34}H_{28}ClF_3IN_3O_2S$: C, 53.59; H, 3.70; found: C, 53.33; H, 3.63.

*4.1.14 1-(((1-(3-(2-(7-Chloroquinolin-2-yl)ethenyl)-phenyl)-3-(3-trimethylstannyl-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl)propyl)thio)methyl)cyclopropaneacetic acid (**22**)*

To a solution of acid **4** (112 mg, 0.147 mmol) in 1,4-dioxane (2 ml) was added $Pd(PPh_3)_4$ (17 mg, 0.015 mmol) and $Me_3SnSnMe_3$ (480 mg, 1.46 mmol). The mixture was degassed at rt and stirred for 12 h at 50°C. The resulting suspension was poured in aqueous NH_4OAc (10 ml, 25%) and extracted with EtOAc (3×10 ml). The combined organics extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by HPLC using a Prep Nova-Pak® HR C18 column (MeOH 89.9%/H_2O 10%/AcOH 0.1%) to give the title compound (85 mg) in 72% yield; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.31 (d, J=8.5 Hz, 1H), 8.01 (d, J=2.1 Hz, 1H), 7.92 (d, J=8.7 Hz, 1H), 7.89 (d, J=16.3 Hz, 1H), 7.83 (d, J=8.6 Hz, 1H), 7.71 (s, 1H), 7.60 (d, J=7.4 Hz, 1H), 7.52 (d, J=2.1 Hz, 1H), 7.50 (d, J=2.1 Hz, 1H), 7.48 (d, J=16.6 Hz, 1H), 7.40 (t, J=7.5 Hz, 1H), 7.36 (dt, J=7.7, 1.4 Hz, 1H), 7.22 (t, J=0.9 Hz, 1H), 7.05 (s, 1H), 3.94 (t, J=7.5 Hz, 1H), 2.78-2.68 (m, 2H), 2.58 (d, J=13.2 Hz, 1H), 2.54 (d, J=13.2 Hz, 1H), 2.43 (d, J=16.0 Hz, 1H), 2.38 (d, J=16.0 Hz, 1H), 2.78-2.22 (m, 2H), 0.53-0.31 (m, 4H), 0.27 (s, 9H); ^{13}C NMR (100 MHz, acetone-d_6) δ 173.5, 157.9, 149.4, 145.1, 144.5, 142.8, 138.6, 137.6, 137.2, 135.9, 135.7, 131.8, 130.3, 129.9, 129.4, 129.3, 128.8, 128.5, 127.8, 127.5, 127.2, 126.9, 126.8, 123.3 (q, J=274, 1), 121.1, 49.7, 40.0, 39.6, 39.0, 34.3, 29.2 (q, J=44, 1), 17.5, 12.9, 12.6, −9.5; IR (KBr) 1715, 1610, 1500, 1155 cm^{-1}; Anal.

calcd for $C_{37}H_{37}ClF_3N_3O_2SSn$: C, 55.63; H, 4.67; found: C, 55.70; H, 4.97.

4.1.15 1-(((1-(3-(2-(7-Chloroquinolin-2-yl)-ethenyl)phenyl)-3-(3-iodo^{125}-5-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl)propyl)thio)methyl)cyclopropaneacetic acid (^{125}I-L745310)

To a solution of **22** (1.0 mg, 1.3 μmol) in DMF (250 μl) was added a phosphate buffer (50 μl, 0.2 M, NaH_2PO_4 + NaOH, pH 7) then NaI^{125} (40 μl, 5mCi/100 μl, 2 mCi) and finally a solution of Chloramine-T™(N-chloro-*p*-toluenesulfoamide, sodium salt) (7 μl, 5.0 mg/ml DMF, 0.13 μmol). The final mixture was stirred 1 h at 25°C. The reaction was quenched using aqueous $Na_2S_2O_5$ (10 μl, 2.0 g/ml) and then diluted with MeOH (150 μl). The desired ^{125}I-L-745310 was purified by HPLC using a Prep Nova-Pak® HR C18 columm (MeOH 89.89%/H_2O 10%/AcOH 0.1%/mercaptoethanol 0.01%) and recuperated in a 2 ml fraction. Based on the activity of this fraction (2 μl = 1721889 cpm) the quantum yield is 49% (0.98 mCi).

4.2 Photoaffinity labeling of guinea-pig lung membrane by ^{125}I-L-745310

Guinea-pig lung membrane were prepared according to established procedures [7]. Photoaffinity labeling was conducted under equilibrium binding assay conditions. Experiments were performed in a final volume of 2 ml of HEPES/KOH buffer (pH 7.4), containing 10 mM $CaCl_2$, 20 mM l-penicillamine (cysteinyl glycyl dipeptidase inhibitor; prevents LTD_4 metabolism), 0.03% taurocholic acid, 100 μg/ml guinea-pig lung membrane preparation and ^{125}I-L-745310 (≈400,000 cpm). Non-specific labeling was determined in the presence of 1 μM LTD_4. The resulting mixture was incubated at rt for 45 min in the dark prior to irradiation. A sample (1.2 ml) was then transferred to a 12 well Petri dish, placed on an aluminum block frozen in liquid nitrogen and irradiated for 90 s using a 40-watt ultraviolet lamp (Phillips, λ_{max} = 350 nm) at a distance of 10 cm. The labeling was quenched with 400 μl MeOH. The samples were thawed and the guinea-pig lung membranes were recovered from a 1.3 ml aliquot by centrifugation (150,000 *g*) at 4°C for 15 min. The membrane pellets were dried for 45 min, solubilized in sodium dodecyl-polyacrylamide gel electrophoresis sample buffer and finally resolved by SDS–PAGE. Protein band were visualized using Coomassie Blue staining while photo-labeled protein were identified by autoradiography of dried gel and quantified by laser densitometry.

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