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Modeling, Synthesis and Biological Evaluation of Potential Retinoid X Receptor (RXR) Selective Agonists: Novel Analogues of 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic Acid (Bexarotene)

Carl E. Wagner,*,† Peter W. Jurutka,† Pamela A. Marshall,† Thomas L. Groy,‡ Arjan van der Vaart,§ Joseph W. Ziller,∥ Julie K. Furmick,† Mark E. Graeber,† Erik Matro,† Belinda V. Miguel,† Ivy T. Tran,† Jungeun Kwon,† Jamie N. Tedeschi,† Shahram Moosavi, Amina Danishyar,† Joshua S. Philp,† Reina O. Khamees, Jevon N. Jackson,† Darci K. Grupe,† Syed L. Badshah,‡ and Justin W. Hart†

†*Division of Mathematical and Natural Sciences, New College of Interdisciplinary Arts and Sciences, Arizona State University, 4701 West Thunderbird Road, Glendale, Arizona 85306,* ‡*Department of Chemistry and Biochemistry, College of Liberal Arts and Sciences, Arizona State University, Tempe, Arizona 85287,* §*Department of Chemistry, University of South Florida, 4202 East Fowler Avenue, CHE 205, Tampa, Florida 33620, and* ∥*Department of Chemistry, University of California, Irvine, 576 Rowland Hall, Irvine, California 92697*

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This report describes the synthesis of analogues of 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic acid (**1**), commonly known as bexarotene, and their analysis in acting as retinoid X receptor (RXR)-specific agonists. Compound **1** has FDA approval to treat cutaneous T-cell lymphoma (CTCL); however, its use can cause side effects such as hypothyroidism and increased triglyceride concentrations, presumably by disruption of RXR heterodimerization with other nuclear receptors. The novel analogues in the present study have been evaluated for RXR activation in an RXR mammalian-2-hybrid assay as well as an RXRE-mediated transcriptional assay and for their ability to induce apoptosis as well as for their mutagenicity and cytotoxicity. Analysis of 11 novel compounds revealed the discovery of three analogues that best induce RXR-mediated transcriptional activity, stimulate apoptosis, have comparable K_i and EC_{50} values to **1**, and are selective RXR agonists. Our experimental approach suggests that rational drug design can develop new rexinoids with improved biological properties.

Introduction

Retinoids are a class of small molecule compounds that play vital roles in the regulation of cellular processes, including transcription of genes, differentiation, and proliferation. Two classes of proteins that bind to retinoids, retinoic acid receptors (RARs) and retinoid X receptors (RXRs), have been studied in detail, and three subtypes, α, β, and γ, have been identified for both RAR and RXR[a] proteins.[1] The receptors for retinoids, as well as for other small lipophilic hormonal ligands, belong to the larger superfamily of receptors for steroids, as well as for thyroid hormone receptor (TR) and vitamin D receptor (VDR), which all function as transcription factors. All of the receptor proteins have an "endogenous ligand" that binds to a specific pocket within the protein, altering the protein's conformation and inducing the protein to bind to a specific molecular scaffold on DNA. Most of these proteins, once bound to a signaling lipophilic ligand, interact directly with DNA sequences known as hormone response elements (HREs). Most HREs consist of minimal core hexad sequences that exist as half-sites separated by variable length nucleotide spacers between direct, inverted, or everted repeats[2] and are found within the promoter region of target genes. To activate transcription, nuclear receptors bind to the HREs as homodimers or heterodimers, with each partner binding to a half-site of the element. The association of the nuclear receptor protein with the DNA results in regulation of target gene expression, ultimately leading to a physiological effect or bioresponse.

Although originally proposed to act as homodimers,[3] TR, RAR, and VDR high affinity DNA binding is mediated via a heterodimer of RXR and the appropriate receptor.[4] RXR also binds to a natural endogenous ligand, 9-*cis* retinoic acid (9-*cis*-RA; see below), and functions as a homodimer when bound to its cognate ligand or can function as an unliganded heterodimeric partner for other nuclear receptors including VDR.[5] Thus, the RXR "master" partner is central to the function of many nuclear receptors because the RXR protein can form heterodimer complexes with many members of this superfamily that result in specific physiological responses as a result of modulation of gene expression.[6]

Interestingly, ligand-induced transcriptional activity for the RXR homodimer is suppressed in most but not all cases when RXR is complexed with a ligand-bound partner such as VDR and TR, and these heterodimers prevent the binding of RXR to its ligand, suggesting that TR and VDR are "nonpermissive" heteropartners for RXR, in

*To whom correspondence should be addressed. Phone: (602) 543-6937. Fax: (602) 543-6073. E-mail: Carl.Wagner@asu.edu.

[a]Abbreviations: RXR, retinoid X receptor; RAR, retinoic acid receptor; CTCL, cutaneous T-cell lymphoma; RXRE, retinoid X receptor element; HRE, hormone response element; TR, thyroid receptor; VDR, vitamin D receptor; SNuRMs, specific nuclear receptor modulators.

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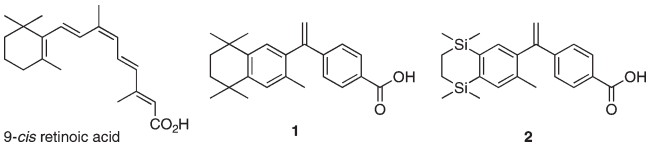
which the "primary" receptor (TR or VDR) and its ligand play a dominant role over the "subordinate" RXR coreceptor.[5] However, in the case of RAR, which is a primary partner activated by all-*trans*-retinoic acid (see structure below), the RXR heteropartner is still able to bind 9-*cis*-RA and the two retinoids synergistically enhance transactivation from retinoic acid response elements (RAREs). Conversely, when 9-*cis*-RA or synthetic RXR ligands (rexinoids) are present in excess in the case of VDR-RXR or VDR-TR, these rexinoids divert RXR monomers away from forming heterodimers, instead facilitating RXR homodimers with a resulting attenuation of $1,25(OH)_2D_3$ or thyroid hormone responsiveness. It is now generally recognized that by modifying the structure of nuclear receptor (NR) ligands (and especially the RXR master partner ligand), one can produce specific NR modulators (collectively termed the SNuRMs) with unique new properties that can influence the activity of the NR in novel ways.[7]

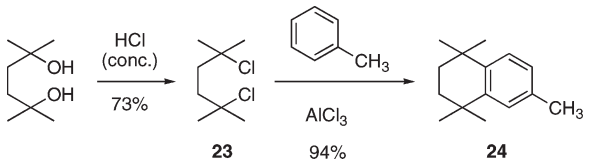
SNuRMs such as RXR selective molecules (rexinoids) have recently been targets of interest because the selective activation of RXR proteins versus RAR proteins might confer cancer chemotherapeutic effects[21] without inciting concurrently negative side effects by interacting with the RAR proteins.[8] The structure of the endogenous 9-*cis* retinoic acid ligand for RAR and RXR is shown below. Ligand Pharmacueticals, Inc., developed an RXR selective agonist, 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-ethynyl]benzoic acid (**1**),[9] commonly known as bexarotene, after lengthy SAR (structure−activity relationship) studies of several analogous compounds.[10] Another analogue that was later synthesized that had nearly identical response profiles to **1** was compound **2**[11] (disilabexarotene).



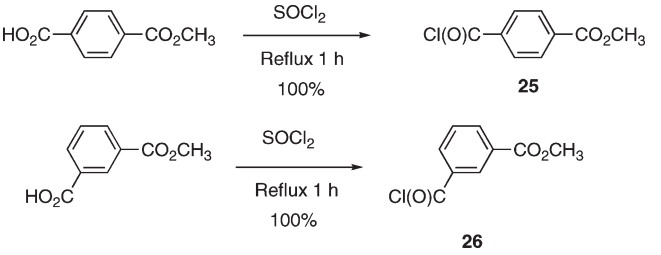
Compound **1** is an FDA approved drug, effective in the treatment of cutaneous T-cell lymphoma (CTCL), and it is being explored for treatment of breast cancer,[12] lung cancer,[13] colon cancer,[14] and other diseases of uncontrolled cellular proliferation because activation of RXR and "up-regulation" (or expression) of the genes RXR regulates seems to have a therapeutic effect by slowing or arresting cellular proliferation in these conditions. Analogues of **1** and compound **1** itself have also been explored as possible treatments for noninsulin-dependent diabetes mellitus (NIDDM) in mouse models.[15] Despite specific activation of RXR by **1** versus RAR, three drawbacks to the use of **1** include hypothyroidism,[16] because there may be an unintentional antagonism of the TR receptor with ligand activated RXR,[17] hyperlipidemia, and cutaneous toxicity as a result of residual RAR agonism at the dose concentration. Thus, there is motivation to pursue novel RXR agonist molecules that avoid these side effects.

There are several reports of compounds analogous to **1** in the literature. In addition to the disilabexarotene, the trifluoromethyl bexarotene (**3**),[18] the cyclopropyl dienoic acid (**4**),[19] and a host of novel aza-retinoids, including compound **5**,[20]

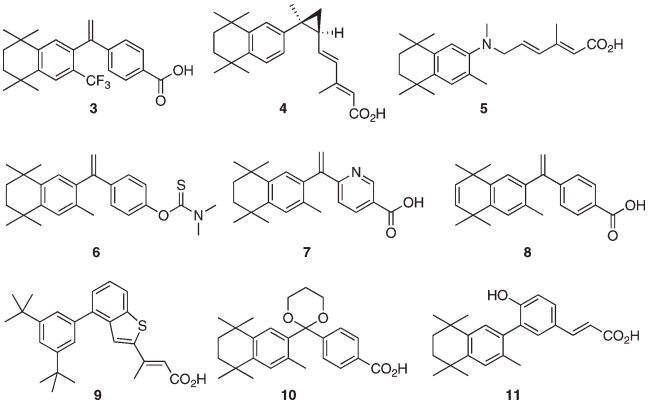
Scheme 1



Scheme 2

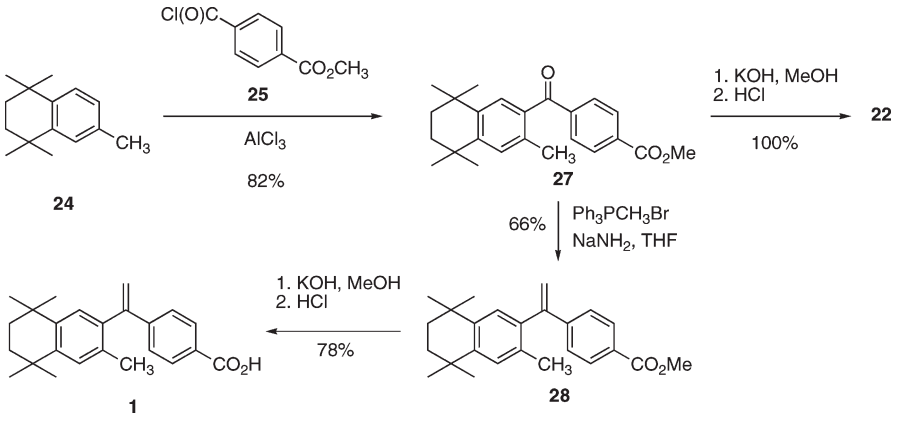


as well as amide retinoids[21] have been reported. The thiocarbamate analogue **6**[22] was shown to induce apoptosis in leukemia HL-60 cells. Several pyridine containing analogues, as in compound **7**,[23] and unsaturated analogues, such as compound **8**,[24] were synthesized and shown to be potent RXR selective agonists. Boehm and co-workers published a series of papers that developed RXR selective agonists based on aryl-trienoic acids locked by none,[25] one,[26] and two rings,[27] the last of which is demonstrated by compound **9**.[27] Notably, addition of fluorine in proximity to the carboxylic acid of the trienoic acids locked by no rings resulted in improved pharmacological profiles[28] and we hypothesize that analogues of **1** possessing a fluorine atom close to the carboxylic acid group will possess similarly improved RXR agonist characteristics. Compound **10**[29] and analogous compounds were identified as potent RXR selective agonists. Finally, Gronemeyer and co-workers used the RXR selective agonist **11**[6] as well as a model compound to design RXR modulating antagonists, several of which were cocrystallized in the ligand binding domain of hRXRα. However, despite the wealth of different RXR agonist compounds that incorporate structural motifs from **1**, there are, as yet, no analogues of **1** that contain additional functional moieties such as a nitro group or a halogen atom substituted for hydrogen atoms on the aromatic ring that bears the carboxylic acid.

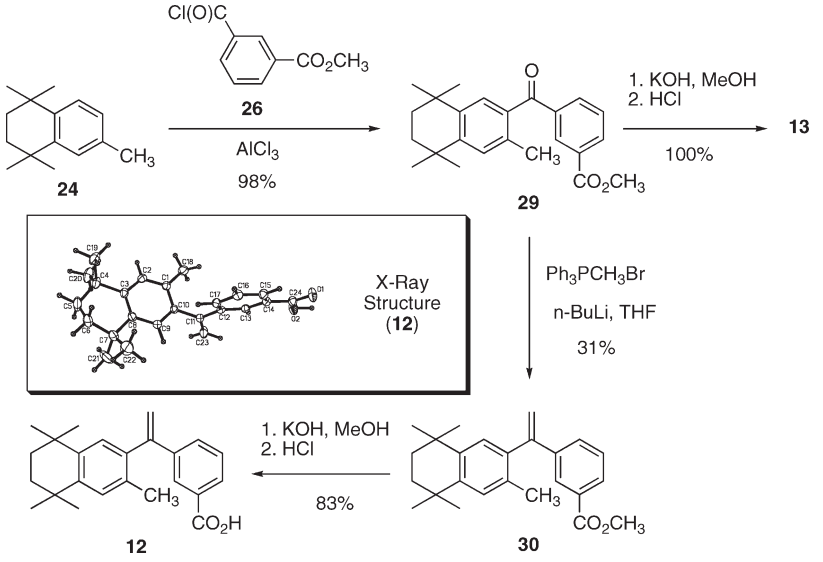


Therefore, studies in our laboratories have focused on the synthesis, modeling, and biological evaluation of novel

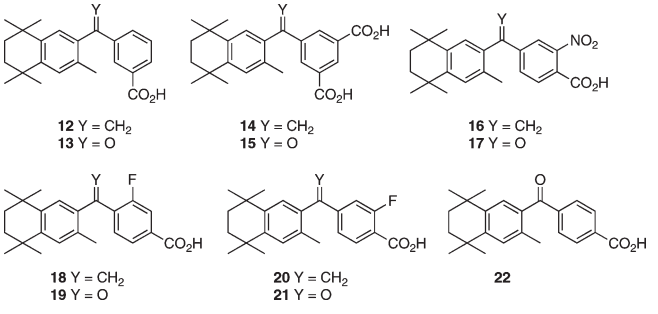
Scheme 3



Scheme 4



compounds analogous to **1** such as compounds **12** and **14**−**21**. We have compared these novel analogues to **1** and its ketone analogue (**22**).[9]



In the present study, we have synthesized compounds **1** and **12**−**22** and evaluated these in mammalian 2-hybrid assays and RXR- and RAR-response element (RXRE and RARE) transcriptional activation systems using cultured human cells, as well as in apoptosis, cytotoxicity, and mutagenicity assays.

Results and Discussion

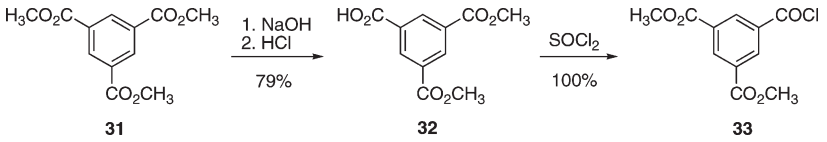
Chemistry. Synthesis of 1 and Isomer 12. To prepare a standard sample of **1**, as well as the novel isomer (**2**, 2, 5-dimethyl-2,5-hexanediol was converted to 2,5-dichloro-2,5-dimethylhexane (**23**)[9] in 73% recovered yield by treatment with concentrated hydrochloric acid, and the dihalide **23** was reacted with toluene in the presence of aluminum chloride to provide 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene (**24**)[9] in 94% yield following the procedure in literature (Scheme 1).

To make the Friedel−Crafts acylation coupling partners for **24** en route to **1** and its structural isomer (**12**), commercially available mono methyl terephthalate and mono methyl isophthalate were converted to the corresponding acid chlorides, **25** and **26**, respectively, by reaction with thionyl chloride (Scheme 2).

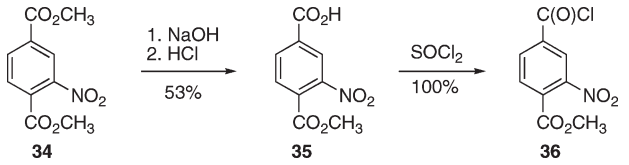
Compound **1** was prepared according to the method of Boehm and co-workers.[9] The Friedel−Crafts acylation of **24** with **25** provided ketone **27**[9] in 82% yield. Ketone **27** was converted to alkene-ester **28**[9] in 66% yield by following the Wittig reaction with triphenylphosphonium methylide, prepared by the treatment of triphenylphosphonium methyl bromide with sodium amide in THF. Alkene-ester **28** was saponified by treatment with potassium hydroxide in methanol, followed by acidification with hydrochloric acid to give **1** in 78% yield (Scheme 3).

Analogue **12** was prepared by a slightly modified route as reported for **1** (Scheme 4).

Scheme 5



Scheme 6



Despite the report that ketone **13**[23] is an inactive RXR agonist, we prepared isomer **12**.

Synthesis of Analogues 14−21. To prepare analogues **14−21**, the appropriate acid chloride Friedel−Crafts acylation coupling partners for **26** were synthesized. Thus, trimethyl-1,3,5-benzenetricarboxylate (**31**) was converted to the monoacid diester (**32**)[30] according to literature procedures, and compound **32** was refluxed in excess thionyl chloride to give acid chloride **33** (Scheme 5).

Dimethyl-nitro-terephthalate (**34**) was converted to the monoacid ester (**35**),[31] and compound **35** was refluxed in excess thionyl chloride to give acid chloride **36** (Scheme 6).

To prepare the analogues of **1** with fluorine, the method of Kishida and co-workers was used.[32] 3-Fluoro-4-methylbenzoic acid (**37**) was dibrominated with NBS and catalytic benzoylperoxide to give compound **38**,[32] which was subsequently treated with silver nitrate in ethanol and water to give aldehyde **39**.[32] Aldehyde **39** was either converted to the methyl-ester **40**,[32] followed by oxidation to the methyl-fluoro-terephthalic acid **41**[32] and conversion to acid chloride **42**, or **39**[32] was benzylated to give benzyl-ester **43**.[32] Benzyl-ester **43** was oxidized with sodium hypochlorite to acid **44**,[32] which was esterified to methyl-ester **45**,[32] debenzylated to give acid **46**,[32] and converted to acid chloride **47**[32] (Scheme 7).

The analogues **14−21** were synthesized according to a route analogous to the one used to give analogues **13** and **14** (Scheme 8).

The X-ray crystal structures of ketones **49**, **50**, and **51** are shown in Figure 1.

The X-ray crystal structures of fluorinated analogues **18** and **20** are shown in Figure 2.

Biological Assays and Rationale. A Mammalian Two-Hybrid Assay Reveals that Several Novel Analogues Induce RXR Homodimerization as Well as 1 Binds to RXR. Biological assessment of a subset of analogues described above (compounds **12−22**) was first carried out by employing the mammalian two-hybrid assay in human colon cancer (Caco-2) cells (Figure 3). This assay tests for homodimerization and ligand binding to a recombinant RXR. If the ligand−receptor complex then homodimerizes with an RXR-Gal4 fusion protein, luciferase will be transcribed, as the luciferase gene is downstream of Gal4p DNA binding elements.

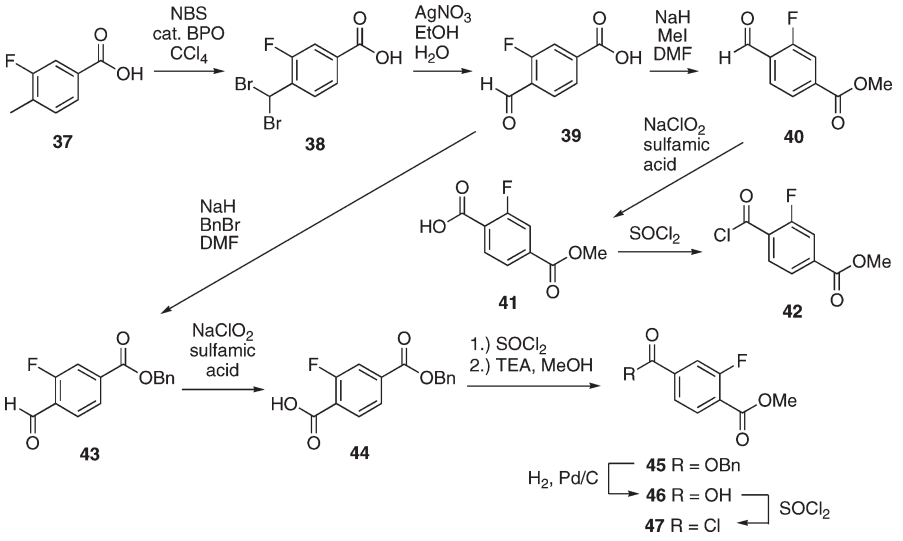
This initial evaluation revealed that five compounds exhibited at least some activity in the same order of magnitude as **1**. More importantly, the initial array of analogues shows a range of receptor binding and RXR homodimerization ability: **16** and **18** bind and mediate homodimerization about half as well as **1**, whereas compound **20** binds and activates transcription better than **1** (for compound **20**, using a one-tailed heteroscadastic *t* test $P = 0.049$, indicating that compound **20** is significantly better than **1**). Additionally, ketones **21** and **22** also display a small degree of RXR binding and homodimerization relative to **1**. These results imply that compounds modeled after **1** can be synthesized successfully and can possess RXR binding properties, and we are interested in elucidating the factors responsible for eliciting the different response-ranges observed. Moreover, these data suggest that construction of additional analogues of **1** is warranted, especially those compounds that preserve the carboxylic acid position but substitute non-hydrogen atom groups on the aromatic ring that bears the carboxylic acid.

Novel Analogues of 1 Bind to RXR and Mediate Transactivation. It is important to point out that the use of the mammalian two-hybrid assay as an initial screen for agonist-induced homodimerization of RXR is useful because of the speed, convenience, and sensitivity of the assay. However, an additional and vital question in testing RXR agonists is the role of the correct biologically relevant DNA platform, or retinoid X receptor response element (RXRE), that specifically associates with the RXR homodimer in vivo. The RXRE DNA sequence is present in the upstream promoter region of genes controlled by the RXR homodimer in response to the endogenous 9-*cis* RA ligand or when RXR is bound to a synthetic rexinoid. It is possible that the RXRE may influence the affinity and/or selectivity of the RXR protein toward potential ligands. Thus, a second screening protocol for our collection of possible RXR agonists included transfection of Caco-2 cells with an expression vector for wild-type human RXRα along with a reporter construct that contains an RXRE driving the expression of the luciferase reporter gene. The results in Figure 4 reveal that of all the compounds tested (**12−22**), only analogue **16**, **18**, and **20** displayed transcriptional activity significantly above the ethanol control levels (*P* values of < 0.001 for all, using a one-tailed heteroscedastic *t* test).

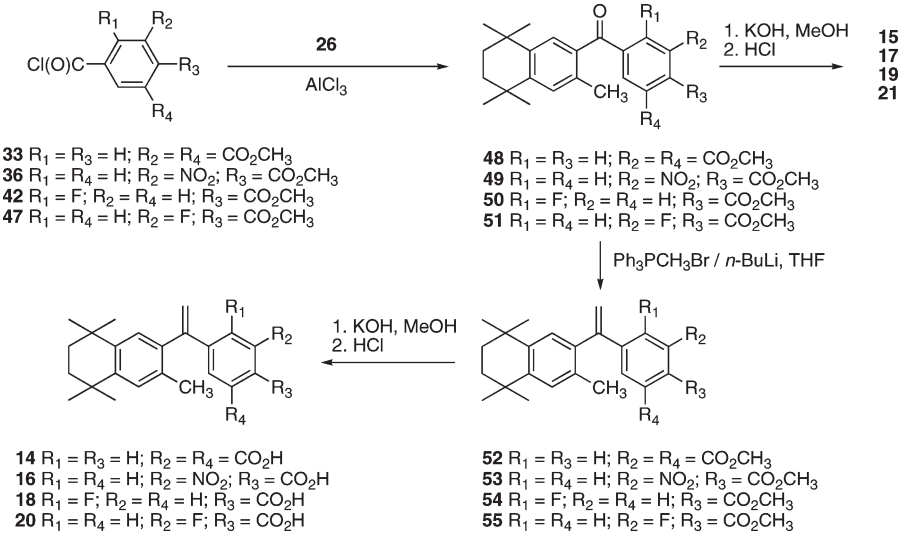
These same 3 compounds were also active in the mammalian two-hybrid assay described above (Figure 3).

Determination of RXR Binding Affinity, EC_{50} Values, and Quantitation of RAR Agonist Activity by the Most Active Rexinoids. To better quantitate the affinity and efficacy of the most active novel analogues for RXR binding, we utilized both a ligand binding assay with overexpressed human RXRα as well as the mammalian two-hybrid assay in human colon cancer cells (Figure 3) to evaluate a much larger array of **1** and analogue concentrations. The binding affinities (K_i values) of the most active rexinoids were determined by performing competition binding studies via displacement of 10 nM [^{3}H]-9-*cis*-retinoic acid essentially as described previously.[33] The transcriptional efficacy of these same compounds was tested in mammalian two-hybrid dose−response assays carried out with ligand concentrations ranging from 10×10^{-10} M up to 0.5×10^{-5} M. Utilizing this collection of binding affinity and dose−response experiments, we were able to calculate K_i and EC_{50} values,

Scheme 7



Scheme 8



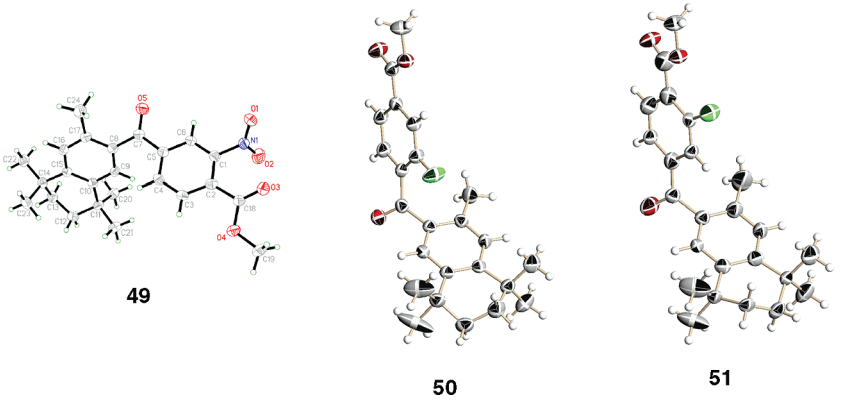


Figure 1. The X-ray crystal structures of ketone-esters **49**, **50**, and **51**. Compound **50** displays twist isomerism in the aliphatic ring, hence, only one of the two isomers in the crystal structure is shown.

which are listed in Table 1. The K_i value, which is an estimate of ligand affinity for RXR, is similar to that obtained previously for **1** by another group.[9] Moreover, the data in Figures 3 and 4 suggest that compounds **16** and **18** possess slightly lower RXR binding activity while compound **20** is slightly more active, observations that are entirely consistent with both the K_i and EC_{50} values in Table 1.

We also performed an analysis of the "residual" retinoic acid receptor (RAR) agonist activity of the parent compound

1, as well as analogues **16**, **18**, and **20** versus the authentic RAR ligand (all-trans retinoic acid). The results of this assay, which employed expression of the human RAR and a retinoic acid responsive element (RARE)-luciferase reporter system, revealed that compound **16** possess slightly greater RAR agonist activity, analogue **20** is approximately equal to **1** in its activation of RAR, and compound **18** possesses significantly lower RAR binding. Taken together, these results suggest that modification of **1** with a halogen atom on the aromatic ring that bears the carboxylic acid may reduce the activation of RAR (compound **18**) or increase its ability to activate RXR (analogue **20**).

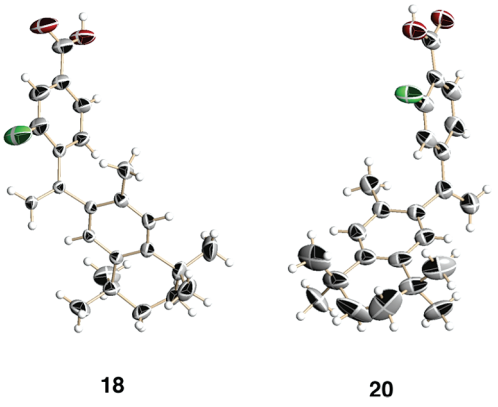


Figure 2. The X-ray crystal structures of fluorinated bexarotene analogues **18** and **20**. Compound **18** crystallized in such a way that a channel whose boundaries were formed by the carboxylic acid groups of **18** incorporated a solvent that could not be identified by the model, although likely candidates such as ethyl acetate (the crystallization solvent) were examined. Compound **20** displayed twist-isomerism in the aliphatic ring, as well as partial-filled occupancies of the fluorine atom at both ortho-positions to the carboxylic acid group due to carbon−carbon single bond rotation. Additionally, the carbonyl carbonoxygen bond of the carboxylic acid group of **20** could not be clearly resolved. Hence, one twist isomer, as well a given rotational isomer for the fluorine and carboxylic acid groups, has been displayed.

Compound 1 and Novel Analogues Induce Apoptosis in a CTCL System. It has been hypothesized that **1** treats

Table 1. Determination of Binding Affinity, EC_{50} Values, and Quantitation of RAR Agonist Activity

compd	RXRα binding affinity (K_i,[a] nM (±SD)	EC_{50} value[b] nM (±SD)	% RAR agonist activity[c] at 100 nM (±SD)	% RAR agonist activity at 1 μM (±SD)
compd **1**	21(3)	52 (6)	23 (5)	25 (4)
analogue **16**	81(12)	200 (28)	30 (4)	35 (6)
analogue **18**	161 (28)	420 (63)	13 (2)	14 (1)
analogue **20**	12 (2)	43 (5)	25 (6)	26 (2)

[a] Binding affinities (K_i, values) were determined by competition of 10 nM [^{3}H]-9-*cis*-retinoic acid (RA) with unlabeled test rexinoids as described in Experimental Section. [b] EC_{50} values were determined from full dose−response curves ranging from 10^{-10} to 10^{-5} M in transfected Caco-2 cells using an RXR mammalian two-hybrid system. [c] RAR agonist activity was derived from an PAR/RARE reporter system in transfected Caco-2 cells treated with analogue or all-trans PA at 100 nM or 1 μM. The activity with analogue (or compound **1**) divided by the activity with all-trans RA expressed as a percentage represents the PAR agonist activity.

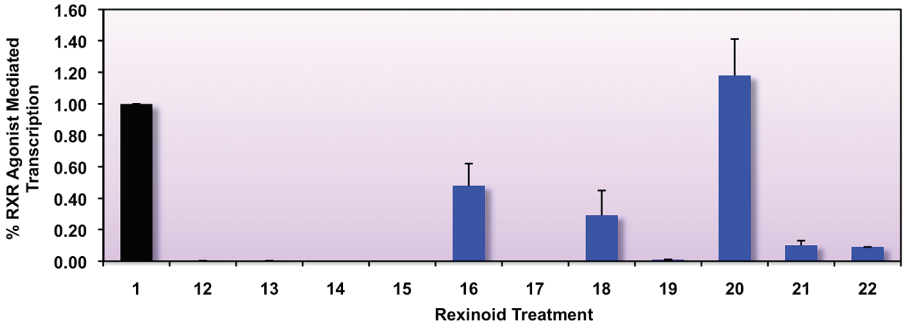


Figure 3. Identification of potential RXR agonists via a mammalian two-hybrid screening assay in human colon cancer cells. Caco-2 human colon cancer cells were cotransfected using both a pCMVhRXR binding domain vector (BD) as well as an hRXR-activation domain (AD) plasmid along with a pFR-Luc reporter gene containing BD-binding sites and renilla control plasmid. Cells were transfected for six hours utilizing a liposome-mediated transfection protocol and then treated with ethanol vehicle or 10−7 M of the indicated compound. After a 24 h incubation, cells were lysed and a luciferase assay was completed. Analogue-mediated RXR binding and homodimerization, as measured by luciferase output, was compared to the RXR agonist parent compound **1** (value set to 1.0).

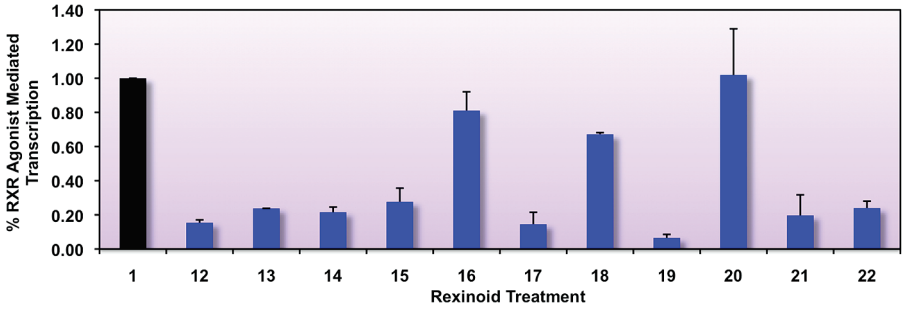


Figure 4. Identification of potential RXR agonists via an RXRE-luciferase reporter-based screening assay in human colon cancer cells. Caco-2 cells were transfected with hRXRα, an RXREluciferase reporter gene, renilla control plasmid, and carrier DNA (pTZ18U). Cells were transfected for six hours utilizing a liposome-mediated transfection protocol, and then treated with ethanol vehicle or 10−7 M compound **1** or the indicated analogue (**12**−**22**). After a 24 h incubation, cells were lysed and a luciferase assay was completed. Analogue-stimulated, RXR-mediated transcription, as measured by luciferase output, was compared to the RXR agonist parent compound **1** (value set to 1.0).

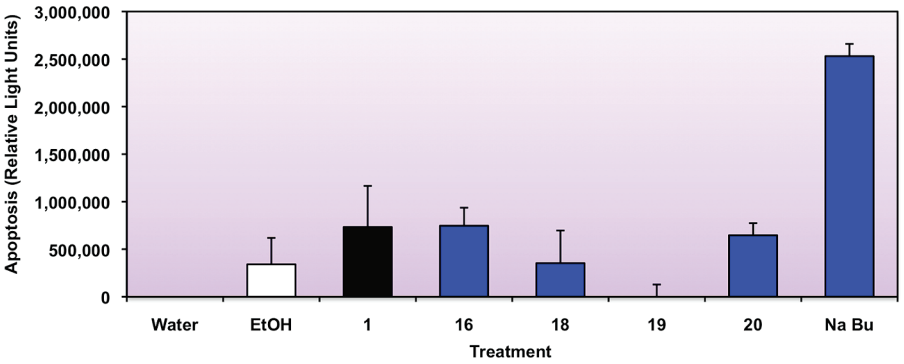


Figure 5. Evaluation of bexarotene (compound **1**) and selected analogues for apoptotic activity utilizing a caspase 3/7 assay in CTCL cells. Human T-cell lymphoma cells (CTCL) were plated and immediately dosed with the indicated treatments. Cells were allowed to incubate for 24 h, and the level of apoptosis was measured with a commercial kit (see Experimental Section). Sodium butyrate (Na Bu), a known inducer of apoptosis, was used as a positive control.

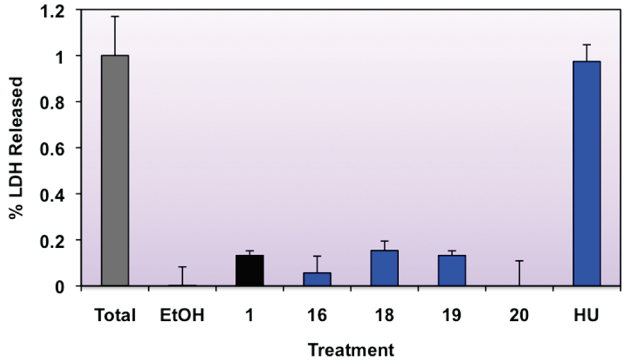


Figure 6. Cytotoxocity Analysis of Select Compounds. Cytotoxicity was assayed by determining lactate dehydrogenase (LDH) release. CTCL cells were left untreated or were treated for 48 h with ethanol vehicle, 10−7 M of the indicated compounds, or 10 mM hydroxyurea (HU, positive control). After a 48 h incubation, the supernatant from cells was removed and assayed for LDH activity (Promega CytoTox 96 nonradioactive cytotoxicity assay). The total cell activity was determined by lysing untreated cells and assaying the whole cell lysates. Cytotoxicity was determined as percentage of LDH released, as compared to total activity (total activity set to 1.0) after normalizing for background.

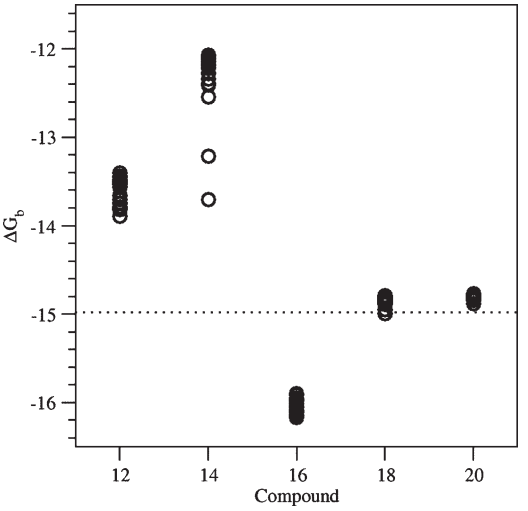


Figure 7. AutoDock binding free energies for compounds **12**, **14**, **16**, **18**, and **20**. Shown are the 20 lowest energies for each compound. The calculated binding free energy of **1** is shown by the dotted line.

CTCL effectively because it induces apoptosis and/or cytotoxicity in the T-lymphocyte.[34] Thus, we next tested CTCL cells treated with **1** and promising analogues for their ability to induce classic apoptosis, using an assay for caspases 3 and 7, two executioner caspases in apoptosis.[35] We included compounds **16**, **18**, and **20** because they possess the most potent RXR binding and activation profile, as well as compound **19**, which does not bind to RXR and serves as a negative control. Figure 5 illustrates the results of the apoptosis assay and suggests that some of the analogues that bind and activate RXR (compounds **16** and **20**) also possess apoptotic activity that is statistically significantly greater than the ethanol vehicle control (using a one-tailed heteroscedastic *t* test, $P = 0.003$ for compound **16**, and $P = 0.002$ for compound **20**), while compound **19**, which displays almost no apoptotic activity, also does not bind to RXR. A potent known apoptotic inducer (sodium butyrate, NaBu) serves as a positive control in this system.

Compound 1 and the Active Analogues Display a Low Level of Cytotoxicity. Cytotoxicity results for **1** and active analogues are shown in Figure 6.

Compound **1** is statistically significantly more cytotoxic in CTCL cells than ethanol vehicle alone ($P = 0.009$, unpaired *t* test, one tail, unequal variance); however, the level of cytotoxicity is low (13.2% of total) compared to hydroxyurea (97% of total), a positive control. This is in line with previously reported data for the cytotoxicity of **1**.[36] All of the analogues are no more cytotoxic than **1** ($P < 0.01$, unpaired *t* test, one tail, unequal variance), indicating that these compounds are similar in biological activity to **1**.

Compound 1 and the Novel Analogues are Not Mutagenic. Mutagenicity of all compounds was tested in a *Saccharomyces cerevisiae* assay in order to determine if the compounds are potentially suitable to administer in animal models. This assay utilizes a strain of *S. cerevisiae* in which three phenotypic readouts have been engineered[37,38] in order to determine if the compounds are potentially suitable to administer in animal models. All compounds were tested for mutagenicity compared to DMSO vehicle, and none were mutagenic.

Molecular Modeling. Docking Studies of 1 and the Novel Analogues Predict that Analogues 16, 18, and 19 Will Have the Best Binding Aaffinities. We performed docking studies of **1** and compounds **12**, **14**, **16**, **18**, and **20** using the X-ray structure of human RXRα in complex with **56**[39] (BMS 649) as template. The molecular frameworks of **56**, **1**, and the

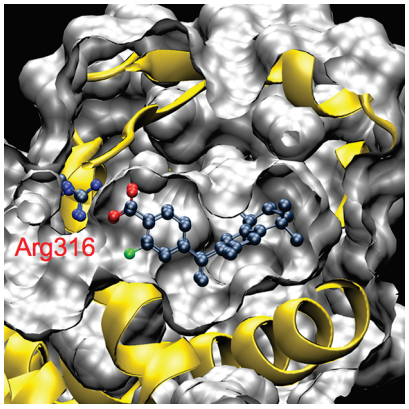
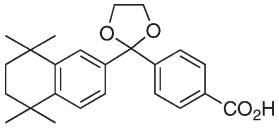


Figure 8. Docked structures of **1** and compound **20** to human RXRα.

compounds are very similar, with 21 carbon atoms at identical positions.



BMS649

Docking predicted low binding energies for compounds **16**, **18**, and **20** (Figure 7). The calculated average binding free energies were −14.9 and −14.8 kcal/mol for compound **18** and **20**, respectively; this was identical to the calculated binding energy of the docked **1** (−14.9 kcal/mol). Compound **16** was predicted to be the best binder, with an average binding free energy of −16.0 kcal/mol. This lower energy was mostly a result of a more favorable desolvation energy. Overall, the binding of **1** and compounds **16**, **18**, and **20** were very similar to the binding of **56**, with C atom root-mean-square deviations (rmsds) of 0.35 ± 0.08, 0.50 ± 0.10, 0.43 ± 0.10, and 0.33 ± 0.08 Å for the 20 lowest energy structures, respectively. The main deviations stemmed from the benzene ring, which slightly rotated to best accommodate hydrogen bonding between the carboxylate and Arg316. The ligands bound in a large hydrophobic pocket (Figure 8), with the fluoride atom of compounds **18** and **20**, and the nitro group of compound **16** pointing toward the C terminus of the H2 helix.

Compounds **12** and **14** were predicted to have significantly higher binding energies than **1** (Figure 7). Structural analyses of the docked structures showed that the increase in binding energy was mainly due to the carboxylate at the ortho position. To enable hydrogen bonding between the carboxylate and Arg316, relatively large readjustments of the ligands were needed, resulting in C atom rmsds of 0.95 ± 0.16 and 1.25 ± 0.15 Å with **56** for compound **12** and **14**, respectively. The readjustments not only involved the benzene ring but also shifted the rest of the ligand out of the hydrophobic pocket. Such shifts were particularly pronounced for the two lowest energy structures of compound **14** (with binding free energies of −13.7 and −13.2 kcal/mol, see Figure 7). These structures have C atom rmsds of 1.63 and 1.45 Å. Although the hydrophobic contacts were less optimal in these structures, overall decreases in binding energy compared to the other docked structures of compound **14** were obtained from better hydrogen bonding with Arg316. Despite these rearrangements, the best docked structures still had much larger binding free energies than **1** or compounds **16**, **18**, and **20**. Therefore, our modeling studies are predictive of the observation that compounds **16**, **18**, and **20** possessed the greatest RXR/RXRE-mediated transcriptional activation of all novel analogues tested in the RXRE assay, while analogues **12** and **14** did not display significant RXR binding and activation.

Conclusions

We have modeled, synthesized and evaluated several novel analogues of **1** with new functional groups substituting hydrogen atoms on the aromatic ring that bears the carboxylic acid. Biological assays employing these compounds in human Caco-2 and CTCL cells have identified compounds that bind and activate RXR slightly below or near the levels of **1** (**16**, **18**, **21** and **22**) and we have also synthesized and identified a compound (**20**) that possesses an apparent RXR binding affinity that is 75% greater than **1** and that displays a 20% increase in efficacy based on EC_{50} values (Table 1). Our results suggest that additional novel analogues of **1** that substitute non-hydrogen functional groups on the aromatic ring bearing the carboxylic acid will likely serve as effective, and perhaps more potent, ligands for RXR, which may also have reduced RAR agonist activity (Table 1); thus, these compounds may also possess less detrimental side-effects in cutaneous T-cell lymphoma patients.

Experimental Section

Mammalian Two-Hybrid Assay. Mammalian two-hybrid experiments were conducted using Caco-2 human colon cancer cells. Cells were plated at 90000 cells/well in a 24 well plate and maintained in minimum essential medium (MEM) (Invitrogen, Carlsbad, CA), supplemented with 20% fetal bovine serum (FBS) (Invitrogen), 1 mM sodium pyruvate (Invitrogen), 100 units/mL penicillin, and 100 μg/mL streptomycin. The cells were cotransfected utilizing RXR-bait (BD) and RXR-prey (AD) fusion constructs, pFR-Luc reporter gene, and renilla control plasmid via liposome-mediated transfection with Lipofectamine LTX and PLUS reagent (Invitrogen). The cells were incubated with the transfection mixture overnight and then treated with ethanol vehicle, **1**, or analogues at concentrations ranging from 10×10^{-10} M up to 0.5×10^{-5} M. After incubation with ligands, cells were collected and the amount of reporter gene product (luciferase) produced in the cells was measured using the Dual-Luciferase reporter assay system according to the manufacturer's protocol (Promega, Madison, WI) in a Sirus FB12 luminometer (Berthold Detection Systems). Independent experiments were conducted with triplicate samples for each treatment group.

RXRE-Mediated Transcription Assay. Caco-2 human colon cancer cells were plated at 90000 cells/well in a 24-well plate and maintained as described above. The transfection procedure was adapted from the manufacturer's protocol (Invitrogen). Briefly, each well received 1 μL of Lipofectamine reagent, 2 μL of Plus reagent, 500 ng of pTZ18U carrier DNA plasmid, and 20 ng of pRL-null (constitutively expressing low levels of *Renilla reniformis* luciferase) to monitor transfection efficiency. Each well also received 250 ng of pLuc-MCS plasmid (Stratagene, La Jolla, CA) containing an oligonucleotide (cloned between the *Hin*dIII and *Bgl*II sites) with two copies of the retinoid X receptor response element (RXRE) upstream of the firefly (*Photinus pyralis*) luciferase gene. The RXRE was based on a naturally occurring double repeat responsive element from the rat cellular retinol binding protein II gene. The sequence used

was AAAATGAACTGTGACCTGTGACCTGTGACCTGTGAC, with the half elements underlined. The cells were incubated with the transfection mixture overnight and then treated with ethanol vehicle, 10^{-7} M **1**, or analogues for 24 h. After incubation with ligands, cells were collected and the amount of reporter gene product (luciferase) produced in the cells was measured using a luminometer.

RXR Binding Assay. [^{3}H]-9-*cis*-Retinoic acid (60 Ci/mmol) was obtained from Perkin-Elmer (Waltham, MA). Assays were carried out essentially as described previously.[9,33] Briefly, Caco-2 cells (500000 cells/60 mm plate) transfected with 50 ng human wild-type RXRα expression plasmid were lysed in KETZD-0.3 buffer (0.3 M KCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.3 mM $ZnCl_2$, 5 mM DTT) containing 0.5% Triton X-100 and supplemented with HALT protease inhibitors (Thermo Scientific, Rockford, IL). Lysates were clarified by centrifugation for 15 min at 16000×*g* at 4 °C and then 40 μg of total protein lysate was incubated with 10 nM [^{3}H]-9-*cis*-retinoic acid and varying concentrations of competing ligand for 16 h at 2 °C. Bound and free hormone were separated with dextran-coated charcoal for subsequent analysis of ligand binding.

RAR/RARE-Agonist Activity Assay. Caco-2 human colon cancer cells were plated at 90000 cells/well in a 24-well plate and maintained as described above. The transfection procedure employed 1 μL of Lipofectamine reagent, 2 μL of Plus reagent, 50 ng of pTZ18U carrier DNA plasmid, 20 ng of pRL-null (constitutively expressing low levels of *Renilla reniformis* luciferase) to monitor transfection efficiency, and pCMX-human RARα expression vector. Each well also received 250 ng of the pTK-DR5(X2)-Luc plasmid containing an oligonucleotide with two copies of the retinoic acid response element (RARE) upstream of the firefly (*Photinus pyralis*) luciferase gene. This RARE is an optimized element that has been described previously[40] and is responsive to the RAR ligand, all-trans retinoic acid. The sequence of the double RARE is (5′-AAAGGTCACCGAAAGGTCACCATCCCGGGAAAAGGTCACCGAAAGGTCACC-3′), with the half elements underlined. The cells were incubated with the transfection mixture overnight and then treated with ethanol vehicle, all-trans retinoic acid, or analogues (retinoic acid or analogue concentrations ranged from 1×10^{-8} M to 5×10^{-6} M) for 24 h. After incubation with ligands, cells were collected and the amount of reporter gene product (luciferase) produced in the cells was measured using a luminometer.

Apoptosis Assay. Human T-cell lymphoma (CTCL) cells (Hut78) were plated in a 24-well plate and immediately dosed with the indicated treatments, including ethanol, compound **1**, analogue, water, or sodium butyrate. Cells were allowed to incubate for 24 h, and then lysis was initiated by the addition of a Caspase-Glo lysis/substrate mix. Upon programmed cell death, caspases released by the lysed cells cleave the added substrate and generate a measurable luminescent signal, assayed in a luminometer. Analogue-induced apoptosis was compared to the parent compound, **1**. Sodium butyrate (Na Bu), a known inducer of apoptosis, was used as a positive control.

Cytotoxicity Assay. Cytotoxicity was measured in the CTCL cells (Hut78) by performing a lactate dehydrogenase (LDH) assay (Cytotox 96 nonradioactive cytotoxicity assay, Promega, Madison, WI), whereby induction of LDH leakage is an indication of cytotoxicity. LDH levels are indicated by a change in a tetrazolium salt into a red formazan compound, read by a microtiter plate reader (Bio-Tek Instruments, EL_X808), and 15000 CTCL cells in 50 μL were seeded into 96-well plates in RPMI-1640 media supplemented with 10% FBS. Cells were then treated with 0.5 mL of compound or ethanol vehicle alone or were left untreated. Compound **1** or analogue final concentrations were 10^{-7} M. Hydroxurea, a known cytotoxic compound, final concentration was 10 mM. Cells were incubated for 48 h. Untreated cells were incubated with a final concentration 0.8% Triton-X 100 for 1 h at 37 °C to lyse the cells and release all LDH; this was used as the total cell LDH level. For the remainder of the cells, the supernatant was removed and centrifuged at 500*g* to remove residual cells; these supernatants were then assayed for LDH activity. All assays were carried out in a 96-well plate and included 25 μL of total cell lysate or supernatant plus 25 μL of water and 50 μL of assay reagent. The assay was incubated in the dark for 30 min, and then 50 μL of stop solution was added. The assay was read in a Biotek microtiter plate reader at 490 nm. Cytotoxicity was graphed in Figure 6 for select analogues. The total cell LDH was set to 100%, and the no treatment control was used as 0%. All points treatments were performed six times to generate the data set.

Mutagenicity. Mutagenicity of all compounds was tested in a *Saccharomyces cerevisiae* assay in order to determine if the compounds are potentially suitable to administer in animal models. None were mutagenic in this assay. All compounds were tested for mutagenicity with an incubation time of 3 h; the highest concentration in the dose response curve was 0.15% w/v, and compounds were dissolved in DMSO and compared to the nonmutagenic DMSO control.

Instrumentation. All ^{1}H NMR spectra were acquired at 400 or 500 MHz on Bruker or Varian spectrometers. Chemical shifts (δ) are listed in ppm against deuterated solvent peaks as an internal reference. Coupling constants (*J*) are reported in Hz, and the abbreviations for splitting include: s, single; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; br, broad. All ^{13}C NMR spectra were acquired on Bruker instruments at 125.8 or 100.6 MHz. Chemical shifts (δ) are listed in ppm against solvent carbon peaks as an internal reference. Infrared spectra (IR) were assayed on a Perkin-Elmer 1600 series FTIR. High resolution mass spectra were recorded using either a JEOL GCmate(2004), a JEOL LCmate(2002) high resolution mass spectrometer, or an ABI Mariner (1999) ESI-TOF mass spectrometer. Melting points were assayed on a Thomas−Hoover capillary melting point apparatus.

General Procedures. Tetrahydrofuran, methylene chloride, diethyl ether, and benzene were dried by filtration through alumina according to the procedure described by Grubbs.[41] All other solvents were distilled from CaH_2 prior to use. Removal of volatile solvents transpired under reduced pressure using a Buchi rotary evaporator and is referred to as removing solvents in vacuo. Thin layer chromatography was conducted on precoated (0.25 mm thickness) silica gel plates with 60F-254 indicator (Merck). Column chromatography was conducted using 230−400 mesh silica gel (E. Merck reagent silica gel 60). All tested compounds were analyzed for purity by combustion analysis through Columbia Analytical Services (formerly Desert Analytics in Tucson, AZ) and were found to be >95% pure.

2,5-Dichloro-2,5-dimethylhexane (23). A slightly modified method of Boehm and co-workers[9] was followed to make **23**. To 2,5-dimethyl-2,5-hexanediol (5.0 g, 34 mmol) in a 100 mL round-bottom flask was added concentrated hydrochloric acid (40.0 mL), slowly with gentle swirling. The diol slowly dissolved and a white precipitate formed simultaneously within 10 min. After sitting 2 h, the heterogeneous mixture was filtered and washed with copious amounts of water and a small amount of methanol to give crude **23** that was dried under vacuum for 30 min to yield a white crystalline solid, mp 63−65.8 °C (lit.[42] 63−66.5 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (s, 4H), 1.59 (s, 12H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 70.3, 41.1 32.5.

1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalene (24). To a three-neck, 500 mL round-bottom flask charged with **23** (10.0 g, 54.6 mmol) and dry DCM (50.0 mL), fitted with a spiral water condenser, was added aluminum chloride (0.50 g, 3.7 mmol) in small scoops. The colorless, homogeneous solution of **23** in DCM turned canary yellow concurrent with gas evolution as the aluminum chloride was added. The reaction solution was stirred at room temperature (30 min), and an additional amount of aluminum chloride (100 mg) was added and the reaction solution was heated to reflux and stirred for 15 min. TLC indicated that the reaction

was complete. The reaction solution was cooled in an ice bath and quenched with a 20% HCl solution (50 mL). The reaction solution was extracted with hexanes (200 mL, twice), dried over sodium sulfate, concentrated in vacuo, and the crude oil was purified by column chromatography (SiO_2, Hexanes) to give **24** (10.4 g, 94%) as a white solid, mp 34−36 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (d, J = 8.0, 1H), 7.13 (s, 1H), 6.97 (d, J = 8.4, 1H), 2.31 (s, 3H), 1.68 (s, 4H), 1.29 (s, 6H), 1.28 (s, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 144.6, 141.8, 134.7, 126.9, 126.5, 126.4, 35.1, 35.1, 34. 1, 33.8, 31.9, 31.8, 21.1; GC-EI-MS (M+) calcd for $C_{15}H_{22}$ 202.1722, found 202.1751.

Methyl-4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoate (27). Compound **27** was synthesized according to the methods of Boehm and co-workers.[9] Monomethylterephthalic acid chloride (**25**) was synthesized by refluxing monomethylterephthalic acid (10.1 g, 56.2 mmol) in thionyl chloride (120 mL, 1.65 mol) in a 500 mL one-neck round-bottom flask fitted with a water cooled reflux condenser. Excess thionyl chloride was removed in vacuo to give crude **25** as an off-white solid, and this solid was dissolved in dry benzene (ca. 40 mL) and evaporated to dryness three times to remove residual thionyl chloride. The acid chloride **25** was dried on high vacuum to remove residual benzene. To a three-neck, 500 mL round-bottom flask equipped with a reflux condenser and magnetic stir-bar was added **24** (10.0 g, 49.4 mmol), followed by a solution of crude acid chloride **25** (56.2 mmol) in DCM (50 mL). Aluminum chloride (14.0 g, 105 mmol) was added to the reaction solution at room temperature slowly, with stirring, and the reaction solution turned from colorless to red accompanied by the evolution of gas and heat. The reaction was stirred for 30 min and then heated to reflux for 15 min. The reaction was judged to be complete by TLC, and the solution was poured into an ice solution (200 mL) acidified with a 20% HCl solution (50 mL) and ethyl acetate was added (100 mL). The aqueous and organic layers were separated, and the aqueous layer was extracted with ethyl acetate (100 mL, twice). The combined organics were washed with water and brine, dried over sodium sulfate, filtered, and rotevapped to give crude **27**. Crude **27** was purified by dissolving the crude material in hot ethyl acetated (53 mL), followed by the addition of methanol (100 mL) and slowly cooling the solution to room temperature to yield square plate crystals of **27** (12.86 g, 71%) that were filtered, mp 143−144 °C (lit.[9] 142−143 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, J = 6.8 Hz, 2H), 7.89 (d, J = 6.8, 2H), 7.30 (s, 1H), 7.25 (s, 1H), 4.00 (s, 3H), 2.39 (s, 3H), 1.74 (s, 4H), 1.35 (s, 6H), 1.24 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 197.7, 166.4, 148.4, 141.9, 141.8, 134.7, 134.5, 133.4, 129.9, 129.5, 129.4, 128.4, 52.4, 34.9, 34.8, 34.3, 33.8, 31.7, 31.6, 20.0.

Methyl 4-(1-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoate (28). The method of Boehm and co-workers[9] was followed to prepare compound **28**. To a 100 mL round-bottom flask charged with methyltriphenylphosphonium bromide (4.72 g, 13.2 mmol) and dry THF (15 mL) under nitrogen was slowly added sodium amide (0.72 g, 18.5 mmol). The heterogeneous solution was allowed to stir for 46 h, and it was slowly added to a solution of **27** (3.14 g, 8.62 mmol) in dry THF (20 mL) over 15 min. The reaction solution was stirred for 45 min and then poured into water (200 mL). The aqueous solution was extracted with ethyl acetate (200 mL, twice), and the combined organic layers were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give a rouge powder. Crude **28** was dissolved in hot ethyl acetate (10 mL), hot methanol (40 mL) was added, and the solution was allowed to cool to room temperature to give pure **28** as a white powder that was filtered (2.06 g, 66%), mp 160−161 °C (lit.[9] 160−161 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.4, 2H), 7.34 (d, J = 8.8, 2H), 7.13 (s, 1H), 7.08 (s, 1H), 5.81 (d, J = 1.2, 1H), 5.33 (d, J = 1.2, 1H), 3.91 (s, 3H), 1.94 (s, 3H), 1.71 (s, 4H), 1.31 (s, 6H), 1.27 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 166.9, 149.1, 145.5, 144.3, 142.3, 137.9, 132.7, 129.6, 128.9, 128.1, 128.0, 126.5, 116.8, 52.0, 35.1, 33.9, 33.8, 31.9, 31.8, 19.9.

4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-ethynyl]benzoic Acid (1). Compound **1** was synthesized according to the method of Boehm and co-workers.[9] To a 100 mL round-bottom flask charged with **28** (1.48 g, 4.08 mmol) and methanol (20 mL) was added a 5 M aqueous solution of potassium hydroxide (2 mL, 10 mmol). A reflux condenser was fitted to the round-bottom flask and the reaction solution was refluxed and monitored by TLC. After 70 min at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (250 mL). The aqueous solution was extracted with ethyl acetate (200 mL, twice) and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **1**. Crude **1** was purified by dissolving the crude material in hot ethyl acetate (19 mL), adding warm hexanes (19 mL), and allowing the solution to cool to room temperature to give crystals of **1** (1.12 g, 78%) mp 224−226 °C (lit.[9] 234 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.2, 2H), 7.37 (d, J = 8.2, 2H), 7.14 (s, 1H), 7.09 (s, 1H), 5.84 (s, 1H), 5.36 (s, 1H), 1.94 (s, 3H), 1.70 (s, 4H), 1.32 (s, 6H), 1.29 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.2, 149.4, 146.7, 144.7, 142.6, 138.1, 132.9, 130.5, 128.3, 128.2, 126.9, 117.4, 105.0, 35.4, 35.4, 34.2, 34.1, 32.1, 32.1, 20.1. LC-APCI-MS (M+) calcd for $C_{24}H_{29}O_2$ 349.2168, found 349.2161. Anal. Calcd for $C_{24}H_{29}O_2$: C 82.72; H 8.10. Found: C 82.59; H 8.01.

4-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid (22). Compound **22** was synthesized according to the method of Boehm and co-workers.[9] To a 100 mL round-bottom flask charged with **27** (0.505 g, 1.39 mmol) and methanol (6.8 mL) was added a 5 M aqueous solution of potassium hydroxide (0.71 mL, 3.6 mmol). A reflux condenser was fitted to the round-bottom flask, and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (20 mL). The aqueous solution was extracted with ethyl acetate (25 mL, twice) and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **22**. Crude **22** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **22** (0.429 g, 88%) as a white crystalline solid, mp 198−199 °C (lit.[9] 198−199 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (dd, J = 6.8, 1.8 Hz, 2H), 7.90 (d, J = 8.5, 1.8 Hz, 2H), 7.26 (s, 1H), 7.22 (s, 1H), 2.36 (s, 3H), 1.70 (s, 4H), 1.32 (s, 6H), 1.21 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 197.7, 171.3, 148.5, 142.8, 141.9, 134.7, 134.6, 132.4, 130.1, 130.0, 129.5, 128.5, 34.9, 34.8, 34.4, 33.9, 31.7, 31.6, 20.0. Anal. Calcd for $C_{23}H_{26}O_3$: C 78.83; H 7.48. Found: C 78.91; H 7.48.

Methyl 3-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoate (29). Compound **29**[23] was synthesized according to a slightly modified method of Boehm and co-workers.[9] Monomethylisophthalic acid chloride (**26**) was synthesized by refluxing monomethylisophthalic acid (1.30 g, 7.24 mmol) in thionyl chloride (12.0 mL, 165 mmol) in a 100 mL one-neck round-bottom flask fitted with a water cooled reflux condenser. Excess thionyl chloride was removed in vacuo to give crude **26** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness three times to remove residual thionyl chloride. The acid chloride **26** was dried on high vacuum to remove residual benzene. To a two-neck, 50 mL round-bottom flask equipped with a reflux condenser and magnetic stir-bar was added **24** (1.35 g, 6.67 mmol) followed by a solution of crude acid chloride **26** (7.24 mmol) in DCM (15 mL). Aluminum chloride (2.0 g, 15 mmol) was added to the reaction solution at room temperature slowly, with stirring, and the reaction solution turned from colorless to red accompanied by the evolution of gas and heat. The reaction was stirred for 5 min then heated to reflux for 15 min. The reaction was judged to be complete by TLC, and the solution was poured into an ice solution (25 mL) acidified with a 20% HCl solution (8 mL) and ethyl acetate was added (13 mL). The aqueous and organic

layers were separated, and the aqueous layer was extracted with ethyl acetate (15 mL, twice). The combined organics were washed with water and brine, dried over sodium sulfate, filtered, and rotevapped to give crude **29**. Crude **29** was purified by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 95:5) to give **29** (2.21 g, 93%) as an oil that crystallized into a solid 110−111 °C (lit.[23] 110−112 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.24 (d, J = 8.0, 1H), 8.04 (d, J = 7.6, 1H), 7.55 (t, J = 7.6, 1H), 7.28 (s, 1H), 7.21 (s, 1H); 3.91 (s, 3H), 2.35 (s, 3H), 1.69 (s, 4H), 1.31(s, 6H), 1.21(s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 197.5, 166.5, 148.4, 142.0, 138.8. 134.8, 134.5, 133.7, 131.6, 130.6, 129.7, 128.8, 128.7, 52.5, 35.1, 35.0, 34.5, 34.1, 31.8, 20.2. LC-APCI-MS (M+) calcd for $C_{24}H_{29}O_3$ 365.2117, found 365.2133.

Methyl 3-(1-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoate (30). To a 100 mL round-bottom flask charged with **29** (0.96 g, 2.63 mmol) and dry THF (5 mL) at room temperature was slowly added by equal parts separated by 45 min a triphenylphosphonium methylide solution prepared as follows: methyltriphenylphosphonium bromide (1.9 g, 5.32 mmol) suspended in dry THF (16 mL) in a 100 mL round-bottom flask equipped with a stir-bar was stirred for 30 min at room temperature after the addition of a 2.5 M solution of *n*-butyl lithium in hexanes (2.2 mL, 5.5 mmol), which provided a homogeneous dark-yellow ylide solution. The reaction was monitored by TLC, and when the reaction was judged to be complete, the reaction solution was poured into water (70 mL) and the aqueous solution was extracted with ethyl acetate (70 mL, twice). The combined organic extracts were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **30**, which was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 97.5:2.5) to give **30** (0.26 g, 27%) as a white solid, mp 89−91 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.92 (d, J = 6.8, 1H), 7.36 (m, 2H), 7.15 (s, 1H), 7.08 (s, 1H), 5.77 (d, J = 1.2, 1H), 5.28 (d, J = 1.2, 1H), 3.91 (s, 3H), 1.96 (s, 3H), 1.71 (s, 4H), 1.31 (s, 6H), 1.28 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 167.4, 149.3, 144.4, 142.5, 142.4, 141.8, 138.3, 132.9, 131.6, 131.4, 130.5, 128.7, 128.5, 128.4, 128.3, 127.7, 116.2, 52.3, 35.5, 35.4, 34.2, 34.1, 32.2, 32.1, 30.9, 30.5, 20.2. LC-APCI-MS (M+) calcd for $C_{25}H_{31}O_2$ 363.2329, found 363.2324.

3-(1-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoic Acid (12). Compound **12** was synthesized from **30** according to the representative procedure for the synthesis of **1** from **28**. To a 100 mL round-bottom flask charged with **30** (0.3278 g, 0.90 mmol) and methanol (5 mL) was added a 5 M aqueous solution of potassium hydroxide (0.50 mL, 2.5 mmol). A reflux condenser was fitted to the round-bottom flask, and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (55 mL). The aqueous solution was extracted with ethyl acetate (50 mL, twice) and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **12**. Crude **12** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **12** (0.2623 g, 83%) as a white crystalline solid, mp 195−196 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.02 (m, 1H), 7.42 (m, 2H), 7.15 (s, 1H), 7.09 (s, 1H), 5.79 (s, 1H), 5.30 (s, 1H), 1.96 (s, 3H), 1.71 (s, 4H), 1.31 (s, 6H), 1.29 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.5, 149.2, 144.6, 142.5, 141.9, 138.2, 132.8, 132.5, 129.6, 129.3, 128.7, 128.4, 128.3, 116.4, 35.5, 34.3, 34.2, 32.2, 32.1, 20.2. LC-APCI-MS (M+) calcd for $C_{24}H_{29}O_2$ 349.2168, found 349.2149. Anal. Calcd for $C_{24}H_{28}O_2$: C 82.72; H 8.10. Found: C 82.26; H 7.84.

3-[(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-carbonyl]benzoic Acid (13). Compound **13**[23] was synthesized according to the method of Boehm and co-workers.[9] To a 100 mL round-bottom flask charged with **29** (0.27 g, 0.74 mmol) and methanol (5 mL) was added a 5 M aqueous solution of potassium hydroxide (0.64 mL, 3.2 mmol). A reflux condenser was fitted to the round-bottom flask, and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (20 mL). The aqueous solution was extracted with ethyl acetate (25 mL, twice) and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **13**. Crude **13** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **13** (0.2573 g, 99%) as a white crystalline solid, mp 192−195 °C (lit.[23] 192−194 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.32 (d, J = 7.5, 1H), 8.12 (d, J = 8, 1H), 7.61 (t, J = 8, 1H), 7.30 (s, 1H), 7.24 (s, 1H), 2.37 (s, 3H), 1.71 (s, 4H), 1.34 (s, 6H), 1.22 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 197.1, 171.4, 148.4, 141.9, 138.8, 135.1, 134.6, 134.4, 133.9, 132.0, 129.5, 129.4, 128.5, 34.9, 34.8, 34.3, 31.6, 20.0. Anal. Calcd for $C_{23}H_{26}O_3$: C 78.83; H 7.48. Found: C 79.13; H 7.68.

3,5-Di(methoxycarbonyl)benzoic Acid (32). Compound **32** was synthesized by the method of Dimick and co-workers.[30] To a 250 mL round-bottom flask charged with trimethyl 1,3,5-benzenetricarboxylate (**31**) (1.10 g, 4.36 mmol) and methanol (100 mL) was added a 1 M aqueous solution of sodium hydroxide (3.95 mL, 3.95 mmol). The reaction solution was stirred for 18 h, the solvent was removed in vacuo, and the residual solid material was dissolved in saturated $NaHCO_3$ (150 mL) and washed with DCM (75 mL). The aqueous layer was acidified with conc HCl (18 mL) until the pH ~ 2.0, and the heterogeneous solution was extracted with ethyl acetate (75 mL, twice). The combined extracts were dried over sodium sulfate, and the solvents were removed in vacuo to give crude **32**. Crude **32** was purified by recrystallization from boiling water (500 mL/5 g) to give pure **32** (0.817 g, 79%) as a white powder, mp 264 °C (decomposition). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 3H), 3.99 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.0, 165.3, 135.4, 135.1, 131.4, 130.3, 52.7.

4-(Methoxycarbonyl)-3-nitrobenzoic Acid (35). Compound **35** was synthesized and purified according to a slightly modified method of Keenan and co-workers.[31] To a 250 mL round-bottom flask charged with dimethylnitroterephthalate (12 g, 50.2 mmol) and dioxane (100 mL) was added 1 M sodium hydroxide (50 mL, 50 mmol) dropwise over 30 min at room temperature. The reaction solution was stirred overnight, water was added (100 mL), and the solution was washed with diethyl ether (100 mL, twice). The aqueous layer was acidified with 1 M HCl (56 mL) to pH ~ 1−2, then extracted with ethyl acetate (150 mL, thrice). The combined organic extracts were dried over sodium sulfate and removed in vacuo to give crude (**35**). Crude **35** was recrystallized in water (600 mL/12 g) to give pure **35** as white crystals (6.0 g, 53%), mp 177−179 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.39 (d, J = 8.0, 1H), 7.84 (d, J = 8.0, 1H), 3.97 (s, 3H).

3-Fluoro-4-formylmethylbenzoate (40). Compound **40** was synthesized according to the methods of Kishida and co-workers.[32] To a 500 mL round-bottom flask charged with 3-fluoro-4-methylbenzoic acid (**37**) (9.0 g, 58.4 mmol) was added NBS (25.0 g, 140 mmol), benzoylperoxide (0.66 g, 2.73 mmol), and carbon tetrachloride (112 mL). The reaction solution was heated to reflux under magnetic stirring for 36 h, cooled to room temperature, and solids were filtered and washed with carbon tetrachloride (~20 mL). The filtrate solvent was removed in vacuo and the crude 4-(dibromomethyl)-3-fluorobenzoic acid (**38**) was dried on high vacuum and used without further purification. To a 500 mL round-bottom flask charged with crude **38** (10.44 g, 58.4 mmol) was added ethanol (148 mL), and a solution of silver nitrate (20.5 g, 120.7 mmol) in warm water (28 mL) was added dropwise while the reaction solution was stirred in an oil bath preheated to 50−55 °C. Upon addition of the silver nitrate solution, a green precipitate formed. After stirring at 50 °C for 45 min, the reaction solution was cooled to room temperature and filtered to remove the green precipitate. The filtrate solvent was concentrated in vacuo, extracted

with ethyl acetate (130 mL), and the combined organic extracts were washed with water and brine, dried over sodium sulfate, and removed in vacuo to give crude 3-fluoro-4-formylbenzoic acid (**39**) (8.68 g, 87%) that was used without further purification. To a 500 mL round-bottom flask charged with **39** (9.87 g, 58.7 mmol) was added dry dimethylformamide (190 mL) and a 60 wt % suspension of NaH in mineral oil (2.75 g, 68.8 mmol) in small aliquots over 20 min. The reaction solution was stirred an additional 20 min, and methyliodide (4.32 mL, 69.4 mmol) was added to the red heterogeneous solution. After stirring 5 h, the reaction solution had become homogeneous, and it was poured into 1N HCl (490 mL), extracted with ethyl acetate (150 mL, twice), and the combined organic extracts were washed with saturated $NaHCO_3$ (75 mL) and brine, dried over sodium sulfate, and removed in vacuo to give crude **40**. Crude **40** was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 4:1) to give **40** (10.24 g, 95%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.41 (s, 1H), 7.94 (m, 2H), 7.85 (m, 1H), 3.95 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 186.6, 165.2, 164.9, 163.1, 137.2, 132.1, 128.8, 126.8, 125.5, 124.8, 117.9, 117.7, 52.8; LC-APCI-MS (M+) calcd for $C_9H_7O_3F$ 182.0379, found 182.0336.

4-(Methoxycarbonyl)-2-fluorobenzoic acid (41). Compound **41** was synthesized by the method of Kishida and co-workers.[32] To a 250 mL round-bottom flask charged with compound **40** (9.22 g, 50.5 mmol) and sulfamic acid (5.40 g, 55.6 mmol) in water (21 mL) and ACN (42 mL) was added a solution of 80% $NaClO_2$ (4.92 g, 53.8 mmol) in water (21 mL) dropwise at room temperature. After stirring for 1 h, the reaction solution was poured into a saturated, aqueous solution of Na_2SO_3 (75 mL) and 1 N HCl (150 mL) and the resulting solution was extracted with ethyl acetate (75 mL, thrice). The combined organic extracts were washed with brine, dried over sodium sulfate, and the solvents were removed in vacuo to give crude **41** (7.56 g, 75%) as a white solid. A small sample was recrystallized from hot ethyl acetate to give pure **41** as white crystals, mp 154−155 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.5 (br s, 1H), 8.10 (t, J = 7.8, 1H), 7.89 (d, J = 8.2, 1H), 7.82 (d, J = 11.0, 1H), 3.97 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 168.6, 168.5, 165.0, 164.9, 163.4, 160.8, 136.7, 136.6, 132.8, 124.9, 124.8, 121.3, 121.2, 118,4, 118.1, 52.8. LC-APCI-MS (M+) calcd for $C_9H_7O_4F$ 198.0328, found 198.0331.

Benzyl-3-fluoro-4-formylbenzoate (43).[32] To a 100 mL round-bottom flask charged with a crude sample of 3-fluoro-4-formylbenzoic acid (**39**) (2.51 g, 14.9 mmol) and dry DMF (45 mL) was slowly added with stirring 60 wt % NaH in mineral oil (0.726 g, 18.2 mmol). The reaction solution was stirred for 45 min, benzyl bromide (2.2 mL, 18.4 mmol) was added dropwise, and the reaction solution was stirred for an additional 5 h. TLC indicated the completion of the reaction, and the reaction solution was poured into 1N HCl (80 mL) and extracted with ethyl acetate (50 mL, twice). The combined organic extracts were washed with saturated $NaHCO_3$ and brine, dried over sodium sulfate, and removed in vacuo to give crude **43** as a yellow oil. Crude **43** was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 4:1) to give **43** (3.84 g, 99%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.31 (s, 1H), 7.83 (m, 2H), 7.76 (m, 1H), 7.31 (m, 5H), 5.29 (s, 2H);^{13}C NMR (100.6 MHz, $CDCl_3$) δ 218.2, 186.6, 186.5, 165.2, 164.3, 164.2, 163.1, 137.3, 137.2, 135.2, 132.1, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 127.0, 126.9, 125.7, 125.6, 124.8, 118.2, 118.0, 117.8, 67.6, 67.4; LC-APCI-MS (M+) calcd for $C_{15}H_{12}O_3F$ 259.0771, found 259.0751.

4-((Benzyloxy)carbonyl)-2-fluorobenzoic Acid (44). Compound **44** was prepared according to the method of Kishida and co-workers.[32] To a 100 mL round-bottom flask charged with **43** (3.29 g, 12.7 mmol), sulfamic acid (1.235 g, 12.7 mmol), water (20 mL), and ACN (10 mL) was added a solution of 80% $NaClO_2$ (1.16 g, 12.8 mmol) in water (6.8 mL). After stirring for 1 h, the reaction solution was poured into saturated Na_2SO_3 (25 mL) and 1N HCl (50 mL), and the resulting solution was extracted with ethyl acetate (50 mL, thrice). The combined organic extracts were washed with brine, dried over sodium sulfate, and removed in vacuo to give crude **44** (3.14 g, 90%) that was used without further purification. A small sample was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 1:1) to give pure **44** as a white powder, mp 127−128 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.91 (br s, 1H), 8.09 (t, J = 7.8, 1H), 7.93 (d, J = 9.2, 1H), 7.85 (d, J = 10.9, 1H), 7.39 (m, 5H), 5.40 (s, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 168.8, 168.7, 164.4, 164.3, 163.4, 160.8, 136.8, 136.7, 135.2, 132.8, 128.7, 128.5, 128.3, 125.0, 124.9, 121.3, 121.2, 118.4, 118.2, 67.6.

4-Benzyl 1-Methyl 2-Fluorobenzene-1,4-dioate (45). Compound **45** was synthesized according to the method of Kishida and co-workers.[32] To a 100 mL round-bottom flask charged with compound **44** (2.91 g, 10.6 mmol) was added $SOCl_2$ (9.0 mL, 124 mmol) and the reaction solution was refluxed for 1 h. The reaction solution was cooled to room temperature and the excess thionyl chloride was removed in vacuo to give crude 4-chlorocarbonyl-2-fluorobenzoic acid benzyl ester. The crude 4-chlorocarbonyl-2-fluorobenzoic acid in dry toluene (4.5 mL) was added dropwise to a solution of triethylamine (2.9 mL, 20.9 mmol) in methanol (29.8 mL, 736 mmol) over 10 min. The reaction solution was stirred 1 h and poured into 1N HCl (80 mL) and extracted with ethyl acetate (80 mL, thrice). The combined organic extracts were washed with brine, dried over sodium sulfate, and removed in vacuo to give crude **45**. Crude **45** was purified by column chromatography (150 mL SiO_2, hexanes:ethyl acetate 95:5) to give pure **45** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (t, J = 7.8, 1H), 7.88 (d, J = 8.2, 1H), 7.81 (d, J = 10.9, 1H), 7.41 (m, 5H), 5.37 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 164.5, 164.4, 164.2, 164.1, 162.7, 160.1, 135.7, 135.6, 135.2, 132.2, 128.7, 128.5, 124.9, 124.8, 122.5, 122.4, 118.3, 118.0, 67.4, 52.6; LC-APCI-MS (M+) calcd for $C_{16}H_{14}O_4F$ 289.0876, found 289.0886.

4-(Methoxycarbonyl)-3-fluorobenzoic Acid (46).[32] A three-neck 250 mL round-bottom flask charged with **45** (1.87 g, 6.49 mmol), 10% Pd/C (0.191 g), ethanol (11.0 mL), and ethyl acetate (11.0 mL) was evacuated and backfilled with hydrogen gas from a balloon three times, and the reaction solution was allowed to stir under hydrogen at room temperature overnight. The reaction solution was filtered through celite, and the solvents were removed in vacuo to give crude **46** (1.23 g, 95%) as a white crystalline solid that was used without further purification. A small sample of crude **46** was purified by recrystallization from hot ethyl acetate to give pure **46**, mp 210−211 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.61 (br s, 1H), 7.98 (t, J = 7.8, 1H), 7.83 (d, J = 8.2, 1H), 7.74 (d, J = 11.2, 1H), 3.87 (s, 3H). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 165.9, 165.8, 163.9, 163.8, 162.2, 159.6, 137.4, 137.3, 132.5, 125.6, 125.5, 122.1, 122.0, 117.9, 117.7, 53.0. LC-APCI-MS (M+) calcd for $C_9H_7O_4F$ 198.0328, found 198.0371.

Dimethyl 5-(2-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzene-1,3-dioate (48). Compound **48** was synthesized according to the representative procedure for the synthesis of compound **29**. Dimethyl 5-(chlorocarbonyl)benzene-1,3-dioate (**33**) was synthesized by refluxing 3,5-di(methoxycarbonyl)benzoic acid (**32**) (1.71 g, 7.17 mmol) in thionyl chloride (12.0 mL, 165 mmol) in a 100 mL one-neck round-bottom flask fitted with a water cooled reflux condenser. Excess thionyl chloride was removed in vacuo to give crude **33** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness three times to remove residual thionyl chloride. The acid chloride **33** was dried on high vacuum to remove residual benzene. To a two-neck, 50 mL round-bottom flask equipped with a reflux condenser and magnetic stir-bar was added **24** (1.39 g, 6.86 mmol), followed by a solution of crude acid chloride **33** (7.17 mmol) in DCM (15 mL). Aluminum chloride (2.0 g, 15 mmol) was added to the reaction solution at room temperature slowly, with stirring, and the reaction solution turned from colorless to red accompanied by the evolution of gas and heat. The reaction was stirred for 5 min then heated to reflux

for 15 min. The reaction was judged to be complete by TLC, and the solution was poured into an ice solution (25 mL) acidified with a 20% HCl solution (8 mL) and ethyl acetate was added (13 mL). The aqueous and organic layers were separated, and the aqueous layer was extracted with ethyl acetate (15 mL, twice). The combined organics were washed with water and brine, dried over sodium sulfate, filtered, and removed in vacuo to give crude **48**. Crude **48** was purified by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 95:5 to 9:1) to give **48** (1.29 g, 44%) as a white powder, mp 143−145 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.65 (s, 1H), 7.27 (s, 1H), 7.22 (s, 1H), 3.95 (s, 6H), 2.37 (s, 3H), 1.68 (s, 4H), 1.31 (s, 6H), 1.19 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 196.2, 165.8, 149.0, 142.2, 139.4, 135.3, 131.2, 130.0, 129.1, 52.8, 35.0, 34.6, 34.1, 31.8, 20.3. LC-APCI-MS (M + H)+ calcd for $C_{26}H_{31}O_5$ 423.2171, found 423.2163.

Dimethyl 5-(1-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzene-1,3-dioate (52). Compound **52** was synthesized following the representative procedure to synthesize compound **30**. To a 100 mL round-bottom flask charged with **48** (0.92 g, 2.18 mmol) and dry THF (5 mL) at room temperature was slowly added by equal parts separated by 45 min a triphenylphosphonium methylide solution prepared as follows: methyltriphenylphosphonium bromide (1.65 g, 4.62 mmol) suspended in dry THF (10 mL) in a 100 mL round-bottom flask equipped with a stir-bar was stirred for 30 min at room temperature after the addition of a 2.5 M solution of *n*-butyl lithium in hexanes (1.86 mL, 4.65 mmol), which provided a homogeneous dark yellow ylide solution. The reaction was monitored by TLC, and when the reaction was judged to be complete, the reaction solution was poured into water (70 mL) and the aqueous solution was extracted with ethyl acetate (70 mL, twice). The combined organic extracts were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **52**, which was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 97.5:2.5) to give **52** (0.14 g, 16%) as a white solid, mp 138 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.16 (s, 2H), 7.15 (s, 1H), 7.09 (s, 1H), 5.81 (s, 1H), 5.35 (s, 1H), 3.93 (s, 6H), 1.95 (s, 3H), 1.73 (s, 4H), 1.31 (s, 6H), 1.29 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 166.5, 148.6, 144.8, 142.6, 137.6, 132.7, 132.2, 130.9, 129.7, 128.5, 128.3, 117.5, 52.6, 35.5, 35.4, 34.2, 34.1, 32.1, 32.0, 20.3. LC-APCI-MS (M + H)+ calcd for $C_{27}H_{33}O_4$ 421.2379, found 421.2375.

5-(1-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzene-1,3-dioic Acid (14). Compound **14** was synthesized following the representative procedure for the synthesis of compound **12**. To a 100 mL round-bottom flask charged with **52** (0.15 g, 0.36 mmol) and methanol (5 mL) was added a 5 M aqueous solution of potassium hydroxide (0.43 mL, 2.15 mmol). A reflux condenser was fitted to the round-bottom flask, and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (49 mL). The precipitate product was filtered to give crude **14** that appeared to be pure by TLC. A small sample of crude **14** was purified by recrystallization from hot ethyl acetate to give pure **14** as a white crystalline solid, mp 258−260 °C. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.55 (s, 1H), 8.12 (s, 2H), 7.15 (s, 2H), 5.87 (s, 1H), 5.28 (s, 1H), 1.95 (s, 3H), 1.72 (s, 4H), 1.30 (s, 6H), 1.28 (s, 6H). ^{13}C NMR (100.6 MHz, MeOH-d_4) δ 167.5, 148.9, 144.5, 142.4, 142.1, 137.9, 132.5, 131.6, 131.5, 129.6, 128.2, 127.8, 116.0, 35.2, 35.1, 33.7, 33.6, 31.1, 31.0, 18.9. LC-APCI-MS (M − H)− calcd for $C_{25}H_{27}O_4$ 391.1909, found 391.1942. Anal. Calcd for $C_{25}H_{28}O_4$: C 76.50; H 7.19. Found: C 74.10; H 6.96.

5-(1-(3,5,5,8,8-Pentamethylnaphthalen-2-yl)carbonyl)benzene-1,3-dioic Acid (15). Compound **15** was synthesized following the representative procedure for the synthesis of compound **13**. To a 100 mL round-bottom flask charged with **48** (0.31 g, 0.74 mmol and methanol (5 mL) was added a 5 M aqueous solution of potassium hydroxide (0.87 mL, 4.4 mmol). A reflux condenser was fitted to the round-bottom flask, and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (20 mL). The precipitate was filtered to give crude **15** (0.28 g, 97%) that appeared to be pure by TLC. A sample of **15** was purified by recrystallization in hot ethyl acetate to give pure **15** as a white crystalline solid, mp 301−303 °C. ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.85 (s, 1H), 8.57 (s, 2H), 7.33 (s, 1H), 7.31 (s, 1H), 2.34 (s, 3H), 1.73 (s, 4H), 1.34 (s, 6H), 1.22 (s, 6H). ^{13}C NMR (100.6 MHz, MeOH-d_4) δ 196.8, 166.6, 148.7, 142.1, 139.1, 134.7, 134.6, 134.2, 132.0, 129.6, 128.5, 106.4, 105.0, 34.8, 34.7, 34.2, 33.7, 30.8, 18.8. Anal. Calcd for $C_{24}H_{26}O_5$: C 73.08; H 6.64. Found: C 72.95; H 6.75.

Methyl 4-(1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)-2-nitrobenzoate (49). Compound **49** was synthesized following the representative procedure for the synthesis of compound **29**. Methyl 4-(chlorocarbonyl)-2-nitrobenzoate (**36**) was synthesized by refluxing 4-(methoxycarbonyl)-3-nitrobenzoic acid (**35**) (2.46 g, 10.9 mmol) in thionyl chloride (22.0 mL, 302 mmol) in a 100 mL one-neck round-bottom flask fitted with a water-cooled reflux condenser. Excess thionyl chloride was removed in vacuo to give crude **36** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness three times to remove residual thionyl chloride. The acid chloride **36** was dried on high vacuum to remove residual benzene. To a two-neck, 50 mL round-bottom flask equipped with a reflux condenser and magnetic stir-bar was added **24** (2.55 g, 12.6 mmol), followed by a solution of crude acid chloride **36** (10.9 mmol) in DCM (15 mL). Aluminum chloride (4.0 g, 30 mmol) was added to the reaction solution at room temperature slowly, with stirring, and the reaction solution turned from colorless to red accompanied by the evolution of gas and heat. The reaction was stirred for 5 min then heated to reflux for 15 min. The reaction was judged to be complete by TLC, and the solution was poured into an ice solution (50 mL) acidified with a 20% HCl solution (16 mL) and ethyl acetate was added (13 mL). The aqueous and organic layers were separated, and the aqueous layer was extracted with ethyl acetate (25 mL, twice). The combined organics were washed with water and brine, dried over sodium sulfate, filtered, and removed in vacuo to give crude **49**. Crude **49** was purified by column chromatography (250 mL SiO_2, hexanes:ethyl acetate 95:5 to 9:1) to give **49** (4.08 g, 91%) as a yellow, crystalline solid, mp 115−116 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.09 (d, J = 8, 1H), 7.82 (d, J = 8, 1H), 7.25 (m, 1H), 3.95 (s, 3H), 2.36 (s, 3H), 1.69 (s, 4H), 1.31 (s, 6H), 1.20 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 194.8, 165.5, 149.6, 148.2, 142.5, 141.7, 135.4, 134.2, 133.3, 130.7, 130.2, 128.9, 125.6, 53.7, 35.0, 34.9, 34.7, 34.1, 31.9, 31.7, 20.3; LC-APCI-MS (M + H)+ calcd for $C_{24}H_{28}NO_5$ 410.1967, found 410.1959.

Methyl 4-(1-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)-2-nitrobenzoate (53). Compound **53** was synthesized following the representative procedure for the synthesis of compound **30**. To a 100 mL round-bottom flask charged with **52** (0.895 g, 2.19 mmol) and dry THF (5 mL) at room temperature was slowly added by equal parts separated by 45 min a triphenylphosphonium methylide solution prepared as follows: methyltriphenylphosphonium bromide (1.7 g, 4.76 mmol) suspended in dry THF (16 mL) in a 100 mL round-bottom flask equipped with a stir-bar was stirred for 30 min at room temperature after the addition of a 2.5 M solution of *n*-butyl lithium in hexanes (1.86 mL, 4.65 mmol), which provided a homogeneous dark-yellow ylide solution. The reaction was monitored by TLC, and when the reaction was judged to be complete, the reaction solution was poured into water (70 mL) and the aqueous solution was extracted with ethyl acetate (70 mL, twice). The combined organic extracts were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **53**, which was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 97.5:2.5) to give **53** (0.10 g, 11%) as an off-white solid.: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.68 (d, J = 8, 1H), 7.50 (d, J = 7.6,

1H), 7.10 (m, 2H), 5.87 (s, 1H), 5.43 (s, 1H), 3.90 (s, 3H), 1.95 (s, 3H), 1.70 (s, 4H), 1.30 (s, 6H), 1.27 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.5, 149.0, 147.1, 145.4, 145.0, 142.7, 136.5, 132.4, 130.3, 130.1, 128.4, 128.0, 125.2, 121.5, 118.8, 104.7, 53.1, 35.1, 35.0, 34.0, 33.9, 31.9, 31.8, 19.9. LC-APCI-MS (M + H)+ calcd for $C_{25}H_{30}NO_4$ 408.2175, found 408.2169.

4-(1-(1,2,3,4-Tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)-2-nitrobenzoic Acid (16). Compound **16** was synthesized following the representative procedure for the synthesis of compound **12**. To a 100 mL round-bottom flask charged with **53** (0.099 g, 0.24 mmol) and methanol (5 mL) was added a 5 M aqueous solution of potassium hydroxide (0.12 mL, 0.61 mmol). A reflux condenser was fitted to the round-bottom flask, and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (15 mL). The aqueous solution was extracted with ethyl acetate (50 mL, twice) and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **16**. Crude **16** was purified by column chromatography (25 mL SiO_2, ethyl acetate) to give **16** (0.077 g, 80%) as an off-white crystalline solid, mp 212−214 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (br s, 1H), 7.86 (d, J = 8, 1H), 7.71 (s, 1H), 7.52 (d, J = 8, 1H), 7.12 (s, 1H), 7.11 (s, 1H), 5.92 (s, 1H), 5.47 (s, 1H), 1.98 (s, 3H), 1.72 (s, 4H), 1.32 (s, 6H), 1.29 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 169.9, 150.1, 147.3, 146.8, 145.4, 143.0, 136.7, 132.7, 131.2, 130.1, 128.7, 128.3, 123.6, 121.7, 119.4, 35.4, 35.3, 34.3, 34.2, 32.1, 32.0, 20.2. LC-APCI-MS (M − H)− calcd for $C_{24}H_{26}NO_4$ 392.1862, found 392.1872. Anal. Calcd for $C_{24}H_{27}O_4N$: C 73.26; H 6.92; N 3.56. Found: C 72.92; H 6.71; N 3.76.

4-(1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)-2-nitrobenzoic Acid (17). Compound **17** was synthesized following the representative procedure for the synthesis of compound **13**. To a 100 mL round-bottom flask charged with **52** (0.30 g, 0.73 mmol) and methanol (5 mL) was added a 5 M aqueous solution of potassium hydroxide (0.37 mL, 1.85 mmol). A reflux condenser was fitted to the round-bottom flask, and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (20 mL). The aqueous solution was extracted with ethyl acetate (25 mL, twice) and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **17**. Crude **17** was purified by column chromatography (25 mL SiO_2, ethyl acetate) to give **17** (0.28 g, 97%) as a yellow crystalline solid, mp 167−169 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (br s, 1H), 8.29 (s, 1H), 8.11 (d, J = 7.5, 1H), 7.97 (d, J = 7.5, 1H), 7.26 (m, 2H), 2.39 (s, 3H), 1.71 (s, 4H), 1.33 (s, 6H), 1.22 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 194.7, 169.2, 149.5, 148.5, 142.4, 142.0, 135.2, 133.7, 133.0, 130.4, 130.0, 129.2, 128.7, 125.2, 34.8, 34.7, 34.4, 33.9, 31.7, 31.5, 31.4, 20.1. Anal. Calcd for $C_{23}H_{25}O_5N$: C 69.86; H 6.37; N 3.54. Found: C 68.35; H 6.75; N 3.34.

Methyl 3-Fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoate (50). Compound **50** was synthesized following the representative procedure for the synthesis of compound **29**. Methyl 4-(chlorocarbonyl)-3-fluorobenzoate (**42**) was synthesized by refluxing 4-(methoxycarbonyl)-2-fluorobenzoic acid (**41**) (1.34 g, 6.76 mmol) in thionyl chloride (12.0 mL, 165 mmol) in a 100 mL one-neck round-bottom flask fitted with a water-cooled reflux condenser. Excess thionyl chloride was removed in vacuo to give crude **42** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness three times to remove residual thionyl chloride. The acid chloride **42** was dried on high vacuum to remove residual benzene. To a two-neck, 50 mL round-bottom flask equipped with a reflux condenser and magnetic stir-bar was added **24** (1.35 g, 6.67 mmol), followed by a solution of crude acid chloride **42** (6.25 mmol) in DCM (15 mL). Aluminum chloride (2.0 g, 15 mmol) was added to the reaction solution at room temperature slowly, with stirring, and the reaction solution turned from colorless to red accompanied by the evolution of gas and heat. The reaction was stirred for 5 min and then heated to reflux for 15 min. The reaction was judged to be complete by TLC, and the solution was poured into an ice solution (25 mL) acidified with a 20% HCl solution (8 mL) and ethyl acetate was added (13 mL). The aqueous and organic layers were separated, and the aqueous layer was extracted with ethyl acetate (15 mL, twice). The combined organics were washed with water and brine, dried over sodium sulfate, filtered, and rotevapped to give crude **50**. Crude **50** was purified by column chromatography (250 mL SiO_2, hexanes: ethyl acetate 95:5 to 92.5:7.5) to give **50** (2.50 g, 97%) as a colorless crystalline solid, mp 114−117 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 8.0, 1H), 7.80 (d, J = 10.1, 1H), 7.60 (t, J = 7.4, 1H), 7.32 (s, 1H), 7.20 (s, 1H), 3.96 (s, 3H), 2.51 (s, 3H), 1.67 (s, 4H), 1.29 (s, 6H), 1.14 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ193.9, 165.4, 165.3, 161.1, 158.6, 149.9, 142.2, 136.0, 134.4, 134.3, 134.0, 132.4, 132.3, 131.0, 130.9, 130.3, 129.9, 125.2, 125.1, 117.5, 117.3, 52.6, 34.8, 34.7, 34.4, 33.8, 31.5, 31.4, 20.9. LC-APCI-MS (M + H)+ calcd for $C_{24}H_{28}O_3F$ 383.2023, found 383.2021.

Methyl 3-Fluoro-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoate (54). Compound **54** was synthesized following a slightly modified procedure for the synthesis of compound **30**. To a 20-dram vial containing **50** (0.79 g, 2.07 mmol) and dry THF (3 mL) at room temperature was slowly added a triphenylphosphonium methylide solution prepared as follows: to a 20-dram vial equipped with a Teflon magnetic stir-bar and contining dry THF (2.0 mL) was added ^{i}Pr$_2$NH (0.66 mL, 4.67 mmol) and a 2.5 M solution of *n*-butyl lithium in hexanes (1.7 mL, 4.25 mmol), and the solution was stirred for 30 min at room temperature, at which point methyl triphenylphosphonium bromide (1.13 g, 3.19 mmol) was added and the solution was stirred an additional 20 min to provide a homogeneous dark-yellow ylide solution. The reaction was monitored by TLC, and when the reaction was judged to be complete, the reaction solution was poured into water (50 mL) and the aqueous solution was extracted with ethyl acetate (50 mL, twice). The combined organic extracts were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **54** which was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 97.5:2.5) to give **54** (0.254 g, 32%) as a white solid, mp 107−109 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 10.8, 1H), 7.70 (d, J = 7.8, 1H), 7.15 (s, 1H), 7.10 (t, J = 8.0, 1H), 7.05 (s, 1H), 5.86 (s, 1H), 5.56 (s, 1H), 3.91 (s, 3H), 1.98 (s, 3H), 1.69 (s, 4H), 1.29 (s, 6H), 1.28 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.9, 165.8, 161.0, 158.5, 144.3, 143.5, 142.3, 138.3, 133.8, 133.7, 132.3, 130.7, 130.6, 130.5, 130.4, 128.0, 127.8, 125.0, 124.9, 121.3, 121.2, 117.2, 117.0, 52.2, 35.2, 35.1, 33.9, 33.8, 31.9, 31.8, 19.7. LC-APCI-MS (M + H)+ calcd for $C_{25}H_{30}O_2F$ 381.2230, found 381.2220.

3-Fluoro-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoic Acid (18). Compound **18** was synthesized following the representative procedure for the synthesis of compound **12**. To a 100 mL round-bottom flask charged with **54** (0.2541 g, 0.90 mmol) and methanol (5 mL) was added a 5 M aqueous solution of potassium hydroxide (0.35 mL, 1.74 mmol). A reflux condenser was fitted to the round-bottom flask and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (42 mL). The aqueous solution was extracted with ethyl acetate (50 mL, twice), and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **18**. Crude **18** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **18** (0.24 g, 98%) as a white crystalline solid, mp 188−189 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.80 (br s, 1H), 7.80 (d, J = 9.9, 1H), 7.78 (d, J = 7.0, 1H), 7.15 (s, 1H), 7.13 (t, J = 8.1, 1H), 7.07 (s, 1H), 5.89 (s, 1H), 5.59 (s, 1H), 1.99 (s, 3H), 1.70 (s, 4H), 1.30 (s, 6H), 1.29 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 171.0, 170.9, 161.0, 158.5, 144.4, 143.4, 142.4, 138.2, 134.8, 134.7, 132.3, 130.8, 130.7, 129.5, 129.4, 128.1,

127.8, 125.7, 125.6, 121.7, 121.6, 117.8, 117.6, 35.1, 33.9, 33.8, 31.9, 31.8, 19.7. LC-APCI-MS (M + H)+ calcd for $C_{24}H_{28}O_2F$ 367.2073, found 367.2032. Anal. Calcd for $C_{24}H_{27}O_2F$: C 78.66; H 7.43; F 5.18. Found: C 78.34; H 7.52; F 4.8.

3-Fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoic Acid (19). Compound **19** was synthesized following the representative procedure used to synthesize compound **13**. To a 100 mL round-bottom flask charged with **50** (0.50 g, 1.31 mmol) and methanol (5 mL) was added a 5 M aqueous solution of potassium hydroxide (0.74 mL, 3.71 mmol). A reflux condenser was fitted to the round-bottom flask, and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (20 mL). The aqueous solution was extracted with ethyl acetate (25 mL, twice), and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **19**. Crude **19** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 4:1 to 3:2) to give **19** (0.409 g, 84%) as a white crystalline solid, mp 228−229 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.58 (br s, 1H), 7.88 (d, J = 8.0, 1H), 7.76 (d, J = 10.4, 1H), 7.64 (t, J = 7.4, 1H), 7.32 (s, 1H), 7.28 (s, 1H), 2.40 (s, 3H), 1.61 (s, 4H), 1.26 (s, 6H), 1.09 (s, 6H). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 193.5, 165.6, 160.4, 157.9, 149.4, 141.9, 135.6, 135.5, 135.1, 133.9, 131.4, 131.3, 131.0, 129.7, 129.5, 125.3, 116.9, 116.6, 34.2, 34.1, 33.4, 31.2, 31.1, 20.3. LC-APCI-MS (M + H)+ calcd for $C_{23}H_{26}O_3F$ 369.1866, found 369.1877. Anal. Calcd for $C_{23}H_{25}O_3F$: C 74.98; H 6.84; F 5.16. Found: C 74.92; H 7.21; F 4.9.

Methyl 2-Fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoate (51). Compound **51** was synthesized following the representative procedure for the synthesis of compound **29**. Methyl 4-(chlorocarbonyl)-2-fluorobenzoate (**47**) was synthesized by refluxing 4-(methoxycarbonyl)-3-fluorobenzoic acid (**46**) (1.21 g, 6.11 mmol) in thionyl chloride (12.0 mL, 165 mmol) in a 100 mL one-neck round-bottom flask fitted with a water-cooled reflux condenser. Excess thionyl chloride was removed in vacuo to give crude **47** as an off-white solid, and this solid was dissolved in dry benzene (ca. 20 mL) and evaporated to dryness three times to remove residual thionyl chloride. The acid chloride **47** was dried on high vacuum to remove residual benzene. To a two-neck, 50 mL round-bottom flask equipped with a reflux condenser and magnetic stir-bar was added **24** (1.34 g, 6.61 mmol), followed by a solution of crude acid chloride **47** (6.11 mmol) in DCM (15 mL). Aluminum chloride (2.0 g, 15 mmol) was added to the reaction solution at room temperature slowly, with stirring, and the reaction solution turned from colorless to red accompanied by the evolution of gas and heat. The reaction was stirred for 5 min and then heated to reflux for 15 min. The reaction was judged to be complete by TLC, and the solution was poured into an ice solution (25 mL) acidified with a 20% HCl solution (8 mL) and ethyl acetate was added (13 mL). The aqueous and organic layers were separated, and the aqueous layer was extracted with ethyl acetate (15 mL, twice). The combined organics were washed with water and brine, dried over sodium sulfate, filtered, and concentrated to give crude **51**. Crude **51** was purified by column chromatography (250 mL SiO_2, hexanes: ethyl acetate 95:5 to 92.5:7.5) to give **51** (1.76 g, 75%) as a white, crystalline solid, mp 105−106 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (t, J = 7.4, 1H), 7.59 (d, J = 8.4, 1H), 7.56 (d, J = 11.6, 1H), 7.24 (s, 1H), 7.21 (s, 1H), 3.96 (s, 3H), 2.34 (s, 3H), 1.69 (s, 4H), 1.31 (s, 6H), 1.20 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 196.1, 164.3, 164.2, 162.8, 160.2, 148.8, 143.9, 143.8, 142.0, 134.7, 134.0, 132.1, 129.5, 128.4, 125.3, 125.2, 121.9, 121.8, 118.3, 118.1, 52.6, 34.8, 34.7, 34.3, 33.8, 31.6, 31.5, 20.0. LC-APCI-MS (M + H)+ calcd for $C_{24}H_{28}O_3F$ 383.2023, found 383.2036.

Methyl 2-Fluoro-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoate (55). Compound **55** was synthesized following a representative procedure for the synthesis of compound **54**. To a 20 dram vial containing **51** (0.79 g, 2.07 mmol) and dry THF (3 mL) at room temperature was slowly added a triphenylphosphonium methylide solution prepared as follows: to a 20 dram vial equipped with a Teflon magnetic stir-bar and contining dry THF (2.0 mL) was added ^{i}Pr$_2$NH (0.66 mL, 4.67 mmol) and a 2.5 M solution of *n*-butyl lithium in hexanes (1.7 mL, 4.25 mmol), and the solution was stirred for 30 min at room temperature, at which point methyl triphenylphosphonium bromide (1.13 g, 3.19 mmol) was added and the solution was stirred an additional 20 min to provide a homogeneous dark-yellow ylide solution. The reaction was monitored by TLC, and when the reaction was judged to be complete, the reaction solution was poured into water (50 mL) and the aqueous solution was extracted with ethyl acetate (50 mL, twice). The combined organic extracts were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **55** which was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 97.5:2.5) to give **54** (0.301 g, 38%) as a white solid, mp 130−132 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (t, J = 8.0, 1H), 7.14 (d, J = 8.2, 1H), 7.12 (d, J = 6.0, 1H), 7.02 (d, J = 12.4, 1H), 5.82 (s, 1H), 5.36 (s, 1H), 3.93 (s, 3H), 1.95 (s, 3H), 1.70 (s, 4H), 1.31 (s, 6H), 1.27 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 164.8, 164.7, 163.3, 160.7, 148.1, 147.8, 147.7, 144.6, 142.4, 137.2, 132.6, 132.0, 128.1, 128.0, 122.0, 117.7, 117.1, 117.0, 115.0, 114.8, 52.2, 35.2, 35.1, 33.9, 33.8, 31.9, 31.8, 19.8. LC-APCI-MS (M + H)+ calcd for $C_{25}H_{30}O_2F$ 381.2230, found 381.2254.

2-Fluoro-4-(1-(1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalen-7-yl)vinyl)benzoic Acid (20). Compound **20** was synthesized following the representative procedure for the synthesis of compound **12**. To a 100 mL round-bottom flask charged with **55** (0.25 g, 0.66 mmol) and methanol (5 mL) was added a 5 M aqueous solution of potassium hydroxide (0.35 mL, 1.76 mmol). A reflux condenser was fitted to the round-bottom flask, and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (42 mL). The aqueous solution was extracted with ethyl acetate (50 mL, twice), and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **20**. Crude **20** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **12** (0.24 g, 99%) as a white crystalline solid, mp 212−214 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.12 (br s, 1H), 7.97 (t, J = 7.9, 1H), 7.18 (d, J = 8.3, 1H), 7.11 (s, 1H), 7.10 (s, 1H), 7.06 (d, J = 12.3, 1H), 5.86 (s, 1H), 5.39 (s, 1H), 1.96 (s, 3H), 1.71 (s, 4H), 1.31 (s, 6H), 1.28 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 169.5, 169.4, 164.0, 161.4, 149.0, 148.9, 148.0, 144.7, 142.5, 137.1, 132.7, 132.6, 128.1, 128.0, 122.2, 122.1, 118.1, 115.9, 115.8, 115.1, 114.9, 35.2, 35.1, 34.0, 33.8, 31.9, 31.8, 19.8. LC-APCI-MS (M + H)+ calcd for $C_{24}H_{28}O_2F$ 367.2073, found 367.2097. Anal. Calcd for $C_{24}H_{27}O_2F$: C 78.66; H 7.43; F 5.18. Found: C 78.45; H 7.45; F 5.0.

2-Fluoro-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl)benzoic Acid (21). Compound **21** was synthesized following the representative procedure for the synthesis of compound **13**. To a 100 mL round-bottom flask charged with **51** (0.5065 g, 1.34 mmol) and methanol (5 mL) was added a 5 M aqueous solution of potassium hydroxide (0.73 mL, 3.66 mmol). A reflux condenser was fitted to the round-bottom flask and the reaction solution was refluxed and monitored by TLC. After 1 h at reflux, the reaction solution was cooled to room temperature and quenched with 20% HCl (20 mL). The aqueous solution was extracted with ethyl acetate (25 mL, twice) and the organic extracts were combined, washed with water and brine, dried over sodium sulfate, and concentrated in vacuo to give crude **21**. Crude **21** was purified by column chromatography (25 mL SiO_2, hexanes:ethyl acetate 9:1) to give **21** (0.477 g, 97%) as a white crystalline solid, mp 174−175 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ

11.00 (br s, 1H), 8.14 (t, J = 7.5, 1H), 7.63 (d, J = 9.1, 1H), 7.60 (d, J = 12.3, 1H), 7.27 (s, 1H), 7.22 (s, 1H), 2.36 (s, 3H), 1.70 (s, 4H), 1.32 (s, 6H), 1.22 (s, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 196.1, 168.3, 168.7, 163.5, 160.9, 148.9, 144.9, 144.8, 142.1, 134.8, 133.8, 132.7, 129.6, 128.5, 125.3, 125.2, 120.7, 120.6, 118.5, 118.3, 34.8, 34.7, 34.4, 33.9, 31.6, 31.5, 20.0. LC-APCI-MS (M + H)+ calcd for $C_{23}H_{26}O_3F$ 369.1866, found 369.1867. Anal. Calcd for $C_{23}H_{25}O_3F$: C 74.98; H 6.84; F 5.16. Found: C 74.95; H 6.98; F 5.0.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of all compounds reported in the Experimental Section as well as X-ray data for compounds **12**, **18**, **20**, **49**, **50**, and **51**. The X-ray data can be found at the Cambridge Crystallographic Data Centre under the identification numbers: 738925−73830. This material is available free of charge via the Internet at http://pubs.acs.org.

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