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Article

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# Bis-Michael Acceptors as Novel Probes to Study the Keap1/Nrf2/ARE Pathway

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KEYWORDS. Nrf2, Keap1, Michael acceptor, Nazarov acceptor, antioxidant response element, bis-electrophile, luciferase assay, Oxidative/Electrophilic stress.

ABSTRACT. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a master regulator that promotes the transcription of cytoprotective genes in response to oxidative/electrophilic stress. Various Michael–type compounds were designed and synthesized and their potency to activate the Keap1/Nrf2/ARE pathway was evaluated. Compounds bearing two Michael–type acceptors

proved to be the most active. Tether length and rigidity between the acceptors was crucial. This study will help to understand how this feature disrupts the interaction between Keap1 and Nrf2.

## INTRODUCTION

Oxidative/electrophilic stress (OES) is the consequence of an abnormal production of reactive oxygen and nitrogen species and/or electrophiles in the cells.[1] OES stress is linked to over 200 diseases and conditions such as cancers, diabetes, pulmonary infections and neurodegenerative disorders. The Keap1/Nrf2/ARE pathway plays a central role in the cellular response to OES since the Antioxidant Response Element (ARE) is an essential component of the cellular antioxidant defense and is under the transcriptional control of Nuclear factor erythroid 2-related factor 2 (Nrf2).[2–4]

Because Kelch like ECH Associated Protein 1 (Keap1) regulates Nrf2-mediated transcription, the former is recognized as a therapeutic target with high potential. Indeed, the genes targeted by the Keap1/Nrf2/ARE pathway are involved in the synthesis and conjugation of glutathione and antioxidant proteins and enzymes.[5] Understanding the Keap1/Nrf2/ARE pathway has thus become extremely important and the focus of drug discovery programs recently initiated by several research groups.[6–18]

In the basal state, Nrf2 is sequestered in the cytosol via its interaction with a Keap1 dimer. When this Keap1-Nrf2 complex binds to the Keap1-dependent E3-ubiquitin ligase (Cullin 3), Nrf2 is then degraded by the proteasome and the Keap1 dimer is free to bind to a newly translated Nrf2. This maintains low concentrations of free Nrf2 in the nucleus and transcription of genes related to ARE at basal levels. When Keap1 is targeted by small molecules, the conformation of the Keap1

dimer is modified and its interactions with Nrf2 are tighter. Accordingly, the ubiquitination of Nrf2 is stopped and newly synthesized Nrf2 can accumulate in the nucleus and turn on the expression of cytoprotective genes. According to Dinkova-Kostova *et al.*, this mode of action is proper to electrophilic inducers.[19]

Keap1 comprises five domains: 1) the N-terminal region, 2) the Broad complex, Tramtrack, Bric-a-Brac (BTB) domain (exposing Cys151, responsible for the formation of the Keap1 dimer), 3) the Intervening region (IVR, exposing Cys273, Cys288, and 6 others), 4) the Kelch repeat domain (responsible for the recognition of Nrf2), and 5) the C-terminal region.[20] Among strategies used to disrupt the Keap1-Nrf2 interaction, non-covalent protein-protein interaction inhibitors have recently been reported.[6–10] Several crystal structures reveal that these molecules target the binding site of Nrf2 on Keap1 (Kelch repeat domain).[7–9] Another strategy consists of using electrophiles from synthetic or natural sources.[11–18] The latter proved particularly successful (Figure 1) and are believed to target some of the cysteine (Cys) residues of Keap1. Among them, Cys151 (BTB domain) as well as Cys273 and Cys288 (IVR domain) appear to be the most sensitive to electrophiles.[21]

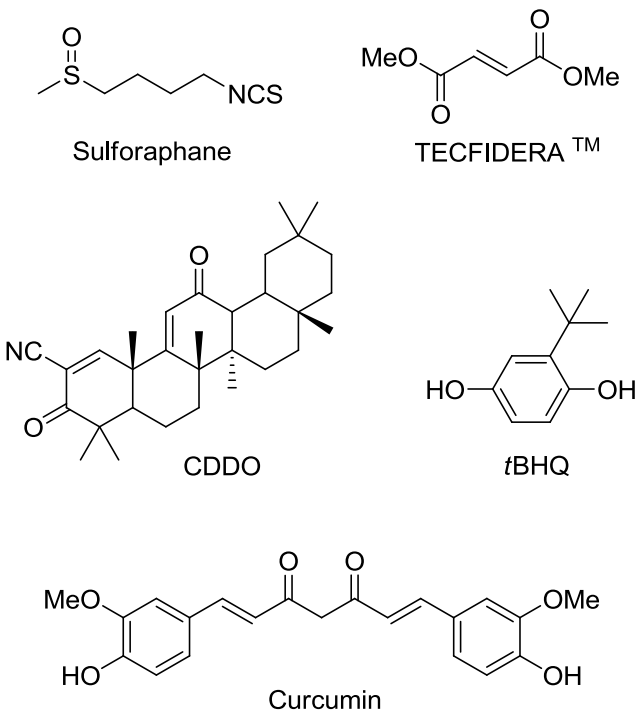



**Figure 1**. Electrophilic modulators of the Keap1/Nrf2/ARE pathway.

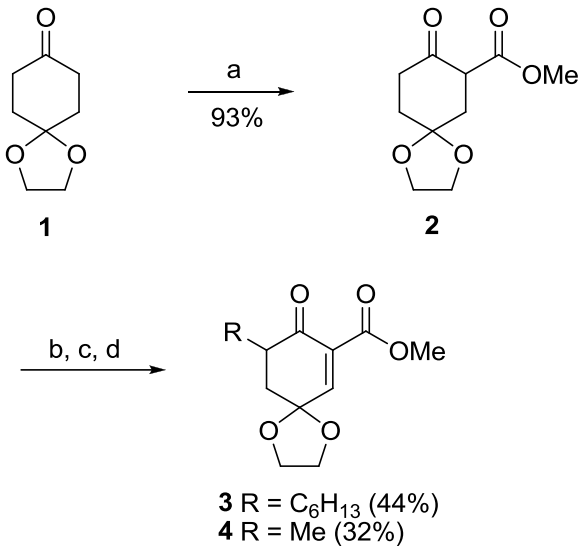
Despite potential toxicity issues associated with off-target interactions, covalent drugs nonetheless offer certain advantages over traditional drugs such as low administration doses and frequency, selectivity, and potentially longer action times.[22,23] Most of the time, covalent drugs are designed in a way that a warhead reacts irreversibly with the targeted host. This renders off-target interactions hard to circumvent except by modulating the reactivity of the warhead. We decided to tackle this issue in a completely different way: since Keap1 offers a unique profile with its multi-cysteine domains, we rather opted to design new agonists bearing two moderately reactive reversible cysteine acceptors. Dual cysteine trapping should significantly increase the target residence time: once the first cysteine is anchored, trapping of a second cysteine becomes a kinetically favored intramolecular process that should also be thermodynamically beneficial due

to enthalpy gain and weak entropy variation. Proper distance and/or nature of the acceptors, combined with traditional secondary non-covalent interactions, should provide selectivity to either the BTB or the IVR domains for these new agonists. Herein, we report the synthesis of series of single and double Michael-type acceptor compounds and their biological evaluation toward the Keap1/Nrf2/ARE pathway. For the first time, a systematic study of the effect of the length and rigidity of the tether between the Michael acceptors, as well as the effect of the electrophilicity of these acceptors offers new insight into how this therapeutic target reacts to multi-electrophile compounds.[24]

## SYNTHESIS

**Single-electrophilic compounds.** α,β'-Unsaturated β-dicarbonyle compounds were synthesized in three to four steps from the corresponding ketones. First, the methoxycarbonylation of monoprotected cyclohexane-1,4-dione **1** gave the β-ketoester **2** (Scheme 1). Upon deprotonation with two equivalents of LDA, the resulting dianion was reacted with a series of electrophiles.[25] Finally, selenation and oxidative elimination[26] generated the Michael acceptors **3** and **4**.[27,28]

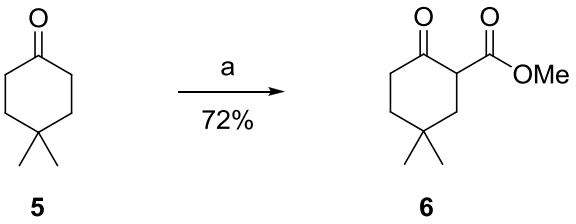
**Scheme 1**[α]



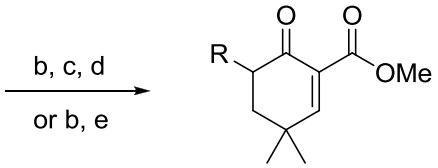

[α]Reagents and conditions: (a) NaH, $Me_2CO_3$, reflux; (b) LDA, R-I, rt; (c) PhSeBr, pyridine, $CH_2Cl_2$, 0 °C; (d) $H_2O_2$, $CH_2Cl_2$, 0 °C.

In order to vary the lipophilicity of the compounds, the same sequence was applied to the gem-dimethyl analog **5** (Scheme 2). For β-ketoesters bearing an unsaturation (cf. compounds **10** and **12**), final oxidation gave best results using DDQ.

**Scheme 2**[α]




**5** **6**




**7** R = H (57%)
**8** R = $C_6H_{13}$ (34%)
**9** R = Me (39%)
**10** R = allyl (39%)
**11** R = Bn (40%)
**12** R = propargyl (34%)
**13** R = $CH_2CF_3$ (7%)
**14** R = $CH_2CO_2tBu$ (19%)
**15** R = $CH_2CN$ (9%)
**16** R = $CH_2C(O)Me$ (4%)
**17** R = $(CH_2)_2CO_2Me$ (16%)

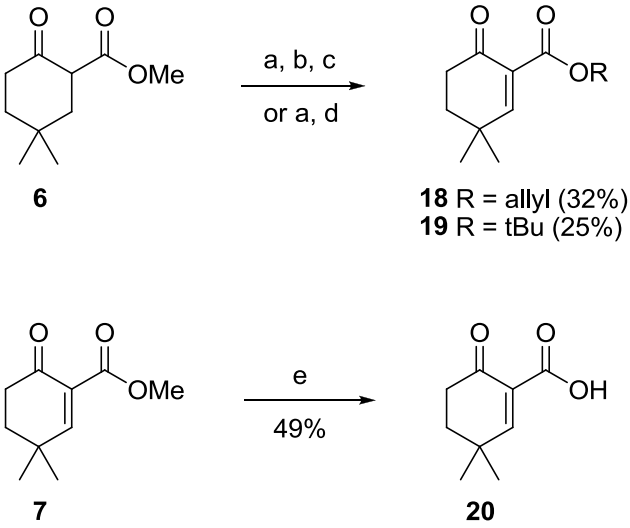
[α]Reagents and conditions: (a) NaH, KH, $Me_2CO_3$, THF, reflux; (b) LDA, R-X, rt; (c) PhSeBr, pyridine, $CH_2Cl_2$, 0 °C; (d) $H_2O_2$, $CH_2Cl_2$, 0 °C; (e) DDQ, 1,4-dioxane, rt.

To study the influence of the ester portion, transesterification[29] of **6** using allyl alcohol or *tert*-butanol as the solvent gave mixtures of esters. After oxidation, **7** and **18** or **7** and **19**, respectively, could be isolated (Scheme 3). We also prepared the corresponding acid **20** by hydrolysis of unsaturated keto-ester **7**.[13]

To test the influence of the electrophilicity of the Michael acceptor, several variations were made (Scheme 4). First, a reduction of **7** in Luche conditions afforded the alcohol **21**.[30] Chloro- and iodoenones **23** and **24** were prepared using known procedures.[31,32] Morita-Baylis-Hillman reaction on **22** with paraformaldehyde gave the allylic alcohol **25**.[33] Further transesterification afforded **26**.[34] Classic methoxycarbonylation of enone **22** provided keto-ester **27** which, upon α-

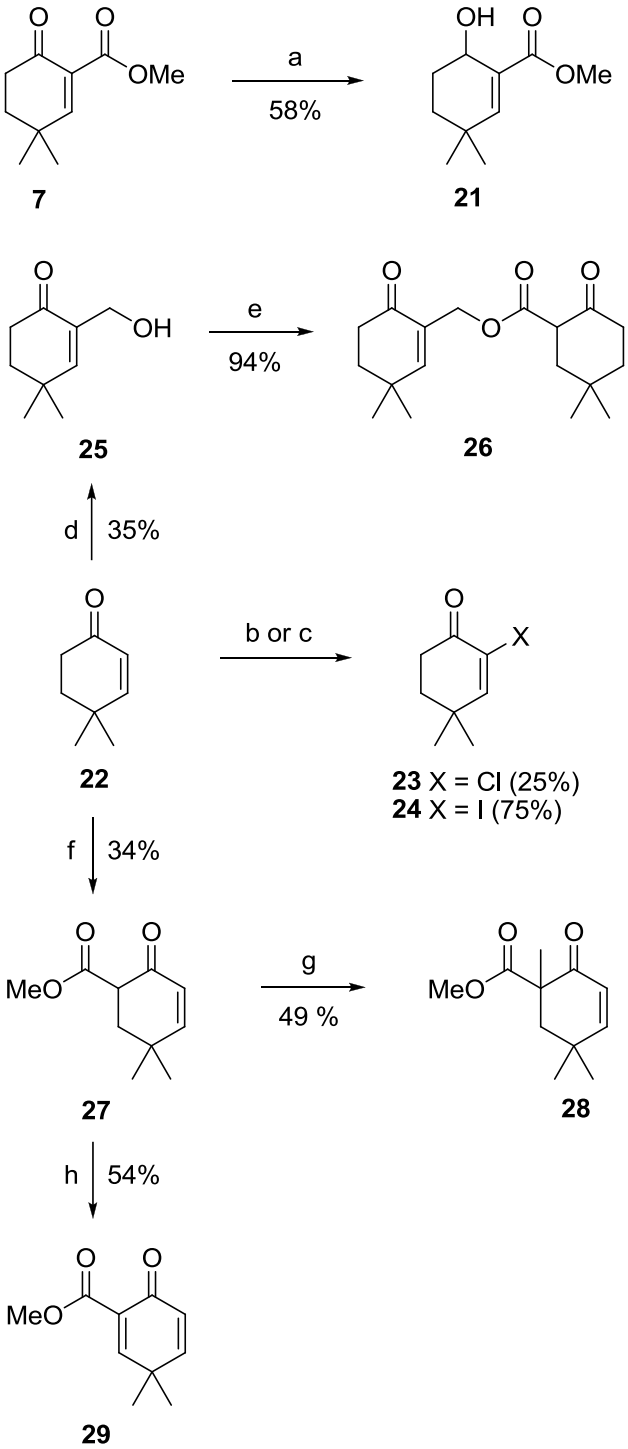
methylation, afforded **28** as a racemic mixture.[35] On the other hand, oxidation of keto-ester **27** by selenium dioxide gave dienone **29**.[36]

**Scheme 3**[α]




[α]Reagents and conditions: (a) DMAP, 4 Å MS, ROH, reflux; (b) PhSeBr, Pyridine, $CH_2Cl_2$, 0 °C; (c) $H_2O_2$, $CH_2Cl_2$, 0 °C; (d) $K_2CO_3$, DDQ, 1,4-dioxane, rt; (e) KOH, $H_2O$/MeOH, reflux.

**Scheme 4**[α]

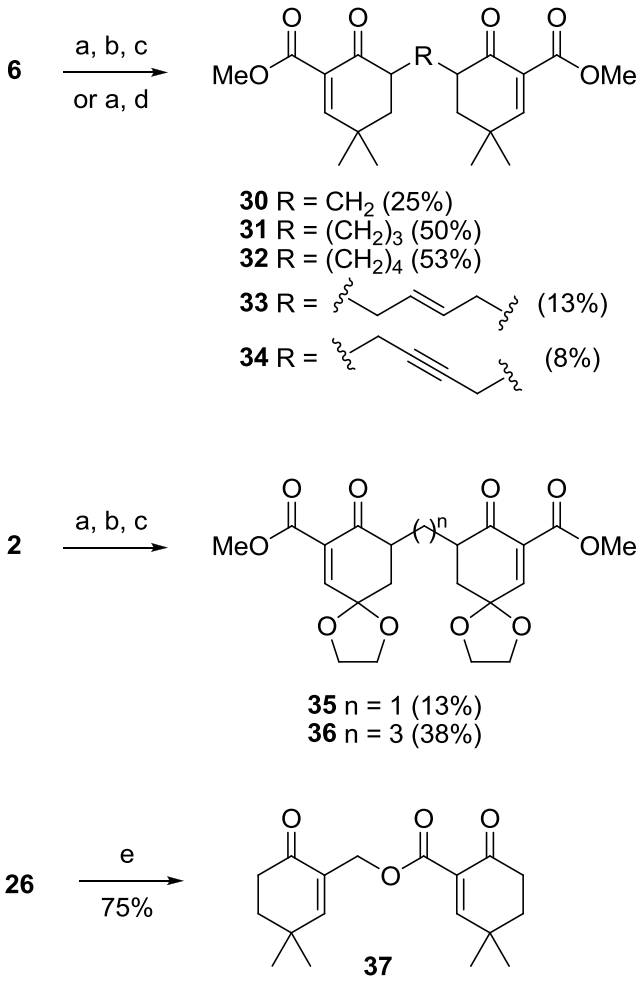



[α]Reagents and conditions: (a) $CeCl_3$, $NaBH_4$, MeOH, rt; (b) $PhI(OAc)_2$, pyridine·HCl, $CH_2Cl_2$, rt; (c) $I_2$, pyridine, $CCl_4$, rt; (d) Paraformaldehyde, $NaHCO_3$, imidazole, THF, rt; (e) **6**, $Et_3N$,

toluene, Dean-Stark; (f) NaH, $Me_2CO_3$, 1,4-dioxane, reflux; (g) MeI, $K_2CO_3$, acetone, 40 °C; (h) $SeO_2$, AcOH, *t*-BuOH, reflux.

**Bis-electrophilic compounds.** In order to attach two Michael acceptors on the same molecule and assess the influence of rigidity on activity, we used tethers of different length (1-4 carbons, **31**-**32**) and rigidity (alkane, alkene **33**, and alkyne **34**, Scheme 5). These tethers were incorporated by bis-alkylation with the dianion of **6**. No significant amount of single alkylation bi-products was observed. The ensuing tandem oxidations afforded the desired products **30**-**34**, albeit in low yield. A series of compounds bearing a dioxolane instead of the gem dimethyl was also prepared for comparison (**35**, **36**). Finally, unsymmetrical bis-electrophilic compound **37** was obtained by oxidation of **26** with DDQ.

**Scheme 5**[α]




[α]Reagents and conditions: (a) LDA, R-X, rt; (b) PhSeBr, pyridine, $CH_2Cl_2$, 0 °C; (c) $H_2O_2$, $CH_2Cl_2$, 0 °C; (d) DDQ, 1,4-dioxane, rt; (e) DDQ, $K_2CO_3$, 1,4-dioxane, rt.

## RESULTS AND DISCUSSION

**ARE inducing activity and cytotoxicity.** To evaluate the ARE-inducing activity, all compounds, at concentrations ranging from 0 to 100 μM, were incubated with ARE-Luc-transfected human embryonic kidney cells (HEK-293T) for 24 h. Suforaphane (10 μM) was used as a positive control. Also, NAD(P)H:quinone oxidoreductase (NQO1) induction was measured by quantitative polymerase chain reaction (qPCR) in RAW 264.7 cells using sulforaphane as a

positive control. In addition, cytotoxicity was evaluated for each molecule in a lactate dehydrogenase (LDH) assay in HEK-293T cells. The data are reported as Mean ± SEM based on three separated experiments.

**Lipophilicity as an off-target indicator.** Compounds lacking any substitution at the C4 position (see **7**, with two hydrogens instead of gem-dimethyl) were too electrophilic. Difficulties in their isolation and purification compromised the validity of their evaluation.

To counterbalance for the level of reactivity of these electrophiles, steric hindrance around C4 was increased. We chose commercially available ketone **1** as a starting point for comparison. Analogues with a dioxolane moiety (**3**, **4**, **35** and **36**) showed moderate activity and significant toxicity at the higher concentrations. (Figure 2).

We hypothesized that toxicity was due to the hydrophilicity associated to the dioxolane moiety.[14,37] To test this hypothesis, we increased the compounds' lipophilicity. Replacing the dioxolane with a gem-dimethyl provided an increased activity with no toxicity even at high concentrations (e.g., **7**). Further exploration of the substitution at C6 was essential to generate unambiguous biological data (Table 1). The single electrophiles showed better activities at 100 μM (compounds **7**–**12**), with no apparent toxicity. They were much more lipophilic than their dioxolane analogues according to their clogP values, which tends to support our hypothesis.

**Importance of electrophilicity.** In order to induce the transcription of genes controlled by ARE, a first cysteine nucleophilic addition to the electrophilic compounds has to occur. Analogs **7** – **12** suggest that unsaturated keto esters are indeed suitable electrophiles to study Keap1 reactivity. Replacement of the methyl ester by larger groups (allyl **18** or *t*-Bu **19**) gave a slight increase in activity, although accompanied with increased toxicity, whereas the corresponding acid (**20**) retained most of the activity. Replacement of the methyl ester by a chloride (**23**) had no significant

effect, but the iodide derivative (**24**) was not reactive enough. This effect was also observed with other poor electrophiles, such as those lacking the ketone (**21**) or the ester (**25**, **26**, and **28**), with the exception of compound **27**. This comes as a surprise because it is hard to evaluate the combined participation of the carbonyls in **27** while we expected **29** to be much more potent.[38]

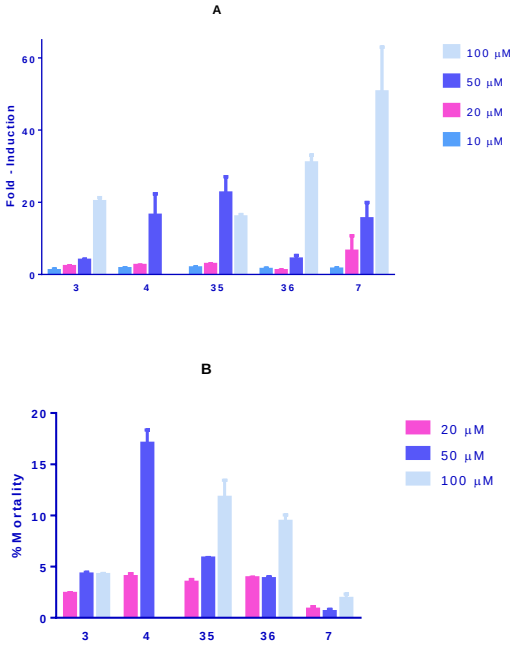


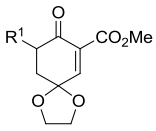
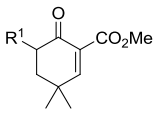

**Figure 2**. (A) Fold induction of ARE-luciferase gene expression; (B) % cell mortality induced by compounds **3**, **4**, **35**, **36** and **7** at 10, 20, 50 and 100 µM

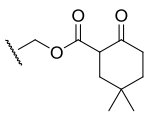
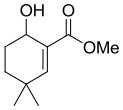
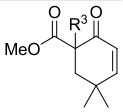
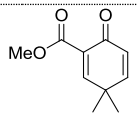
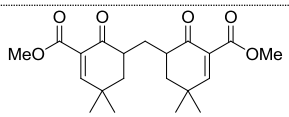
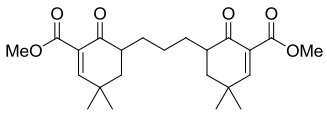
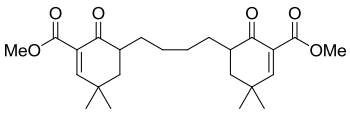
**Boost in activity with a second Michael acceptor.** In order to take advantage of the numerous cysteine residues of Keap1,[39] we tested compounds with two Michael-type acceptors. Right away, this second class of molecules turned out to be very potent (**30**, **31**, and **38**), with an astonishing 167-fold increase from **7** to **31** at 10 µM. Even though solubility and toxicity became an issue at higher concentrations, the study of similar compounds provided new insights about the target.

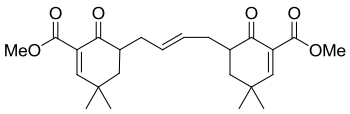
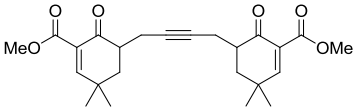
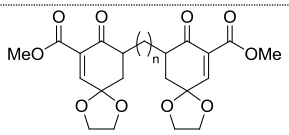
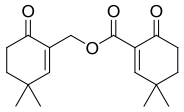
First, the addition of a second unsaturated keto-ester enhanced the fold-induction drastically. Second, the spatial position between the two electrophilic parts is important to modulate activity. Indeed, going from a simple methylene (**30**) to a propane linker (**31**) resulted in a ca. 70-fold increase (at 20 μM), whereas an additional factor 2 was gained with a butane tether (**32**).

So far, it was not clear whether the tether length optimization reflected an optimal distance between the two Michael acceptors for reaction with two cysteine residues in the binding site, or the result of a gain in hydrophobic interactions. To answer this, we decided to rigidify the tether. From butane (**32**) to *E*-butene (**33**) to butyne (**34**) tethers, increased rigidity resulted in a significant increase in activity, with compound **34** being the best compound so far (50-fold-induction at only 1 μM). These results strongly suggest an optimal distance and conformation between the Michael acceptors for interaction with Keap1.

**Table 1**. Fold-induction of ARE-luciferase gene expression[α]

| Compound | | Fold-induction at: [β] | | | clogP[γ] |
|---|---|---|---|---|---|
| | | 1 μM | 20 μM | 100 μM | |
| **3**  | $R^1$ = Hex | 1.40 ± 0.25 | 2.19 ± 0.19 | 20.19 ± 1.10 | 2.49 |
| **4** | $R^1$ = Me | 0.98 ± 0.04 | 2.49 ± 0.08 | N/A | -0.16 |
| **7** | $R^1$ = H | 1.36 ± 0.34 | 6.46 ± 4.26 | 50.58 ± 12.39 | 1.82 |
| **8** | $R^1$ = Hex | 1.15 ± 0.04 | 2.00 ± 0.43 | 64.65 ± 14.31 | 4.98 |
| **9**  | $R^1$ = Me | 1.17 ± 0.11 | 2.75 ± 0.22 | 4.60 ± 0.10 | 2.34 |
| **10** | $R^1$ = allyl | 1.34 ± 0.51 | 2.05 ± 0.06 | 25.32 ± 4.03 | 2.91 |
| **11** | $R^1$ = Bn | 0.95 ± 0.12 | 0.92 ± 0.04 | 48.53 ± 14.74 | 3.91 |
| **12** | $R^1$ = propargyl | 1.14 ± 0.12 | 1.48 ± 0.44 | 34.16 ± 2.62 (7%) | 2.39 |

| | | | | | |
|---|---|---|---|---|---|
| **13** | $R^1 = CH_2CF_3$ | 0.54 ± 0.07 | 1.54 ± 0.22 | 17.90 ± 6.18 (11%) | 2.58 |
| **14** | $R^1 = CH_2CO_2t$-Bu | 0.65 ± 0.09 | 2.00 ± 0.17 | 1.75 ± 0.18 | 3.26 |
| **15** | $R^1 = CH_2CN$ | 1.03 ± 0.39 | 2.22 ± 1.13 | 17.13 ± 2.52 | 1.40 |
| **16** | $R^1 = CH_2C(O)Me$ | 0.49 ± 0.06 | 2.20 ± 0.07 | 8.71 ± 1.01 | 1.72 |
| **17** | $R^1 = (CH_2)_2CO_2Me$ | 0.68 ± 0.08 | 1.24 ± 0.09 | 5.96 ± 1.17 | 2.30 |
| **18** | $R^2 = CO_2All$ | 1.39 ± 0.27 | 4.36 ± 0.50 | 94.79 ± 22.67 (17%) | 2.59 |
| **19** | $R^2 = CO_2t$-Bu | 1.05 ± 0.05 | 3.89 ± 0.39 | 106.40 ± 20.32 (16%) | 3.06 |
| **20** | $R^2 = CO_2H$ | 2.00 ± 0.19 | 3.11 ± 0.73 | 29.56 ± 1.28 | 1.39 |
| **23** | $R^2 = Cl$ | 1.33 ± 0.18 | 4.42 ± 0.69 | 43.41 ± 3.32 | 2.34 |
| **24** | $R^2 = I$ | 1.22 ± 0.16 | 1.16 ± 0.23 | 17.35 ± 0.47 | 2.71 |
| **25** | $R^2 = CH_2OH$ | 0.72 ± 0.05 | 0.77 ± 0.17 | 1.46 ± 0.13 | 0.87 |
| **26** | $R^2 =$  | 2.98 ± 0.36 | 3.79 ± 1.10 | 4.02 ± 0.18 | 3.40 |
| **21** |  | 1.00 ± 0.07 | 1.85 ± 0.37 | 1.29 ± 0.34 | 1.91 |
| **27** |  $R^3 = H$ | 1.05 ± 0.11 | 3.20 ± 0.35 | 41.93 ± 7.94 | 1.43 |
| **28** | $R^3 = Me$ | 1.47 ± 0.13 | 2.43 ± 0.09 | 4.56 ± 0.41 | 1.95 |
| **29** |  | 1.35 ± 0.21 | 3.39 ± 0.41 | 10.32 ± 0.72 | 1.96 |
| **30** |  | 4.49 ± 0.43 | 3.60 ± 0.48 | 101.10 ± 15.76 (24%) | 3.79 |
| **31** |  | 4.71 ± 1.11 | 247.70 ± 17.80 | Not tested | 4.51 |
| **32** |  | 8.23 ± 0.38 | Not tested | Not tested | 5.04 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **33** |  | | 15.20 ± 0.79 | Not tested | Not tested | 4.56 |
| **34** |  | | 52.46 ± 3.01 | Not tested | Not tested | 4.38 |
| **35** |  | n = 1 | 0.94 ± 0.03 | 2.76 ± 0.15 | 15.94 ± 0.67 (12 %) | -1.20 |
| **36** | | n = 3 | 1.00 ± 0.04 | 1.05 ± 0.15 | 30.91 ± 2.14 (9%) | -0.48 |
| **37** |  | | 2.02 ± 0.40 | 3.78 ± 0.56 | 121.1 ± 20.60 (15%) | 3.28 |

[α] Sulforaphane has a fold-induction of 9.80 ± 1.00 at 10 μM. [β] % Mortality evaluated in the LDH assay are reported when over 5 %. [γ] Predicted values by ChemDraw 12.0.

These results raise the question whether the second Michael acceptor on **30**-**34** acts as a cysteine trap. It turns out that the Michael acceptors on these molecules are similar to the α,β'-unsaturated β-cyanoketone of CDDO, which was co-crystallized in the BTB domain of Keap1.[20] We have thus docked our molecules in the crystal structure and set the distance between the nucleophilic sulfur and the electrophilic carbon to 1.8 Å as in CDDO. Interestingly, there is no other cysteine residue in the active site of the BTB domain that could act as a partner for a second nucleophilic addition. In return, the ketone and the ester functions were able to make hydrogen bonds with other residues in the active site and within the electronic density area. Hence, in the absence of a crystal of Keap1 with any of compounds **30**-**34**, we cannot answer the question on the nature of the interaction of the second Michael acceptor with Keap1. As suggested by docking, it could be a H-bond in the BTB domain, but it could also be that these molecules rather target the IVR region. Unfortunately, this region of Keap1 has not been crystallized yet and we cannot answer this hypothesis clearly at this point.

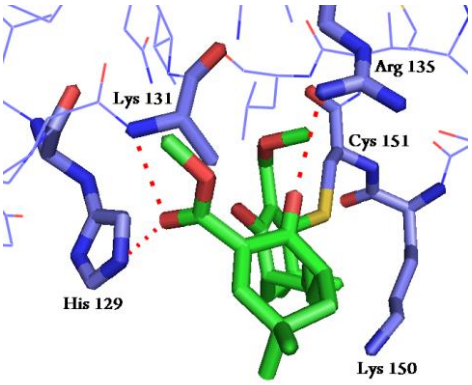



**Figure 3**. Docking of **31** in BTB domain of Keap1 using PyMol and NRGsuite

**Added functionality reduces activity.** In order to prove whether only an H-bond acceptor is needed in addition to the electrophile, we installed different chemical functions (**13** – **17**). None of these compounds showed activity. Even **17** is particularly inactive, although it presents a distance between the ester side chain and the Michael-type acceptor that resembles the distance between Michael-type acceptors in **30**. Having H-bond acceptors on the side chain (**14**-**17**) is even more detrimental than simple alkyl side chains (**8**, **10**-**12**), suggesting that this part of the molecule also plays a role in lipophilic interactions with the active site.

**NQO1 expression.** Michael-type acceptors are listed as pan assay interference compounds (PAINs).[40] In order to address this eventuality in the case of the present class of molecules, we measured the expression of NQO1, a Nrf2 positively regulated gene, by qPCR in RAW 264.7 cells for sulforaphane and 14 compounds of interest at 10 μM (supporting information, Table S1).[5,11,13,15,18] We observed the same tendencies as those observed in the luciferase ARE reported assay (Figure 4). Indeed, an aliphatic side chain (**8**) is preferred over a functionalized one (**17**), the addition of a Michael acceptor enhances activity (cf. **8** and **32**), and an increased rigidity between

Michael acceptors is beneficial (cf. **32**, **33**, and **34**). This confirms that the observed activity is indeed dependent on the Nrf2 pathway, and not on a promiscuous activity. Moreover, these Michael acceptors are much more potent than the naturally occurring sulforaphane.

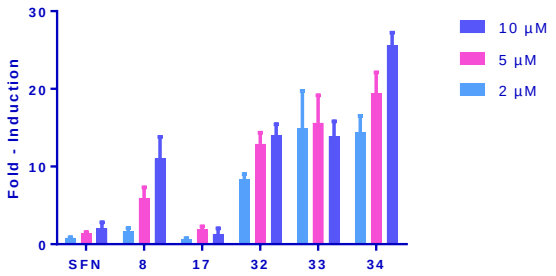



**Figure 4.** Fold induction of NQO1 expression by sulforaphane (SFN), compounds **8**, **17**, **27**, **32**, **33** and **34** at 2, 5 and 10 µM

## CONCLUSION

In this study, we report herein the synthesis and biological evaluations of 32 Michael acceptors toward the Keap1/Nrf2/ARE pathway for the transcription of antioxidant cellular responses. This study gives several insights on how reversible covalent modulators interact with Keap1. The electrophilic part of the molecule has to be sufficiently lipophilic to show some activity. Furthermore, the electrophile has to be powerful enough to react with the targeted cysteine, an essential element in the design of Keap1 ligands to stop the ubiquitination and subsequent degradation of Nrf2. The 1-methoxycarbonyl-3,3-dimethylcyclohex-2-enone motif answered these two criteria. Addition of a second Michael acceptor had a crucial impact on activity, up to a 50-fold-induction at only 1 µM. The distance between the Michael acceptors as well as the rigidity of the tether were optimized to generate our best compound (**34**) in this family.

Our results suggest that the role of the second Michael acceptor is unlikely to be a hydrogen bond acceptor as none of the compounds with heteroatoms at the end of the side chain showed activity. We could hypothesize that the second Michael acceptor rather serves as a second cysteine trap. This remains to be proven, but the consequences of such a hypothesis, should it turn out to be true, are of high importance. Indeed, trapping of several cysteine residues would imply that the BTB domain is not the target, but probably the IVR region of Keap1.

We believe our study will serve to increase the understanding of this target toward the generation of new drug leads. Our data also highlight the importance to design appropriate electrophiles toward covalent drug. Investigations on the nature and exact role of the second electrophile are in progress and the results will be published in due course.

## EXPERIMENTAL SECTION

**Biology. Cell lines**. Human Embryonic Kidney 293 cells (HEK-293T; ATCC CRL-1573) and the murine macrophage cell line (RAW 264.7; ATCC TIB-71) were obtained from American Type Culture Collection (Manassas, VA). Cells were cultured using standard methods at 37°C in 5% $CO_2$ – 95% air in Dulbecco's modified eagle medium (DMEM) (Wisent, Canada) supplemented with 10% fetal bovine serum (FBS), antibiotics (streptomycin at 100 mg/ml and penicillin at 100 U/ml), 5% sodium pyruvate, 5% nonessential amino acids, and 2 mM L-glutamine (complete DMEM).

**Luciferase assays.** The day before transfection, Human Embryonic Kidney HEK 293T cells ($1 \times 10^5$) were plated in 24-well tissue culture-treated plates. Cells were transiently transfected with 0.1 μg of ARE-luc (Nrf2 response element) plasmid using FuGENE6 transfection reagent (Promega, Madison, WI; E2691). The day after transfection, cells were treated with Keap1 ligands

at different concentrations or with sulforaphane at 10 μM. After 24 h, cell lysates were than assayed for luciferase activity using the luciferase assay system from Promega (E1500).

**Real-time PCR analyses.** RAW 264.7 cells were treated with 2, 5 and 10 μM of different compounds or with 10 μM of sulforaphane for 5 hours. Total RNA was then extracted using Ribozol reagent according to the manufacturer's protocol. A total of 500 ng of the resulting RNA was then reverse transcribed using iScript reverse transcription supermix for the reverse transcriptase quantitative PCR (RTqPCR) kit (Bio-Rad). Real-time PCRs were performed with the iQ SYBR green supermix (Bio-Rad). Amplification plots were generated using the Rotorgene 6000 Application software version 1.7 (Corbett Research), and fold induction was calculated using the threshold cycle ($2^{\Delta\Delta Ct}$) method and using 18S expression for normalization. Primer sequences are:

Human/Mouse 18S:

FW: 5′-AGGAATTGACGGAAGGGCAC-3′

RV: 5′-GGACATCTAAGGGCATCACA-3′

Mouse NQO1:

FW: 5′-TCACAGGTGAGCTGAAGGAC-3′

RV: 5′-CTTCCAGCTTCTTGTGTTCG -3′

**Cell viability assay.** The cytotoxicity of Keap1 ligands on cells was determined using the LDH assay. Briefly, HEK 293T cells were treated with increasing concentrations of Keap1 ligands. After 24 hours, supernatants were collected and cells were lysed in triton 1%. To measure lactate dehydrogenase (LDH) activity, buffer containing L-(+)-Lactic acid and Nicotinamide adenine dinucleotide (NAD) (Sigma-Aldrich; L-1750 and N7004 respectively) was added to the supernatant and the cells lysate, followed by measurement of absorbance at 340 nm.

**Chemistry. General**. All reactions requiring anhydrous conditions were conducted in flame-dried glassware under a dry nitrogen or argon atmosphere. THF was distilled from Na and benzophenone under nitrogen immediately prior to use. 1,4-dioxane was distilled from Na and benzophenone under argon and kept on 4 Å molecular sieves. DCM, MeOH, toluene, *i*-$Pr_2NH$, $Et_3N$, and pyridine were distilled from $CaH_2$ under nitrogen at atmospheric pressure immediately prior to use. All other required fine chemicals were used directly without purification. All reference to "water" correspond to deionized water. All references to "brine" refer to a saturated aqueous sodium chloride solution. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. All new compounds showed chemical purity of ≥95% as assessed by $^1$H NMR. All NMR spectra were recorded on an AV300 Bruker (300 MHz for $^1$H and 75 MHz for $^{13}$C) or an AS400 Variant (400 MHz for $^1$H and 100 MHz for $^{13}$C). Chemical shifts are referenced to δ 7.26 signal of $CHCl_3$ ($^1$H NMR) and 77.16 signal of $CDCl_3$ ($^{13}$C NMR) as internal standards for deuterated chloroform and to δ 5.30 signal of $CH_2Cl_2$ ($^1$H NMR) and δ 53.52 signal of $CD_2Cl_2$ ($^{13}$C NMR) as internal standards for deuterated DCM. Data for proton spectra are reported as follows: chemical shift in ppm (multiplicity [singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m)], integration, coupling constants [Hz]). Carbon spectra were recorded with complete proton decoupling and the chemical shifts are reported in ppm. High resolution mass spectrometry data were obtained by the ESI-Q-Tof (maXis). TLC was conducted with pre-coated 60 Å 250 μm silica gel plates with F-254 indicator and visualized using a combination of UV and potassium permanganate staining. Flash column chromatography was performed using silica gel (230–400 mesh). IR spectra were recorded with a FTIR instrument by applying substrates neat.

**Usual Reaction Work-up and Purification**. After addition of the indicated aqueous solution, layers were separated. The aqueous phase was extracted with the indicated solvent, and the

combined organic phases were washed with the indicated aqueous solution (if needed), dried over anh $MgSO_4$, filtered, and concentrated under reduced pressure using a rotary evaporator. The crude material was purified by flash chromatography using silica gel with the indicated eluent.

**Methyl 8-oxo-1,4-dioxaspiro[4.5]decane-7-carboxylate (2).** A solution of **1** (3.0 g, 19.2 mmol) in $Me_2CO_3$ (20 mL) was added dropwise to a suspension of NaH (60% in oil, 1.54 g, 38.4 mmol) in $Me_2CO_3$ (10 mL) at rt. The reaction was heated to reflux for 4 h, then cooled to 0 °C. Saturated aq $NH_4Cl$ (30 mL) was added and the usual work-up (EtOAc; brine) and purification (5-15% EtOAc in hexanes) afforded compound **2** (3.81 g, 93%) as a pale yellow oil. Spectral data was consistent with that previously reported.[41]

**Methyl 9-hexyl-8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate (3).** LDA (2.86 mmol) was prepared by the addition of *n*-BuLi (2.5 M in hexanes, 1.15 mL, 2.86 mmol) to a precooled (–78 °C) solution of diisopropylamine (402 μL, 2.86 mmol) in THF (1.0 mL). The mixture was strirred for 30 min then a solution of **2** (201.3 mg, 0.94 mmol) in THF (0.5 mL + 0.5 mL for rinsing) was added dropwise via canula. The mixture was stirred for 1 h at 0 °C then 1-iodohexane (0.69 mL, 4.68 mmol) was added. The resulting mixture was allowed to warm up to rt and stirred overnight. Water (15 mL) was added and the usual work-up (EtOAc) and purification (silica gel saturated with $Et_3N$, 0 to 5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (158.7 mg confirmed by mass spectrometry) as a yellow oil. A solution of the latter and pyridine (0.12 mL, 1.48 mmol) in $CH_2Cl_2$ (2.0 mL) was added to a solution of PhSeCl (269.9 mg, 1.41 mmol) in $CH_2Cl_2$ (2.0 mL) at rt. The mixture was stirred for 3 h then 1 N aqueous HCl (10 mL) was added. The usual work-up ($CH_2Cl_2$) and purification (10% $Et_2O$ in hexanes) gave the corresponding phenylselanyl-β-ketoester (197.3 mg, 46% over 2 steps) as a yellow oil: $^1$H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.57 (d, 2H, $J$ = 7.3 Hz), 7.40 (t, 1H, $J$ = 7.3 Hz), 7.30 (t, 2H, $J$ = 7.4

Hz), 3.89 (m, 4H), 3.65 (s, 3H), 2.93 (m, 1H), 2.50 (dd, 1H, *J* = 3.6, 13.7 Hz), 2.17 (d, 1H, *J* = 13.7 Hz), 2.01 (m, 1H), 1.88 (m, 1H), 1.79 (t, 1H, *J* = 13.5 Hz), 1.28 (br s, 9H), 0.87 (t, 3H, *J* = 6.7 Hz). $^{13}$C NMR ($CDCl_3$, 100 MHz) δ (ppm) 204.72, 170.07, 138.76, 129.68, 128.77, 126.38, 106.88, 64.93, 64.23, 59.69, 52.74, 46.61, 43.54, 41.75, 31.43, 29.41, 29.20, 26.80, 22.72, 14.22. HRMS (ESI-Q-Tof) calcd for $C_{22}H_{30}O_5SeNa$: 477.1152, found: 477.1152. IR (neat) ν 2952, 2926, 2856, 1713, 1436 cm$^{-1}$. An aqueous $H_2O_2$ solution (30 %, 1.0 mL) was added to a solution of phenylselanyl-β-ketoester (196.1 mg, 0.43 mmol) in $CH_2Cl_2$ (3.0 mL) at rt. The mixture was vigorously stirred for 90 min then water (1 mL) and $CH_2Cl_2$ (3 mL) were added. The usual work-up ($CH_2Cl_2$) afforded pure **3** (122.8 mg, 95%) as a pale yellow oil: $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.06 (d, 1H, *J* = 2.0 Hz), 4.07 (m, 4H), 3.81 (s, 3H), 2.72 (m, 1H), 2.21 (m, 1H), 2.05 (dd, 1H, *J* = 12.3, 13.2 Hz), 1.86 (m, 1H), 1.27 (br s, 9H), 0.87 (t, 3H, *J* = 6.8 Hz). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 196.20, 164.59, 147.61, 132.55, 103.63, 65.31, 64.92, 52.31, 45.48, 38.37, 31.54, 29.14, 28.91, 26.39, 22.46, 13.96. HRMS (ESI-Q-Tof) calcd for $C_{16}H_{24}O_5Na$+MeOH: 351.1778, found: 351.1785. IR (neat) ν 2926, 1746, 1436, 1256, 1083 cm$^{-1}$.

**Methyl 9-methyl-8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate (4).** Following the procedure used to prepare **3**, **2** (503.7 mg, 2.35 mmol) was treated with LDA (4.99 mmol) and iodomethane (0.16 mL, 2.57 mmol) then quenched with saturated aq $NH_4Cl$ (15 mL) and water (2 mL). The usual work-up (EtOAc) and purification (5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (278.5 mg confirmed by mass spectrometry) as a pale yellow oil. The latter was treated with pyridine (0.11 mL, 1.36 mmol) and PhSeBr (319.4 mg, 1.35 mmol) then quenched with 1 N aq HCl (10 mL). The usual work-up ($CH_2Cl_2$; brine) and purification (20% EtOAc in hexanes) gave the corresponding phenylselanyl-β-ketoester (109.2 mg, 32% over 2 steps) as a colorless oil: $^1$H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.58 (d, 2H, *J* = 7.2 Hz), 7.39 (t, 1H, *J* = 7.3

Hz), 7.30 (t, 2H, *J* = 7.5 Hz), 3.85 (m, 4H), 3.64 (s, 3H), 3.06 (m, 1H), 2.50 (dd, 1H, *J* = 3.8, 13.7 Hz), 2.15 (d, 1H, *J* = 13.7 Hz), 1.99 (m, 1H), 1.83 (t, 1H, *J* = 13.4 Hz), 1.13 (d, 3H, *J* = 6.5 Hz). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 170.17, 138.79, 129.71, 128.79, 126.46, 106.79, 64.97, 64.30, 59.43, 52.78, 43.69, 43.51, 40.75, 14.86. HRMS (ESI-Q-Tof) calcd for $C_{17}H_{20}O_5SeNa$: 407.0369, found: 407.0371. IR (neat) ν 2896, 1736, 1709, 1219 $cm^{-1}$. Phenylselanyl-β-ketoester (44.4 mg, 0.12 mmol) was treated with aq $H_2O_2$ (50 wt. %, 0.1 mL) then water (4 mL) and $CH_2Cl_2$ (10 mL) were added. The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$; brine) afforded pure **4** (27.1 mg, quant.) as a pale yellow oil. $^1$H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.07 (d, 1H, *J* = 2.2 Hz), 4.05 (m, 4H), 3.80 (s, 3H), 2.86 (m, 1H), 2.17 (ddd, 1H, *J* = 2.2, 4.8, 13.5 Hz), 2.07 (t, 1H, *J* = 13.5 Hz), 1.16 (d, 3H, *J* = 6.7 Hz). $^{13}$C NMR ($CDCl_3$, 100 MHz) δ (ppm) 196.62, 164.77, 148.17, 132.62, 103.71, 65.52, 65.16, 52.58, 41.27, 40.83, 14.81. HRMS (ESI-Q-Tof) calcd for $C_{11}H_{14}O_5Na$: 249.0733, found: 249.0740. IR (neat): 2955, 2934, 2891, 1744, 1690, 1435, 1251 $cm^{-1}$.

**4,4-Dimethylcyclohexanone (5).** A suspension of Pd/C (5% wt., 729 mg, 0.4 mol%) in ethanol (20 mL) was added to a solution of **22**[42] (11.6 g, 93.4 mmol) in ethanol (60 mL) at rt. The mixture was cooled to 0 °C, the flask was purged with hydrogen and the reaction was stirred at rt for 2 h. Complete conversion was assured by $^1$H NMR. The resulting mixture was filtered on celite and washed with $CH_2Cl_2$. The solvent was removed under vacuum to afford **5** (10.7 g, 91%) as a white solid. Spectral data was consistent with that previously reported.[43]

**Methyl 5,5-dimethyl-2-oxocyclohexanecarboxylate (6).** Following the procedure used to prepare **2**, **5** (9.6 g, 76 mmol) was treated with NaH (60% in oil, 12.2 g, 305 mmol), $Me_2CO_3$ (16.0 mL, 190 mmol), and KH (30% in oil, catalytic amount) then quenched with aq 3 M AcOH (120 mL) and poured into brine (100 mL). The usual work-up (EtOAc, 5 × 100 mL) followed by

distillation (< 1 Torr, 74-76°C) afforded **6** (10.1 g, 72%) as a colorless oil. Spectral data was consistent with that previously reported.[44]

**Methyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (7).**

Following the procedure used to prepare **3**, **6** (102.6 mg, 0.56 mmol) was treated with pyridine (60 μL, 0.74 mmol) and PhSeBr (163.5 mg, 0.69 mmol). The usual work-up ($CH_2Cl_2$) gave the corresponding phenylselanyl-β-ketoester. The latter was treated with aq $H_2O_2$ (50% wt., 0.5 mL, 8.65 mmol) then water (10 mL) was added. The usual work-up ($CH_2Cl_2$; aqueous saturated $NaHCO_3$; brine) and purification (10 to 20% EtOAc in hexanes) afforded **7** (58.2 mg, 57%) as a yellow oil. Spectral data was consistent with that previously reported.[43]

**Methyl 5-hexyl-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (8).** Following the procedure used to prepare **3**, **6** (204.1 mg, 1.11 mmol) was treated with LDA (2.40 mmol) in THF (1.0 mL) 1-iodohexane (0.25 mL, 1.69 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc) and purification (5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (256.8 mg confirmed by mass spectrometry) as a yellow oil. A solution of the latter (49.7 mg, 0.19 mmol) in THF (0.3 mL + 0.2 mL for rinsing) was added to a solution of DDQ (57.2 mg, 0.25 mmol) in THF (0.5 mL) via canula at rt. The mixture was stirred for 24 h then water (2 mL) was added. The usual work-up (EtOAc; brine) and purification (10% EtOAc in hexanes) afforded **8** (19.8 mg, 34% over two steps) as a yellow oil: $^1$H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.25 (d, 1H, $J$ = 2.0 Hz), 3.79 (s, 3H), 2.46 (m, 1H), 1.90 (m, 2H), 1.65 (t, 1H, $J$ = 13.7 Hz), 1.29 (m, 9H), 1.24 (s, 3H), 1.20 (s, 3H), 0.87 (t, 3H, $J$ = 6.7 Hz). $^{13}$C NMR ($CDCl_3$, 100 MHz) δ (ppm) 197.09, 165.79, 163.13, 130.24, 52.35, 43.59, 41.83, 33.91, 31.87, 30.36, 29.53, 29.03, 26.88, 25.40, 22.77, 14.24. HRMS (ESI-Q-Tof) calcd for $C_{16}H_{26}O_3Na$: 289.1774, found: 289.1784. IR (neat) ν 2955, 2925, 2857, 1745, 1719, 1686 cm$^{-1}$.

**Methyl 3,3,5-trimethyl-6-oxocyclohex-1-enecarboxylate (9).** Following the procedure used to prepare **3**, **6** (215.8 mg, 1.17 mmol) was treated with LDA (2.34 mmol) and iodomethane (80 μL, 1.29 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc) and purification (5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (150.0 mg, confirmed by mass spectrometry) as a yellow oil. The latter (100.0 mg, 0.50 mmol) was treated with pyridine (50 μL, 0.62 mmol) and PhSeBr (140.1 mg, 0.59 mmol) then quenched with 1 N aq HCl (5 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl; brine) gave the corresponding phenylselanyl-β-ketoester as a yellow oil. The latter was treated with aq $H_2O_2$ (50 wt. %, 0.5 mL, 8.65 mmol). The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$; brine) and purification (5 to 10% EtOAc in hexanes) afforded **9** (58.4 mg, 39% over two steps) as a yellow oil: $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.25 (s, 1H), 3.78 (s, 3H), 2.62 (m, 1H), 1.83 (ddd, 1H, $J$ = 2.3, 4.9, 13.5 Hz), 1.69 (t, 1H), 1.25 (s, 3H), 1.18 (s, 3H), 1.12 (d, 3H, $J$ = 6.6 Hz). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 197.31, 165.72, 163.38, 129.98, 52.30, 44.61, 38.67, 33.97, 30.28, 25.43, 14.87. HRMS (ESI-Q-Tof) calcd for $C_{11}H_{16}O_3$: 219.992, found: 219.1000. IR (neat) ν 2962, 1743, 1718, 1686, 1285, 1258 cm$^{-1}$.

**Methyl 5-allyl-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (10).** Following the procedure used to prepare **3**, **6** (203.0 mg, 1.10 mmol) was treated with LDA (2.40 mmol) and allyl bromide (0.15 mL, 1.73 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc) and purification (10% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (210.7 mg, confirmed by mass spectrometry) as a yellow oil. Following the procedure used to prepare **8**, alkylated β-ketoester (101.5 mg, 0.45 mmol) was treated with DDQ (57.2 mg, 0.25 mmol) and $K_2CO_3$ (76.5 mg, 0.55 mmol) then quenched with water (4 mL). The usual work-up (EtOAc; brine) and purification (5 to 10% EtOAc in hexanes) afforded **10** (45.9 mg, 39% over two steps) as a yellow oil. Spectral data was consistent with that previously reported.[45]

**Methyl 5-benzyl-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (11).** Following the procedure used to prepare **3**, **6** (203.9 mg, 1.11 mmol) was treated with LDA (2.40 mmol) and benzyl bromide (0.20 mL, 1.68 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc) and purification (5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (303.4 mg, confirmed by mass spectrometry) as a yellow oil. Following the procedure used to prepare **8**, alkylated β-ketoester (102.6 mg, 0.37 mmol) was treated with DDQ (97.8 mg, 0.43 mmol) in 1,4-dioxane then hexanes were added, the suspension was filtered and the solvent was evaporated. The usual and purification (5 to 10% EtOAc in hexanes) afforded **11** (40.5 mg, 40% over two steps) as a pale brown oil: $^{1}$H NMR ($CD_2Cl_2$, 400 MHz) δ (ppm) 7.22 (m, 6H), 3.73 (s, 3H), 3.35 (dd, 1H, *J* = 4.0, 14.0 Hz), 2.78 (m, 1H), 2.43 (dd, 1H, *J* = 9.0, 14.0 Hz), 1.71 (m, 1H), 1.59 (t, 1H, *J* = 13.7 Hz), 1.12 (s, 3H), 1.11 (s, 3H). $^{13}$C NMR ($CD_2Cl_2$, 100 MHz) δ (ppm) 196.17, 165.32, 163.32, 140.22, 130.23, 129.46, 128.86, 126.37, 52.27, 45.45, 41.36, 35.35, 33.99, 30.17, 25.02. HRMS (ESI-Q-Tof) calcd for $C_{17}H_{20}O_3$: 295.1305, found: 295.1314. IR (neat) ν 2958, 1742, 1717, 1690, 1271, 1258 cm$^{-1}$.

**Methyl 3,3-dimethyl-6-oxo-5-(prop-2-yn-1-yl)cyclohex-1-enecarboxylate (12).** Following the procedure used to prepare **3**, **6** (199.3 mg, 1.08 mmol) was treated with LDA (2.41 mmol) and propargyl bromide (80% in toluene, 185 μL, 1.66 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc) and purification (5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (176.8 mg confirmed by mass spectrometry) as a yellow oil. Following the procedure used to prepare **8**, alkylated β-ketoester (98.0 mg, 0.44 mmol) was treated with DDQ (159.7 mg, 0.70 mmol) and $K_2CO_3$ (165.7 mg, 1.20 mmol) in 1,4-dioxane then quenched water (10 mL). The usual work-up (EtOAc; brine) and purification (10% EtOAc in hexanes) afforded **12** (44.7 mg, 34% over two steps) as a white solid: $^{1}$H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.34 (d, 1H,

*J* = 2.2 Hz), 3.80 (s, 3H), 2.81 (ddd, 1H, *J* = 2.7, 4.0, 17.0 Hz), 2.72 (m, 1H), 2.30 (ddd, 1H, *J* = 2.6, 8.7, 17.0 Hz), 2.17 (ddd, 1H, *J* = 2.3, 4.6, 13.4 Hz), 1.98 (t, 1H, *J* = 2.7 Hz), 1.78 (t, 1H, *J* = 13.8 Hz), 1.29 (s, 3H), 1.24 (s, 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 194.59, 165.30, 164.37, 129.58, 81.98, 70.11, 52.43, 42.82, 41.09, 34.03, 30.27, 25.31, 18.86. HRMS (ESI-Q-Tof) calcd for $C_{13}H_{16}O_3$: 243.0992, found: 243.0995. IR (neat) ν 3262, 2967, 1716, 1684, 1259 cm$^{-1}$.

**Methyl 3,3-dimethyl-6-oxo-5-(2,2,2-trifluoroethyl)cyclohex-1-enecarboxylate (13).** Following the procedure used to prepare **3**, **6** (203.8 mg, 1.11 mmol) was treated with LDA (2.42 mmol) and 1,1,1-trifluoro-2-iodoethane (130 μL, 1.33 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc; brine) and purification (5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (136.3 mg) as a yellow oil. The latter (136.3 mg) was treated with pyridine (50 μL, 0.62 mmol) and PhSeBr (138.1 mg, 0.59 mmol) then quenched with 1 N aq HCl (5 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl; brine) afforded the corresponding phenylselanyl-β-ketoester as an orange oil. The latter was treated with aq $H_2O_2$ (50 wt. %, 0.5 mL, 8.65 mmol). The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$; brine) and purification (5% EtOAc in hexanes) afforded **13** (20.1 mg, 7% over two steps) as a yellow oil: $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.35 (d, 1H, *J* = 2.3 Hz), 3.81 (s, 3H), 3.09 (m, 1H), 2.88 (m, 1H), 2.14 (ddd, 1H, *J* = 13.0, 4.2, 1.7 Hz), 1.94 (m, 1H), 1.76 (t, 1H, *J* = 13.9 Hz), 1.30 (s, 3H), 1.23 (s, 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 193.23, 164.99, 164.10, 129.22, 125.40, 52.51, 41.93, 38.76 (q, *J* = 2.2 Hz), 33.97, 32.74 (q, *J* = 28.9 Hz), 30.18, 24.99. HRMS (ESI-Q-Tof) calcd for $C_{12}H_{15}F_3O_3$: 287.0866, found: 287.0870. IR (neat) ν 2957, 1745, 1717, 1690, 1252 cm$^{-1}$.

**Methyl 5-(2-(*tert*-butoxy)-2-oxoethyl)-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (14).** Following the procedure used to prepare **3**, **6** (210.4 mg, 1.14 mmol) was treated with LDA (2.47 mmol) and *tert*-butyl bromoacetate (250 μL, 1.69 mmol) then quenched with aq 1 N HCl

(10 mL). The usual work-up (EtOAc; brine) and purification (5 to 20% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (164.0 mg confirmed by mass spectrometry) as a yellow oil. The latter (93.5 mg) was treated with pyridine (61 μL, 0.75 mmol) and PhSeBr (175.4 mg, 0.74 mmol) then quenched with 1 N aq HCl (5 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl; brine) afforded the corresponding phenylselanyl-β-ketoester as a yellow oil. The latter was treated with aq $H_2O_2$ (50 wt. %, 0.5 mL, 8.65 mmol) then water (5 mL) was added. The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$; brine) and purification (10 to 20% EtOAc in hexanes) afforded **14** (36.0 mg, 19% over two steps) as a white solid: mp = 47-49°C; $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.30 (d, 1H, *J* = 2.2 Hz), 3.79 (s, 3H), 3.10 – 2.97 (m, 1H), 2.86 (dd, 1H, *J* = 16.6, 5.2 Hz), 2.15 (dd, 1H, *J* = 16.6, 7.4 Hz), 1.90 (ddd, 1H, *J* = 13.3, 4.8, 2.2 Hz), 1.76 (t, 1H, *J* = 13.6 Hz), 1.46 (s, 9H), 1.29 (s, 3H), 1.21 (s, 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 195.07, 171.62, 165.37, 163.92, 129.56, 80.82, 52.41, 41.93, 40.84, 35.52, 34.08, 30.26, 28.25, 25.16. HRMS (ESI-Q-Tof) calcd for $C_{16}H_{24}O_5Na$: 319.1516, found: 319.1525. IR (neat) ν 2976, 1724, 1364, 1147, 1125 $cm^{-1}$.

**Methyl 5-(cyanomethyl)-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (15).** Following the procedure used to prepare **3**, **6** (828.8 mg, 4.50 mmol) was treated with LDA (9.88 mmol) and bromoacetonitrile (0.63 mL, 9.04 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc; brine) and purification (5 to 20% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (145.2 mg confirmed by mass spectrometry) as an orange oil. The latter (139.0 mg) was treated with pyridine (0.29 mL, 3.13 mmol) and PhSeBr (588.4 mg, 2.49 mmol) then quenched with 1 N aq HCl (5 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl; brine) afforded the corresponding phenylselanyl-β-ketoester as a brown oil. The latter was treated with aq $H_2O_2$ (50 wt. %, 0.5 mL, 8.65 mmol) then water (5 mL) was added. The usual work-up ($CH_2Cl_2$; saturated

aq $NaHCO_3$; brine) and purification (20 to 30% EtOAc in hexanes) afforded **15** (84.8 mg, 9% over two steps) as a yellow oil: $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.40 (d, 1H, *J* = 2.2 Hz), 3.80 (s, 3H), 2.91 (m, 2H), 2.49 (dd, 1H, *J* = 18.1, 9.5 Hz), 2.15 (ddd, 1H, *J* = 13.4, 4.4, 2.2 Hz), 1.86 (t, 1H, *J* = 13.6 Hz), 1.33 (s, 3H), 1.26 (s, 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 192.61, 164.93, 164.62, 128.92, 118.22, 52.56, 41.21, 40.67, 34.19, 30.12, 25.25, 17.96. HRMS (ESI-Q-Tof) calcd for $C_{12}H_{15}NO_3Na$: 244.0944, found: 244.0950. IR (neat) ν 2959, 2249, 1737, 1684, 1275, 1256, 1218 cm$^{-1}$.

**Methyl 3,3-dimethyl-6-oxo-5-(2-oxopropyl)cyclohex-1-enecarboxylate (16).** Following the procedure used to prepare **3**, **6** (801.1 mg, 4.35 mmol) was treated with LDA (9.62 mmol) and chloroacetone (0.69 mL, 8.67 mmol) then quenched with aq 1 N HCl (30 mL). The usual work-up (EtOAc; brine) and purification (5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (215.4 mg confirmed by mass spectrometry) as an orange oil. The latter (171.7 mg) was treated with pyridine (0.33 mL, 3.56 mmol) and PhSeBr (680.1 mg, 2.88 mmol) then quenched with 1 N aq HCl (5 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl; brine) afforded the corresponding phenylselanyl-β-ketoester as a brown oil. The latter was treated with aq $H_2O_2$ (50 wt. %, 0.5 mL, 8.65 mmol) then water (5 mL) was added. The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$; brine) and purification (20 to 30% EtOAc in hexanes) afforded **16** (37.8 mg, 4% over two steps) as a white solid: mp = 53-55°C; $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.29 (d, 1H, *J* = 2.3 Hz), 3.80 (s, 3H), 2.69 (dd, 1H, *J* = 14.6, 4.5 Hz), 2.64 (d, 1H, 4.4 Hz), 2.55 (d, 1H, *J* = 4.4 Hz), 1.89 (ddd, 1H, *J* = 13.5, 4.5, 2.3 Hz), 1.57 (t, 1H, 14.0 Hz), 1.47 (s, 3H), 1.25 (s, 3H), 1.22 (s, 3H). $^{13}$C NMR ($CDCl_3$, 100 MHz) δ (ppm) 194.21, 165.23, 163.69, 130.12, 55.98, 52.41, 50.94, 49.20, 37.46, 33.92, 30.33, 25.04, 21.87. HRMS (ESI-Q-Tof) calcd for $C_{13}H_{18}O_4Na$: 261.1097, found: 261.1113. IR (neat) ν 2944, 1709, 1682, 1265, 1226 cm$^{-1}$.

**Methyl 5-(3-methoxy-3-oxopropyl)-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (17).** Following the procedure used to prepare **3**, **6** (201.3 mg, 1.09 mmol) was treated with LDA (3.50 mmol) and methyl acrylate (0.15 mL, 1.67 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc; brine) and purification (2% to 20% EtOAc in hexanes) afforded the corresponding alkylated β-ketoester (162.6 mg confirmed by mass spectrometry) as an orange oil. The latter (143.3 mg) was treated with pyridine (0.17 mL, 1.84 mmol) and PhSeBr (400.1 mg, 1.70 mmol) then quenched with 1 N aq HCl (5 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl; brine) afforded the corresponding phenylselanyl-β-ketoester as a brown oil. The latter was treated with aqueous $H_2O_2$ (50 wt. %, 0.5 mL, 8.65 mmol) then water (5 mL) was added. The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$; brine) and purification (5% to 30% EtOAc in hexanes) afforded **17** (40.7 mg, 16% over two steps) as a colorless oil: $^1$H NMR ($CD_2Cl_2$, 300 MHz) δ (ppm) 7.20 (s, 1H), 3.72 (s, 3H), 3.62 (s, 3H), 2.55 (m, 1H), 2.39 (m, 2H), 2.12 (m, 1H), 1.84 (m, 1H), 1.68 (d, 1H, $J$ = 13.8 Hz), 1.56 (m, 1H), 1.22 (s, 3H), 1.17 (s, 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 196.29, 173.71, 165.19, 162.79, 130.00, 51.97, 51.46, 42.63, 41.88, 33.82, 31.45, 29.94, 24.91, 24.70. HRMS (ESI-Q-Tof) calcd for $C_{14}H_{20}O_5Na$: 291.1203, found: 291.1211. IR (neat) ν 2954, 1733, 1685, 1267, 1218, 1168 cm$^{-1}$.

**Allyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (18).** In a sealed tube equipped with a septum, DMAP (213.3 mg, 1.75 mmol) and 4 Å molecular sieves (500.8 mg) were added to a solution of **6** (219.2 mg, 1.19 mmol) in allyl alcohol (5.0 mL). The tube was sealed, the mixture was refluxed for 48 h, allowed to cool down to rt, then aqueous 1 N HCl (10 mL) was added. The usual work-up (EtOAc; brine) and purification (2% EtOAc in hexanes) gave a mixture of **6** (0.09 mmol) and the corresponding allyl ester (0.68 mmol) as a pale yellow oil (158.1 mg). The mixture was dissolved in 1,4-dioxane (2.0 mL) then $K_2CO_3$ (169.9 mg, 1.23 mmol) and DDQ (233.1 mg,

1.02 mmol) were added. The reaction mixture was heated to 100 °C for 4 h, cooled to rt and quenched with water (10 mL). The usual work-up (EtOAc; brine) and purification (5 to 10% EtOAc in hexanes) afforded **18** (78.8 mg, 32% over two steps) as an orange oil: $^{1}$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.34 (s, 1H), 5.96 (m, 1H), 5.38 (ddd, 1H, *J* = 1.5, 3.0, 17.2 Hz), 5.26 (ddd, 1H, *J* = 1.3, 2.6, 10.4 Hz), 4.70 (dt, 2H, *J* = 1.4, 5.7 Hz), 2.53 (t, 2H, *J* = 6.8 Hz), 1.89 (t, 2H, *J* = 6.78 Hz), 1.23 (s, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 194.40, 164.58, 164.36, 131.99, 130.24, 118.76, 65.88, 35.59, 35.35, 33.64, 27.51. HRMS (ESI-Q-Tof) calcd for $C_{12}H_{16}O_3Na$: 231.992, found: 231.1003. IR (neat) ν 2962, 1740, 1714, 1686, 1267, 1224 cm$^{-1}$.

***tert*-Butyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (19).** Following the procedure usedd to prepare **18**, **6** (204.7 mg, 1.11 mmol) was treated DMAP (200.7 mg, 1.64 mmol) and 4 Å molecular sieves (365.8 mg) in *tert*-butanol (5.0 mL) then was quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc; brine) and purification (silica gel saturated with $Et_3N$, 100% hexanes) gave a mixture of **6** (0.36 mmol) and the corresponding *tert*-butyl ester (0.40 mmol) as a colorless oil (156.1 mg). Following the procedure used to prepare **3**, the mixture was treated with pyridine (155 µL, 1.67 mmol) and PhSeBr (375.4 mg, 1.59 mmol) then quenched with 1 N aq HCl (10 mL). The usual work-up ($CH_2Cl_2$; 1 N aq HCl; brine) afforded the corresponding phenylselanyl-β-ketoester as a brown oil. The latter was treated with aq $H_2O_2$ (50% wt, 0.5 mL, 8.65 mmol) then aq saturated $NaHCO_3$ (5 mL) was added. The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$) and purification (10 to 20% EtOAc in hexanes) afforded **19** (61.7 mg, 25% over two steps) as a white solid: mp = 28 – 30 °C; $^{1}$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.17 (s, 1H), 2.50 (t, 2H, *J* = 6.8 Hz), 1.86 (t, 2H, *J* = 6.8 Hz), 1.50 (s, 9H), 1.21 (s, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 194.73, 164.23, 162.53, 131.73, 81.83, 35.69, 35.41, 33.43, 28.23, 27.59. HRMS (ESI-Q-Tof) calcd for $C_{13}H_{20}O_3Na$: 247.1305, found: 247.1311. IR (neat) ν 2957, 1725, 1673, 1367, 1290, 1237 cm$^{-1}$.

**3,3-Dimethyl-6-oxocyclohex-1-enecarboxylic acid (20).** A solution of KOH (85%, 894.0 mg, 15.9 mmol) in water (1.5 mL) was added to a solution of **7** (54.0 mg, 0.30 mmol) in MeOH (5.0 mL) at rt. The mixture was refluxed for 1 h, cooled to rt, diluted with EtOAc (30 mL) and quenched with aqueous 3 N HCl (10 mL). The usual work-up (EtOAc; brine) and purification (30% EtOAc in hexanes) afforded **20** (24.8 mg, 49%) as a white solid: mp = 68-70 °C; $^{1}$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 12.47 (s, 1H), 8.11 (s, 1H), 2.66 (m, 2H), 1.94 (m, 2H), 1.27 (s, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 202.86, 173.76, 164.08, 124.53, 35.05, 34.61, 34.35, 27.01. HRMS (ESI-Q-Tof) calcd for $C_9H_{12}O_3Na$: 191.0679, found 191.0677. IR (neat) ν 2960, 2761, 1742, 1637, 1607, 1439, 1223, 1156 cm$^{-1}$.

**Methyl 6-hydroxy-3,3-dimethylcyclohex-1-enecarboxylate (21).** $CeCl_3$ (heptahydrate, 189.3 mg, 0.51 mmol) and then $NaBH_4$ (20.6 mg, 0.54 mmol) were added to a solution of **7** (91.8 mg, 0.50 mmol) in MeOH (2.0 mL) at rt. The solution was stirred (gas evolution) for 20 h then aq 1 N HCl (2 mL) was added dropwise followed by water (3 mL). The usual work-up (EtOAc) and purification (10 to 20% EtOAc in hexanes) afforded **21** (53.2 mg) as a pale yellow oil: $^{1}$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 6.76 (s, 1H), 4.48 (t, 1H, $J$ = 5.1 Hz), 3.77 (s, 3H), 3.71 (s, 1H), 1.93 – 1.75 (m, 2H), 1.64 (ddd, 1H, $J$ = 13.5, 9.9, 3.6 Hz), 1.42 (m, 1H), 1.11 (s, 3H), 1.03 (s, 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 176.21, 168.22, 151.90, 129.89, 71.59, 63.87, 51.96, 51.92, 47.48, 40.75, 37.24, 33.22, 32.25, 32.21, 30.46, 29.72, 28.85, 27.74, 27.25, 24.31. HRMS (ESI-Q-Tof) calcd for $C_{10}H_{16}O_3Na$: 207.0992, found 207.0993. IR (neat) ν 3445, 2949, 2865, 1702, 1361, 1281, 1251 cm$^{-1}$.

**2-Chloro-4,4-dimethylcyclohex-2-enone (23).** (Diacetoxyiodo)benzene (320.0 mg, 0.99 mmol) and pyridine hydrochloride (238.9 mg, 2.07 mmol) were added to a solution of **22** (100.1 mg, 0.81 mmol) in $CH_2Cl_2$ (4.0 mL) at rt. The mixture was stirred for 6 h then

(diacetoxyiodo)benzene (334.0 mg, 1.04 mmol) and pyridine hydrochloride (233.6 mg, 2.02 mmol) were added again. The mixture was stirred overnight then quenched with aq 1 N HCl (10 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl) and purification (5% EtOAc in hexanes) afforded **23** (32.7 mg, 25%) as a yellow oil. Spectral data was consistent with that previously reported.[46]

**2-Iodo-4,4-dimethylcyclohex-2-enone (24).** A solution of iodine (868.1 mg, 3.42 mmol) and pyridine (3.0 mL) in $CCl_4$ (3.0 mL) was added dropwise to a solution of **22** (233.7 mg, 1.88 mmol) and pyridine (3.0 mL) in $CCl_4$ (3.0 mL) at 0 °C. The resulting mixture was allowed to warm up to rt, stirred for 3 h then quenched with aq 1 N HCl (10 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl; aq saturated $NaHCO_3$) and purification (5% EtOAc in hexanes) afforded **24** (351.6 mg, 75%) as a yellow oil. Spectral data was consistent with that previously reported.[47]

**2-(Hydroxymethyl)-4,4-dimethylcyclohex-2-enone (25).** A solution of **22** (1.6 g, 12.9 mmol) in THF (2.0 mL + 2.0 mL for rinsing) was added to a mixture of paraformaldehyde (194.6 mg, 6.4 mmol) and imidazole (441.6 mg, 6.5 mmol) in 1 N $NaHCO_3$ (25.6 mL) and THF (2.4 mL) ar rt. The mixture was stirred for 50 h then quenched with aq 1 N HCl (25 mL). The usual work-up ($CH_2Cl_2$) and purification (10 to 30% EtOAc in hexanes) afforded 4,4-dimethylcyclohex-2-enone (548.2 mg, 4.4 mmol) and **25** (453.8 mg, 35% corrected) as a colorless oil. Spectral data was consistent with that previously reported.[48]

**(3,3-Dimethyl-6-oxocyclohex-1-en-1-yl)methyl 5,5-dimethyl-2-oxocyclohexanecarboxylate (26).** A solution of **25** (129.5 mg, 0.84 mmol) in toluene (2.0 mL + 2 × 1.0 mL for rinsing) was added dropwise to a solution **6** (300.4 mg, 1.63 mg) in toluene (5.0 mL) at rt. Triethylamine (0.23 mL, 1.65 mmol) was then added and the resulting mixture was refluxed in a Dean-Stark apparatus for 24 h. The solution was allowed to cool down to rt, washed with brine (3 × 5 mL), dried over anh $Na_2SO_4$, filtered and concentrated. The usual purification (2 to 5% EtOAc in hexanes) afforded

methyl 5,5-dimethyl-2-oxocyclohexanecarboxylate (120.8 mg) and **26** (241.1 mg, 94% corrected) as a colorless oil: $^{1}$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 12.12 (s, 1H), 6.61 (s, 1H), 4.78 (d, 2H, *J* = 0.9 Hz), 2.50 (m, 2H), 2.29 (t, 2H, *J* = 6.7 Hz), 2.03 (s, 2H), 1.87 (t, 2H, *J* = 6.8 Hz), 1.44 (t, 2H, *J* = 6.7 Hz), 1.18 (s, 6H), 0.95 (s, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 198.08, 197.86, 172.44, 171.72, 169.96, 157.95, 156.99, 131.57, 131.07, 96.49, 61.90, 61.01, 53.82, 42.06, 39.40, 38.08, 36.13, 36.00, 34.60, 34.49, 33.15, 33.10, 30.92, 30.22, 29.11, 27.99, 27.92, 27.85, 27.81, 26.79, 24.73. HRMS (ESI-Q-Tof) calcd for $C_{18}H_{26}O_4Na$: 329.1723, found: 329.1732. IR (neat) ν 2969, 1669, 1634, 1606, 1230, 1200, 1174 $cm^{-1}$.

**Methyl 5,5-dimethyl-2-oxocyclohex-3-enecarboxylate (27).** Following the procedure used to prepare **2**, **22** (534.0 mg, 4.30 mmol) was treated with NaH (60% in oil, 505.5 mg, 12.6 mmol) and $Me_2CO_3$ (1.7 mL, 20.2 mmol) in refluxing 1,4-dioxane (3 mL) overnight then quenched with water (2 mL) and aq 3 M AcOH (2 mL). The usual work-up ($Et_2O$) and purification (5% EtOAc in hexanes) gave **27** (265.9 mg, 34%) as a yellow oil. The spectral data was consistent with that previously reported.[49]

**Methyl 1,5,5-trimethyl-2-oxocyclohex-3-enecarboxylate (28).** Iodomethane (30 μL, 0.48 mmol) was added dropwise to a solution of **27** (54.5 mg, 0.30 mmol) in acetone (3.0 mL) at 0 °C, followed by $K_2CO_3$ (115.0 mg, 0.83 mmol). The resulting mixture was stirred overnight at 40 °C, was allowed to cool down to rt the solvent was removed under vacuum. Aq 1 N HCl (5 mL) was added then the usual work-up ($CH_2Cl_2$; brine) and purification (5% EtOAc in hexanes) afforded **28** (31.7 mg, 54%) as a yellow oil: $^{1}$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 6.59 (dd, 1H, *J* = 1.9, 10.2 Hz), 5.95 (d, 1H, *J* = 10.2 Hz), 3.68 (s, 3H), 2.43 (dd, 1H, *J* = 1.9, 14.2 Hz), 1.81 (d, 1H, *J* = 14.2 Hz), 1.38 (s, 3H), 1.14 (s, 3H), 1.07 (s, 3H). $^{13}$C NMR ($CDCl_3$, 100 MHz) δ (ppm) 197.16,

174.89, 157.96, 126.31, 52.61, 51.24, 47.37, 33.40, 31.47, 26.84, 23.07. HRMS (ESI-Q-Tof) calcd for $C_{11}H_{16}O_3Na$: 219.992, found: 219.0993. IR (neat) ν 2958, 1731, 1682, 1249, 1228 $cm^{-1}$.

**Methyl 3,3-dimethyl-6-oxocyclohexa-1,4-dienecarboxylate (29).** Selenium dioxide (193.8 mg, 1.75 mmol) was added to a solution of **27** (104.0 mg, 0.57 mmol) in *tert*-butanol (5% wt. AcOH, 10.0 mL) at 30 °C. The resulting mixture was stirred at reflux for 20 h and allowed to cool down to rt. $Et_2O$ (10 mL) was added, layers were separated and the organic layer was washed with aq saturated $NaHCO_3$ (3 × 5 mL). The usual work-up ($Et_2O$; brine) and purification (10 to 50% EtOAc in hexanes) gave the product contaminated with selenium. This material was vaporized under vacuum with a heat gun and the vapor was collected in another flask to afford pure **29** (50.0 mg, 49%) as a yellow oil. Spectral data was consistent with that previously reported.[49]

**Dimethyl 5,5'-methylenebis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (30).** Following the procedure used to prepare **3**, **6** (400.5 mg, 2.17 mmol) was treated with LDA (4.80 mmol) and diiodomethane (105 μL, 1.30 mmol) then quenched with saturated aq 1 N HCl (20 mL). The usual work-up (EtOAc; brine) and purification (10% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (374.8 mg confirmed by mass spectrometry) as an orange oil. The latter (102.3 mg, 0.27 mmol) was treated with pyridine (70 μL, 0.87 mmol) and PhSeBr (192.9 mg, 0.82 mmol) then quenched with aq 1 N HCl (5 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl; brine) afforded the corresponding phenylselanyl-β-ketoester as an orange oil. The latter was treated with aq $H_2O_2$ (50 wt. %, 0.5 mL, 8.65 mmol) then saturated aq $NaHCO_3$ (5 mL) was added. The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$; brine) and purification (20 to 25% EtOAc in hexanes) afforded **30** (28.9 mg, 25% over two steps) as a yellowish solid: mp = 132-134°C; $^1H$ NMR ($CD_2Cl_2$, 400 MHz) δ (ppm) 7.19 (br s, 2H), 3.71 (s, 6H), 2.79 (m, 2H), 1.88 (m, 2H), 1.66 (m, 4H), 1.26 (s, 6H), 1.17 (s, 6H). $^{13}C$ NMR ($CD_2Cl_2$, 100 MHz) δ (ppm) 197.69, 165.48, 163.03,

130.25, 52.22, 43.18, 42.13, 34.20, 30.68, 30.18, 25.27. HRMS (ESI-Q-Tof) calcd for $C_{21}H_{28}O_6Na$: 399.1778, found: 399.1785. IR (neat) ν 2967, 1709, 1692, 1259, 1235 $cm^{-1}$.

**Dimethyl 5,5'-(propane-1,3-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (31).** Following the procedure used to prepare **3**, **6** (404.1 mg, 2.19 mmol) was treated with LDA (4.80 mmol) and 1,3-diiodopropane (150 μL, 1.31 mmol) then quenched with saturated aq $NH_4Cl$ (20 mL). The usual work-up (EtOAc; brine) and purification (5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (355.8 mg confirmed by mass spectrometry) as a yellow oil. The latter (100.8 mg, 0.25 mmol) was treated with pyridine (90 μL, 1.11 mmol) and PhSeBr (237.2 mg, 1.01 mmol) then quenched with 1 N aq HCl (5 mL). The usual work-up ($CH_2Cl_2$; brine) gave the corresponding phenylselanyl-β-ketoester. The latter was treated with aq $H_2O_2$ (50 wt. %, 0.5 mL, 8.65 mmol). The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$; brine) and purification (20 to 25% EtOAc in hexanes) afforded **31** (63.0 mg, 50% over two steps) as a yellow oil: $^1H$ NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.27 (s, 2H), 3.79 (s, 6H), 2.50 (m, 2H), 1.89 (ddd, 4H, $J$ = 2.2, 4.7, 13.4 Hz), 1.67 (t, 2H, $J$ = 18.3 Hz), 1.39 (m, 4H), 1.25 (s, 6H), 1.21 (s, 6H). $^{13}C$ NMR ($CDCl_3$, 75 MHz) δ (ppm) 197.01, 196.97, 165.67, 163.30, 163.27, 130.16, 52.33, 52.30, 43.46, 43.43, 42.02, 41.97, 33.98, 30.33, 29.44, 29.30, 25.38, 24.30, 24.23. HRMS (ESI-Q-Tof) calcd for $C_{23}H_{32}O_6Na$: 427.2091, found: 427.2109. IR (neat) ν 2955, 1742, 1717, 1682, 1270, 1215 $cm^{-1}$.

**5,5'-(butane-1,4-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (32).** Following the procedure used to prepare **3**, **6** (201.3 mg, 1.09 mmol)was treated with LDA (2.50 mmol) and 1,4-diiodobutane (87 μL, 0.66 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc; brine) and purification (5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (199.5 mg confirmed by mass spectrometry) as a white gum. The latter (106.7 mg, 0.25 mmol) was treated with pyridine (90 μL, 1.11 mmol) and PhSeBr (233.8 mg, 0.99 mmol) then

quenched with aq 1 N HCl (5 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl; brine) gave the corresponding phenylselanyl-β-ketoester as a yellow oil. The latter was treated with aq $H_2O_2$ (50% wt, 1.0 mL, 17.3 mmol) then saturated aq $NaHCO_3$ (5 mL) was added. The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$; brine) and purification (30% EtOAc in hexanes) afforded **32** (64.3 mg, 53% over two steps) as a white solid: mp = 84-86°C; $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.25 (d, 2H, *J* = 2.1 Hz), 3.79 (s, 6H), 2.48 (m, 2H), 1.89 (m, 4H), 1.65 (td, 2H, *J* = 0.9, 13.9 Hz), 1.34 (m, 6H), 1.25 (s, 6H), 1.20 (s, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 196.97, 165.74, 163.19, 130.22, 52.34, 43.58, 41.90, 33.92, 30.35, 29.05, 29.03, 27.17, 27.14, 25.40. HRMS (ESI-Q-Tof) calcd for $C_{24}H_{34}O_6Na$: 441.2248, found: 441.2251. IR (neat) ν 2936, 1717, 1675, 1259, 1237 cm$^{-1}$.

**(*E*)-Dimethyl 5,5'-(but-2-ene-1,4-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (33).** Following the procedure used to prepare **3**, **6** (201.3 mg, 1.09 mmol) was treated with LDA (2.50 mmol) and (2*E*)-1,4-dibromobut-2-ene (146.7 mg, 0.69 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc; brine) and purification (10% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (201.9 mg confirmed by mass spectrometry) as a yellow oil. Following the procedure used to prepare **8**, alkylated β-ketoester (89.9 mg, 0.21 mmol) was treated with DDQ (106.5 mg, 0.47 mmol) in 1,4-dioxane then quenched with saturated aq $Na_2CO_3$ (10 mL). The mixture was poured into brine (10 mL). The usual work-up (EtOAc) and purification (10% to 30% EtOAc in hexanes) gave a yellow solid that was triturated in $Et_2O$ to obtain **33** (12.9 mg, 13% over two steps) as a white solid: mp = 126-128°C; $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.27 (d, 2H, *J* =2.2 Hz), 5.43 (m, 2H), 3.79 (s, 6H), 2.54 (m, 4H), 2.08 (m, 2H), 1.85 (m, 2H), 1.61 (td, 2H, *J* = 3.7, 13.6 Hz), 1.23 (s, 6H), 1.19 (s, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 196.19, 196.16, 165.61, 163.55, 130.08, 130.05, 129.95, 52.35, 43.53, 43.51, 41.39, 41.37, 33.96, 33.95,

32.33, 32.31, 30.33, 25.34. HRMS (ESI-Q-Tof) calcd for $C_{24}H_{32}O_6Na$: 439.2091, found: 439.2094. IR (neat) ν 2963, 1717, 1674, 1273, 1252 $cm^{-1}$.

**Dimethyl 5,5'-(but-2-yne-1,4-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (34).** Following the procedure used to prepare **3**, **6** (214.7 mg, 1.17 mmol) was treated with LDA (2.64 mmol), freshly distilled HMPA (200 μL, 1.15 mmol), and 1,4-dichloro-2-butyne (64 μL, 0.65 mmol) then quenched with aq 1 N HCl (10 mL). The usual work-up (EtOAc; brine) and purification (5% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (127.4 mg confirmed by mass spectrometry) as a yellow oil. Following the procedure used to prepare **8**, alkylated β-ketoester (121.4 mg, 0.29 mmol) was treated with DDQ (150.1 mg, 0.66 mmol) in 1,4-dioxane then quenched with saturated aq $Na_2CO_3$ (10 mL). The usual work-up (EtOAc; brine) and purification (20% to 40% EtOAc in hexanes) gave a brown residue that was triturated in $Et_2O$ to afford **34** (17.5 mg, 8% over two steps) as a light brown solid: mp = 132-134°C; $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.31 (d, 2H, *J* = 2.1 Hz), 3.78 (s, 6H), 2.68 (m, 4H), 2.27 (m, 2H), 2.11 (m, 2H), 1.75 (t, 2H, *J* = 13.7 Hz), 1.26 (s, 6H), 1.23 (s, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 194.89, 194.87, 165.36, 165.34, 164.22, 164.15, 129.71, 129.67, 79.25, 79.24, 52.35, 43.19, 43.17, 41.21, 41.19, 33.97, 30.27, 25.33, 19.16. HRMS (ESI-Q-Tof) calcd for $C_{24}H_{30}O_6Na$: 437.1935, found: 437.1938. IR (neat) ν 2956, 1731, 1670, 1281, 1255 $cm^{-1}$.

**Bis(methyl 8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate) methylene (35).** Following the procedure used to prepare **3**, **2** (196.7 mg, 0.92 mmol) was treated with LDA (1.93 mmol) and dibromomethane (32 μL, 0.46 mmol) then quenched with aq 1 N HCl (10 mL) and water (2 mL). The usual work-up ($Et_2O$, brine) and purification (30% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (52.6 mg confirmed by mass spectrometry) as a pale yellow solid: mp = 148-150 °C. The latter (47.5 mg, 0.11 mmol) was treated with pyridine (50 μL, 0.62 mmol) and PhSeBr

(107.6 mg, 0.46mmol) then quenched with aq 1 N HCl (3 mL). The usual work-up ($CH_2Cl_2$; aq 1 N HCl) and purification (20 to 40% EtOAc in hexanes) gave the corresponding phenylselanyl-β-ketoester (37.7 mg, 13% over 2 steps) as a white solid. The latter (23.1 mg, 0.03 mmol) was treated with aq $H_2O_2$ (50 wt. %, 0.05 mL) then water (1 mL) was added. The usual work-up ($CH_2Cl_2$; saturated aq $NaHCO_3$; brine) afforded pure **36** (15.0 mg, quant.) as a brown solid: mp = 196°C; $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.07 (d, 2H, *J* = 1.8 Hz), 4.06 (m, 8H), 3.80 (s, 6H), 3.04 (m, 2H), 2.44 (m, 2H), 2.12 (d, 1H, *J* = 29.2 Hz), 2.07 (d, 1H, *J* = 25.8 Hz), 1.81 (t, 2H, *J* = 6.4 Hz). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 196.72, 164.62, 148.48, 132.53, 103.69, 65.64, 65.23, 52.62, 44.22, 39.81, 30.39. HRMS (ESI-Q-Tof) calcd for $C_{21}H_{24}O_{10}Na$: 459.1262, found: 459.1269. IR (neat) ν 2956, 1742, 1717, 1690, 1257, 1219 cm$^{-1}$.

**Bis(methyl 8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate) propylene (36).** Following the procedure used to prepare **3**, a solution of **2** (101.7 mg, 0.48 mmol) in THF (1.0 mL) was treated with a previously prepared solution of LDA (1.0 mmol) in THF (1.0 mL), then with 1,3-diiodopropane (34 μL, 0.29 mmol). Aqueous 1 N HCl (3 mL) was added and the usual work-up (EtOAc; brine) and purification (10 to 20% EtOAc in hexanes) gave the corresponding alkylated β-ketoester (63.7 mg confirmed by mass spectrometry) as a colorless oil. A solution of PhSeCl (106.9 mg, 0.56 mmol) in $CH_2Cl_2$ (1.0 mL) was treated with pyridine (39 μL, 0.48 mmol) at rt for 30 min, then with a solution of the alkylated β-ketoester (54.5 mg, 0.12) in $CH_2Cl_2$ (1.0 mL) at rt for 8 h. $CH_2Cl_2$ (4 mL), water (1 mL), and 1 N aqueous HCl (1 mL) were added layers were separated. The usual work-up ($CH_2Cl_2$; brine) and purification (0 to 20% EtOAc in hexanes) afforded the corresponding phenylselanyl-β-ketoester (62.7 mg confirmed by mass spectrometry, 38% over 2 steps) as a pale yellow solid. A solution of the latter (60.5 mg, 0.08 mmol )in $CH_2Cl_2$ (1.0 mL) was treated with aqueous $H_2O_2$ (30 wt. %, 0.04 mL). Water (5 mL) was added and the

usual work-up ($CH_2Cl_2$; brine) afforded **37** (37.1 mg, quant.) as a white solid: mp = 138-140 °C; $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.06 (d, 2H, *J* = 1.9 Hz), 4.18 – 3.95 (m, 8H), 3.81 (s, 6H), 2.83 – 2.66 (m, 1H), 2.22 (ddd, 2H, *J* = 13.5, 4.7, 2.0 Hz), 2.13 – 1.99 (m, 2H), 1.97 – 1.77 (m, 2H), 1.49 – 1.32 (m, 4H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 196.21, 164.79, 148.05, 132.80, 103.88, 65.60, 65.20, 52.59, 45.60, 38.67, 29.22, 23.84. HRMS (ESI-Q-Tof) calcd for $C_{23}H_{28}O_{10}Na$: 487.1575, found: 487.1582. IR (neat) ν 2952, 2891, 1741, 1683, 1436, 1260 cm$^{-1}$.

**(3,3-dimethyl-6-oxocyclohex-1-en-1-yl)methyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (37).** $K_2CO_3$ (110.0 mg, 0.80 mmol) and DDQ (103.5 mg, 0.46 mmol) were added to a solution of **26** (97.2 mg, 0.32 mmol) in 1,4-dioxane (2.0 mL). The solution was stirred at refluxed for 4 h and allowed to cool down to rt. Water (10 mL) was added then the usual work-up (EtOAc; brine) and purification (10 to 20% EtOAc in hexanes) afforded **37** (72.9 mg, 75%) as a white solid: mp = 40-42°C; $^1$H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.32 (app. s, 1H), 6.84 (app. s, 1H), 4.85 (d, 2H, *J* = 1.3 Hz), 2.51 (m, 4H), 1.88 (m, 4H), 1.22 (s, 6H), 1.19 (s, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz) δ (ppm) 198.07, 194.52, 164.79, 164.57, 157.47, 131.45, 130.30, 62.12, 36.04, 35.59, 35.36, 34.64, 33.67, 33.15, 27.90, 27.50. HRMS (ESI-Q-Tof) calcd for $C_{18}H_{24}O_4Na$: 327.1567, found: 327.1575. IR (neat) ν 2956, 1710, 1667, 1269, 1227, 1177, 1160 cm$^{-1}$.

## ASSOCIATED CONTENT

### Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.???????

NQO1 induction data, LDH assay data, NMR spectra for new compounds (PDF).

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**Author Contributions**

LJD synthesized new compounds, HT tested for biological activity, LJD, GB and EM designed compounds, HT, MR designed biological experiments, all authors reviewed and approved manuscript contents. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; ARE, antioxidant response element; HEK, human embryonic kidney; ROS, reactive oxygen species; RNS, reactive nitrogen species; FDA, food and drug administration; tBHQ, *tert*-butyl hydroquinone; CDDO, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid; BTB, broad complex, tramtrack, bric-a-brac; IVR, intervening region; LDA, lithium diisopropylamine; DDQ,

2,3-dichloro-5,6-dicyano-p-benzoquinone; LDH, lactate dehydrogenase; SEM, standard error of the mean; GSH, glutathione; clogP, calculated partition coefficient.

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