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One-Pot Regio- and Stereoselective Synthesis of α′-Methoxy-γ-pyrones: Biological Evaluation as Mitochondrial Respiratory Complex Inhibitors

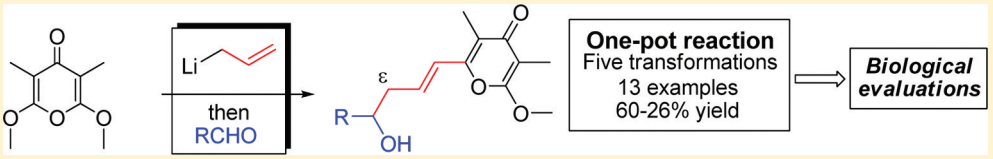
Helena Rosso,[†,‡] Michaël De Paolis,*[,†] Valérie C. Collin,[§] Sriloy Dey,[§] Sidney M. Hecht,[§] Cristina Prandi,[‡] Vincent Richard,[†] and Jacques Maddaluno[†]

[†]Laboratoire des Fonctions Azotées et Oxygénées Complexes de l'IRCOF, CNRS UMR 6014 & FR 3038, Université de Rouen, Mont Saint-Aignan, France

[‡]Dipartimento di Chimica Generale e Chimica Organica, Universita di Torino, via P. Giuria 7, 10125, Torino, Italy

[§]Center for BioEnergetics, The Biodesign Institute, and Department of Chemistry, Arizona State University, Tempe, Arizona 85287, United States

Supporting Information



ABSTRACT: The one-pot construction of functionalized α′-methoxy-γ-pyrones is detailed. Starting from α,α′-dimethoxy-γ-pyrone, molecular diversity is attained by a regio- and stereoselective desymmetrization using allyllithium followed by vinylogous aldol reaction. Mechanistic considerations including density functional theory calculations and insightful experiments have been gathered to shed light on this complex multistep process. To illustrate the versatility of this methodology, some of the molecules prepared were evaluated for their ability to inhibit NADH-oxidase and NADH-ubiquinone oxidoreductase. In the process, a potent new inihibitor of NADH-oxidase activity (IC_{50} 44 nM) was identified.

INTRODUCTION

In recent years, several new natural products containing the α′-methoxy-γ-pyrone scaffold have been isolated and shown to exhibit interesting bioactivities.[1] The modification of natural products through diverted total synthesis is a powerful tool in the discovery of analogues.[2] In addition to having improved bioactivities, these new molecules can also help in understanding the interactions involved between the natural products and the biological targets. In this context, verticipyrone constitutes a simple and interesting case of diverted total synthesis (see Figure 1a). Verticipyrone ((*E*)-2-methoxy-3,5-dimethyl-6-(3-methyl-2-undecenyl)-4*H*-pyran-4-one) was isolated from the fungus *Verticillium* sp. FKI-1083 in a screen for anthelmintic agents.[3] The molecule was reported to inhibit *Ascaris suum* NADH-fumarate reductase and bovine heart NADH oxidase activity and NADH-ubiquinone oxidoreductase. Structurally related natural products such as cyercene A[4] and salinipyrone,[5] shown in Figure 1a, feature conjugated diene side chains connected to α′-methoxy-γ-pyrone or γ-hydroxy-α′-pyrone scaffolds. More recently, synthetic analogues of verticipyrone have been prepared and tested on the biological targets mentioned above,[6,7] and products **1** and **2**, which exhibited greater inhibition than verticipyrone, were discovered (see Figure 1b).

When it is possible, the one-pot reaction is an attractive strategy for the rapid preparation of bioactive molecules.[8] We have recently introduced α,α′-dimethoxy-γ-pyrone **3** as a new building block for the expedient synthesis of verticipyrone via the direct preparation of α-allyl-α′-methoxy-γ-pyrone **4** from **3** (see Scheme 1).[9] Product **4** results from a complex, multistep process, beginning with the desymmetrization of electrophile **3** by the conjugate addition of allyllithium. Since product **4** is relatively acidic, its formation is followed by its rapid deprotonation with allyllithium leading to lithiated α-allyl-α′-methoxy-γ-pyrone **5**. Being electronically rich, this intermediate behaves as a nucleophile and prevents further nucleophilic attack of allyllithium to the γ-pyrone ring. Although the protonation of **5** during the workup delivered **4** for the purpose of the synthesis of verticipyrone, we anticipated that modulation of the electrophiles used to intercept **5** would allow the one-pot synthesis of biomolecules belonging to this family of natural products. In terms of molecular diversity, this strategy would constitute a unique tool in reaching, in one step, valuable biomolecules from α,α′-dimethoxy-γ-pyrone **3**.

In continuation of our preliminary work, we report now a stereocontrolled *one-pot preparation* of new molecules bearing the α′-methoxy-γ-pyrone core and functionalized side chains from **3** and commercially or readily available starting materials.

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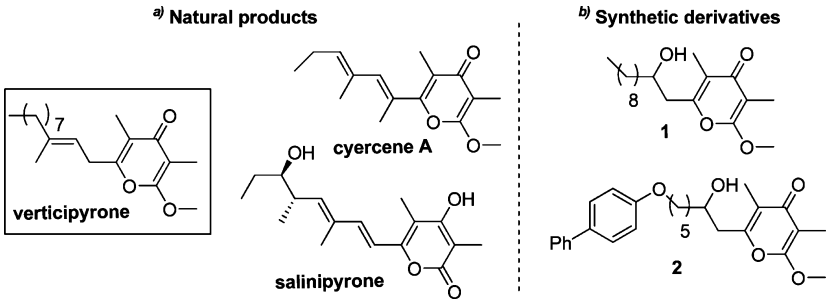
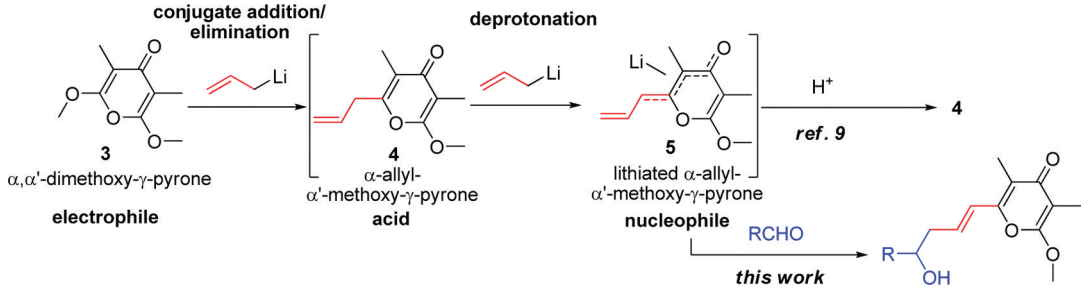
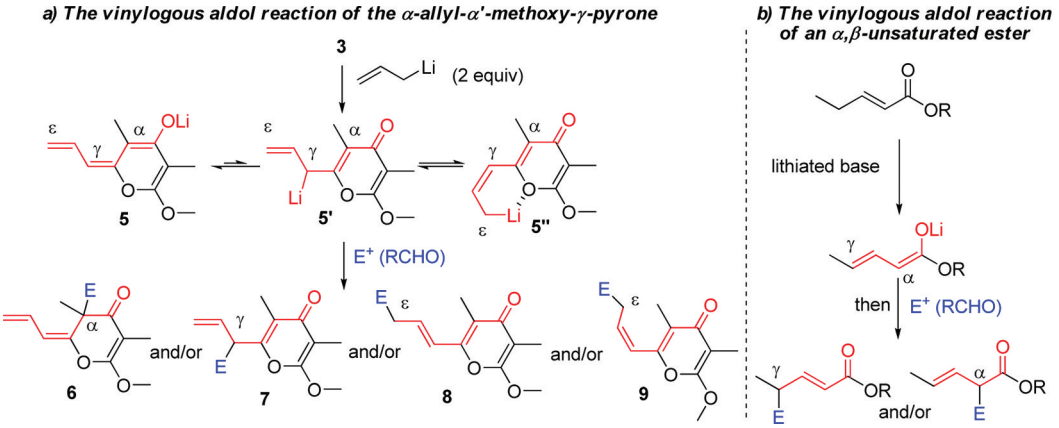


Figure 1. Verticipyrone and structurally related cyercene A and salinipyrone and selected bioactive synthetic derivatives **1** and **2**.

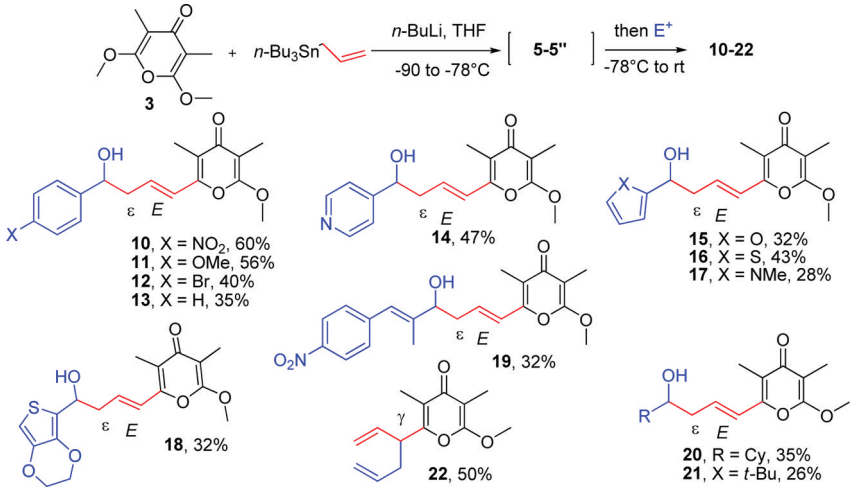
Scheme 1. Desymmetrization of 3 by Allyllithium Leading to 4 via 5 and Interception of 5 with Carboxaldehydes



Scheme 2. Analogy of Regioselectivity Issues Arising from the Vinylogous Aldol Reaction of the α-Allyl-α′-methoxy-γ-pyrone and an α,β-Unsaturated Ester



Scheme 3. One-Pot Preparation of α′-Methoxy-γ-pyrones 10−22 from 3



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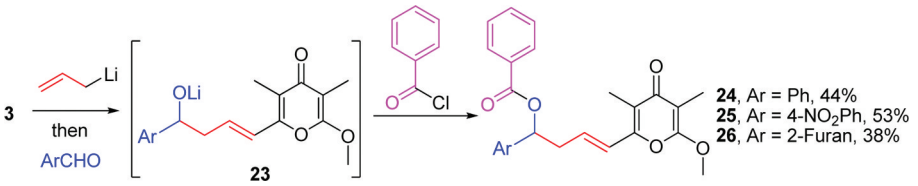
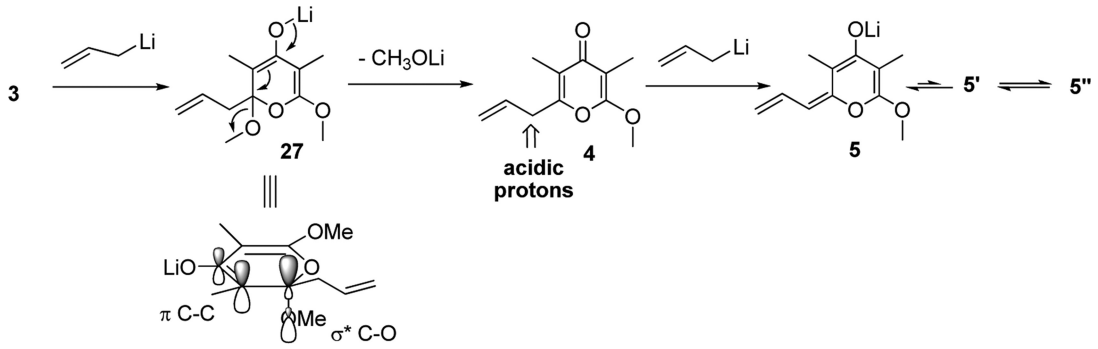
Scheme 4. One-Pot Preparation of Esters 24−26 from 3



Scheme 5. Putative Pathway and Intermediates



Mechanistic insights into this process and biological evaluations of some products are also disclosed.

RESULTS AND DISCUSSION

Although the use of metalated α,α'-dialkyl-γ-pyrone for alkylation or aldolisation is well documented,[10] the reactivity of the lithium enolate of the α-allyl-α'-methoxy-γ-pyrone **5** and the delocalized forms **5′** and **5″** had not been examined (see Scheme 2a). The unknown preparation of **5** and the polytopicity of isomers **5**−**5″** may explain this situation. Indeed, a mixture of isomers **6**−**9** are expected to be produced after treatment of **5**−**5″** with an electrophile. Apart from the *O*/*C* chemoselectivity and the *E*/*Z* configuration of the double bond, the nucleophilic attack may occur at three different positions, α-, β-, or γ-, delivering several products of condensation.

Actually, these problems of regioselectivity are similar to those encountered in the versatile and well-studied vinylogous aldol reaction of an α,β-unsaturated ester (see Scheme 2b), where the γ-functionalized α,β-unsaturated ester may be obtained as a mixture with the α-isomer after quenching the enolate with an aldehyde.[11,12] The fact that the α'-methoxy-γ-pyrone core is present in several natural products prompted our interest in the reactivity of **5** with aldehydes.

In our previous study, we showed that the conjugate addition of 2 equiv of in situ generated allyllithium to **3** is a simple and efficient way to access lithiated α-allyl-α'-methoxy-γ-pyrone **5**. Using this procedure to prepare **5**, its reactivity was evaluated with aromatic aldehydes (see Scheme 3). The regio- and stereoselectivity of the condensation were particularly good because only ε-selectivity was observed and *E*-olefins **10**−**22** were obtained in yields ranging from 60 to 35%. 4-Nitrobenzaldehyde gave the best result, delivering the ε-aldol product **10** in 60% yield. An electron-rich benzaldehyde such as 4-methoxybenzaldehyde or 4-bromobenzaldehyde and hetero-aromatic carboxaldehydes were also suitable electrophiles, yielding the corresponding benzylic alcohols **11**−**18** (56−28% yields).

Furthermore, the chemistry was found to tolerate an α,β-unsaturated aldehyde to afford the allylic alcohol **19** (32%). Even hindered aliphatic aldehydes such as cyclohexylcarboxaldehyde and pivalic aldehyde were reacted with **5** to produce the secondary alcohols **20** and **21** in 35 and 26% yields, respectively. Importantly, only ε-products were obtained with carbonylated electrophiles.

Interestingly, γ-selectivity was observed when an alkylating reagent such as allyl bromide was employed and resulted in the formation of the unconjugated α-allyl-α'-methoxy-γ-pyrone **22** in 50% yield. The simplicity of the procedure deserves to be highlighted here: α,α'-Dimethoxy-γ-pyrone **3** and tri(*n*-butyl)-allylstannane were mixed together prior to the addition of *n*-BuLi at low temperature. After 30 min, the electrophile was added and the reaction was slowly warmed to room temperature. The resulting lithium hydroxylates were quenched with H_3O^+ to yield the products **10**−**22** or with another type of electrophile such as benzoyl chloride, which afforded esters **24**−**26** in 53−38% yields, thus introducing a supplementary point of molecular diversity (see Scheme 4).

The one-pot preparation of a variety of substituted α'-methoxy-γ-pyrones constitutes a substantial improvement over known strategies, which involve several steps for the elaboration of the functionalized γ-pyrone core and the side chain.[13] Since the α,α'-dimethoxy-γ-pyrone **3** is readily available in two steps on a multigram scale from dimethyl ketodicarboxylate,[9] this strategy opens a handy access to a large family of compounds.

The putative mechanism of this transformation is depicted in Scheme 5. The formation of allyllithium, from the reaction of *n*-BuLi with tri(*n*-butyl)allylstannane,[14] is the first step in this process followed by the 1,4-addition to **3**, affording the enolate **27**. From **27**, the primary expulsion of MeOLi can provide **4**. After deprotonation of **4** by the second equivalent of allyllithium, **5** is expected to be formed. In **27**, the molecular orbitals alignement of the π-electrons of the enolate and the orbital σ^*C−O of the methoxy group favors the elimination of MeOLi.

On the other hand, when the anion **28** generated by the action of LiC-KOR base (*t*-BuOK/*n*-BuLi) upon diethoxybut-2-ene was treated with **3** in the presence of CuCl, the trienic keto ester **30** was isolated in 55% yield without the expected

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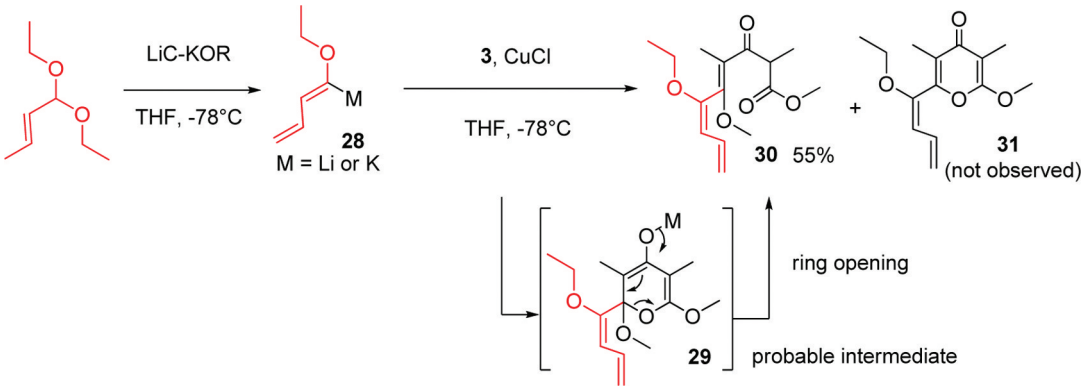
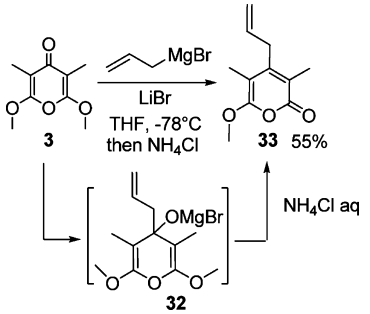
Scheme 6. Reaction of Metal Alkoxydiene 28 to 3



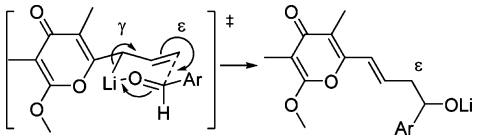
product **31** (see Scheme 6).[15,16] This trienic product could arise from the ring-opening of the adduct **29** taking place instead of the elimination of MeOLi. With an access to the polyenic keto ester **30**, the methodology of desymmetrization demonstrated some versatility when the nature of the nucleophile and the metal employed was modulated. Using allylmagnesium bromide as a nucleophile illustrated further the versatility of the methodology and the importance of the initial 1,4-addition. Hence, when compound **3** was treated with allylmagnesium bromide in the presence of LiBr, the polysubstituted γ-allyl-α'-methoxy-α-pyrone **33** was isolated in 55% yield (see Scheme 7). The formation of **33** may find its origin in the 1,2-addition of allylmagnesium bromide to **3**. The highly unstable diallylic tertiary alcohol **32** obtained may undergo, upon workup, a Stork–Danheiser rearrangement to produce **33**.[17] Consequently, it seems that the 1,4-addition of the nucleophile to **3** is a mandatory step for the successful formation of substituted α'-methoxy-γ-pyrones **10**–**22**.

Scheme 7. Reaction of Allylmagnesium Bromide with 3



Density functional theory (DFT) calculations have been carried out at the B3LYP/6-31G** level to investigate the relative stability of the isomers **5**–**5″**. In an effort to increase the realism of the theoretical model, two discrete molecules of THF were taken into account (see Figure 2). As expected, enolate **5** appears to be more stable than **5′** (+10.6 kcal mol^{-1}) and **5″** (+18.9 kcal mol^{-1}). If **5** cannot be ruled out as a possible reacting intermediate, the ε-selectivity of the aldolisation reaction may be explained by the higher reactivity of intermediate **5′**. Although it does not seem possible to go through a Zimmerman–Traxler transition state involving **5** and the aldehyde because of the rigid conformation of **5**, such a classical transition state does seem possible with **5′** and the aldehyde, as illustrated in Scheme 8.

Scheme 8. Putative Zimmerman–Traxler Transition State 5′/ArCHO Explaining the ε-Selectivity



In order to deepen our understanding on the origin of the ε-selectivity, the relative stability of the ε-(**23**) and γ-(**23′**) isomers of the lithium hydroxylates arising from the aldol reaction was investigated because an equilibrium between these two isomers through retro-aldolisation/aldolisation processes

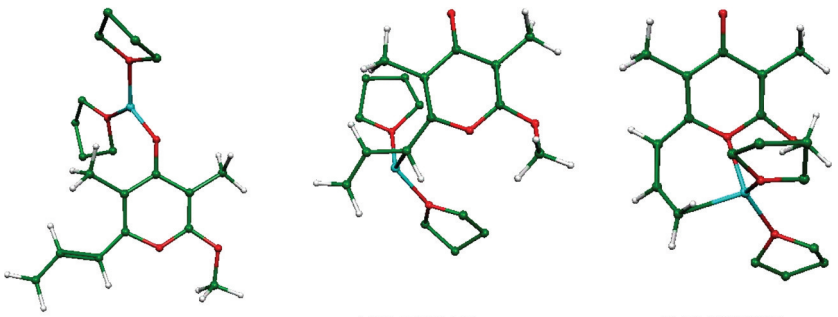


Figure 2. DFT calculations of **5**–**5″** with two discrete molecules of THF.

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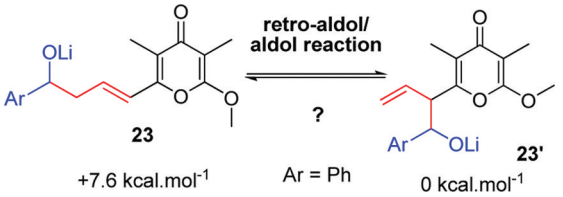
could be considered (see Scheme 9).[18] This equilibrium was likely to modify the distribution of isomers toward the thermodynamic product when the reaction reached room temperature. This hypothesis implies that **23**, the only isolated product, is the most stable one. However, DFT computations, ran at the same level as above on a model including two discrete molecules of THF, led to the conclusion that **23′** is more stable than **23** by 7.6 kcal mol^{-1} (see the Supporting Information for details). Parallel to these computations, a complementary reaction was run in which the temperature was maintained at −40 °C after addition of the aldehyde. In these conditions, the ε-isomer **23** was the sole product observed.[19] In light of these concording data, two conclusions can be drawn: the equilibrium between **23** and **23′** seems unlikely and the formation of **23** is under kinetic control.

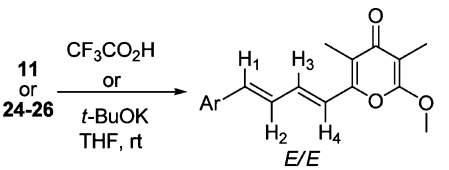
Scheme 9. Relative Stability of 23 and Its Isomer 23′ Calculated by DFT



Therefore, the one-pot process described here involves probably at least five transformations: formation of allyllithium, conjugate addition to the γ-pyrone, elimination of lithium methoxide, deprotonation, and, finally, aldolisation or alkylation. The aldol reaction may take place via a Zimmermam−Traxler transition state. With regard to the number of transformations involved, the yields ranging from 60 to 26% remain synthetically useful.

The conversion of some of the aldol products to dienes was next carried out. Connected to the α′-methoxy-γ-pyrone core, these conjugated products could be seen as analogues of cyercene A or salinipyrone. Hence, the dehydration of electron-rich **11** was conducted in CF_3CO_2H to deliver the (*E*/*E*)-diene **34** in 90% yield (see Scheme 10). Alternatively, the elimination of the benzoate of **21**, **22**, and **23** took place in the presence of *t*-BuOK at rt in lower yields (55−65%) and led stereoselectively to (*E*/*E*)-dienes **35**−**37**. The configurations of the double bonds of products **34**−**37** have been determined by ^{1}H NMR analysis.[20]

Scheme 10. Conversion of Alcohol 11 and Benzoates 24−26 to Dienes 34−37



34, Ar = 4-MeOPh, 90%, *E/E:E/Z* = 20 : 1
35, Ar = Ph, 60%, *E/E:E/Z* = 6 : 1
36, Ar = 4-NO_2Ph, 55%, *E/E:E/Z* = 6 : 1
37, Ar= furan, 65%, *E/E:E/Z* = 20 : 1

The potential bioactivities of these compounds were of interest. Indeed, verticipyrone is a known inhibitor of bovine heart mitochondrial respiratory chain function having reported IC_{50} values of 1.3 nM and 46 nM[6] or 64.9 ± 1.7 nM and 230.9 ± 5.7 nM[7] for the inhibition of NADH oxidase and NADH-ubiquinone oxidoreductase activities, respectively.

In this study, the inhibition of complex I by seven additional synthetic derivatives of verticipyrone was measured by monitoring their effects on NADH-ubiquinone oxidoreductase activity. NADH oxidase activity measures electron transport between complex I and IV of the mitochondrial electron transport chain.

Electrons are initially transported from complex I (NADH: ubiquinone oxidoreductase) to complex III (ubiquinol: cytochrome *c* oxidoreductase) by coenzyme Q_{10} and then to complex IV (cytochrome *c* oxidase) by the peripheral membrane protein cytochrome *c*. Of the analogues tested in the present study, structurally related compounds **10** and **11** showed the greatest affinity for complex I (Table 1).

Table 1. Respiratory Chain Inhibition by Verticipyrone Analogues

	NADH oxidase	NADH-ubiquinone oxidoreductase[a]		
compound	IC_{50} μM	IC_{50} (μM)	Imax (%)	half Imax (μM)
10	1.1 ± 0.08	3.9 ± 1.1	76.2 ± 3.7	2.7 ± 0.1
11	0.044 ± 0.005	2.8 ± 0.6	91.4 ± 1.1	2.5 ± 0.5
16	3.4 ± 0.3	21.5 ± 1.7	87.9 ± 1.1	17.0 ± 1.4
17	>20	56.3 ± 7.2	89.9 ± 0.9	46.8 ± 5.7
19	0.6 ± 0.1	4.7 ± 0.4	99.7 ± 1.7	4.7 ± 0.4
21	1.4 ± 0.2	59 ± 15	90.4 ± 1.1	54 ± 16
36	3.7 ± 0.8	51.3 ± 7.4	89.9 ± 6.3	44.8 ± 7.6

[a]The maximum activity of NADH-ubiquinone oxidoreductase was 1.03 ± 0.06 μmol min^{-1} mg^{-1} ± SD and was determined from at least five measurements.

The Imax value for compound **11** (91.4 ± 1.1%) was slightly greater than that observed for compound **10** (76.2 ± 1.1%), whereas the half Imax values for the two compounds were quite similar.

In the NADH oxidase assay, compound **11** was the most potent of the species studied (IC_{50} 0.044 ± 0.005 μM); its potency was comparable to the best of the analogues reported previously.[7] Interestingly, compound **10** exhibited much less inhibition (IC_{50} 1.1 ± 0.08 μM) in the NADH oxidase assay. The differences in results for these compounds in the two assays underscores the roles that small differences in inhibitor structure can have on the overall inhibitory effect on mitochondrial electron transport chain function.

The greater potency of compound **11** on NADH oxidase activity may reflect additional binding interactions for this compound with complex III or IV or possibly differences in the qualitative nature of its interaction with complex I.

Formal insertion of a —C(CH_3)=CH— group within the aliphatic side chain of **10** (to afford compound **19**) slightly decreased the affinity of the molecule for complex I and resulted in a greater half Imax value, but the Imax value itself was actually higher (99.7 ± 1.7%). Compound **19** also exhibited strong inhibition of NADH oxidase. In an earlier report, a compound with a shorter side chain was found to be >100-fold less inhibitory to complex I and more than 300-fold to the NADH oxidase activity.[7] In contrast, in the present study the effect of side chain length was much less pronounced, possibly because of the presence of unsaturation within the side chain, which must significantly reduce conformational flexibility.

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Replacement of the benzene ring by a *tert*-butyl group (compound **18**) led to a dramatic loss of complex I inhibition and to a smaller loss of NADH oxidase inhibitory activity. In contrast, Leiris et al.[7] found that removal of a phenyl ring within the side chain had more minimal effects. The differences may be due to overall chain length or to the presence of para substituents in the present case.

The formal dehydration of compound **10** produces compound **36**, which exhibited much less potent inhibition of NADH-ubiquinone oxidoreductase (IC_{50} 51.3 ± 7.4 μM). The hydroxyl group of **10** is thus essential for its affinity toward complex I. Compound **34** also exhibited less inhibition of electron transport through complex IV (IC_{50} 3.7 ± 0.8 μM).

For compounds **16** and **17**, the replacement of the benzene ring by a thiophene and an *N*-methylpyrrole led to a dramatic loss of affinity for complex I. The IC_{50} values determined were 21.5 ± 1.7 μM and 56.3 ± 7.2 μM, respectively. The Imax were comparable for both compounds (87.9 ± 1.1 and 89.9 ± 0.9%, respectively) and significantly higher than for **10**. The half Imax values (17.0 ± 1.4 and 46.8 ± 5.7 μM, respectively) reflected a greater loss of affinity for the analogues having a methylated pyrrole (**17**) than for the analogues having a thiophene ring (**16**). In the NADH oxidase assay, compound **17** was essentially inactive as an inhibitor (IC_{50} >20 μM), whereas compound **16** retained some inhibitory activity (IC_{50} 3.4 ± 0.2 μM).

■ CONCLUSIONS

The one-pot preparation of molecules containing the α′-methoxy-γ-pyrone core has been achieved. This procedure is remarkably simple, short, and open to molecular diversity. The whole transformation is based upon the unprecedented reactivity of lithiated α-allyl-α′-methoxy-γ-pyrone **5**; the latter reacts regio- and stereoselectively with electrophiles such as carboxaldehydes or allyl bromide. Furthermore, the conjugated dienes α′-methoxy-γ-pyrones have been prepared without transition metals from the aldol products. Interestingly, several of the compounds, including **10**, **11**, and **19**, are strong inhibitors of NADH oxidase and NADH-ubiquinone oxidoreductase activities.

■ EXPERIMENTAL SECTION

THF was distilled from sodium/benzophenone ketyl, and argon was passed through a pad of anhydrous $CaSO_4$/crystals of silica gel prior to use. α,α′-Dimethoxy-γ-pyrone,[9] tri(*n*-butyl)allylstannane,[21] 3,4-ethylenedioxythiophene-2-carboxaldehyde,[22] and (*E*)-2-methyl-3-(4-nitrophenyl)acrylaldehyde[23] were synthesized according to literature procedures. All reactions requiring anhydrous conditions were performed in oven (120 °C) and/or heat gun dried glassware under an atmosphere of desiccated argon. Syringes and needles were dried (90−120 °C) and cooled to rt prior use in desiccators containing P_2O_5 or anhydrous $CaSO_4$/crystals of SiO_2 as desiccants. ^{1}H NMR (300 and 200 MHz) and ^{13}C NMR (75 and 50.2 MHz) spectra were recorded at 293 K in $CDCl_3$ unless otherwise mentioned and using $(CH_3)_4Si$ and residual $CHCl_3$ as internal references. Chemical shifts (δ) are expressed in parts per million (ppm) and the coupling constants in hertz (Hz). Thin layer chromatography was performed on aluminum-backed plates precoated with silica gel (Merck, Silica Gel 60 F_{254}). Compounds were visualized by exposure to UV light (254 nm) and by dipping the plates in a solution of potassium permanganate ($KMnO_4$) in water. Unless otherwise stated, chromatographic separations were carried out under pressure on Merck silica gel 60 Å (70−230 mesh) pretreated, in some cases, with Et_3N (1%). The neutral alumina employed was Broeckmann grade III.

General Procedure I for the Syntheses of Compounds 10−22. In a Schlenck vessel under an argon atmosphere, a solution of α,α′-dimethoxy-γ-pyrone **3** (100 mg, 0.54 mmol) and tributyl(allyl)-stannane (360 mg, 337 μL, 1.08 mmol, 2 equiv) in THF (5 mL, 0.1 M) was cooled to −90 °C using a toluene/liquid nitrogen bath. After *n*-BuLi (1.6 M in hexanes, 747 μL, 2.2 equiv) was added dropwise, the solution went from colorless to shining red. After 20 min at this temperature, the electrophile (0.65 mmol, 1.2 equiv) was added dropwise. The resulting mixture was stirred between −90 and −78 °C for 30 min, after which time the temperature was allowed to rise to rt. Then a saturated NH_4Cl solution (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL), washed with brine (2 × 10 mL), and dried with anhydrous $MgSO_4$. After filtration and evaporation of the solvent, the crude products were purified by trituration or by flash chromatography.

(E)-2-(4-Hydroxy-4-(4-nitrophenyl)but-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***10.*** The residue was purified by trituration with CH_2Cl_2/pentane to give **10** (118 mg, 63%) as a gray powder: mp 158−159 °C; ^{1}H NMR (300 MHz) δ 8.20 (d, *J* = 8.7 Hz, 2H), 7.58 (d, *J* = 8.7 Hz, 2H), 6.70−6.12 (m, 2H), 5.01 (dd, *J* = 7.7, 4.6 Hz, 1H), 3.96 (s, 3H), 3.71 (bs, 1H), 3.14−2.36 (m, 2H), 1.93 (s, 3H), 1.82 (s, 3H); ^{13}C NMR (75 MHz) δ 181.2, 161.9, 151.4, 147.3, 132.1 (2C), 126.6 (2C), 123.7 (2C), 122.9, 118.2, 99.5, 72.3, 55.4, 42.9, 9.6, 7.0; IR (KBr disk) ν 3392, 2916, 1664, 1630, 1568, 1514, 1340, 1261, 1166, 1055, 854. HRMS (ESI) for $C_{18}H_{20}NO_6$ Calcd: 346.1291 $[M + H]^+$. Found: 346.1299.

(E)-2-(4-Hydroxy-4-(4-methoxyphenyl)but-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***11.*** The residue was purified by flash column chromatography to give **11** (100 mg, 56%) as a white solid: R_f = 0.4 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; mp 149−150 °C; ^{1}H NMR (300 MHz) δ 7.21 (d, *J* = 8.7 Hz, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.37−6.29 (m, 2H), 4.71 (dd, *J* = 7.3, 5.3 Hz, 1H), 3.84 (s, 3H), 3.70 (s, 3H), 3.38 (bs, 1H), 2.80−2.30 (m, 2H), 1.84 (s, 3H), 1.72 (s, 3H); ^{13}C NMR (75 MHz) δ 181.2, 161.8, 159.0, 151.7, 136.2, 133.5, 127.0 (2C), 122.0, 117.7, 113.7 (2C), 99.2, 73.0, 55.3 (2C), 42.8, 9.5, 7.0; IR (KBr disk) ν 3375, 1666, 1578, 1468, 1261, 1241, 1175. HRMS (ESI) for $C_{19}H_{23}O_5$ Calcd: 331.1545 $[M + H]^+$. Found: 331.1546.

(E)-2-(4-(4-Bromophenyl)-4-hydroxybut-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***12.*** The residue was purified by trituration with CH_2Cl_2/pentane to give **12** as a white powder (82 mg, 40%): mp 137−138 °C; ^{1}H NMR (300 MHz) δ 7.37 (d, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 8.4 Hz, 2H), 6.35−6.28 (m, 2H), 4.75 (t, *J* = 6.2 Hz, 1H), 3.86 (s, 3H), 3.73 (bs, 1H), 2.63−2.52 (m, 2H), 1.84 (s, 3H), 1.73 (s, 3H); ^{13}C NMR (75 MHz) δ 181.3, 161.8, 151.6, 143.1, 132.9, 131.4 (2C), 127.6 (2C), 122.3, 121.2, 117.8, 99.3, 72.6, 55.3, 42.8, 9.5, 7.0; IR (KBr disk) ν 3352, 1663, 1596, 1573, 1466, 1263, 1163. HRMS (ESI) for $C_{18}H_{20}BrO_4$ Calcd: 379.0545 $[M + H]^+$. Found: 379.0559.

(E)-2-(4-Hydroxy-4-phenylbut-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***13.*** The residue was purified by flash column chromatography to give **13** (78 mg, 48%) as a pale yellow solid: R_f = 0.6 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; mp 128.5−129.0 °C; ^{1}H NMR (200 MHz) δ 7.39−7.06 (m, 5H), 6.40−6.18 (m, 2H), 4.75 (t, *J* = 6.3 Hz, 1H), 3.81 (s superimposed to a bs, 4H), 2.71−2.47 (m, 2H), 1.82 (s, 3H), 1.71 (s, 3H); ^{13}C NMR (50.2 MHz) δ 181.1, 161.4, 151.3, 143.9, 133.3 (2C), 127.8, 127.4, 125.3, 121.8 (2C), 117.3, 99.1, 73.0, 54.9, 42.6, 9.1, 6.6; IR (KBr disk) ν 3316, 1667, 1633, 1570, 1467, 1417, 1259, 1168. HRMS (ESI) for $C_{18}H_{21}O_4$ Calcd: 301.1440 $[M + H]^+$. Found: 301.1427.

(E)-2-(4-Hydroxy-4-(pyridin-4-yl)but-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***14.*** The residue was purified by an acid/base workup (2 M HCl or citric acid/10% w/w NaOH) followed by ammonia-impregnated preparative TLC to give **14** (76 mg, 47%) as a light brown oil: R_f = 0.2 (CH_2Cl_2/MeOH/NH_3, 9:1) [UV, $KMnO_4$]; ^{1}H NMR (300 MHz) δ 8.60 (s br, 2H), 7.34 (d br, *J* = 3.5 Hz, 2H), 6.47 (d, *J* = 16.0 Hz, 1H), 6.38 (m, 1H), 4.90 (m, 1H), 3.95 (s, 3H), 2.70 (m, 2H), 1.97 (s, 3H), 1.85 (s, 3H); ^{13}C NMR (50.2 MHz) δ 180.9, 161.6, 153.7, 151.4, 149.1, 132.5, 122.2, 120.8, 117.5, 99.0, 71.2, 55.0, 42.2, 9.2, 6.7; IR (film) ν 3332, 3054, 1665, 1602, 1416, 1265, 1166. HRMS (ESI) for $C_{17}H_{20}NO_4$ Calcd: 302.1392 $[M + H]^+$. Found: 302.1387.

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(E)-2-(4-(Furan-2-yl)-4-hydroxybut-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***15.*** The residue was purified by flash column chromatography to give **15** (50 mg, 32%) as a yellow-orange solid: R_f = 0.4 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; mp 116.5−118.6 °C; ^{1}H NMR (200 MHz) δ 7.34 (dd, J = 1.8, 0.9 Hz, 1H), 6.45−6.36 (m, 2H), 6.31−6.25 (m, 2H), 4.85 (t, J = 6.4 Hz, 1H), 3.92 (s, 3H), 3.66 (bs, 1H), 2.82−2.76 (m, 2H), 1.91 (s, 3H), 1.79 (s, 3H); ^{13}C NMR (50.2 MHz) δ 180.9, 161.6, 155.9, 151.4, 141.7, 132.4, 122.1, 117.7, 110.0, 106.0, 99.1, 66.6, 55.2, 39.2, 9.3, 6.7; IR (KBr disk) ν 2926, 1667, 1572, 1470, 1422, 1341, 1263, 1170. HRMS (ESI) for $C_{16}H_{18}O_5$ Calcd: 291.1232 [M + H]$^+$. Found: 291.1225.

(E)-2-(4-Hydroxy-4-(thiophen-2-yl)but-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***16.*** The residue was purified by flash column chromatography to give **16** (71 mg, 43%) as a pale yellow solid: R_f = 0.55 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; mp 127.5−128.8 °C; ^{1}H NMR (200 MHz) δ 7.15 (dd, J = 4.8, 1.4 Hz, 1H), 7.05−6.77 (m, 2H), 6.92−6.82 (m, 2H), 5.04 (t, J = 6.3 Hz, 1H), 3.86 (s, 3H) superimposed to 4.1−3.8 (bs, 1H), 2.85−2.63 (m, 2H), 1.85 (s, 3H), 1.73 (s, 3H); ^{13}C NMR (50.2 MHz) δ 181.0, 161.7, 151.5, 147.9, 132.6, 126.4, 124.3, 123.4, 122.2, 117.7, 99.1, 69.1, 55.1, 42.7, 9.3, 6.8; IR (KBr disk) ν 3313, 1666, 1632, 1594, 1571, 1468, 1422, 1170, 1038. HRMS (ESI) for $C_{16}H_{19}O_4S$ Calcd: 307.1004 [M + H]$^+$. Found: 307.1004.

(E)-2-(4-Hydroxy-4-(1-methyl-1H-pyrrol-2-yl)but-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***17.*** The residue was purified by flash column chromatography to give **17** (46 mg, 28%) as a pale yellow solid: R_f = 0.55 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; mp 104.3−106.1 °C; ^{1}H NMR (200 MHz) δ 6.63−6.56 (m, 1H), 6.54−6.39 (m, 2H), 6.11 (dd, J = 3.6, 1.8 Hz, 1H), 6.08−6.00 (m, 1H), 4.80 (t, J = 6.7 Hz, 1H), 3.96 (s, 3H), 3.69 (s, 3H), 2.84 (t, J = 6.3 Hz, 2H), 2.71 (bs, 1H), 1.96 (s, 3H), 1.82 (s, 3H); ^{13}C NMR (50.2 MHz) δ 180.9, 161.6, 151.4, 133.6, 133.0, 123.1, 122.0, 117.7, 106.4, 106.0, 99.1, 65.6, 55.2, 40.0, 34.0, 9.3, 6.8; IR (KBr disk) ν 3311, 1664, 1380, 1340, 1261, 1169, 1039. HRMS (ESI) for $C_{17}H_{22}NO_4$ Calcd: 304.1549 [M + H]$^+$. Found: 304.1553.

(E)-2-(4-(2,3-Dihydrothieno[3,4-b][1,4]dioxin-5-yl)-4-hydroxybut-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***18.*** The residue was purified by flash column chromatography to give **18** (67 mg, 28%) as a white solid: R_f = 0.3 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; mp 79.2−80.0 °C; ^{1}H NMR (200 MHz) δ 6.49 (m, 2H), 6.26 (s, 1H), 5.10 (t, J = 6.2 Hz, 1H), 4.19 (s, 3H), 3.97 (s, 3H), 2.85−2.74 (m superimposed to 2.71 bs, 3H), 1.98 (s, 3H), 1.84 (s, 3H); ^{13}C NMR (50.2 MHz) δ 181.0, 161.6, 151.5, 141.1, 137.2, 132.7, 122.0, 120.2, 117.6, 99.0, 97.3, 66.0, 64.5, 55.1, 41.0, 9.3, 6.8; IR (KBr disk) ν 3312, 1664, 1560, 1437, 1382, 1261, 1166, 1065. HRMS (ESI) for $C_{18}H_{21}O_6S$ Calcd: 365.1059 [M + H]$^+$. Found: 365.1075.

2-((1E,5E)-4-Hydroxy-5-methyl-6-(4-nitrophenyl)hexa-1,5-dienyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***19.*** The residue was purified by flash column chromatography to give **19** (64 mg, 32%) as a pale yellow solid: R_f = 0.4 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; mp 147−148 °C; ^{1}H NMR (300 MHz) δ 8.14 (d, J = 8.6 Hz, 2H), 7.36 (d, J = 8.6 Hz, 2H), 6.64 (s, 1H), 6.50−6.42 (m, 2H), 4.36 (dd, J = 7.2, 4.5 Hz, 1H), 3.92 (s, 3H), 3.57 (bs, 1H), 2.78−2.47 (m, 2H), 1.94 (s, 6H), 1.80 (s, 3H); ^{13}C NMR (75 MHz) δ 180.2, 160.9, 150.7, 145.0, 143.4, 143.3, 132.4 (2C), 128.5 (2C), 122.5 (2C), 121.1, 116.8, 98.3, 74.9, 54.3, 38.2, 13.6, 8.6, 6.0; IR (KBr disk) ν 3352, 1664, 1595, 1514, 1341, 1260, 1167. HRMS (ESI) for $C_{21}H_{23}NO_6$ Calcd: 386.1604 [M + H]$^+$. Found: 386.1602.

(E)-2-(4-Cyclohexyl-4-hydroxybut-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***20.*** The residue was purified by flash column chromatography to give **20** (55 mg, 33%) as a pale yellow solid: R_f = 0.6 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; mp 123.4−125.2 °C; ^{1}H NMR (200 MHz) δ 6.65−6.22 (m, 2H), 3.94 (s, 3H), 3.47 (ddd, J = 9.0, 5.6, 3.5 Hz, 1H), 2.78 (bs, 1H), 2.57−2.19 (m, 2H), 1.91 (s, 3H), 1.78 (s, 3H) superimposed to 1.84−1.52 (m, 6H), 1.49−0.87 (m, 5H); ^{13}C NMR (50.2 MHz) δ 181.0, 161.6, 151.6, 134.6, 121.4, 117.2, 98.9, 74.8, 55.1, 43.5, 37.0, 28.9, 28.0, 26.2, 26.0, 25.9, 9.3, 6.8; IR (KBr disk) ν 3315, 2925, 1665, 1601, 1461, 1337, 1261, 1166. HRMS (ESI) for $C_{18}H_{27}O_4$ Calcd: 307.1909 [M + H]$^+$. Found: 307.1907.

(E)-2-(4-Hydroxy-5,5-dimethylhex-1-enyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, ***21.*** The residue was purified by flash column chromatography to give **21** (39 mg, 26%) as a white solid: R_f = 0.5 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; mp 137.1−138.0 °C; ^{1}H NMR (200 MHz) δ 6.52−6.36 (m, 2H), 3.98 (s, 3H), 3.37 (dd, J = 10.4, 2.1 Hz, 1H), 2.49 (ddd, J = 14.5, 5.7, 2.1 Hz, 1H), 2.23 (m, 2H), 1.96 (s, 3H), 1.83 (s, 3H), 0.95 (s, 9H); ^{13}C NMR (50.2 MHz) δ 180.9, 161.6, 151.6, 135.4, 121.3, 117.3, 99.0, 78.5, 55.1, 35.4, 34.9, 25.5, 9.3, 6.8; IR (KBr disk) ν 3435, 2961, 1596, 1575, 1471, 1344, 1264, 1163. HRMS (ESI) for $C_{16}H_{25}O_4$ Calcd: 281.1753 [M + H]$^+$. Found: 281.1752.

3-(Hexa-1,5-dien-3-yl)-5-methoxy-2,6-dimethylcyclohexa-2,5-dienone, ***22.*** The residue was purified by flash column chromatography to give **22** (65 mg, 51%) as a brown oil: R_f = 0.4 (neutral alumina, Et_2O/pentane 7/3) [UV, $KMnO_4$]; ^{1}H NMR (300 MHz) δ 5.85 (ddd, J = 15.3, 10.3, 7.1 Hz, 1H), 5.68 (ddt, J = 17.1, 10.1, 7.0 Hz, 1H), 5.16−4.95 (m, 4H), 3.92 (s, 3H), 3.60 (dt, J = 15.3, 7.0 Hz, 1H), 2.58−2.36 (m, 2H), 1.93 (s, 3H), 1.81 (s, 3H); ^{13}C NMR (75 MHz), δ 180.9, 162.0, 157.5, 135.9, 134.6, 118.9, 117.3, 116.8, 99.3, 55.3, 44.6, 36.3, 9.6, 6.9; IR (film) ν 2928, 1746, 1667, 1601, 1463, 1324, 1252; MS (IE) 234 (M$^+$, 37), 193 (35), 165 (100), 105 (41), 83 (46), 77 (36). HRMS (IE, 70 eV) for $C_{14}H_{18}O_3$ Calcd: 234.1256 [M]$^+$. Found: 234.1262.

General Procedure II for the Syntheses of Esters 24−26. Following the general procedure I with the exception of the workup, which is as follows: The solution was cooled to 0 °C and treated with benzoyl chloride (153 mg, 126 μL, 1.08 mmol, 2 equiv). The mixture was stirred for 4 h, during which time the temperature was allowed to rise to rt. Then a saturated $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL), washed with brine (2 × 10 mL), and dried with anhydrous $MgSO_4$. After filtration and removal of the solvent under reduced pressure, the crudes were purified by flash column chromatography.

(E)-4-(6-Methoxy-3,5-dimethyl-4-oxo-4H-pyran-2-yl)-1-(4-nitrophenyl)but-3-enyl Benzoate, ***24.*** The title product was obtained as a yellow solid (54 mg, 54%): R_f = 0.7 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; mp 72.5−73.2 °C; ^{1}H NMR (200 MHz) δ 8.19 (d, J = 8.8 Hz, 2H), 8.03 (dd, J = 8.4, 1.3 Hz, 2H), 7.71−7.50 (m, 3H), 7.50−7.34 (m, 2H), 6.55−6.26 (m, 2H), 6.19 (dd, J = 7.6, 5.5 Hz, 1H), 3.84 (s, 3H), 3.09−2.79 (m, 2H), 1.89 (s, 3H), 1.78 (s, 3H); ^{13}C NMR (50.2 MHz) δ 180.6, 165.2, 161.5, 150.7, 147.5, 146.7, 133.5, 129.6, 129.4, 129.1, 128.4, 126.9, 123.7, 123.4, 118.4, 99.3, 74.0, 55.0, 39.6, 9.2, 6.7; IR (film) ν 1722, 1666, 1608, 1347, 1267, 1108. HRMS (ESI) for $C_{25}H_{24}NO_7$ Calcd: 450.1553 [M + H]$^+$. Found: 450.1564.

(E)-4-(6-Methoxy-3,5-dimethyl-4-oxo-4H-pyran-2-yl)-1-phenylbut-3-enyl Benzoate, ***25.*** The title product was obtained as a yellow oil (51 mg, 47%): R_f = 0.7 (AcOEt/pentane 7/3, 1% Et_3N) [UV, $KMnO_4$]; ^{1}H NMR (200 MHz) δ 8.05 (dd, J = 8.1, 1.2 Hz, 2H), 7.60−7.20 (m, 8H), 6.54−6.22 (m, 2H), 6.13 (dd, J = 7.4, 5.9 Hz, 1H), 3.80 (s, 3H), 3.08−2.76 (m, 2H), 1.90 (s, 3H), 1.80 (s, 3H); ^{13}C NMR (50.2 MHz) δ 180.7, 165.4, 161.5, 150.9, 139.3, 133.0, 130.8, 129.8, 129.3, 128.4, 128.3, 128.1, 126.1, 122.7, 118.0, 99.1, 75.0, 54.9, 39.8, 9.2, 6.7; IR (film) ν 3061, 2955, 1719, 1634, 1464, 1269, 1110. HRMS (ESI) for $C_{25}H_{25}O_5$ Calcd: 405.1702 [M + H]$^+$. Found: 405.1695.

(E)-1-(Furan-2-yl)-4-(6-methoxy-3,5-dimethyl-4-oxo-4H-pyran-2-yl)but-3-enyl Benzoate, ***26.*** The title product was obtained as a pale yellow oil (81 mg, 38%): R_f = 0.6 (AcOEt/pentane 3/2, 1% Et_3N) [UV, $KMnO_4$]; ^{1}H NMR (200 MHz) δ 8.07−7.95 (m, 2H), 7.59−7.24 (m, 4H), 6.52−6.25 (m, 4H), 6.20 (t, J = 7.0 Hz, 1H), 3.83 (s, 3H), 3.03 (dt, J = 7.0, 2.7 Hz, 2H), 1.90 (s, 3H), 1.79 (s, 3H); ^{13}C NMR (50.2 MHz) δ 180.7, 165.4, 161.5, 151.3, 150.9, 142.6, 133.1, 130.2, 129.5, 129.4, 128.2, 122.9, 118.1, 110.2, 108.9, 99.1, 67.8, 54.9, 36.0, 9.2, 6.7; IR (film) ν 2955, 1719, 1667, 1609, 1263, 1168. HRMS (ESI) for $C_{23}H_{23}O_6$ Calcd: 395.1495 [M + H]$^+$. Found: 395.1479.

(4Z,6E)-Methyl-6-ethoxy-5-methoxy-2,4-dimethyl-3-oxonona-4,6,8-trienoate, ***30.*** In a dry Schlenck tube, a solution of freshly sublimated *t*-BuOK (245 mg, 2.3 mmol, 2.5 equiv) in THF (7 mL) was cooled to −78 °C under N_2 atmosphere. (*E*)-1,1-Diethoxybut-2-ene (130 mg, 0.9 mmol, 1 equiv) in THF (2 mL) and *n*-BuLi (1.4 mL,

2.3 mmol, 1.6 M in hexane, 2.5 equiv) were successively added, and the mixture was stirred for 2 h, during which the temperature rose to −40 °C. Simultaneously, in a 25 mL flask, a mixture of **3** (284 mg, 1 mmol, 1.1 equiv) and CuCl (99 mg, 1 mmol, 1.1 equiv) was stirred in THF (8 mL) under N_2 atmosphere at rt for 2 h. The Schlenck tube was refrigerated to −78 °C, and the mixture contained in the flask was transferred into the Schlenck tube. The resulting mixture was stirred at −78 °C for 1 h. Saturated NH_4Cl solution (10 mL) was added, and the mixture was extracted with AcOEt (3 × 10 mL), washed with water (10 mL), brine (2 × 10 mL), and dried over anhydrous K_2CO_3. After filtration and evaporation of the solvent, the crude product was purified by flash chromatography (petroleum ether/AcOEt 80:20 and 1% of Et_3N) to give **30** (140 mg, 55%) as a pale yellow oil: R_f = 0.6 [UV, $KMnO_4$)]; ^{1}H NMR (300 MHz) δ 6.18 (dt, J = 16.7, 10.4 Hz, 1H), 5.70 (d, J = 10.4 Hz, 1H), 5.15 (d, J = 16.7 Hz, 1H), 4.97 (d, J = 10.4 Hz, 1H), 4.13 (m, 1H), 3.85 (q, J = 7.0 Hz, 2H), 3.69 (s, 3H), 3.59 (s, 3H), 1.70 (s, 3H), 1.35 (m, 6H); ^{13}C NMR (75 MHz) δ 197.3, 172.6, 156.2, 149.4, 131.5, 117.0, 115.5, 108.6, 63.6, 56.0, 52.7, 51.9, 14.6, 14.1, 13.5; IR (film) ν 2943, 1740, 1666, 1455, 1374, 1202. HRMS (ESI) for $C_{15}H_{23}O_5$ Calcd: 283.1545 [M + H]$^+$. Found: 283.1538.

*4-Allyl-6-methoxy-3,5-dimethyl-2H-pyran-2-one, **33**.* In a 25 mL Schlenck tube, a mixture of **3** (284 mg, 1 mmol) and flame-dried LiBr (435 mg, 5 mmol, 5 equiv) was stirred in THF (8 mL) under an argon atmosphere at rt for 1 h. The suspension was cooled to −78 °C, a solution of allylmagnesium bromide (1.5 mL, 1.5 mmol, 1.5 equiv, 1 M in Et_2O) was added dropwise, and the reaction mixture was stirred at −78 °C for 2 h. Saturated NH_4Cl solution (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL), washed sequentially with water (10 mL) and brine (2 × 10 mL), and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent, the crude product was purified by flash chromatography (pentane/AcOEt 4/1, 1% Et_3N) to give **33** as a white solid (107 mg, 55%): mp 75−76 °C; R_f = 0.8 (pentane/AcOEt 4/1) [UV, $KMnO_4$]; ^{1}H NMR (200 MHz) δ 5.86−5.66 (m, 1H), 5.03 (m, 1H), 4.88 (m, 1H), 3.89 (s, 3H), 3.43−3.33 (m, 2H), 2.10 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (50.2 MHz) δ 157.8, 157.3, 150.3, 133.5, 115.6, 109.6, 108.2, 54.0, 33.5, 10.8, 10.6; IR (film) ν 2928, 1693, 1643, 1469, 1134, 915; MS (EI, 70 eV) m/z [M − 1]$^+$ 193 (100), 178, 160, 150, 132, 122, 107. HRMS (EI): for $C_{11}H_{13}O_3$ Calcd: 193.0865 [M − 1]$^+$. Found: 193.0860.

*2-((1E,3E)-4-(Furan-2-yl)buta-1,3-dienyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, **34**.* In 25 mL, single-neck round-bottom flask, the (*E*)-2-(4-hydroxy-4-(4-methoxyphenyl)but-1-enyl)-6-methoxy-3,5-dimethyl-4*H*-pyran-4-one **11** (100 mg, 0.3 mmol) was stirred in the presence of TFA (2 mL) at 0 °C. The temperature was then allowed to rise to rt. After 30 min, an aqueous solution of NaOH (10% w/v) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL), washed with brine (10 mL), and dried with anhydrous $MgSO_4$. After filtration and evaporation of the solvent, the product **34** was isolated without further purification as a yellow solid (90 mg, 95%) without distinguishable other isomers of the double bonds by ^{1}H NMR analysis: R_f = 0.7 (AcOEt/pentane 7/3) [UV, $KMnO_4$]; mp 156−152 °C; ^{1}H NMR (200 MHz) δ 7.40 (d, J = 8.7 Hz, 2H), 7.01 (dd, J = 15.0, 9.6 Hz, 1H), 6.89 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 9.7 Hz, 1H), 6.75 (d, J = 15.3 Hz, 1H), 6.54 (d, J = 15.1 Hz, 1H), 4.07 (s, 3H), 3.83 (s, 3H), 2.06 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (50.2 MHz) δ 180.9, 161.7, 160.1, 152.3, 136.8, 134.1, 129.3, 128.2, 125.6, 119.9, 118.5, 114.3, 99.6, 55.4, 55.4, 9.7, 7.0; IR (KBr disk) ν 1654, 1608, 1510, 1407, 1255, 1179, 1033, 979. HRMS (ESI) for $C_{19}H_{21}O_4$ Calcd: 313.1440 [M + H]$^+$. Found: 313.1456.

General Procedure III for the Syntheses of Dienes 35−37. In a 25 mL single necked round-bottom flask, a solution of ester (0.3 mmol) in THF (3 mL) was stirred in the presence of *t*-BuOK (0.4 mmol, 45 mg, 1.2 equiv) at rt, and the reaction progress monitored by TLC. After 2 h a small amount of water was added. The mixture was extracted with CH_2Cl_2 (3 × 10 mL), washed with brine (10 mL), and dried with anhydrous $MgSO_4$. After filtration and evaporation of the solvent, the crude was purified by flash chromatography.

*2-Methoxy-3,5-dimethyl-6-((1E,3E)-4-phenylbuta-1,3-dienyl)-4H-pyran-4-one, **35**.* The title product was obtained from **25** as a white solid (51 mg, 60%): R_f = 0.6 (AcOEt/pentane 3/2, 1% Et_3N) [UV, $KMnO_4$]; mp 245.6−247.5 °C; ^{1}H NMR (200 MHz) δ 7.49−7.29 (m, 5H), 7.11−6.90 (m, 2H), 6.81 (d, J = 15.0 Hz, 1H), 6.61 (d, J = 14.3 Hz, 1H), 4.09 (s, 3H), 2.07 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (50.2 MHz) δ 180.6, 161.5, 151.9, 136.9, 136.3, 133.4, 128.6, 128.4, 127.4, 126.6, 120.9, 118.8, 99.5, 55.2, 9.5, 6.8; IR (KBr disk) ν 1653, 1595, 1464, 1413, 1262. HRMS (ESI) for $C_{18}H_{19}O_3$ Calcd: 283.1334 [M + H]$^+$. Found: 283.1314.

*2-Methoxy-3,5-dimethyl-6-((1E,3E)-4-(4-nitrophenyl)buta-1,3-dienyl)-4H-pyran-4-one, **36**.* The title product was obtained from **26** as a yellow solid (54 mg, 55%): R_f = 0.7 (AcOEt/pentane 3/2, 1% Et_3N) [UV, $KMnO_4$]; mp 209.1−210.0 °C; ^{1}H NMR (200 MHz) δ 8.22 (d, J = 8.8 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 7.21−6.90 (m, 2H), 6.84 (d, J = 14.2 Hz, 1H), 6.74 (d, J = 14.2 Hz, 1H), 4.09 (s, 3H), 2.09 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (50.2 MHz) δ 180.4, 161.5, 151.2, 147.0, 142.6, 133.8, 132.1, 131.7, 127.0, 124.0, 123.8, 120.2, 99.9, 55.3, 9.6, 6.9; IR (KBr disk) ν 1653, 1595, 1335, 1259. HRMS (ESI) for $C_{18}H_{18}NO_5$ Calcd: 328.1185 [M + H]$^+$. Found: 328.1175.

*2-((1E,3E)-4-(Furan-2-yl)buta-1,3-dienyl)-6-methoxy-3,5-dimethyl-4H-pyran-4-one, **37**.* The title product was obtained from **27** as a pale yellow solid (53 mg, 65%) without distinguishable other isomers of the double bonds by ^{1}H NMR analysis: R_f = 0.6 (AcOEt/pentane 3/2, 1% Et_3N) [UV, $KMnO_4$]; mp 135.8−136.0 °C; ^{1}H NMR (200 MHz) δ 7.40 (s, 1H), 7.00−6.75 (m, 2H), 6.55 (d, J = 15.0 Hz, 1H), 6.53 (d, J = 15.0 Hz, 1H), 6.46−6.34 (m, 2H), 4.03 (s, 3H), 2.02 (s, 3H), 1.85 (s, 3H); ^{13}C NMR (50.2 MHz) δ 180.6, 161.4, 152.3, 151.9, 143.0, 133.0, 125.8, 123.7, 120.7, 118.7, 111.9, 110.5, 99.4, 55.2, 9.5, 6.8; IR (KBr disk) ν 1653, 1602, 1415, 1339, 1284, 1168, 991. HRMS (ESI) for $C_{16}H_{17}O_4$ Calcd: 273.1127 [M + H]$^+$. Found: 273.1132.

Submitochondrial Particle (SMP) Preparation. Mitochondria were prepared as described.[24,25] One bovine heart was ground and blended in sucrose buffer (0.25 M sucrose, 10 mM Tris-HCl, pH 7.8, containing 0.2 mM EDTA) at 4 °C. Cellular debris was removed by centrifugation at 1200*g* for 20 min. The supernatant was filtered through two layers of cheesecloth. The mitochondria were harvested by centrifugation at 26000*g* for 15 min and then homogenized in the same buffer. The mitochondria were harvested by centrifugation at 12000*g* for 30 min and then stored at −80 °C in a sucrose buffer.

Submitochondrial particles (SMPs), were prepared as described.[25,26] The mitochondria were sonicated with a Sonic Dismembrator (Fisher Scientific) in 0.25 M sucrose containing 5 mM $MgCl_2$, 1 mM ATP, 10 mM $MnCl_2$, and 1 mM sodium succinate in 10 mM Tris-HCl, pH 7.8, at 4 °C. Cell debris was pelleted by centrifugation at 20000*g* for 7 min at 4 °C. The SMPs were harvested by centrifugation at 152000*g* for 30 min at 4 °C and stored at −80 °C in 0.25 M sucrose cotaining 5 mM $MgCl_2$, 2 mM ATP, 2 mM glutathione, and 1 mM sodium succinate in 10 mM Tris-HCl, pH 7.5. Protein concentration was determined by BCA titration (Pierce) using albumin for the standard curve.

NADH: Ubiquinone Oxidoreductase Activity Measured Using SMPs. The inhibition of NADH-ubiquinone oxidoreductase activity was determined under the same experimental conditions reported.[7,27] Twenty-five micrograms of SMPs were incubated at 39 °C for 5 min with the test compound in 1 mL of 0.25 M sucrose containing 1 mM $MgCl_2$, 2 μM antimycin A, 2 mM KCN, and 50 mM phosphate buffer, pH 7.4. The reaction was initiated by the addition of 50 μM NADH and 50 μM coenzyme Q_1. The enzymatic activity, at 39 °C, was monitored at 340 nm.

NADH Oxidase Activity Assay. Bovine heart mitochondria were obtained by a large-scale procedure.[24] Inverted submitochondrial particles (SMPs) were prepared by the method of Matsuno-Yagi and Hatefi[28] and stored in a buffer containing 0.25 M sucrose in 10 mM Tris-HCl, pH 7.4, at −80 °C. The inhibitory effects of test compounds on bovine heart mitochondrial complex I, II, and IV were evaluated. The compounds were dissolved in dimethylsulfoxide (DMSO) and then used to make serial dilutions. Maximal DMSO concentrations never exceeded 2% and had no influence on the control enzymatic activity. Bovine heart SMPs were diluted to 0.5 mg/mL. After the pre-equilibration of 30 μg/mL of SMP with inhibitor for 5 min at 30 °C in

2.5 mL of 50 mM Hepes, pH 7.5, and 5 mM $MgCl_2$, the oxidation of 50 μM of NADH at 30 °C was monitored spectrophotometrically with a Beckman Coulter DU-530 (340 nm, ε 6.22 mM^{-1} cm^{-1}). The initial rates were calculated from the linear portion of the traces.

■ ASSOCIATED CONTENT

Supporting Information

^1H and ^{13}C NMR spectra of new compounds and absolute energies and atom coordinates for DFT calculations of **5**−**5**″ and **23**−**23**′. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: michael.depaolis@univ-rouen.fr.

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