Synthesis of Multifunctional Radical Quenchers (MRQ’s)

By

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Doctor of Philosophy

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ABSTRACT

Mitochondria produce most of the ATP needed for the cell as an energy source. It is well known that cellular respiration results in oxidative damage to the cell due to the production of reactive oxygen species (ROS). Mitochondrial dysfunction is believed to contribute to a number of degenerative diseases; because of this the mitochondrial respiratory chain is considered as potential drug target.

A few series of idebenone analogues with quinone, pyridinol and pyrimidinol redox cores have been synthesized and evaluated as antioxidants able to protect cellular integrity and, more specifically, mitochondrial function. The compounds exhibited a range of activities. The activities observed were used for the design of analogues with enhanced properties as antioxidants. Compounds were identified which provide better protection against oxidative stress than idebenone, and it is thought that they do so catalytically.
ACKNOWLEDGEMENTS

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<tbody>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>APCI</td>
<td>atmospheric pressure chemical ionization</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>aq</td>
<td>aqueous</td>
</tr>
<tr>
<td>BDE</td>
<td>bond dissociation energy</td>
</tr>
<tr>
<td>br s</td>
<td>broad singlet</td>
</tr>
<tr>
<td>br m</td>
<td>broad multiplet</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>conc</td>
<td>concentrated</td>
</tr>
<tr>
<td>C⁵¹¹ BODIPY⁵⁸¹/⁵⁹¹</td>
<td>4,4-difluoro-5-(4-phenyl-1,3-butenyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid</td>
</tr>
<tr>
<td>CoQ⁰</td>
<td>coenzyme Q⁰</td>
</tr>
<tr>
<td>CoQ¹</td>
<td>coenzyme Q¹</td>
</tr>
<tr>
<td>CoQ¹⁰</td>
<td>coenzyme Q¹⁰</td>
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<tr>
<td>CV</td>
<td>cyclic voltametry</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
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<td>dd</td>
<td>doublet of doublet</td>
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<tr>
<td>dt</td>
<td>doublet of triplet</td>
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<tr>
<td>ddd</td>
<td>doublet of doublet of doublet</td>
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<tr>
<td>ddt</td>
<td>doublet of doublet of triplet</td>
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<tr>
<td>DCF</td>
<td>2',7'-dichlorofluorescein</td>
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DCFH 2',7'-dichlorodihydrofluorescein
DCFH-DA 2',7'-dichlorodihydrofluorescein diacetate
DCM dichloromethane
DEM diethyl maleate
DMF $N,N$-dimethylformamide
DMSO dimethylsulfoxide
EI electronic ionization
ESR electron spin resonance
FAB fast atomic bombardment
FACS fluorescence-activated cell sorting
FADH flavin adenine dinucleotide
FBS fetal bovine serum
FCCP carbonyl cyanide-$p$-trifluoromethoxyphenylhydrazone
FRDA Friedreich’s ataxia
g grams
GSH glutathione
h hour(s)
Hz hertz
IC inhibitory concentration
IP ionization potential
$J$ coupling constant
LDL low density lipoproteins
M molar
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<tr>
<td>M+</td>
<td>molecular ion</td>
</tr>
<tr>
<td>MELAS</td>
<td>mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>mg</td>
<td>milligram(s)</td>
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<tr>
<td>mL</td>
<td>milliliter(s)</td>
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<tr>
<td>mM</td>
<td>millimolar</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole(s)</td>
</tr>
<tr>
<td>MOM</td>
<td>methoxymethyl</td>
</tr>
<tr>
<td>mp</td>
<td>melting point</td>
</tr>
<tr>
<td>N</td>
<td>normal</td>
</tr>
<tr>
<td>NADH</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NBS</td>
<td>( N)-bromosuccinimide</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer(s)</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffer saline</td>
</tr>
<tr>
<td>PIDA</td>
<td>phenyliodine diacetate</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>( R_f )</td>
<td>ratio of fronts</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>satd</td>
<td>saturated</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
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<td>------------</td>
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<tr>
<td>S.E.M.</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SMPs</td>
<td>submitochondrial particles</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMEDA</td>
<td>$N,N,N',N'$-tetramethylethylenediamine</td>
</tr>
<tr>
<td>TMRM</td>
<td>tetramethylrhodamine methyl ester</td>
</tr>
<tr>
<td>α-TOH</td>
<td>α-tocopherol</td>
</tr>
<tr>
<td>tris</td>
<td>tris(hydroxymethyl)aminomethane</td>
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CHAPTER 1

1. INTRODUCTION

Mitochondria are organelles that are found in the eukaryotic cells; they are responsible for various processes within the cell, such as production of energy and cell signaling.\textsuperscript{1-3} Mitochondria produce most of the ATP needed by the cell as an energy source. ATP is synthesized by the electron transport chain, which consists of a series of transmembranal proteins located in the inner mitochondrial membrane (Figure 1).\textsuperscript{4}

\textbf{Figure 1:} Electron transport chain.

Electrons are first transferred from NADH and succinate (via FADH\textsubscript{2}) to complex I and complex II, respectively. The electrons are then transferred from complexes I and II to coenzyme Q\textsubscript{10} (CoQ\textsubscript{10}) (Figure 2) and transported to complex III. Complex III then transfers the electrons to cytochrome c, which
transports them to complex IV. Finally complex IV transfers the electrons to oxygen to form water. When electrons pass through complexes I, III and IV there is a translocation of protons from the matrix to the intermembrane space. This proton gradient is then used by ATP synthase to synthesize ATP from ADP and inorganic phosphorus.\textsuperscript{5,6}

\textbf{Figure 2:} Reduction-oxidation cycle of CoQ\textsubscript{10}.

In normal mitochondria, when two electrons are transported through complex I, four protons are translocated into the mitochondrial intermembrane space. When these two electrons are transported through complex III and complex IV, six more protons are transported to the intermembrane space, two when they pass through complex III and four when transported through complex IV. In total, ten protons are transported to the intermembrane space for each two electrons from NADH. It is interesting that when two electrons are transported through complex II no protons are translocated; thus only six protons are transported to the intermembrane space. NADH and succinate are metabolites produced in glycolysis and the citric acid cycle when carbohydrates, lipids and amino acids are metabolized to generate energy. ATP synthase utilizes about three protons to
generate a molecule of ATP, thus oxidation of one NADH and one succinate produces ~ 3 ATP’s and 2 ATP’s respectively.

The large production of ATP by the electron transport chain requires an elevated consumption of oxygen; in fact, 80% of the oxygen consumption related to ATP production occurs in mitochondria. Molecular oxygen is a vital substance but at the same time could be converted to very lethal toxins, namely reactive oxygen species (ROS). Mitochondria are considered the principal source of reactive oxygen species in the cell. These reactive oxygen species are produced from 1-2 % of the oxygen consumed during cellular respiration in normal mitochondria. As shown in Figure 3, ROS are formed when an electron is transferred to molecular oxygen, usually at complex I and III sites, to form superoxide. Superoxide could be further converted to hydrogen peroxide by means of superoxide dismutase (SOD).

**Figure 3:** Mitochondrial formation and disposal of superoxide. Adapted from Ref. 11.
Superoxide and hydrogen peroxide are not harmful by themselves; actually they are essential to the function of a number of enzymes. However hydrogen peroxide can be converted to hydroxyl radicals in the presence of metal ions such as copper and iron by the Fenton reaction (Figure 4).  

\[
\text{HOOH} + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \cdot \text{OH} + \cdot \text{OH}
\]

**Figure 4**: Formation of hydroxyl radicals by the Fenton reaction.

Hydroxyl radicals are highly reactive chemical species and can react with any macromolecule in the mitochondria including DNA, RNA, lipid membranes and proteins. Normally, cells are detoxified by a combination of antioxidant enzymes including superoxide dismutase, glutathione peroxidase and catalase (Figure 3); they are also protected by non-enzymatic antioxidants like ubiquinol-10 (reduced form of CoQ\(_{10}\)), ascorbic acid, glutathione (GSH) and \(\alpha\)-tocopherol (Figure 5).

Since mitochondria are the organelles in charge of producing most of the energy required by cell, and are involved in several other processes, is not surprising that mitochondrial dysfunction is strongly linked to the pathogenesis of a number of human diseases (e.g. diabetes, cardiovascular diseases and neurodegenerative diseases).
Figure 5: Structure of some non-enzymatic antioxidants.

Defects in any of the complexes in the electron transport chain can cause a mitochondrial disorder. Such defects could be the product of mutations in mitochondrial DNA or genes located in the nucleus that encode proteins used in mitochondria. Also, mitochondrial function could be affected by factors exogenous to the mitochondria such as side effects of drugs, infections, or other environmental factors. It is thought that mitochondrial dysfunction is closely related to an impairment of the electron flow through the mitochondrial complexes. This leads to an increased production of ROS, exhausting the capacity of the cell for detoxification.

One very well known reaction of hydroxyl radicals is the peroxidation of lipid membranes (Figure 6). The hydroxyl radical abstracts a hydrogen from the lipids forming a carbon centered radical. This radical can react with molecular oxygen to form a peroxyl radical. The peroxyl radical is also reactive enough to
abstract another hydrogen from another lipid molecule, forming a new carbon centered radical. This process occurs in a chain reaction manner producing many lesions in membranes from a single hydroxyl radical.

\[
\begin{align*}
\cdot\text{OH} + \text{RH} & \rightarrow \text{R}^\cdot + \text{H}_2\text{O} & \text{Initiation} \\
\text{R}^\cdot + \text{O}_2 & \rightarrow \text{ROO}^\cdot & \\
\text{ROO}^\cdot + \text{RH} & \rightarrow \text{ROOH} + \text{R}^\cdot & \text{Propagation}
\end{align*}
\]

**Figure 6:** Free radical chain reaction leading to lipid peroxidation.

As mentioned above, the antioxidant mechanism in the cell involves a series of non-enzymatic scavengers of ROS. The most abundant antioxidants in humans are water soluble like glutathione and ascorbic acid, and hydrophobic like ubiquinol-10 and α-tocopherol.

Coenzyme Q_{10} is the most abundant ubiquinone present in the inner membrane of the mitochondria and is the factor normally used to transport electrons from complex I and II to complex III. As a reduced form (ubiquinol-10), CoQ_{10} is one of the most potent antioxidants in cell membranes and is fully capable of quenching lipid peroxidation.\textsuperscript{42-44} When ubiquinol-10 is oxidized, it is reduced back again by effects of the electron transport chain as shown previously in Figure 2. α-Tocopherol is part of the vitamin E family (Figure 7) and is also a potent lipophilic antioxidant with excellent properties in quenching lipid peroxidation.\textsuperscript{45}
Quenching peroxidation of lipid membranes could be accomplished by inhibition of the initiation step or propagation step. Phenolic antioxidants (like ubiquinol-10 and α-tocopherol) are able to inhibit lipid peroxidation by transferring their phenolic hydrogen to the carbon centered or to the peroxyl radical (Figure 8).

It has been shown that α-TOH reacts much faster with peroxyl radicals than carbon centered radicals during lipid peroxidation,\(^{46-49}\) hence one can assume that phenolic antioxidants should act in the same manner. Also, oxygen reacts with carbon center radicals in a diffusion controlled process.

When α-TOH transfers the phenolic hydrogen to a peroxyl radical, a radical is formed within the antioxidant molecule (Figure 9). The α-TOH radical is stabilized by resonance, which contributes to its facile formation. This
delocalization of the unpaired electron in the aromatic core makes α-TOH radical less reactive for abstraction of another hydrogen from a membrane lipid, therefore quenching the propagation step of lipid peroxidation.

![Chemical structure of α-TOH and its radical form](image)

**Figure 9:** Quenching of lipid peroxidation by α-TOH and recycling of α-TOH by ascorbic acid and NADH.

The formed α-TOH radicals can be reduced back to α-TOH by other redox molecules, such as ascorbic acid and NADH (Figure 9).50

The appropriate performance of the mitochondria, especially the electron transport chain, is extremely important for human health. Thus, the synthesis of CoQ10 and α-TOH analogues with enhanced properties as antioxidants and as electron carriers through the electron transport chain is an important goal, especially for treating individuals with impaired mitochondrial function.
CHAPTER 2

2. SYNTHESIS AND CHARACTERIZATION OF IDEBENONE ANALOGUES AS MEDIATORS OF OXYGEN CONSUMPTION IN MITOCHONDRIA

2.1 INTRODUCTION

Mitochondria dysfunction is believed to make an important contribution to a number of degenerative diseases. Because of this, mitochondria, and more specifically the electron transport chain, are considered potential drug targets. In order to study and treat the impaired electron flow in affected mitochondria, it is necessary to create drugs able to reach the inner mitochondrial membrane where the electron transport chain is located.

Coenzyme Q\textsubscript{10} (Figure 10a) is one of the electron carriers in the electron transport chain, but the effect of using it as therapeutic agent could be expected to be weak due to its extreme hydrophobicity (octanol/water partition $> 10^{20}$), and therefore its poor bioavailability.

![Coenzyme Q\textsubscript{10}, MitoQ's, Idebenone](image)

**Figure 10:** Structure of a) coenzyme Q\textsubscript{10}, b) mitoQ’s and c) idebenone.
In order to overcome this problem mitochondrial targeted ubiquinone antioxidants with varying carbon side chains bearing lipophilic cations at the end, such as the triphenylphosphonium group, have been developed (Figure 10b).\textsuperscript{54,55} These molecules, called mitoQ’s, accumulate several hundred fold within the mitochondria because of the large mitochondrial membrane potential (~150 to ~170 mV, negative inside). However, accumulation of these cationic species might cause depolarization of the mitochondrial membrane and subsequent failure of the respiration.\textsuperscript{56-58} Also, it has been shown that once in the reduce form, the mitoQ’s are not able to transfer electrons to complex III.\textsuperscript{59}

Idebenone is another analogue of CoQ\textsubscript{10} that consists of the same redox core as coenzyme Q\textsubscript{10} but has a shorter aliphatic side chain. (Figure 10c) The side chain used in idebenone is only 10 carbons long and bears a hydroxyl group at the end. It is thus less lipophilic than coenzyme Q\textsubscript{10} but lipophilic enough to interact with lipid bilayers. Idebenone is currently in clinical trials phase III for the treatment of Friedreich’s ataxia;\textsuperscript{60} however, studies have revealed that, idebenone inhibits complex I in the respiratory chain.\textsuperscript{61,62}

As part of our efforts to define the structural elements of coenzyme Q\textsubscript{10} essential to support mitochondrial respiration, a family of ubiquinones was developed based on idebenone scaffold as a coenzyme Q\textsubscript{10} surrogate (Figure 11). Thus, the ubiquinones synthesized consisted of analogues containing the same lipophilic side chain and having methyl and methoxyl groups at varying positions on the redox core.
The effect of varying groups (methyl and methoxyl) attached to the quinone cores of the analogues synthesized here was then evaluated by their ability to support oxygen consumption in the mitochondrial respiratory chain and by their ability to confer protection from oxidative stress to cultured CEM cells treated with diethyl maleate.

2.2 RESULTS

2.2.1 Synthesis of idebenone analogues

In order to synthesize idebenone and analogues bearing different redox cores, a short pathway was developed (Figure 12). The strategy followed consisted of a radical addition of the aliphatic chain to the non-substituted carbon of the corresponding quinone core. The radical on the alkyl chain was generated from the silver salt of the corresponding carboxylic acid.63
The syntheses of idebenone and analogues 5-(10-hydroxydecyl)-2,3,6-trimethyl-p-benzoquinone (2.1), 2-(10-hydroxydecyl)-5-methoxy-3,6-dimethyl-p-benzoquinone (2.2) and 2-(10-hydroxydecyl)-6-methoxy-3,5-dimethyl-p-benzoquinone (2.3) commenced with the preparation of their corresponding quinone precursors 2,3,5-trimethyl-p-benzoquinone (2.4), 2-methoxy-3,6-dimethyl-p-benzoquinone (2.7) and 2-methoxy-3,5-dimethyl-p-benzoquinone (2.11) (Scheme 1).

**Figure 12:** Retrosynthetic analysis of quinone analogues.
Scheme 1: Route employed for the synthesis of quinone cores 2.4, 2.7 and 2.11.

Thus, oxidation of 2,3,5-trimethylphenol using a mixture of iodine, sulfuric acid, and aqueous hydrogen peroxide\textsuperscript{64} at 23 °C afforded compound 2.4 in 94% yield. Treatment of 2,5-dimethyl-\textit{p}-benzoquinone with acetic anhydride and boron trifluoride-etherate\textsuperscript{65} at 40 °C afforded 1,2,4-triacetoxy-3,6-
dimethylbenzene (2.5) in 92% yield; compound 2.5 was then treated with sodium hydroxide and dimethyl sulfate in methanol\textsuperscript{65} at 23 °C to provide 1,2,4-trimethoxy-3,6-dimethylbenzene (2.6) in 82% yield. Finally, compound 2.6 was oxidized using phenyliodine diacetate (PIDA)\textsuperscript{66} to obtain compound 2.7 in 65% yield. Jones oxidation of 2,6-dimethylphenol provided 2,6-dimethyl-p-benzoquinone (2.8) in 94% yield.\textsuperscript{67} Quinone 2.8 was then subjected to the same synthetic procedures as for the preparation of compound 2.7. Treatment of compound 2.8 with acetic anhydride and boron trifluoride etherate at 40 °C afforded 1,2,4-triacetoxy-3,5-dimethylbenzene (2.9) in 82% yield; compound 2.9 was then treated with sodium hydroxide and dimethyl sulfate in methanol at 23 °C to provide 1,2,4-trimethoxy-3,5-dimethylbenzene (2.10) in 24% yield. Finally, compound 2.10 was oxidized using phenyliodine diacetate (PIDA) to obtain compound 2.11 in 66% yield.

The aliphatic side chain was then introduced (Scheme 2). First, commercially available 11-bromoundecanoic acid was treated with the sodium salt of benzyl alcohol in DMF at 75 °C to afford 11-benzyloxyundecanoic acid (2.12) in 60% yield. Treatment of CoQ\textsubscript{0} and of compound 2.4 with compound 2.12, silver nitrate, and potassium persulfate, in acetonitrile–water\textsuperscript{63} in the dark at 75 °C afforded 5-(10-benzyloxydecyl)-2,3-dimethoxy-6-methyl-p-benzoquinone (2.13) and 5-(10-benzyloxydecyl)-2,3,6-trimethyl-p-benzoquinone (2.14) in 17% and 37% yields, respectively. Cleavage of the benzyl ether protecting group of compounds 2.13 and 2.14 by catalytic hydrogenation using palladium-on-carbon
in methanol led to idebenone and to compound 2.1 in 82% and 53% yields, respectively.

**Scheme 2**: Route employed for the synthesis of idebenone and analogue 2.1.

For the synthesis of compounds 2.2 and 2.3, 11-hydroxyundecanoic acid (2.15) was used directly without any further protection of the hydroxyl group (Scheme 3). First, commercially available 11-bromoundecanoic acid was treated with aqueous potassium hydroxide at reflux to afford compound 2.15 in 96% yield. Treatment of compound 2.7 and compound 2.11 with compound 2.15, silver nitrate, and potassium persulfate, in acetonitrile–water in the dark at 75 °C afforded compounds 2.2 and 2.3 in 10% and 15% yields, respectively.
Scheme 3: Route employed for the synthesis of idebenone analogues 2.2 and 2.3.

2.2.2 Electrochemical evaluation of the redox cores

Cyclic voltammetric analyses of the redox cores 2.4, 2.7 and 2.11 are presented in comparison with CoQ0. Figure 13 shows that the redox cores of 2.7 and 2.11 have similar redox potentials and that they are harder to reduce than CoQ0. However, CoQ0 shows a second process at more negative potentials than compounds 2.7 and 2.11, which might correspond to the second electron reduction process. Compound 2.4 showed a more negative reduction potential than any of the other cores evaluated.
**Figure 13:** Cyclic voltammetry of CoQ₀ (green), 2.4 (red), 2.7 (blue) and 2.11 (brown) in 0.1 M tetrabutylammonium perchlorate in acetonitrile using Ag/AgCl electrode as a reference. Experiment performed by Manikandadas M. Madathil.

**2.2.3 Biochemical and biological evaluation of idebenone analogues**

In order to evaluate the structural effects of the prepared analogues of idebenone 2.1-2.3, the compounds were tested for oxygen consumption and in cell viability assays.

**2.2.3.1 Oxygen consumption assay**

C2C12 cells pretreated with 25 nM rotenone were further treated with 10µM of compounds 2.1-2.3 and idebenone, either in the presence or absence of 5
µM of FCCP (carbonylcyanide p-trifluoromethoxyphenylhydrazone). Oxygen consumption was monitored for 2 h and quantified. As shown in Figure 14, rotenone + idebenone stimulated mitochondrial oxygen consumption about 50% above the basal level. This was consistent for each of the idebenone analogues as well, and all four compounds stimulated maximal O$_2$ consumption about 3-fold.

![Figure 14: Effects of idebenone and compounds 2.1-2.3 in supporting mitochondrial oxygen consumption in C2C12 cells. Modified from Ref. 68. This experiment was performed by Robert A. Schoenfeld.](image)

**2.2.3.2 Cytoprotective effects of idebenone analogues**

The promising effects noted with idebenone and its analogues 2.1-2.3 in supporting mitochondrial oxygen consumption encouraged us to assay these compounds for their ability to protect mammalian cells from the effects of
oxidative stress. Accordingly, cultured CEM leukemia cells were treated with 5 mM diethyl maleate to deplete cellular glutathione, thus exposing the cells to the effects of oxidative stress. Pretreatment of the cells with idebenone and 2.1-2.3 prior to diethyl maleate treatment actually did confer protection to the cells, as summarized in Table 1. As shown in Table 1 compound 2.1, containing two methyl groups in place of the methoxyl groups in idebenone, was actually the most effective, conferring virtually complete protection against depletion of glutathione when employed at a concentration of 2.5 µM and affording the best protection when used at 0.1 µM concentration. Compounds 2.1 and 2.2 showed similar protection at 0.5 µM concentration. Compound 2.3 was the least effective at all concentrations tested.

Table 1: Cytoprotective effects of idebenone and 2.1-2.3 on cultured CEM cells treated with diethyl maleate. Experiment performed by Nidhi Raghav

<table>
<thead>
<tr>
<th>Compound</th>
<th>0.1 µM</th>
<th>0.5 µM</th>
<th>2.5 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>idebenone</td>
<td>24.6 ± 1.2</td>
<td>35.8 ± 3.3</td>
<td>86.2 ± 4.1</td>
</tr>
<tr>
<td>2.1</td>
<td>34.8 ± 3.4</td>
<td>77.3 ± 2.8</td>
<td>96.3 ± 3.7</td>
</tr>
<tr>
<td>2.2</td>
<td>22.9 ± 0.8</td>
<td>74.7 ± 7.7</td>
<td>82.3 ± 3.7</td>
</tr>
<tr>
<td>2.3</td>
<td>8.27 ± 0.7</td>
<td>21.2 ± 1.0</td>
<td>77.1 ± 1.4</td>
</tr>
</tbody>
</table>
2.3 DISCUSSION

The key step for the synthesis of idebenone and analogues 2.1-2.3 was the radical addition of the aliphatic chain. For the synthesis of idebenone and analogue 2.1 the hydroxyl group of the aliphatic chain was protected giving modest yields; however, having the hydroxyl group deprotected did not affect the yields for the radical addition. The substitution of methoxyl groups on the quinone ring for methyl groups causes a shift of the reduction potential to more negative regions, which means that they are harder to reduce.

While the errors associated with mitochondrial oxygen consumption measurements do not permit a more detailed analysis of those structural elements within the quinone moiety, they do suggest that some variation in substituents on the quinone moiety can be tolerated, and thus support the concept that exogenously supplied coenzyme Q analogues may be envisioned as mechanistically and therapeutically relevant probes. Idebenone and each of its analogues 2.1-2.3 promoted basal mitochondrial $\mathrm{O}_2$ consumption by about 50%, and maximal mitochondrial $\mathrm{O}_2$ consumption by about 300%, i.e. much more than in the presence of rotenone itself. The method by which idebenone and its analogues increased mitochondrial $\mathrm{O}_2$ consumption could be either through uncoupling mitochondria, or through supporting maximal electron transport activity. Since rotenone + FCCP only produced a 50% increase in $\mathrm{O}_2$ over rotenone alone, whereas idebenone and analogues + FCCP produced a 300% increase, the latter hypothesis (facilitation of maximal electron transport activity) is better supported. However, further studies of absolute mitochondrial
productivity (like ATP synthesis and interaction with the components of the
electron transport chain) are necessary to confirm this idea.

2.4 EXPERIMENTAL

Reactions were carried out under an atmosphere of argon unless specified
otherwise. The glassware was dried in an oven at 110 °C prior to use. All other
solvents were of analytical grade and were used without further purification. Flash
column chromatography was carried out using silica gel (Silicycle R10030B, 60
Å particle size, 230-400 mesh), applying a low pressure stream of nitrogen or air.
Analytical thin layer chromatographic separations were carried on glass plates
coated with silica gel (60 Å particle size, 250 µm thickness, F-254, Silicycle).
The TLC chromatograms were developed using iodine vapor, or by immersing the
plates either in 2.5% phosphomolybdic acid in ethanol, or in 2.0% anisaldehyde in
ethanol/sulfuric acid/acetic acid, followed by heating (heat gun). \(^1\)H NMR
chemical shifts were reported relative to residual CHCl\(_3\) at 7.26 ppm; \(^{13}\)C NMR
chemical shifts were reported relative to the central line of CDCl\(_3\) at 77.0 ppm.
High resolution mass spectra were obtained in the Arizona State University CLAS
High Resolution Mass Spectrometry Laboratory.
Trimethyl-\(p\)-benzoquinone (2.4).\textsuperscript{64} To a stirred solution containing 1.00 g (6.57 mmol) of trimethyl-\(p\)-hydroquinone in 20 mL of methanol at 23 °C was added 83.3 mg (0.33 mmol) of iodine followed by 329 µL (2.90 mmol) of 30% aq hydrogen peroxide and 329 µL (0.93 mmol) of sulfuric acid. The reaction mixture was stirred at 23 °C for 3 h and was then diluted with 150 mL of ether. The organic layer was washed with three 75-mL portions of water, then with one 75-mL portion of satd aq sodium thiosulfate and finally with 75 mL of brine. The organic layer was dried (MgSO\(_4\)) and concentrated under diminished pressure to afford compound 2.4 as yellow crystals: yield 930 mg (94%); mp 29-30 ºC; silica gel TLC \(R_f\) 0.65 (3:1 hexanes–ethyl acetate); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 2.03 (m, 9H) and 6.56 (s, 1H); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 12.0, 12.3, 15.9, 133.0, 140.7, 140.9, 145.3, 187.5 and 187.9.

\[
\begin{array}{c}
\text{OAc} \\
\text{OAc} \\
\text{OAc}
\end{array}
\]

1,2,4-Acetoxy-3,6-dimethylbenzene (2.5).\textsuperscript{65} To a stirred solution at 23 °C containing 1.00 g (7.34 mmol) of 2,5-dimethyl-\(p\)-benzoquinone in 8.0 mL of acetic anhydride was added 400 µL (3.13 mmol) of boron trifluoride etherate. The reaction mixture was stirred at 40 °C for 48 h and was then poured into 100 mL of water. The formed precipitate was collected by filtration, and was dried under diminished pressure to afford compound 2.5 as a colorless solid: yield 1.89 g (92%); mp 97-98 ºC; silica gel TLC \(R_f\) 0.5 (1:1 hexanes–ethyl acetate); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 1.95 (s, 3H), 2.14 (s, 3H), 2.29 (s, 9H) and 6.84 (s, 1H); \(^{13}\)C
NMR (CDCl$_3$) δ 10.1, 16.0, 20.2, 20.7, 20.8, 121.4, 122.5, 129.5, 139.2, 141.8, 146.7, 167.8, 167.9 and 168.8; mass spectrum (EI), m/z 280.0927 (M)$^+$ (C$_{14}$H$_{16}$O$_6$ requires 280.0947).

1,2,4-Trimethoxy-3,6-dimethylbenzene (2.6). To a stirred solution at 23 °C containing 1.84 g (6.74 mmol) of compound 2.5 in 6.0 mL of methanol was added 5.0 mL (6.7 g; 52 mmol) of dimethyl sulfate followed by the slow addition of a solution containing 2.17 g (54.3 mmol) of sodium hydroxide in 2.5 mL of water. The reaction mixture was stirred at 23 °C for 16 h. The reaction mixture was poured into 60 mL of water and was then extracted with 80 mL of 1:1 diethyl ether–hexanes and then with 60 mL of hexanes. The combined organic layer was washed with 80 mL of water and then with 80 mL of brine. The solution was dried (MgSO$_4$) and was then concentrated under diminished pressure. The residue was applied to a silica gel column (10 × 6 cm); elution with 3:1 hexanes–ethyl acetate afforded compound 2.6 as a colorless oil; yield 1.08 g (82%); silica gel TLC $R_f$ 0.62 (4:1 hexanes–diethyl ether); $^1$H NMR (CDCl$_3$) δ 2.12 (s, 3H), 2.27 (s, 3H), 3.78 (s, 3H), 3.79 (s, 3H), 3.83 (s, 3H) and 6.43 (s, 1H); $^{13}$C NMR (CDCl$_3$) δ 8.7, 16.0, 55.7, 60.3, 60.3, 107.5, 118.0, 128.5, 145.3, 151.8 and 153.8; mass spectrum (EI), m/z 196.1094 (M)$^+$ (C$_{11}$H$_{16}$O$_3$ requires 196.1100).
2-Methoxy-3,6-dimethyl-p-benzoquinone (2.7). To a stirred solution containing 900 mg (4.85 mmol) of compound 2.6 in 30 mL of 9:1 water–methanol at 23 °C was added 2.21 g (6.86 mmol) of phenyliodine diacetate (PIDA). The reaction mixture was stirred at 40 °C for 16 h, and then poured into 100 mL of water. The product was extracted with 150 mL of ether. The organic layer was washed with 100 mL of water, then with 100 mL of satd aq sodium bicarbonate solution and 100 mL of brine, and was then dried (MgSO₄) and concentrated under diminished pressure. The residue was applied to a silica gel column (10 × 6 cm); elution with 3:1 hexanes–ethyl acetate afforded compound 2.7 as a yellow solid: yield: 543 mg (65%); mp 58-59 °C; silica gel TLC \( R_f \) 0.46 (4:1 hexanes–ether); \(^1\)H NMR (CDCl₃) \( \delta \) 1.93 (s, 3H), 2.03 (s, 3H), 3.98 (s, 3H) and 6.51 (s, 1H); \(^{13}\)C NMR (CDCl₃) \( \delta \) 8.8, 15.7, 60.8, 128.8, 133.1, 143.7, 155.5, 183.6 and 188.5; mass spectrum (EI), \( m/z \) 166.0632 (M)\(^+\) (C₉H₁₀O₃ requires 166.0630).

2,6-Dimethyl-p-benzoquinone (2.8). To a stirred solution at 23 °C containing 2.00 g (16.4 mmol) of 2,6-dimethylphenol in 20 mL of diethyl ether was added dropwise a solution containing 11.0 g (36.9 mmol) of sodium dichromate-
dihydrate and 7 mL of sulfuric acid in 20 mL of water (Jones’ reagent). The reaction mixture was stirred at 23 °C for 16 h, and was then poured into 150 mL of water. The product was extracted using three 150-mL portions of ether. The combined organic layer was washed with 150 mL of water, then with 150 mL of brine, and was then dried (MgSO₄) and concentrated under diminished pressure to afford compound 2.8 as a yellow solid; yield 2.10 g (94%); mp 65-66 °C; silica gel TLC $R_f$ 0.60 (5:1 hexanes–ethyl acetate); $^1$H NMR (CDCl₃) $\delta$ 2.05 (s, 6H) and 6.55 (s, 2H); $^{13}$C NMR (CDCl₃) $\delta$ 16.0, 17.0, 133.3, 145.8, 187.6 and 188.2.

1,2,4-Acetoxy-3,5-dimethylbenzene (2.9). To a stirred solution containing 1.10 g (8.08 mmol) of compound 2.8 in 8.0 mL of acetic anhydride at 23 °C was added 360 µL (3.23 mmol) of boron trifluoride etherate. The reaction mixture was stirred at 40 °C for 48 h. The reaction mixture was poured into 100 mL of water and the product was extracted with two 75-mL portions of ethyl acetate. The combined organic layer was washed with 60 mL of water, then with 60 mL of satd aq sodium bicarbonate and 60 mL of brine, and was dried (MgSO₄) and concentrated under diminished pressure. The residue was applied to a silica gel column (12 × 3 cm); elution with 3:1 hexanes–ethyl acetate afforded compound 2.9 as a colorless solid; yield 1.86 g (82%); mp 94-95 °C; silica gel TLC $R_f$ 0.25 (3:1 hexanes/ethyl acetate); $^1$H NMR (CDCl₃) $\delta$ 1.97 (s, 3H), 2.13 (s, 3H), 2.25 (s,
1,2,4-Trimethoxy-3,5-dimethylbenzene (2.10). To a stirred solution containing 1.77 g (6.31 mmol) of compound 2.9 in 5.0 mL of methanol at 23 °C was added 5.0 mL (6.7 g; 52 mmol) of dimethyl sulfate followed by the slow addition of a solution containing 5.25 g (56.3 mmol) of sodium hydroxide in 6 mL of water. The reaction mixture was stirred at 23 °C for 16 h, and was then poured into 60 mL of water. The product was extracted with 80 mL of 1:1 ethyl acetate–hexanes and then with 60 mL of hexanes. The combined organic layer was washed with 80 mL of water, then with 80 mL of brine, and was dried (MgSO₄) and concentrated under diminished pressure. The residue was applied to a silica gel column (12 × 3 cm); elution with 3:1 hexanes–ethyl ether afforded compound 2.10 as a colorless oil: yield 292 mg (24%); silica gel TLC $R_f$ 0.5 (9:1 hexanes–ether); $^1$H NMR (CDCl₃) δ 2.21 (s, 3H), 2.52 (s, 3H), 3.66 (s, 3H), 3.77 (s, 3H), 3.81 (s, 3H) and 6.56 (s, 1H); $^{13}$C NMR (CDCl₃) δ 9.4, 16.0, 55.9, 59.9, 60.3, 111.5, 125.2, 125.7, 145.8, 148.9 and 150.6; mass spectrum (EI), m/z 196.1094 (M)$^+$ (C₁₁H₁₆O₃ requires 196.1100).
2-Methoxy-3,5-dimethyl-\textit{p}-benzoquinone (2.11). To a stirred solution containing 500 mg (2.55 mmol) of compound 2.10 in 15 mL of 9:1 water–methanol at 23 °C was added 1.22 g (3.78 mmol) of PIDA. The reaction mixture was stirred at 40 °C for 16 h, and was then poured into 50 mL of water. The product was extracted with 150 mL of ether. The organic layer was washed with 100 mL of water, then with 100 mL of satd aq sodium bicarbonate, and with 100 mL of brine, and was dried (MgSO\(_4\)) and concentrated under diminished pressure. The residue was applied to a silica gel column (12 × 3 cm); elution with 3:1 hexanes–diethyl ether afforded compound 2.11 as a yellow solid: yield 282 mg (66%); mp 55–56 °C; silica gel TLC \(R_f\) 0.47 (4:1 hexanes–diethyl ether); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 1.95 (s, 3H), 2.03 (s, 3H), 4.01 (s, 3H) and 6.43 (s, 1H); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 8.8, 15.7, 60.8, 128.8, 131.4, 145.7, 155.5, 183.6 and 188.5; mass spectrum (EI), m/z 166.0625 (M\(^+\)) (C\(_9\)H\(_{10}\)O\(_3\) requires 166.0630).

11-Benz oxyundecanoic acid (2.12). To a stirred solution containing 330 mg (8.25 mmol) of sodium hydride in 15 mL of anhydrous DMF at 23 °C was added dropwise 490 \(\mu\)L (510 mg; 4.71 mmol) of anhydrous benzyl alcohol followed by
1.00 g (3.77 mmol) of 11-bromoundecanoic acid. The reaction mixture was stirred at 75 °C for 3 h, and was then poured into 100 mL of ether. The formed precipitate was collected by filtration. The solid was then dissolved in 70 mL of water and the pH was adjusted to ~1. The product was extracted with 75 mL of dichloromethane. The organic layer was washed with two 60-mL portions of water, then with 60 mL of brine, and was then dried (MgSO₄) and concentrated under diminished pressure to afford compound 2.12 as a colorless wax: yield 660 mg (60%); silica gel TLC $R_f$ 0.15 (4:1 hexanes–ethyl acetate); $^{1}$H NMR (CDCl₃) δ 1.28 (m, 12H), 1.62 (m, 4H), 2.34 (t, 2H, $J = 7.6$ Hz), 3.46 (t, 2H, $J = 6.8$ Hz), 4.50 (s, 2H), 6.98 (m, 1H) and 7.03 (m, 4H); $^{13}$C NMR (CDCl₃) δ 24.7, 26.1, 29.0, 29.2, 29.3, 29.4, 29.5, 29.7, 34.0, 70.5, 72.8, 127.4, 127.6, 127.6, 128.3, 128.3, 138.7 and 179.7; mass spectrum (APCI), $m/z$ 293.2106 (M+H)$^+$ (C$_{18}$H$_{29}$O$_3$ requires 293.2117).

5-(10-Benzylkoxydecyl)-2,3-dimethoxy-6-methyl-p-benzoquinone (2.13). To a stirred solution containing 900 mg (3.07 mmol) of compound 2.12 and 500 mg (2.74 mmol) of coenzyme Q₀ in 50 mL of acetonitrile at 23 °C was added 464 mg (2.74 mmol) of silver nitrate. The reaction mixture was heated to 75 °C, then 900 mg (3.32 mmol) of potassium persulfate in 50 mL of water was added very slowly. The reaction mixture was stirred at 75 °C for 4 h and was then cooled to
23 °C, and poured into 25 mL of water. The product was extracted with 50 mL of ethyl acetate. The organic layer was washed with 25 mL of satd aq sodium bicarbonate, then with 25 mL of brine, and was then dried (MgSO₄) and concentrated under diminished pressure. The residue was applied to a silica gel column (12 × 4 cm); elution with 3:1 hexanes–ethyl acetate afforded compound 2.13 as an orange oil: yield 200 mg (17%); silica gel TLC $R_f$ 0.75 (4:1 hexanes–ethyl acetate); $^1$H NMR (CDCl₃) δ 1.34 (m, 14H), 1.60 (quint, 2H, $J = 5.2$ Hz), 2.04 (s, 3H), 2.44 (t, 2H, $J = 8.4$ Hz), 3.46 (t, 2H, $J = 6.4$ Hz), 3.98 (s, 6H), 4.50 (s, 2H), 7.27 (m, 1H), 7.34 (m, 4H); $^{13}$C NMR (CDCl₃) δ 11.9, 26.2, 26.4, 28.7, 29.3, 29.4, 29.5, 29.8, 61.1, 70.5, 72.8, 127.44, 127.6, 128.3, 138.6, 138.7, 143.1, 144.29, 144.30, 184.1 and 184.7; mass spectrum (APCI), $m/z$ 429.2637 (M+H)$^+$ (C$_{26}$H$_{37}$O$_5$ requires 429.2641).

![Image of idebenone](image.png)

**Idebenone.** To a stirred solution containing 200 mg (467 µmol) of compound 2.13 in 5 mL of anh methanol at 23 °C was added 15 mg of 10 % palladium-on-carbon in one portion. The reaction mixture was stirred at 23 °C under an atmosphere of hydrogen for 24 h. Air was then bubbled through the reaction mixture at 23 °C for 24 h. The suspension was filtered through Celite® and the filtrate was concentrated under diminished pressure to afford idebenone as an orange solid: yield 130 mg (82%); mp 46-47 °C; $^1$H NMR (CDCl₃) δ 1.34 (m,
14H), 1.60 (quint, 2H, \( J = 7.6 \) Hz), 2.04 (s, 3H), 2.44 (t, 2H, \( J = 8.0 \) Hz), 3.63 (t, 2H, \( J = 6.8 \) Hz) and 3.99 (s, 6H); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 11.9, 25.7, 26.4, 28.7, 29.3, 29.4, 29.5, 29.8, 32.7, 61.1, 63.0, 138.6, 143.1, 144.3, 184.1 and 184.7.

5-(10-Benzoyloxydecyl)-2,3,6-trimethyl-\( p \)-benzoquinone (2.14). To a stirred solution containing 600 mg (3.11 mmol) of 2.12 and 450 mg (3.00 mmol) of compound 2.4 in 50 mL of acetonitrile at 23 °C was added 509 mg (3.00 mmol) of silver nitrate. The reaction mixture was heated to 75 °C and then 981 mg (3.63 mmol) of potassium persulfate in 50 mL of water was added very slowly. The reaction mixture was stirred at 75 °C for 3 h in the dark, and was then allowed to cool to 23 °C and poured into 25 mL of water. The product was extracted with 50 mL of ethyl acetate. The organic layer was washed with 25 mL of water, then with 25 mL of satd aq sodium bicarbonate, and 25 mL of brine, then dried (MgSO\(_4\)). Concentration was then carried out under diminished pressure. The residue was purified by chromatography on silica gel column (13 × 4 cm); elution with 3:1 hexanes–ethyl acetate afforded compound 2.14 as an orange oil: yield 330 mg (37%); silica gel TLC \( R_f \) 0.75 (3:1 hexanes–ethyl acetate); \(^1\)H NMR (CDCl\(_3\)) \( \delta \) 1.26 (m, 14H), 1.59 (quint, 2H, \( J = 7.2 \) Hz), 2.00 (m, 9H), 2.43 (t, 2H, \( J = 8.0 \) Hz), 3.44 (t, 2H, \( J = 6.4 \) Hz), 4.50 (s, 2H), 7.25 (m, 1H) and 7.31 (m, 4H); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 12.1, 12.3, 26.2, 26.6, 28.8, 29.3,
29.4, 29.5, 29.8, 29.9, 70.5, 72.8, 127.4, 127.6, 128.3, 138.7, 140.0, 140.3, 144.5, 187.1 and 187.8; mass spectrum (EI), \(m/z\) 296.2676 (M)\(^+\) (C\(_{26}\)H\(_{36}\)O\(_3\) requires 396.2665).

5-(10-Hydroxydecal)-2,3,6-trimethyl-\(p\)-benzoquinone (2.1). To a stirred solution containing 330 mg (0.832 mmol) of compound 2.14 in 6 mL of anhydrous methanol at 23 °C was added 20 mg of 10% palladium-on-carbon. The reaction mixture was stirred at 23 °C under a hydrogen atmosphere for 24 h, then air was bubbled through the reaction mixture at 23 °C for 24 h. The suspension was filtered through Celite and the filtrate was concentrated under diminished pressure. The residue was applied to a silica gel column (13 × 4 cm); elution with 3:1 hexanes–ethyl acetate afforded compound 2.1 as a yellow solid: yield 130 mg (51%); mp 61-62 °C; silica gel TLC \(R_f\) 0.4 (3:1 hexanes–ethyl acetate); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 1.26 (m, 14H), 1.53 (quint, 2H, \(J = 6.4\) Hz), 2.00 (m, 9H), 2.43 (t, 2H, \(J = 8.0\) Hz) and 3.61 (t, 2H, \(J = 6.8\) Hz); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 12.1, 12.3, 25.7, 26.6, 28.8, 29.31, 29.34, 29.4, 29.5, 29.8, 32.7, 63.0, 140.0, 140.3, 140.4, 144.5, 187.2 and 187.9; mass spectrum (APCI), \(m/z\) 307.2270 (M+H)\(^+\) (C\(_{19}\)H\(_{31}\)O\(_3\) requires 307.2273).
**11-Hydroxyundecanoic Acid (2.15).** To 70 mL of 2 M aq potassium hydroxide solution was added 1.60 g (6.08 mmol) of 11-bromoundecanoic acid. The reaction mixture was stirred at 100 °C for 16 h, and was then cooled to 23 °C. The pH was adjusted to ~1 by addition of conc HCl. The formed precipitate was collected by filtration and was then dried under diminished pressure to afford compound 2.15 as a colorless solid: yield 1.18 g (96%); mp 65-66 °C; \(^1H\) NMR (CDCl\(_3\)) \(\delta\) 1.28 (m, 12H), 1.62 (m, 4H), 2.34 (t, 2H, \(J = 7.6\) Hz) and 3.63 (t, 2H, \(J = 6.8\) Hz); \(^{13}C\) NMR (CDCl\(_3\)) \(\delta\) 24.6, 25.6, 29.0, 29.1, 29.3, 32.7, 34.0, 63.0 and 179.3; mass spectrum (APCI), \(m/z\) 203.1651 (M+H)\(^+\) (C\(_{11}\)H\(_{23}\)O\(_3\) requires 203.1647).

![Image of 11-Hydroxyundecanoic Acid](image)

**2-(10-Hydroxydecy)-5-methoxy-3,6-dimethyl-p-benzoquinone (2.2).** To a stirred solution containing 110 mg (0.54 mmol) of compound 2.15 and 85.0 mg (0.51 mmol) of compound 2.7 in 2.5 mL of acetonitrile at 23 °C was added 87.0 mg (0.51 mmol) of silver nitrate. The reaction mixture was heated to 75 °C, then 167 mg (0.61 mmol) of potassium persulfate in 2.5 mL of water was added very slowly. The reaction mixture was stirred at 75 °C for 4 h, and was then allowed to cool to 23 °C, and poured into 50 mL of water. The product was extracted with 75 mL of dichloromethane. The organic layer was washed with 75 mL of water, then with 75 mL of satd aq sodium bicarbonate and 75 mL of brine, and was then dried (MgSO\(_4\)) and concentrated under diminished pressure. The residue was
applied to a silica gel column (10 × 3 cm); elution with 19:1 dichloromethane–ethyl ether afforded compound 2.2 as an orange solid: yield 25 mg (15%); mp 58-59 °C; silica gel TLC $R_f$ 0.32 (3:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $\delta$
1.28 (m, 14H), 1.59 (quint, 2H, $J = 7.0$ Hz), 1.93 (s, 3H), 2.01 (s, 3H), 2.44 (t, 2H, $J = 7.0$ Hz), 3.62 (t, 2H, $J = 6.5$ Hz) and 3.95 (s, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 8.8, 12.2, 26.16, 26.22, 28.7, 29.4, 29.5, 29.8, 60.9, 63.0, 72.8, 128.6, 140.3, 143.1, 155.4, 183.4 and 188.7; mass spectrum (APCI), $m/z$ 322.2217 (M+H)$^+$ (C$_{19}$H$_{31}$O$_4$ requires 322.2222).

![Chemical Structure](image)

2-(10-Hydroxydecyl)-6-methoxy-3,5-dimethyl-\(p\)-benzoquinone (2.3). To a stirred solution containing 364 mg (1.79 mmol) of compound 2.15 and 282 mg (1.69 mmol) of compound 2.11 in 10 mL of acetonitrile at 23 °C was added 288 mg (1.69 mmol) of silver nitrate. The reaction mixture was heated to 75 °C, then 552 mg (2.04 mmol) of potassium persulfate in 10 mL of water was added dropwise. The reaction mixture was stirred at 75 °C for 4 h, then allowed to cool to 23 °C, and then poured into 50 mL of water. The mixture was extracted with 75 mL of dichloromethane. The organic layer was washed with 75 mL of water, then with 75 mL satd aq sodium bicarbonate, and 75 mL of brine, and was then dried (MgSO$_4$) and concentrated under diminished pressure. The residue was applied to a silica gel column (12 × 3 cm); elution with 19:1 dichloromethane–ether afforded
compound 2.3 as an orange wax: yield 56 mg (10%); silica gel TLC $R_f$ 0.33 (3:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $\delta$ 1.28 (m, 14H), 1.59 (quint, 2H, $J$ = 7.2 Hz), 1.93 (s, 3H), 2.01 (s, 3H), 2.43 (t, 2H, $J$ = 8.0 Hz), 3.62 (t, 2H, $J$ = 6.8 Hz) and 3.95 (s, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 8.8, 12.2, 26.2, 28.7, 29.4, 29.5, 29.8, 60.9, 63.0, 72.8, 128.6, 140.3, 143.1, 155.4, 183.4 and 188.7; mass spectrum (APCI), $m/z$ 323.2229 (M+H)$^+$ (C$_{19}$H$_{31}$O$_4$ requires 323.2222).

**Measurement of oxygen consumption**

C2C12 cells were grown in DMEM supplemented with 10% fetal bovine serum and 2nM glutamine. Mitochondrial O$_2$ consumption was performed essentially as described in literature$^{70}$ with minor modifications. Briefly, C2C12 cells were trypsinized and resuspended in phenol red-free growth medium, treated with 25 nM of rotenone and plated at 200,000 cells/well in a 96-well BD Oxygen Biosensor plate. Compounds 2.1-2.3 and idebenone (10 $\mu$M) were added and fluorescence was monitored for 2 h in a PerSeptive Biosystems Cytofluor Series 4000 plate reader (ex 485 nm; em 620 nm). Oxygen consumption was quantified by calculating M/T, that is, the slope of 50% maximum divided by the time to reach 50% maximum. This experiment was performed by Robert A. Schoenfeld, UC Davis.

**Measurement of cytoprotective effects of idebenone analogues**

CEM cells were grown in RPMI medium 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone South Logan Utah) and 1%
penicillin-streptomycin solution (Cellgro, Manassas, VA). In a 12-well cell culture plate 500,000 cells per well were seeded and treated with compounds 2.1-2.3 and idebenone for 18 h. Cells were then treated with DEM (diethyl meleate) at 37 °C for 4 h in a humidified atmosphere of 5% CO₂ in air. The viability of the cells was measured by staining with 0.4% trypan blue. Viable cells exclude the dye and non-viable cells take-up the dye. Cell viability is calculated as percentage of positive control (cells not treated with DEM or the idebenone analogues). Data are expressed as mean ± SDE (n=3). This experiment was performed by Nidhi Raghav, ASU.

Cyclic voltammetry

Cyclic voltammetry (CV) studies were carried out using a Model 1030 multi-potentiostat from CH Instruments. The platinum working electrode (2 mm diameter, model CHI102), platinum wire counter electrode (model CHI115), and Ag/AgCl reference electrode (model CHI111) were obtained from CH Instruments. Cyclic voltammetry measurements were performed in a precut 20 mL vial at room temperature in 2 mL of a 0.1 M tetrabutylammonium perchlorate solution in acetonitrile containing the quinone analyte at a 1 mM (CoQ₀, compounds 2.7 and 2.11) and 2 mM (compound 2.4) concentrations. The analytes were dissolved in acetonitrile. The vial was covered with a Teflon cap (CHI223) and sealed using parafilm. All samples were purged with nitrogen for 5 min to remove any oxygen. The substrate was then added with a syringe and stirred for 1 minute. Stirring was stopped and the nitrogen stream was focused
above the sample so that only the headspace was streamed with nitrogen. The cell was allowed to sit for one minute to allow for the diffusion layer to set up and come to equilibrium. The parameters used for the starting and vertex potential were \(-1.5\) V and \(1.5\) V respectively, and the scan rate was \(100\) mV/s. The cyclic voltammograms of the quinone cores were recorded using an initial reductive sweep. Between each CV experiment, the electrodes were rinsed with dichloromethane. The platinum working electrode was then rinsed with water and polished with 0.05 micron aluminum powder and distilled water on a polishing pad (CHI120). Immediately before each CV was performed, all electrodes were rinsed with acetonitrile. This experiment was performed by Manikandadas Mathilakathu Madathil.
3. SYNTHESIS AND CHARACTERIZATION OF PYRIDINOL AND PYRIMIDINOL AS MULTIFUNCTIONAL RADICAL QUENCHERS

3.1 INTRODUCTION

It is well known that cellular respiration is the process that produces more oxidative damage to the cell due to the production of ROS. Increased production of ROS is linked to mitochondrial dysfunction, more specifically to an inefficient electron transport through the respiratory chain. Several human neurodegenerative diseases including Alzheimer’s disease, MELAS syndrome, and Friedreich’s ataxia are strongly linked to mitochondrial dysfunction.

One of the most common autosomal recessive ataxia in the Caucasian population is Friedreich’s ataxia (FRDA); approximately, 1 in 50,000 people in the United States are affected. Currently there are no effective therapeutic strategies to treat this disease. Friedreich’s ataxia is caused by a reduced production of the protein frataxin, approximately in the range of 5% to 30% of normal levels. It is believed that frataxin is involved in trafficking of iron within the mitochondria and the assembly of iron sulfur (Fe-S) clusters. Low levels of frataxin leads to iron accumulation, inappropriate function of Fe-S cluster containing enzymes, and a high incidence of Fenton’s type reactions producing high levels of ROS.
Idealone (Catena®), a synthetic analogue of coenzyme Q10, is currently the only compound that has reached phase III clinical trials for the treatment of FRDA.60

It has been shown that a major source of superoxide production during respiration occurs at complex III. The redox cycle in this complex involve the formation of ubisemiquinone radical, which could be oxidized back by oxygen generating superoxide (Figure 15).11,12 Then, it can be assume that quinone analogues of CoQ10 could generate superoxide in the same fashion as CoQ10.

![Figure 15: Oxidation of ubisemiquinone radical by molecular oxygen.](image)

α-TOH is one of the best lipophilic antioxidants; although, it has been shown that the α-TOH radical formed during inhibition of lipid peroxidation in low density lipoproteins (LDL) particles is capable of promoting oxidation of lipids under mild conditions by initiating the peroxidation chain reaction.76,77

The activity of phenolic antioxidants in quenching lipid membrane peroxidation relies on their ability to transfer the phenolic hydrogen to a carbon or peroxyl radical and on the stability of the phenoxy radical formed. Thus, novel synthetic and more potent antioxidants than α-TOH are currently under development.
The stability of phenoxyl radicals formed could be measured by O-H bond dissociation energy (O-H BDE), the lower the BDE, the more stable the radical formed. It has been shown that increasing the ring electronic density of phenolic antioxidants lowers the hydroxyl BDE, thus their activity as antioxidants is enhanced. Nonetheless, some antioxidants with strong electron donating groups are unstable to air. Air stability is correlated to the ionization potential (IP) of molecules, lower the IP, easier is to abstract an electron from the molecule. If an antioxidant has a very low IP, it could transfer an electron to molecular oxygen, losing its properties as an antioxidant and becoming a source superoxide.

Recognizing that the O-H BDE depends mostly on π-interactions with ortho and para substituents and IP depends on σ-induced electron density, a series of aminopyridinols has been synthesized and evaluated as antioxidants (Figure 16). Their particular structure confers upon them greater inhibition rate constants than α-tocopherol in quenching lipid peroxidation and enhanced air stability. These properties are due to the presence of electron donating groups attached to the aromatic core that lowers the O-H BDE and the presence of nitrogen atoms as part of the aromatic ring that increase the IP (more stable towards oxidation).

![Figure 16](image_url) Pyridinols with enhanced activities as antioxidants.
As part of the effort to synthesize compounds with enhanced antioxidant activity a family of pyridinols (Figure 17) and pyrimidinols (Figure 18) with different substituents attached to the core was developed. Also, to study their ability to reach mitochondria and their interaction with the electron transport chain, different lipophilic side chains were attached.

**Figure 17**: Series of pyridinol analogues synthesized and evaluated.
Figure 18: Series of pyrimidinol analogues synthesized and evaluated.

The effect of varying groups and lipophilic side chains attached to pyridinol and pyrimidinol cores of the analogues synthesized here was then evaluated by their ability to confer protection from oxidative stress to cultured FRDA lymphocytes cells treated with diethyl maleate. The effect of the analogues
synthesized towards interaction with the electron transport chain complexes was studied using submitochondrial particles (SMP) prepared from bovine heart.

3.2 RESULTS

In order to synthesize a first generation of pyridinol and pyrimidinol analogues having a methyl group at the position ortho to the phenolic hydroxyl group, a concise strategy was followed (Figure 19). The strategy consisted of synthesizing a fully protected pyridine and pyrimidine core followed by attachment of the aliphatic chain. These intermediates were then deprotected and modified in order to synthesize the proposed analogues.

X
O
H
R1
R2

Br
R1

X
N
O
Bn

N
2

X
=C
H
N
R1=a
liphatic side chain

Figure 19: Retrosynthetic analysis for the synthesis of pyridinol and pyrimidinol analogues with a methyl substituent at the position ortho to the phenolic hydroxyl group.

For the synthesis of those pyridinol and pyrimidinol analogues containing a methoxyl group at position ortho of the phenolic hydroxyl group, a different approach was followed (Figure 20). The strategy consisted of attaching the
lipophilic side chain to the pyridine or pyrimidine core prior to its corresponding modification to the final pyridinol or pyrimidinol analogue.

Figure 20: Retrosynthetic analysis for the synthesis of pyridinol and pyrimidinol analogues with a methoxyl substituent at the position ortho to the phenolic hydroxyl group.

In order to create a larger library of pyrimidinol analogues, a different strategy was proposed (Figure 21). This strategy consisted of synthesizing a fully protected core containing a short side chain with a functional group that could work as anchoring point for different lipophilic side chains. A terminal alkene was chosen as the functionality for the attachment of other side chains by cross metathesis.
3.2.1 Synthesis and evaluation of pyridinol and pyrimidinol analogues.

3.2.1.1 Synthesis of pyridinol analogues

6-((N,N-dimethylamino)-2-(10-hydroxydecyl)-4-methylpyridin-3-ol (3.1) was synthesized in eight steps (Scheme 4). First, 2-amino-5-bromo-4,6-dimethylpyridine (3.26) was prepared in 84% yield by treating commercially available 2-amino-4,6-dimethylpyridine with N-bromosuccinimide.84,85 Compound 3.26 was then treated with 2,5-hexanedione in presence of p-toluensulfonic acid to obtain 3-bromo-6-(2,5-dimethyl-1H-pyrrol-1-yl)-2,4-dimethylpyridine (3.27) in 62% yield.84,86 3-(Benzyloxy)-6-(2,5-dimethyl-1H-pyrrol-1-yl)-2,4-dimethylpyridine (3.28) was prepared in 82% yield by treating compound 3.27 with potassium hydroxide in presence of Pd2dba3 and 2-di-tert-butylphosphino-2,4,6-triisopropylbiphenyl (L1),87 followed by protection of the hydroxyl group by means of benzyl ether. The fully protected core 3.28, was then treated with phenyllithium in the presence of 1-bromo-9-(methoxymethoxy)nonane (3.25) to obtain 3-(benzyloxy)-6-(2,5-dimethyl-1H-pyrrol-1-yl)-2,4-dimethylpyridine (3.28).
pyrrolo[1-yl]-2-(10-(methoxymethoxy)decyl)-4-methylpyridine (3.29) in 60% yield.

Scheme 4: Route employed for the synthesis of pyridinol analogue 3.1.
Compound 3.25 was previously prepared by treating 9-bromo-1-nonanol with methoxymethyl chloride in presence of sodium hydride in 70% yield.\textsuperscript{90}

Compound 3.29 was deprotected by treating it with hydroxylamine hydrochloride in the presence of potassium hydroxide to obtain 2-amino-5-(benzyloxy)-6-(10-(methoxymethoxy)decal)-4-methylpyridine (3.30) in 56% yield.\textsuperscript{84,86} Then, compound 3.30 was treated with methyl iodide in presence of sodium hydride to obtain 3-(benzyloxy)-6-(N,N-dimethylamino)-2-(10-(methoxymethoxy)decal)-4-methylpyridine (3.31) in 21% yield.\textsuperscript{84} 6-(N,N-Dimethylamino)-2-(10-hydroxydecal)-4-methylpyridin-3-ol (3.1) was obtained in 77% yield by treating compound 3.31 first with HCl and then with palladium hydroxide under a hydrogen atmosphere.

6-Amino-2-(10-hydroxydecal)-4-methylpyridin-3-ol (3.2) (Scheme 5) was synthesized from compound 3.30 by deprotection of the aliphatic hydroxyl group using acidic conditions followed by hydrogenolysis in 29% yield over two steps.

![Scheme 5: Route employed for the synthesis of pyridinol analogue 3.2.](image)

6-(2,5-Dimethyl-1H-pyrrol-1-yl)-2-(10-hydroxydecal)-4-methylpyridin-3-ol (3.3) and 6-(2,5-dimethylpyrrolidin-1-yl)-2-(10-hydroxydecal)-4-
methylpyridin-3-ol (3.4) (Scheme 6) were synthesized using the same conditions in 17% and 5% yields, respectively, by treating compound 3.29 under acidic conditions followed by hydrogenolysis.

Scheme 6: Route employed for the synthesis of pyridinol analogues 3.3 and 3.3.

In order to synthesize 2-(10-hydroxydecyl)-6-methoxy-4-methylpyridin-3-ol (3.5) (Scheme 7), compound 3.30 was treated with sodium nitrite under aqueous conditions followed by selective O-methylation using methyl iodide in presence of silver carbonate to obtain 3-(benzyloxy)-6-methoxy-2-(10-(methoxymethoxy)decyl)-4-methylpyridine (3.32) in 26% yield over two steps.\textsuperscript{85} Then compound 3.32 was treated under acidic conditions followed by hydrogenolysis to obtain compound 3.5 in 67% yield.
Scheme 7: Route employed for the synthesis of pyridinol analogue 3.5.

6-(N,N-Dimethylamino)-2-(10-hydroxydecyl)-4-methoxypyridin-3-ol (3.6) (Scheme 8) was synthesized in eight steps. First, dimethyl 2-(1-aminoethylidene)malonate (3.33) was obtained in 60% yield by treating dimethyl malonate with acetonitrile in presence of tin tetrachloride. Then compound 3.33 was treated with N,N’-dimethylacetamide dimethyl acetal in presence of a strong base, sodium tert-butoxide, to obtain methyl 6-(N,N-dimethylamino)-4-hydroxy-2-methylnicotinate (3.34) in 51% yield. Then the hydroxyl group of compound 3.34 was methylated in presence of sodium carbonate and methyl iodide to obtain methyl 6-(N,N-dimethylamino)-4-methoxy-2-methylnicotinate (3.35) in 47% yield. Compound 3.35 was then hydrolyzed followed by decarboxylation via thermolysis to obtain 2-(N,N-dimethylamino)-4-methoxy-6-methylpyridine (3.36) in 34% yield. 2-(N,N-Dimethylamino)-4-methoxy-6-(10-(methoxymethoxy)decyl)pyridine (3.37) was obtained in 70% yield by treating compound 3.36 with n-BuLi in the presence of compound 3.25. Then compound 3.37 was brominated at position 5 using N-bromosuccinimide to obtain
5-bromo-2-(N,N-dimethylamino)-4-methoxy-6-(10-(methoxymethoxy)decyl)pyridine (3.38) in 44% yield.

Scheme 8: Route employed for the synthesis of pyridinol analogue 3.6.

The bromine at position 5 was then replaced by a hydroxyl group by treating compound 3.38 with n-BuLi, then with trimethyl borate, and finally with hydrogen peroxide in subsequent steps in order to obtain 6-(N,N-dimethylamino)-
4-methoxy-2-(10-(methoxymethoxy)decyl)pyridin-3-ol (3.39) in 21% yield. Finally, the hydroxyl group on the aliphatic chain was deprotected by treating compound 3.39 with dilute HCl to obtain compound 3.6 in 40% yield.

6-(N,N-Dimethylamino)-2-(10-hydroxydecyl)-4,5-dimethylpyridin-3-ol (3.7) was synthesized in seven steps (Scheme 9). First, 6-amino-2,4,5-trimethylpyridin-3-ol (3.40) was synthesized according to a literature procedure. Then the exocyclic amine of compound 3.40 was protected by treating it with 2,5-hexanediol in presence of p-toluenesulfonic acid to obtain 6-(2,5-dimethyl-1H-pyrrol-1-yl)-2,4,5-trimethylpyridin-3-ol (3.41) in 75% yield. 3-(Benzyloxy)-6-(2,5-dimethyl-1H-pyrrol-1-yl)-2,4,5-trimethylpyridine (3.42) was prepared in 78% yield by treating compound 3.41 with sodium hydride and then with benzyl bromide. The fully protected core, compound 3.42, was then treated with n-BuLi in the presence of TMEDA (N,N,N',N'-tetramethylethylenediamine) followed by compound 3.25 to obtain 3-(benzyloxy)-6-(2,5-dimethyl-1H-pyrrol-1-yl)-2-(10-(methoxymethoxy)decyl)-4,5-dimethylpyridine (3.43) in 41% yield. The previously protected amine moiety of compound 3.43 was deprotected by treating it with hydroxylamine hydrochloride in presence of potassium hydroxide to obtain 2-amino-5-(benzyloxy)-6-(10-(methoxymethoxy)decyl)-4,5-dimethylpyridine (3.44) in 13% yield. Compound 3.44 was then treated with a formaldehyde solution and sodium cyanoborohydride in presence of acetic acid to obtain 3-(benzyloxy)-6-(N,N-dimethylamino)-2-(10-(methoxymethoxy)decyl)-4,5-dimethylpyridine (3.45) in 77% yield. 6-(N,N-Dimethylamino)-2-(10-hydroxydecyl)-4,5-dimethylpyridin-3-ol (3.7) was obtained in 79% yield by
treating compound 3.45 first with HCl in methanol and then with palladium hydroxide-on-carbon under a hydrogen atmosphere.

Scheme 9: Route employed for the synthesis of pyridinol analogue 3.7.

In order to synthesize compounds 3.8 and 3.9 (Scheme 10), compound 3.42 was first alkylated at the methyl group of the pyridine ring by treating it with \( n\)-BuLi followed by 1-bromopentadecane to obtain 3-(benzyloxy)-6-(2,5-dimethyl-1H-pyrrol-1-yl) - 2-hexa3decyl-4,5-dimethylpyridine (3.46) in 48% yield. The exocyclic amine of compound 3.46 was then deprotected by treating it
with hydroxylamine hydrochloride and potassium hydroxide in order to obtain 5-(benzyloxy)-6-hexadecyl-3,4-dimethylpyridin-2-amine (3.47) in 61% yield.

Then the exocyclic amine of compound 3.47 was methylated using formalin in presence if sodium cyanoborohydride and acetic acid in order to obtain 3-(benzyloxy)-6-(N,N-dimethylamino)-2-hexadecyl-4,5-dimethylpyridine (3.48) in 70% yield. Finally, compound 3.8 was obtained in 98% yield by treating compound 3.48 with hydrogen in presence of a palladium catalyst. 3-(Acetoxy)-6-(N,N-dimethylamino)-2-hexadecyl-4,5-dimethylpyridine (3.9) was obtained in
82% yield by treating compound 3.8 with acetic anhydride in presence of potassium carbonate.

In order to study the redox properties of the pyridinol analogues, three representative pyridinol cores were synthesized (Scheme 11). The redox core of compound 3.1 was synthesized in two steps starting from 2-amino-5-bromo-4,6-dimethylpyridine (3.26). First, compound 3.26 was treated with methyl iodide followed by sodium hydride to obtain 3-bromo-6-(N,N-dimethylamino)-2,4-dimethylpyridine (3.49) in 55% yield. Then compound 3.49 was treated with potassium hydroxide in presence of Pd2dba3 and 2-di-tert-butylphosphino-2,4,6-triisopropylbiphenyl (L1) to obtain 6-(N,N-dimethylamino)-2,4,6-dimethylpyridin-3-ol (3.50) in 35% yield. The redox core of analogue 3.6 was synthesized in two steps starting from 2-(N,N-dimethylamino)-4-methoxy-6-methylpyridine (3.36). 3-Bromo-6-(N,N-dimethylamino)-4-methoxy-2-methylpyridine (3.51) was obtained in 95% yield by treating compound 3.36 with N-bromosuccinimide. Then compound 3.51 was treated with n-BuLi followed by trimethyl borate and hydrogen peroxide to obtain 6-(N,N-dimethylamino)-4-methoxy-2-methylpyridin-3-ol (3.52) in 11% yield. The redox core of compound 3.7 was synthesized from 6-amino-2,4,5-trimethylpyridin-3-ol (3.40) by treatment with formalin in presence of sodium cyanoborohydride and acetic acid to obtain 6-(N,N-dimethylamino)-2,4,5-trimethylpyridin-3-ol (3.53) in 50% yield.
Scheme 11: Routes employed for the synthesis of pyridinol redox cores.

3.2.1.2 Synthesis of pyrimidinol analogues

The synthesis of 2-(N,N-dimethylamino)-4-(10-hydroxydecyl)-6-methylpyrimidin-5-ol (3.10) was completed in nine steps (Scheme 12). First guanidine sulfate was treated with 2,4-pentanedione in presence of sodium carbonate to obtain 2-amino-4,6-dimethylpyrimidine (3.54) in 95% yield.\textsuperscript{84,96} 2-Amino-5-bromo-2,6-dimethylpyridine (3.55) was obtained in 83% yield after the
treatment of compound 3.54 with N-bromosuccinimide.\textsuperscript{84,85} The exocyclic amine of compound 3.55 was protected by treating it with 2,5-hexanedione in presence of p-toluenesulfonic acid to obtain 5-bromo-2-(2,5-dimethyl-1\textit{H}-pyrrol-1-yl)-4,6-dimethylpyrimidine (3.56) in 81\% yield.\textsuperscript{84,86} Then, compound 3.56 was treated with potassium hydroxide in the presence of Pd\textsubscript{2}dba\textsubscript{3} and 2-di-\textit{tert}-butylphosphino-2,4,6-triisopropylbiphenyl (L\textsubscript{1})\textsuperscript{87} followed by benzyl protection of the hydroxyl group to obtain 5-(benzyloxy)-2-(2,5-dimethyl-1\textit{H}-pyrrol-1-yl)-4,6-dimethylpyrimidine (3.57) in 76\% yield. 5-(Benzyloxy)-2-(2,5-dimethyl-1\textit{H}-pyrrol-1-yl)-4-(10-(methoxymethoxy)decyl)-6-methylpyrimidine (3.58) was obtained in 56\% yield by treatment of compound 3.57 with \textit{n}-BuLi in presence of 3.25.\textsuperscript{88,89} The previously protected amine of compound 3.58 was deprotected by treating it with hydroxylamine hydrochloride in presence of potassium hydroxide to obtain 2-amino-5-(benzyloxy)-4-(10-(methoxymethoxy)decyl)-6-methylpyrimidine (3.59) in 76\% yield.\textsuperscript{84,86} The exocyclic amine of compound 3.59 was dimethylated and the MOM protecting group was cleaved by treatment with a solution of 1:1 35\% aq formaldehyde–formic acid to obtain 5-(benzyloxy)-2-(\textit{N},\textit{N}-dimethylamino)-4-(10-hydroxydecyl)-6-methylpyrimidine (3.60) in 44\% yield.\textsuperscript{84,85} Finally, the benzyl ether group of compound 3.60 was cleaved by the treatment with hydrogen in presence of palladium hydroxide in methanol to obtain 2-(\textit{N},\textit{N}-dimethylamino)-4-(10-hydroxydecyl)-6-methylpyrimidin-5-ol (3.10) in quantitative yield, which after column purification gave a 26\% recovery.
Scheme 12: Route employed for the synthesis of pyrimidinol analogue 3.10.

6-Amino-2-(10-hydroxydecyl)-4-methylpyrimidin-3-ol (3.11) (Scheme 13) was synthesized from 2-amino-5-(benzyloxy)-6-(10-(methoxymethoxy)decyl)-4-methylpyrimidine (3.59) by deprotection of the
aliphatic hydroxyl group under acidic conditions followed by hydrogenolysis. The yield was 69% over two steps.

\[
\text{Scheme 13: Route employed for the synthesis of pyrimidinol analogue 3.11.}
\]

In order to synthesize 2-(10-hydroxydecyl)-6-methoxy-4-methylpyridin-3-ol (3.12) (Scheme 14), compound 3.59 was treated with tert-butyl nitrite in presence of benzyltrimethyl ammonium chloride to obtain 3-(benzyloxy)-6-chloro-2-(10-(methoxymethoxy)decyl)-4-methylpyrimidine (3.61) in 16% yield. Then compound 3.61 was treated with sodium methoxide to obtain 3-(benzyloxy)-6-methoxy-2-(10-(methoxymethoxy)decyl)-4-methylpyrimidine (3.62) in 74% yield.

\[
\text{Scheme 14: Route employed for the synthesis of pyrimidinol analogue 3.12.}
\]
Compound 3.62 was then treated under acidic conditions followed by hydrogenolysis to obtain compound 3.12 in 97% yield over two steps. 6-(2,5-Dimethyl-1H-pyrrol-1-yl)-2-(10-hydroxydecyl)-4-methylpyrimidin-3-ol (3.13) and 6-(2,5-dimethylpyrrolidin-1-yl)-2-(10-hydroxydecyl)-4-methylpyridin-3-ol (3.14) (Scheme 15) were synthesized using the same conditions in 8% and 14% yields, respectively, by treating compound 3.58 under acidic conditions followed by hydrogenolysis.

Scheme 15: Route employed for the synthesis of pyrimidinol analogues 3.13 and 3.14.

The synthesis of 2-\((N,N\text{-dimethylamino})\)-4-(10-hydroxydecyl)-6-methoxypyrimidin-5-ol (3.15) was accomplished in five steps (Scheme 16). First, 2-amino-4-methoxy-6-methylpyrimidine was treated with methyl iodide in presence of sodium hydride to obtain 2-\((N,N\text{-dimethylamino})\)-4-methoxy-6-methylpyrimidine (3.63) in 80% yield. Then the side chain was attached by treating compound 3.63 with \(n\)-BuLi in presence of compound 3.25 to obtain 2-\((N,N\text{-dimethylamino})\)-4-methoxy-6-(10-(methoxymethoxy)decyl)pyrimidine (3.64) in 71% yield. Then compound 3.64 was brominated at position 5 using \(N\)-
bromosuccinimide to obtain 5-bromo-2-(N,N-dimethylamino)-4-methoxy-6-(10- (methoxymethoxy)decyl)pyrimidine (3.65) in 53% yield. The bromine at position 5 was then replaced by a hydroxyl group by treating compound 3.65 with n-BuLi, then with trimethyl borate, and finally with hydrogen peroxide in subsequent steps in order to obtain 6-(N,N-dimethylamino)-4-methoxy-2-(10- (methoxymethoxy)decyl)pyrimidin-3-ol (3.66) in 51% yield. Finally, the hydroxyl group on the aliphatic chain was deprotected by treating compound 3.66 with dilute HCl to obtain compound 3.15 in 40% yield.

Scheme 16: Route employed for the synthesis of pyrimidinol analogue 3.15.

Compounds 2-(N,N-dimethylamino)-4-methyl-6-pentylpyrimidin-5-ol (3.16), 2-(N,N-dimethylamino)-4-decyl-6-methylpyrimidin-5-ol (3.17) and 2-(N,N-dimethylamino)-4-hexadecyl-6-methylpyrimidin-5-ol (3.18) were synthesized in analogy with the synthesis of compound 3.10 (Scheme 17). First,
the fully protected pyrimidinol 3.57 was monoalkylated on one of the methyl
groups by generating the carbanion with n-BuLi in presence of butyl bromide,
nonyl bromide and pentadecyl bromide to afford 5-(benzyloxy)-2-(2,5-dimethyl-
1H-pyrrol-1-yl)-4-methyl-6-pentylpyrimidine (3.67), 5-(benzyloxy)-4-decyl-2-
(2,5-dimethyl-1H-pyrrol-1-yl)-6-methylpyrimidine (3.68) and 5-(benzyloxy)-2-
(2,5-dimethyl-1H-pyrrol-1-yl)-4-hexadecyl-6-methylpyrimidine (3.69) in 69%,
53% and 24% yields, respectively. The exocyclic amines were then deprotected
by treatment of compounds 3.67, 3.68 and 3.69 with hydroxylamine
hydrochloride to afford 2-amino-5-(benzyloxy)-4-methyl-6-pentylpyrimidine
(3.70), 2-amino-5-(benzyloxy)-4-decyl-6-methylpyrimidine (3.71) and 2-amino-
5-(benzyloxy)-4-hexadecyl-6-methylpyrimidine (3.72) in 80%, 75% and 100%
yields, respectively. The exocyclic amines of compounds 3.70, 3.71 and 3.72
were then dimethylated using formalin and sodium cyanoborohydride to afford 5-
(benzyloxy)-2-N,N-dimethylamino-4-methyl-6-pentylpyrimidine (3.73), 5-
(benzyloxy)-4-decyl-2-N,N-dimethylamino-6-methylpyrimidine (3.74) and 5-
(benzyloxy)-2-N,N-dimethylamino-4-hexadecyl-6-methylpyrimidine (3.75) in
41%, 46% and 41% yields, respectively. Finally, compounds 3.16, 3.17 and 3.18
were obtained quantitatively by respective hydrogenolysis of compounds 3.73,
3.74 and 3.75 over Pd(OH)$_2$/C in methanol.
Scheme 17: Route employed for the synthesis of pyrimidinol analogues 3.16, 3.17 and 3.18.

The syntheses of 4-decyl-2-(N,N-dimethylamino)-6-methoxypyrimidin-5-ol (3.19) and 2-(N,N-dimethylamino)-4-hexadecyl-6-methoxypyrimidin-5-ol (3.20) were accomplished in analogy with the synthesis of 3.15 (Scheme 18). First, 2-(N,N-dimethylamino)-4-methoxy-6-methylpyrimidine (3.63) was monoalkylated on one of the methyl groups by generating the carbanion with n-BuLi in presence of nonyl bromide and pentadecyl bromide to afford 4-decyl-2-N,N-dimethylamino-6-methoxypyrimidine (3.76) and 2-N,N-dimethylamino-4-hexadecyl-6-methoxypyrimidine (3.77) in 66% and 62% yields, respectively. The pyrimidines 3.76 and 3.77 were then brominated at position 5 to obtain 5-bromo-4-decyl-2-N,N-dimethylamino-6-methoxypyrimidine (3.78) and 5-bromo-2-N,N-dimethylamino-4-hexadecyl-6-methoxypyrimidine (3.79) in 82% and 95% yields, respectively. Finally, compounds 3.19 and 3.20 were obtained in 27% and 55%
yields, respectively by treating compounds 3.78 and 3.79 with n-BuLi in presence of TMEDA, then with trimethyl borate and finally with hydrogen peroxide in subsequent steps. Compound 3.20 was then O-acetylated in order to obtain 5-acetoxy-2-N,N-dimethylamino-4-hexadecyl-6-methoxypyrimidine (3.21) in 80% yield.

Scheme 18: Route employed for the synthesis of pyrimidinol analogues 3.19, 3.20 and 3.21.

In order to create a large library of compounds containing the same core, 5-(benzyloxy)-4-(γ-butenyl)-2-N,N-dimethylamino-6-methoxypyrimidine (3.83) was synthesized as a common intermediate (Scheme 19). First, compound 3.63 was treated with n-BuLi in presence of TMEDA to form the corresponding carbanion on the methyl group and then treated with allyl bromide to obtain 4-(γ-butenyl)-2-N,N-dimethylamino-6-methoxypyrimidine (3.80) in 50% yield. Then,
the pyrimidine core of compound 3.80 was brominated at position 5 using N-bromosuccinimide to obtain 5-bromo-4-(γ-butenyl)-2,N,N-dimethylamino-6-methoxypyrimidine (3.81). Compound 3.81 was then converted to 4-(γ-butenyl)-2-(N,N-dimethylamino)-6-methoxypyrimidin-5-ol (3.82) in 70% yield by first treating it with n-BuLi in presence of TMEDA, then with trimethyl borate and finally with hydrogen peroxide in subsequent steps. Finally, the hydroxyl group of compound 3.82 was benzyllated using benzyl bromide in presence of potassium carbonate to obtain compound 3.83 in 98% yield.

Scheme 19: Route employed for the synthesis of intermediate 3.83.

For the synthesis of 2-(N,N-dimethylamino)-4-methoxy-6-tetradecylpyrimidin-5-ol (3.22), 2-(N,N-dimethylamino)-4-methoxy-6-(10-methyladamantyloxydecyl)pyrimidin-5-ol (3.23) and 2-(N,N-dimethylamino)-4-methoxy-6-octadecylpyrimidin-5-ol (3.24) a different approach was used (Scheme 20). Compound 3.83 was attached to 1-dodecene, 1-methyladamantyloxy-γ-
octene (3.84) and 1-hexadecene by cross metathesis reaction using 2nd generation Grubb’s catalyst followed by hydrogenation in presence of palladium-on-carbon to obtain 3.22, 3.23 and 3.24 in 40%, 11% and 36% yields, respectively. Compound 3.84 was previously synthesized by treating 8-bomo-1-octene with 1-(hydroxymethyl)adamantane and sodium hydride in 58% yield.

The redox cores of compounds 3.10 and 3.15 were synthesized in three and four steps, respectively (Scheme 21). First, the exocyclic amine of compound 3.57 was deprotected by treatment with hydroxylamine hydrochloride to obtain 2-amino-5-(benzyloxy)-4,6-dimethylpyrimidine (3.85) in 98% yield. Then,
compound 3.85 was dimethylated using a mixture of formalin and formic acid to obtain 5-(benzyloxy)-2-\(N,N\)-dimethylamino-4,6-dimethylpyrimidine (3.86) in 23% yield. Finally, the hydroxyl group of compound 3.86 was deprotected quantitatively using palladium-on-carbon under a hydrogen atmosphere to obtain 2-(\(N,N\)-dimethylamino)-4,6-dimethylpyrimidin-5-ol (3.87). 2-(\(N,N\)-Dimethylamino)-4-methoxy-6-methylpyrimidin-5-ol (3.89) was prepared in two steps starting from intermediate 3.63. First, compound 3.63 was brominated at position 5 using \(N\)-bromosuccinimide to obtain 5-bromo-2-(\(N,N\)-dimethylamino)-4-methoxy-6-methylpyrimidine (3.88) in 73% yield. The bromine of compound 3.88 was then replaced by a hydroxyl group by first treating it with \(n\)-BuLi in presence of TMEDA, then with trimethyl borate, and finally with hydrogen peroxide in subsequent steps to obtain 2-(\(N,N\)-dimethylamino)-4-methoxy-6-methylpyrimidin-5-ol (3.89) in 8% yield.

**Scheme 21:** Route employed for the synthesis of redox cores 3.87 and 3.89.
3.2.1.3 Evaluation of pyridinol and pyrimidinol analogues

3.2.1.3.1 Electrochemical evaluation of pyridinol and pyrimidinol redox cores

The ability of compound 3.1 to transfer its phenolic hydrogen atom and form a stabilized radical has been studied by the use of electron spin resonance (ESR). Figure 22A shows the signal obtained from the radical activation of compound 3.1 in the presence of illuminated di-tert-butyl peroxide. The hyperfine splitting constants are as follows: $a_H$ (3H, o-methyl) = 3.5 G, $a_H$ (2H, o-carbon chain) = 3.3 G, $a_H$ (1H, o-carbon chain) = 3.4 G, $a_H$ (1H, m-ring) = 1.9 G, $a_H$ (6H, $p$-NMe$_2$) = 4.2 G, $a_N$ (1N, ring) = 1.1 G, $a_N$ (1N, $p$-NMe$_2$) = 5.9 G, and $g$ = 2.00493

![Figure 22A](image)

**Figure 22**: A) ESR signal and simulation of compound 3.1 (5 mM) in dry de-aerated benzene at 25 °C. B) Resonance stabilized 3-pyridinoxyl radical resulting from abstraction of the O-H hydrogen atom. $^{98}$ Experiment performed by Dr. Ruth Goldschmidt and Manikandadas M. Madathil.
The simulation corresponds to the experimental signal with a relative error of $R = 0.983$. Figure 22B shows the resonance contributors of the stabilized 3-pyridinoxyl radical putatively formed.

**Cyclic voltammetry**

Cyclic voltammetric analyses of the redox cores of compounds 3.1, 3.6 and 3.7 (compounds 3.50, 3.52 and 3.53, respectively) are presented in Figure 23.

**Figure 23:** Cyclic voltammetry of compounds 3.50 (green), 3.52 (red) and 3.53 (blue) in 0.1 M tetrabutylammonium perchlorate in acetonitrile using Ag/AgCl electrode as a reference. Experiment performed by Manikandadas M. Madathil.
Figure 23 shows that the redox cores 3.50, 3.52 and 3.53 are more easily reduced than CoQ0 (cf. Figure 13). Compound 3.50 showed a more negative reduction potential than any of the other pyridinol cores evaluated.

Figure 24 shows that the redox core of compound 3.1 (compound 3.50) is unstable to repetitive redox cycles while the redox core of compound 3.7 (compound 3.53) was completely unstable.

**Figure 24**: Normalized peak current (\(i_{p(0)} - i_{p(1)}\)) plotted against time in continuous cyclic voltammetric analysis of compound 3.50 (black) versus compound 3.53 (red). Experiment performed by Manikandadas M. Madathil.

Cyclic voltammetric analyses of the redox cores of compounds 3.10 and 3.15 (compounds 3.88 and 3.89, respectively) are presented in Figure 25. The figure shows that the redox cores 3.88 and 3.89 are more easily reduced than
CoQ$_0$ (cf. Figure 13). The redox core 3.88 is more easily reduce than the redox core 3.89.

![Cyclic voltammetry of compounds](image)

**Figure 25:** Cyclic voltammetry of compounds 3.88 (blue) and 3.89 (red) in 0.1 M tetrabutylammonium perchlorate in acetonitrile using Ag/AgCl electrode as a reference. Experiment performed by Manikandadas M. Madathil.

### 3.2.1.3.2 Biochemical evaluation of pyridinol and pyrimidinol analogues

**Inhibition of lipid peroxidation**

The ability of the pyridinol analogues to quench lipid peroxidation has been studied in FRDA lymphocytes depleted of glutathione by treatment with diethyl maleate (DEM). The cells were pre-incubated with the test compounds for 16 h prior to DEM treatment. Lipid peroxidation was measured by a quantitative
FACS analysis using the fluorescent probe, \( C_{11}\text{-BODIPY}^{581/591} \), reported to be highly accurate in measuring lipid peroxidation.\(^{99}\) The results, presented in Table 2, show the most potent activity for compounds 3.4 and 3.7 among the pyridinol analogues with idebenone side chain. Compound 3.1, lacking a methyl group at position 6, was slightly less potent than idebenone, and better than compound 3.2. However, the redox core of compound 3.1 (compound 3.50) lacked significant activity. Finally, compound 3.3 had the lowest activity for suppressing lipid peroxidation among the analogues having a 10-hydroxydecyl side chain. Compounds 3.5 and 3.6 had activities similar to compound 3.3 (data not shown).

**Table 2:** Suppression of lipid peroxidation by pyridinol antioxidants in cultured FRDA lymphocytes treated with DEM. Experiment performed by Dr. Omar M. Khdour.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Lipid peroxidation quenching activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5 ( \mu \text{M} )</td>
</tr>
<tr>
<td>idebenone</td>
<td>68 ± 6.4</td>
</tr>
<tr>
<td>3.1</td>
<td>71 ± 6.0</td>
</tr>
<tr>
<td>3.2</td>
<td>62 ± 6.4</td>
</tr>
<tr>
<td>3.3</td>
<td>20 ± 10</td>
</tr>
<tr>
<td>3.4</td>
<td>90 ± 2.9</td>
</tr>
<tr>
<td>3.7</td>
<td>87 ± 5.1</td>
</tr>
<tr>
<td>3.50</td>
<td>3.5 ± 2.3</td>
</tr>
</tbody>
</table>
Pyrimidinol analogues 3.17 and 3.19 exhibited better activity as compared to compounds 3.10 and 3.15, respectively. In general, pyrimidinol analogues with long aliphatic side chains exhibited good quenching activity, except for compound 3.24 that had a comparable activity as 3.15. Compound 3.16 had a poor activity even at higher concentrations.

**Table 3:** Suppression of lipid peroxidation by pyrimidinol antioxidants in cultured FRDA lymphocytes treated with DEM. Experiment performed by Dr. Omar M. Khdour.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Lipid peroxidation quenching activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 µM</td>
</tr>
<tr>
<td>3.10</td>
<td>1.3 ± 1.6</td>
</tr>
<tr>
<td>3.15</td>
<td>12 ± 1.6</td>
</tr>
<tr>
<td>3.16</td>
<td>-</td>
</tr>
<tr>
<td>3.17</td>
<td>-</td>
</tr>
<tr>
<td>3.18</td>
<td>-</td>
</tr>
<tr>
<td>3.19</td>
<td>42 ± 6.0</td>
</tr>
<tr>
<td>3.20</td>
<td>94 ± 0.6</td>
</tr>
<tr>
<td>3.22</td>
<td>92 ± 3.8</td>
</tr>
<tr>
<td>3.23</td>
<td>88 ± 2.7</td>
</tr>
<tr>
<td>3.24</td>
<td>15 ± 4.4</td>
</tr>
</tbody>
</table>
Reactive oxygen species

The ability of the pyridinol and pyrimidinol analogues to suppress ROS induced by depletion of glutathione was evaluated in CEM cells and FRDA lymphocytes. ROS was measured in a quantitative FACS experiment, using dichlorofluorescin diacetate (DCFH-DA) as a substrate for determining intracellular oxidant production. DCFH-DA is hydrolyzed by esterases to afford 2,7-dichlorodihydrofluorescin (DCFH), the latter of which is trapped within the cell. This non-fluorescent molecule is then oxidized to fluorescent dichlorofluorescin (DCF) by the action of cellular oxidants. Protection of CEM cells, presented in Table 4, shows that analogues 3.8 and 3.4 were more potent than compound 3.1, which was more potent than idebenone. Similar (Table 5), most of the pyrimidinol analogues were more potent than idebenone. Compounds 3.15, 3.20, 3.22 and 3.23 were more potent than their parent compound 3.10 at 2.5 \( \mu \)M concentration. At 250 nM concentration compounds 3.17, 3.18, 3.20 and 3.23 were the most potent analogues tested. Compound 3.20 was the most potent analogue tested at 50 nM concentration. Compound 3.16 did not show any activity at 250 nM concentration.

Because the use of DCF fluorescence as an endpoint has been shown to lack specificity under certain circumstances,\(^\text{100,101}\) it was shown in the present study that the increase in DCF fluorescence induced by DEM was completely reversed by superoxide dismutase + catalase, or by the antioxidant N-acetylcysteine (data not shown).
Table 4: Suppression of ROS by pyridinol antioxidants in cultured CEM lymphocytes treated with DEM. Experiment performed by Dr. Ruth Goldschmidt.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ROS suppression activity (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.25 µM</td>
</tr>
<tr>
<td>idebenone</td>
<td></td>
</tr>
<tr>
<td>decylubiquinone</td>
<td></td>
</tr>
<tr>
<td><strong>3.1</strong></td>
<td>42 ± 4</td>
</tr>
<tr>
<td><strong>3.2</strong></td>
<td>28 ± 8</td>
</tr>
<tr>
<td><strong>3.3</strong></td>
<td>22 ± 6</td>
</tr>
<tr>
<td><strong>3.4</strong></td>
<td>77 ± 3</td>
</tr>
<tr>
<td><strong>3.6</strong></td>
<td></td>
</tr>
<tr>
<td><strong>3.7</strong></td>
<td>47 ± 5</td>
</tr>
<tr>
<td><strong>3.8</strong></td>
<td>85 ± 2</td>
</tr>
<tr>
<td><strong>3.9</strong></td>
<td>40 ± 2</td>
</tr>
</tbody>
</table>


Table 5: Suppression of ROS by pyrimidinol antioxidants in cultured CEM lymphocytes treated with DEM. Experiment performed by Dr. Ruth Goldschmidt.

<table>
<thead>
<tr>
<th>Compound</th>
<th>0.05 µM</th>
<th>0.25 µM</th>
<th>0.5 µM</th>
<th>2.5 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>74 ± 6.0</td>
</tr>
<tr>
<td>3.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>3.16</td>
<td>-</td>
<td>0 ± 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.17</td>
<td>-</td>
<td>89 ± 3.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.18</td>
<td>-</td>
<td>90 ± 1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.19</td>
<td>-</td>
<td>54 ± 4.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.20</td>
<td>71 ± 3.0</td>
<td>89 ± 7.0</td>
<td>90 ± 7.0</td>
<td>89 ± 5.0</td>
</tr>
<tr>
<td>3.21</td>
<td>52 ± 4.0</td>
<td>75 ± 4.0</td>
<td>89 ± 7.0</td>
<td>88 ± 8.0</td>
</tr>
<tr>
<td>3.22</td>
<td>31 ± 4.0</td>
<td>40 ± 2.0</td>
<td>63 ± 3.0</td>
<td>90 ± 5.0</td>
</tr>
<tr>
<td>3.23</td>
<td>-</td>
<td>82 ± 3.0</td>
<td>-</td>
<td>91 ± 5.0</td>
</tr>
<tr>
<td>3.24</td>
<td>-</td>
<td>49 ± 5.0</td>
<td>-</td>
<td>87 ± 6.0</td>
</tr>
</tbody>
</table>

Protection of FRDA lymphocytes presented in Table 6 shows that analogue 3.8 was more potent than compound 3.1. Similarly, compound 3.20 was more potent that its parent compound 3.10. Compounds 3.8 and 3.20 showed almost complete protection at 250 nM concentration.
Table 6: Suppression of ROS by pyridinol and pyrimidinol antioxidants in cultured FRDA lymphocytes treated with DEM. Experiment performed by Dr. Omar M. Khdour.

<table>
<thead>
<tr>
<th>Compound</th>
<th>0.25 µM</th>
<th>0.5 µM</th>
<th>2.5 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>idebenone</td>
<td>21 ± 4.8</td>
<td>38 ± 4.2</td>
<td>75 ± 4.3</td>
</tr>
<tr>
<td>3.1</td>
<td>29 ± 4.4</td>
<td>40 ± 5.0</td>
<td>78 ± 4.3</td>
</tr>
<tr>
<td>3.8</td>
<td>86 ± 5.1</td>
<td>92 ± 3.0</td>
<td>-</td>
</tr>
<tr>
<td>3.10</td>
<td>35 ± 4.4</td>
<td>48 ± 4.2</td>
<td>83 ± 4.0</td>
</tr>
<tr>
<td>3.20</td>
<td>87 ± 3.3</td>
<td>94 ± 0.7</td>
<td>-</td>
</tr>
</tbody>
</table>

Preservation of mitochondrial membrane potential

The ability of the pyridinol and pyrimidinol analogues to preserve mitochondrial membrane potential under conditions of oxidative stress was studied. Mitochondrial membrane potential, Δψm, is an important parameter of mitochondrial integrity and is essential for maintaining the physiological function of the respiratory chain in ATP synthesis. Δψm was estimated using the cationic fluorescent probe tetramethylrhodamine methyl ester (TMRM), which preferentially accumulates in the mitochondria due to the negative membrane potential across the inner mitochondrial membrane, in accordance with the Δψm Nernst potential. The red fluorescent signal in mitochondria decreases when Δψm is impaired, and can be measured using flow cytometry. A representative flow cytometric two-dimensional color density dot plot analyses of the...
mitochondrial membrane potential measurements with and without incubation in the presence of selected compounds are shown in Figure 26.

<table>
<thead>
<tr>
<th>untreated control</th>
<th>Treated control</th>
<th>FCCP</th>
<th>no TMRM</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
</tr>
<tr>
<td>3.1 (0.5 μM)</td>
<td>3.4 (0.5 μM)</td>
<td>3.7 (0.5 μM)</td>
<td>Idebenone (0.5 μM)</td>
</tr>
<tr>
<td><img src="image5" alt="Graph" /></td>
<td><img src="image6" alt="Graph" /></td>
<td><img src="image7" alt="Graph" /></td>
<td><img src="image8" alt="Graph" /></td>
</tr>
<tr>
<td>3.1 (1 μM)</td>
<td>3.4 (1 μM)</td>
<td>3.7 (1 μM)</td>
<td>Idebenone (1 μM)</td>
</tr>
<tr>
<td><img src="image9" alt="Graph" /></td>
<td><img src="image10" alt="Graph" /></td>
<td><img src="image11" alt="Graph" /></td>
<td><img src="image12" alt="Graph" /></td>
</tr>
<tr>
<td>3.1 (2.5 μM)</td>
<td>3.4 (2.5 μM)</td>
<td>3.7 (2.5 μM)</td>
<td>Idebenone (2.5 μM)</td>
</tr>
<tr>
<td><img src="image13" alt="Graph" /></td>
<td><img src="image14" alt="Graph" /></td>
<td><img src="image15" alt="Graph" /></td>
<td><img src="image16" alt="Graph" /></td>
</tr>
</tbody>
</table>

**Figure 26:** A representative flow cytometric two-dimensional color density dot plot analyses of the mitochondrial membrane potential measurements. Experiment performed by Dr. Omar M. Khdour.

The percentage of cells with intact mitochondrial membrane potential appears in the top right quadrant of individual treatments. Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), a commonly used uncoupler of oxidative phosphorylation in mitochondria, was employed to dissipate the chemiosmotic proton gradient ($\Delta\mu H^+$). The lower levels of TMRM fluorescence resulting from FCCP treatment reflect the depolarization of mitochondrial inner membrane potential. Figure 27 summarizes the relative geometric mean

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fluorescence intensity (GMFI) of the flow cytometric profiles of some pyridinol analogues. It shows clearly that compound 3.4 had the greatest potency among pyridinol analogues, and acted in a dose-dependent manner; compound 3.7 was almost as good although not strictly dose-dependent in this experiment. These three compounds were clearly better than idebenone, and better than compounds 3.1 and 3.2, which had activity in the same range as idebenone. Finally, compound 3.3 had the lowest activity and the redox core of compound 3.1 was completely inactive.

Figure 27: Mitochondrial membrane potential protection of some pyridinol analogues. 

For pyrimidinol analogues it was found that compounds with longer aliphatic side chains, like compounds 3.20 and 3.22, clearly have better activities protecting the mitochondrial membrane potential (Figure 28). Also, it was found
that more electron donating groups present in the redox core like in compound 3.19 provides enhanced protection.

Interestingly, compound 3.24 showed the lowest protection of the mitochondrial membrane potential among the analogues with long aliphatic side chains. Suggesting that there is an optimal side chain length to support this property.

![Figure 28: Mitochondrial membrane potential protection of some pyrimidinol analogues. Experiment performed by Dr. Omar M. Khdour.](image)

**Cytoprotection**

Cytoprotection was measured initially using cultured FRDA lymphocytes that were treated with diethyl maleate to induce oxidative stress through depletion of glutathione. As shown in Table 7, compounds 3.1, 3.4 and 3.7 were the most effective pyridinol analogue having an idebenone side chain at the concentrations tested. Compound 3.8 was the most active of the pyridinol analogues. In general pyrimidinol analogues had better protective activities than pyridinols and idebenone as shown in Table 8. Compounds with long aliphatic side chains, like...
compounds 3.18, 3.20, 3.22, and 3.23, were the most effective pyrimidinol analogues tested at 100 nM concentration. Compound 3.24 had the lowest activity of the pyrimidinol analogues tested.

**Table 7:** Cytoprotective effects of pyridinol antioxidants on the viability of cultured FRDA lymphocytes treated with DEM. Experiment performed by Jennifer Jaruvangsanti.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Viable cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 µM</td>
</tr>
<tr>
<td>idebenone</td>
<td>31 ± 0.6</td>
</tr>
<tr>
<td>decylubiquinone</td>
<td>-</td>
</tr>
<tr>
<td>3.1</td>
<td>59 ± 5.3</td>
</tr>
<tr>
<td>3.2</td>
<td>59 ± 2.4</td>
</tr>
<tr>
<td>3.3</td>
<td>17 ± 4.0</td>
</tr>
<tr>
<td>3.4</td>
<td>49 ± 9.0</td>
</tr>
<tr>
<td>3.6</td>
<td>33 ± 2.0</td>
</tr>
<tr>
<td>3.7</td>
<td>38 ± 7.0</td>
</tr>
<tr>
<td>3.8</td>
<td>80 ± 10</td>
</tr>
<tr>
<td>3.9</td>
<td>19 ± 9.0</td>
</tr>
</tbody>
</table>
Table 8: Cytoprotective effects of pyrimidinol antioxidants on the viability of cultured FRDA lymphocytes treated with DEM. Experiment performed by Jennifer Jaruvangsanti.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Viable cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 µM</td>
</tr>
<tr>
<td>3.10</td>
<td>66 ± 7.0</td>
</tr>
<tr>
<td>3.14</td>
<td>68 ± 11</td>
</tr>
<tr>
<td>3.15</td>
<td>62 ± 7.4</td>
</tr>
<tr>
<td>3.16</td>
<td>50 ± 3.6</td>
</tr>
<tr>
<td>3.17</td>
<td>60 ± 4.2</td>
</tr>
<tr>
<td>3.18</td>
<td>68 ± 0.9</td>
</tr>
<tr>
<td>3.19</td>
<td>45 ± 2.4</td>
</tr>
<tr>
<td>3.20</td>
<td>83 ± 4.0</td>
</tr>
<tr>
<td>3.21</td>
<td>52 ± 6.0</td>
</tr>
<tr>
<td>3.22</td>
<td>72 ± 5.0</td>
</tr>
<tr>
<td>3.23</td>
<td>76 ± 6.0</td>
</tr>
<tr>
<td>3.24</td>
<td>28 ± 6.0</td>
</tr>
</tbody>
</table>

Mitochondrial electron transport chain function

The inhibitory effects of the test compounds on bovine heart mitochondrial complexes I, III and IV were evaluated using submitochondrial particles (SMP), by measuring NADH oxidase activity assay. The results
presented in Table 9 show that compounds 3.1, 3.2, 3.3, and 3.4 were all less inhibitory to respiratory chain function of complexes I, III and IV than idebenone. Compound 3.7 was the most inhibitory and compound 3.8 the least inhibitory of the pyridinol analogues.

Table 9: Inhibitory effects of pyridinol analogues on bovine heart mitochondrial NADH oxidase activity. Experiment performed by Sriloy Dey.

<table>
<thead>
<tr>
<th>Compound</th>
<th>NADH oxidase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 µM</td>
</tr>
<tr>
<td>idebenone</td>
<td>65 ± 2.5</td>
</tr>
<tr>
<td>decylubiquinone</td>
<td>93 ± 6.9</td>
</tr>
<tr>
<td>3.1</td>
<td>85 ± 2.5</td>
</tr>
<tr>
<td>3.2</td>
<td>74 ± 2.2</td>
</tr>
<tr>
<td>3.3</td>
<td>71 ± 4.5</td>
</tr>
<tr>
<td>3.4</td>
<td>78 ± 4.0</td>
</tr>
<tr>
<td>3.6</td>
<td>-</td>
</tr>
<tr>
<td>3.7</td>
<td>27 ± 0.8</td>
</tr>
<tr>
<td>3.8</td>
<td>92 ± 11</td>
</tr>
</tbody>
</table>

Table 10 shows that compounds 3.17 and 3.19 were stronger inhibitors of NADH oxidase activity than idebenone and compound 3.10, which exhibited
inhibition similar to idebenone. Compound 3.15 exhibited less inhibition than compound 3.10 but more than compounds 3.16, 3.18, 3.20, 3.22, 3.23 and 3.24.

**Table 10:** Inhibitory effects of pyrimidinol analogues on bovine heart mitochondrial NADH oxidase activity. Experiment performed by Sriloy Dey.

<table>
<thead>
<tr>
<th>Compound</th>
<th>NADH oxidase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 μM</td>
</tr>
<tr>
<td>3.10</td>
<td>49 ± 4.3</td>
</tr>
<tr>
<td>3.15</td>
<td>63 ± 3.0</td>
</tr>
<tr>
<td>3.16</td>
<td>85 ± 7.4</td>
</tr>
<tr>
<td>3.17</td>
<td>40 ± 2.1</td>
</tr>
<tr>
<td>3.18</td>
<td>96 ± 2.6</td>
</tr>
<tr>
<td>3.19</td>
<td>38 ± 2.1</td>
</tr>
<tr>
<td>3.20</td>
<td>84 ± 4.0</td>
</tr>
<tr>
<td>3.22</td>
<td>-</td>
</tr>
<tr>
<td>3.23</td>
<td>-</td>
</tr>
<tr>
<td>3.24</td>
<td>-</td>
</tr>
</tbody>
</table>

For complex I inhibition (Table 11), compounds 3.1, 3.4, 3.5 and 3.7 were better inhibitors than idebenone. Compounds 3.1 and 3.7 were the most inhibitory. Compound 3.8 was the least inhibitory among of the pyridinol analogues.
**Table 11:** Inhibitory effect of pyridinol analogues on bovine heart mitochondrial complex I. Experiment performed by Dr. Valérie C. Collin.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
<th>Iₘₐₓ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>idebenone</td>
<td>6.3 ± 2.5</td>
<td>84 ± 2.3</td>
</tr>
<tr>
<td>decylubiquinone</td>
<td>&gt; 200</td>
<td>ND</td>
</tr>
<tr>
<td>3.1</td>
<td>1.3 ± 0.4</td>
<td>76 ± 3.0</td>
</tr>
<tr>
<td>3.2</td>
<td>7.8 ± 0.1</td>
<td>81 ± 0.6</td>
</tr>
<tr>
<td>3.3</td>
<td>7.8 ± 0.3</td>
<td>84 ± 2.9</td>
</tr>
<tr>
<td>3.4</td>
<td>2.6 ± 0.1</td>
<td>81 ± 4.4</td>
</tr>
<tr>
<td>3.5</td>
<td>4.0 ± 0.2</td>
<td>84 ± 2.0</td>
</tr>
<tr>
<td>3.7</td>
<td>0.70 ± 0.02</td>
<td>90 ± 0.7</td>
</tr>
<tr>
<td>3.8</td>
<td>&gt; 500</td>
<td>ND</td>
</tr>
</tbody>
</table>

As shown in Table 12 pyrimidinol analogues 3.10, 3.12, 3.14, 3.17, 3.22 and 2.23 were more inhibitory of complex I than idebenone. Compounds 3.15, 3.16, 3.19 and 3.20 were less inhibitory than idebenone but more inhibitory than compound 3.18. Compound 3.24 was the least inhibitory among the pyrimidinol analogues.
Table 12: Inhibitory effect of pyrimidinol analogues on bovine heart mitochondrial complex I. Experiment performed by Dr. Valérie C. Collin.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
<th>$I_{\text{max}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.10</td>
<td>1.2 ± 0.1</td>
<td>85 ± 3.0</td>
</tr>
<tr>
<td>3.12</td>
<td>3.0 ± 0.3</td>
<td>82 ± 0.2</td>
</tr>
<tr>
<td>3.13</td>
<td>7.4 ± 0.6</td>
<td>91 ± 1.5</td>
</tr>
<tr>
<td>3.14</td>
<td>1.0 ± 0.2</td>
<td>89 ± 0.3</td>
</tr>
<tr>
<td>3.15</td>
<td>63± 3.0</td>
<td>50 ± 2.1</td>
</tr>
<tr>
<td>3.16</td>
<td>38 ± 3.0</td>
<td>98 ± 1.0</td>
</tr>
<tr>
<td>3.17</td>
<td>1.7 ± 0.8</td>
<td>88 ± 2.0</td>
</tr>
<tr>
<td>3.18</td>
<td>198 ± 12</td>
<td>84 ± 0.6</td>
</tr>
<tr>
<td>3.19</td>
<td>17 ± 5.0</td>
<td>91 ± 1.0</td>
</tr>
<tr>
<td>3.20</td>
<td>19 ± 3.0</td>
<td>86 ± 9.0</td>
</tr>
<tr>
<td>3.22</td>
<td>3 ± 0.3</td>
<td>69 ± 3.0</td>
</tr>
<tr>
<td>3.23</td>
<td>1.3 ± 0.3</td>
<td>71 ± 1.0</td>
</tr>
<tr>
<td>3.24</td>
<td>&gt; 500</td>
<td>ND</td>
</tr>
</tbody>
</table>

ATP production

The effects of pyridinol and pyrimidinol analogues on cellular ATP levels were also studied. All of the cells were adapted to growth on galactose for at least one week prior to measuring ATP levels. As shown in Table 13, idebenone
strongly diminished ATP levels in Friedreich’s ataxia lymphocytes in a concentration dependent fashion. In the presence of 25 µM idebenone, only residual ATP concentrations were detected. Compound 3.7 showed a similar behavior as idebenone. Compounds 3.4, 3.8 and its acetate form (3.9) were the most effective among pyridinol analogues (Table 13).

**Table 13:** Total ATP concentration in Friedreich’s ataxia lymphocytes, upon incubation with the pyridinol compounds for 48 h. Experiment performed by Dr. Omar M. Khdour.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total ATP concentration (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 µM</td>
</tr>
<tr>
<td>idebenone</td>
<td>-</td>
</tr>
<tr>
<td>decylubiquinone</td>
<td>-</td>
</tr>
<tr>
<td>3.4</td>
<td>105 ± 3.0</td>
</tr>
<tr>
<td>3.7</td>
<td>96 ± 3.0</td>
</tr>
<tr>
<td>3.8</td>
<td>99 ± 3.3</td>
</tr>
<tr>
<td>3.9</td>
<td>98 ± 2.3</td>
</tr>
</tbody>
</table>

Pyrimidinols provided better support for ATP production than idebenone and in FRDA lymphocytes at 25 µM concentration (Table 14). Compounds 3.16, 3.18, 3.19, 3.20, 3.21, 3.22, 3.23 and 3.24 provided the best support for ATP synthesis among the pyrimidinol analogues. Encouragingly, when compounds 3.20, 3.21, 3.22 and 3.23 were used at 5 µM, there was an increase of about 9% in
the ATP production. Compounds 3.22 and 3.23 afforded an increase in the ATP at about 12% when used at 1 μM concentration.

**Table 14**: Total ATP concentration in Friedreich’s ataxia lymphocytes, upon incubation with pyrimidinol analogues for 48 h. Experiment performed by Dr. Omar M. Khdour.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total ATP concentration (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 μM</td>
</tr>
<tr>
<td>3.10</td>
<td>-</td>
</tr>
<tr>
<td>3.15</td>
<td>-</td>
</tr>
<tr>
<td>3.16</td>
<td>-</td>
</tr>
<tr>
<td>3.17</td>
<td>-</td>
</tr>
<tr>
<td>3.18</td>
<td>-</td>
</tr>
<tr>
<td>3.19</td>
<td>-</td>
</tr>
<tr>
<td>3.20</td>
<td>-</td>
</tr>
<tr>
<td>3.21</td>
<td>-</td>
</tr>
<tr>
<td>3.22</td>
<td>112 ± 3.2</td>
</tr>
<tr>
<td>3.23</td>
<td>112 ± 2.3</td>
</tr>
<tr>
<td>3.24</td>
<td>104 ± 2.3</td>
</tr>
</tbody>
</table>
3.3 DISCUSSION

In our efforts to create a potent antioxidant that could be delivered to mitochondria, a number of pyridinol and pyrimidinol analogues were synthesized. These series of compounds contained idebenone side chains as well as hydrocarbon side chains of varying lengths. They also had methyl and methoxyl groups at varying positions on the pyridinol and pyrimidinol redox scores. For the synthesis of those analogues containing a methyl group ortho to the phenolic OH, the alkylation was done by selective lithiation of the methyl group next to the pyridine or pyrimidine nitrogen in the fully protected pyridinol or pyrimidinol core,88,89 followed by the addition of the corresponding alkyl bromide. When a methoxyl group was placed at the ortho position of the phenolic OH, lithiation of the methyl group next to the pyridine or pyrimidine nitrogen did not afford the desired product; only decomposition was observed. To solve this problem it was logical to perform the lithiation on less electron-rich cores, so the alkylation step was done before installing the phenolic OH group.

It was also noticed that for the conversion of the brominated core to the corresponding pyridinol or pyrimidinol, the effect of the substituent at the position para to the bromide was important. The first strategy involved using a palladium catalyst and a phosphorous ligand when the exocyclic amine was protected as the dimethyl pyrrole derivative. When the exocyclic amine was protected as a dimethylamino group, the conversion of the bromide could not be successfully accomplished by this method. Then, a lithium/bromine exchange followed by
boronation and subsequent oxidation afforded the corresponding pyridinol and pyrimidinol compounds.

**Pyridinols**

The antioxidant properties of the new pyridinol analogues were tested and analyzed in selected biological and biochemical assays. Cultured Friedreich’s ataxia lymphocytes were challenged with diethyl maleate, which depletes cellular glutathione.\(^{104-106}\) Diethyl maleate induces greater oxidative stress than is likely to be encountered physiologically, permitting the identification of compounds anticipated to function robustly under pathophysiological conditions.

Preliminary results showed the electronic effect of the substituents plays a very important role in their activity. It was observed that when the dimethylamino group at the *para* position of phenolic OH was exchanged for a methoxyl group (compound 3.5), the activity as antioxidant decreased considerably. The same result was observed when a dimethyl pyrrole group (compound 3.3) was used. These results are in agreement with the fact that more electron donating groups lower the bond dissociation energy of the O-H bond and, therefore, their ability to protect against oxidative stress is superior. However, when a methoxyl group replaced the methyl group in compound 3.1 to afford compound 3.6, activity decreased considerably, possibly due to instability towards air oxidation or decomposition of the oxidized form. The greater antioxidant potency of pyridinols having a strong donating group at the position *para* of the phenolic OH group is
entirely consistent with the reduction of their redox core at more oxidizing potentials than the redox core of idebenone (and coenzyme Q\textsubscript{10}).

The results indicated that compound \textbf{3.4} performed better than the other analogues containing the idebenone side chain. This presumably reflects the steric effects of the C-methyl groups of the pyrrolidine moiety in preventing side reactions at the unsubstituted position of the pyridine ring of compound \textbf{3.4}. Logically, this prompted us to insert a methyl group at the free position of the core of compound \textbf{3.1}, affording compound \textbf{3.7}.

Many compounds have been reported to inhibit mitochondrial complexes I\textsuperscript{107-110} and III\textsuperscript{111,112} and these invariably strongly diminish cell viability. Since idebenone has been found to significantly inhibit complex I,\textsuperscript{61,62} it seemed logical to consider that this property might limit its useful antioxidant properties. Accordingly, we evaluated most pyridinol analogues for this property using a biochemical assay that measures the activity of NADH oxidase, which encompasses mitochondrial complexes I, III, and IV. In fact, most pyridinols were found to be superior to idebenone in that they displayed less inhibition of NADH oxidase activity (Table 9). While these experiments do not definitively establish the biochemical locus of inhibition by these compounds, complex I seems likely to be the relevant locus.

Interestingly, it appears that compound \textbf{3.7} had better antioxidant activity than \textbf{3.1}, despite the fact that it was more inhibitory to NADH-ubiquinone oxidoreductase (Table 11). This is undoubtedly due to the low stability of
compound 3.1 to the redox cycling (Figure 24), which it is thought to be essential to its antioxidant function.

Previously, studies performed in our research group demonstrated that quinones having a lipophilic side chain with a polar substituent at the end were more inhibitory than those having only hydrocarbons chains, as shown with idebenone and decylubiquinone (Tables 9 and 11). So, it was logical to synthesize compounds in which the side chain had no hydroxyl group at the end. When the side chain of compound 3.7 was changed to a more lipophilic hydrocarbon chain (16 carbons) lacking a hydroxyl group at the end, the activity of the antioxidant was enhanced several fold. It was observed that compound 3.8 displayed very low inhibition of NADH oxidase (Table 9) and NADH-ubiquinone oxidoreductase (Table 11), which is presumably the reason that this compound was among the most potent antioxidants in the pyridinol series.

As predicted for this family of pyridinol molecules, the mechanism for the scavenging of ROS and lipid radicals is through the formation of a pyridinoxyl radical. To test this hypothesis, we studied the ability of compound 3.1 to form a radical upon hydrogen abstraction of the hydroxyl group at position 3. As described above, the radical derived from compound 3.1 was formed by excitation of di-tert-butyl peroxide, and the signal obtained was measured using an ESR spectrophotometer (Figure 22A). The signal was characterized by its g factor of 2.0049 and the small hyperfine coupling of the pyridine ring nitrogen (1.1G). In agreement with the work of Pratt et al., this is attributed to the electron donating
character of $N(CH_3)_2$ responsible for an increase in contribution to stability arising from the higher energy polar structures (Figure 22B).

It is interesting that the best pyridinol analogues function more effectively than idebenone as regards the several parameters studied, and were more potent overall as cytoprotective agents. Idebenone has also been reported to form a hydroquinone \textit{in situ} via the action of NAD(P)H-quinone reductases, which enables the transfer of electrons to complex III.\textsuperscript{113} When pyridinol analogues were tested for supporting ATP production in FRDA lymphocytes, compounds 3.4, 3.8 and 3.9 showed the best activity in this assay. These results are in complete agreement with their enhanced antioxidant activity and their lesser inhibition of the respiratory chain.

\textbf{Pyrimidinols}

A series of pyrimidinols was subjected to the same biological and biochemical assays as were employed for the pyridinol analogues. The antioxidant properties of pyrimidinol analogues were affected similarly as for pyridinol analogues. The effect of the electron donating group at the position \textit{para} to the phenoxy OH was very important for their activity. Compounds containing a methoxyl group (3.12) or a dimethyl pyrrole group (3.13) at this position had poor activity as antioxidants. Contrary to the pyridinols, when a methoxyl group was utilized instead of the methyl group \textit{ortho} to the phenolic OH the antioxidant activity was increased significantly. It is well known that introduction of nitrogen atoms as part of the six-membered aromatic ring increases the stability of the
redox cores.\textsuperscript{81-83} Also, pyrimidinol analogues do not have free positions on the redox core susceptible to nucleophilic attack upon the oxidized forms. Another interesting observation was that when the methyl group \textit{ortho} to the phenolic OH was replaced by a methoxyl group the inhibition of the electron transport chain decreased. These properties may be the reason why compound 3.15 was the most potent pyrimidinol analogue bearing the idebenone side chain.

Removal of the hydroxyl group at the end of the lipophilic side chain had different effects on the activity of the pyrimidinol analogue activities as regards of inhibition of the respiratory chain, and consequently in their activity as antioxidants. It was observed that when the OH group was removed from lipophilic side chain of compound 3.10 to afford compound 3.17, the inhibition of NADH oxidase was greater (Table 10), but for NADH-ubiquinone oxidoreductase was similar (Table 12). On the other hand, removal of the OH group of the lipophilic side chain of compound 3.15 to afford compound 3.19, resulted in increased inhibition of NADH oxidase and NADH-ubiquinone oxidoreductase activities. After observing this behavior it was decided to create a series of analogues containing aliphatic side chains of different lengths. The effectiveness of the pyrimidinol analogues is highly dependent on the length of their lipophilic side chain. The inhibition of the respiratory chain was decreased when aliphatic chains longer and shorter than 10 carbons were used, as observed for pyrimidinol analogues 3.16, 3.17 and 3.18. Compound 3.16 did not perform well in other biological assays even when it was not a strong inhibitor of the electron transport chain. This lack of activity was attributed to its low interaction with lipid
membranes. To the contrary compounds 3.17 and 3.18 were potent antioxidant pyrimidinol analogues; however 3.17 was a strong inhibitor of the electron transport chain. Exploration of the effects of aliphatic chains longer that 10-carbons was then pursued. It was observed that when an aliphatic side chain of 18 carbon atoms was present (compound 3.24), the antioxidant properties were diminished as compared to the corresponding analogues with a 16-carbon (compound 3.20) or 14-carbon (compound 3.22) aliphatic side chain. From this one could assume that the optimal side chain length was 14-16 carbon atoms.

Interestingly, when a 16-carbon side chain was used, the replacement of the methyl groups on the pyrimidinol ring (compound 3.18) for a methoxyl group (compound 3.20) produced an increase in the inhibition of the respiratory chain.

Previously, it was concluded that ubiquinones interact with complex I at different sites according to the lipophilicity of their aliphatic side chains. The wide range of effects observed for the inhibition of the respiratory chain by pyrimidinol and pyridinol analogues could suggest different interactions with the components of the electron transport chain as well.

It is interesting to note that when glutathione, present in ~ 1-10 mM concentration in cells, is completely depleted by diethyl maleate, the best pyridinol and pyrimidinol analogues afford protection at the level of nanomolar concentrations. These compounds could be recycled in the cell by the action of other redox active compounds, just as α-TOH is recycled by vitamin C and NADH. The reduction of pyridinol and pyrimidinol analogues at oxidizing
potentials suggests that these compounds could be regenerated by superoxide as well.\(^{116}\)

The wide range of activities presented by the analogues here suggested a strategy for the synthesis of a large library of compounds. To accomplish this goal, a strategy in which a terminal alkene is used as anchoring point, was developed and validated by synthesizing compounds 3.22-3.24.

3.4 EXPERIMENTAL

Reactions were carried out under an atmosphere of argon unless specified otherwise. The glassware was dried in an oven at 110 °C prior to use. Tetrahydrofuran was distilled from sodium/benzophenone, dichloromethane was distilled from calcium hydride, benzene and toluene were distilled from sodium, and triethylamine and diisopropylamine were distilled from potassium hydroxide. All other solvents were of analytical grade and were used without further purification. Flash column chromatography was carried out using silica gel (Silicycle R10030B, 60 Å particle size, 230-400 mesh), applying a low pressure stream of nitrogen or air. Analytical thin layer chromatographic separations were carried on glass plates coated with silica gel (60 Å particle size, 250 µm thickness, F-254, Silicycle). The TLC chromatograms were developed using iodine vapor, or by immersing the plates either in 2.5% phosphomolybdic acid in ethanol, or in 2.0% anisaldehyde in ethanol/sulfuric acid/acetic acid, followed by heating (heat gun). \(^1\)H NMR chemical shifts were reported relative to residual CHCl\(_3\) at 7.26 ppm or DMSO at 3.31ppm or methanol-\(d_4\) at 3.31 ppm or
acetonitrile-$d_3$ at 2.10 ppm and 116.4 ppm; $^{13}$C NMR chemical shifts were reported relative to the central line of CDCl$_3$ at 77.0 ppm, DMSO-$d_6$ at 39.5 ppm, methanol-$d_4$ at 49.0 ppm, or acetonitrile-$d_3$ at 1.89 ppm and 116.4 ppm. High resolution mass spectra were obtained in the Arizona State University CLAS High Resolution Mass Spectrometry Laboratory.

1-Bromo-9-(methoxymethoxy)nonane (3.25). To a stirred solution containing 5.00 g (22.4 mmol) of 9-bromo-1-nonanol in 60 mL of anh THF was added 5.10 mL (5.40 g; 67.1 mmol) of MOM-Cl followed by 1.79 g (44.8 mmol) of a 60% suspension of NaH in mineral oil. The reaction mixture was stirred at 23 °C for 16 h. The reaction mixture was carefully quenched with satd aq sodium bicarbonate, poured into 200 mL of water and extracted with two 150-mL portions of ether. The combined organic solution was washed with 200 mL of brine, dried (MgSO$_4$) and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 9:1 hexanes–ethyl acetate afforded compound 3.10 as colorless oil: yield 4.21 g (70%); silica gel TLC $R_f$ 0.45 (9:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $\delta$ 1.28-1.39 (br, 10H), 1.56 (quint, 2H, $J$ = 7.2 Hz), 1.82 (quint, 2H, $J$ = 7.2 Hz), 3.33 (s, 3H), 3.37 (t, 2H, $J$ = 6.8 Hz), 3.48 (t, 2H, $J$ = 6.8 Hz) and 4.59 (s, 2H); $^{13}$C NMR (CDCl$_3$) $\delta$ 26.1, 28.1, 28.7, 29.28, 29.33, 29.7, 32.8, 34.0, 55.0, 67.7 and 96.4; mass spectrum (APCI), m/z 267.0953 (M+H)$^+$ (C$_{11}$H$_{24}$O$_2$Br requires 267.0960).
6-Amino-3-bromo-2,4-dimethylpyridine (3.26). To a stirred solution containing 2.00 g (16.3 mmol) of 2-amino-4,6-dimethylpyridine in 25 mL of acetonitrile was added 2.90 g (16.3 mmol) of N-bromosuccinimide. The reaction mixture was stirred at room temperature for 5 h. The formed precipitate was filtered and dried to afford compound 3.26 as a colorless solid: yield 2.76 g (84%); mp 143-145 °C; silica gel TLC \( R_f \) 0.15 (2:1 hexanes–ethyl acetate); \(^1\)H NMR (CDCl\(_3\)) \( \delta \) 2.25 (s, 3H), 2.48 (s, 3H), 4.39 (br s, 2H), and 6.22 (s, 1H); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 23.3, 25.1, 108.1, 112.3, 148.6, 155.2 and 156.3; mass spectrum (APCI), \( m/z \) 201.0032 (M+H)+ (C\(_7\)H\(_{10}\)N\(_2\)Br requires 201.0027).

3-Bromo-6-(2,5-dimethyl-1\(H\)-pyrrol-1-yl)-2,4-dimethylpyridine (3.27). To a stirred solution containing 2.76 g (13.8 mmol) of compound 3.26 in 25 mL of toluene was added 2.02 mL (1.98 g; 17.2 mmol) of 2,5-hexanediode followed by 130 mg (0.68 mmol) of \( p \)-toluenesulfonic acid. The reaction mixture was stirred at reflux for 14 h. The reaction mixture was poured into 150 mL of water and then extracted with 200 mL of ethyl acetate. The organic phase was washed with 150
mL of brine, dried (MgSO₄) and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 6 cm). Elution with 6:1 hexanes–ethyl acetate afforded compound 3.27 as an orange oil: yield 2.37 g (62%); silica gel TLC Rf 0.70 (6:1 hexanes–ethyl acetate); ¹H NMR (CDCl₃) δ 2.12 (s, 6H), 2.47 (s, 3H), 2.70 (s, 3H), 5.88 (s, 2H), and 6.93 (s, 1H); ¹³C NMR (CDCl₃) δ 13.1, 23.4, 25.6, 106.9, 121.16, 121.24, 122.5, 128.4, 149.6 and 157.4; mass spectrum (APCI), m/z 279.0502 (M+H)⁺ (C₁₃H₁₆N₂Br requires 279.0497).

![Chemical Structure](image)

3-(Benzyl)oxy)-6-(2,5-dimethyl-1H-pyrrol-1-yl)-2,4-dimethylpyridine (3.28). To a stirred solution containing 3.13 g (11.2 mmol) of compound 3.27 in 50 mL of 1:1 degassed dioxane–water was added 612 mg (0.67 mmol) of Pd₂dba₃ followed by 284 mg (0.67 mmol) of 2-di-tert-butylphosphino-2’,4’,6’-triisopropylbiphenyl (L₁) and 1.88 g (33.6 mmol) of KOH. The reaction mixture was stirred at 100 °C for 3 h. The reaction mixture was poured into 200 mL of water and extracted with two 100-mL portions of ethyl acetate. The aqueous layer was acidified with HCl (to pH 2-3) and then extracted with two 100-mL portions of ethyl acetate. The combined organic layer was washed with 100 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was dissolved in 50 mL of anh THF and 1.99 mL (2.86 g; 16.8 mmol) of benzyl
bromide followed by 807 mg (22.4 mmol) of 60% sodium hydride suspension in mineral oil were added. The reaction mixture was stirred at 23 °C for 16 h. The reaction mixture was poured into 150 mL of water and extracted with two 100-mL portions of diethyl ether. The combined organic layer was washed with 100 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 6 cm).

Elution with hexanes (removal of unreacted benzyl bromide) and then with 4:1 hexanes–diethyl ether afforded compound 3.28 as a yellowish oil: yield 2.82 g (82%); silica gel TLC Rf 0.65 (5:1 hexanes–ethyl acetate); ¹H NMR (CDCl₃) δ 2.17 (s, 6H), 2.37 (s, 3H), 2.58 (s, 3H), 4.94 (s, 2H), 5.91 (s, 2H), 6.98 (s, 1H), and 7.40-7.50 (m, 5H); ¹³C NMR (CDCl₃) δ 13.1, 16.2, 19.5, 74.6, 106.4, 121.9, 127.9, 128.31, 128.34, 128.6, 136.7, 142.0, 146.6, 151.0 and 152.0; mass spectrum (APCI), m/z 307.1801 (M+H)⁺ (C₂₀H₂₃N₂O requires 307.1810).

3-(Benzyloxy)-6-(2,5-dimethyl-1H-pyrrol-1-yl)-2-((10-(methoxymethoxy)decal)-4-methylpyridine (3.29). To a stirred solution at −78 °C containing 906 mg (2.95 mmol) of compound 3.28 in 20 mL of anh THF was added 789 mg (2.95 mmol) of compound 3.25 followed by 1.99 mL (3.54 mmol) of a 1.80 M solution of PhLi in hexane. The reaction mixture was stirred at −78 °C for 30 min then the reaction mixture was allowed to warm slowly to 23 °C; the
reaction mixture was then stirred at 23 °C for 30 min. The reaction was quenched with satd aq ammonium chloride and then poured into 80 mL of water. The mixture was then extracted with two 80-mL portions of ethyl acetate. The combined organic layer was then washed with 100 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 4:1 hexanes–ethyl acetate afforded compound 3.29 as a light yellow oil: yield 877 mg (60%); silica gel TLC $R_f$ 0.30 (9:1 hexanes–ethyl acetate); $^1$H NMR (CDCl₃) δ 1.18-1.22 (br m, 12H), 1.50 (quint, 2H, $J$ = 6.8 Hz), 1.65 (m, 2H), 2.06 (s, 6H), 2.26 (s, 3H), 2.75 (dd, 2H, $J$ = 7.6 and 7.6 Hz), 3.28 (s, 3H), 3.43 (t, 2H, $J$ = 6.8 Hz), 4.53 (s, 2H), 4.82 (s, 2H), 5.78 (s, 2H), 6.79, (s, 1H) and 7.29-7.40 (m, 5H); $^{13}$C NMR (CDCl₃) δ 13.3, 16.4, 26.2, 28.9, 29.40, 29.48, 29.50, 29.53, 29.6, 29.7, 32.1, 55.1, 67.9, 75.2, 96.4, 106.4, 121.7, 127.7, 128.3, 128.6, 128.9, 138.8, 142.0, 146.8, 150.8 and 155.9; mass spectrum (APCI), $m/z$ 493.3426 (M+H)$^+$ (C₃₁H₄₃N₂O₃ requires 493.3430).

**2-Amino-5-(benzyloxy)-6-(10-(methoxymethoxy)decyl)-4-methylpyridine (3.30).** To a stirred solution containing 240 mg (0.49 mmol) of compound 3.29 in 10 mL of 9:1 ethanol–water was added 338 mg (4.87 mmol) of hydroxylamine hydrochloride followed by 273 mg (4.87 mmol) of KOH. The reaction mixture
was stirred at reflux for 6 h, and then a second portion of 338 mg (4.87 mmol) of hydroxylamine hydrochloride was added, followed by 273 mg (4.87 mmol) of KOH. The reaction mixture was stirred at reflux for 16 h. The cooled reaction mixture was poured into 50 mL of water and extracted with two 50-mL portions of dichloromethane. The combined organic layer was washed with 60 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 9:1 dichloromethane–methanol afforded compound 3.30 as a yellowish oil: yield 113 mg (56%); silica gel TLC Rₚ 0.45 (9:1 dichloromethane–methanol); ¹H NMR (CDCl₃) δ 1.24-1.31 (br m, 12H), 1.53-1.63 (m, 4H), 2.16 (s, 3H), 2.62 (dd, 2H, J = 8.0 and 8.0 Hz), 3.33 (s, 3H), 3.48 (t, 2H, J = 6.8 Hz), 4.24 (br s, 2H), 4.59 (s, 2H), 4.71 (s, 2H), 6.16 (s, 1H) and 7.31-7.43 (m, 5H); ¹³C NMR (CDCl₃) δ 16.7, 29.2, 29.40, 29.46, 29.48, 29.6, 29.7, 29.8, 32.2, 53.4, 55.1, 67.9, 75.4, 96.3, 108.1, 127.7, 128.0, 128.5, 137.4, 142.5, 144.9, 153.5 and 154.1; mass spectrum (APCI), m/z 415.2957 (M+H)⁺ (C₂₅H₃₉N₂O₃ requires 415.2961).

3-(Benzyloxy)-6-(N,N-dimethylamino)-2-(10-(methoxymethoxy)decy)-4-methylpyridine (3.31). To a stirred solution containing 218 mg (0.53 mmol) of compound 3.30 in 3 mL of anh DMF was added 100 µL (228 mg; 1.59 mmol) of methyl iodide followed by 64.0 mg (1.59 mmol) of a 60% suspension of NaH in
mineral oil. The reaction mixture was stirred at 23 °C for 3 h. The reaction was quenched with 0.5 mL of water and concentrated under diminished pressure. The residue was dissolved in 50 mL of dichloromethane and washed with 50 mL of brine. The organic solution was dried (MgSO₄) and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (8 × 3 cm). Elution with 3:1 hexanes–ethyl acetate afforded compound 3.31 as a yellowish oil: yield 27 mg (21%); silica gel TLC Rf 0.30 (3:1 hexanes–ethyl acetate); ¹H NMR (CDCl₃) δ 1.22-1.34 (br m, 12H), 1.56 (quint, 2H, J = 6.8 Hz), 1.71 (quint, 2H, J = 7.6 Hz), 2.21 (s, 3H), 2.68 (dd, 2H, J = 7.6 and 7.6 Hz), 3.01 (s, 6H), 3.43 (s, 3H), 3.49 (t, 2H, J = 6.8 Hz), 4.60 (s, 2H), 4.71 (s, 2H), 6.16 (s, 1H) and 7.32-7.45 (m, 5H); ¹³C NMR (CDCl₃) δ 16.7, 26.2, 28.7, 29.4, 29.5, 29.6, 29.73, 29.76, 31.6, 32.0, 38.3, 55.0, 67.9, 75.3, 96.4, 104.9, 127.8, 127.9, 128.5, 137.7, 141.2, 143.4, 152.9 and 155.7; mass spectrum (APCI), m/z 443.3290 (M+H)+ (C₂₇H₄₃N₂O₃ requires 443.3274).

6-(N,N-Dimethylamino)-2-(10-hydroxydecyl)-4-methylpyridin-3-ol (3.1). To a stirred solution containing 27.0 mg (0.09 mmol) compound 3.31 in 3 mL of methanol was added one drop of concentrated HCl. The reaction mixture was stirred at reflux for 16 h. Then, 3 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/N) was added to the cooled reaction mixture, which was
stirred at 23 °C under a H₂ atmosphere for 15 min. The reaction mixture was
filtered through Celite and the filtrate was concentrated under diminished
pressure. The residue was purified by chromatography on a silica gel column (10
× 1 cm). Elution with 9:1 dichloromethane–methanol afforded compound 3.1 as a
colorless oil: yield 10 mg (77%); silica gel TLC Rf 0.50 (9:1 dichloromethane–
methanol); ¹H NMR (methanol-ᴅ₄) δ 1.28-1.34 (br m, 12H), 1.49 (m, 2H), 1.63
(m, 2H), 2.18 (s, 3H), 2.66 (dd, 2H, J = 7.6 and 7.6 Hz), 2.93 (s, 6H), 3.51 (t, 2H,
J = 6.4 Hz) and 6.31 (s, 1H); ¹³C NMR (methanol-ᴅ₄) δ 15.5, 25.5, 28.2, 29.16,
29.20, 29.22, 29.24, 29.3, 31.2, 32.2, 38.1, 67.9, 106.0, 138.1, 141.0, 147.1 and
154.2; mass spectrum (APCI), m/z 309.2553 (M+H)⁺ (C₁₈H₃₃N₂O₂ requires
309.2542).

6-Amino-2-(10-hydroxydecyl)-4-methylpyridin-3-ol (3.2). To a stirred solution
containing 113 mg (0.27 mmol) of compound 3.30 in 10 mL of methanol was
added two drops of concentrated HCl. The reaction mixture was stirred at reflux
for 16 h. To the reaction mixture was added 5 mg of 20% palladium hydroxide-
on-carbon (Degussa type E101 NE/N). The reaction mixture was stirred at 23 °C
under a H₂ atmosphere for 15 min. The reaction mixture was filtered through
Celite and the filtrate was concentrated under diminished pressure. The residue
was purified by chromatography on a silica gel column (10 × 1 cm). Elution with
17:3 dichloromethane–methanol afforded compound 3.2 as a colorless solid: yield 22 mg (29%); mp 138-139 °C; silica gel TLC Rf 0.20 (17:3 dichloromethane–methanol); 1H NMR (methanol-d4) δ 1.28-1.35 (m, 12H), 1.48-1.58 (m, 4H), 2.13 (s, 3H), 2.60 (dd, 2H, J = 7.6 and 7.6 Hz), 3.51 (t, 2H, J = 6.8 Hz) and 6.25 (s, 1H); 13C NMR (methanol-d4) δ 15.2, 25.5, 28.8, 29.15, 29.20, 29.22, 29.3, 31.5, 32.2, 61.6, 108.4, 139.2, 141.7, 146.9 and 152.5; mass spectrum (APCI), m/z 281.2231 (M+H)⁺ (C16H29N2O2 requires 281.2229).

6-(2,5-Dimethyl-1H-pyrrol-1-yl)-2-(10-hydroxydecyl)-4-methylpyridin-3-ol (3.3). To a stirred solution containing 321 mg (0.65 mmol) of compound 3.29 in 5 mL of methanol was added two drops of concentrated HCl. The reaction mixture was stirred at reflux for 16 h. To the cooled reaction mixture was added 10 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/E) and the reaction mixture was then stirred at 23 °C under a H2 atmosphere for 15 min. The reaction mixture was filtered through Celite and the filtrate was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (13 × 3 cm). Elution with 1:1 hexanes–ethyl acetate afforded compound 3.3 as a colorless solid: yield 40 mg (17%); mp 98-99 °C; silica gel TLC Rf 0.42 (1:1 hexanes–ethyl acetate); 1H NMR (CDCl3) δ 1.29-1.35 (m, 12H), 1.53 (quint, 2H, J = 6.8 Hz), 1.68 (quint, 2H, J = 6.8 Hz), 2.03 (s, 6H), 2.24 (s, 3H), 2.77 (dd,
2H, J = 7.6 and 7.6 Hz), 3.60 (t, 2H, J = 6.8 Hz), 5.80 (s, 2H) and 6.80 (s, 1H);

$^{13}$C NMR (CDCl$_3$) δ 12.9, 15.9, 25.6, 28.2, 29.24, 29.25, 29.30, 29.36, 29.38, 32.0, 32.6, 63.0, 106.0, 121.8, 128.5, 134.4, 143.5, 147.9 and 148.3; mass spectrum (APCI), m/z 359.2697 (M+H)$^+$ (C$_{22}$H$_{35}$N$_2$O$_2$ requires 359.2699).

6-(2,5-Dimethyl-1H-pyrrolidin-1-yl)-2-(10-hydroxydecyl)-4-methylpyridin-3-ol (3.4). To a stirred solution containing 479 mg (0.65 mmol) of compound 3.29 in 5 mL of methanol were added two drops of concentrated HCl. The reaction mixture was stirred at reflux for 16 h. To the mixture was added 10 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/E) and the reaction mixture was then stirred at 23 °C under a H$_2$ atmosphere for 16 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (13 × 3 cm). Elution with 1:1 toluene–ethyl acetate afforded compound 3.4 as a colorless oil: yield 17 mg (5%); silica gel TLC $R_f$ 0.42 (1:1 hexanes–ethyl acetate); $^1$H NMR (CD$_3$CN) δ 1.24-1.34 (m, 18H), 1.46 (m, 2H), 1.68 (m, 4H), 2.01 (m, 2H), 2.25 (s, 3H), 2.59 (t, 2H, J = 7.2 Hz), 3.47 (t, 2H, J = 6.4 Hz), 3.87 (m, 2H) and 6.11 (s, 1H); $^{13}$C NMR (CD$_3$CN) δ 16.1, 16.8, 19.6, 22.5, 22.6, 26.6, 29.0, 30.2, 30.24, 30.27, 30.33, 30.9, 32.6, 32.8, 33.6, 56.1, 62.6, 106.5, 136.9,
141.3, 143.6 and 152.9; mass spectrum (APCI), m/z 363.3018 (M+H)^+
(C_{22}H_{39}N_{2}O_{2} requires 363.3012).

3-(Benzyloxy)-6-methoxy-2-(10-(methoxymethoxy)decy1)-4-methylpyridine (3.32). To a stirred solution at 0 °C containing 65.0 mg (0.16 mmol) of compound 3.30 in 2 mL of 1:1 water–50% aq H_3PO_4 was added 17.0 mg (0.24 mmol) of sodium nitrite. The reaction mixture was stirred at 0 °C for 30 min and then at 23 °C for 16 h. The reaction mixture was neutralized (pH ~ 7) with aqueous NaOH and stirred for 2 h. The reaction mixture was poured into 50 mL of water and extracted with two 40-mL portions of CH_2Cl_2. The combined organic layer was dried (MgSO_4) and concentrated under diminished pressure. The residue was dissolved into 5 mL of anh CH_2Cl_2. To the solution were added 100 µL (228 mg; 1.60 mmol) of methyl iodide followed by 66.0 mg (0.24 mmol) of Ag_2CO_3. The reaction mixture was stirred overnight at 23°C and protected from light. The reaction mixture was filtered and the filtrate was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (8 × 1 cm). Elution with 3:1 hexanes–ethyl acetate afforded compound 3.32 as colorless oil: yield 18 mg (26%); silica gel TLC R_f 0.55 (4:1 hexanes–ethyl acetate) \(^1\)H NMR (CDCl_3) δ 1.24-1.34 (m, 12H), 1.56 (quint, 2H, J = 7.6 Hz), 1.70 (quint, 2H, J = 7.6 Hz), 2.22 (s, 3H), 2.70 (dd, 2H, J = 7.6, 7.6 Hz), 3.34 (s,
3H), 3.49 (t, 2H, $J = 6.8$ Hz), 3.86 (s, 3H), 4.60 (s, 2H), 4.74 (s, 2H), 6.37 (s, 1H) and 7.33-7.44 (m, 5H); $^{13}$C NMR (CDCl$_3$) $\delta$ 16.4, 26.2, 28.7, 29.4, 29.52, 29.54, 29.6, 29.65, 29.73, 31.8, 53.3, 55.1, 67.9, 75.3, 96.4, 108.8, 127.8, 128.1, 128.5, 137.3, 143.3, 146.6, 152.3 and 159.4; mass spectrum (APCI), $m/z$ 430.2965 (M+H)$^+$ (C$_{26}$H$_{39}$NO$_4$ requires 430.2957).

![Chemical structure](image)

2-(10-Hydroxydecyl)-6-methoxy-4-methylpyridin-3-ol (3.5). To a stirred solution containing 18 mg (0.04 mmol) of compound 3.32 in 3 mL of methanol was added two drops of concentrated HCl. The reaction mixture was stirred at reflux for 16 h. To the mixture was added 3 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/N). The reaction mixture was stirred at room temperature under a hydrogen atmosphere for 15 min. The reaction mixture was filtered through Celite and the filtrate was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (5 × 1 cm). Elution with 3:1 hexanes–ethyl acetate afforded compound 3.5 as white solid: yield 8 mg (67%); mp 80-81 °C; silica gel TLC $R_f$ 0.50 (3:1 hexanes–ethyl acetate); $^1$H NMR (CD$_3$OD) $\delta$ 1.27 (br s, 12H), 1.49 (br s, 2H), 1.64 (br s, 2H), 2.41 (br s, 3H), 2.81 (br s, 2H), 3.51 (br s, 3H), 4.02 (br s, 1H) and 7.05 (br s, 1H); $^{13}$C NMR (CD$_3$OD) $\delta$ 16.6, 25.5, 28.1, 28.6, 28.9, 29.0, 29.09, 29.14, 29.2, 29.73, 53.3, 55.1, 67.9, 75.3, 96.4, 108.8, 127.8, 128.1, 128.5, 137.3, 143.3, 146.6, 152.3 and 159.4; mass spectrum (APCI), $m/z$ 430.2965 (M+H)$^+$ (C$_{26}$H$_{39}$NO$_4$ requires 430.2957).
32.2, 56.4, 61.6, 107.6, 142.3, 145.3, 148.7 and 155.1; mass spectrum (APCI), \( m/z \) 296.2220 (M+H)\(^+\) (C\(_{17}\)H\(_{30}\)NO\(_3\) requires 296.2226).

![Dimethyl 2-(1-Aminoethylidene)malonate (3.33)](image)

**Dimethyl 2-(1-Aminoethylidene)malonate (3.33).**

To a stirred solution containing 4.36 mL (5.04 g; 38.2 mmol) of dimethyl malonate and 2.00 mL (1.57 g; 38.3 mmol) of acetonitrile in 20 mL of 1,2-dichloroethane was added 8.90 mL (19.8 g; 76.0 mmol) of SnCl\(_4\). The reaction mixture was stirred at reflux for 2 h. The reaction mixture was then concentrated under diminished pressure and the residue was dissolved in 100 mL of acetone. Then, 70 mL of satd aq sodium carbonate was added and the mixture was stirred for 20 minutes. The mixture was poured into 200 mL of satd aq sodium bicarbonate and extracted with two 200-mL portions of CH\(_2\)Cl\(_2\). The combined organic solution was washed with 200 mL of brine, dried (MgSO\(_4\)) and concentrated under diminished pressure. The residue was recrystallized from ethyl acetate—hexanes to afforded compound 3.33 as colorless crystals: yield 3.96 g (60%); mp 81-82°C; silica gel TLC \( R_f \) 0.5 (1:1 ethyl acetate–hexanes); \(^1\)H NMR (CDCl\(_3\)) \( \delta \) 2.10 (s, 3H), 3.66 (s, 3H), 3.69 (s, 3H), 5.29 (br s, 1H) and 8.94 (br s, 1H); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 22.0, 51.0, 51.6, 92.4, 164.0, 168.7 and 168.9; mass spectrum (APCI), \( m/z \) 174.0760 (M+H)\(^+\) (C\(_7\)H\(_{12}\)NO\(_4\) requires 174.0766).
Methyl 6-(N,N-Dimethylamino)-4-hydroxy-2-methylnicotinate (3.34). A solution containing 1.10 g (6.36 mmol) of compound 3.33 and 1.40 mL (1.28 g; 9.57 mmol) of N,N’-dimethylacetamide dimethyl acetal in 10 mL of anh dioxane was stirred at reflux for 3 h then 1.24 g (12.7 mmol) of sodium tert-butoxide was added and the reaction mixture was stirred at reflux for 3h. The reaction mixture was poured into 100 mL of water and extracted with two 80-mL portions of ethyl acetate. The combined organic solution was washed with 80 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 4 cm). Elution with 3:1 hexanes–ethyl acetate afforded compound 3.34 as a colorless solid: yield 689 mg (51%); mp 61-62 °C; silica gel TLC R_f 0.47 (3:1 ethyl acetate–hexanes); ^1H NMR (CDCl₃) δ 2.58 (s, 3H), 3.06 (s, 6H), 3.87 (s, 3H), 5.97 (s, 1H) and 11.88 (s, 1H); ^13C NMR (CDCl₃) δ 27.5, 37.4, 51.6, 88.6, 99.3, 160.3, 161.9, 169.3 and 171.9; mass spectrum (APCI), m/z 211.1081 (M+H)^+ (C_{10}H_{15}N_2O_3 requires 211.1083).

Methyl 6-(N,N-Dimethylamino)-4-methoxy-2-methylnicotinate (3.35). To a stirred solution containing 689 mg (3.27 mmol) of compound 3.34 in 10 mL of
anh DMF was added 1.91 g (18.0 mmol) of anh sodium carbonate followed by 611 mL (1.39 g; 9.81 mmol) of methyl iodide. The reaction mixture was stirred at 23 °C for 16 h. Then the reaction mixture was poured into 80 mL of water and extracted with two 80-mL portions of ethyl acetate. The combined organic solution was washed with 80 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 1:1 hexanes–diethyl ether afforded compound 3.35 as a colorless solid: yield 340 mg (47%); mp 65-66°C; silica gel TLC $R_f$ 0.15 (1:1 hexanes–diethyl ether); $^1$H NMR (CDCl₃) δ 2.37 (s, 3H), 3.07 (s, 6H), 3.80 (s, 3H), 3.82 (s, 3H) and 5.72 (s, 1H); $^{13}$C NMR (CDCl₃) δ 23.5, 37.8, 51.7, 55.2, 84.5, 107.6, 156.7, 160.3, 165.2 and 168.4; mass spectrum (APCI), $m/z$ 225.1246 (M+H)$^+$ (C_{11}H_{17}N_{2}O_{3} requires 225.1239).

![Structure](image)

**2-(N,N-dimethylamino)-4-methoxy-6-methylpyridine (3.36).** A solution containing 340 mg (1.52 mmol) of compound 3.35 in 5 mL of 6 N aq HCl was stirred at reflux for 16 h. The reaction mixture was poured into 50 mL of water and the pH was adjusted to 12. The mixture was extracted with two 40-mL portions of ethyl acetate. The combined organic solution was washed with 40 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (6 × 2 cm). Elution with 9:1 dichloromethane–methanol afforded compound 3.36 as a colorless oil: yield
87 mg (34%); silica gel TLC $R_f$ 0.25 (9:1 dichloromethane–methanol); $^1$H NMR (CDCl$_3$) $\delta$ 2.33 (s, 3H), 3.02 (s, 6H), 3.75 (s, 3H), 5.77 (s, 1H) and 6.03 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 24.8, 38.0, 54.7, 87.4, 98.5, 157.9, 160.8 and 167.5; mass spectrum (APCI), $m/z$ 167.1183 (M+H)$^+$ (C$_9$H$_{15}$N$_2$O requires 167.1184).

2-($N,N$-dimethylamino)-4-methoxy-6-(10-(methoxymethoxy)decyl)-$N,N$-dimethylpyridine (3.37). To a stirred solution containing 156 mg (0.94 mmol) of compound 3.36 in 5 mL of anh THF was added 252 mg (0.94 mmol) of compound 3.25 followed by 650 µL (1.04 mmol) of 1.6 M n-BuLi in pentane. The reaction mixture was stirred at 23°C for 15 min. The reaction was quenched with satd aq ammonium chloride and poured into 50 mL of water. The mixture was extracted with two 50-mL portions of ethyl acetate. The combined organic solution was washed with 50 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 1:1 hexanes–ethyl acetate afforded compound 3.37 as yellowish oil: yield 235 mg (70%); silica gel TLC $R_f$ 0.47 (1:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $\delta$ 1.24 (br m, 12H), 1.55 (quint, 2H, $J = 6.4$ Hz), 1.68 (quint, 2H, $J = 7.6$ Hz), 2.55 (dd, 2H, $J = 7.6$ Hz and 7.6 Hz), 3.03 (s, 6H), 3.46 (s, 3H), 3.49 (t, 2H, $J = 6.8$ Hz), 3.76 (s, 3H), 4.59 (s, 2H), 5.77 (s, 1H) and 6.01 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 26.2, 29.3, 29.4, 29.48, 29.50, 29.55, 29.60,
29.62, 29.7, 38.0, 54.6, 55.0, 67.9, 87.4, 96.4, 97.9, 160.8, 162.1 and 167.4; mass spectrum (APCI), m/z (M+H)\(^+\) 353.2808 (C\(_{20}\)H\(_{37}\)N\(_2\)O\(_3\) requires 353.2804).

![Chemical Structure](image)

5-Bromo-2-((N,N-dimethylamino)-4-methoxy-6-(10-(methoxymethoxy)decyl)pyridine (3.38). To a stirred solution containing 213 mg (0.60 mmol) of compound 3.37 in 5 mL of acetonitrile was added 107 mg (0.60 mmol) of N-bromosuccinimide. The reaction mixture was stirred at 23 °C protected from light for 3 h. The reaction mixture was poured into 50 mL of satd aq sodium bicarbonate and extracted with two 50-mL portions of ethyl acetate. The combined organic layer was washed with 50 mL of brine, dried (MgSO\(_4\)) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 4:1 hexanes–ethyl acetate afforded compound 3.38 as a yellowish oil: yield 114 mg (44%); silica gel TLC \(R_f\) 0.45 (4:1 hexanes–ethyl acetate); \(^1\)H NMR (CDCl\(_3\)) δ 1.29 (br m, 12H), 1.56 (quint, 2H, \(J = 7.2\) Hz), 1.68 (quint, 2H, \(J = 7.2\) Hz), 2.55 (dd, 2H, \(J = 7.6\) Hz and 7.6 Hz), 3.03 (s, 6H), 3.33 (s, 3H), 3.48 (t, 2H, \(J = 6.8\) Hz), 3.85 (s, 3H), 4.59 (s, 2H) and 5.76 (s, 1H); \(^{13}\)C NMR (CDCl\(_3\)) δ 26.2, 28.1, 29.4, 29.47, 29.51, 29.57, 29.65, 29.72, 37.3, 38.05, 55.0, 55.7, 67.9, 86.5, 96.4, 97.3, 158.8, 159.1 and 162.6; mass spectrum (APCI), m/z (M+H)\(^+\) 431.1913 (C\(_{20}\)H\(_{36}\)N\(_2\)O\(_3\)Br requires 431.1909).
6-(N,N-Dimethylamino)-4-methoxy-2-(10-(methoxymethoxy)decyl)pyridin-3-ol (3.39). To a stirred solution containing 114 mg (0.26 mmol) of compound 3.38 in 5 mL of anh THF was added 413 µL (0.66 mmol) of 1.6 M n-BuLi in pentane. The reaction mixture was stirred at 23 °C for 10 min. Then was added 89.0 µL (82.9 mg; 0.79 mmol) of trimethylborate and the reaction mixture was stirred at 23 °C for 30 min. Then was added 684 µL (12.6 mmol) of 35% aq H₂O₂ followed by 684 µL of 1 N NaOH. The reaction mixture was stirred at 23 °C for 2 h and then poured into 20 mL of water. The mixture was extracted with two 20-mL portions of ethyl acetate. The combined organic layer was washed with 20 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 2 cm). Elution with 1:1 hexanes–ethyl acetate afforded compound 3.39 as a yellowish oil: yield 20 mg (21%); silica gel TLC Rf 0.1 (1:1 hexanes–ethyl acetate); ¹H NMR (CD₃OD) δ 1.29 (br s, 12H), 1.56 (br m, 4H), 2.66 (dd, 2H, J = 7.6 Hz and 7.6 Hz), 3.00 (s, 6H), 3.29 (s, 3H), 3.48 (t, 2H, J = 6.8 Hz), 3.90 (s, 3H), 4.56 (s, 2H) and 6.11 (s, 1H); ¹³C NMR (CD₃OD) δ 25.9, 28.1, 29.07, 29.11, 29.19, 29.24, 29.4, 30.1, 35.2, 38.2, 53.9, 54.9, 67.5, 87.9, 96.0, 132.8, 143.7, 153.9 and 157.4; mass spectrum (APCI), m/z (M+H)⁺ 369.2760 (C₂₀H₃₇N₂O₄ requires 369.2753)
6-(N,N-Dimethylamino)-2-(10-hydroxydecyl)-4-methoxypyridin-3-ol (3.6). To a stirred solution containing compound 3.39 in 5 mL of methanol was added a drop of conc HCl. The reaction mixture was stirred at reflux for 2 h. A drop of conc NH₄OH was added and the mixture was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 9:1 dichloromethane–methanol afforded compound 3.6 as a yellowish oil: yield 7 mg (40%); silica gel TLC R_f 0.2 (9:1 dichloromethane–methanol); ^1H NMR (CD₃OD) δ 1.29 (br m, 12H), 1.49 (quint, 2H, J = 6.8 Hz), 1.62 (quint, 2H, J = 7.2 Hz), 2.66 (dd, 2H, J = 7.6 Hz and 7.6 Hz), 3.00 (s, 6H), 3.49 (t, 2H, J = 6.8 Hz), 3.90 (s, 3H) and 6.11 (s, 1H); ^13C NMR (CD₃OD) δ 25.5, 28.1, 29.08, 29.13, 29.14, 29.2, 29.3, 30.1, 32.2, 38.2, 54.9, 61.6, 87.9, 132.8, 143.9, 154.0 and 157.3; mass spectrum (APCI), m/z (M+H)^+ 325.2489 (C₁₈H₃₃N₂O₃ requires 325.2491).

6-Amino-2,4,5-trimethylpyridin-3-ol (3.40). To a stirred solution containing 10.0 g (48.6 mmol) of 4,5-bis(hydroxymethyl)-2-methylpyridin-3-ol hydrochloride in 37 mL of thionyl chloride was added 370 µL (372 mg; 0.51
mmol) of DMF. The reaction mixture was stirred at reflux for 2 h. The cooled reaction mixture was treated with 20 mL of diethyl ether. The resulting suspension was stirred for 1 h and the formed precipitate was filtered and washed with 20 mL of ether. The solid so obtained was dissolved in 42 mL of glacial acetic acid and 9.80 g (150 mmol) of zinc dust was added in three portions. The resulting suspension was stirred and heated at reflux for 2 h. The cooled reaction mixture was filtered and washed with glacial acetic acid. The filtrate was neutralized with 6 M NaOH and the formed precipitate was filtered and washed with a small amount of brine. The orange precipitate was dissolved in 10 N HCl solution and the HCl adduct was salted out with NaCl. The solid so obtained was dissolved in 200 mL of satd aq sodium bicarbonate. Then a diazonium salt, freshly prepared by slowly mixing 4.00 mL (4.09 g; 43.9 mmol) of aniline in 40 mL of 6 N HCl at 0°C with a solution containing 3.00 g (44.0 mmol) of NaNO₂ in 15 mL of water, was added to the reaction mixture dropwise. After 1 h, a red precipitate that had formed was filtered. The diazo intermediate was dissolved in 80 mL of 1:1 methanol–formic acid and 14.1 g (220 mmol) of zinc dust was added in three portions. The reaction mixture was then stirred at reflux for 2 h. The cooled reaction mixture was then filtered and the filtrate was washed with hot MeOH. The MeOH was concentrated and the resulting white precipitate was filtered, washed with ether, dried, and then dissolved in hot water and adjusted to pH 8.0 with 6 M NaOH. Upon cooling the solution, a white precipitate formed. The precipitate was filtered, dissolved in EtOH and filtered through a silica gel pad. The filtrate was concentrated under diminished pressure to afford compound
3.40 as a pale orange solid; yield 1.54 g (21%); mp 169-171 °C; silica gel TLC $R_f$ 0.20 (9:1 dichloromethane–methanol); $^1$H NMR (DMSO-$d_6$) $\delta$ 1.88 (s, 3H), 2.00 (s, 3H), 2.12 (s, 3H), 4.86 (br s, 2 H), and 7.40 (br s, 1H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 12.9, 13.4, 19.4, 112.7, 135.0, 140.2, 141.4 and 151.3.

6-(2,5-Dimethyl-1H-pyrrol-1-yl)-2,4,5-trimethylpyridin-3-ol (3.41). To a stirred solution containing 1.36 g (8.94 mmol) of compound 3.40 in 50 mL of toluene was added 1.32 mL (1.28 g; 11.2 mmol) of 2,5-hexanedione followed by 86.0 mg (0.45 mmol) of $p$-toluenesulfonic acid. The reaction mixture was stirred and heated at reflux for 14 h using a Dean-Stark apparatus. The reaction mixture was poured into 80 mL of water and then extracted with 100 mL of ethyl acetate. The organic solution was washed with 80 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 6 cm). Elution with 2:1 hexanes–ethyl acetate afforded compound 3.41 as colorless needles: yield 1.54 g (75%); mp 218-219 °C; silica gel TLC $R_f$ 0.25 (2:1 hexanes–ethyl acetate); $^1$H NMR (CD$_3$OD) $\delta$ 1.75 (s, 3H), 1.86 (s, 6H), 2.24 (s, 3H), 2.38 (s, 3H) and 5.77 (s, 2H); $^{13}$C NMR (CD$_3$OD) $\delta$ 12.4, 12.8, 13.6, 18.6, 106.7, 129.1, 131.1, 136.7, 142.7, 144.0 and 151.2; mass spectrum (APCI), $m/z$ (M+H)$^+$ 231.1492 (C$_{14}$H$_{19}$N$_2$O requires 231.1497).
3-(Benzyloxy)-6-(2,5-dimethyl-1H-pyrrol-1-yl)-2,4,5-trimethylpyridine (3.42).

To a stirred solution containing 1.54 g (6.69 mmol) of compound 3.41 in 50 mL of anh THF was added 1.19 mL (1.71 g; 10.0 mmol) of benzyl bromide followed by 803 mg (20.1 mmol) of 60% sodium hydride suspension in mineral oil. The reaction mixture was stirred at 23 °C for 16 h. The reaction mixture was poured into 150 mL of water and extracted with two 100-mL portions of ether. The combined organic layer was washed with 100 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 6 cm). Elution with hexanes (separation of unreacted benzyl bromide) and then with 4:1 hexanes–diethyl ether afforded compound 3.42 as yellowish crystals: yield 1.67 g (78%); mp 64-65 °C; silica gel TLC $R_f$ 0.60 (2:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $\delta$ 1.83 (s, 3H), 1.95 (s, 6H), 2.27 (s, 3H), 2.52 (s, 3H), 4.89 (s, 2H), 5.87 (s, 2H) and 7.40-7.50 (m, 5H); $^{13}$C NMR (CDCl$_3$) $\delta$ 12.5, 13.3, 13.8, 19.5, 74.9, 105.8, 128.0, 128.1, 128.5, 128.8, 129.7, 136.7, 141.2, 145.8 149.5 and 151.6; mass spectrum (APCI), $m/z$ (M+H)$^+$ 321.1969 (C$_{21}$H$_{25}$N$_2$O requires 321.1967).
3-(Benzyloxy)-6-(2,5-dimethyl-1H-pyrrol-1-yl)-2-[10-(methoxymethoxy)decyl]-4,5-dimethylpyridine (3.43). To a stirred solution at −78 °C containing 670 mg (2.09 mmol) of compound 3.42 in 30 mL of anh THF was added 314 mL (243 mg; 2.09 mmol) of N,N,N′,N′-tetramethylethylenediamine (TMEDA) followed by 1.00 mL (2.50 mmol) of a 2.50 M solution of n-BuLi in hexane. The reaction mixture was stirred for 5 min at −78 °C and then 615 mg (2.30 mmol) of compound 3.25 was added. The reaction mixture was stirred at −78 °C for 30 min, then the reaction mixture was warmed to 23 °C slowly and stirred for 30 min. The reaction was quenched with satd aq ammonium chloride and then poured into 50 mL of water. The mixture was then extracted with two 80-mL portions of ether. The combined organic layer was then washed with 100 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 3 cm). Elution with 4:1 hexanes–ether afforded compound 3.43 as a colorless oil: yield 432 mg (41%); silica gel TLC Rf 0.25 (4:1 hexanes–ether); ¹H NMR (CDCl₃) δ 1.21-1.32 (br m, 12H), 1.55 (m, 2H), 1.68 (m, 2H), 1.81 (s, 3H), 1.92 (s, 6H), 2.25 (s, 3H), 2.82 (dd, 2H, J = 8.0 Hz and 7.6 Hz), 3.33 (s, 3H), 3.48 (t, 2H, J = 6.7 Hz), 4.59 (s, 2H), 4.85 (s, 2H), 5.85 (s, 2H) and 7.38-7.50 (m, 5H); ¹³C NMR (CDCl₃) δ 12.6, 13.4, 13.8, 26.3, 29.45, 29.54, 29.63, 29.65, 29.68,
29.7, 29.9, 32.4, 55.2, 68.0, 75.6, 96.5, 105.8, 127.8, 128.1, 128.4, 128.8, 129.4, 137.0, 141.2, 146.0, 151.3 and 153.4; mass spectrum (APCI), m/z (M+H)+ 507.3590 (C_{32}H_{47}N_{2}O_{3} requires 507.3587).

2-Amino-5-(benzyloxy)-6-[10-(methoxymethoxy)decyl]-4,5-dimethylpyridine (3.44). To a stirred solution containing 849 mg (1.68 mmol) of compound 3.43 in 10 mL of 9:1 ethanol–water was added 2.34 g (33.6 mmol) of hydroxylamine hydrochloride followed by 2.07 g (37.0 mmol) of potassium hydroxide. The reaction mixture was stirred and heated at reflux for 16 h. The reaction mixture was then poured into 50 mL of water and extracted with two 50-mL portions of ethyl acetate. The combined organic layer was washed with 60 mL of brine, dried (MgSO_{4}) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 9:1 dichloromethane–methanol afforded compound 3.44 as a colorless oil: yield 96 mg (13%); silica gel TLC R_{f} 0.45 (9:1 dichloromethane–methanol); ^{1}H NMR (CDCl_{3}) δ 1.21-1.32 (br m, 12H), 1.55 (m, 2H), 1.67 (m, 2H), 2.01 (s, 3H), 2.18 (s, 3H), 2.64 (dd, 2H, J = 8.0 Hz and 8.0 Hz), 3.34 (s, 3H), 3.50 (t, 2H, J = 6.7 Hz), 4.45 (br s, 2H), 4.60 (s, 2H), 4.69 (s, 2H) and 7.32-7.45 (m, 5H); ^{13}C NMR (CDCl_{3}) δ 12.9, 13.1, 21.9, 26.3, 29.46, 29.53, 29.54, 29.63, 29.66, 29.79, 29.87, 32.2, 55.1, 67.9, 96.4, 113.6, 127.8, 128.0, 128.6, 137.5, 140.1, 144.9, 150.1 and
152.8; mass spectrum (APCI), \( m/z \) (M+H)\(^+\) 429.3114 (C\(_{26}\)H\(_{41}\)N\(_2\)O\(_3\) requires 429.3117).

![Chemical Structure](image)

3-Benzylxoy-6-(N,N-dimethylamino)-2-[10-(methoxymethoxy)decyl]-4,5-dimethylpyridine (3.45). To a stirred solution containing 96.0 mg (0.22 mmol) of compound 3.44 in 3 mL of 1:1 formalin–acetonitrile was added 83.0 mg (1.32 mmol) of sodium cyanoborohydride followed by 46.0 µL (48.2 mg; 0.80 mmol) of glacial acetic acid. The reaction mixture was stirred at 23 °C for 16 h and then poured into 20 mL of satd aq sodium bicarbonate. The mixture was then extracted with 20 mL of ether. The organic phase was washed with 20 mL of brine, dried (MgSO\(_4\)) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 2 cm). Elution with 4:1 hexanes–ether afforded compound 3.45 as a colorless oil: yield 78 mg (77%); silica gel TLC \( R_f \) 0.30 (4:1 hexanes–diethyl ether); \(^1\)H NMR (CDCl\(_3\)) \( \delta \) 1.21-1.32 (br m, 12H), 1.55 (m, 2H), 1.73 (m, 2H), 2.18 (s, 3H), 2.19 (s, 3H), 2.64 (m, 8H), 3.36 (s, 3H), 3.50 (t, 2H, \( J = 6.8 \) Hz), 4.62 (s, 2H), 4.73 (s, 2H) and 7.32-7.45 (m, 5H); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 13.2, 15.0, 26.4, 28.8, 29.6, 29.70, 29.74, 29.8, 29.9, 32.0, 42.7, 55.2, 68.0, 75.4, 96.5, 121.5, 127.9, 128.1, 128.7, 137.7, 140.3, 147.4, 149.6 and 158.2; mass spectrum (APCI), \( m/z \) (M+H)\(^+\) 457.3437 (C\(_{28}\)H\(_{45}\)N\(_2\)O\(_3\) requires 457.3430).
6-(N,N-dimethylamino)-2-(10-hydroxydecyl)-4,5-dimethylpyridin-3-ol (3.7).

To a stirred solution containing 76.0 mg (0.17 mmol) of compound 3.45 in 3 mL of methanol was added 1 drop of concentrated HCl. The reaction mixture was stirred at reflux for 2 h. The cooled reaction mixture was concentrated under diminished pressure and the residue was partitioned between 20 mL of satd aq NaHCO$_3$ and 20 mL of ethyl acetate. The organic layer was washed with 20 mL of brine, dried over MgSO$_4$ and concentrated under diminished pressure. The residue was dissolved in 2 mL methanol, then 2 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/N) was added and the reaction mixture was stirred at 23 °C under a H$_2$ atmosphere for 15 min. The reaction mixture was filtered through Celite® and the filtrate was concentrated under diminished pressure to afford compound 3.7 as a yellowish oil: yield 41 mg (79%); silica gel TLC $R_f$ 0.50 (9:1 dichloromethane–methanol); $^1$H NMR (CD$_3$OD) $\delta$ 1.28-1.34 (br m, 12H), 1.51 (m, 2H), 1.65 (m, 2H), 2.15 (s, 3H), 2.17 (s, 3H), 2.66 (s, 6H), 2.72 (dd, 2H, $J = 8.0$ Hz and 7.2 Hz) and 3.51 (t, 2H, $J = 6.8$ Hz); $^{13}$C NMR (CD$_3$OD) $\delta$ 12.9, 14.6, 26.9, 29.5, 30.46, 30.56, 30.59, 30.66, 30.69, 30.74, 32.7, 33.7, 43.4, 63.0, 123.7, 136.9, 145.8, 146.4 and 156.3; mass spectrum (APCI), $m/z$ 323.2694 (M+H)$^+$ (C$_{19}$H$_{35}$N$_2$O$_2$ requires 323.2699).
3-(Benzylxy)-6-(2,5-dimethyl-1H-pyrrol-1-yl)-2-hexadecyl-4,5-dimethylpyridine (3.46). To a stirred solution at −78 °C containing 1.00 g (3.12 mmol) of compound 3.42 in 50 mL of anh THF was added 512 µL (397 mg; 3.41 mmol) of TMEDA followed by 1.44 mL (3.59 mmol) of a 2.50 M solution of n-BuLi in hexane. The mixture was stirred for 5 min at −78 °C and then 977 µL (981 mg; 3.37 mmol) of 1-bromopentadecane was added. The reaction mixture was stirred at −78 °C for 30 min. The reaction mixture was warmed to 23 °C slowly, and then stirred for 30 min. The reaction was quenched with satd aq ammonium chloride and then poured into 50 mL of water. The mixture was then extracted with two 80-mL portions of diethyl ether. The combined organic layer was then washed with 100 mL of brine, dried (MgSO₄) and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 4:1 hexanes–diethyl ether afforded compound 3.46 as a light yellow oil: yield 800 mg (48%); silica gel TLC Rf 0.5 (4:1 hexanes–diethyl ether); ¹H NMR (CDCl₃) δ 0.89 (t, 3H, J = 6.8 Hz) 1.21-1.32 (br m, 26H), 1.71 (quint, 2H, J = 7.6 Hz), 1.84 (s, 3H), 1.95 (s, 6H), 2.28 (s, 3H), 2.82 (dd, 2H, J = 7.6 Hz and 7.6 Hz), 4.89 (s, 2H), 4.88 (s, 2H) and 7.38-7.50 (m, 5H); ¹³C NMR (CDCl₃) δ 12.6, 13.4, 13.8, 14.3, 22.8, 29.46, 29.49, 29.66, 29.72, 29.74, 29.77, 29.78, 29.83, 32.1, 32.4, 75.6, 96.4, 105.8, 127.9,
128.2, 128.4, 128.8, 129.4, 137.0, 141.3, 146.0, 151.3 and 153.4; mass spectrum (APCI), \( m/z \) 531.4300 (M+H)\(^+\) (C\textsubscript{36}H\textsubscript{55}N\textsubscript{2}O requires 531.4314).

![Chemical Structure](image)

**2-Amino-5-(benzyloxy)-6-hexadecyl-4,5-dimethylpyridine (3.47).** To a stirred solution containing 800 mg (1.51 mmol) of compound 3.46 in 10 mL of 9:1 ethanol–water was added 785 mg (11.3 mmol) of hydroxylamine hydrochloride followed by 615 mg (11.3 mmol) of potassium hydroxide. The reaction mixture was then stirred at reflux 6 h. A second portion of 785 mg (11.3 mmol) of hydroxylamine hydrochloride followed by 615 mg (11.3 mmol) of potassium hydroxide was added and the mixture was stirred at reflux for 16 h. The reaction mixture was then poured into 50 mL of water and extracted with two 50-mL portions of ethyl acetate. The combined organic layer was washed with 60 mL of brine, dried (MgSO\textsubscript{4}) and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 9:1 dichloromethane–methanol afforded compound 3.47 as a colorless solid: yield 416 mg (61%); mp 50-52 °C; silica gel TLC \( R_f \) 0.65 (9:1 dichloromethane–methanol); \(^1\)H NMR (CDCl\textsubscript{3}) \( \delta \) 0.86 (t, 3H, \( J = 6.8 \) Hz) 1.18-1.32 (br m, 26H), 1.64 (quint, 2H, \( J = 7.6 \) Hz), 2.01 (s, 3H), 2.18 (s, 3H), 2.63 (dd, 2H, \( J = 8.0 \) Hz and 8.0 Hz), 4.25 (br s, 2H), 4.69 (s, 2H) and 7.33-7.45 (m, 5H); \(^1^3\)C NMR (CDCl\textsubscript{3}) \( \delta \) 12.9, 13.1, 14.1, 22.7, 29.3, 29.5, 29.60, 29.62, 29.64,
29.7, 29.99, 29.89, 31.9, 32.2, 75.7, 113.6, 127.7, 128.0, 128.5, 137.4, 140.0, 145.0, 150.2 and 152.5; mass spectrum (APCI), \( m/z \) 453.3843 (M+H)⁺

\[(C_{30}H_{49}N_2O \text{ requires 453.3845})\]

3-(Benzyloxy)-6-(\(N,N\)-dimethylamino)-2-hexadecyl-4,5-dimethylpyridine (3.48). To a stirred solution containing 416 mg (0.92 mmol) of compound 3.47 in 10 mL of 1:1 formalin–acetonitrile were added 347 mg (5.52 mmol) of sodium cyanoborohydride followed by 190 µL (199 mg; 3.32 mmol) of glacial acetic acid. The reaction mixture was stirred at 23 °C for 16 h and then poured into 50 mL of satd aq sodium bicarbonate. The mixture was extracted with 80 mL of ether. The organic solution was washed with 80 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 4:1 hexanes–diethyl ether afforded compound 3.48 as colorless oil: yield 310 mg (70%); silica gel TLC \( R_f \) 0.60 (4:1 hexanes–diethyl ether); \(^1\)H NMR (CDCl₃) \( \delta \) 0.86 (t, 3H, \( J = 6.4 \) Hz) 1.21-1.32 (br m, 26H), 1.72 (quint, 2H, \( J = 7.2 \) Hz), 2.17 (s, 3H), 2.18 (s, 3H), 2.71-2.75 (m, 8H), 4.72 (s, 2H) and 7.34-7.48 (m, 5H); \(^{13}\)C NMR (CDCl₃) \( \delta \) 13.1, 14.1, 14.5, 22.6, 22.7, 28.6, 29.3, 29.62, 29.63, 29.7, 31.6, 31.89, 31.90, 42.6, 75.3, 121.4, 127.7, 128.0, 128.5, 137.5, 140.2, 147.2, 149.5 and 158.1; mass spectrum (APCI), \( m/z \) 481.4159 (M+H)⁺ (C\(_{32}\)H\(_{53}\)N\(_2\)O requires 481.4158).
6-(N,N-Dimethylamino)-2-(hexadecyl)-4,5-dimethylpyridin-3-ol (3.8). To a stirred solution containing 310 mg (0.64 mmol) of compound 3.48 in 15 mL of methanol was 3.0 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/N) and the reaction mixture was stirred at 23 °C under hydrogen atmosphere for 15 min. The reaction mixture was filtered through Celite® and the filtrate was concentrated under diminished pressure to afford compound 3.8 as a colorless solid: yield 246 mg (98%); mp 86-88 °C; silica gel TLC Rf 0.28 (4:1 hexanes–diethyl ether); $^1$H NMR (CD$_3$OD) $\delta$ 0.89 (t, 3H, $J = 6.4$ Hz), 1.28-1.36 (br m, 26H), 1.67 (quint, 2H, $J = 7.2$ Hz), 2.27 (s, 6H), 2.82 (dd, 2H, $J = 7.6$ Hz and 7.6 Hz) and 2.95 (s, 6H); $^{13}$C NMR (CD$_3$OD) $\delta$ 12.3, 13.1, 13.3, 22.3, 28.0, 29.1, 29.2, 29.3, 29.37, 29.40, 29.7, 31.7, 42.0, 72.8, 124.1, 127.8, 128.1 and 146.7; mass spectrum (APCI), m/z 391.3690 (M+H)$^+$ (C$_{25}$H$_{47}$N$_2$O requires 391.3688).

3-(Acetoxy)-6-(N,N-dimethylamino)-2-hexadecyl-4,5-dimethylpyridine (3.9). To a stirred solution containing 100 mg (0.26 mmol) of compound 3.8 in 2 mL of anh DMF was added 108 mg (0.78 mmol) of potassium carbonate followed by 37 µL (40 mg; 0.39 mmol) of acetic anhydride. The reaction mixture was stirred at
23 °C for 1h. The reaction mixture was poured into 30 mL of diethyl ether and washed with 20 mL of satd aq sodium bicarbonate, 20 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 4:1 hexanes–diethyl ether afforded compound 3.9 as a colorless oil: yield 92 mg (82%); silica gel TLC Rᵣ 0.45 (4:1 hexanes–diethyl ether); ¹H NMR (CDCl₃) δ 0.88 (t, 3H, J = 6.8 Hz) 1.26-1.32 (br m, 26H), 1.72 (quint, 2H, J = 7.2 Hz), 2.01 (s, 3H), 2.18 (s, 3H), 2.33 (s, 3H), 2.55 (dd, 2H, J = 7.6 Hz and 7.6 Hz) and 2.75 (s, 6H); ¹³C NMR (CDCl₃) δ 13.4, 14.2, 15.1, 20.7, 22.8, 28.1, 29.5, 29.6, 29.7, 29.75, 29.79, 29.84, 32.1, 32.2, 42.6, 121.3, 139.3, 139.8, 148.1, 159.8 and 169.5; mass spectrum (APCI), m/z 433.3785 (M+H)⁺ (C₂₇H₄₉N₂O requires 433.3794).

![5-Bromo-2-(N,N-dimethylamino)-4,6-dimethylpyridine](image)

5-Bromo-2-(N,N-dimethylamino)-4,6-dimethylpyridine (3.49). To a stirred solution containing 1.12 g (5.57 mmol) of compound 3.26 in 20 mL of anhydrous THF was added 1.04 mL (2.37 g; 16.71 mmol) of methyl iodide followed by 668 mg (16.7 mmol) of a 60 % suspension of sodium hydride in mineral oil. The reaction mixture was stirred at 23 °C for 18 h. The reaction mixture was poured into 100 mL of water and extracted with two 100-mL portions of ether. The combined organic solution was dried (MgSO₄) and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel
column (10 × 3 cm). Elution with 9:1 hexanes–diethyl ether afforded compound 3.49 as a colorless oil: yield 700 mg (55%); silica gel TLC $R_f$ 0.45 (3:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $\delta$ 2.30 (s, 3H), 2.51 (s, 3H), 3.01 (s, 6H) and 6.22 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 23.7, 25.6, 37.9, 37.9, 105.1, 110.2, 147.4, 154.6 and 178.5.

![Image of compound 3.49](image)

6-(N,N-Dimethylamino)-2,4-dimethylpyridin-3-ol (3.50). To a stirred solution containing 1.12 g (5.57 mmol) of compound 3.49 in 10 mL of 1:1 degassed dioxane–water was added 56 mg (0.06 mmol) of Pd$_2$dba$_3$ followed by 45 mg (0.10 mmol) of 2-di-tert-butylphosphino-2’,4’,6’-triisopropylbypheynyl (L$_1$) and 512 mg (9.15 mmol) of potassium hydroxide. The reaction mixture was stirred at 100 °C during 4 h. The reaction mixture was poured into 100 mL of water and extracted with 75 mL of ethyl ether. The aqueous layer was neutralized (pH ~7.0) with HCl and then extracted with two 75-mL portions of ethyl acetate. The combined organic solution was washed with 75 mL of brine, dried (MgSO$_4$) and then concentrated under diminished pressure to afford compound 3.50 as a light brownish solid: yield 200 mg (35%); mp 130-131 °C; silica gel TLC $R_f$ 0.15 (3:1 hexanes–ethyl acetate); $^1$H NMR (CD$_3$OD) $\delta$ 2.19 (s, 3H), 2.30 (s, 3H), 2.93 (s, 6H) and 6.31 (s, 1H); $^{13}$C NMR (CD$_3$OD) $\delta$ 16.8, 18.9, 39.5, 107.4, 139.0, 142.7, 144.9 and 155.9.
5-Bromo-2-(N,N-dimethylamino)-4-methoxy-6-methylpyridine (3.51). To a stirred solution containing 87.0 mg (0.52 mmol) of compound 3.36 in 4 mL of acetonitrile was added 93.0 mg (0.52 mmol) of N-bromosuccinimide. The reaction mixture was stirred at 23 °C protected from light for 4 h. The mixture was poured into 20 mL of ethyl acetate and washed with two 20-mL portions of satd aq sodium bicarbonate. The organic layer was dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 1:2 hexanes–ethyl acetate afforded compound 3.51 as a colorless solid: yield 121 mg (95%); mp 58-59°C; silica gel TLC Rf 0.37 (2:1 hexanes–ethyl acetate); ¹H NMR (CDCl₃) δ 2.47 (s, 3H), 3.03 (s, 6H) 3.85 (s, 3H) and 5.75 (s, 1H); ¹³C NMR (CDCl₃) δ 25.1, 38.0, 38.0, 55.7, 86.5, 97.4, 155.7, 158.8 and 165.6; mass spectrum (APCI), m/z 254.0287 (M+H)⁺ (C₉H₁₄N₂OBr requires 254.0290).

6-(N,N-Dimethylamino)-4-methoxy-2-methylpyridin-5-ol (3.52). To a stirred solution at –78 °C containing 121 mg (0.49 mmol) of compound 3.51 in 3 mL of anh THF was added 763 µL (1.22 mmol) of 1.6 M solution of n-BuLi in hexanes.
The reaction mixture was stirred at –78 °C for 20 min. To the mixture was added 163 µL (152 mg; 1.44 mmol) of trimethyl borate and the reaction mixture was stirred at 23 °C for 1 h. To the reaction mixture was added 1.08 mL of 30% aq H₂O₂. The reaction mixture was stirred for 30 min and poured into 20 mL of water. The aq mixture was neutralized with dilute aq HCl and extracted with two 30-mL portions of ethyl acetate. The combined organic solution was washed with 40 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 2 cm). Elution with 9:1 dichloromethane–methanol afforded compound 3.52 as a colorless oil: yield 10 mg (11%); silica gel TLC $R_f$ 0.10 (9:1 dichloromethane–methanol); $^1$H NMR (CD₃OD) δ 2.27 (s, 3H), 3.00 (s, 6H), 3.90 (s, 3H) and 6.10 (s, 1H); $^{13}$C NMR (CD₃OD) δ 15.6, 38.1, 38.1, 54.9, 87.9, 133.0, 139.67, 154.0 and 157.3; mass spectrum (APCI), m/z 183.1133 (M+H)$^+$ (C₉H₁₅N₂O₂ requires 183.1134).

![Compound 3.52](image)

2-(N,N-Dimethylamino)-5-hydroxy-3,4,6-trimethylpyridine (3.53). To a stirred solution containing 500 mg (3.29 mmol) of compound 3.40 in 10 mL of 1:1 formalin–acetonitrile was added 860 mg (13.7 mmol) of sodium cyanoborohydride followed by 529 µL (621 mg; 10.3 mmol) of glacial acetic acid. The reaction mixture was stirred at 23 °C for 2 h and then poured into 20
mL of satd aq sodium bicarbonate. The mixture was then extracted with 100 mL of ethyl acetate. The organic phase was washed with 50 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 2 cm). Elution with 2:1 hexanes–ethyl acetate afforded compound 3.53 as a colorless solid: yield 78 mg (77%); mp 128–129 °C; silica gel TLC $R_f$ 0.40 (2:1 hexanes–ethyl acetate); $^1$H NMR (CD$_3$OD) $\delta$ 2.14 (s, 3H), 2.18 (s, 3H), 2.37 (s, 3H) and 2.69 (s, 6H); $^{13}$C NMR (CD$_3$OD) $\delta$ 12.5, 14.7, 18.9, 43.0, 122.7, 133.9, 138.6, 144.6 and 156.0.

2-Amino-4,6-dimethylpyrimidine (3.54). To a stirred solution containing 4.00 g (37.0 mmol) of guanidine sulfate and 8.40 g (79.3 mmol) of sodium carbonate in 25 mL of water was added 6.00 mL (5.88 g; 58.7 mmol) of 2,4-pentanedione. The reaction mixture was stirred at 100 °C for 16 h. The reaction mixture was poured into 150 mL of water and then extracted with two 150-mL portions of dichloromethane. The combined organic phase was washed with 150 mL of brine, dried (MgSO$_4$) and then concentrated under diminished pressure to afford compound 3.54 as a colorless solid: yield 4.31g (95%); mp 152-153 °C; silica gel TLC $R_f$ 0.50 (9:1 dichloromethane–methanol); $^1$H NMR (CDCl$_3$) $\delta$ 2.24 (s, 6H), 5.39 (br s, 2H) and 6.33 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 23.7, 110.5, 162.9 and 167.7; mass spectrum (APCI), $m/z$ 124.0869 (M+H)$^+$ (C$_6$H$_{10}$N$_3$ requires 124.0875).
2-Amino-5-bromo-4,6-dimethylpyrimidine (3.55). To a stirred solution containing 4.31 g (34.8 mmol) of compound 3.54 in 150 mL of acetonitrile was added 6.15 g (52.1 mmol) of \(N\)-bromosuccinimide. The reaction mixture was stirred at 23 °C for 3 h. The formed precipitate was filtered and dried to afford compound 3.55 as a colorless solid: yield 5.93 g (83%); mp 183-185 °C; silica gel TLC \(R_f\) 0.15 (2:1 ethyl acetate–hexanes); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 2.44 (s, 6H) and 5.19 (br s, 2H); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 24.7, 109.6, 160.7 and 166.3; mass spectrum (APCI), \(m/z\) 201.9982 (M+H\(^+\)) (C\(_6\)H\(_9\)N\(_3\)Br requires 201.9980).

5-Bromo-2-(2,5-dimethyl-1\(H\)-pyrrol-1-yl)-4,6-dimethylpyrimidine (3.56). To a stirred solution containing 2.00 g (9.89 mmol) of compound 3.55 in 16 mL of anh toluene was added 1.36 mL (1.32 g; 11.6 mmol) of 2,5-hexanediol followed by 96 mg (0.50 mmol) of \(p\)-toluenesulfonic acid. The reaction mixture was heated and stirred at reflux for 12 h using a Dean-Stark apparatus. The reaction mixture was poured into 150 mL of water and then extracted with 200 mL of ethyl acetate. The organic solution was washed with 150 mL of brine, dried (MgSO\(_4\)) and
concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 5:1 hexanes–ethyl acetate afforded compound 3.56 as light yellow crystals: yield 2.23 g (81%); mp 64-65 °C; silica gel TLC $R_f$ 0.65 (6:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $\delta$ 2.34 (s, 6H), 2.67 (s, 6H) and 5.89 (s, 2H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.5, 24.9, 108.7, 118.6, 129.5, 129.5, 155.3 and 166.9; mass spectrum (APCI), $m/z$ 280.0458 (M+H)$^+$ (C$_{12}$H$_{15}$N$_3$Br requires 280.0449).

![Image of compound structure](image)

**5-(Benzyloxy)-2-(2,5-dimethyl-1H-pyrrol-1-yl)-4,6-dimethylpyrimidine (3.57).** To a stirred solution containing 4.87 g (17.4 mmol) of compound 3.56 in 50 mL of 1:1 dioxane–degassed water was added 632 mg (0.69 mmol) of Pd$_2$dba$_3$ followed by 293 mg (0.69 mmol) of 2-di-tert-butylphosphino-2',4',6'-triisopropylbiphenyl (L$_1$) and 2.92 g (52.1 mmol) of KOH. The reaction mixture was stirred at 100 °C for 3 h. The cooled reaction mixture was poured into 200 mL of water and extracted with 100 mL of ethyl acetate. The aqueous layer was acidified with HCl (pH 2-3) and then extracted with two 150-mL portions of ethyl acetate. The combined organic layer was washed with 150 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was dissolved in 50 mL of anh THF and treated with 3.10 mL (4.46 g; 26.1 mmol) of benzyl bromide followed by 1.40 g (34.8 mmol) of a 60% suspension of NaH in mineral
oil. The reaction mixture was stirred at 23 °C for 48 h. The reaction mixture was quenched with satd aq sodium bicarbonate and poured into 150 mL of water and extracted with two 150-mL portions of ether. The combined organic layer was washed with 150 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (20 × 6 cm). Elution with 9:1 hexanes–ethyl acetate afforded compound 3.57 as a light yellow oil: yield 4.09 g (76%); silica gel TLC $R_f$ 0.6 (6:1 hexanes–ethyl acetate); $^1$H NMR (CDCl₃) δ 2.29 (s, 6H), 2.47 (s, 6H), 4.92 (s, 2H), 5.86 (s, 2H) and 7.42 (m, 5H); $^{13}$C NMR (CDCl₃) δ 14.1, 14.1, 19.1, 19.1, 75.3, 107.9, 107.9, 128.3, 128.6, 128.6, 129.0, 129.0, 129.2, 129.2, 136.0, 147.7, 152.3, 152.3 and 161.6; mass spectrum (APCI), $m/z$ 307.1675 (M)$^+$ (C₁₉H₂₁N₃O requires 307.1685).

5-(Benzyloxy)-2-(2,5-dimethyl-1H-pyrrol-1-yl)-4-(10-(methoxymethoxy)decyl)-6-methylpyrimidine (3.58). To a stirred solution containing 486 mg (1.58 mmol) of compound 3.57 and 281 mg (1.05 mmol) of compound 3.25 in 10 mL of anh THF at −78 °C was added 987 µL (1.58 mmol) of a 1.6 M solution of $n$-BuLi in pentane. The reaction mixture was stirred under argon atmosphere at 23 °C for 30 min. The reaction was quenched with satd aq ammonium chloride and then poured into 50 mL of water. The mixture was then
extracted with two 50-mL portions of ethyl acetate. The combined organic layer was washed with 80 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 3 cm). Elution with 5:1 hexanes–ethyl acetate afforded compound 3.58 as a light yellow oil: yield 289 mg (56%); silica gel TLC R_f 0.55 (5:1 hexanes–ethyl acetate); ¹H NMR (CDCl₃) δ 1.33 (m, 12H), 1.60 (m, 2H), 1.82 (m, 2H), 2.33 (s, 6H), 2.49 (s, 3H), 2.79 (dd, 2H, J = 7.6 Hz and 7.6 Hz), 3.36 (s, 3H), 3.52 (t, 2H, J = 6.4 Hz), 4.62 (s, 2H), 4.90 (s, 2H), 5.87 (s, 2H) and 7.41 (m, 5H); ¹³C NMR (CDCl₃) δ 14.4, 14.4, 19.3, 26.2, 27.7, 29.4, 29.4, 29.5, 29.5, 29.6, 29.8, 31.6, 55.1, 67.9, 75.7, 96.4, 108.0, 108.0, 128.0, 128.58, 128.62, 128.7, 129.3, 136.2, 147.4, 147.4, 152.6, 161.6, 161.6 and 165.1; mass spectrum (APCI), m/z 494.3395 (M+H)⁺ (C₃₀H₄₄N₃O₃ requires 494.3383).

2-Amino-5-(benzyloxy)-4-(10-(methoxymethoxy)decyl)-6-methylpyrimidine (3.59). To a stirred solution containing 230 mg (0.47 mmol) of compound 3.58 in 15 mL of 9:1 ethanol–water was added 327 mg (4.70 mmol) of hydroxylamine hydrochloride followed by 263 mg (4.70 mmol) of KOH. The reaction mixture was then heated and stirred at reflux for 5 h. A second portion of 327 mg (4.70 mmol) of hydroxylamine hydrochloride followed by 263 mg (4.70 mmol) of KOH was added and the reaction mixture was heated and stirred at reflux for 12 h. The
reaction mixture was poured into 70 mL of water and then treated with 1N NaOH until pH 9-10 was reached. The reaction mixture was extracted with two 70-mL portions of ethyl acetate. The combined organic layer was washed with 70 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 2:1 hexanes–ethyl acetate afforded compound 3.59 as a colorless oil: yield 133 mg (76%); silica gel TLC $R_f$ 0.76 (9:1 dichloromethane–methanol); $^1$H NMR (CDCl$_3$) $\delta$ 1.27 (m, 12H), 1.53-1.63 (m, 4H), 2.26 (s, 3H), 2.54 (dd, 2H, $J = 7.6, 7.6$ Hz), 3.31 (s, 3H), 3.46 (t, 2H, $J = 6.8$ Hz), 4.57 (s, 2H), 4.69 (s, 2H), 5.19 (brs, 2H) and 7.35-7.37 (m, 5H); $^{13}$C NMR (CDCl$_3$) $\delta$ 18.9, 26.2, 28.4, 29.36, 29.40, 29.5, 29.67, 29.70, 31.9, 55.0, 67.8, 75.9, 96.3, 127.9, 128.3, 128.6, 128.6, 136.8, 142.8, 158.9, 161.1, 161.1 and 164.8; mass spectrum (APCI), $m/z$ 416.2908 (M+H)$^+$ (C$_{24}$H$_{38}$N$_3$O$_3$ requires 416.2913).

\[ \text{5-} \text{(Benzyloxy)-2-} \text{(N,N-dimethylamino)-4-} \text{(10-hydroxydeyl)-6-methylpyrimidine (3.60).} \]

A stirred solution containing 180 mg (0.43 mmol) of compound 3.59 in 10 mL of 35% aq 1:1 formaldehyde–formic acid was heated and stirred at reflux for 16 h. The reaction mixture was poured into 20 mL of water and extracted with two 40-mL portions of ethyl acetate. The combined organic layer was washed with 40 mL of satd aq NaHCO$_3$ and then 40 mL of
brine. The organic solution was dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (8 × 3 cm). Elution with 1:2 acetone–hexanes afforded compound 3.60 as a colorless oil: yield 44 mg (44%); silica gel TLC $R_f$ 0.58 (1:2 acetone–hexanes); $^1$H NMR (CD$_3$OD) δ 1.27 (m, 12H), 1.53-1.73 (m, 4H), 2.26 (s, 3H), 2.55 (dd, 2H, $J = 7.6$ Hz and 7.6 Hz), 3.09 (s, 6H), 3.50 (t, 2H, $J = 6.8$ Hz), 4.71 (s, 2H) and 7.35-7.37 (m, 5H); $^{13}$C NMR (CD$_3$OD) δ 14.0, 17.8, 25.5, 27.6, 29.1, 29.2, 29.3, 31.3, 32.3, 36.3, 36.3, 61.6, 75.5, 127.9, 128.3, 128.3, 128.6, 128.6, 137.1, 141.0, 158.4, 160.5, 161.5 and 163.7; mass spectrum (APCI), $m/z$ 400.2969 (M+H)$^+$ (C$_{24}$H$_{38}$N$_3$O$_2$ requires 400.2964).

![Structure](image.png)

2-(N,N-Dimethylamino)-4-(10-hydroxydecyl)-6-methylpyrimidin-5-ol (3.10). To a stirred solution containing 44.0 mg (0.11 mmol) of compound 3.60 in 3 mL of methanol was added 3 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/N). The reaction mixture was stirred at 23 °C for 15 min under a hydrogen atmosphere. The reaction mixture was filtered through Celite and the filtrate was concentrated under diminished pressure to afford compound 3.10 as colorless oil: yield 33 mg (100%). An analytical sample was obtained by chromatography on a silica gel column (10 × 1 cm). Elution with 2:1 toluene–ethyl acetate afforded the purified product as a colorless oil; silica gel TLC $R_f$
0.25 (2:1 toluene–ethyl acetate); \(^1\)H NMR (CD\(_3\)OD) \(\delta\) 1.29-1.35 (m, 12H), 1.49 (m, 2H), 1.65 (m, 2H), 2.26 (s, 3H), 2.62 (dd, 2H, \(J = 7.2\) Hz and 7.2 Hz), 3.06 (s, 6H) and 3.51 (t, 2H, \(J = 6.4\) Hz); \(^13\)C NMR (CD\(_3\)OD) \(\delta\) 17.8, 25.5, 27.3, 29.13, 29.15, 29.18, 29.3, 31.2, 32.2, 36.5, 36.5, 61.6, 138.0, 155.6, 157.3 and 159.4; mass spectrum (APCI), \(m/z\) 310.2490 (M+H)\(^+\) (C\(_{17}\)H\(_{32}\)N\(_3\)O\(_2\) requires 310.2495).

![Chemical structure](image)

**2-Amino-4-(10-hydroxydecyl)-6-methylpyrimidin-5-ol (3.11).** To a stirred solution containing 133 mg (0.48 mmol) of compound 3.59 in 10 mL of methanol was added two drops of concentrated HCl and the reaction mixture was stirred at reflux for 16 h. To the cooled mixture was added 10 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/N). The reaction mixture was stirred at 23 °C for 15 min under a hydrogen atmosphere. The reaction mixture was filtered through Celite® and the filtrated was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (13 \(\times\) 3 cm). Elution with 17:3 dichloromethane–methanol afforded compound 3.11 as colorless solid: yield 68 mg (69%); mp 103-104 °C; silica gel TLC \(R_f\) 0.20 (17:3 dichloromethane–methanol); \(^1\)H NMR (CD\(_3\)OD) \(\delta\) 1.29-1.35 (m, 12H), 1.50 (quint, 2H, \(J = 6.8\) Hz), 1.64 (quint, 2H, \(J = 7.6\) Hz), 2.37 (s, 3H), 2.71 (dd, 2H, \(J = 7.6\) Hz and 7.6 Hz) and 3.51 (t, 2H, \(J = 6.4\) Hz); \(^13\)C NMR (CD\(_3\)OD) \(\delta\) 16.4,
25.5, 27.1, 29.00, 29.02, 29.08, 29.13, 29.3, 30.7, 32.2, 61.6, 139.0, 153.5, 157.6 and 160.8; mass spectrum (APCI), m/z 282.2170 (M+H)^+ (C_{15}H_{28}N_{3}O_{2} requires 282.2182).

5-(Benzyloxy)-2-chloro-4-(10-(methoxymethoxy)decyl)-6-methylpyrimidine (3.61). To a stirred solution containing 240 mg (0.58 mmol) of compound 3.54 in 10 mL of anh CH$_2$Cl$_2$ was added 1.10 g (5.80 mmol) of benzyltrimethylammonium chloride followed by 696 µL (603 mg; 5.85 mmol) of t-butyl nitrite. The reaction mixture was stirred at 23 °C for 15 h protected from light. The mixture was filtered and the filtrate was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 x 3 cm). Elution with 4:1 hexanes–ethyl acetate afforded compound 3.61 as a colorless oil: yield 44 mg (16%); silica gel TLC $R_f$ 0.45 (4:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) δ 1.24 (br s, 12H), 1.55 (quint, 2H, $J = 7.2$ Hz), 1.65 (quint, 2H, $J = 6.8$ Hz), 2.42 (s, 3H), 2.67 (dd, 2H, $J = 8.0$ Hz and 8.0 Hz), 3.33 (s, 3H), 3.47 (t, 2H, $J = 6.8$ Hz), 4.59 (s, 2H), 4.83 (s, 2H) and 7.37 (m, 5H); $^{13}$C NMR (CDCl$_3$) δ 19.3, 26.2, 28.1, 29.3, 29.4, 29.47, 29.51, 29.7, 31.9, 55.0, 67.9, 76.0, 96.4, 128.1, 128.7, 128.8, 135.7, 149.0, 154.2, 163.8 and 167.4; mass spectrum (APCI), m/z (M+H)$^+$ 435.2411 (C$_{24}$H$_{36}$N$_2$O$_3$Cl requires 435.2415)
5-(Benzyloxy)-2-methoxy-4-(10-(methoxymethoxy)decyl)-6-methylpyrimidine (3.62). To a stirred solution containing 41.0 mg (0.09 mmol) of compound 3.61 in 5 mL of anhydrous methanol was added 22 mg (0.94 mmol) of sodium. The reaction mixture was then stirred at reflux for 16 h. The cooled reaction mixture was quenched with water and poured into 50 mL of water. The mixture was extracted with two 50-mL portions of ethyl acetate. The combined organic solution was washed with 50 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (6 × 2 cm). Elution with 1:1 hexanes–ethyl acetate afforded compound 3.62 as a colorless oil: yield 30 mg (74%); silica gel TLC $R_f$ 0.65 (1:1 hexanes–ethyl acetate); $^1$H NMR (CDCl₃) δ 1.24 (br m, 12H), 1.55 (quint, 2H, $J = 6.8$ Hz), 1.68 (quint, 2H, $J = 7.6$ Hz), 2.37 (s, 3H), 2.65 (dd, 2H, $J = 7.6, 7.6$ Hz), 3.33 (s, 3H), 3.48 (t, 2H, $J = 6.8$ Hz), 3.93 (s, 3H), 4.59 (s, 2H), 4.83 (s, 2H) and 7.37 (m, 5H); $^{13}$C NMR (CDCl₃) δ 19.2, 26.2, 27.9, 29.4, 29.4, 29.51, 29.53, 29.7, 31.7, 54.7, 55.0, 67.9, 75.8, 96.4, 127.5, 128.4, 128.6, 136.5, 145.1, 160.6, 162.4 and 165.9; mass spectrum (APCI), $m/z$ (M+H)+ 431.2910 ($C_{25}H_{30}N_2O_4$ requires 431.2910).
4-(10-Hydroxydecyl)-2-methoxy-6-methylpyrimidin-5-ol (3.12). To a stirred solution containing 30.0 mg (0.07 mmol) of compound 3.62 in 3 mL of methanol was added a drop of concentrated HCl. The mixture was stirred at reflux for 16 h. Then was added 2 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/N) and the reaction mixture was stirred at 23°C for 20 min. The mixture was filtered through Celite® and concentrated under diminished pressure to afford compound 3.12 as a yellowish oil: yield 20 mg (97%); silica gel TLC $R_f$ 0.45 (4:1 hexanes–ethyl acetate); $^1$H NMR (methanol) $\delta$ 1.30 (br m, 12H), 1.50 (br m, 2H), 1.74 (br m, 2H), 2.56 (s, 3H), 2.89 (dd, 2H, $J = 7.6$ Hz and 7.6 Hz), 3.51 (t, 2H, $J = 6.4$ Hz) and 4.15 (s, 3H); $^{13}$C NMR (methanol) $\delta$ 16.2, 25.5, 26.8, 28.9, 29.0, 29.1, 29.18, 29.22, 30.8, 32.2, 56.1, 61.6, 143.1, 153.0 and 156.6 and 163.0; mass spectrum (APCI), $m/z$ (M+H)$^+$ 297.2180 (C$_{16}$H$_{29}$N$_2$O$_3$ requires 297.2178).
2-(2,5-Dimethyl-1H-pyrrol-1-yl)-4-(10-hydroxydecyl)-6-methylpyrimidin-5-ol (3.13). To a stirred solution containing 240 mg (0.48 mmol) of compound 3.58 in 10 mL of methanol was added two drops of concentrated HCl and the mixture was stirred at reflux overnight. To the mixture was added 10 mg of 20% palladium hydroxide–on–carbon (Degussa type E101 NE/E). The reaction mixture was stirred at 23 °C for 15 min under a hydrogen atmosphere. The reaction mixture was filtered through Celite® and the filtrate was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (13 × 3 cm). Elution with 2:1 toluene–ethyl acetate compound 3.13 product as colorless oil: yield 13 mg (8%); silica gel TLC $R_f$ 0.40 (2:1 toluene–ethyl acetate); $^1$H NMR (CD$_3$OD) $\delta$ 1.29-1.35 (m, 12H), 1.49 (m, 2H), 1.71 (m, 2H), 2.11 (s, 6H), 2.45 (s, 3H), 2.80 (dd, 2H, $J = 7.2$ Hz and 7.2 Hz), 3.50 (t, 2H, $J = 6.4$ Hz) and 5.74 (s, 2H); $^{13}$C NMR (CD$_3$OD) $\delta$ 12.0, 17.5, 25.5, 27.3, 28.9, 29.05, 29.12, 29.18, 29.22, 31.0, 32.2, 61.6, 106.3, 128.4, 146.2, 148.8, 155.4 and 158.9; mass spectrum (APCI), $m$/z 360.2659 (M+H)$^+$ (C$_{21}$H$_{34}$N$_3$O$_2$ requires 360.2651).

2-(2,5-Dimethylpyrrolidin-1-yl)-4-(10-hydroxydecyl)-6-methylpyrimidin-5-ol (3.14). This compound was obtained as a side product in the reaction for the preparation of compound 3.13 as a colorless oil: yield 25 mg (14%); silica gel
TLC $R_f$ 0.46 (2:1 toluene–ethyl acetate); $^1$H NMR (CD$_3$OD) $\delta$ 1.31 (m, 16H), 1.51 (m, 2H), 1.69 (m, 4H), 1.98 (m, 2H), 2.25 (s, 3H), 2.61 (dd, 2H, $J = 7.2$ Hz and 7.2 Hz), 3.51 (t, 2H, $J = 6.8$ Hz) and 4.11 (m, 2H); $^{13}$C NMR (CD$_3$OD) $\delta$ 17.6, 21.0, 25.5, 27.1, 29.08, 29.14, 29.2, 29.3, 31.0, 31.5, 32.2, 54.5, 61.6, 137.8, 141.0, 155.5 and 159.1; mass spectrum (APCI), $m/z$ 364.2954 (M+H)$^+$ (C$_{21}$H$_{38}$N$_3$O$_2$ requires 364.2964).

2-($N,N$-Dimethylamino)-2-methoxy-6-methylpyrimidine (3.63). To a stirred solution containing 1.00 g (7.19 mmol) of 2-amino-4-methoxy-6-methylpyrimidine in 10 mL of anh DMF was added 1.35 mL (3.08 g; 21.7 mmol) of methyl iodide followed by 853 mg (21.6 mmol) of a 60% suspension of sodium hydride in mineral oil. The reaction mixture was stirred at 23 °C for 20 min. The reaction mixture was quenched with water and concentrated under diminished pressure in order to remove as much DMF as possible. The residue was dissolved in 70 mL of ethyl acetate and washed with 50 mL of water and 50 mL brine. The organic solution was dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 $\times$ 5 cm). Elution with 4:1 hexanes–ethyl acetate afforded compound 3.63 as a colorless oil: yield 755 mg (63%); silica gel TLC $R_f$ 0.30 (4:1 hexanes–ethyl ether); $^1$H NMR (CDCl$_3$) $\delta$ 2.22 (s, 3H), 3.12 (s, 6H), 3.83 (s, 3H) and 5.76 (s, 1H); $^{13}$C NMR
(CDCl$_3$) δ 24.1, 36.8, 52.7, 93.7, 162.2, 167.7 and 170.2; mass spectrum (APCI), m/z 168.1135 (M+H)$^+$ (C$_8$H$_{14}$N$_3$O requires 168.1137).

2-(N,N-Dimethylamino)-4-methoxy-6-[10-]
(methoxymethoxy)decy|pyrimidine (3.64). To a stirred solution containing 933 mg (5.58 mmol) of compound 3.63 and 1.5 g (5.58 mmol) of compound 3.25 in 20 mL of anh THF was added 5.23 mL (8.37 mmol) of 1.6 M n-BuLi in hexanes. The reaction mixture was stirred at 23 °C for 20 min. The reaction mixture was quenched with satd aq ammonium chloride and poured into 100 mL of water. The compound was extracted with two 80-mL portions of ethyl acetate. The combined organic layer was washed with 80 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 9:1 hexanes–ethyl acetate afforded compound 3.64 as a colorless oil: yield 1.41 g (71%); silica gel TLC $R_f$ 0.45 (9:1 hexanes–ethyl acetate); $^1$H-NMR (CDCl$_3$) δ 1.27 (br m, 10H), 1.82 (m, 4H), 2.48 (dd, 2H, $J = 8.0$ Hz and 8.0 Hz), 3.14 (s, 6H), 3.33 (s, 3H), 3.48 (t, 2H, $J = 6.8$ Hz), 3.85 (s, 3H), 4.59 (s, 2H) and 5.76 (s, 1H); $^{13}$C NMR (CDCl$_3$) δ 26.2, 28.5, 28.7, 29.3, 29.4, 29.45, 29.54, 29.7, 36.8, 37.8, 52.7, 55.0, 67.9, 93.0, 96.4, 162.4, 170.3 and 172.0; mass spectrum (APCI), m/z 354.2766 (M+H)$^+$ (C$_{19}$H$_{36}$N$_3$O$_3$ requires 254.2757).
**5-Bromo-2-(N,N-dimethylamino)-4-methoxy-6-(10-(methoxymethoxy)decyl)pyrimidine (3.65).** To a stirred solution containing 1.41 g (3.99 mmol) of compound 3.64 in 10 mL of acetonitrile was added 852 mg (4.79 mmol) of N-bromosuccinimide. The reaction mixture was stirred at 23 °C for 5 h and protected from light. The reaction mixture was concentrated under diminished pressure and the residue was dissolved into 100 mL of ethyl acetate. The organic solution was washed with 100 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column. Elution with 9:1 hexanes–ethyl acetate afforded compound 3.65 as colorless oil: yield 910 mg (53%); silica gel TLC \( R_f \) 0.65 (9:1 hexanes–ethyl acetate); \(^1\)H NMR (CDCl₃) \( \delta \) 1.27 (br m, 10H), 1.59 (m, 4H), 2.67 (dd, 2H, \( J = 7.6 \text{ Hz} \) and 7.6 Hz), 3.11 (s, 6H), 3.33 (s, 3H), 3.48 (t, 2H, \( J = 6.8 \text{ Hz} \)), 3.92 (s, 3H) and 4.59 (s, 2H); \(^{13}\)C NMR (CDCl₃) \( \delta \) 26.2, 27.6, 29.3, 29.4, 29.5, 29.6, 36.7, 36.9, 54.0, 55.0, 67.9, 91.2, 96.4, 160.1, 165.1 and 169.0; mass spectrum (APCI), \( m/z \) 432.1857 (M+H)+ \((\text{C}_{19}\text{H}_{35}\text{N}_{3}\text{O}_{3}\text{Br})\) requires 432.1862).
2-(N,N-Dimethylamino)-4-methoxy-6-(10-(methoxymethoxy)decyl)pyrimidin-5-ol (3.66). To a stirred solution at −5 °C containing 120 mg (0.25 mmol) of compound 3.65 in 3 mL of anh THF was added 390 µL (0.62 mmol) of 1.6 M solution of n-BuLi in hexanes. The reaction mixture was stirred at −5 °C for 20 min. To the mixture was added 84 µL (78 mg; 0.75 mmol) of trimethyl borate and the reaction mixture was stirred for 1 h. To the reaction mixture was added 0.55 mL of 30% aq H₂O₂ followed by 0.18 mL of 3 N aq NaOH. The reaction mixture was stirred for 30 min and poured into 50 mL of water. The aq mixture was neutralized with dilute aq HCl and extracted with two 50-mL portions of ethyl acetate. The combined organic solution was washed with 80 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 2:1 hexanes–ethyl acetate afforded compound 3.66 as a colorless oil: yield 47 mg (51%); silica gel TLC Rf 0.50 (2:1 hexanes–ethyl acetate); ¹H NMR (CDCl₃) δ 1.25 (br m, 10H), 1.59 (m, 4H), 2.58 (br s, 2H), 3.07 (s, 6H), 3.33 (s, 3H), 3.48 (t, 2H, J = 6.8 Hz), 3.92 (s, 3H) and 4.59 (s, 2H); ¹³C NMR (CDCl₃) δ 26.1, 27.7, 29.3, 29.37, 29.42, 29.45, 29.52, 29.7, 31.2, 37.2, 53.2, 55.0, 67.9, 96.3, 126.9, 155.0, 155.9 and 158.0; mass spectrum (APCI), m/z 370.2700 (M+H)⁺ (C₁₉H₃₆N₃O₃ requires 370.2706).
2-(N,N-Dimethylamino)-4-(10-hydroxydecyl)-6-methoxypyrimidin-5-ol (3.15). To a stirred solution containing 20 mg (0.054 mmol) of compound 3.66 in 5 mL of methanol was added 2 drops of concentrated HCl. The reaction mixture was stirred at reflux for 16 h. The reaction mixture was concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (8 × 1 cm). Elution with 1:2 hexanes–ethyl acetate afforded compound 3.15 as colorless solid: yield 7 mg (40%); mp 104-105 °C; silica gel TLC \( R_f \) 0.15 (1:2 hexanes–ethyl acetate); \(^1\)H NMR (CD\(_3\)OD) \( \delta \ 1.30 \) (br m, 10H), 1.49 (m, 2H), 1.64 (m, 2H), 2.76 (dd, 2H, \( J = 7.6 \) Hz and 7.6 Hz), 3.22 (s, 6H), 3.51 (t, 2H, \( J = 6.8 \) Hz) and 4.07 (s, 3H); \(^{13}\)C NMR (CD\(_3\)OD) \( \delta \ 25.6, 27.5, 27.7, 29.02, 29.03, 29.17, 29.19, 29.3, 32.3, 37.2, 54.5, 61.6, 128.1, 146.4, 151.3 \) and 162.7; mass spectrum (APCI), \( m/z \) 326.2442 (M+H)\(^+\) (C\(_{17}\)H\(_{32}\)N\(_3\)O\(_3\) requires 326.2444).

![Structure](image)

5-(Benzyloxy)-2-(2,5-dimethyl-1H-pyrrol-1-yl)-4-methyl-6-pentylpyrimidine (3.67): To a stirred solution at −78 °C containing 1.00 g (3.25 mmol) of compound 3.57 and 233 \( \mu \)L (296 mg; 2.16 mmol) of 1-bromobutane in 30 mL of anh THF was added 2.70 mL (4.32 mmol) of a 1.6 M solution of \( n \)-BuLi in pentane. The reaction mixture was stirred at 23 °C for 30 min. The reaction was quenched with satd aq ammonium chloride and then poured into 70 mL of water.
The mixture was then extracted with two 70-mL portions of diethyl ether. The combined organic layer was then washed with 100 mL of brine, dried (MgSO₄) and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 4:1 hexanes–diethyl ether afforded compound 3.67 as a colorless oil: yield 533 mg (69%); silica gel TLC *R*ₐ 0.50 (4:1 hexanes–diethyl ether); ¹H NMR (CDCl₃) δ 0.90 (t, 3H, *J* = 7.2 Hz), 1.34 (m, 4H), 1.77 (m, 2H), 2.33 (s, 6H), 2.49 (s, 3H), 2.79 (dd, 2H, *J* = 7.6 Hz and 7.6 Hz), 4.91 (s, 2H), 5.88 (s, 2H) and 7.42 (m, 5H); ¹³C NMR (CDCl₃) δ 14.0, 14.3, 19.3, 22.5, 27.4, 31.6, 31.7, 75.7, 107.9, 128.0, 128.2, 128.7, 129.3, 136.2, 147.4, 152.6, 161.6 and 165.1; mass spectrum (APCI), *m/z* 364.2394 (M+H)⁺ (C₂₃H₃₀N₃O requires 364.2389).

![Chemical Structure](image)

5-(Benzyloxy)-4-decyl-2-(2,5-dimethyl-1H-pyrrol-1-yl)-6-methylpyrimidine (3.68). To a stirred solution at −78 °C containing 1.00 g (3.25 mmol) of compound 3.57 and 415 µL (452 mg; 2.16 mmol) of 1-bromononane in 30 mL of anh THF was added 2.70 mL (4.32 mmol) of a 1.60 M solution of *n*-BuLi in pentane. The reaction mixture was stirred at 23 °C for 30 min. The reaction was quenched with satd aq ammonium chloride and then poured into 70 mL of water. The mixture was extracted with two 70-mL portions of ether. The combined organic layer was then washed with 100 mL of brine, dried (MgSO₄) and concentrated under
diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 4:1 hexanes–ether afforded compound 3.68 as a colorless oil: yield 502 mg (53%); silica gel TLC $R_f$ 0.60 (4:1 hexanes–ether); $^1$H NMR (CDCl$_3$) $\delta$ 0.89 (t, 3H, $J = 7.2$ Hz), 1.25 (m, 14H), 1.75 (m, 2H), 2.31 (s, 6H), 2.48 (s, 3H), 2.77 (dd, 2H, $J = 7.6$ Hz and 7.6 Hz), 4.90 (s, 2H), 5.86 (s, 2H) and 7.42 (m, 5H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.1, 14.3, 19.3, 22.7 27.7, 29.3, 29.4, 29.51, 29.57, 31.62, 31.9, 75.7, 107.9, 128.0, 128.6, 128.7, 129.3, 136.2, 147.4, 152.6, 161.6 and 165.1; mass spectrum (APCI), $m/z$ 434.3172 (M+H)$^+$ (C$_{28}$H$_{40}$N$_3$O requires 434.3171).

![Chemical Structure](image)

**5-(Benzyloxy)-2-(2,5-dimethyl-1H-pyrrol-1-yl)-4-hexadecyl-6-methylpyrimidine (3.69).** To a stirred solution at −78 °C containing 1.00 g (3.25 mmol) of compound 3.57 and 630 µL (630 mg; 2.16 mmol) of 1-bromopentadecane in 20 mL of anh THF was added 2.70 mL (4.32 mmol) of a 1.60 M solution of n-BuLi in pentane. The reaction mixture was stirred at 23 °C for 30 min. The reaction was quenched with satd aq ammonium chloride and then poured into 70 mL of water. The mixture was then extracted with two 70-mL portions of ether. The combined organic layer was washed with 100 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 4:1
hexanes–ether afforded compound **3.69** as a colorless oil: yield 269 mg (24%);
silica gel TLC $R_f$ 0.5 (4:1 hexanes–ether); $^1$H NMR (CDCl$_3$) $\delta$ 0.89 (t, 3H, $J = 7.2$
Hz), 1.25 (br m, 26H), 1.75 (quint, 2H, $J = 7.2$ Hz), 2.29 (s, 6H), 2.47 (s, 3H),
2.76 (dd, 2H, $J = 8.0$ Hz and 8.0 Hz), 4.89 (s, 2H), 5.85 (s, 2H) and 7.35 (m, 5H);
$^{13}$C NMR (CDCl$_3$) $\delta$ 14.1, 14.30, 14.30, 19.3, 22.7, 27.7, 29.3, 29.4, 29.50, 29.55,
29.63, 29.67, 31.6, 31.9, 75.7, 107.9, 128.0, 128.5, 128.7, 129.3, 136.2, 147.4,
152.6, 161.6 and 165.1; mass spectrum (APCI), $m/z$ 518.4113 (M+H)$^+$
(C$_{34}$H$_{52}$N$_3$O requires 518.4110).

![](image.png)

**2-Amino-5-(benzylxoy)-4-methyl-6-pentylpyrimidine (3.70).** To a stirred
solution containing 533 mg (1.46 mmol) of compound **3.67** in 10 mL of 9:1
ethanol–water was added 1.00 g (14.6 mmol) of hydroxylamine hydrochloride.
The reaction mixture was then stirred at reflux during 5 h. A second portion of 1.0
g (14.6 mmol) of hydroxylamine hydrochloride was added and the reaction
mixture was stirred at reflux for 16 h. The reaction mixture was then poured into
70 mL of water and adjusted to pH 9-10 with 1 N aq NaOH. The mixture was
extracted with two 70-mL portions of ethyl acetate. The combined organic layer
was washed with 70 mL of brine, dried (MgSO$_4$) and concentrated under
diminished pressure. The residue was purified by chromatography on a silica gel
column (10 × 3 cm). Elution with 2:1 hexanes–ethyl acetate afforded compound
3.70 as a colorless oil: yield 334 mg (80 %); silica gel TLC $R_f$ 0.25 (2:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $\delta$ 0.86 (t, 3H, $J = 6.8$ Hz), 1.27 (m, 4H), 1.63 (m, 2H), 2.28 (s, 3H), 2.56 (dd, 2H, $J = 8.0$ Hz and $8.0$ Hz), 4.71 (s, 2H), 5.15 (brs, 2H) and 7.36 (m, 5H); $^{13}$C NMR (CDCl$_3$) $\delta$ 13.9, 18.9, 22.4, 28.0, 31.9, 75.9, 127.9, 128.3, 128.6, 136.8, 142.8, 158.8, 161.1 and 164.9; mass spectrum (APCI), $m/z$ 286.1914 (M+H)$^+$ (C$_{17}$H$_{23}$N$_3$O requires 286.1919).

![Chemical Structure](image)

2-Amino-5-(benzylcyloxy)-4-decyl-6-methylpyrimidine (3.71). To a stirred solution containing 502 mg (1.15 mmol) of compound 3.68 in 10 mL of 9:1 ethanol–water was added 800 mg (11.5 mmol) of hydroxylamine hydrochloride. The reaction mixture was stirred at reflux for 5 h. A second portion of 800 mg (11.5 mmol) of hydroxylamine hydrochloride was added and the reaction mixture was stirred at reflux for 16 h. The cooled reaction mixture was then poured into 70 mL of water and then adjusted to pH 9-10 with 1 N aq NaOH. The mixture was extracted with two 70-mL portions of ethyl acetate. The combined organic layer was washed with 70 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 $\times$ 3 cm). Elution with 3:2 hexanes–ethyl acetate afforded compound 3.71 as a colorless oil: yield 305 mg (75%); silica gel TLC $R_f$ 0.25 (3:2 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $\delta$ 0.85 (t, 3H, $J = 6.4$ Hz), 1.26 (m, 14H), 1.61
(m, 2H), 2.27 (s, 3H), 2.56 (dd, 2H, J = 8.0 Hz and 8.0 Hz), 4.71 (s, 2H), 5.24 (br s, 2H) and 7.36 (m, 5H); $^{13}$C NMR (CDCl$_3$) δ 13.9, 18.9, 22.6, 28.4, 29.3, 29.4, 29.5, 29.6, 29.7, 31.87, 31.88, 75.9, 127.9, 128.2, 128.6, 136.8, 142.8, 158.9, 161.1 and 162.8; mass spectrum (APCI), m/z 356.2704 (M+H$^+$) (C$_{22}$H$_{34}$N$_3$O requires 356.2702).

![Structure of 2-Amino-5-(benzyloxy)-4-hexadecyl-6-methylpyrimidine](image)

**2-Amino-5-(benzyloxy)-4-hexadecyl-6-methylpyrimidine (3.72).** To a stirred solution containing 269 mg (0.52 mmol) of compound 3.69 in 10 mL of ethanol was added 723 mg (10.4 mmol) of hydroxylamine hydrochloride. The reaction mixture was stirred at reflux for 5 h. A second portion of 723 mg (10.4 mmol) of hydroxylamine hydrochloride was added and the reaction mixture was stirred at reflux for 16 h. The reaction mixture was then poured into 70 mL of water and then adjusted to pH 9-10 with 1 N aq NaOH. The mixture was extracted with two 70-mL portions of ethyl acetate. The combined organic layer was washed with 70 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 3:2 hexanes–ethyl acetate afforded compound 3.72 as a colorless oil: yield 231 mg (100%); silica gel TLC $R_f$ 0.25 (1:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) δ 0.85 (t, 3H, J = 6.8 Hz), 1.26 (m, 26H), 1.61 (quint, 2H, J = 7.2 Hz), 2.28 (s, 3H), 2.56 (dd, 2H, J = 7.6 Hz and 7.6 Hz), 4.72 (s, 2H), 5.03 (br s,
2H) and 7.37 (m, 5H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.1, 18.9, 22.7, 28.4, 29.3, 29.4, 29.5, 29.62, 29.63, 29.67, 29.73, 31.9, 75.9, 127.9, 128.2, 128.6, 136.8, 142.9, 158.8, 161.1 and 164.9; mass spectrum (APCI), $m/z$ 440.3646 (M+H)$^+$ (C$_{28}$H$_{46}$N$_3$O requires 440.3641).

![Chemical structure](image)

5-(Benzyloxy)-2-(N,N-dimethylamino)-4-methyl-6-pentylpyrimidine (3.73).

To a stirred solution containing 334 mg (1.17 mmol) of compound 3.71 in 4 mL of methanol was added 4 mL of 35% aq formaldehyde followed by 588 mg (9.35 mmol) of NaCNBH$_3$. The reaction mixture was stirred at 23 °C for 3 h. The reaction mixture was quenched with acetic acid until bubbling ceased then poured into 20 mL of water and extracted with two 40-mL portions of ethyl acetate. The combined organic layer was washed with 40 mL of satd aq NaHCO$_3$ and 40 mL of brine. The organic solution was dried (MgSO$_4$) and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (8 × 3 cm). Elution with 2:1 hexanes–ethyl acetate afforded compound 3.73 as colorless oil: yield 150 mg (41%); silica gel TLC $R_f$ 0.7 (2:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $\delta$ 0.88 (t, 3H, $J = 7.2$ Hz), 1.33 (m, 4H), 1.69 (quint, 2H, $J = 7.6$ Hz), 2.32 (s, 3H), 2.62 (dd, 2H, $J = 8.0$ Hz and 8.0 Hz), 3.15 (s, 6H), 4.70 (s, 2H) and 7.37 (m, 5H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.0, 19.3, 22.5, 27.5,
5-(Benzyloxy)-4-decyl-2-(N,N-dimethylamino)-6-methylpyrimidine (3.74). To a stirred solution containing 305 mg (0.86 mmol) of compound 3.71 in 3 mL of methanol was added 3 mL of 35% aq formaldehyde, followed by 271 mg (4.30 mmol) of NaCNBH₃. The reaction mixture was stirred at 23 °C for 3 h. The reaction was quenched with acetic acid until bubbling ceased, then poured into 20 mL of water and extracted with two 40-mL portions of ethyl acetate. The combined organic layer was washed with 40 mL of satd aq NaHCO₃ and 40 mL of brine. The organic solution was dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (8 × 3 cm). Elution with 2:1 hexanes–ethyl acetate afforded compound 3.74 as a colorless oil: yield 154 mg (46%); silica gel TLC Rf 0.8 (2:1 hexanes–ethyl acetate); ¹H NMR (CDCl₃) δ 0.87 (t, 3H, J = 6.4 Hz), 1.33 (m, 14H), 1.69 (quint, 2H, J = 7.2 Hz), 2.32 (s, 3H), 2.61 (dd, 2H, J = 7.6 Hz and 7.6 Hz), 3.14 (s, 6H), 4.70 (s, 2H) and 7.37 (m, 5H); ¹³C NMR (CDCl₃) δ 14.1, 19.3, 22.7, 27.9, 29.3, 29.50, 29.55, 29.59, 29.6, 31.7, 31.9, 37.2, 75.7, 127.9, 128.1, 128.5, 137.2, 141.3, 158.6, 159.0 and 163.5; mass spectrum (APCI), m/z 384.3003 (M+H)⁺ (C₂₄H₃₈N₃O requires 384.3015).
5-(Benzyloxy)-2-(N,N-dimethylamino)-4-hexadecyl-6-methylpyrimidine (3.75). To a stirred solution containing 230 mg (0.52 mmol) of compound 3.72 in 4 mL of methanol was added 4 mL of 35% aq formaldehyde, followed by 263 mg (4.18 mmol) of NaCNBH₃. The reaction mixture was stirred at 23 °C for 3 h. The reaction was quenched with acetic acid until bubbling ceased, then poured into 20 mL of water and extracted with two 40-mL portions of ethyl acetate. The combined organic layer was washed with 40 mL of satd aq NaHCO₃ and 40 mL of brine. The organic solution was dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (8 × 3 cm). Elution with 4:1 hexanes–ethyl acetate afforded compound 3.75 as a colorless oil: yield 100 mg (41%); silica gel TLC Rₜ 0.75 (4:1 hexanes–ethyl acetate); ¹H NMR (CDCl₃) δ 0.86 (t, 3H, J = 6.8 Hz), 1.25 (br m, 26H), 1.68 (quint, 2H, J = 6.8 Hz), 2.31 (s, 3H), 2.61 (dd, 2H, J = 7.6 Hz and 7.6 Hz), 3.14 (s, 6H), 4.69 (s, 2H) and 7.43 (m, 5H); ¹³C NMR (CDCl₃) δ 14.1, 19.3, 22.6, 27.8, 29.3, 29.50, 29.55, 29.62, 29.64, 29.66, 31.7, 31.9, 37.2, 75.7, 127.9, 128.2, 128.5, 137.2, 141.3, 158.6, 159.0 and 163.6; mass spectrum (APCI), m/z 468.3950 (M+H)⁺ (C₃₀H₅₀N₃O requires 468.3954).
2-(N,N-Dimethylamino)-4-methyl-6-pentylpyrimidin-5-ol (3.16). To a stirred solution containing 150 mg (0.48 mmol) of compound 3.73 in 5 mL of methanol was added 5 mg of 20% palladium hydroxide-on-carbon (Degussa typy E101 NE/E). The reaction mixture was stirred at 23 °C under hydrogen atmosphere for 15 min. The reaction mixture was filtered through Celite® and the filtrate was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (8 × 3 cm). Elution with 2:1 hexane–ethyl acetate afforded compound 3.16 as a colorless solid: yield 60 mg (56%); mp 61-62 °C; silica gel TLC Rf 0.50 (2:1 hexanes–ethyl acetate); \(^{1}\)H NMR (CD\(_3\)OD) δ 0.89 (t, 3H, \(J = 5.6\) Hz), 1.33 (m, 4H), 1.66 (quint, 2H, \(J = 7.2\) Hz), 2.26 (s, 3H), 2.62 (dd, 2H, \(J = 7.6\) Hz and 7.6 Hz) and 3.05 (s, 6H); \(^{13}\)C NMR (CD\(_3\)OD) δ 12.9, 17.5, 22.3, 27.0, 31.2, 31.4, 36.5, 138.0, 155.6, 157.3 and 159.4; mass spectrum (APCI), \(m/z\) 224.1769 (M+H)\(^+\) (C\(_{12}\)H\(_{22}\)N\(_3\)O requires 224.1763).

4-Decyl-2-(N,N-dimethylamino)-6-methylpyrimidin-5-ol (3.17). To a stirred solution containing 150 mg (0.48 mmol) of compound 3.74 in 5 mL of methanol
was added 5 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/E). The reaction mixture was stirred at 23 °C under a hydrogen atmosphere for 15 min. The reaction mixture was filtered through Celite® and the filtrate was concentrated under diminished pressure to afford compound 3.17 as a colorless solid: yield 140 mg (100%). An analytical sample was obtained by chromatography on a silica gel column (8 × 1 cm). Elution with 4:1 hexanes–ethyl acetate afforded compound 3.17 as a colorless solid; mp 71-72 °C; silica gel TLC $R_f$ 0.25 (4:1 hexanes–ethyl acetate); $^1$H NMR (methanol-$d_4$) $\delta$ 0.87 (t, 3H, $J$ = 6.8 Hz), 1.26 (m, 14H), 1.65 (quint, 2H, $J$ = 7.2 Hz), 2.26 (s, 3H), 2.62 (dd, 2H, $J$ = 7.2 Hz and 7.2 Hz) and 3.05 (s, 6H); $^{13}$C NMR (methanol-$d_4$) $\delta$ 13.0, 17.6, 22.3, 27.3, 29.0, 29.15, 29.17, 29.26, 29.29, 31.2, 31.6, 36.52, 36.52, 138.0, 155.6, 157.3 and 159.4; mass spectrum (APCI), $m/z$ 294.2554 (M+H)$^+$ (C$_{17}$H$_{32}$N$_3$O requires 294.2545).

2-(N,N-Dimethylamino)-4-hexadecyl-6-methylpyrimidin-5-ol (3.18). To a stirred solution containing 100 mg (0.21 mmol) of compound 3.75 in 5 mL of methanol was added 5 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/E). The reaction mixture was stirred at 23 °C under a hydrogen atmosphere for 15 min. The reaction mixture was filtered through Celite and the filtrate was concentrated under diminished pressure to afford compound 3.18 as a
colorless solid: yield 79 mg (100%). An analytical sample was obtained by chromatography on a silica gel column (8 × 1 cm). Elution with 4:1 hexanes–ethyl acetate afforded compound 3.18 as a colorless solid; mp 86-87 °C; silica gel TLC $R_f$ 0.45 (4:1 hexanes–ethyl acetate); $^1$H NMR (methanol-$d_4$) δ 0.89 (t, 3H, $J = 6.8$ Hz), 1.30 (m, 26H), 1.67 (quint, 2H, $J = 7.2$ Hz), 2.28 (s, 3H), 2.63 (dd, 2H, $J = 7.6$ Hz and 7.6 Hz) and 3.07 (s, 6H); $^{13}$C NMR (methanol-$d_4$) δ 13.0, 17.5, 22.3, 27.2, 29.0, 29.10, 29.12, 29.20, 29.29, 29.30, 31.2, 31.6, 36.50, 138.0, 155.6, 157.3 and 159.4; mass spectrum (APCI), $m/z$ 378.3491 (M+H)$^+$ (C$_{23}$H$_{44}$N$_3$O requires 378.3484).

![Chemical Structure](image)

4-Decyl-2-(N,N-dimethylamino)-6-methoxypyrimidine (3.76). To a stirred solution containing 1.06 g (6.34 mmol) of compound 3.63 and 1.22 mL (1.32 g; 6.34 mmol) of 1-bromononane in 20 mL of anh THF was added 5.15 mL (8.34 mmol) of 1.6 M $n$-BuLi in hexanes. The reaction mixture was stirred at 23 °C for 20 min. The reaction mixture was quenched with satd aq ammonium chloride and poured into 100 mL of water. The solution was extracted with two 80-mL portions of ethyl acetate. The combined organic layer was washed with 80 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 9:1 hexanes–ethyl acetate afforded compound 3.76 as a colorless oil: yield 1.22 g (66%); silica gel TLC $R_f$ 0.7 (9:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) δ 0.85
(t, 3H, $J = 6.8$ Hz), 1.25 (br m, 14H), 1.82 (quint, 2H, $J = 7.2$ Hz), 2.46 (dd, 2H, $J = 7.6$ Hz and 7.6 Hz), 3.14 (s, 6H), 3.85 (s, 3H) and 5.77 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.1, 22.7, 28.5, 29.31, 29.32, 29.48, 29.54, 29.56, 31.9, 36.8, 37.8, 52.8, 93.0, 162.3, 170.3 and 173.9; mass spectrum (APCI), $m/z$ 294.2541 (M+H)$^+$ (C$_{17}$H$_{32}$N$_3$O requires 245.2545).

2-(N,N-Dimethylamino)-4-hexadecyl-6-methoxypyrimidine (3.77). To a stirred solution containing 1.12 g (6.70 mmol) of compound 3.63 and 2.61 mL (2.62 g; 6.70 mmol) of 1-pentadecane in 20 mL of anh THF was added 6.28 mL (10.1 mmol) of 1.6 M $n$-BuLi in hexanes. The reaction mixture was stirred for 20 min at 23 °C. The reaction mixture was quenched with satd aq ammonium chloride and poured into 100 mL of water. The compound was extracted with two 80-mL portions of ethyl acetate. The combined organic layer was washed with 80 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 9:1 hexanes–ethyl acetate afforded compound 3.77 as a colorless solid: yield 1.58g (62%); mp 49-50 °C; silica gel TLC $R_f$ 0.5 (9:1 hexanes–ethyl ether); $^1$H NMR (CDCl$_3$) $\delta$ 0.86 (t, 3H, $J = 6.8$ Hz), 1.25 (br m, 26H), 1.63 (quint, 2H, $J = 7.2$ Hz), 2.46 (dd, 2H, $J = 7.6$ Hz and 7.6 Hz), 3.14 (s, 6H), 3.85 (s, 3H) and 5.77 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.1, 22.7, 28.5, 29.3, 29.5, 29.55, 29.64, 29.7, 31.9,
36.8, 37.8, 52.7, 93.0, 162.3, 179.3 and 171.9; mass spectrum (APCI), \( m/z \) 378.3483 (M+H)\(^+\) (C\(_{23}\)H\(_{44}\)N\(_3\)O requires 378.3484).

![Chemical Structure](image)

**5-Bromo-4-decyl-2-(N,N-dimethylamino)-6-methoxypyrimidine (3.78).** To a stirred solution containing 1.22 g (4.16 mmol) of compound 3.76 in 20 mL of acetonitrile was added 808 mg (4.54 mmol) of N-bromosuccinimide. The reaction mixture was stirred at 23 °C and protected from light for 5 h. The reaction mixture was concentrated under diminished pressure and the residue was dissolved in 100 mL of ethyl acetate. The organic solution was washed with 100 mL of brine, dried (MgSO\(_4\)) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 9:1 hexanes–ethyl ether afforded compound 3.78 as white solid: yield 1.27 g (82%); mp 45-46 °C; silica gel TLC \( R_f \) 0.75 (9:1 hexanes–ethyl ether); \(^1\)H NMR (CDCl\(_3\)) \( \delta \) 0.86 (t, 3H, \( J = 6.8 \) Hz), 1.28 (br m, 14H), 1.65 (quint, 2H, \( J = 7.2 \) Hz), 2.68 (dd, 2H, \( J = 7.6 \) Hz and 7.6 Hz), 3.12 (s, 6H) and 3.92 (s, 3H); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 14.1, 22.7, 27.6, 29.3, 29.4, 29.5, 29.57, 29.60, 31.9, 36.7, 36.9, 36.9, 54.0, 91.2, 160.1, 165.1 and 169.05; mass spectrum (APCI), \( m/z \) 372.1654 (M+H)\(^+\) (C\(_{17}\)H\(_{31}\)N\(_3\)OBr requires 372.1650).

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5-Bromo-2-(N,N-dimethylamino)-4-hexadecyl-6-methoxypyrimidine (3.79).

To a stirred solution containing 1.56 g (4.13 mmol) of compound 3.77 in 30 mL of acetonitrile was added 888 mg (4.99 mmol) of N-bromosuccinimide. The reaction mixture was stirred at 23 °C for 5 h and protected from light. The reaction mixture was concentrated under diminished pressure and the residue was dissolved in 100 mL of ethyl acetate. The organic solution was washed with 100 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm).

Elution with 9:1 hexanes–ethyl ether afforded compound 3.79 as colorless solid:
yield 1.80 g (95%); mp 66–67 °C; silica gel TLC Rᵣ 0.60 (9:1 hexanes–ethyl ether); ¹H NMR (CDCl₃) δ 0.86 (t, 3H, J = 6.8 Hz), 1.23 (br m, 26H), 1.65 (quint, 2H, J = 7.2 Hz), 2.68 (dd, 2H, J = 7.6 Hz and 7.6 Hz), 3.12 (s, 6H) and 3.93 (s, 3H); ¹³C NMR (CDCl₃) δ 14.1, 26.7, 27.6, 29.3, 39.4, 29.5, 29.57, 29.63, 29.65, 29.68, 31.9, 36.7, 37.0, 54.0, 91.2, 160.1, 165.1 and 169.1; mass spectrum (APCI), m/z 456.2579 (M+H)⁺ (C₂₃H₄₃N₅OBr requires 456.2589).
4-Decyl-2-(N,N-dimethylamino)-6-methoxypyrimidin-5-ol (3.19). To a stirred solution at \(-5\, ^\circ\text{C}\) containing 500 mg (1.34 mmol) of compound 3.78 in 10 mL of anh THF was added 202 \(\mu\text{L}\) (156 mg; 1.34 mmol) of TMEDA followed by 2.09 mL (3.35 mmol) of a 1.6 M solution of \(n\)-BuLi in hexanes. The reaction mixture was stirred at \(-5\, ^\circ\text{C}\) for 20 min. To the mixture was added 440 \(\mu\text{L}\) (410 mg; 3.94 mmol) of trimethyl borate and the reaction mixture was stirred at 23 \(^\circ\text{C}\) for 1 h. To reaction mixture was added 3.46 mL of 30 \% aq \(\text{H}_2\text{O}_2\). The reaction mixture was then stirred for 30 min and poured into 100 mL of water. The mixture was neutralized with dilute aq HCl and extracted with two 100-mL portions of ethyl acetate. The combined organic solution was washed with 100 mL of brine, dried (MgSO\(_4\)) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 \(\times\) 3 cm). Elution with 4:1 hexanes–ethyl acetate afforded compound 3.20 as white solid: yield 111 mg (27\%); mp 59-60 \(^\circ\text{C}\); silica gel TLC \(R_f\) 0.60 (4:1 hexanes–ethyl acetate); \(^1\)H NMR (CD\(_3\)OD) \(\delta\) 0.87 (t, 3H, \(J = 6.4\) Hz), 1.27 (br m, 14H), 1.62 (quint, 2H, \(J = 7.2\) Hz), 2.58 (dd, 2H, \(J = 7.6\) Hz and 7.6 Hz), 3.09 (s, 6H) and 3.91 (s, 3H); \(^13\)C NMR (CD\(_3\)OD) \(\delta\) 13.1, 22.3, 27.6, 29.1, 29.15, 29.17, 29.29, 29.31, 30.6, 31.7, 36.3, 52.2, 127.0, 155.9, 155.9 and 159.5; mass spectrum (APCI), \(m/z\) 310.2500 (M+H)\(^+\) (C\(_{17}\)H\(_{32}\)N\(_3\)O\(_2\) requires 310.2495).
2-(N,N-Dimethylamino)-4-hexadecyl-6-methoxypyrimidin-5-ol (3.20). To a stirred solution at −5 °C containing 500 mg (1.10 mmol) of compound 3.79 in 10 mL of anh THF was added 166 µL (128 mg; 1.10 mmol) of TMEDA followed by 1.72 mL (2.75 mmol) of 1.6 M solution of n-BuLi in hexanes. The reaction mixture was stirred at −5 °C for 20 min. Then to the mixture was added 369 µL (344 mg; 3.31 mmol) of trimethyl borate and the reaction mixture was stirred at 23 °C for 1 h. Then to reaction mixture was added 2.84 mL of 30 % aq H₂O₂. The reaction mixture was then stirred for 30 min and poured into 100 mL of water. The mixture was neutralized with dilute aq HCl and extracted with two 100-mL portions of ethyl acetate. The combined organic solution was washed with 100 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 4:1 hexanes–ethyl acetate afforded compound 3.20 as a colorless solid: yield 240 mg (55%); mp 78-79 °C; silica gel TLC Rf 0.50 (4:1 hexanes–ethyl acetate); ¹H NMR (DMSO-d₆) δ 0.81 (t, 3H, J = 6.0 Hz), 1.21 (br m, 26H), 1.52 (quint, 2H, J = 6.8 Hz), 2.46 (dd, 2H, J = 7.2 Hz and 7.2 Hz), 2.97 (s, 6H), 3.82 (s, 3H) and 7.77 (s, 1H); ¹³C NMR (DMSO-d₆) δ 14.4, 22.5, 27.7, 29.1, 29.27, 29.32, 29.38, 29.43, 29.5, 31.2, 31.7, 37.3, 53.1, 127.1, 155.5, 156.8 and 159.7; mass spectrum (APCI), m/z 394.3430 (M+H)⁺ (C₂₅H₄₄N₃O₂ requires 394.3434).
5-(Acetoxy)-2-(N,N-dimethylamino)-4-methoxy-6-hexadecylpyrimidine (3.21): To a stirred solution containing 15.0 mg (0.04 mmol) of compound 3.20 in 1 mL of anh DMF was added 11.0 mg (0.08 mmol) of potassium carbonate followed by 5.00 µL (5.40 mg: 0.05 mmol) of acetic anhydride. The reaction mixture was stirred at 23 °C for 1 h. The reaction mixture was poured into 10 mL of diethyl ether and washed with 10 mL of satd aq sodium bicarbonate, 10 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 9:1 hexanes–diethyl ether compound 3.21 product as colorless solid: yield 94.0 mg (80%); mp 45-46 °C; silica gel TLC $R_f$ 0.60 (9:1 hexanes–diethyl ether); $^1$H NMR (CDCl₃) δ 0.87 (t, 3H, $J = 6.4$ Hz), 1.25 (br m, 26H), 1.65 (quint, 2H, $J = 6.8$ Hz), 2.27 (s, 3H), 2.42 (dd, 2H, $J = 7.6$ Hz and 7.6 Hz), 3.12 (s, 6H) and 3.89 (s, 3H); $^{13}$C NMR (CDCl₃) δ 14.1, 20.4, 22.7, 27.6, 29.3, 29.40, 29.42, 29.5, 29.6, 29.66, 29.67, 31.6, 31.9, 37.0, 53.3, 121.8, 159.0, 161.2, 162.1 and 169.1; mass spectrum (APCI), m/z 436.3539 (M+H)$^+$ (C$_{25}$H$_{46}$N$_3$O$_2$ requires 436.3531).
2-((N,N-Dimethylamino)-4-(γ-butenyl)-6-methoxypyrimidine (3.80). To a stirred solution containing 1.42 g (8.49 mmol) of compound 3.63 in 80 mL of anh THF at \(-78^\circ\text{C}\) was added 1.36 mL (1.05 g; 9.07 mmol) of TMEDA followed by 5.1 mL (12.7 mmol) of a 2.5 M solution of \(n\)-BuLi in hexanes. The reaction mixture was stirred at \(-78^\circ\text{C}\) for 15 min then was added 1.10 mL (1.54 g; 12.7 mmol) of allyl bromide. The reaction mixture was stirred at \(-78^\circ\text{C}\) for 1 h. The reaction mixture was quenched with satd aq ammonium chloride and poured into 100 mL of water. The compound was extracted with two 80-mL portions of diethyl ether. The combined organic layer was washed with 80 mL of brine, dried (MgSO\(_4\)) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 \(\times\) 5 cm). Elution with 4:1 hexanes–diethyl ether afforded compound 3.80 as colorless oil: yield 878 mg (50%); silica gel TLC \(R_f\) 0.50 (4:1 hexanes–diethyl ether); \(^1\text{H NMR (CDCl}_3\) \(\delta\) 2.44 (dt, 2H, \(J = 14.0\) Hz and 6.9 Hz), 2.59 (dd, 2H, \(J = 8.8\) Hz and 6.6 Hz), 3.15 (s, 6H), 3.86 (s, 3H), 4.94 (m, 1H), 5.05 (ddd, 1H, \(J = 17.1\) Hz, 3.2 Hz and 1.6 Hz), 4.79 (s, 1H) and 5.86 (ddt, 1H, \(J = 16.8\) Hz, 10.2 Hz and 6.5 Hz); \(^{13}\text{C NMR (CDCl}_3\) \(\delta\) 32.5, 36.9, 37.1, 52.8, 93.4, 114.8, 138.2, 162.4, 170.4 and 170.8; mass spectrum (FAB), \(m/z\) 208.1449 (M+H\(^+\)) (C\(_{11}\)H\(_{18}\)N\(_3\)O requires 208.1450).
**5-Bromo-2-(N,N-dimethylamino)-4-(γ-butenyl)-6-methoxypyrimidine (3.81).**

To a stirred solution containing 1.15 g (5.54 mmol) of compound 3.80 in 30 mL of acetonitrile was added 986 mg (5.54 mmol) of N-bromosuccinimide. The reaction mixture was stirred at 23 °C and protected from light for 2 h. The reaction mixture was concentrated under diminished pressure and the residue was dissolved into 100 mL of ethyl acetate. The organic solution was washed with 100 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (15 × 5 cm). Elution with 4:1 hexanes–diethyl ether afforded compound 3.81 as a colorless oil: yield 1.45 g (92%); silica gel TLC Rf 0.55 (4:1 hexanes–diethyl ether); ¹H NMR (CDCl₃) δ 2.46 (m, 2H), 2.81 (dd, 2H, J = 8.8 Hz and 6.7 Hz), 3.16 (s, 6H), 3.95 (s, 3H), 4.97 (dddt, 1H, J = 11.5 Hz, 10.2 Hz, 2.0 Hz and 1.4 Hz), 5.05 (ddd, 1H, J = 17.1 Hz, 3.2 Hz and 1.6 Hz) and 5.90 (ddt, 1H, J = 16.8 Hz, 10.2 Hz and 6.5 Hz); ¹³C NMR (CDCl₃) δ 31.6, 36.1, 37.1, 54.2, 91.4, 114.9, 138.2, 160.2, 165.3 and 168.0; mass spectrum (EI), m/z 285.0481 (M)⁺ (C₁₁H₁₆N₃OBr requires 285.0477).

![Chemical Structure](image)

**2-(N,N-dimethylamino)-4-(γ-butenyl)-6-methoxypyrimidin-5-ol (3.82).** To a stirred solution at –78 °C containing 1.45 g (5.07 mmol) of compound 3.81 in 50 mL of anh THF was added 830 µL (643 mg; 5.53 mmol) of TMEDA followed by...
3.04 mL (7.61 mmol) of a 2.5 M solution of \(n\)-BuLi in hexanes. The reaction mixture was stirred at \(-78^\circ\text{C}\) for 40 min. To the mixture was added 845 \(\mu\text{L}\) (787 mg; 7.57 mmol) of trimethyl borate and the reaction mixture was stirred for 30 min. The reaction mixture was cooled to \(-78^\circ\text{C}\) and 740 \(\mu\text{L}\) of 30\% aq \(\text{H}_2\text{O}_2\) was added. The reaction mixture was stirred for 30 min at \(-78^\circ\text{C}\) and then poured into 50 mL of water. The mixture was extracted with two 50-mL portions of ethyl acetate. The combined organic solution was washed with 80 mL of brine, dried (\(\text{MgSO}_4\)) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 \(\times\) 5 cm). Elution with 1:1 hexanes–diethyl ether afforded compound 3.82 as a colorless solid: yield 790 mg (70\%); mp 59-61\(^\circ\text{C}\); silica gel TLC \(R_f\) 0.50 (1:1 hexanes–diethyl ether); \(^1\text{H}\) NMR (\(\text{CDCl}_3\)) \(\delta\) 2.46 (dt, 2H, \(J = 14.0\) Hz and 7.0 Hz), 2.81 (t, 2H, \(J = 7.7\) Hz), 3.09 (s, 6H), 3.93 (s, 3H), 4.60 (br s, 1H), 4.87 (ddd, 1H, \(J = 10.2\) Hz, 3.3 Hz and 1.3 Hz), 4.98 (ddd, 1H, \(J = 17.2\) Hz, 3.6 Hz and 1.6 Hz) and 5.83 (ddt, 1H, \(J = 16.8\) Hz, 10.2 Hz and 6.5 Hz); \(^{13}\text{C}\) NMR (\(\text{CDCl}_3\)) \(\delta\) 30.6, 36.7, 37.4, 53.4, 114.6, 127.1, 138.6, 154.0, 156.1 and 158.3; mass spectrum (APCI), \(m/z\) 224.1393 (M+H\(^+\)) (\(\text{C}_{11}\text{H}_{18}\text{N}_3\text{O}_2\) requires 224.1399).

![Structure of 5-Benzyloxy-2-(N,N-dimethylamino)-4-(\(\gamma\)-butenyl)-6-methoxypyrimidine (3.83)](image)

**5-Benzyloxy-2-(N,N-dimethylamino)-4-(\(\gamma\)-butenyl)-6-methoxypyrimidine (3.83).** To a stirred solution containing 790 mg (3.54 mmol) of compound 3.82 in
15 mL of anh DMF was added 1.47 g (10.6 mmol) of potassium carbonate followed by 631 µL (907 mg; 5.31 mmol) of benzyl bromide. The reaction mixture was stirred at room temperature for 3h. The reaction mixture was poured into 50 mL of satd aq sodium bicarbonate and extracted with two 50-mL portions of ethyl acetate. The combined organic solution was washed with 80 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 5 cm). Elution with 9:1 hexanes–diethyl ether gave compound **8.83** as yellowish oil: yield 1.09 g (98%); silica gel TLC *R*ₚ 0.50 (9:1 hexanes–diethyl ether); ¹H NMR (CDCl₃) δ 2.37 (dt, 2H, *J* = 14.1 Hz and 7.0 Hz), 2.62 (m, 2H), 3.14 (s, 6H), 3.97 (s, 3H), 4.83 (s, 2H), 4.93 (ddt, 1H, *J* = 10.2 Hz, 2.2 Hz and 1.2 Hz), 5.01 (ddd, 1H, *J* = 17.1 Hz, 3.6 Hz and 1.6 Hz), 5.85 (ddt, 1H, *J* = 16.8 Hz, 10.2 Hz and 6.5 Hz) and 7.43-7.31 (m, 5H); ¹³C NMR (CDCl₃) δ 30.6, 31.9, 37.1, 53.1, 75.1, 114.5, 128.0, 128.4, 129.1, 137.4, 138.5, 157.6, 161.0, 161.8 and 162.5; mass spectrum (APCI), *m/z* 314.1875 (M+H)⁺ (C₁₈H₂₄N₃O₂ requires 314.1869).

**8-Methyladamantylxyoct-1-ene (3.84).** To a stirred solution containing 362 mg (2.18 mmol) of 1-adamantane methanol and 438 µL (499 mg; 2.61 mmol) of 8-bromo-1-octene in 10 mL of anh DMF at 0 °C was added 174 mg (4.36 mmol) of sodium hydride as a 60% suspension in mineral oil. The reaction mixture was
stirred at room temperature for 16 h. The reaction mixture was poured into 50 mL of satd aq sodium bicarbonate and extracted with two 50-mL portions of ethyl acetate. The combined organic solution was washed with 80 mL of brine, dried (MgSO$_4$) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 95:5 hexanes–ethyl acetate gave compound 8.84 as yellowish oil: yield 350 mg (58%); silica gel TLC $R_f$ 0.50 (95:5 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) $d$ 1.31 (m, 6H), 1.56 (m, 8H), 1.65 (dt, 6H, $J$ = 24.6 Hz and 7.9 Hz), 1.95 (s, 3H), 2.05 (dd, 2H, $J$ = 14.1 Hz and 6.9 Hz), 2.95 (s, 2H), 3.37 (t, 2H, $J$ = 6.6 Hz), 4.98 (ddd, 2H, $J$ = 20.4 Hz, 11.0 Hz and 5.9 Hz) and 5.81 (ddt, 1H, $J$ = 17.0 Hz, 10.2 Hz and 6.7 Hz); $^{13}$C NMR (CDCl$_3$) $d$ 26.2, 28.5, 29.0, 29.1, 29.7, 33.9, 37.4, 39.9, 71.8, 82.0, 114.3 and 139.3; mass spectrum (EI), $m/z$ 276.2450 (M$^+$) (C$_{19}$H$_{32}$O requires 276.2453)

![Chemical structure of 2-(N,N-Dimethylamino)-4-methoxy-6-tetradecylpyrimidin-5-ol (3.22).](image)

2-(N,N-Dimethylamino)-4-methoxy-6-tetradecylpyrimidin-5-ol (3.22). To a stirred solution containing 100 mg (0.32 mmol) of compound 3.83 and 142 µL (108 mg; 0.64 mmol) of 1-dodecene in 2.00 mL of anh CH$_2$Cl$_2$ was added 27 mg (16 µmol) of Grubb’s 2$^{nd}$ generation catalyst. The reaction mixture was stirred at reflux for 16 h. The reaction mixture was concentrated under diminished pressure

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and the residue was partially purified on a short silica gel pad. The crude mixture was concentrated under diminished pressure and the residue was dissolved in 2 mL of methanol. Then 3 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/N) was added and the reaction mixture was stirred at 23 °C for 15 min under a hydrogen atmosphere. The reaction mixture was filtered through Celite® and the filtrate was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 2 cm). Elution with 4:1 hexanes–diethyl ether gave compound 3.22 as a colorless solid: yield 47 mg (40%); mp 70–71 °C; silica gel TLC Rf 0.40 (4:1 hexanes–diethyl ether); 1H NMR (CDCl3) δ 0.88 (t, 3H, J = 6.7 Hz), 1.30 (br m, 22H), 1.65 (m, 2H), 2.60 (m, 2H), 3.10 (s, 6H) and 3.95 (s, 3H); 13C NMR (CDCl3) δ 14.3, 22.9, 27.9, 29.5, 29.68, 29.70 29.8, 29.9, 31.4, 32.0, 37.4, 53.5, 127.1, 155.0, 156.1 and 158.2; mass spectrum (APCI), m/z 366.3111 (M+H)+ (C21H40N3O2 requires 366.3121).

[Chemical structure image]

2-(N,N-Dimethylamino)-4-methoxy-6-(10-methyladamantyloxydecyl)pyrimidin-5-ol (3.23). To a stirred solution containing 50.0 mg (0.16 mmol) of compound 3.83 and 88 mg (0.32 mmol) of compound 3.84 in 1.00 mL of anh CH2Cl2 was added 14 mg (16 µmol) of
Grubb’s 2nd generation catalyst. The reaction mixture was stirred at reflux for 16 h. The reaction mixture was concentrated under diminished pressure and the residue was partially purified on a short silica gel pad. The crude mixture was concentrated under diminished pressure and the residue was dissolved in 1 mL of methanol. Then 3 mg of 20% palladium hydroxide-on-carbon (Degussa type E101 NE/N) was added and the reaction mixture was stirred at 23 °C for 15 min under a hydrogen atmosphere. The reaction mixture was filtered through Celite® and the filtrated was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 4:1 hexanes–diethyl ether gave compound 3.23 as yellowish oil: yield 8.00 mg (11%); silica gel TLC $R_f$ 0.20 (4:1 hexanes–diethyl ether); $^1$H NMR (CDCl$_3$) $\delta$ 1.30 (br m, 12H), 1.53 (m, 6H), 1.62 (br m, 8H), 1.95 (s, 2H), 2.61 (s, 2H), 2.95 (s, 2H), 3.10 (s, 6H), 3.36 (t, 2H, $J = 6.7$ Hz), 3.96 (s, 3H) and 4.31 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.3, 22.8, 26.3, 27.8, 28.5, 29.6, 29.66, 29.69, 29.74, 31.4, 31.7, 31.8, 34.2, 37.4, 40.0, 53.5, 71.9, 82.0, 127.1, 155.0, 156.1 and 158.1; mass spectrum (APCI), m/z 474.3703 (M+H)$^+$ (C$_{28}$H$_{48}$N$_3$O$_3$ requires 474.3690).

2-(N,N-Dimethylamino)-4-methoxy-6-octadecylpyrimidin-5-ol (3.24). To a stirred solution containing 100 mg (0.32 mmol) of compound 3.83 and 142 µL (111 mg; 0.50 mmol) of 1-hexadecene in 2.00 mL of anh CH$_2$Cl$_2$ was added 27
mg (16 µmol) of Grubb’s 2\textsuperscript{nd} generation catalyst. The reaction mixture was stirred at reflux for 16 h. The reaction mixture was concentrated under diminished pressure and the residue was partially purified on a short silica gel pad. The crude mixture was concentrated under diminished pressure and the residue was dissolved in 2 mL of methanol. Then 3 mg of 20\% palladium hydroxide-on-carbon (Degussa type E101 NE/N) was added and the reaction mixture was stirred at 23 °C for 15 min under a hydrogen atmosphere. The reaction mixture was filtered through Celite\textregistered and the filtrated was concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 1 cm). Elution with 4:1 hexanes–diethyl ether gave compound 3.24 as a colorless solid: yield 49 mg (36\%); mp 78-79 °C; silica gel TLC \( R_f \) 0.45 (4:1 hexanes–diethyl ether); \(^1\text{H NMR} (\text{CDCl}_3) \delta 0.88 (\text{t, } 3\text{H, } J = 6.8 \text{ Hz}), 1.27 (\text{br m, } 30\text{H}), 1.66 (\text{br m, } 2\text{H}), 2.62 (\text{m, } 2\text{H}), 3.10 (\text{s, } 6\text{H}) \text{ and } 3.95 (\text{s, } 3\text{H}). \(^{13}\text{C NMR} (\text{CDCl}_3) \delta 14.3, 15.4, 22.8, 22.9, 27.9, 29.5, 29.68, 29.71, 29.78, 29.82, 29.9, 31.4, 31.8, 32.1, 37.4, 53.5, 66.0, 127.1, 155.0, 156.1 \text{ and } 158.2; \text{ mass spectrum (APCI), } m/z 422.3741 (M+H)^+ (C_{25}H_{48}N_3O_2 \text{ requires } 422.3747). \)

\[
\text{N} \quad \text{N} \\
\text{N} \quad \text{N} \\
\text{O} \quad \text{Bn} \\
\text{NH}_2
\]

\textbf{2-Amino-5-(benzyloxy)-4,6-dimethylpyrimidine (3.85).} To a stirred solution containing 2.85 g (9.27 mmol) of compound 3.57 in 30 mL of 1:1 ethanol–water was added 6.44 g (93 mmol) of hydroxylamine hydrochloride. The reaction
mixture was stirred at reflux for 18 h. The solution was then adjusted to pH~10 and diluted in 20 mL of water. The mixture was extracted with two 30-mL portions of dichloromethane. The combined organic solution was dried (MgSO₄) and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 4 cm). Elution with 1:2 acetone–hexanes gave compound 3.85 as a colorless solid: yield: 2.10 g (98%); mp 123-125°C; silica gel TLC Rᵣ 0.25 (1:2 acetone–hexanes); ¹H NMR (CDCl₃) δ 2.29 (s, 6H), 4.74 (s, 2H), 4.80 (br s 2H) and 7.37 (m, 5H); ¹³C NMR (CDCl₃) δ 18.8, 75.5, 128.1, 128.4, 128.6, 136.6, 143.2, 158.5 and 161.2; mass spectrum (APCI), m/z 230.1299 (M+H)⁺ (C₁₃H₁₆N₃O requires 230.1293).

![5-Benzylino-2-(N,N-dimethylamino)-4,6-dimethylpyrimidine (3.86)](image)

**5-Benzylino-2-(N,N-dimethylamino)-4,6-dimethylpyrimidine (3.86).** A stirred solution containing 500 mg (2.18 mmol) of compound 3.85 in 20 ml of 1:1 formalin–formic acid was stirred at reflux for 18 h. The reaction mixture was poured into 100 mL of 1 N NaOH and extracted with two 100-mL portions of dichloromethane. The combined organic solution was dried (MgSO₄) and then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 3:1 hexanes–ethyl acetate gave compound 3.86 as a colorless oil: yield: 130 mg (23%); silica gel TLC Rᵣ 0.60 (3:1 hexanes–ethyl acetate); ¹H NMR (CDCl₃) δ 2.31 (s, 6H),
3.14 (s, 6H), 4.74 (s, 2H) and 7.38 (m, 5H); $^{13}$C NMR (CDCl$_3$) δ 19.2, 37.3, 75.3, 128.1, 128.2, 128.6, 137.0, 141.5, 145.5, 158.5 and 160.0; mass spectrum (APCI), m/z 258.1614 (M+H)$^+$ (C$_{15}$H$_{20}$N$_3$O requires 258.1614).

2-(N,N-Dimethylamino)-4,6-dimethylpyrimidin-5-ol (3.87). To a stirred solution containing 130 mg (0.50 mmol) of compound 3.86 in 4 ml of methanol was added 5 mg Pd/C (10%). The reaction mixture was stirred at 23 °C for 5 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite® and concentrated under diminished pressure to afford compound 3.87 as a colorless solid: yield 82 mg (100%); mp 147-148 °C; silica gel TLC $R_f$ 0.15 (3:1 hexanes–ethyl acetate); $^1$H NMR (CDCl$_3$) δ 2.35 (s, 6H) and 3.13 (s, 6H); $^{13}$C NMR (CDCl$_3$) δ 18.7, 37.9, 138.3, 155.4, 155.4 and 155.6.

5-Bromo-2-(N,N-dimethylamino)-4-methoxy-6-methylpyrimidine (3.88). To a stirred solution containing 600 mg (3.59 mmol) of compound 3.63 in 20 mL of acetonitrile was added 703 mg (3.95 mmol) of N-bromosuccinimide. The reaction mixture was stirred at 23°C and protected from light for 18 h. The mixture was poured into 75 mL of ethyl acetate and washed with two 75-mL portions of satd
aq sodium bicarbonate. The organic layer was dried (MgSO₄) and concentrated under diminished pressure to afford compound 3.88 as a colorless solid: yield 1.15 g (73%); mp 40-41°C; silica gel TLC \( R_f \) 0.55 (4:1 hexanes–ethyl acetate); \(^1\)H NMR (CDCl₃) \( \delta \) 2.39 (s, 3H), 3.12 (s, 6H) and 3.93 (s, 3H); \(^{13}\)C NMR (CDCl₃) \( \delta \) 24.4, 37.0, 37.0, 53.9, 91.3, 160.0, 165.0 and 165.8; mass spectrum (APCI), \( m/z \) 246.0249 (M+H)\(^+\) (C₈H₁₃N₃OBr requires 246.0242).

![Chemical Structure](image)

### 2-(N,N-Dimethylamino)-5-hydroxy-4-methoxy-6-methylpyrimidine (3.89)

To a stirred solution at -5 °C containing 362 mg (1.47 mmol) of compound 3.88 in 15 mL of anh THF was added 2.29 mL (3.67 mmol) of 1.6 M solution of \( n \)-BuLi in hexanes. The reaction mixture was stirred at -5 °C for 20 min. To the mixture was added 493 \( \mu \)L (459 mg; 4.42 mmol) of trimethyl borate and the reaction mixture was stirred for 1 h. To the reaction mixture was added 3.23 mL of 30% aq H₂O₂ followed by 1.05 mL of 3 N aq NaOH. The reaction mixture was stirred for 30 min and poured into 50 mL of water. The mixture was neutralized with dilute aq HCl and extracted with two 50-mL portions of ethyl acetate. The combined organic solution was washed with 80 mL of brine, dried (MgSO₄) and concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (10 × 3 cm). Elution with 1:1 hexanes–ethyl acetate afforded compound 3.89 as a colorless oil: yield 22 mg (8%); silica
gel TLC $R_f$ 0.15 (4:1 hexanes–ethyl acetate); $^1$H NMR (CD$_3$OD) $\delta$ 2.23 (s, 3H), 3.07 (s, 6H) and 3.92 (s, 3H); $^{13}$C NMR (CD$_3$OD) $\delta$ 16.3, 36.3, 52.2, 127.3, 152.1, 155.9 and 159.9; mass spectrum (APCI), $m/z$ 184.1089 (M+H)$^+$ (C$_8$H$_{14}$N$_3$O$_2$ requires 184.1086).

**Cyclic voltammetry**

Cyclic voltammetry (CV) studies were carried out using a Model 1030 multi-potentiostat from CH Instruments. The platinum working electrode 2 mm diameter (model CHI102), platinum wire counter electrode (model CHI115), and Ag/AgCl reference electrode (model CHI111) were obtained from CH Instruments. Cyclic voltammetry measurements were performed in a precut 20 mL vial at room temperature in 2 mL of a 0.1 M tetrabutylammonium perchlorate solution in acetonitrile containing the pyridinol and pyrimidinol analyte at a 1 mM concentration. The analytes were dissolved in acetonitrile. The vial was covered with a Teflon cap (CHI223) and sealed using parafilm. All samples were purged with nitrogen for 5 min to remove any oxygen. The substrate was then added with a syringe and stirred for 1 minute. Stirring was stopped and the stream of nitrogen was focused above the sample so that only the headspace was streamed with nitrogen. The cell was allowed to sit for one minute to allow for the diffusion layer to set up and come to equilibrium. The parameters used for the starting and vertex potential were –1.5 V and 1.5 V respectively, and the scan rate was 100 mV/s. The cyclic voltammogram of CoQ$_0$ was recorded using an initial reductive sweep; those of the pyridinols and pyrimidinols involved initial
oxidative sweeps. Between each CV experiment, the electrodes were rinsed with dichloromethane. The platinum working electrode was then rinsed with water and polished with 0.05 micron aluminum powder and distilled water on a polishing pad (CHI120). Immediately before each CV was performed, all electrodes were rinsed with acetonitrile. This experiment was performed by Manikandadas Mathilakathu Madathil, ASU.

**Electron spin resonance study (ESR)**

Compound 3.1 was dissolved in deoxygenated benzene solution for analysis to a final concentration of 10-20 mM. Then, di-tert-butylperoxide (98%, Aldrich) was added to a final concentration of 0.1 M. The sample was inserted in the ESR cavity and the mercury lamp was turned on. Illumination was done directly in the cavity by inserting an optic fiber that was connected to the lamp. The ESR spectra was recorded at regular intervals on a X-band Bruker ELEXSYS E580 spectrometer using the following settings: microwave frequency 9.70–9.77 GHz, power 64 mW, modulation amplitude 0.1G, center field 3462 G, sweep time 168 s, and time constant 40 ms. The simulations were done using the Winsim software. Control experiments (dark sample, and sample without test compounds) were measured and gave no signal. This experiment was performed by Dr. Ruth Goldschmidt and Manikandadas M. Madathil, ASU.
Cell culture experiments

Friedreich’s ataxia lymphocytes (Coriell, GM158150) were cultured in RPMI (Gibco, Grand Island, NY, USA) with 15% fetal bovine serum (Fisher Scientific, TX, USA), 2 mM glutamine (HyClone, South Logan, Utah, USA) and 1% penicillin–streptomycin mix (Cellgro). Cells were maintained in log phase at a concentration between $1 \times 10^5$ and $1 \times 10^6$ cells/mL.

Lipid peroxidation assay

A quantitative FACS analysis of lipid peroxidation of FRDA lymphocytes, which had been treated with diethyl maleate following incubation in the presence and absence of the test compounds, was measured as described previously. Briefly, FRDA lymphocytes ($5 \times 10^5$ cell/mL) were treated with the test compounds at final concentrations of 2.5 and 5 µM and incubated at 37 °C for 16 h in a humidified atmosphere containing 5% CO₂ in air. Cells were treated with 1 µM C₁₁BODIPY₅₈₁/₅₉₁ in phenol red-free RPMI-1640 media and incubated at 37 °C in the dark for 30 min. Oxidative stress was induced with 5 mM DEM in phenol red-free RPMI-1640 media for 2 h. Treated cells were collected by centrifugation at 300 × g for 3 min and then washed with phosphate buffered saline. Cells were resuspended in 250 mL of phosphate buffered saline and analyzed by FACS (FACS Calibur flow cytometer, Becton Dickinson) to monitor the change in intensity of the C₁₁BODIPY₅₈₁/₅₉₁-green (oxidized) fluorescence signal. In each analysis, 10,000 events were recorded. Results obtained were
verified by running duplicates and repeating experiments in three independent experiments. This experiment was performed by Dr. Omar M. Khdour, ASU.

**NADH oxidase activity assay**

Mitochondria were prepared as described. One beef 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. Cell debris were removed by centrifugation at 1200 × g for 20 min. The supernatant was filtered through two layers of cheesecloth. Mitochondria were harvested by centrifugation at 26000 × g for 15 min and then homogenized in the same buffer with a Dounce homogenizer. Mitochondria were harvested by centrifugation at 12000 × g for 30 min, and stored at –80 °C in sucrose buffer. Submitochondrial particles (SMPs) were prepared as described by Linnane and Titchener. Mitochondria were sonicated with a Sonic Dismembrator (Fisher Scientific) in 0.25 M sucrose, 5 mM MgCl₂, 1 mM ATP, 10 mM MnCl₂, 1 mM sodium succinate, 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. SMPs were harvested at 152000 × g for 30 min at 4 °C and stored at –80 °C in 10 mM Tris-HCl, pH 7.5, containing 0.25 M sucrose, 5 mM MgCl₂, 2 mM ATP, 2 mM glutathione, and 1 mM sodium succinate. The protein concentration was determined by BCA titration (Pierce) and the sample was diluted as described below.

The inhibitory effects of the tested compounds on bovine heart mitochondrial complex I, III 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. The enzymatic activities were assayed at 30 °C and monitored spectrophotometrically with a Beckman Coulter DU-530 (340 nm, ε₆.₂₂ mM⁻¹ cm⁻¹). NADH oxidase activity was determined in a reaction medium (2.5 mL total volume) containing 50 mM Hepes, pH 7.5, and 5 mM MgCl₂. The final mitochondrial protein concentration was 30 µg/mL. After the pre-equilibration of SMP with inhibitor for 5 min, the initial rates were calculated from the linear portion of the traces. This experiment was performed by Sriloy Dey and Dr. Omar M. Khdour, ASU.

**NADH-ubiquinone oxidoreductase activity assay**

The inhibition of NADH-Ubiquinone oxidoreductase activity was determined using the same experimental conditions described previously.¹²¹,¹²² Twelve µg of SMP’s were incubated at 39 °C for 5 min with the test compound in 1 mL of 50 mM phosphate buffer, pH 7.4, containing 0.25 M sucrose, 1 mM MgCl₂, 2 µM antimycin A and 2 mM KCN. The reaction was initiated by the addition of 50 µM NADH and 50 µM of coenzyme Q₁. Enzymatic activity, measured by the loss of NADH absorbance, was monitored at 340 nm. This experiment was performed by Dr. Valérie C. Collin, ASU.
**Cell viability (trypan blue exclusion assay)**

A hemocytometer-based assay was used to determine the number of viable cells present in the cell suspensions. Briefly, FRDA cells were seeded at a density of $5 \times 10^5$ cells/mL and treated with different concentrations of the test compounds. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO$_2$ in air for 17 h. Oxidative stress was then induced by incubation with 5 mM DEM for 6 h followed by evaluation of cytoprotection. Cell viability was determined by the use of 0.4% trypan blue. Cytoprotection by the test compounds was assessed with respect to the untreated controls. Cells not treated with DEM had > 90% cell viability whereas DEM treatment reduced cell viability to < 20%. The ability of the test compounds to protect the cells against the effects of DEM was determined. Cell viability was expressed as the percentage of untreated control. Data are expressed as means ± S.E.M. (n = 3). This experiment was performed by Jennifer Jaruvangsanti, ASU.

**Scavenging of reactive oxygen species (ROS)**

Intracellular ROS production was measured using the oxidant-sensitive fluorescent probe 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA) (Molecular Probes, Eugene, OR, USA). One mL of CEM lymphocytes ($2.5 \times 10^5$ cell/mL) were plated in a 24-well plate, treated with the test compounds and incubated for 16 h at 37 °C, in a humidified atmosphere containing 5% CO$_2$ in air. Cells were treated with 5 mM diethyl maleate (DEM) for 1 h, collected by centrifugation at 300 × g for 3 min and then washed twice with phosphate
buffered saline (PBS) (Invitrogen, NY). Cells were resuspended in PBS containing 10 mM glucose and incubated at 37 °C in the dark for 25 min with 10 µM DCFH-DA. Cells were collected by centrifugation at 300 × g for 3 min and then washed twice with PBS. The samples were analyzed immediately by flow cytometry using a 488 nm excitation laser and FL1-H channel 538 nm emission filter. In each analysis, 10,000 events were recorded after cell debris were electronically gated. The generation of ROS, mainly peroxides, was detected as a result of the oxidation of DCFH ($\lambda_{ex}$ 488 nm; $\lambda_{em}$ 515-540 nm). Results obtained were verified by running duplicates and repeating experiments in three independent experiments. Hydrogen peroxide was used to produce the positive control. This experiment was performed by Dr. Omar M. Khdour and Dr. Ruth Goldschmidt, ASU.

**Maintenance of mitochondrial membrane potential ($\Delta \psi_m$)**

Briefly, cells were pre-treated with or without the test compounds for 16 h. The cells were treated with 5 mM DEM for 140 min, collected by centrifugation at 300 × g for 3 min and then washed twice with phosphate buffered saline. The cells were resuspended in PBS containing 20% glucose and incubated at 37 °C in the dark for 15 min with 250 nM TMRM. Cells were collected by centrifugation at 300 × g for 3 min and were then washed with phosphate buffered saline. The samples were analyzed immediately by flow cytometry using a 488 nm excitation laser and the FL2-H channel. The results obtained were verified in three independent experiments. FCCP, a mitochondrial uncoupler was used to produce
a negative control. In each analysis, 10,000 events were recorded. This experiment was performed by Dr. Omar M. Khdour

**Cellular ATP concentration**

Briefly, lymphocytes (2 × 10^5 cell/ mL) were plated (1 mL in 12-well plates) and treated with the test compounds at final concentrations of 5, 10 and 25 µM, and then incubated at 37 °C for 48 h in a humidified atmosphere containing 5% CO₂ in air. Cells were transferred (100 µL) to 96-well microtiter black-walled cell culture plates (Costar, Corning, NY). The total intracellular ATP level was measured in a luminator (Clarity™ luminescence microplate reader) using an ATP Bioluminescence Assay Kit (ViaLight®.Plus ATP monitoring reagent kit, Lonza, Walkersville, MD) following the manufacturer’s protocol. The total ATP level was expressed as a percentage of untreated control. To compare the ATP content between normal and CoQ₁₀ deficient lymphocytes, fixed number of lymphocytes (1×10⁶ cell/ mL) were incubated for two hours under the above culture conditions. Cells were washed in PBS, harvested and lysed immediately in 0.5% Triton X-100, 10 mM Tris-HCl, pH 7.5, containing 1 mM EDTA, and incubated for 10 min on ice. After removal of cell debris by centrifugation (12,000 × g, 15 min, 4 °C), the ATP level in the resulting supernatant was determined by luciferase bioluminescent assay, using appropriate ATP standards. ATP content measured in total cell lysates was expressed as nmoles/mg cellular protein. The protein content was determined by the bicinchoninic acid assay method (micro-BCA kit, Pierce)
using bovine serum albumin (BSA) as reference. This experiment was performed by Dr. Omar M. Khdour.
REFERENCES

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APPENDIX A

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