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CHAPTER 1. INTRODUCTION

1.1 Introduction to HNO

1.1.1 The unique qualities of HNO

HNO (nitrosyl hydride, nitroxyl, azanone, Figure 1.1) is a small inorganic molecule with unique chemical characteristics and has attracted considerable interest for its functions in biological systems. HNO has also been rapidly emerging as a novel entity with distinct pharmacology and therapeutic advantages and, it has been discovered that HNO has different chemical and biochemical activities than its more widely studied redox sibling, nitric oxide (NO).\(^1\)

\[\begin{array}{c}
\text{H} \\
\text{N} \equiv \text{O}
\end{array}\]

Figure 1.1: Lewis structure of HNO

HNO is the reduced form of NO and is thought to be formed by the enzyme-catalyzed reduction of NO from enzyme catalysts such as NOS catalysts.\(^1\) It has been postulated that HNO can be produced directly from NOS-dependent sources in the absence of tetrahydrobiopterin (BH\(_4\), THB) cofactor, which is a naturally occurring cofactor for the enzymatic degradation of the aromatic amino acids; there is speculation that BH\(_4\) is required for the enzyme to serve as an NO rather than an HNO synthase.\(^1\) HNO is also
thought to be produced after oxidation of the NOS intermediates \( \text{N-hydroxy-L-arginine} \) and hydroxylamine (Figure 1.2).

\[ \text{L-arginine} \xrightarrow{\text{NADPH, O}_2} \text{N-hydroxy-L-arginine} \xrightarrow{\text{ONOO}^-, \text{BH}_4} \text{L-citrulline} + \text{HNO} \]

**Figure 1.2: Possible biosynthetic pathway to \( \text{HNO} \) from the nitric oxide synthase (NOS) intermediate \( \text{N-hydroxy-L-arginine} \) (reproduced from Irvine)**

This has shown potential as a physiologically relevant process since \( \text{N-hydroxy-L-arginine} \) has been detected at significant levels (~20 \( \mu \text{m} \)) and is released by some cells \textit{in vivo}. However, there is a lack of strong evidence for endogenous \( \text{HNO} \) generation, which can be accredited to the fact that there is currently no efficient, specific, and/or sensitive \( \text{HNO} \) detection method available for use in biological systems. However, \textit{in vitro} experiments have exhibited evidence that \( \text{HNO} \) could be generated endogenously.

The \( \text{pK}_a \) value for deprotonation of \( \text{HNO} \) to give \( \text{NO}^- (+ \text{H}^+) \) is ~ 11.4, making \( \text{HNO} \) the biologically relevant species. The acid–base equilibrium of \( \text{HNO} \) is not typical because the conjugate acid and conjugate base have different electronic spin states. With typical acid–base reactions, it is normally assumed that proton transfer is
extremely fast such that, the equilibrium constant ($K_a$) is an effective indication of the ratio of acid and conjugate base present. However, the ground state of HNO is a singlet while NO$^-$ is a ground state triplet.$^{3,6}$ Since the ground states for HNO and NO$^-$ are different, proton transfer requires a spin state change, which is forbidden by quantum mechanics. Spin-forbidden processes have a low probability of occurring and thus are kinetically slow compared to spin-allowed processes.$^{3,6,7}$ The consequence of the high kinetic barrier is that proton transfer becomes slow enough to impede the rapid establishment of an acid–base equilibrium between HNO and NO$^-$. Also, since, both HNO and NO$^-$ are also highly reactive species, pathways other than proton transfer could be more kinetically favorable.

Another unique characteristic of HNO is that it is resistant to scavenging by superoxide ("O$^2\cdot$).$^{1,5}$ In contrast, NO reacts with superoxide to form toxic peroxynitrite/peroxynitrous acid (ONOO(H)).$^1$ This property of HNO makes it more resistant to reaction with possible radical byproducts formed during biological processes. HNO, like NO, elicits vasorelaxation through cell signaling transduction.$^2,5$ However, NO eventually reaches a tolerance level in therapeutic applications, while HNO does not show any development of vascular tolerance and can have positive effects at low concentrations.$^{1,5}$ This makes it a more attractive agent for pharmacological and medicinal studies. HNO was discovered in the Nagasawa laboratory to have the potential to serve as a treatment for alcoholism.$^3,6$ In their studies, they found that cyanamide, an HNO-donating drug for treating alcoholism, is oxidatively bioactivated to produce HNO
which then inhibits the enzyme aldehyde dehydrogenase by interacting with the cysteine thiolate active site. There has been some evidence showing that the interaction of HNO and cyanamide also produces hydrogen cyanide (HCN) as a byproduct. However, there is literature stating that pharmacological use of cyanamide does not appear to be associated with cyanide toxicity, providing some evidence that HNO is an extremely potent inhibitor of aldehyde dehydrogenase, which then produces HCN at a threshold well below that of cyanide poisoning. In clinical trials performed on adult male dogs at Johns Hopkins University, HNO was also found to target cardiac sarcoplasmic ryanodine receptors to increase myocardial contractility by acting as a cardiac inotrope, which increases the force of contractions. HNO also interacts directly with thiols and other thiol-containing species, while NO cannot. The reaction of HNO with thiols leads to an initial formation of a putative N-hydroxysulfenamide via attack of a nucleophilic sulfur atom on the electrophilic nitrogen atom of HNO (Figure 1.3a). The N-hydroxysulfenamide can further react with excess thiol (or a vicinal protein thiol) to give the corresponding disulfide and hydroxylamine (Figure 1.3b). Competing with this reaction is a process whereby the N-hydroxysulfenamide rearranges to a sulfonamide (Figure 1.3c).
\[
RSH + HNO \rightarrow RS-NHOH \quad (a)
\]
\[
RSNHOH + R'SH \rightarrow RSSR' + NH_2OH \quad (b)
\]
\[
RSNHOH \rightarrow RS(O)NH \quad (c)
\]

**Figure 1.3: Sequence of reactions for HNO and thiols**

With all of the intriguing and unique characteristics of HNO and the potential therapeutic uses of the molecule, it does possess some unfortunate disadvantages.

1.1.2 **Drawbacks and issues of HNO**

HNO is not very stable in aqueous solution.\(^4\) HNO is a metastable species that rapidly forms dimers with other molecules of HNO \((k \sim 8 \times 10^6 \text{ M}^{-1} \text{ s}^{-1} \text{ (22 °C)})\). The resulting hyponitrous acid then decomposes to nitrous oxide \((\text{N}_2\text{O})\) and water (see Figure 1.4).\(^7,11\)

\[
HNO + HNO \rightarrow [HO-N=N-OH] \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O}
\]

**Figure 1.4: Equation showing the formation of the HNO dimer and subsequent products**

Aside from not being very stable, HNO is quite difficult to detect and measure. Detection of HNO and discrimination from NO currently is done through indirect methods. For example, the amount of HNO can be calculated by measuring existing amounts of N\(_2\)O after a reaction has been completed through gas chromatography (GC) head-space analysis, by reductive nitrosylation of oxidized metal complexes such as
metmyoglobin, or by the detection of reaction products of HNO reacting with thiols.\textsuperscript{4,6,12} GC head-space analysis is less specific and convenient in bioassays because N\textsubscript{2}O can be formed without the intermediacy of HNO. Thus, the collection of N\textsubscript{2}O can be a misleading measure of HNO generation.\textsuperscript{3} Furthermore, HNO consumption \textit{in vivo} is likely to occur largely by pathways other than dimerization (e.g., by reaction with glutathione (GSH) and metal- or critical-thiol proteins).\textsuperscript{3} The high reactivity of HNO with thiols also provides an additional indication of HNO formation due to the susceptibility to signal quenching by thiols such as GSH in assays.\textsuperscript{3} Miranda and coworkers utilized both GSH and urate as scavengers of HNO.\textsuperscript{3,13} Many of these assays utilize biomolecules or have been adapted for \textit{in vitro} use; these techniques cannot yet be applied to intact cells and they also need to be able to detect HNO and discriminate its actions from NO.\textsuperscript{4} As previously mentioned, at concentrations above low nanomolar values, HNO will kinetically undergo dimerization over deprotonation to NO\textsuperscript{−}.\textsuperscript{4} Thus, studies examining HNO are commonly performed using HNO donors (\textit{vide infra}).\textsuperscript{4}

\subsection{1.1.3 Importance of HNO donors}

Due to HNO’s inherent instability in aqueous solution, ‘HNO donors’ are required for biological and chemical studies of HNO reactivity and as potential therapeutics.\textsuperscript{9} HNO donors have been used as sources of HNO, which can induce biochemical reactions in biological systems. In order to fully exploit HNO as a pharmacological agent, a variety of donors need to be developed as replacements and/or complements to Angeli's salt as the primary HNO donor in biological/pharmacological
Use of new HNO donors can help to confirm that the observed biological activity is in fact due to HNO, rather than to the HNO donor itself or other reactive nitrogen species. There are a wide variety of HNO donors that have been synthesized and studied experimentally.

### 1.2 Examples of HNO donors

#### 1.2.1 Angeli’s Salt (AS)

Angeli’s salt (AS, Na$_2$N$_2$O$_3$, Figure 1.5), has been used in clinical trials for treatment of acute heart failure. Angeli’s salt has been studied most extensively in terms of structure, thermodynamics and decomposition mechanism.

![Lewis structure of Angeli’s salt](image)

**Figure 1.5: The Lewis structure of Angeli’s salt**

Angeli’s salt has been the mainstay of the HNO donor field by being the most convenient, well-studied and utilized HNO donor. Angeli’s salt is a pH dependent donor (Figure 1.6) which spontaneously decomposes above pH 4 to generate
Figure 1.6: Decomposition of Angeli’s salt to form HNO

The rate of the thermal decay of Angeli’s salt is pH-independent from pH 4–8 and then successively decreases with elevated pH. These properties are extremely useful for practical purposes since stock solutions of Angeli’s salt are relatively stable at high pH while the HNO release profile is pH insensitive near physiological conditions (i.e. pH 7.4). At pH values less than 4, Angeli’s salt converts to an NO donor. However, its short half-life and concomitant release of nitrite confer limitations.

1.2.2 Piloty’s Acid (PA)

N-hydroxysulfonamides (e.g. Piloty’s acid, PA) are another class of HNO donor that has been investigated for generation of HNO. Figure 1.7 displays how Piloty’s acid, when deprotonated, can produce HNO and benzenesulfinate.
In contrast to Angeli’s salt, Piloty’s acid, and its related chemical species, are stable at low pH and decompose with rates that are accelerated as the pH is elevated.\(^6\)\(^{17}\)

The rate constant for decomposition of Piloty’s acid at pH 13 (4.2 × 10\(^{-4}\) s\(^{-1}\) at 25 °C and 1.8 × 10\(^{-3}\) s\(^{-1}\) at 35 °C) is similar to that for Angeli’s salt at pH 4–8.\(^4\) Piloty’s acid decomposes at a pH above 8, which makes significant HNO release typically occur only at pH values well beyond biological conditions.\(^18\) At physiological pH (7.4), PA is oxidized and subsequently releases NO rather than HNO and the decomposition of PA is very slow.\(^18\) One attractive feature of Piloty’s acid is its capacity for broad substituent modification, enabling structural optimization of physicochemical and pharmacological properties.

Piloty’s acid can be altered to make derivatives that can generate HNO more rapidly. Miyata synthesized several PA derivatives to observe the half-life and generation of HNO in a Tris buffer/20% DMSO (dimethyl sulfoxide) solution at pH 7.5 (Figure 1.8).\(^{19}\)
Figure 1.8: Piloty acid derivatives for rapid HNO release (reproduced from Miyata\textsuperscript{19})

The leftmost figure shows the half-life of PA in the Tris buffer/20\% DMSO solution at 270.2 minutes. However, with the addition of an ortho-positioned substituent, such as bromine (middle) or nitro (NO\textsubscript{2}, rightmost) group, the half-life was significantly reduced.

Toscano also investigated PA derivatives through barbiturate analogs (Figure 1.9).\textsuperscript{20} The analogs were able to achieve HNO release with half-lives of 0.7 min and 9.5 min respectively in a phosphate-buffered saline (PBS) solution at pH 7.4.
Figure 1.9: Barbiturate analogs for the rapid release of HNO (reproduced from Toscano)\textsuperscript{20}

Toscano studied the synthesis of Piloty’s acid derivatives and patented his findings into the chemistry of their various compounds.\textsuperscript{20} Figure 1.10 shows the general structure of Piloty’s acid analogs with the R groups designating variable atoms and functional groups.

Figure 1.10: Toscano patent for Piloty’s acid derivatives (reproduced from Toscano)\textsuperscript{21}

Toscano patented these compounds based on the potential therapeutic advantages of Piloty’s acid as an HNO donor. Toscano also partnered with Cardioxyl Pharmaceuticals to patent some promising PA analogs that have undergone clinical
CXL-1020 was the first generation HNO donor and is currently in a Phase I/IIa
dose-escalation study to study the safety and tolerability of CXL-1020. Cardioxyl also
now has a second-generation HNO donor in Phase II clinical testing named CXL-1427.
Structure elucidation was not entirely clear however, by checking with U.S. patents
submitted by Toscano, the estimated structures of two of the PA analogs are shown
in Figure 1.11.

![Figure 1.11: Toscano’s clinical trial Piloty’s acid analogs](image)

1.2.3 HNO-generating diazeniumdiolates

Although primarily known as NO donors, diazeniumdiolates (NONOates) can be
designed to generate HNO as well. In fact, Angeli’s salt is a diazeniumdiolate analog
with an oxygen atom replacing the amine function (see Figure 1.5).

$$[RNH-N(O)=NO^- \rightleftharpoons [RN=N(O)-NHO^-] \rightarrow HNO + RNNO^-]$$

![Figure 1.12: General reaction scheme for NONOate generation of HNO](image)

NO-releasing diazeniumdiolates are made using secondary amines, while
diazeniumdiolates made with primary amines can release HNO at neutral pH and have
been shown to possess biological activity similar to Angeli's salt (see Figure 1.1).\textsuperscript{24} The byproduct of primary amine diazeniumdiolate decomposition is a nitrosamine, a species which is of toxicological concern.\textsuperscript{26}

1.2.4 Cyanamide

The use of cyanamide as an anti-alcoholism drug has predated recent interest in HNO donors as pharmacological agents for the treatment of cardiovascular conditions and other applications.\textsuperscript{6} Cyanamide is bioactivated via oxidation by the enzyme catalase, leading to the generation of an intermediate, \textit{N}-hydroxycyanamide.\textsuperscript{6} This species spontaneously decomposes to release cyanide and HNO (Figure 1.13).\textsuperscript{27}

![](image)

\begin{figure}
\includegraphics[width=0.5\textwidth]{cyanamide_bioactivation}
\caption{Equation demonstrating the bioactivation of cyanamide}
\end{figure}

However, this catalase-catalyzed reaction represents a minor pathway in the overall disposition of cyanamide \textit{in vivo}.

Cyanamide demonstrates both the feasibility of clinical use of HNO donors and the ability of such species to selectively target protein thiols, such as in aldehyde dehydrogenase (AlDH).\textsuperscript{6,27} Cyanamide does not inhibit AlDH directly, but must be bioactivated \textit{in vivo} to an active inhibitory species.\textsuperscript{27}
1.2.5 Nitrosocarbonyls

Compounds that generate nitrosocarbonyls are another HNO donor class. The nitrosocarbonyl functionality is inherently unstable in aqueous solution and spontaneously hydrolyzes to HNO and the corresponding carboxylic acid (Figure 1.14).⁶

\[
\begin{align*}
\text{R} - \text{N} = \text{O} + \text{H}_2\text{O} & \rightarrow \text{R} - \text{O} - \text{H} + \text{HNO} \\
\end{align*}
\]

**Figure 1.14: Equation showing the instability of nitrosocarbonyl compounds**

_In situ_ generation of nitrosocarbonyls can occur via a retro-Diels-Alder reaction or through oxidation of hydroxamic acids.²⁸,²⁹,³⁰ Nagasawa attempted to generate nitroxyl by preparing a series of _N_,_O_-bisacylated derivatives. Such prodrugs of nitroxyl undergo a disproportionation reaction following ester hydrolysis to give an unstable acylnitro intermediate that rapidly hydrolyzes to nitroxyl.³¹
Nagasawa proposed that the mechanism shown above (Figure 1.15) would proceed through Path A, via the intermediacy of the de-esterified compound 2. Elimination of arenesulfinic acid from compound 2 produces benzoynitroxyl, which spontaneously hydrolyzes to give nitroxyl.\textsuperscript{31} In contrast, direct hydrolysis of the sulfonate group (Path B) produces the corresponding benzenesulfonic acid 5 and $O$-acylated benzoxyhydroxamic acid 6, which hydrolyzes to benzoxyhydroxamic acid (compound 7).\textsuperscript{31} In summary, Nagasawa found that $N$-acyloxy-$O$-arenesulfonylated benzenecarboximidic acids represent a new series of nitroxyl generators that require enzymatic activation (ester hydrolysis) before nitroxyl can be liberated. However, for optimal bioactivation,
compounds much more water-soluble than the present series are required.\textsuperscript{31} To date, nitrosocarbonyl precursors that generate HNO \textit{in situ} have not been used extensively for biological studies.\textsuperscript{6}

1.2.6 \textit{Hydroxylamine}

Another example of a potential HNO donor is hydroxylamine. It has been hypothesized that a two electron oxidation of NH$_2$OH could serve as a pathway to HNO.\textsuperscript{32} The use of NH$_2$OH as a precursor to HNO has not been exploited as a strategy for HNO generation in biological studies. However, there has been a report from the Donzelli research group where they have indirectly demonstrated that oxidation of NH$_2$OH by HRP (horseradish peroxidase) leads to release of HNO, which is trappable by GSH (glutathione).\textsuperscript{6,33}

1.2.7 \textit{α-Acylxoy-C-nitroso compounds}

α-Acylxoy-C-nitroso compounds are another version of HNO donors, which were recently investigated by the King research group (Figure 1.16).\textsuperscript{34}

\textbf{Figure 1.16: Reaction showing the generation of HNO by α-acyloxy-C-nitroso hydrolysis}
These compounds are readily synthesized from oxidation of oximes and have been shown to be capable of releasing HNO under physiological conditions. As depicted in Figure 1.16, the release of HNO has been postulated to occur either by spontaneous hydrolysis of the ester or via esterase activity. Moreover, the release rate of HNO can be varied by altering the appending organic “R” groups. This may prove to be important in the future development of drugs with proper pharmacokinetics and distribution profiles.

1.2.8 Photolabile HNO and NO donors

Flash photolysis is widely used for rapid in situ generation of small molecules of biological interest, including NO. In contrast, photochemical processes for the generation of HNO are not very well developed. However, photomediated methods for HNO generation offer potential advantages over traditional approaches, whereby site-specific irradiation with a thermally-stable HNO donor could achieve both high spatial and temporal control during HNO generation. An example of this approach was reported by Toscano where HNO generation was photochemically induced through generation of an acyl nitroso intermediate, which then underwent another transformation en route to producing HNO (Figure 1.17).
Figure 1.17: Photodecomposition of ethyl nitrodiazoacetate in 75:25 acetonitrile/water (reproduced from Toscano)\textsuperscript{37}

They reported that photolytic generation of acyl nitroso compounds was on the nanosecond (ns) timescale.\textsuperscript{37} Figure 1.17 shows nucleophilic attack from water present in solution; however, acyl nitroso compounds can also react with \textit{in situ} amine\textsuperscript{38} or thiolate\textsuperscript{37} nucleophiles to generate HNO.

The Nakagawa research group reported completion of photochemically mediated retro-hetero-Diels-Alder chemistry to generate an acyl nitroso intermediate compound which then can undergo hydrolytic cleavage to generate HNO in aqueous solution (Figure 1.18).\textsuperscript{36, 39, 40, 41} However, Nakagawa suggested that the chemistry occurred thermally via a non-radiative relaxation process since the Woodward-Hoffman rules forbid the retro-cycloaddition.\textsuperscript{36} Other drawbacks were that the rate of HNO generation was quite slow ($t_{1/2}$ \textasciitilde mins) and only partial decomposition of the cycloreversion precursor occurred.\textsuperscript{36, 40}
In contrast to the dearth of photoactivatable HNO donors, several photoactivatable NO donors have been developed which possess photocleavable protecting groups. After the removal of the protecting group by photoirradiation, the small NO molecule can be released spontaneously. For example, Tsien performed experiments where NONOates were caged by photocleavable protecting groups in order to prevent them from spontaneously decomposing to produce NO in aqueous solution. This so-called “switch-on” type NO donor proved very effective for time-lapse imaging of NO effects with fluorescence or luminescence methods.

Unfortunately, limitations of photocontrollable NO donors do exist. One such limitation is the use of UVA (blue light) irradiation, which is needed to activate certain photoactivatable donors for biological applications. Light in this wavelength range can be cytotoxic and shows poor penetration into cells and tissues. Nakagawa and Ford worked on employing two-photon-absorbing NO donors to overcome this limitation.
through the use of longer-wavelength pulse laser irradiation to provide greater penetration into biological tissues. Nakagawa found that some of his donor molecules were quite stable in the dark, while others were stable in ambient light without irradiation.

1.3 Our research group’s family of current HNO donors

1.3.1 Previous work on first family of photoactivatable HNO donors

A member of the Brasch/Sampson/Seed research group has pursued the development of a new class of photoactivated HNO donors for rapid HNO generation. The design for this HNO donor utilized the photolabile (3-hydroxy-2-naphthyl)methyl (HNM) OH protecting group which is shown in Figure 1.19.

\[
\text{HNM-O-NHSO}_2\text{CF}_3 \xrightarrow{\text{hv}} \text{H-N=O}
\]

Figure 1.19: General strategy for photodeprotection of the HNM group

A wide variety of photoprotecting groups exist for OH groups. Among the most popular is the \(o\)-nitrobenzyl ether group, which exhibits relatively slow
photorelease.\textsuperscript{47,48,49} In contrast, Popik has reported that the HNM group undergoes very rapid photocleavage ($k_{\text{release}} \sim 10^5 \text{ s}^{-1}$) with good quantum ($\Phi = 0.17-0.26$) and chemical yields (>90%) (Figure 1.20).\textsuperscript{50}

\begin{center}
\begin{tikzpicture}
\node (n1) at (0,0) {8} ;
\node (n2) at (1,0) {9} ;
\node (n3) at (2,0) {10} ;
\node (n4) at (3,0) {11} ;
\node (n5) at (4,0) {12} ;
\node (n6) at (0.5,1) {\text{hv}} ;
\node (n7) at (3.5,1) {\text{H}_2\text{O}} ;
\node (n8) at (4.5,0) {\text{ms timescale}} ;
\draw [->] (n1) -- (n2) node[midway, above] {$\text{OR}$} ;
\draw [->] (n2) -- (n3) node[midway, above] {$\text{OR}$} ;
\draw [->] (n3) -- (n4) node[midway, above] {$\text{OR}$} ;
\draw [->] (n4) -- (n5) node[midway, above] {\text{OR}} ;
\end{tikzpicture}
\end{center}

\textbf{Figure 1.20: Mechanistic depiction of the photocleavage of the HNM Group}\textsuperscript{50}

The photocleavage proceeds via photodeprotonation and rapid displacement of the OR group to afford a benzoxete intermediate 9. Electroyclic ring opening affords an $o$-naphthoquinone methide byproduct (10)\textsuperscript{51} which then, reacts on the millisecond (ms) timeframe with water\textsuperscript{52} to afford 11. There is also an alternative approach: if the former phenolic byproduct is not desirable, trapping the quinone methide via a hetero-Diels-Alder cycloaddition using a simple alkyl vinyl ether can produce a chemically benign hemiacetal adduct (12).

The decision to use the HNM photoprotecting group, based on Popik’s results\textsuperscript{50}, was because they can be cleaved in the absence of added nucleophiles and are thermally stable. Photodeprotection under neutral pH conditions should proceed rapidly. An anionic intermediate (CF$_3$SO$_2$NHO$^-$) will be formed which will rapidly decompose after the
initial photocleavage of the HNM group to produce HNO and a substituted sulfinate anion (Figure 1.21).

Figure 1.21: Deprotection of HNM photoprotecting group

1.3.2 Previous synthesis of target photolabile HNO donors

Three different HNMONHSO₂R targets were synthesized, via the common synthetic strategy outlined in Figure 1.22.
Starting with commercially available methyl 2-hydroxy-3-naphthoate (13), 14 was synthesized by triisopropylsilyl chloride (TIPS-Cl) protection of the phenolic –OH. Then, 14 underwent reduction of the ester functional group using LiAlH₄, which gave the corresponding alcohol 15. Unfortunately, the yields for this reaction were highly capricious and limited product output. The alcohol was then brominated with PBr₃ (16) and nucleophilic substitution was effected using N-hydroxyphthalimide in basic media to
afford 17. Hydrazine monohydrate was then used to produce amine 18 in high yields. N-Sulfonation with RSO₂Cl, DMAP, and pyridine afforded 19. Removal of the TIPS protecting group was completed using TBAF (Bu₄NF) in anhydrous THF to complete the synthesis of the 1st HNO donor 22a (room temperature, 1 hr).\(^{53, 54}\) Shorter reaction times (~ 10 min) revealed that the initial removal of the TIPS protecting group was successful; however, there was formation of an N-O triflyl migrated adduct 20. Figure 1.23 shows the structures of the desired HNO donor (22a) and the shorter reaction time adduct with accompanying NMR chemical shifts.

![Figure 1.23: Depiction of reaction time effect on deprotection of silyl ether 19](image.png)

Interestingly, 20 converts to 22a if the reaction is allowed to react longer (~ 1 hr). 22a can also be generated from 19 by using potassium fluoride in tetaethylene glycol (KF/TEG) which promotes rapid cleavage of the TIPS protecting group (rt, 30 mins, 57% yield) with no evidence of forming 20. Figure 1.22 also shows a minor byproduct pathway which is possibly from F⁻-mediated oxidation/N-O bond cleavage forming 21. Two related HNO donors 22b and 22c were also synthesized. This permitted a study of the sulfinate leaving groups on the HNO donor abilities of these targets. N-Sulfonation of 18 resulted in good yields for MeSO₂Cl (78% yield) and PhSO₂Cl (80% yield), and
cleavage of the TIPS protecting group also resulted in good yields (KF/TEG, 86-89% yield). No evidence of the byproduct 21 was observed in either case.

1.4 Photolysis results from HNO donors 22

1.4.1 Photolysis results of first HNO donor 22a

The photolysis sample was prepared by dissolving 1.00 mg of HNO donor 22a in a mixture of CD$_3$CN and phosphate buffer (0.16 mL CD$_3$CN/0.64 mL phosphate buffer (0.1 M, pH 7.00)). The sample solution was placed in a screw-cap NMR tube in the glovebox to ensure no oxygen was present during the transfer. The NMR tube was capped, and the sample solution was irradiated under a mini-photo reactor (Rayonet RMR-600) with 350 nm bulbs (8 lamps) for 35 min. The sample was monitored periodically every 5 minutes by $^{19}$F NMR spectroscopy after irradiation (Figure 1.24). Based on the $^{19}$F NMR spectrum above, CF$_3$SO$_2$NH$_2$ and CF$_3$SO$_2^-$ were formed. The integration ratio between CF$_3$SO$_2$NH$_2$ and CF$_3$SO$_2^-$ was slightly decreased during the photolysis process. Another intermediate ($\delta$ -75.64) was also formed during the photolysis process which began to fade as photolysis was continued. The presence of CF$_3$SO$_2^-$ suggested that HNO generation was indeed occurring based on the photolysis mechanism shown (Figure 1.25).
Figure 1.24: Photolysis of HNM-ONHSO$_2$CF$_3$ (22a) monitoring by $^{19}$F NMR spectroscopy
1.4.2 Proposed photolysis mechanism

Based on the general scheme for photoremovable of the HNM group in Figure 1.18 we proposed that the photochemistry of 22a should lead to release of HNO via the mechanism shown in Figure 1.25 (Pathway A).

Figure 1.25: Photolysis mechanism from 22a showing the wanted and unwanted photolytic reactions and the products formed.
The proposed reaction was intended to proceed via photodeprotonation of the phenolic OH, followed by intramolecular elimination to form the quinone methide (10) and an anionic intermediate (27). A series of steps then occurs to finally produce the diol byproduct (11) and the anionic species (27) decomposes rapidly to form HNO and the trifluoromethanesulfinate anion (28) (Pathway A). During photolysis of HNO donor molecule 22a, it was found that the generation of HNO was successful based on product characterization by fluorine (19F) NMR of the resulting leaving group, trifluoromethanesulfinate (28). In addition, the expected diol byproduct 11 was characterized by 1H NMR spectroscopy. However, another chemical species was found which accounted for 32-70% of the 19F NMR spectrum, depending on the solvent that was used for photolysis. It was postulated that an oxidative side reaction occurred that did not produce HNO, but instead formed a trifluoromethanesulfonamide byproduct 25 and an aldehyde species 21 (Pathway B).

This oxidative elimination pathway is the main concern that needs to be addressed in order to move this project forward. A solution to halt Pathway B was critical to the development of this new class of photolabile HNO donor.
1.5 Thesis project goal

1.5.1 Thesis project

The primary aim of the thesis project was to synthesize a compound that, during photolysis, might not follow the oxidative elimination pathway (see Pathway B, Figure 1.25). The target molecule incorporated an additional methyl substituent at the α-methine position (notified as an asterisk (*) on compound 29, Figure 1.26). It was of interest to understand how such a structural change would impact the selectivity between the different competing photolysis pathways. If competition from Pathway B could be suppressed, it was anticipated that clean formation of HNO via Pathway A could be achieved.

![Comparison of original and new classes of HNO donors](image)

Figure 1.26: Comparison of original and new classes of HNO donors

Literature investigations revealed two possible synthetic strategies that seemed feasible for making the desired compound 29. The first strategy, strategy A, involved the use of a directed ortho-lithiation approach which utilizes the chemistry of a directing metalation group (DMG which is designated as –PG in Figure 1.27). Such groups assist
with regioselective proton abstraction\textsuperscript{56} and in our case, we were hoping to explain a *ortho*-directing metalation group to selectively lithiate A at C-3 to form the intermediate X. Electrophilic trapping with acetaldehyde would then afford key intermediate Z.

Strategy B involved a longer synthesis that proceeded via reduction of B and reaction of Y with a methyl-organometallic nucleophile to afford the same common alcohol target Z.

More synthetic detail into the two strategies will be discussed later in the discussion section of the thesis.

*Figure 1.27: Retrosynthetic sequence for two proposed synthetic strategies for developing HNO donor 29.*
1.5.2 Proposed photolysis mechanism

It was of interest to probe how the incorporation of an additional methyl substituent at the benzylic position in 29 would impact the selectivity of the subsequent photochemistry. Our group postulated that the additional substituent at the α-methine carbon center (see Figure 1.26, compound 29) might inhibit the oxidative elimination pathway (see Figure 1.25, pathway B) that was troublesome in our 1st generation studies. If this was achieved, the new class of HNO donor should then only generate compound 30 to produce HNO and the innocuous diol byproduct after hydrolysis (see Figure 1.28);
however, the ultimate impact of the additional methyl substituent was uncertain, and it remained for us to synthesize HNO donor target 29 and experimentally probe its selectivity for photomediated HNO generation vs the oxidative side reaction.
CHAPTER 2. RESULTS AND EXPERIMENTAL DISCUSSION

2.1 Attempted synthesis of α-methyl-substituted photolabile caged CF$_3$SO$_2$NH-OHNM HNO donor 29

2.1.1 Synthetic strategy A towards α-methyl-substituted CF$_3$SO$_2$NHOHNM HNO donor 29.

As described in the introduction, two synthetic strategies were formulated to target the desired HNO donor compound (see Figure 1.24, compound 29). The first synthetic strategy involved the use of commercially available 2-naphthol and proceeded via the use of directed ortho-metalation (DOM) to incorporate the 2$^{nd}$ aromatic substituent at C-3. Shown below in Figure 2.1, is the synthetic route for this directed-ortho-metalation synthetic strategy.
Figure 2.1: The directed ortho-metalation reaction scheme for producing the α-methyl modified HNO donor molecule

Good DMGs (directing metalation groups) are strong complexing or chelating groups that have the effect of increasing the kinetic acidity of protons which are positioned ortho (or adjacent to) to the DMG. Generally, an arene bearing a DMG reacting with an alkylithium, leads to an ortho-metalated intermediate in solution, which can further react with an electrophile (see Figure 2.2).
Figure 2.2: Schematic showing the role of DMGs$^{55}$ in directing ortho-lithiation

There was literature support to suggest that lithiation could occur selectively with an appropriate DMG.

Figure 2.3: Chowdhury’s experiment with ortho-lithiation (reproduced from Chowdhury)$^{55}$

Chowdhury$^{55}$ suggested that it is possible for selective lithiation at the target site with the use of a methoxymethyl ether (MOM) or a methoxyl substituent.$^{55}$ The MOM group is a strong directing metalator and can enhance the ability of alkyllithium reagents
(or other strong bases) to abstract protons ortho to the MOM group. With alkyllithium reagents as the base, ortho-lithiated species can be formed and this species can then be reacted with an electrophile in one-pot to form a new product. The directing ortho-metallation (DoM) process normally demands the use of a powerful alkyllithium base (e.g. n-BuLi, or tert-BuLi) dissolved in organic solvents in which they exhibit high solubility. For a successful deprotonation to occur, the DMG must serve as a good coordinating site for alkyllithium and a poor electrophilic site for attack by a strong nucleophile. Steric hindrance, charge deactivation, or both may be incorporated into the design of the metallation director.

For the purpose of this project, acetaldehyde was the chosen electrophile to produce the alcohol which could serve as a flexible intermediate en route to target 29.

The first protection reaction involved a simple S_N2 reaction involving deprotonation of 2-naphthol (32) with sodium hydride (NaH) followed by nucleophilic attack on bromomethyl methyl ether. This reaction was highly reproducible and product 33 was obtained in very high yields (96%).

The second step involved ortho-lithiation/electrophilic trapping. Compound 33 was dissolved in anhydrous THF under argon to which a solution of n-BuLi in hexane was added dropwise while being cooled at -78 °C. Afterwards, acetaldehyde was added dropwise to the same reaction flask while being cooled to -78 °C. However, the reaction did not produce the desired product but, instead, two ortho-substituted regioisomers 35a and 35 were generated in a 2:1 ratio.
The main issue with this reaction was that the ortho-lithiation reaction was not selective. Compound 33 possesses two possible sites for ortho-lithiation to occur as noted by asterisks in Figure 2.5.

Both of the sites noted in Figure 2.5 must be lithiated during the reaction, given the formation of the regioisomeric mixture of 35 and 35a (see Figure 2.4). Figure 2.6 shows an expanded view of the $^1$H NMR spectrum of the crude reaction product, showing the $\alpha$-methyl signature of 35 and its regioisomer 35a.
Figure 2.6: Expanded view of the $^1$H NMR chemical shift (ppm) of the $\alpha$-methyl substituent on the regioisomers 35a (leftmost peak) and 35 (rightmost peak).

The chemical shifts at 1.69 and 1.59 ppm correspond to the $\alpha$-methyl substituents and the integration values show a 2.1:1 ratio of regioisomers. The doublet peak at 1.59 ppm is the desired regioisomer based on other indicative peaks in the aromatic region. Also, the spectrum of compound 43 obtained in a later study (see Figure 2.10) confirmed that the 1.59 ppm peaks are indicative of the desired regioisomer 35. The chromatographic separation of the regioisomeric compounds 35 and 35a was not successful because they are structurally very similar and had similar R$_f$ values in a range of solvent systems.
Several solvent systems were investigated, including 20% Et₂O/80% petroleum ether, 10% DCM/90% petroleum ether, and 10%/15%/20% EtOAc/90%/85%/80% petroleum ether. None of the solvent systems showed any separation of the isomers.

Several trials of the reaction were attempted where parameters were altered including varying the reaction temperature, reaction time and the amount of alkyllithium reagent employed. None of these changes proved successful and this initial synthetic route was put on hold.

Later literature searching revealed that the scope of the method was limited by regioselectivity of the initial metalation reaction in such cases where two nonequivalent ortho sites are available. Harvey\textsuperscript{57} and colleagues found that metalation of 2-(methoxymethoxy)-naphthalene with tert-BuLi followed by reaction with dimethylformamide (DMF) furnished two possible ortho-aldehydes in a 4:3 ratio. However, they did acquire their desired regioisomer, 2-hydroxy-3-naphthaldehyde, through the Reimer-Tieman reaction of 2-naphthol.\textsuperscript{57} The Reimer–Tiemann reaction is a reaction used for the ortho-formylation of phenols.\textsuperscript{57}
Figure 2.7: Reimer-Tiemann mechanism for ortho-formylation of phenols

Chloroform is deprotonated by a strong base (normally hydroxide) to form the chloroform carbanion which will quickly alpha-eliminate to give dichlorocarbene. The hydroxide species will also deprotonate the phenol to give a negatively charged phenolate. The negative charge is delocalized into the aromatic ring, making it far more nucleophilic and increasing its ortho selectivity. Nucleophilic attack on the dichlorocarbene from the ortho position gives an intermediate dichloromethyl substituted phenol. After elimination, the desired product is formed.
The Reimer-Tiemann reaction can be highly exothermic which makes it prone to thermal runaways and byproduct formation.\textsuperscript{57}

Other literature searching revealed that metalation of 2-methoxynaphthalene followed by reaction with \textit{N}-methylformanilide is reported to afford the 3-formyl derivative (67\%) accompanied by only 7\% of the 1-formyl isomer.\textsuperscript{57} The claim for this difference in product ratio was attributed to the greater steric bulk of \textit{N}-methylformanilide.\textsuperscript{57} This is unusual because regioselectivity is presumably determined during the initial deprotonation. In any case, this observation suggests an alternative method to direct formylation to a sterically less crowded ring position, which could allow access to the desired secondary alcohol 35 after reaction with acetaldehyde. Unfortunately, time constraints precluded this investigation.
2.2 Reevaluation of synthetic route for producing photolabile caged CF_3SO_2NHOHN HNO donor 29

2.2.1 Second synthetic strategy (B) toward the preparation of photolabile HNO donor 29

![Synthetic pathway diagram]

Figure 2.8: Original synthesis of the first photolabile caged CF_3SO_2NHOHN HNO donor 22

As stated previously in the introduction, a member of the Brasch/Sampson/Seed research group successfully synthesized our first generation of photoactivatable HNO donors (e.g. 22) through a seven step synthesis (see Figure 2.8). The initial reaction involved protection of the phenolic hydroxyl group of 13 through the use of a triisopropylsilyl ether. This reaction proceeded with a good yield to afford compound 14.
However, the reduction procedure utilizing LiAlH₄ did not result in good yields of 15. The most optimized reaction only proceeded in 21% yield and product purification proved difficult. The TIPS protecting group did not survive this reaction, resulting in the formation of depicted diol 41. A literature search revealed that reduction was accompanied by unwanted intramolecular hydride transfer (see Figure 2.9).[^58] [^59]

![Figure 2.9: Mechanism showing the intramolecular hydride transfer during reduction of 14](attachment:image.png)

**Figure 2.9: Mechanism showing the intramolecular hydride transfer during reduction of 14**

Saravanan et al. investigated this peculiar deprotection using varying positions of TBDMS protected phenols. They discovered that the proximity of the ester to silicon was responsible for the cleavage of the vicinal TBDMS ether.[^59] They synthesized a series of substrates where the ester was oriented α, β, and γ to the TBDMS ether, and in the cases of α and β esters, the TBDMS ether was reported to be cleaved.[^59] But with γ positioned esters, the TBDMS ether was only partially cleaved, while remote esters exhibited no cleavage of the TBDMS ether.[^59] It is important to note, as depicted in Figure 2.9, that the Al-complex needs to be present before cleavage of the silyl ether can take place.[^58]
My initial work in this area involved repeating the first two steps of the chemistry outlined in Figure 2.8. The first reaction, involving the protection of methyl 3-hydroxy-2-naphthanoate 13, was carried out in reproducible yields averaging to ~80%. As found by the previous worker in our group, the LiAlH₄ reduction of 14 to 15 did not proceed smoothly. Varying product yields were obtained and many of them were far from acceptable (2-13%). The inconsistency and poor outcome of this reduction step persuaded us to investigate alternative reduction conditions.

2.2.2 Reduction step modification to the synthesis of photolabile HNO donor 29

Figure 2.10: Synthetic strategy B for development of desired HNO donor 29
Our goal was to develop alternate reducing conditions that would not suffer from similar Al-complex hydride transfer side reactions (see Section 2.2.1). We concluded that the use of a milder reducing agent, such as sodium borohydride (NaBH₄), would hopefully improve product recovery and also prevent the loss of the TIPS protecting group during reduction of the ester. The challenge with using NaBH₄ is that it is slow to react with esters by itself. However, literature searching revealed a communication detailing research into the reductive potential of various combinations of additives to reducing agents like KBH₄ and NaBH₄. They found that the combinations of HfCl₄/KBH₄ and HfCl₄/NaBH₄ were more facile, efficient, and chemoselective reducing systems, that reduce esters in good yields under mild conditions. HfCl₄ has been used as a catalyst in organic synthesis, including intramolecular allylsilylation of alkynes, esterification of carboxylic acids, and synthesis of benzimidazole derivatives. Zhang postulated that NaBH₄ is as active as KBH₄ for the reduction of carboxylic acids and their derivatives to the corresponding alcohols. The reactivity of a hydride reducing agent is influenced by the bulkiness of the reagent and the Lewis acidity of the counter-ion. HfCl₄/KBH₄ and HfCl₄/NaBH₄ undergo the formation of Hf(BH₄)₄ first, which is the active species in the reduction process. It is possible that Hf could also act as a Lewis acid and complex to the ester carbonyl oxygen and increase the electrophilicity of the ester. One example reported by Zhang involved the reduction of naphthoate ester 47, which was cleanly reduced to the primary alcohol 11 in 87% yield (Figure 2.11).
Thus, we considered it likely that this method would be possibly useful for our reduction of ester 14, so long as the unwanted hydride transfer reaction could be avoided.

However, when the reduction of ester 14 was carried out exactly as described in the literature source, the yield of product 15 was negligible. Different variables were adjusted for the reduction reaction including molar ratios of reagents, reaction time, and temperature of the reaction. Initially, none of the changes seemed to make a difference to the yield of the reaction. However, an examination of the MSDS (Material Safety Data Sheet) of HfCl₄ revealed that the reagent is highly hygroscopic and degrades to form HCl. Previously we had conducted the reduction reaction under an inert atmosphere but had not taken rigorous precautions to exclude all air during weighing of the HfCl₄. This discovery lead to an attempt to rigorously exclude air and oxygen throughout the entire procedure. All materials were collected and weighed under an inert (argon) atmosphere using a glove box, and the solvent, distilled THF, and starting material were all added by syringe to the reaction flask through a septum. The reaction progress was monitored by TLC to confirm that the starting material had been consumed. Table 2.1 describes all of
the various trials under these conditions and product yields that led to the optimization of the reduction procedure.

![Chemical Reaction](image)

<table>
<thead>
<tr>
<th>Molar equivalents used (HfCl₄/NaBH₄)</th>
<th>Reaction Temperature (°C)</th>
<th>Reaction Time (hours)</th>
<th>Isolated Yield of 15 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.32/5.26</td>
<td>22</td>
<td>1.5</td>
<td>(30%)</td>
</tr>
<tr>
<td>1.4/12.69</td>
<td>22</td>
<td>42</td>
<td>(68%)</td>
</tr>
<tr>
<td>1.4/3.8</td>
<td>24</td>
<td>28</td>
<td>(78%)</td>
</tr>
<tr>
<td>1.25/2.9</td>
<td>24</td>
<td>22</td>
<td>(81%)</td>
</tr>
</tbody>
</table>

**Table 2.1: HfCl₄/NaBH₄ reduction of ester 14: reaction parameters and yields**

From carrying out various trials and scale-ups ranging from 100-300 milligram to gram-scale reactions, the new reduction reaction has proved to be successful and reproducible. The molar ratios of HfCl₄ and NaBH₄ were not as crucial as the reaction time. Zhang’s reduction of naphthoate ester 47 was complete in 1.5 hr at room temperature, while our reaction required ~22 hr to go to completion. The reasons behind this difference are unclear. One possibility could be the bulky nature of the TIPS
protecting group which could possibly slow the progress of the reaction due to steric effects, or that the reduction of 47 proceeds via complex 48 with intramolecular delivery of hydride, which is likely to be faster than an analogous intermolecular reduction (Figure 2.12).

![Intramolecular delivery of hydride to a α-carbonyl ester](image)

**Figure 2.12: Intramolecular delivery of hydride to a α-carbonyl ester**

The next step in our synthesis required oxidation of 15 to aldehyde 42 (Figure 2.10). We found that the use of Dess-Martin periodinane (DMP) was an effective approach. This reaction was reproducible and resulted in good yields averaging about 80%.

The next step involved reaction of aldehyde 42 with CH$_3$MgBr to target secondary alcohol 43. The Grignard reaction was also a high yielding reaction. The original literature procedure (used as a model for the reaction of an aldehyde with CH$_3$MgBr) stated that the addition of the organometallic reagent was performed in two separate equimolar quantities. When reproducing the literature procedure, the yield of the reaction was moderate (~70%). The reaction procedure was adjusted slightly by adding...
the organometallic reagent in a single portion in molar excess and the yield of the product 43 was considerably higher (90%).

2.2.3 Mitsunobu reaction step modification to the synthesis of photolabile HNO donor 29

Previous approaches toward making our first photolabile caged CF$_3$SO$_2$NHOHN HNO donors 22a-c (see Figure 1.22) had employed a sequence involving conversion of primary alcohol 15 to alkyl bromide 16, followed by S$_{N}$2 displacement with an N-alkoxyphthalimide nucleophile to afford the N-alkoxyphthalimide 17 (see Figure 2.8). In the present synthesis, we decided to exploit an alternate shorter route involving a Mitsunobu reaction, which we considered should directly allow the conversion of secondary alcohol 43 to 44. The Mitsunobu reaction allows the conversion of primary and secondary alcohols to esters, phenyl ethers, thioethers and various other compounds. The nucleophile employed should be acidic, since one of the reagents (DIAD, diisopropylazodicarboxylate) must be protonated during the course of the reaction to prevent side reactions. The Mitsunobu reaction (Figure 2.13) is very appealing due to its reaction scope, stereospecificity, mild reaction conditions, and the wide array of commercially available alcohols that can be used as substrates.
Figure 2.13: Mitsunobu reaction mechanism for the formation of compound 44

Typical substrates for the Mitsunobu reaction are primary and secondary alcohols. Chiral secondary alcohols undergo complete inversion of configuration if they are not sterically congested. The nucleophilic component for the Mitsunobu reaction is usually a relatively acidic compound containing an O-H, S-H, or an N-H group with a $pK_a$ value $\leq 15$. Common nucleophiles include carboxylic acids, phenols, imides,
purine/pyrimidine bases, thiols and fluorinated alcohols. Some N-nucleophiles can then be further modified through hydrolysis (in the case of using phthalimide; Gabriel synthesis) or, selectively reduced (in the case of azide formation; Staudinger reaction) to make the corresponding amine accessible.

The Mitsunobu reaction that was investigated began by dissolving triphenyl phosphine (PPh$_3$), N-hydroxyphthalimide, and alcohol 43 in freshly distilled dichloromethane under argon. Then, the reaction flask was cooled in an ice-water bath to 6 °C. After cooling, diisopropylazodicarboxylate (DIAD) was added dropwise to the reaction flask solution.

However, yields for our Mitsunobu reaction (43 to 44) were very poor, ranging from 10-15%. Literature sources stated that secondary benzyl alcohols are susceptible to facile Mitsunobu reactions. However, none of the reactions were acheived in good yield. Trials were performed where reaction temperature was varied but none produced a clean reaction or high yield. Reaction temperatures were kept between 2 – 5 °C and 21 – 25 °C with the cooler temperature range producing slightly higher yielding reactions. Side reaction products were also observed from the reaction but were not fully characterized due to time constraints.

A subsequent literature search revealed an interesting article published by Durand and coworkers. Durand describes the use of different bases to enhance the yield and purity of the Mitsunobu product along with the use of a polymer-based N-hydroxyphthalimide reagent. The reasoning for why they decided to investigate specific
base effects was due to complications from rearrangement reactions during the Mitsunobu reaction and also during subsequent hydrazinolysis of the phthalimide moiety. Durand continually encountered issues with regeneration of starting alcohol and they attributed it to possible Lossen Rearrangement (Figure 2.14).

**Figure 2.14: Lossen Rearrangement mechanism proposed by Durand**

(reproduced from Durand)\(^68\)

Other investigations showed that products having an activated hydroxy group undergo Lossen rearrangement to isocyanates as intermediates, followed by degradation to primary amines in the presence of water.\(^69\) Different reaction conditions were examined, varying the solvent, temperature, the order of reagent addition and reaction time. To solve this issue, Durand and colleagues introduced a spacer between the resin backbone and the N-hydroxyphthalimide moiety in their polymer-supported N-hydroxyphthalimide. The spacer increased the yield of the reaction, possibly due to relief
of steric effects that can limit the effectiveness of the Mitsunobu reaction. Durand also found that certain bases had a profound effect on the resulting yield of the Mitsunobu reaction and the purity of the products. The most intriguing result was when they compared secondary and primary alcohol reactivity with the introduction of specific bases. Using a reagent combination of Ph₃P/DIAD/imidazole (base)/alcohol, products of ~93% purity were obtained in ~100% yield. This result was quite remarkable and Durand could not find any correlation between the pKₐ of the amine base and the activation of the reaction. Swamy compiled a review article which highlighted Durand’s work.

If time constraints were not an issue, the Mitsunobu reaction conditions we employed would have been adjusted to include imidazole to observe if purity and yield would have been improved based on the results that Durand obtained.

However, due to time constraints, we were unable to further optimize the Mitsunobu step. The product of the Mitsunobu reaction (compound 44) can then be subjected to subsequent hydrazinolysis of the phthalimide group to allow access to the N-alkoxyamine intermediate 45. Thus, we continued with 44 accumulated from our unoptimized reactions. Compound 44 was converted to N-alkoxyamine 45 good yield via hydrazinolysis. However, purification of this product proved difficult. Since compound 45 is an N-alkoxyamine, the amine functionality can interact strongly with the silica stationary phase during column chromatography. As a result, the product was never recovered from the chromatography column. Thus, due to time constraints, the product
from the second trial of the hydrazinolysis reaction was not purified and instead was
directly reacted with trifluoromethanesulfonfyl chloride to afford compound 46. The first
time this reaction was carried out, it was not successful. This was due to the temperature
of the reaction not being optimal. Cooling the sulfonation reaction using an ice-salt water
bath was not sufficient to ensure a clean monosulfonation reaction. It appeared that bis-
trifluoromethanesulfonation was occurring on compound 45 if the reaction temperature is
not kept sufficiently low. An acetone-dry ice bath (-78 °C) proved necessary to ensure
that only mono-sulfonation occurs at the nitrogen in 45. After changing the reaction
temperature to -78 °C, the reaction yielded compound 46 in moderate yield (58%). 19F
NMR analysis of 46 showed a clean product after column chromatography with only one
major peak. 1H NMR analysis showed a very clean spectrum of the product. The next
reaction involving cleavage of the TIPS protecting group to afford HNO donor 29 did not
go smoothly. The reaction conditions were very mild; compound 46 was dissolved in a
small amount of dichloromethane and a 0.15 M solution of potassium fluoride in
tetraethylene glycol (KF/TEG) was added and left to stir for an hour. However, when
attempts to carry out using 30% EtOAc/70% petroleum ether as eluent.
Unfortunately, none of the column fractions contained the desired product 29. This may have been due to decomposition on the chromatography column, although this seems unlikely. After flushing the column with a polar solvent (EtOAc), still no desired product was recovered. One possibility for the lack of desired product could be that the extended reaction time could have allowed for the fluoride ion to cleave not only the silyl ether functional group but also the \( N \)-trifluoromethanesulfonate group. Fluoride ions are good nucleophiles in anhydrous conditions. So, the fluorine peak observed in the \(^{19}\text{F} \) NMR spectrum could have been due to the formation of CF\(_3\)SO\(_2\)F as a byproduct (lit. \(^{19}\text{F} \) NMR: \( \delta -113.8 \) (s, 1F, SO\(_2\)F), 2.71 (d, \( J = 17.9 \) Hz, 3F, CF\(_3\))\(^{70} \) from the reaction.

Due to problems with the group’s glove box, we were able to make no further progress with this chemistry prior to the deadline for submission of the thesis. Furthermore, since no appreciable amount of compound 29 could be obtained, we were unable to study the photochemistry of our target compound 29.

### 2.3 Potential future goals

Optimization of the silyl-cleaving reaction and the Mitsunobu reaction are necessary to develop a higher yield of synthetic intermediates and ultimately HNO donor 29. Once the synthetic reactions can be optimized, the photolysis of 29 will be studied. The hope of the thesis was to observe if the oxidative side reaction seen during the photolysis of analogous HNO donor 22 would be eliminated due to the introduction of
the α-methyl substituent. If the photolysis indeed does show promise for eliminating the oxidative pathway, various R substituents can be probed at * to observe if they will generate HNO more cleanly or not, and other RSO₂⁻ leaving groups can also be investigated to see if they impact the rate and selectivity of the photolysis leading to HNO generation.

![Chemical structure](image)

**Figure 2.15: Schematic showing future potential α-substituted HNM-ONHΣO₂CF₃ HNO donors**
CHAPTER 3. EXPERIMENTAL DETAILS

3.1 General considerations

Unless otherwise noted, all chemicals were used as received from the supplier (Sigma-Aldrich, Acros Organics, TCI America, and/or Alfa Aesar). All structural elucidation was performed using $^1$H NMR (400 MHz, Bruker Avance 400 MHz spectrometer running Topspin version 2.1 software with TMS as internal standard) and $^{19}$F NMR (376 MHz) spectroscopy. Reactions were monitored using TLC (aluminum-backed silica gel plates, Sigma-Aldrich, 200 μm layer thickness, 2-25 μm particle size and 60 Å pore size) or previously described NMR techniques. Column chromatography was typically performed under positive air pressure (flash column chromatography) using Fisher Scientific silica gel (SiliaFlash®, 230-400 Mesh). Anhydrous tetrahydrofuran and diethyl ether were distilled from sodium metal/benzophenone, anhydrous dichloromethane was distilled from CaH₂, and all other solvents/reagents not used as received were purified as noted. All anhydrous solvents were distilled under argon except for DMF, which was used directly from the chemical supplier. When glassware is said to be “oven-dried”, the glass was thoroughly washed, rinsed with acetone, dried in an oven overnight (approx. 150° C), and cooled to room temperature. Petroleum ether (bp 66 to 68 °C), ethyl acetate, dichloromethane and diethyl ether used in column chromatography were distilled prior to use.
3.2 Experimental details and schemes

![Synthetic scheme for the preparation of CF₃SO₂NH-OHNH donor](image)

**Figure 3.1**: Synthetic scheme for the preparation of CF₃SO₂NH-OHNH donor

29 (Strategy A)
**2-(Methoxymethoxy)naphthalene (33)**

NaH (0.872 g, 36.3 mmol) was added to a 100 mL 3-neck RB flask under argon in a glove box. Anhydrous THF (24 mL) was added and the flask was clamped in an ice bath with stirring. Some bubbles were produced indicating that the solvent was not entirely dry. 2-Naphthol (2.59 g, 18.0 mmol) was added to a separate 100mL RB flask under nitrogen gas. The compound was dissolved in anhydrous THF (12 mL) and the resulting solution was added dropwise by syringe to the reaction flask containing the NaH solution. Bubbles were observed indicating that hydrogen gas was being produced. The internal temperature of the reaction mixture rose to 18 °C from 0 °C. The reaction was left to stir for 90 minutes in the ice bath at 0°C. After that time, the reaction mixture was allowed to warm to room temperature. The reaction mixture was stirred at room temperature for 30 minutes. As the solution was stirred at room temperature, the solution turned a pale-yellow color. Then, methoxymethyl bromide (1.5 mL, ρ = 1.531 g/mL at 25°C, 18.4 mmol) was added dropwise to the reaction flask with stirring. The cloudy yellow solution changed to a cloudy white solution. The internal temperature of the reaction mixture changed from 8 °C to 28 °C. The reaction was left to stir overnight for 17 hours at room temperature. Once stirring was ceased, the solution was poured into a 500 mL separatory funnel containing diethyl ether (70 mL). The solution was then
washed with water (2 x 20 mL). The aqueous layer was a dark yellow color. The combined organic extracts were then dried with magnesium sulfate, filtered and concentrated in vacuo to leave crude red colored oil (3.57 g). TLC analysis confirmed the starting material had been consumed (10% EtOAc/90% petroleum ether). Column chromatography was used to purify the crude material using 10% EtOAc/90% petroleum ether and 45.6 g of flash silica. After fraction 22, the eluent ratio was changed to 20%EtOAc/80% petroleum ether. Fractions 4-24 were collected and concentrated in vacuo (P₂O₅ and paraffin wax) and the remaining material was flushed with ethyl acetate. After being concentrated, fractions 4-24 resulted in 3.26 g of the title compound as a red-colored oil (96% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.778 (d, J = 2.8 Hz, 1H), 7.752 (s, 1H), 7.747 (d, J = 12.4 Hz, 1H), 7.436 (td, J = 6.8 Hz, 1.2 Hz, 1H), 7.399 (d, J = 2.4 Hz, 1H), 7.350 (td, J = 8 Hz, 1.2 Hz, 1H), 7.217 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 5.299 (s, 2H), 3.526 (s, 3H).

1-(3-(Methoxymethoxy)naphthalen-2-yl)ethanol (35)

2-Methoxymethoxynaphthalene (33) (1.02 g, 5.42 mmol) was added to a 100 mL 3-neck RB flask with a magnetic stir bar. The flask was then flushed with nitrogen gas. The bubbler used was equipped with a cold-temperature reading thermometer and the apparatus was placed in a dry ice/acetone bath to cool the reaction mixture to -78°C. 2-
Methoxymethoxynaphthalene (33) was then dissolved in anhydrous THF (10 mL). \( n \)-BuLi (3.5 mL, 6.1 mmol, 1.73M in cyclohexane) was added to the reaction mixture dropwise by syringe. The internal temperature of the reaction mixture was \(-74°C\) prior to the addition of \( n \)-BuLi; the temperature during addition fluctuated between \(-65°C\) and \(-71°C\). The reaction mixture was left to stir for 30 minutes. After that time, the dry ice/acetone bath was removed and the reaction mixture was allowed to warm to room temperature for 50 minutes while stirring. The solution began as a dull-orange color which transitioned to a blood-red color and then a dark-red color. The reaction mixture was again placed in the dry ice/acetone bath to cool to \(-78°C\). Acetaldehyde (0.32 mL, \( \rho = 0.78 \text{ g/mL} \), 5.7 mmol) dissolved in anhydrous THF (6 mL) was then added dropwise by syringe to the reaction mixture. The internal temperature of the reaction flask fluctuated between \(-73°C\) and \(-68°C\). The reaction mixture was left to stir for 17 hours while warming to room temperature slowly. Stirring and \( \text{N}_2 \) gas protection was ceased and the reaction was quenched with saturated aq. \( \text{NH}_4\text{Cl} \) (80 mL). The mixture was extracted with ethyl acetate (2 x 25 mL). The combined organic extracts were dried over sodium sulfate and the organic solvent was removed \textit{in vacuo} to afford a bright-red colored oil (1.43 g). A second workup was performed to remove additional precipitated impurities. The crude product was then dissolved in ethyl acetate (20 mL) and washed with water (25 mL) and brine (25 mL) in a separatory funnel. The organic layer was dried over sodium sulfate and the organic solvent was removed \textit{in vacuo} to afford a dull-orange oil (1.26 g). Column chromatography was performed on the crude material using 10%
EtOAc/90% petroleum ether. Fractions 2-7 collected the first impurity, fractions 9-14 collected the starting material and the first evidence of the product spot was detected at fraction 17. After concentrating all of the collected fractions in vacuo, \(^1\)H NMR analysis of the fractions containing the product revealed that two regioisomers were present. TLC analysis of the product was tested with different solvent systems (20% Et\(_2\)O/80% petroleum ether, 10% DCM/90% petroleum ether, and 10%/15%/20% EtOAc/90%/85%/80% petroleum ether) with all of them showing a separation of a minor amount of starting material and a collective streak of a major compound. The isomeric products could not be separated and had a collective weight of 0.91 g. The \(^1\)H NMR spectrum showed evidence that two isomers 35 and 35a had formed due to duplicate methylene peaks at 5.3 ppm and 3.5 ppm. The fraction containing the regioisomers was left to dry overnight in a vacuum desiccator. The weight of the regioisomeric alcohol products was 0.908 g (72%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.198 (d, \(J = 8.8\) Hz, 1H), 7.840 (s, 1H), 7.744 (m, 3H), 7.471 (dt, \(J = 8.0\) Hz, 1.2 Hz, 1H), 7.376 (m, 3H), 5.790 (br s, 1H), 5.293 (m, 3H), 4.771 (br s, 1H), 3.533 (s, 3H), 3.509 (s, 1H), 1.687 (d, \(J = 6.8\) Hz, 3H), 1.586 (d, \(J = 6.4\) Hz, 1H). [Solvent and impurity peaks were excluded].

Figure 3.2: Synthesis of (3-(methoxymethoxy)naphthalen-2-yl)methanol 50
**Methyl 3-(methoxymethoxy)-2-naphthoate (49)**

\[
\text{Methyl 3-hydroxynaphthanoate (13) dissolved in anhydrous THF (10 mL) in a separate flask under N}_2\text{. The methyl 3-hydroxynaphthanoate solution was added dropwise (22 °C) by syringe, to the stirred reaction flask. Bubbles were observed indicating the release of hydrogen gas. The solution was left to stir for 10 minutes while cooling in the ice bath (15°C → 18°C). The solution turned a yellow-orange color. Anhydrous THF (5 mL) was used to extract the remaining starting material from the flask which held the starting material and was added to the reaction flask. This caused a dark brown color to be produced which slowly changed back to the original yellow-orange color after the cold water bath was removed and allowed to stir for an hour at room temperature. Methoxymethyl bromide (1.1 mL, ρ = 1.531 g/mL at 25°C, 13 mmol) was added dropwise by syringe to the reaction mixture [21°C → 26°C]. The mixture changed to bright yellow and then cloudy white. The reaction mixture was left to stir for two hours. The reaction mixture was then quenched with water (50 mL).} \]

NaH (0.598 g, 24.9 mmol) was added to a 100 mL 3-neck, RB-flask under argon gas in a glove box along with a magnetic stirring bar. Then, the reaction flask was protected under a flow of N\(_2\) gas. Anhydrous THF (10 mL) was charged to the flask to create a gray suspension. The reaction mixture was chilled to 0°C in an ice water bath.
aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic extracts were dried over MgSO₄, filtered, and the organic solvent was removed in vacuo to afford a yellow crude oil (2.60 g). TLC analysis with an eluent of 10% EtOAc/90% PET ether showed that product and starting material were present. Flash column chromatography was performed with 10% EtOAc/90% petroleum ether. After the 18th fraction, the eluent was changed to 20% EtOAc/80% petroleum ether. The first compound collected was the starting material (1.21 g, 39%) as yellow crystals. The second compound was the title compound (1.83 g, 60%). ¹H NMR (400 MHz, CDCl₃) δ 8.301 (s, 1H), 7.814 (d, J = 8.0 Hz, 1H), 7.737 (d, J = 8.4 Hz, 1H), 7.507 (td, J = 6.0 Hz, 1H), 7.498 (s, 1H), 7.389 (td, J = 6.0 Hz, 1H), 5.358 (s, 2H), 3.945 (s, 3H), 3.562 (s, 3H).

(3-(Methoxymethoxy)naphthalen-2-yl)methanol (50)

To a 3-neck, 250 mL, RB-flask, LiAlH₄ (0.33 g, 8.7 mmol) was suspended in anhydrous THF (10 mL) under argon and stirred and cooled in an ice bath to 0°C. Methyl 3-(methoxymethoxy)-2-naphthanoate (49) (0.65 g, 2.6 mmol) was added to a separate flask and dissolved in anhydrous THF (10 mL) under argon. The methyl 3-(methoxymethoxy)-2-napthanoate solution was added dropwise by syringe at room temperature to the main reaction flask. Bubbles were observed forming from the reaction mixture. The reaction mixture was left to stir for an hour and 45 minutes while warming
up to room temperature. The stirring was halted and the reaction mixture was quenched with chilled water (7 mL) and extracted using dichloromethane (2 x 10 mL). The organic layers were difficult to observe and aq. HCl (0.5 mL, 6M, 3.0 mmol) was added to the separatory funnel mixture which helped to discern between the two separate solvent layers. These layers were separated. The combined organic extracts were washed with water (25 mL) and aq. HCl (1 mL, 6M, 6.0 mmol). The organic extract was dried over MgSO₄, filtered, and concentrated in vacuo. The product obtained was a yellow-colored oil which was dried in a vacuum desiccator (0.39 g, 68%). ¹H NMR (400 MHz, CDCl₃) δ 7.759 (d, J = 8.0 Hz, 1H), 7.763 (s, 1H), 7.727 (d, J = 8.0 Hz, 1H), 7.429 (td, J = 7.7 Hz, 1.2 Hz, 1H), 7.401 (s, 1H), 7.359 (td, J = 7.5 Hz, 1.6 Hz, 1H), 5.351 (s, 2H), 4.851 (s, 2H), 3.520 (s, 3H), 1.119 (br s, 1H).
Figure 3.3: Synthetic scheme for CF$_3$SO$_2$NH-OHNMe donor 29 (Strategy B)

*Methyl 3-(triisopropylsilyloxy)-2-naphthoate (14)*

- Reaction 1: Methyl 3-(triisopropylsilyloxy)-2-naphthoate (14) is prepared with DMAP and imidazole in DMF (80% yield).
- Reaction 2: Compound 14 is treated with HCl and NaBH$_4$ to yield alcohol 15 in THF (77% yield).
- Reaction 3: Compound 15 undergoes a reduction with DMP and Na$_2$S$_2$O$_4$, followed by treatment with H$_2$O (79% yield).
- Reaction 4: Compound 14 is reacted with DIAD in CH$_2$Cl$_2$ (15% yield).
- Reaction 5: Compound 42 is obtained by reacting with CH$_3$MgBr in anhydrous Et$_2$O (90% yield).
- Reaction 6: Compound 43 is transformed into compound 44 via Ph$_2$P-N-hydroxyphthalimide.
- Reaction 7: Compound 44 is treated with CF$_3$SO$_2$Cl and DMAP in pyridine and CH$_2$Cl$_2$ (58% yield).
- Reaction 8: Compound 45 is converted into compound 46 with KF and Tetraethylene glycol.
- Reaction 9: Compound 46 is transformed into compound 29 with CF$_3$SO$_2$Cl and DMAP in pyridine and CH$_2$Cl$_2$ (58% yield).

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Note: The reactions and yields are illustrative and may vary in actual experimental conditions.
Methyl 3-hydroxy-2-naphthoate (13) (3.02 g, 14.9 mmol), N, N-(dimethylamino)pyridine (DMAP) (153 mg, 1.25 mmol), imidazole (1.58 g, 23.2 mmol), and anhydrous DMF (20 mL) were all added to a 3-neck, 250 mL RB flask under argon with stirring. Once all of the starting material and base was dissolved (15 min), TIPS-Cl (5.0 mL, ρ= 0.901 g/mL at 25°C, 23.4 mmol) was added dropwise by syringe at room temperature to the stirred reaction mixture. The solution was a golden-yellow color. The reaction was then left to stir at room temperature for 28 h. A 0.3 mL aliquot of the reaction mixture was taken and quenched with saturated aqueous sodium bicarbonate. Then, the product was extracted using diethyl ether and washed with brine. TLC analysis (using an eluent ratio of 10% EtOAc/90% petroleum ether) of the sample showed that the starting material had been consumed. Stirring of the reaction mixture was halted and the mixture was quenched with saturated aqueous NaHCO₃ (60 mL). After being transferred to a 250 mL separatory funnel, diethyl ether (3 x 20 mL) was then added to the funnel to extract the organic products. The combined organic extracts were washed with brine (60 mL). Afterwards, the organic layer was dried over magnesium sulfate (1.77 g), filtered, and the organic solvent was removed in vacuo. Silica flash column chromatography was performed with 104.5 g of flash silica gel and the eluent used was a mixture of 5% EtOAc/95% petroleum ether. Fractions 11-31 were collected and the solvent was removed in vacuo. After drying in a vacuum desiccator for two days the title compound was obtained as an opaque, yellow-colored oil (4.27 g, 80%). ¹H NMR (400 MHz, CDCl₃) δ 8.237 (s, 1H), 7.796 (d, J = 8.0 Hz, 1H), 7.654 (d, J = 8.0 Hz, 1H), 7.467
(td, J = 7.6 Hz, 1.2 Hz, 1H), 7.342 (td, J = 7.6 Hz, 1.2 Hz, 1H), 7.183 (s, 1H), 3.919 (s, 3H), 1.373 (septet, J = 7.6 Hz, 3H), 1.144 (d, J = 7.6 Hz, 18H).

**(3-(Triisopropylsilyloxy)naphthalen-2-yl)methanol (15)**

A 250 mL 3-neck RB flask was charged with HfCl₄ (1.5 g, 4.7 mmol) and NaBH₄ (0.4 g, 11 mmol) under argon in a glovebox. This mixture was then suspended in anhydrous THF (10 mL) to create a cream-colored suspension. The mixture was stirred for 10 minutes in an ice water bath (3 °C). Then, the reaction mixture was stirred for 2 hours at room temperature (24 °C). A separately prepared solution of methyl 3-triisopropylsilyloxy-2-naphthanoate (14) (1.32 g, 3.68 mmol) in anhydrous THF (20 mL) was added dropwise by syringe to the stirred reaction flask at room temperature. The reaction was then left to stir for 22 hours. After such time, a 0.25 mL aliquot of the reaction mixture was tested for the presence of the desired product by first quenching with water and extracting with EtOAc. Then the organic layer was washed sequentially with small portions of saturated aqueous sodium bicarbonate and brine. TLC analysis of the organic layer confirmed that the starting material had been consumed. The reaction mixture was slowly quenched with chilled water (40 mL) in separate 5 mL portions.
exothermic reaction was observed that was accompanied by the evolution of gas. The mixture was then extracted using EtOAc (3 x 15 mL). The combined organic extracts were then washed with saturated aqueous sodium bicarbonate (40 mL) and brine (40 mL). The organic layer was dried with Na$_2$SO$_4$ (2.9 g), filtered, and the organic solvent was removed in vacuo to leave a slightly yellow opaque oil (1.21 g). The crude product was then left to dry in a vacuum desiccator. After drying, the product weighed 1.09 g.

Flash column chromatography was then performed with 109.8 g of flash silica gel and an eluent of 7% EtOAc/93% petroleum ether. After fraction 24, the eluent was changed to 10% EtOAc/90% petroleum ether and the first observation of the product by TLC, was on fraction 36. Fractions 36-60 were collected and the solvent was removed in vacuo and the resulting product was then placed in a vacuum desiccator to dry overnight, to afford a yellow-tinted oil (0.94 g, 77% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.771 (s, 1H), 7.761 (d, $J$ = 8.4 Hz, 1H), 7.656 (d, $J$ = 8.0 Hz, 1H), 7.403 (td, $J$ = 7.2 Hz, 1.2 Hz, 1H), 7.329 (td, $J$ = 7.6 Hz, 1.2 Hz, 1H), 7.138 (s, 1H), 4.867 (s, 2H), 2.239 (br s, 1H), 1.409 (septet, $J$ = 7.6 Hz, 3H), 1.159 (d, $J$ = 7.6 Hz, 18H).

3-(Triisopropylsilyloxy)-2-naphthaldehyde (42)

![Chemical Structure Image]
To a 250 mL, 3-neck, RB flask equipped with a condenser, an argon gas line and a magnetic stirring bar, was added Dess-Martin periodinane (DMP) (1.48 g, 3.49 mmol). The reaction flask was placed in an ice-water bath. To a separate flask, alcohol 15 (0.94 g, 2.8 mmol) was dissolved in freshly distilled dichloromethane (40 mL) that was then chilled under argon in a separate ice-water bath. The alcohol solution was added dropwise by syringe to the reaction flask in two portions (20 mL) with stirring. The solution turned a cloudy yellow color. The reaction mixture was then left to stir for 30 minutes in the ice-water bath and then, for an hour at room temperature (21 °C). After this time, the condenser and argon gas line were removed and the reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (30 mL) and solid sodium thiosulfate (2.0 g). The reaction mixture was then stirred vigorously for 10 minutes. After this time, the layers were separated in a separatory funnel. The organic layer was washed with water (3 x 50 mL) and the combined aqueous washings were extracted with diethyl ether (3 x 35 mL). The combined organic layers were then dried with Na₂SO₄ (5.0 g), filtered, and concentrated in vacuo to afford an orange-colored oil (0.79 g). Flash column chromatography was performed with 104.33 g of silica and an eluent of 7% EtOAc/ 93% petroleum ether. Fractions 11-31 were collected and concentrated in vacuo. The remaining material was placed in a vacuum desiccator for 24 hours to remove any other residual solvent. Then, the material was placed in a freezer for three days. The resulting product was a bright yellow-colored solid (0.74 g, 79%). ¹H NMR (400 MHz, CDCl₃) δ 10.674 (s, 1H), 8.373 (s, 1H), 7.876 (d, J = 8.0 Hz, 1H), 7.673 (d, J = 8.0 Hz, 1H), 7.509
(td, \(J = 8.4\) Hz, 1.6 Hz, 1H), 7.369 (td, \(J = 8\) Hz, 1.2 Hz, 1H), 7.191 (s, 1H), 1.422 (septet, \(J = 8\) Hz, 3H), 1.167 (d, \(J = 7.6\) Hz, 18H).

\(1\)-(3-((triisopropylsilyl)oxy)naphthalen-2-yl)ethanol (43)

\[\text{OTIPS} \quad \begin{array}{c} \text{H} \\ \text{(i) MeMgBr (2.5 eq)} \\ \text{anhydrous Et}_2\text{O} \quad 0^\circ\text{C} \rightarrow \text{r.t.} \\ \text{(ii) NH}_4\text{Cl (aq)} \end{array} \quad \begin{array}{c} \text{OTIPS} \\ \text{OH} \end{array} \]

A 2-neck 250 mL RB-flask was equipped a condenser, a magnetic stir bar, and a thermometer. The reaction flask was then flushed with argon using a schlenk line. Aldehyde 42 (1.15 g, 3.50 mmol) was dissolved in anhydrous diethyl ether (20 mL) under argon and the reaction flask was then placed in an ice-water bath and chilled to 3 °C. Methylmagnesium bromide (3.0 mL, 7.5 mmol, 3.0M in diethyl ether) was then added dropwise by syringe to the reaction flask (the internal temperature was 3 °C - 12 °C). The reaction mixture was left to stir for an hour and a half while the reaction flask was left to progressively warm to room temperature (internal temperature 12 °C → 22 °C). Then, the reaction was stopped and the argon gas line was shut off. Saturated aqueous ammonium chloride (30 mL) was used to quench the reaction and protonate the alcohol product. The organic and aqueous layers were separated in a 125-mL separatory funnel. The aqueous layer was then extracted with Et2O (3 x 15 mL). The combined organic layers were washed with brine (30 mL), dried with magnesium sulfate and
concentrated in vacuo. The resulting crude yellow-tinted oil was placed in a vacuum desiccator for 24 hours (1.22 g). The crude product was then purified by column chromatography using 92 g of silica gel, and an eluent of 10% EtOAc/90% petroleum ether. Fractions 15-30 were collected and concentrated in vacuo. $^1$H NMR analysis of the product showed that solvent was still present so it was left in a freezer for six days and then dried in a vacuum desiccator. The product formed was a yellow-tinted viscous oil (1.09 g, 90% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.848 (s, 1H), 7.769 (d, $J = 7.6$ Hz, 1H), 7.643 (d, $J = 8.0$ Hz, 1H), 7.394 (dt, $J = 7.6$ Hz, 1.6 Hz, 1H), 7.324 (dt, $J = 7.6$ Hz, 1.2 Hz, 1H), 7.118 (s, 1H), 5.323 (m, 1H), 2.485 (d, $J = 3.6$ Hz, 1H), 1.593 (d, $J = 6.4$ Hz, 3H), 1.435 (septet, $J = 8.0$ Hz, 3H), 1.166 (d, $J = 7.6$ Hz, 18 H).

$N$-(1-(3-(Triisopropylsilyloxy)naphthalen-2-yl)ethoxy)isoindoline-1,3-dione (44)

To a 100 mL, 3-neck RB-flask was added PPh$_3$ (0.39 g, 1.5 mmol) and $N$-hydroxyphthalimide (0.22 g, 1.3 mmol). Then, alcohol 43 (0.47 g, 1.4 mmol) was dissolved in anhydrous dichloromethane (9 mL) and added dropwise at room temperature (22 °C) to the reaction flask under argon. Then, an additional amount of dichloromethane (11 mL) was added to the reaction flask which was then cooled to 3°C in an ice-water bath. Then, diisopropylazodicarboxylate (0.30 mL, 1.5 mmol, 1.027 g/mL at 5 °C, 99%)
was added dropwise by syringe to the reaction flask (the internal temperature was 3 °C - 6 °C. Throughout the addition, the color of the solution changed from cloudy to yellow, to orange and then a deep red color. After fifteen minutes, the solution changed from red-orange to yellow. The reaction was left to stir for 22 hours. The solvent had almost completely evaporated leaving a bright-yellow viscous oil, so an additional 10 mL of anhydrous dichloromethane was added to the reaction flask. After five minutes, a 1 mL aliquot was then taken and worked up using brine (2 mL) and Et₂O (1 mL) after the DCM was evaporated. The addition of the ether to the aliquot caused a white precipitate to appear but, the brine redissolved this solid. TLC analysis showed no evidence of remaining starting material. The reaction was stopped and the dichloromethane was evaporated in vacuo. The crude material was dissolved in anhydrous diethyl ether (20 mL). The organic layer was washed with brine (30 mL) in a separatory funnel. The aqueous layer was then washed with diethyl ether (2 x 15 mL). The combined organic washings were dried with Na₂SO₄ (1.60 g), filtered, and concentrated in vacuo. The resulting product was then left in a freezer for three days. The crude product (1.33 g) was a clumpy yellow solid and was purified by flash column chromatography using 70 g of silica and an eluent of 50% DCM/ 50% petroleum ether. Several fractions were collected (51-105) and concentrated in vacuo to yield 0.36 g of product. ¹H NMR analysis showed that there was still DIAD in the product.

Further TLC test plates were used and an eluent mixture of 20% Et₂O/80% petroleum ether showed a moderate separation of the product and the impurity with Rf
values of 0.29 and 0.21 respectively. 94.6 g of flash silica was then wet-packed into a column and the product from the 1st column was repurified by flash chromatography. The desired product (fractions 109-130) was concentrated in vacuo in a vacuum desiccator for two days. The resulting product was an off-white solid (0.10 g, 15% yield) and \(^1\)H NMR analysis showed that the product was pure. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.334\) (s, 1H), 7.890 (d, \(J = 8.0\) Hz, 1H), 7.730 (dd, \(J = 8.8\) Hz, 2.8 Hz, 2H), 7.664 (dd, \(J = 8.8\) Hz, 2.8 Hz, 2H), 7.605 (d, \(J = 8.4\) Hz, 1H) 7.393 (dt, \(J = 8.0\) Hz, 1.2 Hz, 1H), 7.337 (dt, \(J = 8.4\) Hz, 1.2 Hz, 1H) 7.053 (s, 1H), 6.169 (m, 1H), 1.720 (d, \(J = 6.4\) Hz, 3H), 1.364 (septet, \(J = 7.6\) Hz, 3H), 1.099 (d, \(J = 5.6\) Hz, 9H), 1.081 (d, \(J = 5.6\) Hz, 9H).

\(O-(1-(3-(Triisopropylsilyloxy)naphthalen-2-yl)ethyl)hydroxylamine (45)\)

To a 100 mL-3-neck RB-flask was added the phthalimide starting material 44 (0.25 g, 0.49 mmol) dissolved in HPLC grade dichloromethane (15 mL). The reaction flask was equipped with a thermometer, magnetic stir bar, and a condenser. The reaction flask was then placed in an ice bath and chilled to 6 °C. Then, 98% hydrazine monohydrate (64 % hydrazine) (0.2 mL, 4.1 mmol, \(\rho = 1.032\) g/mL at 25 °C) was added dropwise (6 -12 °C) to the solution. A white precipitate appeared 15 min after the initial
addition of hydrazine monohydrate. The reaction was then left to stir for 30 min on ice (10-12 °C). The ice bath was then removed and the reaction was allowed to warm to room temperature. TLC analysis after one hour and two hours (20%/80% Et₂O/petroleum ether and 50%/50% DCM/petroleum ether respectively) showed no remaining starting material. The reaction was stopped after two hours and 25 min. The precipitate was then filtered off and the organic solvent was removed in vacuo to afford a crude yellow-tinted oil (0.245 g). This was dissolved in Et₂O (1 mL) and the solvent was evaporated in vacuo. Argon gas was flowed into the septum capped flask to remove any atmospheric air and the flask was placed in a freezer for storage. After three days in the freezer, the flask containing the compound was placed in a vacuum desiccator for two days to evaporate any remaining solvent. The flask was then capped with a septum and flushed with argon by using a needle gas inlet and needle vent outlet. The flask was then placed back in the freezer for an extended period of time. A yellow-orange colored residue was obtained and a ¹H NMR spectrum was obtained for the compound (0.196 g, crude). [Purity based on ¹H NMR, 98%] ¹H NMR (400 MHz, CDCl₃) δ 7.826 (s, 1H), 7.775 (d, J = 8.0 Hz, 1H), 7.647 (d, J = 8.0 Hz, 1H), 7.384 (td, J = 7.6 Hz, 1.6 Hz, 1H), 7.315 (td, J = 7.2 Hz, 1.2 Hz, 1H), 7.133 (s, 1H), 5.212 (q, J = 6.4 Hz, 1H), 1.910 (s, 1H), 1.746 (s, 1H), 1.474 (d, J = 3.6 Hz, 3H), 1.416 (m, 3H), 1.164 (d, J = 1.2 Hz, 9H), 1.146 (d, J = 1.2 Hz, 9H).

1,1,1-Trifluoro-N-(1-(3-(triisopropylsilyl)oxy)naphthalen-2-yl)ethoxy)methanesulfonamide (46)
In a 100 mL 3-neck RB-flask equipped with a magenetic stir bar, thermometer
and condenser under an argon atmosphere, was added a mixture of alkoxyamine starting
material 45 (0.05 g, 0.14 mmol, 3.02 mL from a 0.05 M solution of 0.10 g of 45 in 6 mL
of dichloromethane), DMAP (0.017 g, 0.14 mmol), pyridine (0.1 mL, 1.2 mmol, 0.978
g/mL at 25°C) in freshly distilled dichloromethane (6 mL) was prepared within the
reaction flask. The flask was placed in an ethanol/dry ice bath and chilled to -66 °C for 15
minutes. CF₃SO₂Cl (0.019 mL, 0.18 mmol, 1.583 g/mL at 25°C) was added dropwise to
the reaction flask over a time period of 2 minutes [the internal temperature was (-66) – (-
58°C)]. The reaction was then left to warm to room temperature over a period of four
hours [21°C]. TLC analysis was performed using 50%/50% dichloromethane/petroleum
ether and 5%/95% Et₂O/petroleum ether. Both analyses showed that no starting material
remained. The reaction mixture was transferred to a separatory funnel and the organic
layer was washed with aq. CuSO₄· 5 H₂O (25 mL) and distilled H₂O (40 mL). The
organic layer was dried using Na₂SO₄ (2.5 g), filtered, and concentrated in vacuo (P₂O₅
and paraffin wax). An orange residue was obtained (0.05 g crude). Flash column
chromatography was performed using 2-5% ethyl ether/ 98-95% petroleum ether and
flash silica gel (20 g). Fractions 27-51 were collected and concentrated in vacuo over
P₂O₅. The resulting product was an oily colorless residue (0.040 g, 58% yield).
\(^{19}\)F NMR (CDCl\(_3\) with CFCl\(_3\), 400 MHz) \(\delta\) -73.084. \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 7.783-7.759 (m, 2H), 7.665 (d, \(J = 8.4\) Hz, 1H), 7.475 (s, 1H), 7.429 (td, \(J = 8\) Hz, 1.2 Hz, 1H) 7.352 (td, \(J = 8\) Hz, 1.2 Hz, 1H), 7.153 (s, 1H), 5.746 (m, 1H), 1.587 (d, \(J = 6.8\) Hz, 3H), 1.423 (m, 3H), 1.169 (d, \(J = 1.4\) Hz, 9H), 1.151 (d, 1.3 Hz, 9H).

\textit{Attempted preparation of 1,1,1-Trifluoro-N-(1-(3-hydroxynaphthalen-2-yl)ethoxy)methanesulfonamide (29)}

To a 50 mL, two-neck round bottom flask containing the starting material 46 (0.04 g, 0.08 mmol) dissolved in freshly distilled dichloromethane (0.5 mL), was added a magnetic stir bar and a condenser under an argon atmosphere. Then, a solution of KF in tetraethylene glycol (TEG) (0.0095 g, 1.0 mL, 0.16 mmol, 0.15 M in TEG) was added to the reaction flask while stirring. The reaction was left to stir for three and a half hours at which time, TLC analysis (5% Et\(_2\)O/95% petroleum ether) showed no evidence of starting material. The reaction was then stopped and quenched with nanopure water (50 mL) and extracted with distilled EtOAc (3 x 20 mL). The organic layer was dried using MgSO\(_4\) (1.9 g), filtered, and concentrated \textit{in vacuo} (P\(_2\)O\(_5\) and paraffin wax). The crude material obtained was an orange oil (0.064 g). Two purifications using flash column chromatography were run on the crude material. The first column (30 g of silica) was run
using a 10% EtOAc/90% petroleum ether eluent initially and then 30% EtOAc/70% petroleum ether, after fraction 66. The solvent was again changed to 50%/50% EtOAc/petroleum ether after fraction 115. Fractions 69-115 were collected and concentrated as well as further EtOAc flushing fractions; which were lost due to breaking the flask. [No weights could be determined due to the scale not being calibrated correctly.] $^{19}$F NMR analysis showed only one peak consistent with the target compound, however, the $^1$H NMR spectrum was still quite dirty. A second column was performed using 15% EtOAc/ 85% petroleum ether and 19 g of flash silica gel. Column fractions 28-41 were collected and concentrated in vacuo and the product weight was 0.007 g (crude, 26% yield based on mass). $^{19}$F NMR (CDCl$_3$ with CFCl$_3$, 400 MHz) $\delta$ – 74.144. $^1$H NMR (CDCl$_3$, 400 MHz) [Proton NMR sample was contaminated. Compound 29 could not be found in the spectrum.]
References


