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AMINOXY REAGENTS FOR SYNTHESIS AND ANALYSIS: EXPANDING THE ROLE OF OXIMATION

by

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B.S., Ecole Nationale Supérieure de Chimie de Montpellier, 2007

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M.S., University of Louisville, 2012

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A Dissertation Approved on

April 17, 2013

by the Following Dissertation Committee:

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Dr. Christopher T. Burns

Dr. James L. Wittliff

Dr. Xiang Zhang

DEDICATION

To all the wonderful people I met in the Department of Chemistry.

To my parents, my brother, my family, and my friends.

“¡Lúchalo!”

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I have been very fortunate to have Professor Michael H. Nantz as my thesis advisor. His support and his brilliant mind have made these years of hard work among the best I have had. It is fair to say that with him “work time becomes fun time”. He is a natural leader with a nurturing and fair, yet strict, management style. This great balance results in respect and admiration from his students and is what has driven me to give the best I have to the service of chemistry. For the past 5 years, Prof. Nantz has enthusiastically supported my ideas and encouraged independent learning. More importantly, he has pushed me to achieve excellence and develop my full potential. His influence in my scientific education is the one of a father, and over the years of support and advice, “The Baus” has become one of my best friends.

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ABSTRACT

AMINOXY REAGENTS FOR SYNTHESIS AND ANALYSIS: EXPANDING THE ROLE OF OXIMATION

April 17, 2013

Sébastien Laulhé

The reaction between an aminoxy moiety (RONH_2) and a carbonyl group of either an aldehyde or a ketone — known as an oximation reaction — is a versatile click chemistry coupling that generates a robust oxime ether linkage. The oximation reaction is chemoselective and can be performed under mild conditions in a large variety of solvents, including water.

The attractive properties of the aminoxy group and derived oximation reactions, reviewed in Chapter 1, inspired us to use this chemistry as a key feature of our research. Specifically, we prepare functionalized aminoxy compounds so that the oximation chemistry can then serve as a prelude to new synthetic or analytical methods. For example, Chapter 2 presents an improved preparation of *O*-(diphenylphosphinyl)hydroxylamine (DPPH), an aminoxy-containing reagent, using the classic Schotten-Baumann conditions. We show how DPPH can then be used as a chemoselective nitrogen transfer reagent for a one-pot aldehyde-to-nitrile functional group transformation. Sixteen aldehydes were smoothly transformed to their

corresponding nitriles by heating at 85 °C with DPPH in toluene. The reaction can be accomplished in the presence of alcohol, ketone, ester, or amine functionality.

In another application, we use functionalized aminooxy reagents to achieve quantitative multiplexed gas chromatography-mass spectrometry (GC-MS) analysis. Specifically, we chemoselectively derivatize carbonyl (aldehyde and ketone) metabolites using the aminooxy-containing reagents. Chapter 3 presents a focused fundamental study of the propensity of oxime ethers to undergo MS-induced fragmentations, such as the McLafferty rearrangement. In particular, we studied structural factors that promoted α,β -fragmentation in oximes of both ketones and aldehydes, as well as the derived silyl ethers of these adducts. We determined that 1) the propensity of the McLafferty rearrangement was greatly enhanced by oxygen at the b-position of silyl oxime ethers, 2) the McLafferty rearrangement is more prominent for *E*-isomers of oxime and silyl oxime ethers than for the corresponding *Z*-isomers, and 3) *Z*-isomers of silyl oxime ethers with CH₂ at the b-position generate nitrilium ions to a greater extent than their corresponding *E*-isomers.

Chapter 4 describes the 3-step synthesis of a new class of stable isotope-labeled derivatizing reagents — aminooxyethyl propionate reagents (AEP) — that enable multiplexed GC-MS analysis of small molecule carbonyl compounds. The AEP reagents contain 1) an aminooxy moiety, and 2) a propionate ester moiety that generates a reporter isotope-labeled mass spectral tag (MST) in the form of an ethyl carbenium ion via an ester α -cleavage. The AEP MSTs appear in an *m/z* zone of minimal interference (*ZMI*) in the range *m/z* 32-34. This is a key feature in that unobstructed observation of reporter MSTs in this zone significantly improves simultaneous quantitation of carbonyl analytes from multiple samples without recourse to MS peak deconvolution strategies. Also, and

in contrast to known isotope coding reagents for GC-MS, AEP reagents are not affected by the chromatographic isotope effect. The versatility of the technology for carbonyl metabolite profiling and absolute quantification is demonstrated by an analysis of turmeric extract, serving as a representative complex biological sample. A series of analogous methyl ketones were profiled from characteristic MS fragmentations of the AEP-derived oxime ether adducts, and two members, 2-nonanone and 2-undecanone, were quantified using AEP-labeled external standards.

Finally, Chapter 5 concludes with additional demonstrations of click chemistry. We used oximation to ligate linker molecules to fluorophores and gold nanoparticles (AuNPs) to generate a fluorescent nano-entity for breast cancer location and diagnosis. Five homologous linkers, each consisting of a thiol-terminated hydrophobic domain coupled to an aminooxy-terminated PEG-based domain, were prepared using a 6-step synthesis in 7-25% overall yield. The aminooxy end subsequently was reacted with an aldehyde-functionalized cyprate fluorophore, and the thiol end was used for attachment to gold nanoparticles. Linker attachment to cyprate in this manner was superior to previously investigated amide coupling involving linker amines and cyprate carboxylic acid.

Collectively, the results from these investigations demonstrates a novel strategy that employs functionalized aminooxy substrates and reagents to first exploit the high yielding and selective click coupling with carbonyl substrates to set the stage for secondary synthetic or analytical operations. Approaches developed in this multifaceted study appear to be applicable to a variety of synthetic problems ranging from those of a purely chemical nature to other impacting biological systems.

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CHAPTER 1

Click Chemistry: Revising the Role of Oximation Reactions

1.A. Introduction to Click Chemistry

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1.A. Introduction to Click Chemistry

1.A.1 General

The concept of click chemistry was promoted by Prof. K. Barry Sharpless (The Scripps Research Institute) in 2001 as a call to chemists to conduct simple-to-perform, high-yielding reactions between select and complementary functional groups.¹ Click reactions ideally should also generate inoffensive byproducts, be wide in scope, and not require chromatographic purification of products. In particular, the reactions should be insensitive to water or oxygen. Mainly, this concept was promoted as a way to mimic nature's ability to join small molecular units with high efficiency. Instead of attempting traditional synthetic carbon-carbon bond formations, Sharpless advocated forming robust and physiologically stable carbon-heteroatom bonds.

In the click chemistry manuscript by Prof. Sharpless, four main classes of chemical transformations embraced the above criteria: 1) cycloadditions of unsaturated species, such as 1,3-dipolar cycloaddition reactions and also the Diels-Alder family of transformations; 2) carbonyl chemistry of the “non-aldol” type, such as formation of oxime ethers, hydrazones, and amides; 3) nucleophilic substitution chemistry of S_N2 type, like ring-opening reactions of strained heterocyclic electrophiles such as epoxides and aziridines; and 4) additions to carbon-carbon multiple bonds, especially oxidative cases such as epoxidation, dihydroxylation and aziridination, but also thiol-ene and thiol-yne reactions. Figure 1.1 shows reaction examples for each of the four families as defined by Sharpless *et al.*¹

1. Cycloadditions of unsaturated species

Azide-Alkyne Cycloadditions

2. Carbonyl chemistry of the "non-aldol" type

Aminooxy-Carbonyl Oximation

3. Ring opening reactions of strained heterocycles

Epoxide ring-opening

4. Additions to carbon-carbon multiple bonds

Thiol-ene and thiol-yne reactions

Figure 1.1. The four classes of click chemistry transformations and reaction examples.

Although some of the criteria for a click chemistry reaction are subjective, the four main linking reactions that have been consistently associated with the “click” concept are: 1) The azide-alkyne cycloaddition that, in presence of a copper catalyst, generates triazoles at room temperature;² 2) the oximation reaction between aminooxy functionality (ONH₂) and an aldehyde or ketone carbonyl group to form oxime ethers; 3) the nucleophilic ring-opening of epoxides;³ and 4) the thiol-ene and thiol-yne reaction, a sulfur version of hydrosilylation that requires a radical initiator or UV irradiation.⁴

Of these four reactions, the azide-alkyne cycloaddition is by far the most used and the most associated with the term click chemistry. Indeed, many of the starting materials for the azide-alkyne cycloaddition are available commercially, and their cycloaddition reactions selectively give 1,2,3-triazoles. Compared to the original Huisgen 1,3-dipolar cycloaddition that requires elevated temperature and often produces mixtures of two regioisomers,⁵ the copper-catalyzed variant features reaction rates 7 to 8 orders of magnitude higher and can be conducted at room temperature and even under aqueous conditions. Additionally, the copper-catalyzed reaction allows the synthesis of the 1,4-disubstituted regioisomers (Scheme 1.1),⁶ while a later developed ruthenium-catalyzed reaction gives the opposite regioselectivity with the formation of 1,5-disubstituted triazoles.⁷

Scheme 1.1. Copper-catalyzed azide-alkyne cycloaddition mechanism.⁴

The mechanism for the reaction starts with the coordination of Cu(I) to the alkyne to form copper acetylide **1.1**, which then enables the attachment of the azide on ligand

displacement (Scheme 1.1). An unusual six-membered copper(III) metallacycle **1.2** then forms and undergoes a ring contraction to form triazolyl-copper derivative **1.3**. Hydrolysis delivers the triazole product and closes the catalytic cycle.

Recently, Worrell *et al.* proposed a new mechanism for the copper(I)-catalyzed azide-alkyne cycloaddition in which two copper atoms are involved in the catalytic cycle.⁸ Using copper isotopes and mass spectrometric analysis, they were able to determine that both copper atoms bind equivalently in a reaction intermediate.

The interest in these three reactions has been growing constantly over the past decade as a result of the high-yielding ease with which two molecules of interest may be selectively joined. A SciFinder[®] literature search conducted on March 29, 2013 revealed a total of 7451 publications containing the key words “Click Chemistry” or “Click Reaction”.

1.A.2. Aminoxy-Carbonyl Oximation

The oximation reaction between the aminoxy group and carbonyl compounds has the advantage of not requiring a catalyst. Indeed, the aminoxy functionality reacts chemoselectively with aldehyde and ketone carbonyl groups at room temperature in a large variety of solvents, including water, and the resultant oxime ether linkage is extremely robust. The resistance to hydrolysis differentiates the oxime ether from imine or enamine linkages. Table 1.1 shows some of the chemical differences between an aminoxy group and the structurally similar primary amine.

Table 1.1. Comparison between aminoxy and primary amine.

Aminoxy	Primary Amine
$\text{R-OH}_2 \xrightleftharpoons{\text{H}^+} \text{R-OH}_3^+$ <p style="text-align: center;">pKa = 5-6</p>	$\text{R-NH}_2 \xrightleftharpoons{\text{H}^+} \text{R-NH}_3^+$ <p style="text-align: center;">pKa = 9-10</p>
<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> $\text{R}-\overset{\text{O}}{\parallel}-\text{H}$ </div> <div style="text-align: center;"> $\text{R}-\overset{\text{O}}{\parallel}-\text{R}'$ </div> </div> <p>At room temperature and under neutral conditions, the aminoxy moiety reacts chemoselectively with aldehydes and ketones to form <i>E/Z</i> oxime ethers.</p>	<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> $\text{R}-\overset{\text{O}}{\parallel}-\text{H}$ </div> <div style="text-align: center;"> $\text{R}-\overset{\text{O}}{\parallel}-\text{R}'$ </div> <div style="text-align: center;"> $\text{R}-\overset{\text{O}}{\parallel}-\text{O-R}'$ </div> </div> <p>At room temperature and under neutral conditions, primary amines react with aldehydes, ketones to form imines, and react with esters to form secondary amides.</p>
$\text{R-O}-\text{N}=\text{C}(\text{H})\text{R}'' + \text{H}_2\text{O} \xrightarrow[\text{heat}]{\text{Low pH}} \text{R}-\overset{\text{O}}{\parallel}-\text{C}(\text{H})\text{R}''$ <p>The oxime ether linkage is very robust and is not readily hydrolyzed in water.</p>	$\text{R}-\text{N}=\text{C}(\text{H})\text{R}'' + \text{H}_2\text{O} \longrightarrow \text{R}-\overset{\text{O}}{\parallel}-\text{C}(\text{H})\text{R}''$ <p>The imine linkage is reversible and is readily hydrolyzed in water.</p>

These differences in reactivity between primary amine and aminoxy are due to the α -effect.⁹ The term α -effect was first introduced in 1962 by Edwards and Pearson¹⁰ to describe the anomalously high nucleophilic reactivity of compounds having one or more lone pair electrons at an atom adjoining (i.e., in the α -position) the nucleophilic center. Among the major influences of the α -effect in aminoxy compounds and

hydroxylamine is the lower pK_a (i.e. $pK_a = 5-6$)¹¹ of the protonated form, compared to the pK_a of a primary aminium ion (i.e., $pK_a = 9-10$). The α -effect in aminooxy compounds is affected by the electronegative nature of the oxygen. Indeed, despite an α -effect, hydrazines have a higher pK_a (i.e., $pK_a = 8$). Similarly, hydrazine derivatives react with ketones and aldehydes to yield relatively stable hydrazones, but they also react with esters, phthalimides, and anhydrides. Both hydrazones and oximes possess greater intrinsic hydrolytic stability than do imines. This can be explained by the contribution of the resonance form in alkylhydrazones and oxime ethers. As shown in Figure 1.2, the resonance form increases the electron density on carbon C1, hence reducing its electrophilicity and imparting greater hydrolytic stability.¹²

Figure 1.2. Resonance structures of oxime ethers and hydrazones.

Winberg *et al.* also proposed that the greater stability of hydrazones and oximes is due to the reduction of lone-pair repulsion relative to the hydrazine and aminooxy state.¹³ This second explanation better correlates with the fact that oxime ethers are superior to hydrazones with respect to stability since their hydrolytic rate is nearly 10^3 fold lower. Hydrolysis of oximes and oxime ethers occurs at a convenient rate at low pH (pH range 0-4) in concentrated solutions of HCl and HClO₄. A kinetic and mechanistic study by More O'Ferrall *et al.* shows that the hydrolysis reaction is acid-catalyzed above pH 2, but remains pH independent below pH 2.¹⁴

Under neutral conditions at room temperature, the aminoxy group reacts chemoselectively with aldehydes and ketones. This property has been exploited to attach polymers to proteins,¹⁵ and also to ligate small molecule probes to the cell surface ketones. However, for some applications the rates of reaction of the aminoxy group are not sufficient under normal conditions.¹⁶ To overcome this problem, Dirksen *et al.* showed that the reaction rates and yields for oximation were substantially enhanced by aniline catalysis (Scheme 1.2).¹⁷ Under these conditions, they were able to effectively ligate aldehydes at concentrations as low as 10 μM . Thyngensen *et al.* used the aniline catalysis to accelerate the reaction of carbohydrates with aminoxy substrates.¹⁸

Scheme 1.2. Main pathways of aniline catalysis as proposed by Thyngensen *et al.*¹⁸

Recently, Crisalli and Kool have identified water-soluble catalysts that enhance the rates of the oximation reaction.¹⁹ Rashidian *et al.* discovered a new catalyst, *m*-phenylenediamine (mPDA), for oxime ligation.^{19b} According to their study, mPDA is 15 times more efficient than aniline for the catalysis of oximation. Importantly, this new catalyst is more soluble in aqueous media, which makes it very attractive for some biological applications.

Although aminoxy groups undergo chemoselective oximation under mild conditions, there are many examples in the literature of other forms of reactivity for the aminoxy moiety. Due to the α -effect the aminoxy group remains a good nucleophile and can react as such with a variety of electrophiles (Table 1.2). The reactivity trend for reaction with these non-oximation electrophiles is: acyl chlorides > activated carboxylic acids > anhydrides and imides > esters > epoxides > alkyl halides.

Table 1.2. Non-oxime forming reactions of aminoxy species.

Aminoxy reacts readily with acyl chlorides at room temperature and even even at 0 °C in the presence of base. ²⁰	$\text{R}'\text{-ONH}_2 \xrightarrow[\text{Base, 0 } ^\circ\text{C}]{\text{R}-\text{C}(=\text{O})\text{Cl}} \text{R}'\text{-O}-\text{NH}-\text{C}(=\text{O})\text{R}$
Reaction of aminoxy with carboxylic acids requires coupling conditions. Once activated, the carboxylic acid and the aminoxy group react at room temperatures within hours. ²¹	$\text{R}'\text{-ONH}_2 \xrightarrow[\text{rt}]{\text{Coupling conditions}} \text{R}'\text{-O}-\text{NH}-\text{C}(=\text{O})\text{R}$
Anhydrides and imides react at room temperature with aminoxy moieties in aqueous media or polar solvents. The reaction is slow but quantitative yields are obtained within 24h. ²²	$\text{R}'\text{-ONH}_2 \xrightarrow[\text{Base, rt}]{\text{R}-\text{C}(=\text{O})\text{X}-\text{C}(=\text{O})\text{R}} \text{R}'\text{-O}-\text{NH}-\text{C}(=\text{O})\text{R}$ <p style="text-align: center;">$\text{X} = \text{O, N}$</p>
Aminoxy moiety reacts with esters in presence of AlMe_3 at reflux. AlMe_3 reacts with the aminoxy group prior to reaction with the ester. ²³	$\text{R}'\text{-ONH}_2 \xrightarrow[\text{AlMe}_3, \text{ reflux}]{\text{R}-\text{C}(=\text{O})\text{O}-\text{R}''} \text{R}'\text{-O}-\text{NH}-\text{C}(=\text{O})\text{R}$
An epoxide ring-opening reaction can be achieved with aminoxy. This requires base at room temperature for 48h or Lewis acid catalysis for 6 days. ²⁴	$\text{R}'\text{-ONH}_2 \xrightarrow[\text{rt, days}]{\text{Base / Lewis acid}} \text{R}'\text{-O}-\text{NH}-\text{CH}_2\text{-CH}(\text{R})\text{-OH}$
There are some examples of halogen displacement by aminoxy in basic conditions. Yields for these transformations can vary drastically on reaction time and base use, but remain moderate (20-80%). ²⁵	$\text{R}'\text{-ONH}_2 \xrightarrow[\text{Base, rt, 14-20h}]{\text{R}-\text{X}} \text{R}'\text{-O}-\text{NH}-\text{CH}_2\text{-R}$ <p style="text-align: center;">poor-moderate yields</p>

As shown in Table 1.2, the aminoxy group can react as a nucleophile with a variety of different electrophiles. Acylating the aminoxy moiety with protecting groups such as Boc (*tert*-butoxycarbonyl) is common.^{22b,c,d} On the other hand, the reaction of aminoxy with alkyl halides is more rare and low-yielding.²⁵ To achieve the same hydroxylamine intermediates in higher yields, many groups prefer the two-step (oximation and reduction) pathway (Scheme 1.3).²⁶

Scheme 1.3. Oxime ether reduction using NaBH_3CN as performed by Daher *et al.*^{26c}

The aminoxy group can also behave as an electrophile when activated by an electron-withdrawing group, enabling electrophilic amination reactions. This reactivity of the aminoxy moiety will be described in detail in Chapter 2. Briefly, an activated aminoxy group can undergo a nucleophilic attack to transfer the nitrogen to the nucleophile (Figure 1.3), thereby forming primary amines, hydrazines, etc.

Figure 1.3. Representation of an aminoxy-based electrophilic amination reaction.

Finally, beyond the reactivity of the aminoxy group, there are various other hydroxylamine-derived reactions and rearrangements. Oximes, oxime ethers, hydroxamic acids/esters, and other hydroxylamine derivatives can undergo a large variety

of rearrangements. For instance, oximes under acidic conditions or electrophilic activation undergo the classic Beckmann rearrangement.²⁷ This reaction proceeds by protonation of the oxime hydroxyl, which is then eliminated as water by the migration of an adjacent alkyl substituent to nitrogen. The preferred migrating group is *trans* to the oxime hydroxyl group. The N-O bond cleavage happens simultaneously with the expulsion of water, so that formation of a free nitrene is avoided (Figure 1.4). Ganguly *et al.* showed that electrophilic activation of oximes by elemental iodine can also lead to the Beckman rearrangement.^{27c}

Beckmann rearrangement mechanism

Recent example by Ganguly *et al.*^{27c}

Figure 1.4. Beckmann rearrangement mechanism and reaction example.

Activated oxime ethers also rearrange to form 2H-azirines via the Neber rearrangements.²⁸ After activating the oxime with tosylchloride (TsCl), treatment with base generates a carbanion in the α -position, which then reacts to displace the tosylate

group and give a 2H-azirine (Figure 1.5). If an aqueous work up is performed, the 2H-azirine is converted into an alpha-aminoketone.

Neber rearrangement mechanism

Recent example by Ooi et al.^{28c}

Figure 1.5. Neber rearrangement mechanism and reaction example.

Activated hydroxamic acids rearrange via the Lossen rearrangement to form primary amines when the reaction is performed in aqueous media, or carbamates or ureas if the reaction is respectively performed in presence of alcohols or amines (Figure 1.6).²⁹ The reaction proceeds by generating a reactive isocyanate intermediate that undergoes nucleophilic attack by water to form the primary amine; by alcohols to form the corresponding carbamates; or by amines to form the corresponding ureas.

Lossen rearrangement mechanism

Recent example by Dubé et al.^{29c}

Figure 1.6. Lossen rearrangement mechanism and reaction example.

Finally, a less known rearrangement of *N*-phenylhydroxylamines is the Bamberger rearrangement.³⁰ Under acidic conditions the hydroxyl group of aromatic aminooxy groups is protonated and generates an imine cation on loss of water, with the positive charge residing principally in *para*-position. Nucleophilic attack by water is determined by sterics and electronics, leading to the regioselective addition to the *para*-position, to afford the 4-aminophenol (Scheme 1.4).

Bamberger rearrangement mechanism

Scheme 1.4. Bamberger rearrangement mechanism.

1.A.3. Current Uses for the Aminoxy Group

Traditional organic synthesis is not readily adapted to the easy and rapid construction of macromolecules. Moreover, traditional ligation reaction conditions, such as the labeling of a cell surface, are generally not compatible with physiological conditions.³¹ Thus, there is a need for chemoselective ligation reactions between complementary functional groups (electrophile-nucleophile pairs) that proceed under mild and aqueous conditions. Since the oximation reactions—aminoxy-aldehyde or aminoxy-ketone—have such properties, many biological applications have been developed.

In the development of functional proteins, Canne *et al.* performed an oximation reaction for the assembly of two peptide segments for the total synthesis of a transcription factor-related protein (cMyc–Max).³² The strategy (Figure 1.7) initially formed a thioester between peptide segments to form two larger domains, followed by the further ligation between a ketone and an aminoxy group to form the final oxime-linked product in 21% yield.

Figure 1.7. Final step in the chemical synthesis of a modified protein construct derived from the heterodimeric transcription factor cMyc–Max.

Glycopeptide and oligosaccharide couplings have also been accomplished using the click properties of the oximation reaction. Indeed, the synthesis of glycopeptides is a difficult task due to the weakness of the glycosidic linkage and the need for selective protection of several functional groups.³³ Cervigni *et al.* synthesized *N*-terminal aminoxy-peptides and then coupled them with maltotriose to generate glycopeptides in yields ranging from 60% to 75% (Figure 1.8).³⁴ The same peptides were also coupled with decanal to form lipopeptides.

Figure 1.8. Synthesis of glycopeptides as developed by Cervigni *et al.*³⁴

In a similar approach, the ligation properties of the aminoxy group were explored by Lees *et al.* for the bioconjugation of proteins and polysaccharides for the preparation of conjugate vaccines.³⁵ According to the authors, oximation reaction both simplified the synthesis and increased the yields of the conjugate vaccines.

Zeng *et al.* used aminoxy chemistry for the labeling of glycoproteins on living cells.¹⁶ Their method employed mild periodate oxidation to generate an aldehyde on sialic acid, followed by aniline-catalyzed oximation to ligate a biotin derivative

containing an aminooxy moiety. Under such mild conditions, Zeng *et al.* found that the viability of the cells (from the combined periodate and oxime ligation reaction) was 93%. This example shows that the reactions involving the oximation of aldehydes and ketones are so highly selective that they do not affect significantly living cells during the time frame of the experiments. Figure 1.9 is a schematic representation of the experiment conducted by Zeng.

Figure 1.9. Periodate oxidation followed by aniline-catalyzed oximation of surface-aldehydes with biotin- ONH_2 in living cells.

Combining material science and biological applications, Thygesen *et al.* prepared gold nanoparticles functionalized with glycans for the study of protein-carbohydrate interactions.³⁶ They synthesized bifunctional (aminoxy-thiol) linkers to exploit the ligation properties of click chemistry. Reacting the aminoxy moiety of the linker with a carbohydrate, followed by the attachment of the resulting compound onto gold nanoparticles afforded complex gold-glyconanoparticles (Figure 1.10). The oximation

reactions between the linkers and the carbohydrates (glucose, maltose, and maltotriose) were performed at room temperature for 16 hours, and gave the desired products in high yields (74-88%).

Figure 1.10. Reaction of bifunctional aminooxy-thiol linkers with glucose, followed by loading onto gold nanoparticles.

In another example relating to nanoparticles, Chan and Yu used the ligation properties of aminooxy to immobilize gold nanoparticles onto self-assembled monolayers.³⁷ They proposed the use of chemoselective ligation as a means to immobilize a variety of nanoparticles that are specific for the ligands presented on the surface. Ideally, this immobilization can be achieved with spatial control. Chan and Yu decorated their gold nanoparticles using a mixture of dodecanethiol and 11-mercapto-2-undecanone. The thiol functionality of the linkers enabled attachment onto the gold nanoparticle. However, only the ketone functionality of 11-mercapto-2-undecanone would react with the aminooxy moiety present on the linkers of a gold surface in which the nanoparticle monolayer was formed (Figure 1.11). The functionalized gold nanoparticles then were added to the aminooxy functionalized gold surface to form the

self-assembling monolayer. After 12 hours, rinsing the substrate with dichloromethane and ethanol to remove unreacted gold nanoparticles afforded the self-assembled nanoparticle monolayer.

Figure 1.11. Nanoparticle-conjugated self-assembling monolayer using chemoselective oximation.

In analytical applications, aminooxy reagents have been used to trap carbonyl compounds.³⁸ Thus, Jakober *et al.* performed the liquid chromatography-mass spectrometry (LC-MS) analysis of carbonyl compounds in diesel emissions.^{38d} To do so, they used *O*-(2,3,4,5,6-pentafluoro-benzyl)hydroxylamine (PFBHA) as their derivatizing reagent (Figure 1.12). In a second study by Li *et al.*, volatile carbonyls were captured from breath samples using micropillar silicon wafers coated with 2-(aminooxy)ethyl-

N,N,N-trimethylammonium iodide (ATM).^{38f} The direct analysis of the mixtures were analyzed by Fourier transform-ion cyclotron resonance mass spectrometry (FTICR-MS). Figure 1.12 shows both reagents and their respective analytical methods.

Figure 1.12. Examples of aminooxy reagents for mass spectrometry purposes.

The aminooxy group was used to specifically target carbonyl compounds by Hovinen to attach aminooxy-tethered lanthanide(III) chelates (Figure 1.13).³⁹ According to Hovinen, such chelates could be suitable for the introduction of MRI contrast agents or luminescent lanthanide(III) to bioactive carbonyl molecules.

Figure 1.13. Aminooxy-functionalized Lanthanide(III) chelates for carbonyl-group conjugation.

Recently, aminooxy chemistry was found to have a promising role for drug discovery and development. Bahta *et al.* proposed a general approach for the rapid generation of libraries using oximation.⁴⁰ In particular, they applied the concept to multidentate inhibition of protein tyrosine phosphatase, and also to the optimization of peptide-antagonist-targeting interactions between Tsg101 and HIV-1 Gag. The main idea is to use an inexpensive aldehyde as a building block to generate a library of oxime ethers (as suggested by Sharpless so many years ago). Because the reactions are highly chemoselective and the products are sufficiently stable, the solutions can be evaluated biologically without purification. In their work, Bahta *et al.* synthesized 12 different aminooxy-containing peptides (P_1 - P_{12}) that then were subjected to oxime-based library diversification by reacting each peptide with a series of 12 commercially available aldehydes (A_1 - A_{12}) to generate 144 different peptide-oxime ethers (Figure 1.14). As a result of the biological assay and the structure-activity relationship (SAR) studies, they found a peptide-oxime with 15- to 20-fold binding enhancement as compared with the wild-type parent peptide.

Figure 1.14. Design of the Combinatorial Screening Assay based on oximation.

1.B. Transformations Affording the Aminooxy Moiety

1.B.1. Alcohol to Aminooxy Transformations

The transformation of alcohol moieties has been the primary mode for introduction of an aminooxy group into a molecule. The alcohol-to-aminooxy methods can be divided into two main groups: 1) those displacing the hydroxyl group after its activation, and 2) those directly aminating the alcohol functionality.

In the first group, one of the most commonly used transformations is a modification of the Mitsunobu reaction⁴¹ by Grochowski *et al.*⁴² Primary alcohols **1.4**, and to a lesser extent secondary alcohols, are first transformed into the *O*-alkyl phthalimides **1.5**. Hydrazinolysis of the phthalimide group using hydrazine generates the desired aminooxy product (Scheme 1.5).

Scheme 1.5. Alcohol to aminooxy transformation using Mitsunobu conditions followed by hydrazinolysis.

This Mitsunobu approach follows the mechanism shown in Scheme 1.6.⁴³ The initial nucleophilic attack of triphenylphosphine upon diisopropyl azodicarboxylate (DIAD) generates betaine **1.6**, which then proceeds to deprotonate *N*-hydroxyphthalimide to form the ion pair **1.7** (Scheme 1.6). Nucleophilic attack of the phosphonium ion by the primary alcohol leads to the formation of the oxyphosphonium ion **1.8**. S_N2 displacement

of triphenylphosphine oxide **1.9** by *N*-hydroxyphthalimide anion gives the desired product. Hughes *et al.* have found that the formation of the ion pair **1.7** is very fast, and that the rate determining step in the reaction changes depending on the basicity of the carboxylate anion (in this case $^-\text{ONphth}$).⁴⁴ They showed that with weak acid anions, the rate-determining step is the alcohol activation, not the $\text{S}_{\text{N}}2$ step. When using acetic acid, Hughes *et al.* observed the accumulation of oxyphosphonium ion **1.8**, which made the $\text{S}_{\text{N}}2$ attack the rate-determining step. On the other hand, when using trifluoroacetic acid, they did not observe build up of the oxyphosphonium **1.8**, which implied that the rate-determining step was the alcohol activation.

Scheme 1.6. Mitsunobu reaction mechanism.

Using the Mitsunobu reaction, Nicolaou *et al.* added the aminooxy functionality to an oligosaccharide fragment for the click chemistry construction of calicheamicin analogs.⁴⁵ The reaction conditions for this transformation are shown in Scheme 1.7. Reduction of 1,2-enone **1.10** undergoes an expected *in situ* ester migration to **1.11**. Immediate treatment of **1.11** with Mitsunobu conditions affords the β -glycoside phthalimide **1.12** in 53% yield over the two steps. Presumably, the reaction proceeds via

the oxyphosphonium intermediate, which undergoes S_N2 displacement and configuration inversion. Hydrazinolysis and coupling with a ketone gave **1.12** in 92% yield over two steps.

Scheme 1.7. Nicolaou *et al.* synthesis of aminooxy functionalized oligosaccharide.

Karshkela *et al.* doubly functionalized the branching unit of what would become a larger oligonucleotide glycoconjugate (Scheme 1.8).⁴⁶ The reaction yield was 53% for the formation of **1.14**, which averages an alcohol-to-*N*-hydroxyphthalimide transformation at 73% yield per alcohol.

Scheme 1.8. Double aminooxy functionalization.

A very interesting application of the Mitsunobu reaction applied to aminooxy functionalization was developed by Su *et al.*⁴⁷ Acknowledging the increasing demand for aminooxy functionalized molecules, but conscious of the lengthy purification process, they developed a polymer containing reactive *N*-hydroxyphthalimide for a Mitsunobu-based solid phase synthesis of aminooxy molecules (Figure 1.15). The lack of purification needed in solid phase synthesis in both synthetic steps—Mitsunobu and hydrazinolysis—make this approach very appealing for the synthesis of aminooxy products.

Figure 1.15. Polymer-supported *N*-hydroxyphthalimides for solid phase aminooxy functionalization.

A second method involving the displacement of a hydroxyl group of tertiary alcohols was developed by Palandoken *et al.*⁴⁸ This method enables the formation of the tertiary aminooxy groups in good yields. Contrary to the Mitsunobu reaction where the hydroxyl group is displaced via an S_N2 mechanism, the preparation of tertiary aminooxy following Palandoken's method involves an S_N1 mechanism. Such a transformation mechanism leads to a loss of chirality if the starting alcohol was enantiopure (Scheme 1.9). Hydrazinolysis of the phthalimide forms tertiary aminooxy **1.17** (scheme 1.9).

Scheme 1.9. Tertiary aminooxy preparation.

Mixing the tertiary alcohol **1.15** with boron trifluoride at 0 °C allows the formation of the tertiary carbocation that can then be attacked by *N*-hydroxyphthalimide to afford the *O*-alkyl phthalimide **1.16**. Yields for the transformation are good and range from 45-80% (Scheme 1.10). Only in cases where the alcohol elimination would generate a conjugated alkene, were the yields low (0-20%).

Scheme 1.10. Examples of transformations using Palandoken *et al.* procedure.

As a novel approach to the alcohol-to-aminooxy transformation, Choong *et al.*⁴⁹ were the first to propose the use of electrophilic amination reagents for the direct amination of the hydroxyl group of alcohols other than phenoxide. This method involves the electrophilic amination of alkoxide nucleophiles using oxaziridines **1.18** (Scheme

1.11). Primary, secondary, and tertiary alcohols can be transformed into their corresponding nitriles. More importantly, this method has the advantage of retaining the stereochemistry of the alcohol, which is not possible in the case of the Mitsunobu approach or in Palandoken's method.

Scheme 1.11. Aminooxy synthesis using oxaziridines.

It is important to note that the byproduct generated by the oxaziridine after reaction with the alcohol is the highly sterically hindered ketone **1.19**. This feature was cleverly designed by Choong *et al.* to avoid further reaction of the formed aminooxy with the byproduct of the oxaziridine reagent. Indeed, in their search for aminating reagents to generate aminooxy groups, Choong *et al.* realized that the traditionally used cyclohexanespiro-3'-oxaziridine (**1.20**), although capable of forming aminooxy, would generate a byproduct that would react to form oxime ether (Scheme 1.12). To circumvent this problem, they surmised that the oximation reaction could be impeded if the resulting ketone was highly sterically hindered. They were gratified to find that this approach also yielded a more stable oxaziridine that could be stored at room temperature for months without signs of decomposition.

Scheme 1.12. Formation of aminoxy using non-hindered oxaziridines generates oximes.

As shown in Scheme 1.13, the yields for transformation of primary and secondary alcohols using oxaziridine **1.18** are good and range from 51-86%. However, the transformation only afforded 10% yield in the case of tertiary alcohols.

Scheme 1.13. Examples of transformations using Choong *et al.* procedure.

1.B.2. Halogen to Aminoxy Transformation

Aminoxy functionality can also be introduced via S_N2 displacement of halides. In this case, the *N*-hydroxyphthalimide, *N*-hydroxysuccinimide, or other *N*-protected hydroxylamine derivatives is used directly as the nucleophile in a substitution reaction. Selective deprotection affords the corresponding aminoxy derivative.

Boc protected hydroxylamines

Examples by Jones *et al.*⁵⁰

Figure 1.16. Transformation of alkylhalides into *N*-(*tert*-butyloxycarbonyl)aminoxy esters.

Displacement of halogens by *N*-(*tert*-butyloxycarbonyl)aminoxy has been investigated by Jones *et al.* (Figure 1.16).⁵⁰ In their search for developing bifunctional linkers, it appeared that conventional Mitsunobu (halide displacement by *N*-hydroxyphthalimide or *N*-hydroxysuccinimide) was not adaptable, and they needed a different method of accomplishing the transformation. Halide displacement to obtain *O*-

alkylated *N*-(*tert*-butoxycarbonyl)hydroxylamine was therefore developed. The reaction times for these room temperature transformations were long; the yields of the transformations varied from 26% to 80%, with the exception of chloro-alkyl compounds where no product was isolated.

In a similar transformation to obtain bifunctional aminooxy and amine compounds for use in bioconjugation, Carrasco *et al.* functionalized bromo-alkyl chains with Boc-protected aminooxy group (Scheme 1.14).⁵¹ Employing similar conditions but a different solvent, Carrasco obtained good to excellent transformation yields.

Scheme 1.14. Transformation of bromo-alkyl chains by Carrasco *et al.*

In a different approach to displace halogens, Lemieux *et al.*⁵² and Marcaurelle *et al.*⁵³ used *N*-hydroxysuccinimide (NHS) to impart the compounds with the aminooxy moiety (Figure 1.17). It is interesting to note how adaptable the displacement by NHS can be; if the functional groups present in the starting molecule are not sensitive to soft nucleophiles, then the reaction can be performed quickly in presence of base with heat. Otherwise, the reaction can be carried out under mild conditions at room temperature in the presence of Lewis acids.

***N*-hydroxysuccinimide**

Example by Lemieux *et al.*⁵²

Example by Marcaurelle *et al.*⁵³

Figure 1.17. Imparting molecules with the aminooxy moiety using halogen displacement by *N*-hydroxysuccinimide (NHS).

Finally, the last main class of S_N2 displacement of halides for introduction of aminooxy moiety into a molecule is the base-catalyzed alkylation using *N*-hydroxyphthalimide. Kim *et al.*⁵⁴ and Mikola *et al.*⁵⁵ reported conditions for the introduction of a phthalimide-protected aminooxy group using this reaction (Figure 1.18). In a similar manner, halides (bromo- and iodo-compounds) are displaced by *N*-hydroxyphthalimide in presence of base with heat. Deprotection of the phthalimide group is achieved using standard hydrazinolysis conditions.

N-hydroxyphthalimide

Example by Kim *et al.*⁵⁴

Example by Mikola *et al.*⁵⁵

Figure 1.18. Aminooxy synthesis from halogenated compounds.

1.C. Conclusion

It is clear from the above review that aminooxy chemistry can be both versatile and selective. The attractive properties of the aminooxy group and the oximation reaction inspired us to pursue its development as a key feature in our research. Specifically, we aimed to prepare functional-aminooxy compounds and reagents in which oxime ether linkage formation would enable new synthetic and analytical properties. For example, Chapter 2 presents the use of aminooxy reagent *O*-(diphenylphosphinyl)hydroxylamine (DPPH) as a chemoselective nitrogen transfer reagent in nucleophilic fashion (Figure 1.19). DPPH has been used traditionally as an electrophilic amination reagent. We surmised that the possible oximation reaction of this aminooxy reagent with aldehydes would form activated oxime ethers having new and unexplored synthetic properties. Thus, after having developed an improved preparation of DPPH using Schotten-Baumann conditions,⁵⁶ we developed a chemoselective one-pot aldehyde to nitrile transformation (Figure 1.19).⁵⁷

Figure 1.19. Chapter 2 Summary: Synthesis of DPPH and use in aldehyde-to-nitrile transformations.

Chapter 3 and Chapter 4 present a new approach to the quantitative, multiplexed gas chromatography-mass spectrometry (GC-MS) analysis of complex mixtures. Our goal here was to develop a new class of chemoselective reagents that enable the

simultaneous analysis of multiple samples. In particular, we aimed to analyze carbonyl (aldehyde and ketone) metabolites. Once again, to specifically target carbonyl metabolites, we developed an aminoxy reagent to derivatize these metabolites. In this case, the derivatization reagents, and the oxime ether products, are designed for further reaction to impart analytical properties. Since very little was known on the mass spectrometry-induced fragmentation of oxime ethers, we focused research efforts to study the MS-induced fragmentation of oxime ethers, and this work is presented in Chapter 3. Our work revealed that oxime ether fragmentations provide, through McLafferty and the nitrilium ion formation, a great deal of structural information (Figure 1.20).⁵⁸ Thus, the fragmentation properties of oxime ethers could be applied to identify carbonyl metabolites.

Figure 1.20. Chapter 3 Summary: Principal MS-induced fragmentation of carbonyl, oxime and silyloxime ethers.

Results from this fundamental study led us to develop a new class of derivatizing reagents, and this work is described in Chapter 4. As shown in Figure 1.21, our aminooxyethyl propionate (AEP) reagents contain an aminoxy moiety to selectively derivatize carbonyl metabolites as well as a propionate ester to generate an isotopically labeled reporter mass spectral tag (MST) in a zone of minimal interference (*ZMI*) for direct mass-spectral quantitation of the derivatized metabolites. These reagents are

designed to profile the carbonyl substrates in complex biological samples and also to perform the absolute quantification of some these metabolites.

Figure 1.21. Chapter 4 Summary: Development of AEP reagents for chemoselective derivatization of carbonyl metabolites.

Finally, Chapter 5 again explores the click chemistry properties of the aminooxy moiety to ligate linkers and fluorophores to gold nanoparticles (AuNPs) to generate a fluorescent nano-entity for breast cancer location and diagnosis.⁵⁹ In this case, we show how the mild oximation reaction conditions enabled the self-assembly of a nano-entity with the extremely acid-, base-, and nucleophile-sensitive fluorescent probe cypate. To do so, we synthesized peptide linkers and heterobifunctional diblock linkers that contained a thiol functionality at one end, for attachment onto AuNPs, and an aminooxy moiety at the other end, for chemoselective ligation of fluorescent probe cypate bis(aldehyde) (Figure 1.22).

Figure 1.22. Chapter 5 Summary: Synthesis of peptide and spacers containing aminoxy functionality for facile ligation with cypate bis(aldehyde).

Chapter 6 presents all the experimental procedures of the chemistry described in Chapters 2, 3, 4 and 5. Tabulated NMR spectra as well as other spectroscopic data of synthesized compounds are given.

CHAPTER 2

Extending Click-Chemistry to Functional Group Interconversion

2.A. Introduction

2.B. An Improved Preparation of *O*-(diphenylphosphinyl)hydroxylamine

2.B.1. Introduction

2.B.2. Results and Discussion

2.B.3. Conclusion

2.C. A Chemoselective, One-Pot Transformation of Aldehydes to Nitriles

2.C.1. Introduction

2.C.2. Results and Discussion

2.C.3. Conclusion

2.A. INTRODUCTION

Electrophilic amination reagents have been used in a variety of reactions to add nitrogen to nucleophiles.¹ In particular, these reagents are valuable for the synthesis of primary amines and hydrazines. They differ in the electron-withdrawing group used to activate the electrophilic nitrogen. The most common electrophilic amination reagents contain an activated hydroxylamine moiety. These reagents can be separated into four groups based on structural type (Table 2.1). The two main groups are *O*-sulfonylhydroxylamines and *O*-phosphinylhydroxylamines. *O*-Hydroxylamine sulfonic acid (HOSA) is known to have better aminating properties than *O*-(diphenylphosphinyl)hydroxylamine (DPPH).² However, HOSA is also more prone to side reactions and is derived from hazardous starting materials. Moreover, sulfonylhydroxylamine compounds such as *O*-(mesitylenesulfonyl)hydroxylamine (MtsONH₂) tend to decompose spontaneously during drying,¹ and Keller *et al.* also reported that HOSA salts deflagrate violently.³

Table 2.1. Electrophilic amination reagents.

<p style="text-align: center;">Sulfur</p> <div style="display: flex; justify-content: space-around; align-items: center;"> </div> <p style="text-align: center;">MtsONH₂ HOSA</p>	<p style="text-align: center;">Phosphorus</p> <div style="text-align: center;"> </div> <p style="text-align: center;">DPPH</p>
<p style="text-align: center;">Acyl</p> <div style="display: flex; justify-content: space-around; align-items: center;"> </div> <p style="text-align: center;">DnpONH₂ NbzONH₂</p>	<p style="text-align: center;">Aryl</p> <div style="text-align: center;"> </div> <p style="text-align: center;">DnpONH₂</p>

Although some literature claims that DPPH is less versatile than the sulfonyl-based counterparts, DPPH has the most extensive track record for amination chemistry.⁴ The higher stability of DPPH compared to other electrophilic amination reagents allows for long-term storage at low temperatures (0 °C). DPPH can also withstand relatively harsh conditions before its degradation ensues. However, its low solubility in non-polar solvents remains a significant drawback.⁵

Table 2.2 provides some general examples from the literature of the use of DPPH as an electrophilic amination reagent. DPPH is a versatile electrophilic amination reagent that has been used widely for the amination of a variety of nucleophiles,⁶ stable carbanions, Grignard reagents, enols,⁷ and π -electron-rich nitrogen heterocycles.

Table 2.2. Literature examples of DPPH as an electrophilic amination reagent.

Stabilized enolates or enolate-like compounds can be aminated in good to excellent yields. ⁵	$\text{EWG}-\text{CH}_2-\text{R} \xrightarrow[\text{Ph}_2\text{P}(\text{O})\text{ONH}_2]{\text{Base}} \text{EWG}-\text{CH}(\text{NH}_2)-\text{R}$ <p>EWG = CO₂R', PO(OEt)₂ R = CO₂R'', CN, alkyl</p>
Amination of nucleophilic nitrogen in aromatic heterocycles occurs in high yields. ⁸	$\text{Heterocycle} \xrightarrow[\text{Ph}_2\text{P}(\text{O})\text{ONH}_2]{\text{NaH, DMF}} \text{Aminated Heterocycle}$ <p>R = alkyl, aryl R' = CO₂Me</p>
Primary amines can be generated by reaction with Grignard reagents. ⁹	$\text{RMgX} \xrightarrow[2. \text{H}_2\text{O workup}]{1. \text{Ph}_2\text{P}(\text{O})\text{ONH}_2} \text{RNH}_2$
N-N ylides of tertiary amines are generated <i>in-situ</i> for the aziridination of a range of enones. ¹⁰	$\text{Enone} \xrightarrow[\text{NaOH (2 eq.)}]{\text{NMM, Ph}_2\text{P}(\text{O})\text{ONH}_2} \text{Aziridine}$ <p>NMM = </p>

More recently, Armstrong *et al.* have used DPPH for the aziridination of enones by forming a reactive aminimine (Scheme 2.1).¹⁰

Scheme 2.1. Synthesis of aziridines using DPPH by Armstrong *et al.*⁸

Our interest in studying DPPH comes from the common aminoxy moiety, which in all of these reagents serves as the electrophilic nitrogen center. This application of aminoxy chemistry is in contrast to our use of this functionality; we use the aminoxy group as a nucleophile. While only one application has been reported in which DPPH is utilized for purposes other than an amination,¹⁰ we were surprised to find that there are few reports in the literature using the aminoxy functionality of DPPH as a nucleophile.¹¹ We surmised that, given the right conditions, the nitrogen transferring properties of DPPH could be preserved after its initial reaction as a nucleophile with a carbonyl group.

One main goal of our research was to explore more deeply the reactivity of these aminating reagents with the aim to broaden their use. Specifically, we focused on developing new uses for *O*-(diphenylphosphinyl)hydroxylamine (DPPH). Presented in this chapter are an improved synthesis of DPPH and a demonstration of its use as a chemoselective reagent for a one-pot aldehyde to nitrile transformation.

2.B. An Improved Preparation of *O*-(Diphenylphosphinyl)hydroxylamine

2.B.1. Introduction

As previously stated, DPPH is one of the most commonly used electrophilic amination reagents. The main reasons are 1) its chemical stability, making it safe to use and allowing for long-term storage at 0 °C or below, and 2) its low tendency toward side reactions.

Although DPPH is not commercially available, it is prepared in a single-step reaction from hydroxylamine hydrochloride and diphenylphosphonic chloride (**2.1**). Interestingly, although DPPH was first synthesized by Kreutzkamp and Schlindler in 1960,¹² it was mischaracterized as *N*-(diphenylphosphinyl)hydroxylamine (Scheme 2.2). These authors presented no chemical or spectroscopic evidence of the hydroxylamine structure. Not until 1979 when Harger,^{11a} following the Kreutzkamp procedure, and presumably isolating the same compound, was *O*-(diphenylphosphinyl)hydroxylamine properly characterized. Hydroxylamine is a nucleophile exhibiting an α -effect and thus reacts rapidly with acylating reagents. Although both the oxygen and the nitrogen atoms can react as nucleophiles, the attack by oxygen seems to be kinetically preferred.¹³ Indeed, other kinetic studies have also shown that phosphorylating agents undergo initial attack by the hydroxyl group of hydroxylamine.¹⁴

Scheme 2.2. Kinetic reactivity of hydroxylamine with diphenylphosphinyl chloride.

The most widely used procedure to prepare DPPH was developed by Colvin *et al.*¹⁵ It involves the reaction diphenylphosphinyl chloride (**2.1**) in a mixture of water and 1,4-dioxane in the presence of hydroxylamine hydrochloride and sodium hydroxide at -15 °C. The product is then simply filtered and further purified with a base wash. Unfortunately, this method is not reliable. Yields ranging from 42% to a maximum of 70% have been reported using this synthesis.^{6,16} In our hands, we found Colvin's method to be sensitive to reaction scale, with multigram attempts generally delivering DPPH in *ca.* 30–60% yield. The low yields can likely be attributed to the presence of water and sodium hydroxide in 1,4-dioxane. These conditions, in which the organic solvent is miscible with water, may induce hydrolysis of the starting diphenylphosphonic chloride (**2.1**) prior to its reaction with hydroxylamine. In an attempt to minimize hydrolysis, Klötzer *et al.* disclosed an alternative synthesis of DPPH that provides similar yields (*ca.* 70%) but uses anhydrous dichloromethane (DCM) and hydroxylamine at even lower temperature (-30 °C).¹⁷ Unfortunately, there were no apparent advantages using hydroxylamine due to its delicate preparation and handling.¹⁸ Moreover, hydroxylamine is known to decompose rapidly and violently, as evidenced by the explosion of a factory in Japan in the year 2000 leading to the death of four people and injury of 58 others.¹⁹ In particular, under basic conditions, hydroxylamine decomposes into ammonia, water, and dinitrogen.^{19b} Similarly, Kreutzkamp's synthesis also requires the initial generation of hydroxylamine in anhydrous benzene. Such conditions and length of preparation are not convenient. Our interest in aminoxy chemistry led us to develop a reproducible, higher yield synthesis of DPPH. We developed a convenient procedure for the preparation of DPPH on multigram scale.

2.B.2. Results and Discussion

The main challenge for the preparation of *O*-(diphenylphosphinyl)hydroxylamine was to find reagents, solvents, and conditions that would minimize the hydrolysis of the starting phosphinyl chloride without reacting with the freshly formed DPPH. Our first attempts using anhydrous organic solvents were aimed at avoiding the hydrolysis of the starting chloride while exploiting the low solubility of DPPH in organic solvents. Hence, we attempted reactions in anhydrous dichloromethane (DCM) at 0 °C in the presence of imidazole. The combination of hydroxylamine hydrochloride with imidazole generates hydroxylamine *in situ* for reaction with diphenylphosphinyl chloride while forming a poorly nucleophilic, yet soluble, imidazole salt. Due to the low solubility of DPPH in organic solvent, we aimed to filter the product and leave the imidazole salt in solution. Unfortunately, the nucleophilicity of imidazole still led to major consumption of DPPH, presumably forming the hydrazinium salt of imidazole (Scheme 2.3). Also, the poor solubility of hydroxylamine hydrochloride salt in organic solvent slowed the rate of DPPH formation, leading to overall poor yields.

Scheme 2.3. Consumption of DPPH in presence of imidazole.

Therefore, the synthesis of DPPH required use of a non-nucleophilic, non-nitrogen containing base (e.g. sodium hydroxide) to generate hydroxylamine *in situ* and a solvent in which the starting hydroxylamine hydrochloride and the base would be

soluble. Ironically, these two requirements suggest the use of water as a solvent. However, the presence of water in the reaction can cause significant hydrolysis of the starting phosphinyl chloride. Facing the same challenges and to compensate for the low yields of the reported syntheses of DPPH, other groups have developed ways to recover the phosphinic acid generated on hydrolysis of the phosphinyl chloride for recycling (Scheme 2.4).²⁰

Scheme 2.4. Recycling diphenylphosphinic acid, as developed by Higgins.¹⁸

We surmised that the adaptation of Schotten-Baumann conditions²¹ using a two-phase solvent system would help minimize hydrolysis of starting chloride **1** while providing the conditions for the *in situ* generation of hydroxylamine (Scheme 2.5).

Scheme 2.5. Biphasic synthesis of DPPH.²²

The use of the biphasic system water/Et₂O (-15 °C to 0 °C) has proven to be a superior method for preparing DPPH (78% yield on a 10-gram scale). The yield of DPPH formed using these conditions did not vary significantly when the reaction scale was decreased (e.g., 4-gram scale gave an 84% yield of DPPH). The high yields provided by the biphasic system likely could be attributed to both the low solubility of water in Et₂O, which prevents the hydrolysis of phosphinic chloride, as well as the very low solubility of DPPH in Et₂O. This latter property forces the precipitation of the product as it is formed and prevents further reaction with any nucleophiles in solution, such as the reactant hydroxylamine. The precipitate is simply filtered and dried under high vacuum. Figure 2.1 shows the ³¹P NMR of this crude product.

Figure 2.1. A. ³¹P NMR spectrum (CDCl₃) of starting diphenylphosphinic chloride that contains some diphenylphosphinic acid as an impurity. B. ³¹P NMR spectrum of crude DPPH synthesized using Schotten-Baumann conditions before basic wash. Impurities at 32.5 and 29.2 ppm remain unidentified.

^1H and ^{13}C NMR of DPPH and diphenylphosphinic analogs are very similar. However, ^{31}P NMR shows different signals for these products. As shown in Figure 2.1, DPPH generates a signal at 37.5 ppm while diphenylphosphinic acid generates a signal at 28.4 ppm. Diphenylphosphinic chloride (**2.1**) can be identified by its signal at 44.4 ppm. Figure 2.1 also shows that Schotten-Baumann conditions prevent the hydrolysis of **2.1**, as the corresponding diphenylphosphinic acid is the smallest impurity in the crude sample.

To obtain a high purity DPPH, a basic wash can be performed using 120 mL of a 0.25 molar solution of sodium hydroxide in water at 0 °C for 30 minutes. During this process, DPPH remains in suspension while diphenylphosphinic acid is solubilized. Unreacted chloride (**2.1**) is hydrolyzed and solubilized in the solution. Filtration of DPPH from this solution affords purity above 97% as it is shown in Figure 2.2.

Figure 2.2. ^{31}P NMR spectrum (CDCl_3) of DPPH after purification by basic wash. DPPH was synthesized with >97% purity.

To further confirm the NMR data, a high resolution mass spectrometry (HRMS) analysis was performed. Figure 2.3 shows the HRMS of $[\text{DPPH}+\text{Na}]^+$ calculated at 256.04979.

Figure 2.3. HRMS (X-axis: m/z ; Y-axis: intensity) of DPPH after purification. $[\text{DPPH}+\text{Na}]^+$ calculated at 256.04979, and the value obtained is 256.04965.

2.B.3. Conclusion

We developed an improved preparation of *O*-(diphenylphosphinyl)hydroxylamine by using an adaptation of Schotten-Baumann conditions. The biphasic system water/Et₂O afforded good and reproducible yields. This method allows for the product to be simply filtered for further purification with a base treatment. Also, product purity, generally compromised by the presence of Ph₂P(O)OH prior to base treatment, was readily assessed using ³¹P NMR (CDCl₃): DPPH, δ 37.5; Ph₂P(O)OH δ 28.3. Hence, we were able to determine that DPPH was synthesized with >97% purity by ³¹P NMR.

2.C. Chemoselective, One-Pot Transformation of Aldehydes to Nitriles²³

2.C.1. Introduction

Nitriles are versatile intermediates and valued target pharmacophores.²⁴ They can be prepared either through a homologation reaction that will add one extra carbon to the starting functionality (Table 2.3) or through the transformation of carbon-equivalent functional groups (Table 2.4).

Table 2.3. Homologation reactions leading to nitriles.

The reaction of alkyl halides with metal cyanides can generate the corresponding nitrile or the isonitrile depending on the metal.	$\text{R}-\text{X} \xrightarrow{\text{M-CN}} \text{R}-\text{CN} + \text{R}-\text{NC}$ <p>alkyl halide X = Cl, Br</p> <p>nitrile M = Na, K</p> <p>isonitrile M = Ag</p>
Aromatic nitriles can be generated from the starting aromatic halide by reaction with cuprous cyanide.	$\text{C}_6\text{H}_5-\text{X} \xrightarrow{\text{Cu-CN}} \text{C}_6\text{H}_5-\text{CN}$ <p>aryl halide X = Br, I</p>

Homologation reactions are generally simple S_N2 displacements of alkyl halides by alkali cyanides such as sodium cyanide. Metal cyanides are also used in the Kolbe nitrile synthesis, but lead to the formation of isonitriles as byproducts since the reaction partially proceeds via S_N1. Aromatic nitriles are formed via the Rosenmund-von Braun synthesis in which the aryl halide reacts with cuprous cyanide. One of the major problems for these homologation reactions is the highly toxic nature of cyanide reagents.

To avoid the use of toxic cyanides, chemists have preferred exploring the paths of carbon-equivalent syntheses of nitriles. Diverse methodologies and reagents have been developed in the field. As a result, it is now possible to transform alcohols, primary amines, halides, azides, amides, aldehydes, and aldoximes into nitriles. Table 2.3 shows examples of these procedures. Among the most popular approaches is the oxidation-state

neutral conversion of carboxylic acids to nitriles via dehydration of intermediate primary amides (Figure 2.4). They either use traditional dehydrating reagents²⁵ or recently developed metal-mediated procedures.²⁶ With the exception of primary amide dehydration, all the other carbon-equivalent procedures require the oxidation of the carbon center.

Table 2.4. Examples of carbon-equivalent nitrile syntheses.

Oxidative conversion of various benzylic alkyl halides and primary alkyl halides into corresponding nitriles occurs on treatment with iodine.	$\text{Ar}-\text{CH}_2\text{X} \xrightarrow[60\text{ }^\circ\text{C}]{\text{I}_2, \text{aq. NH}_3} \text{Ar}-\text{CN}$ <p>X = Cl, Br, I</p>
The reagent <i>N,N,N',N'</i> -tetrachlorobenzene-1,3-disulfonamide (TCBDA) effects formation of nitriles from primary amines. The direct conversion of primary alcohols into nitriles also was successfully carried out using this reagent in aqueous ammonia.	$\text{R}-\text{CH}_2\text{OH} \xrightarrow[25\text{ }^\circ\text{C}]{\text{TCBDA}, 30\% \text{ aq. NH}_3} \text{R}-\text{CN}$ <p>R = Ar, alkyl</p> <p>TCBDA = </p> $\text{R}-\text{CH}_2\text{NH}_2 \xrightarrow[\text{DMF}, 25\text{ }^\circ\text{C}]{\text{TCBDA}, \text{Et}_3\text{N}} \text{R}-\text{CN}$ <p>R = Ar, alkyl</p>
Primary azides, including benzylic, allylic, and aliphatic, are converted into the corresponding nitriles in the presence of a ruthenium hydroxide catalyst.	$\text{R}-\text{CH}_2\text{N}_3 \xrightarrow[80\text{ }^\circ\text{C}]{\text{Ru(OH)}_x / \text{Al}_2\text{O}_3, \text{toluene, O}_2} \text{R}-\text{CN}$ <p>R = Ar, vinyl, alkyl</p>
The catalytic dehydration of aromatic and aliphatic amides using silanes in the presence of catalytic amounts of fluoride allows the synthesis of a wide range of aliphatic and aromatic nitriles with high selectivity under mild conditions.	$\text{R}-\text{C}(=\text{O})\text{NH}_2 \xrightarrow[100\text{ }^\circ\text{C}]{\text{PhSiH}_3, \text{TBAF}} \text{R}-\text{CN}$ <p>R = Ar, alkyl</p>
Graphite promotes the conversion of aldehydes into nitriles in high yields. The reaction proceeds through the dehydration of the aldoxime intermediate.	$\text{R}-\text{CHO} \xrightarrow[100\text{ }^\circ\text{C}]{\text{HONH}_2 \cdot \text{HCl}, \text{MeSO}_2\text{Cl}} \text{R}-\text{CN}$ <p>R = Ar, alkyl</p>

An efficient complement to amide dehydration is the oxidative transformation of aldehydes to nitriles. Isolation of aldoxime intermediates (Figure 2.4) generally is followed by activation of the oxime hydroxyl group (e.g., as a sulfonate ester derivative) and then its elimination to afford the nitrile.²⁷

Figure 2.4. Common functional group interconversions to prepare nitriles.

The appeal of the aldehyde to nitrile approach has led to several one-pot methods for direct synthesis of nitriles from aldehydes using either hydroxylamine or ammonia in combination with a variety of activating reagents.²⁸

Unfortunately, the accompanying reagents for these one-pot approaches, such as $\text{CuCl}_2/\text{NaOMe}/\text{O}_2$,^{28a} $\text{Pb}(\text{OAc})$,^{28b} oxone,^{28c} H_2O_2 ,^{28h} I_2 ,²⁸ⁱ NBS,^{28j} IBX,^{28k} and NaCl_2 ,^{28l} often are not tolerant of other functional groups or require somewhat harsh conditions to effect the transformation. Ideally, an aldehyde-selective reagent that would facilitate the conversion to the nitrile under neutral conditions would greatly expand the utility of this direct approach. We thus developed the use of *O*-(diphenylphosphinyl)hydroxylamine (DPPH) as such a reagent.

2.C.2. Results and Discussion

Our interest in aminoxy chemistry²⁹ led us to consider the use of DPPH ($\text{Ph}_2\text{P}(\text{O})\text{ONH}_2$) as a possible chemoselective alternative to hydroxylamine or ammonia for introduction of nitrogen onto the carbonyl carbon of aldehydes. Since the reaction of this reagent with an aldehyde would directly form an activated oxime ester as an intermediate (e.g., **2.2**, Scheme 2.6), we reasoned that it should be possible to thermally induce an electrocyclic rearrangement resulting in the elimination of diphenylphosphinic acid (Scheme 2.6).

Scheme 2.6. Electrocyclic rearrangement.

Sharghi *et al.* proposed a similar mechanism involving elimination of methanesulfonic acid for the formation of nitriles from intermediate sulfonylated aldoximes.³⁰ Although DPPH is well appreciated as an electrophilic reagent for the amination of a variety of nucleophiles,⁵⁻⁹ its use as a nucleophilic counterpart, especially in chemoselective “click” transformations, has received limited attention.¹¹ Indeed, there is only one example in the literature showing DPPH condensing with acetone at room temperature. This example was used by Harger to further confirm the structure of DPPH.

To test the action of DPPH as a suitable reagent for oxime ester formation as well as the subsequent elimination to the nitrile, we examined the reaction between DPPH and α -naphthaldehyde (Scheme 2.6).

Figure 2.5. ^{31}P NMR (CDCl_3) spectrum of 1-naphthaldehyde *O*-diphenylphosphoryl oxime (Scheme 2.6 compound **2.2**). Signal at 35.6 ppm.

Reaction of DPPH with naphthaldehyde in THF gave oxime ester **2.2** in 79% yield. Figure 2.5 shows the ^{31}P NMR of compound **2.2** after purification. The NMR signal at 35.6 ppm is close to the ^{31}P NMR signal of DPPH (i.e. 37.5), which confirms the presence of the oxime functionality attached to the phosphorous center. Subsequent heating in toluene revealed that the elimination of diphenylphosphinic acid from **2.2** required warming to above 80 °C to achieve a significant rate of formation of naphthonitrile **2.3** (Scheme 2.6). Of particular note is that no Lewis acid or base was

required to effect the elimination; rather, simple warming under catalyst- and oxidant-free conditions was sufficient to afford **2.3** in 88% yield.

Figure 2.6. A. ^1H NMR (CDCl_3) in the aromatic region of **2.2**. The presence of the aldoximic proton is shown at 9.04 ppm. B. ^1H NMR in the aromatic region of 1-naphthonitrile **2.3** generated from **2.2** after heating in toluene at 85 $^\circ\text{C}$.

Figures 2.6A and 2.6B show the ^1H NMR spectra of compound **2.2** and **2.3** respectively. As expected the electrocyclic rearrangement leads to the elimination of diphenylphosphinic acid by abstracting the aldoximic proton of the phosphonyl aldoxime ester. Thus, the proton signal at 9.04 ppm in Figure 2.6A disappears when the nitrile is generated in Figure 2.6B.

For all the nitriles, ^{13}C NMR was also used to identify the product since the nitrile carbon has a chemical shift ranging from 120 to 116 ppm. For some unknown compounds, such as entry 14 in Table 2.5, we also used infrared (IR) spectroscopy to confirm the functional group interconversion since nitriles have a unique absorption ranging from 2260 to 2220 cm^{-1} .

Table 2.5. Conversion of aldehydes to nitriles using $\text{Ph}_2\text{P}(\text{O})\text{ONH}_2$.^a

Entry	Starting Aldehyde	Method	Major Product	Yield (%) ^b
1	Ph-CHO	A	Ph-CN	82
2		A		80
3		A B		69 78
4		A B		90 87
5		A B		37 58

6		A		80
7		A		96
8		A		81
9		A		75
10		A		85
11		A C		55 64
12		A		92
13		A		90
14		A B C		40 39 44
15		A		57
16		A		78

^a All reactions were performed in toluene at 85 °C for 4-6 h on ≥ 0.5 mmol scale using 1.15 equiv. reagent (method A), 2.0 equiv. reagent at 95 °C for 12h (method B), or 1.15 equiv reagent and 1.1 equiv. trifluoroacetic acid at 95 °C for 12h (method C). ^b Chromato-graphed (SiO₂) yield. Boc = *tert*-butoxycarbonyl; TBS = *tert*-butyldimethylsilyl.

We next examined the possibility of transforming aldehydes directly into nitriles without isolation of the oxime ester adducts. Despite the partial solubility of DPPH in toluene, we were pleased to find that its reactions with a diverse panel of aldehydes in this solvent gave good yields of the corresponding nitriles upon heating at 85 °C (Table 2.5). The conversions to the nitriles proceeded smoothly for aromatic as well as aliphatic aldehydes and accommodated α,β -unsaturation. Importantly, the aminoxy group of DPPH afforded a measure of chemoselectivity that enabled the selective, one-pot transformation of aldehydes to nitriles in the presence of other carbonyl groups, such as ketone, ester (including acetate), and carbamate carbonyls. While in some cases the transformation of electron-rich aldehydes required an additional equivalent of reagent for better overall conversion (entries 3 and 5), the vinylogous formamide carbonyl of entry 14 was only sluggishly transformed into the corresponding nitrile even when using excess reagent.

Figure 2.7. IR spectrum of the corresponding nitrile of entry 14. IR absorption at 2223 cm^{-1} confirms the presence of the nitrile group.

Figure 2.7 shows the IR spectrum obtained after transformation of entry 14 into its corresponding nitrile. The characteristic absorption at 2223 cm^{-1} confirms the presence of the nitrile group. Absorption at 1740 cm^{-1} is characteristic of the ester functionality present in the molecule, which suggests, as observed for entry 10, that our transformation is chemoselective and can be performed in the presence of ester moieties. As shown in Figure 2.8, the ester functionality remains intact from entry 14 to its corresponding nitrile while the aldehydic proton disappears from the ^1H NMR.

Figure 2.8. ^1H NMR spectra (CDCl_3) of aldehyde 14 (top spectrum) and its corresponding nitrile (bottom spectrum). The aldehydic proton (red) disappears in the nitrile. The ester functionality (blue) remains unchanged as well as the CH_2 in α -position of the ester (green).

Given that diphenylphosphinic acid ($\text{pK } 2.32$)³¹ is produced during the course of the reaction, we examined the aldehyde to nitrile conversion in the presence of acid-sensitive N-Boc and silyl ether protection groups. Whereas the Boc group was not affected (entries 12 and 13), the TBS ether was cleaved during the reaction (entry 15). In the entry 15 example, the initially formed desilylated product, 4-hydroxybutyronitrile (isolated in 13% yield), partially cyclized under the reaction conditions to generate γ -butyrolactone (*ca.* 10% yield) after workup. We surmise that this process occurs by generating an acid-catalyzed nitrilium intermediate that undergoes an intramolecular Ritter reaction (Scheme. 2.7). Attempts to buffer the reaction by addition of non-nucleophilic bases (e.g., Na_2CO_3) did not prevent loss of the silyl group.

Scheme 2.7. Generation of γ -butyrolactone via intramolecular Ritter Reaction.

To confirm that the side product of the reaction was indeed γ -butyrolactone, we performed both ^1H NMR and GC-MS analysis. Figure 2.9A shows the electron ionization (EI) mass spectrum obtained after injection in the GC-MS, while Figure 2.9B shows the most similar spectrum matched by the library of compounds, which is γ -butyrolactone. The resemblance between both spectra is 91%.

Figure 2.9. A. EI-MS (X-axis: m/z ; Y-axis: intensity) of γ -butyrolactone obtained as a side product of entry 15. B. EI-MS of γ -butyrolactone matched by the GC-MS library with 91% resemblance.

Since DPPH readily reacts with amines,^{8b-d,11} we noted that an acidification strategy, as in the case of 3-pyridinecarboxaldehyde (entry 11), improved the overall conversion to the nitrile, presumably by *in situ* pyridinium formation preventing electrophilic amination of the pyridine nitrogen. Finally, as a probe to see whether the process could be adapted to polar protic, more green solvents, we examined the reaction of 3-phenylpropionaldehyde with DPPH in water and found that heating at 95 °C for 12 hours afforded the corresponding nitrile in 73% yield. In addition, heating 3-pyridinecarboxaldehyde and DPPH in acetic acid as solvent under similar conditions gave 3-pyridinecarbonitrile in 60% yield.

2.C.3. Conclusion

In conclusion, the present method is applicable for the one-pot conversion of aldehydes to nitriles in the presence of water, alcohols, and other carbonyl functionalities. DPPH is sufficiently selective in its reactions with aldehyde carbonyl groups that chemoselection is achieved on simple mixing at room temperature followed by warming to effect the transformation to the nitrile. Ease of reaction, good yields, and the absence of base or oxidant are other features of this method.

CHAPTER 3

An Approach to Quantitative Multiplexed GC-MS Analysis. Part 1: The MS-Induced Fragmentation of Oxime Ethers.

3.A. Introduction

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-

3.A. Introduction

High-throughput analysis platforms are the technologies of choice for analyzing complex mixtures derived from biological samples, such as blood, urine, and tissue samples. Technologies that facilitate the analysis of these complex mixtures have the potential to become personalized diagnostic tools for a large variety of diseases. The fields of genomics, proteomics and metabolomics have been growing rapidly with the objective of obtaining a “snap-shot” of the chemical composition of biological samples to better understand some metabolic pathways. Such a “snap-shot” could deliver a molecular profile of the sample and facilitate biomarker discovery. For example, the advent of quantitative mass spectrometry proteomics¹ has given researchers an efficient tool for the discovery and study of protein-based biomarkers.

Over the past decade, the field of metabolomics has witnessed dramatic growth. In 2005 the METLIN² Metabolomics Database was established with 5,000 metabolites. As of today this database contains over 42,000 metabolites. The interest in metabolite-based biomarkers comes from the fact that metabolites are the end products of cellular processes and may represent dynamic changes at the cellular level. Their profiling for diseases, such as cancer, is considered a tool for diagnosis of malignancy before changes occur to the cellular architecture.³ Therefore, in such an emerging field, further progress is dependent on the technical evolution of mass spectrometry (MS). Indeed, the ability to analyze multiple extracts in a single assay (the process of multiplexing) and rapidly quantify multiple analytes from different samples (high throughput) would accelerate metabolite-based profiling and biomarker discovery. As previous studies have emulated the stable isotope coding technology used in liquid chromatography-mass spectrometry

(LC-MS) proteomics,⁴ we propose to develop a new technology based on gas chromatography-mass spectrometry (GC-MS) to be compatible with high throughput and multiplex methodologies. By comparison to other existing analytical platforms, comprehensive two-dimensional gas chromatography mass spectrometry (GC×GC-MS) provides increased separation capacity, chemical selectivity, and sensitivity for the analysis of metabolites present in complex samples, even though the samples need to be derivatized first. For example, a GC×GC-MS system can easily detect more than 1,800 analytes from a human plasma extract in a 40-minute analysis.

We propose to develop new derivatizing reagents containing isotopic tags for high throughput multiplexed metabolomics. Initially, the prototype reagents will be fitted with an aminoxy group (-ONH₂) to specifically derivatize carbonyl metabolites. As shown in Scheme 3.1, the reaction of aminoxy compounds with carbonyl groups results in the formation of oxime ether moieties, a process known as oximation. The GC-MS analysis of the oxime ethers separates the different compounds and generates for each one of them an electron ionization (EI) spectrum. This spectrum is composed of the charged fragments that arise from the molecules.

Oximation Reaction of Carbonyl Metabolites

Scheme 3.1. Oximation of carbonyl metabolites and their analysis through GC-MS.

The electron ionization-induced fragmentation of the resulting oxime ethers have not been thoroughly studied.⁵ Consequently, we report here the GC-TOF MS mass spectra for a panel of carbonyl, oxime, and silyl oxime ether substrates. Our goal in this MS study is to understand the different fragmentation pathways that can arise from the electron ionization of oximes and silyl oxime ethers to better predict and help us identify carbonyl metabolites after derivatization using the isotopically labeled aminoxy reagents. We also disclose a systematic computational investigation into the McLafferty rearrangements observed for most of the compound panel to obtain more insight into the intrinsic factors that promote and determine the nature of the McLafferty rearrangement. In particular, we examine the influence of oxygen substitution adjacent to the site of γ -hydrogen atom abstraction in the McLafferty rearrangement.

3.B. Carbonyl, Oxime, and Silyl Oxime Ether Substrates

3.B.1. Significance of the Study of Carbonyl Metabolites

The analysis of cellular aldehyde and ketone species is of considerable importance for the field of metabolomics.⁶ Indeed, carbonyl metabolites tend to represent the extent of oxidative stress often related to diseases including cancer.⁷ Aldehydes and ketones can be formed endogenously by various biochemical pathways. For example, aldehydes can be formed by lipid peroxidation (Scheme 3.2), carbohydrate metabolism, and ascorbate autoxidation as well as by various enzymatic processes such as those involving amine oxidase, cytochrome P-450, or myeloperoxidase.⁸ Malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) and other 4-hydroxyalkenals (4-HDA) are known to be biomarkers of oxidative stress, forming on degradation of polyunsaturated lipids (PUL) by the action of reactive oxygen species (ROS).⁹

Scheme 3.2. Lipid peroxidation cycle and resultant HDA products.

Endogenous ketones, such as acetone and acetoacetic acid, are molecules produced as byproducts when fatty acids are broken down for energy in the liver and

kidney.¹⁰ Other ketones, such as β -ketopentanoate, may be created as a result of the metabolism of synthetic triglycerides.¹¹ Furthermore, ketone bodies, such as acetone, are produced from acetyl-CoA mainly within the mitochondrial matrix of hepatocytes when carbohydrates are so scarce that energy must be obtained from breakdown of fatty acids.¹² Acetone is responsible for the characteristic “fruity” odor of the breath of people in ketoacidosis.¹³

To isolate these carbonyl metabolites for analysis by mass spectrometry, their selective conversion into labeled oximes or oxime ether analogs by water-based click chemistry (i.e., oximation, Scheme 3.2) has become an elegant and highly chemospecific approach.^{5i,5k} Oxime chemistry and subsequent silylations to form silyl oxime ethers have been used to detect and analyze natural and synthetic steroids,^{5j} trisaccharides^{5b,5c,5g,5h} and other classes of compounds^{5d,5f} by gas chromatography-mass spectrometry (GC-MS). Oximation proceeds rapidly under mild conditions and is chemoselective in that the aminooxy reaction with the carbonyl groups of aldehydes and ketones is preferred over the majority of other functionalities. Among other advantages, oxime ethers are potentially suitable as components of dynamic libraries.^{5e} The study of the major MS fragmentations of these carbonyl derivatives, however, has not received due attention. One predictable MS-fragmentation of this carbonyl derivative is the McLafferty rearrangement. The next section describes in detail this fragmentation and our efforts to examine its prevalence among various oxime ethers and silyl oxime ethers.

3.B.2. Introduction to the McLafferty Rearrangement

In 1959, Fred W. McLafferty published¹⁴ an article entitled “Mass Spectrometric Analysis. Molecular Rearrangements” in which mechanistic concepts for mass spectrometry were described that remain valid to this day. The characteristics of the McLafferty rearrangement have been described extensively and in great detail.¹⁵ Briefly, the McLafferty rearrangement involves the transfer of an aliphatic hydrogen atom in γ -position to an acceptor group followed by a β -cleavage (Figure 3.1).

Figure 3.1. Proposed McLafferty fragmentation pathways

Carbonyl groups in ketones, aldehydes, carboxylic acids, esters, amides and other derivatives are the most common acceptor groups, but aromatic and heteroaromatic rings can also act as acceptors. Not only does the γ -hydrogen atom have to be bound to an sp^3 -hybridized carbon atom, it also has to be sterically accessible to the acceptor group. Hydrogen atom transfer is accompanied by the cleavage of the α - β bond, resulting in the formation of an odd-electron positive ion (enol ion) and a neutral molecule (Figure 3.1). In addition, the McLafferty rearrangement is known to occur with a reverse charge

distribution, i.e., generating the neutral enol and the charged alkene fragments.¹⁶

The McLafferty rearrangement can proceed through either a concerted or stepwise mechanism.¹⁵ Various elegant experiments have been performed to probe the mechanistic nature of the McLafferty rearrangement, and to date, in all cases, the results point toward a stepwise mechanism.¹⁷ Various theoretical studies have been performed that confirmed the stepwise mechanism, but some also identified systems that should or could proceed through a concerted mechanism.¹⁸ Finally, in addition to the single hydrogen transfer McLafferty rearrangement, consecutive rearrangements that proceed by a double hydrogen transfer have been reported.¹⁵ In general, one would expect that the McLafferty rearrangement would have to compete with other fragmentation pathways, such as simple bond cleavages that are entropically more favorable.¹⁵ The relative importance of the various fragmentation pathways is determined by their respective kinetics inside the ion source, which is related to the thermochemistry and can be calculated fairly accurately by applying the Rice-Ramsperger-Kassel-Marcus (RRKM) theory.¹⁹ In most cases, the potential energy surfaces of the different fragmentation pathways need to be known, and quantum chemical calculations can provide the input parameters for the RRKM calculations.^{10a}

Although the McLafferty rearrangement²⁰ of carbonyl compounds has been the focus of extensive and continuous research,²¹ studies on oximes and their silyl oxime ether derivatives have been limited.⁵ Surprisingly, to date, only one article has reported the McLafferty rearrangement for ketoximes. Bowen and Maccoll observed both single and double McLafferty rearrangement fragments in the mass spectra of various ketoximes under low-energy (12eV) and low-temperature (350 K) ionization.^{5a}

3.B.3. Synthesis of Carbonyl and Oxime Substrates

We prepared the panel of carbonyl substrates shown in Figure 3.2 and examined their incidence of McLafferty rearrangement to guide the future development of aminooxy derivatizing agents. We examined two key structural variables: (a) ketone vs. aldehyde and (b) β -CH₂ vs. β -O. These variables were examined in both carbonyl and oxime analogs.

Figure 3.2. Carbonyl, oxime, and silyl oxime ether analogs.

We surmized that the α - β bond cleavage of the McLafferty rearrangement might be increased by introduction of an oxygen atom at the β -position (Compounds **3.3-4**, **3.7-8**, **3.11-12**,). Indeed, if the McLafferty rearrangement proceeds via a stepwise radical mechanism (Figure 3.1 and Scheme 3.3), then placing an oxygen in the β -position would stabilize the radical intermediate **X**, and ensure a fast γ -hydrogen transfer. The

McLafferty rearrangement would be expected to result in the β -cleavage of the α -alkoxy oxime as shown in Scheme 3.3.¹¹

Scheme 3.3. Expected McLafferty rearrangement of carbonyls with β -oxygen.

The first set of aldehyde and ketone substrates with a β -CH₂ were synthesized without much difficulty using modifications of known synthetic processes established by Mokhtari *et al.* for the oximation using hydroxylamine hydrochloride,²² and Ortiz-Marciales *et al.* for the silylation of oximes.²³ Starting heptanal (**3.1**) and 2-heptanone (**3.2**) were purchased from Aldrich. Reaction with hydroxylamine hydrochloride in methanol in the presence of base generated the corresponding oximes in 89% yield for the aldehyde and 74% yield for the ketone (Scheme 3.4). The yields in this step, although good but not excellent, may be explained by the decomposition of the hydroxylamine in solution prior to reaction with the carbonyl group. Indeed, it is known that hydroxylamine decomposes into various gases under basic conditions including ammonia, water, and dinitrogen.²⁴ Silylation of the oximes proceeded in dichloromethane in the presence of imidazole and *tert*-butyldimethylsilylchloride (TBSCl). Yields for this step are moderate (65% for the aldoxime and 66% for the ketoxime) probably due to premature hydrolysis of the silyloxime ethers.

Scheme 3.4. Reagents and Conditions: *a*. $\text{HONH}_2 \cdot \text{HCl}$, NaHCO_3 , MeOH, 24h, rt. *b*. TBSCl, imidazole, CH_2Cl_2 , 12h at rt.

NMR analysis of the product mixture provides a relative ratio of both the *E*- and *Z*-isomers. For the oxime and silyloxime ether adducts of hexanal, the downfield aldehydic proton (7.62 ppm) corresponds to the *E*-isomer, while the aldoximic proton at 6.85 ppm corresponds to the *Z*-isomer (Figure 3.3).²⁵ The shielding effect observed for the *Z* isomer is, most likely, the result of the lone pair of electrons on the nitrogen.

Figure 3.3. ^1H NMR spectrum (CDCl_3) showing *E* vs *Z* silylaldoxime ether isomers.

The aldehyde substrates containing a β -O (compounds **3.3**, **3.7** and **3.11**) were obtained in overall poor yield (7% in 4 steps, Scheme 3.5). Propoxy aldehyde **3.3** was prepared by reaction of bromoacetaldehyde dimethyl acetal with sodium hydride and *n*-propanol in THF (25% yield) to afford propoxy acetal **3.13**. Hydrolysis of the acetal using *para*-toluene sulfonic acid (PTSA) in water gave the desired propoxy aldehyde **3.3** (81% yield). Aldoxime **3.7** and silylaldoxime ether **3.11** were prepared using the same standard reaction conditions as described above with similar yields (56% for the oximation and 62% for the silylation step).

Scheme 3.5. Reagents and Conditions: *a.* NaOH, propanol, reflux, 24h. *b.* PTSA (2 equiv.), water, rt, 4h. *c.* HONH₂•HCl, NaHCO₃, MeOH, rt, 24h. *d.* TBSCl, imidazole, CH₂Cl₂, rt, 12h.

Our initial synthesis of propoxy ketone **3.4** iterated closely the steps used to prepare propoxy aldehyde **3.3**. We attempted the S_N2 displacement of bromine from 1-bromo-2,2-dimethoxypropane using sodium propoxide (Scheme 3.6, step *a*) followed by acetal hydrolysis (Scheme 3.6, step *b*) to obtain **3.4**.

Scheme 3.6. Reagents and Conditions: *a.* NaOH, propanol, reflux, 24h. *b.* PTSA (2 equiv.), water, rt, 4h. *c.* NaOH, propanol, reflux, 24h.

Unfortunately, the S_N2 displacement (step *a*, Scheme 3.6) was not achieved. Figure 3.4 shows the Newman projections for the reactions leading to the propoxy aldehyde acetal and to the propoxy ketone acetal. Although the S_N2 displacement occurred to give the propoxy aldehyde acetal in poor yields, steric hindrance from the methyl group apparently deterred S_N2 displacement of bromine. Similarly, the S_N2 displacement of bromine from bromoacetone (Scheme 3.5, step *c*) using the same conditions did not generate propoxy ketone **3.4**.

Figure 3.4. Newman projections for the S_N2 attack of the acetal versus the ketal.

We opted to circumvent the above problems by attempting an S_N2 displacement of bromine from bromoacetic acid (Scheme 3.7). The reaction of bromoacetic acid with *n*-propanol and sodium hydroxide gave the displacement product **3.14** in moderate yield (51% yield, Scheme 3.6, step *a*). The resulting acid **3.14** was converted to methyl ketone **3.4** by treatment with methyl lithium in dry ether at $-78\text{ }^{\circ}\text{C}$ (37% yield, Scheme 3.7, step

b). Standard oximation and silylation conditions described previously generated ketoxime **3.8** and silylketoxime ether **3.12** (44% and 68% yield, respectively).

Scheme 3.7. Reagents and Conditions: *a.* NaOH, propanol, reflux, 24h; *b.* MeLi (2 eq), THF, -78 °C to rt, 4h; *c.* HONH₂•HCl, NaHCO₃, MeOH, rt, 24h; *d.* TBSCl, imidazole, CH₂Cl₂, rt, 12h.

NMR analysis of the product mixture provides a relative ratio of both the *E*- and *Z*-isomers. Silylketoxime ethers can be identified by the CH₂ signal on α-position of the oxime moiety. The downfield CH₂ protons (4.42 ppm) correspond to the *Z*-isomer, while the CH₂ protons at 3.99 ppm correspond to the *E*-isomer (Figure 3.5).

Figure 3.5. ¹H NMR spectrum (CDCl₃) showing *E* vs *Z* silylketoxime ether isomers with β-O.

3.C. Results and Discussion on the MS-Fragmentations

3.C.1. Electron-Ionization Mass Spectral Comparisons

In all, the fragment ions resulting from the McLafferty rearrangement are present to varying extents. Our GC-TOF MS spectra showed high similarities with the known mass spectra of compounds **3.1** and **3.2** from the NIST database. In all mass spectra, except of compound **3.2**, no molecular ions ($M^{+\bullet}$) can be observed. As expected, numerous fragment ions have been generated from the excited molecular ions ($M^{+\bullet}$) that were generated due to electron ionization in the ion source, especially those resulting from simple and favorable bond cleavages. Due to the presence of numerous fragment ions, it is challenging to determine the normalized intensity of McLafferty rearrangement fragment ions and to perform some kind of semiquantification to compare the various entries. However, by comparing compounds **3.1** and **3.2**, it is clear that ketone functionality enhances the McLafferty rearrangement compared with the aldehyde functionality. A similar pattern is observed when comparing compounds **3.3** and **3.4**, and this pattern is in agreement with the relative basicity of the carbonyl oxygens. However, in contrast to expectations, when comparing compounds **3.1** and **3.3**, it seems that introduction of oxygen on the β -position suppresses the McLafferty rearrangement, and a similar observation can be made when comparing compounds **3.2** and **3.4**, although to a much lesser extent. In the case of ketone **3.4**, the β -oxygen appears to promote significant fragmentation via carbonyl alpha cleavage to generate the corresponding acylium ion $H_3CC\equiv O^+$ ($m/z = 43$) by stabilization of the leaving radical fragment $\bullet CH_2On-Pr$.

The synthesis of the oximes **3.5–8** and silyl oxime ethers **3.9–12** gave rise to both the (*E*)- and (*Z*)-isomers (except for ketoximes **3.6** and **3.8** that were formed in lower *E/Z* ratios). As presented in Figure 3.5, unique ¹H NMR signals could be observed and used to determine the *E/Z* ratio. Data are summarized in Table 3.1.

Table 3.1. ¹H NMR ratios of *E*- and *Z*-isomers, their characteristic ¹H NMR shifts, and their GC-MS retention times.

Class	Compound	¹ H NMR ^a <i>E:Z</i> ratio	¹ H NMR shift ^b (ppm)	GC-MS retention time(s) <i>E, Z</i>
Oxime	3.5 aldoxime	1.5 : 1.0	-H _E : δ 7.42 -H _Z : δ 6.72	468, 486
	3.6 ketoxime	1.0 : 0.3	(-CH ₂ -) _E : δ 2.17 (-CH ₂ -) _Z : δ 2.36	506 (<i>E</i>)-isomer only
	3.7 aldoxime	1.2 : 1.0	-H _E : δ 7.50 -H _Z : δ 6.92	510, 515
	3.8 ketoxime	1.0 : 0.17	(-CH ₂ O-) _E : δ 3.98 (- CH ₂ O-) _Z : δ 4.32	527 (<i>E</i>)-isomer only
Silyl-oxime ether	3.9 aldoxime	1.0 : 1.0	-H _E : δ 7.49 -H _Z : δ 6.85	488, 479
	3.10 ketoxime	1.0 : 0.3	(-CH ₂ -) _E : δ 2.17 (-CH ₂ -) _Z : δ 2.34	490, 473
	3.11 aldoxime	1.0 : 1.0	-H _E : δ 7.56 -H _Z : δ 7.03	512, 501
	3.12 ketoxime	1.0 : 0.2	(-CH ₂ O-) _E : δ 3.99 (-CH ₂ O-) _Z : δ 4.31	500, 494

^a 400 MHz, taken in CDCl₃; ^b values given for oximyl-H (**3.5**, **3.7**, **3.9**, **3.11**), C(3) methylene (**3.6**, **3.10**), or C(3) methylene (**3.8**, **3.12**).

In the total ion chromatograms of the GC-TOF MS experiments, two peaks could be observed (except for **3.6** and **3.8**, where only the (*E*)-isomers were detected), originating from the (*E*)- and (*Z*)-isomers. There was only partial agreement between the *E/Z* ratios determined by ¹H NMR and by GC-MS. We concluded that extensive (*Z*)-to-(*E*) isomerization might have taken place in the GC column or in the heated transfer line. In the case of the isolated oxime fragment (aldehyde system), the (*Z*)-isomer is 0.1kcal/mol lower in energy than the (*E*)-isomer. The barrier height for (*E*)-to-(*Z*)

isomerization is predicted to be 16.2 kcal/mol in the ionized state, although it is predicted to be 18.5 kcal/mol for the ketone system [UB3LYP/6-31G(d)]. These results do not confirm or exclude isomerization in the ion source as an alternative. In the case of compounds **3.5–3.8**, the McLafferty rearrangement is more pronounced in the mass spectra of the (*E*)-isomers than in the (*Z*)-isomers. No direct McLafferty rearrangement fragment ions can be observed in the mass spectra of the silyl oxime ethers **3.9–3.12**. However, fragment ions derived from loss of *t*-butyl radical after McLafferty rearrangement are observed in the mass spectra of compounds **3.11** and **3.12** (Table 3.2).

Loss of the *t*-butyl radical followed by McLafferty rearrangement does not seem plausible, for the first loss would create an even electron ion. Although the McLafferty rearrangement has been observed for even electron systems, it seems unlikely to be of great importance for these systems; but it cannot be excluded because we did not investigate this possibility in more detail. The loss of just the *t*-butyl radical was also observed as the peaks at the highest *m/z* values, as well as abundant formation of $[\text{HOSi}(\text{CH}_3)_2]^+$ (*m/z* = 75). These reactions will be discussed in more detail in the next sections.

In addition to the conventional McLafferty rearrangement reaction, the fragment ions corresponding to the McLafferty reverse charge rearrangement were observed. For compound **3.4**, the fragment ions of both reactions have the same *m/z* values and cannot be distinguished, and neither can their relative contributions be estimated. The occurrence of the McLafferty reverse charge rearrangement is an indication that the McLafferty rearrangement actually took place, and so the relative contributions should be added to obtain a quantitative estimate of the overall contribution of the McLafferty

rearrangement reaction. For compounds **3.1,3,5,7** and **3.9–12**, the McLafferty reverse charge rearrangement had contributions of >25% relative to the McLafferty rearrangement. For **3.5** and **3.7**, the relative contributions were different for the (*E*)- and (*Z*)-isomers.

Table 3.2. Identification of McLafferty and reverse-charge McLafferty ions.

Entry	Elemental Composition	M ⁺ <i>m/z</i>	M ⁺ ion count	McLafferty rearrangement fragment <i>m/z</i>	ion count ^a	McLafferty reverse charge rearrangement fragment <i>m/z</i>	ion count ^a
3.1	C ₆ H ₁₂ O	100	not observed	44	1000	56	585
3.2	C ₇ H ₁₄ O	114	< 50	58	420	56	15
3.3	C ₅ H ₁₀ O ₂	102	not observed	44	88	58	20
3.4	C ₆ H ₁₂ O ₂	116	not observed	58	180	58	180 (same <i>m/z</i> as McLafferty)
3.5	C ₆ H ₁₃ ON	115	not observed	59	(<i>E</i>), 1000; (<i>Z</i>), 660	56	(<i>E</i>), 305; (<i>Z</i>), 270
3.6	C ₇ H ₁₅ ON	129	not observed	73	(<i>E</i>), 1000	56	(<i>E</i>), 80
3.7	C ₅ H ₁₁ O ₂ N	117	not observed	59	(<i>E</i>), 1000; (<i>Z</i>), 390	58	(<i>E</i>), 700; (<i>Z</i>), 98
3.8	C ₆ H ₁₃ O ₂ N	131	not observed	73	(<i>E</i>), 1000	58	(<i>E</i>), 120
3.9	C ₁₂ H ₂₇ ONSi	229	not observed	173 116 ^b	(<i>E</i>), not observed; (<i>Z</i>), not observed (<i>E</i>), < 5; (<i>Z</i>), < 5	56	(<i>E</i>), 40; (<i>Z</i>), 30
3.10	C ₁₃ H ₂₉ ONSi	243	not observed	187 130 ^b	(<i>E</i>), not observed; (<i>Z</i>), not observed (<i>E</i>), < 10; (<i>Z</i>), < 5	56	(<i>E</i>), 75; (<i>Z</i>), 70
3.11	C ₁₁ H ₂₅ O ₂ NSi	231	not observed	173 116 ^b	(<i>E</i>), not observed; (<i>Z</i>), not observed (<i>E</i>), 188; (<i>Z</i>), 85	58	(<i>E</i>), 70; (<i>Z</i>), 65
3.12	C ₁₂ H ₂₇ O ₂ NSi	245	not observed	187 130 ^b	(<i>E</i>), not observed; (<i>Z</i>), not observed (<i>E</i>), 170; (<i>Z</i>), 106	58	(<i>E</i>), 68; (<i>Z</i>), 60

^aMajor fragment ion assigned a relative value of 1000. ^bMcLafferty rearrangement fragment minus *t*-Bu[•].

3.C.2. Computational Results and Analysis

Professor Dean Tantillo and his graduate students Osvaldo Gutierrez and Jason Harrison performed all theoretical calculations as part of a collaboration effort.²⁶ Data shows that the oxygen atom at the β -position ($Y=O$, Scheme 3.8) and the aldehyde functionality ($R=H$) strongly favor the McLafferty reverse charge rearrangement energetically compared with $Y=CH_2$ and $R=CH_3$. The identity of the acceptor group ($X=O$, NOH and $NOSiH_3$) was of less importance. Surprisingly, the relative stabilities of the ionic and neutral products of the two reactions do not always determine which process is more favorable or explain the close competitiveness. For **3.1**, **3.3**, and **3.8**, the McLafferty reverse charge rearrangement is energetically more favorable, whereas the McLafferty rearrangement is more abundant for all other entries except **3.9** and **3.10**. However, it was beyond the scope of our study to theoretically investigate the McLafferty reverse charge rearrangement in more detail, which would be needed to provide a more quantitative discussion and comparison.

Scheme 3.8. Computed McLafferty fragmentation. Concerted mechanism in black. Stepwise mechanism in red.

As shown in Scheme 3.8, fragmentation can proceed through a concerted McLafferty rearrangement ($A^{*+} \rightarrow G^{*+} \rightarrow E^{*+} + F$) or via a stepwise hydrogen transfer/cleavage process (red; $A^{*+} \rightarrow B^{*+} \rightarrow C^{*+} \rightarrow D^{*+} \rightarrow E^{*+} + F$). Prof. Tantillo's calculations indicate that for all systems, with the exception of ethoxyethanal ($R=H$, $X=Y=O$; Table 3.3, compound **3.3**), the stepwise pathway is favored.ⁱ Barriers for both steps are somewhat sensitive to the identity of X and Y (Table 3.3). Among the three classes of compounds studied, aldehydes/ketones are predicted to have the largest overall energy barriers, whereas oximes have the lowest. Introduction of a more electronegative group at the β -position is expected to facilitate the cleavage of the α - β bond, and this is the case for all systems examined herein ($Y=CH_2$ vs $Y=O$). Consequently, for the oximes and silyl oxime ethers with $Y=O$, the rate-determining step is predicted to be the initial hydrogen transfer step. Although changing from $Y=CH_2$ to $Y=O$ might be expected to help the hydrogen transfer step as well, because an oxygen can interact favorably with an attached carbon radical, we do not see evidence for this effect. Although fragmentation reactions take place from initially excited molecular ions, an extensive treatment of excitation processes is beyond the scope of this study. Nonetheless, our results reveal the underlying potential energy surfaces for the fragmentation reactions. For entries (**3.9-3.12**), calculations were also performed using the larger 6-311++G(2d,2p) basis set; qualitatively similar results were observed (Table 3.3).

ⁱ (a) All attempts to find concerted transition state structures for the other systems failed at the UB3LYP/6-31G(d) level of theory. (b) For comparison, using SCS-UMP2/6-31G(d) calculations, relative energies for entry (**3.5**) are predicted to be: $A^{*+} = 0.0$, $B^{*+} = -4.7$, $C^{*+} = -14.0$, $D^{*+} = 5.4$ (all in kcal/mol). (c) UM06-2X/6-31G(d) was also used and similar reactivity trends were obtained, although, as shown in Figure 3.5, geometries for hydrogen transfer transition state structures vary slightly.

Table 3.3. Relative free energies and [UB3LYP/6-31 G(d); 298.15 K; kcal/mol; normal text] electronic energies (in parenthesis).

Class	Entry	R	X	Y	A ⁺	B ⁺⁺	C ⁺	D ⁺⁺	$\Delta G^\ddagger$ ($\Delta E^\ddagger$)	RDS
Aldehyde/Ketone	3.1	H	O	CH ₂	0.0 (0.0)	0.3 (1.5)	1.4 (0.8)	8.6 ^a (7.2)	8.6 (7.2)	C–C bond breaking
	3.2	CH ₃	O	CH ₂	0.0 (0.0)	–0.2 (1.2)	–0.3 (–0.8)	14.1 ^a (12.3)	14.1 (12.3)	C–C bond breaking
	3.3	H	O	O	0.0 (0.0)				10.9 (11.3)	<i>Concerted</i>
	3.4	CH ₃	O	O	0.0 (0.0)	8.1 (9.5)	9.1 (9.3)	10.6 (10.8)	10.6 (10.8)	C–C bond breaking
Oxime	3.5	H	NOH	CH ₂	0.0 (0.0)	–0.5 (0.7)	–6.0 (–6.6)	1.4 (3.4)	1.4 (3.4)	C–C bond breaking
	3.6	CH ₃	NOH	CH ₂	0.0 (0.0)	0.3 (1.8)	–6.1 (–6.4)	5.3 (7.3)	5.3 (7.3)	C–C bond breaking
	3.7	H	NOH	O	0.0 (0.0)	0.0 (1.6)	3.4 (–3.8)	1.6 (–1.2)	0.0 (1.6)	H-transfer
	3.8	CH ₃	NOH	O	0.0 (0.0)	0.2 (1.9)	–4.7 (–5.1)	–0.9 (–1.1)	0.2 (1.9)	H-transfer
Silyl-oxime	3.9	H	NOSiH ₃	CH ₂	0.0 (0.0)	1.7 (3.2)	–4.1 (–4.5)	5.1 (7.2)	5.1 (7.2)	C–C bond breaking
	3.10	CH ₃	NOSiH ₃	CH ₂	0.0 (0.0)	0.4 (1.7)	–7.2 (–7.4)	0.2 (2.4)	0.4 (1.7)	C–C bond breaking
	3.11	H	NOSiH ₃	O	0.0 (0.0)	0.9 (2.4)	–3.6 (–4.2)	–1.4 (–1.4)	0.9 (2.4)	H-transfer
	3.12	CH ₃	NOSiH ₃	O	0.0 (0.0)	–0.7 (0.5)	–7.1 (–7.6)	–4.5 (–4.8)	–0.7 (0.5)	H-transfer

^aSingle point energies with UB3LYP/6-31G(d) on geometries optimized with UMP2/6-31G(d); for these structures, convergence problems were encountered when using UB3LYP/6-31G(d). Bold numbers are relative energies calculated using UB3LYP/6-311++G(2d,2p).

Representative computed transition state structures are shown in Figure 3.6. For the one system where a concerted process was predicted to be energetically preferred ($X=Y=O$), this process was found to involve asynchronous hydrogen transfer and cleavage events. In the transition state structure for this process ($3G^{*+}$ in Figure 3.6), hydrogen transfer is nearly complete, whereas the C–O bond cleavage has not yet begun. As mentioned earlier, changing the identity of X and/or Y leads to a stepwise mechanism where hydrogen transfer occurs first. A representative transition state structure for hydrogen transfer ($5B^{*+}$) is also shown in Figure 3.6. In contrast to $3G^{*+}$, this transition state structure is early with respect to the hydrogen transfer event; the migrating hydrogen is closer to the carbon from which it originates than to its destination on nitrogen (note that an even earlier transition state structure is predicted with UM06-2X). Changing from $Y=CH_2$ to $Y=O$ ($7B^{*+}$) leads to a later transition state structure, however, in which the C–H and H–N distances are similar (Figure 3.6). The identity of Y also affects the degree of bond breaking in the transition state structures for cleavage. For example, C–C cleavage is well advanced in $5D^{*+}$ but has barely begun in $7D^{*+}$ (Figure 3.6).

Why are the barriers for oximes and silyl oximes lower than those for aldehydes and ketones? From the energies shown in Table 3.3, it appears that having $X=NOR$ rather than $X=O$ pulls down the energies of B^{*+} , C^{*+} and D^{*+} relative to that of A^{*+} . This is likely due to a combination of effects, whose magnitudes are difficult to assess: differences in O–H and N–H bond strengths (note that N–H bonds tend to be slightly shorter than do the corresponding O–H bonds in intermediates C^{*+}),²⁷ differences in the

ability of OH and HONH groups to stabilize adjacent carbocations, and changes in interactions across the O–N bond upon hydrogen transfer to oximes and silyl oxime ethers. From the energies shown in Table 3.3, it also appears that having Y=O promotes the cleavage process. This is likely due in large part to the greater strength of C=O bonds than C=C bonds, but it may also be due in part to increased $\sigma_{\text{C-R}} \leftrightarrow \sigma^*_{\text{C-Y}}$ interactions, which should help to break the C–Y bond, when Y=O.

Figure 3.6. Representative computed transition state structures.

3.C.3. Mechanistic Summary of the Fragmentations of Oxime Ethers

In the 70eV electron ionization mass spectra of silyl oxime ethers **3.11** (R=H, Scheme 3.9) and **3.12** (R=CH₃), a variety of interesting fragmentation pathways were observed, and the proposed mechanisms are depicted in Scheme 3.9.

Scheme 3.9. The proposed fragmentation mechanisms for silyl oxime ethers **3.11** (R=H) and **3.12** (R=CH₃).

The *tert*-butyl radical (*t*-Bu•) is lost from both isomers of the odd-electron silyl aldoxime and ketoxime species to form corresponding silenium ions (Scheme 3.9, *m/z* 174, 188). The (Z)-silenium ions **A** and (E)-silenium ions **B** are observed in the spectra of the respective (Z)- and (E)-aldoximes as well as ketoximes. Silenium ions **A** are stabilized by the proximal ether functionality, and these intermediates give rise to the iminium dioxosilacycles **C** (*m/z* 132, 146) on loss of the propyl chain either by elimination of propene, as shown, or possibly by loss of the propyl cation. Formation of

C through an ion- neutral complex instead of the 1,2-elimination is also a possibility that cannot be ruled out,²⁸ but we have not investigated this option theoretically in more detail. Finally, it is also possible that **C** could arise via cyclization of the ionized ether linkage [**M**•+ ether] (Scheme 3.9). The observed formation of the α -ether cleavage fragment **D** (m/z 73) supports reaction along this path. Whereas silenium ions **B** are not structurally predisposed to undergo a similar cyclization path, their precursors, the odd electron (*E*)-isomers ($R=H$, $R=CH_3$), can form silacycles **C** by isomerization to the (*Z*)-isomers prior to loss of *t*-Bu•. The isomerization of ionized oximes is known for condensed phase acidic solutions.²⁹ It has to be stated that this might not be applicable in the gas phase and that [**M** + H]⁺ oximes might behave differently than **M**•+ oximes. However, gas-phase ¹H NMR studies on rotational barriers of neutral amides³⁰ and *N,N*-dialkylformamide³¹ showed that barrier heights of ~20 kcal/mol do not preclude isomerization, and so it seems reasonable to assume that the (*E*)-to-(*Z*) isomerization barrier height in the ionized state of 16.2 kcal/mol is not prohibitive in the gas phase. The odd-electron (*E*)-isomers are structurally poised for γ -hydrogen atom abstraction. Subsequent loss of propanal via α,β -bond scission occurs to give the McLafferty rearrangement fragments. The facile loss of *t*-Bu• occurs here as well to yield **E**, which likely forms the isomeric 5-membered silacycles (m/z 116, 130) as depicted, whereas a three-membered silacycle was excluded as a realistic option. The McLafferty rearrangement with loss of *t*-Bu• is observed for both aldoxime isomers and both ketoxime isomers, although the propensity for cleavage via the McLafferty rearrangement is higher for the (*E*)-isomers.

Figure 3.7. The proposed fragmentation mechanism for the N–O bond cleavage followed by loss of RCN from the silyl ketoximes **3.10** and **3.12**.

As reported previously,³² a characteristic fragmentation observed in the mass spectra of silyl ketoxime ethers is the N–O bond cleavage (Figure 3.7). The observed fragment ion is presumably a nitrilium ion derived via a 1,2-shift³³ of the alkyl side-chain concomitant with loss of the silyloxy radical. Nitrilium ions commonly are derived from iminium species.³⁴ Whereas both isomers of silyl ketoxime ether **3.10** undergo major N–O bond cleavage, this mode of fragmentation is not significant for any of the ketoximes (Figure 3.7). Furthermore, the (*Z*)- and (*E*)-isomers of silyl ketoxime ether **3.12** appear to undergo N–O cleavage to only a small extent, possibly because the migratory aptitude of the oxygen-containing side-chain is diminished.²⁸ However, the scarcity of the nitrilium ions for these isomers may be due to the facile loss of acetonitrile, giving the more stable oxonium ion species (*m/z* 73). The *m/z* 73 fragment is also populated by the paths described earlier (e.g. species **D** in Scheme 3.9). Along these lines, the *m/z* 73 fragment derived from oxime **3.8** is the result of the McLafferty rearrangement, which was shown to be the predominant fragmentation for all oximes. The N–O cleavage mode is negligible for all aldoximes and silyl aldoximes (Figure 3.7, where R=H). Figure 3.8 and Figure 3.9 clearly show the impact of oxygen substitution adjacent to the radical-forming

center during McLafferty rearrangement. Indeed, the McLafferty fragment is almost non-existent for the silyl oxime ethers with β -CH₂, while it is clearly present for the analogs containing β -O.

Figure 3.8. EI-MS (X-axis: m/z ; Y-axis: intensity). The influence of β -CH₂ versus β -O on the fragmentation of (*E*)-silyl oxime ethers. The ketoxime (*E*)-isomer (A) do not exhibit significant α,β -bond scission (McLafferty rearrangement), unlike the oxygen-substituted isomer (B).

Figure 3.9. EI- MS (X-axis: m/z ; Y-axis: intensity). The influence of β -CH₂ versus β -O on the fragmentation of (Z)-silyl oxime ethers. The ketoxime (Z)-isomer (A) do not exhibit significant α,β -bond scission (McLafferty rearrangement), unlike the oxygen-substituted isomers (B).

Figures 3.8 and 3.9 also show the differences between (*E*)- and (*Z*)-isomers: the McLafferty rearrangement is favored by the *E*-isomer; the generation of a nitrilium ions is favored by the *Z*-isomer.

In summary, we have shown experimentally and theoretically that a set of model carbonyl compounds comprised of aldehydes and ketones, as well as their derivative oxime and silyl oxime ethers, undergo a McLafferty rearrangement to varying extents upon electron ionization. The silyl oximes that lacked γ -radical stabilization (e.g., β -oxygen substitution) had the lowest incidence of such rearrangement. The McLafferty rearrangement is enhanced, relative to other primary fragmentation processes, by oximes (vs carbonyl), oxygen at the β -position (vs β -methylene) and in ketones (vs aldehydes). The McLafferty reverse charge rearrangement was also observed. The results of density functional calculations indicated that most compounds undergo a stepwise McLafferty rearrangement and that both C–C bond breaking and H-transfer could be the rate-determining step. Only $\text{HC(O)CH}_2\text{OC}_3\text{H}_7$ was predicted to undergo fragmentation via a concerted mechanism. For the silyl oxime ethers, the McLafferty rearrangement was accompanied by the subsequent loss of the *t*-butyl radical. Finally, we observed that oximes and silyl oxime ethers were obtained as both the (*E*)- and (*Z*)-isomers and that the extent of the McLafferty rearrangement was higher in the (*E*)-isomers.

This fundamental study provides essential tools for the identification of carbonyl metabolites that have reacted with aminoxy-containing derivatization reagents. The relative incidence of McLafferty fragments versus nitrilium ions can help assign stereochemistry of the metabolite adducts in future studies. Also, by better understanding the fragmentation of such oxime ether adducts, we will be able to predict MS-induced cleavages that provide structural information on the derivatized metabolite. This information potentially can help identify metabolites that may be molecular biomarkers.

3.D. Discovery of a Zone of Minimal Interference

3.D.1. Mass Spectral Observation of the ZMI

Mass spectral fragments below m/z 50 generally are not noted when analyzing high molecular weight compounds due to the excessive noise generated by ionized gases in this range. Moreover such low molecular weight fragments generally do not provide useful structural information for the identification of the parent compound using the searchable libraries. However, during the course of our oxime ether study, while analyzing the 70eV electron ionization mass spectra of the panel of carbonyl substrates, we noted that the compounds had similar fragmentation behavior within the range m/z 31-37. Shown in Figure 3.10 are the electron ionization (EI) mass spectra of compounds **3.2**, **3.4**, **3.5**, and **3.11**. Highlighted in the red box is a region of the spectrum in which these compounds did not generate any ion fragments. To emphasize the lack of signal generation within this range, we labeled the range m/z 31-37 the Zone of Minimal Interference (ZMI). At first look, the ZMI seems to be independent of the nature of the parent compound, as seen with the spectra of the noted ketone, oxime, and silyloxime ether given in Figure 3.10.

Figure 3.10. EI-MS (X-axis: m/z ; Y-axis: intensity) spectra of compounds **3.11** (A), **3.5** (B), **3.4** (C) and **3.2** (D). The red box delineates a range m/z 31-37 in which the compounds generate few, if any, fragments.

3.D.2. Tabulation of the NIST Library

To better delineate the m/z gap of the *ZMI* and to elucidate whether or not this observation is common among small organic molecules (i.e., $M_w < 500$), professor Xiang Zhang and Dr. Bing Wang consulted the NIST compilation of the EI mass spectra of 163,198 organic compounds and provided a tabulation (Figure 3.11).

Figure 3.11. Delineation of the zone of minimal interference. **A:** Fragment ion frequency for the compound library (NIST/EPA/NIH NIST 08 Mass Spectral Library). **B:** corresponding summed ion count (peak intensity) for a given m/z .

Tabulation of the incidence for every m/z signal in the library of compounds reveals several gaps where the frequency of a signal is minimal (Figure 3.11A). Note that the gap between m/z 19-24 (see Figure 3.11A insert) is sparsely populated, as well as our initially observed *ZMI* gap between m/z 31-37. Although the 31-37 zone is more populated than the 19-24 zone, the intensities of any fragment ions that register within the 31-37 *ZMI* remain quite low as indicated by the summed ion count for each m/z (Figure 3.11B).

To obtain accurate relative quantification information by measuring the intensity of reporter mass spectral tags (MSTs), it is important to have as little background noise as possible to avoid higher than expected intensities. Could these low noise zones be used to observe reporter tags to obtain quantitation information? The gap between m/z 19-24 is too low for synthetic application. However, the zone of minimal interference m/z 31-37 is suitable for engineering a mass spectral tag (MST) based on ^{13}C and ^2H isotopes. Based on this observation, we set out to design a derivatization reagent that generates isotopic MSTs in the mass range m/z 31-37 for distinct observation after electron ionization. This work is described in the next chapter.

CHAPTER 4

An Approach to Quantitative Multiplexed GC-MS Analysis. Part II: Development of Derivatizing Reagents

4.A. Introduction

4.B. Reagent Design and Synthesis

- 4.B.1. Development of POMS Reagents: First Generation
- 4.B.2. Development of POMS-ONH₂ Reagents: Second Generation
- 4.B.3. Development of AEP Reagents

4.C. Results and Applications of AEP Reagent

- 4.C.1. Multiplex Experiment: Proof of Concept
- 4.C.2. Analysis of a Biologically Derived Sample

4.D. Conclusions

- 4.D.1. General
 - 4.D.2. Future Directions
-

4.A. Introduction

With the discovery of a zone of minimal interference (*ZMI*) that enables the unobstructed observation of isotopic reporter ions, we next turn our attention to designing reagents that could generate reporter fragment ions within that region of the mass spectrum. Our goal is to develop reagents for the profiling of endogenous and exogenous small molecules with molecular weights below 1500 Da, known as metabolites.¹ These “metabolites” typically contain diverse chemical structures and functional groups that make analyte extraction, enrichment, separation, and analyses difficult.² Profiling molecules in the search for biomarkers of disease in biological samples, such as blood, urine, or tissue, has led to numerous advances in analytical technologies.³ Mass spectrometry⁴ (MS) and its tandem combinations with principal separation techniques, such as liquid chromatography⁵ (LC-MS) and gas chromatography⁶ (GC-MS), currently are among the most applied analytical tools for metabolomic profiling.⁷

Stable isotope coding is a strategy that has been used to streamline LC-MS analyses in metabolomics.⁸ Analyte samples containing common functional groups are first derivatized with differentially coded isotope reagents and then the derivatized mixtures are combined and analyzed simultaneously by LC-MS. The relative concentrations of the coded analyte adducts are provided by an isotope ratio analysis of the isotopic molecular ions (M^+) obtained on electrospray ionization. Isotopic derivatization reagents have been applied in this way for the quantification of carboxylic acid,^{8a,d} amine,^{8b,c} and fatty acid^{8e} metabolites. Table 4.1 shows the structure of some of the labeled reagents used for isotope coding strategies in LC-MS. These reagents come only as two versions, the light- or heavy-isotope derivative. This binary set of reagents is

used either for absolute quantification from a known standard or for relative quantification between two samples. Whether coded isotopologues co-elute affects how the quantitation analysis can be achieved. If isotopologues exactly co-elute, their isotope ratio from the mass spectra provides the relative quantification between the two adducts. If they do not, each analyte must be quantified independently based on the chromatogram peak area measurements.

Table 4.1. Isotope coding reagents for LC-MS. The location and nature of the isotopes are shown in blue, while the reactive center that enables derivatization is shown in red.

<p>Tsukamoto <i>et al.</i> proposed the use of DBD-PZ-NH₂-(D₆) as a derivatizing reagent for carboxylic acids for the quantification of fatty acids by high performance LC-MS. Shown in red is the primary amine functionality that reacts with the activated carboxylic center.^{8a,b}</p>	<p style="text-align: center;">DBD-PZ-NH₂ (H) or (D)</p>
<p>DBD-PZ was used for the derivatization of ketoprofen and ibuprofen followed by analysis using LC-electrospray MS. DBD-PZ proved to be less reactive than DBD-PZ-NH₂.^{8b}</p>	<p style="text-align: center;">DBD-PZ (H) or (D)</p>
<p>Protonated methyl acetimidate was used as a labeling reagent for amines. Quantification of amino acids from seed extracts was performed via LC-MS.^{8c}</p>	<p style="text-align: center;">* = ¹²C or ¹³C</p>
<p>Cholamine-(d₀) and -(d₉) were used for the relative quantification of carboxylic acids by LC-MS.^{8d}</p>	<p style="text-align: center;">X = H or D</p>
<p>Yang <i>et al.</i> proposed the use of ²H-labeled 2-bromo-1-methylpyridinium iodide (BMP), for the derivatization of fatty acids.^{8e}</p>	<p style="text-align: center;">BMP X = H or D</p>

In contrast with LC-MS, stable isotope coding strategies for GC-MS analyses are scarce.⁹ The ionization method employed in a GC-MS system is electron ionization (EI), in which the electron energy (~70 eV) is so high that metabolite adducts are directly fragmented to yield mass spectra that are considerably more difficult to quantify. Given that GC-MS and comprehensive two-dimensional GCxGC-MS are the principal analytical platforms for metabolomics due to their superior chromatographic resolution and reproducibility,² and that EI enables the use of searchable mass spectra libraries,¹⁰ an isotopic coding strategy for GC-MS addressing the aforementioned quantification challenge is very much needed. Table 4.2 summarizes the currently published isotope coding strategies for GC-MS.

Table 4.2. Isotope coding reagents for GC-MS. The location and nature of the isotopes are shown in blue, while the reactive center that enables derivatization is shown in red.

Huang and Regnier synthesized the (d₆)-labeled version of *tert*-butyldimethylsilyl trifluoroacetamide (MTBS-TFAm). This silicon-based reagent can derivatize alcohol, amine, and carboxylic metabolites. The acid chromatographic isotope effect led to partial resolution of isotopologues via GCxGC-MS.^{9a}

Kvitvang *et al.* used (d₃)-labeled methyl chloroformate (MCF) to derivatize amine metabolites, and quantify amino acids. Although this method uses GC for the separation of complex mixtures, it uses positive chemical ionization to impart a positive charge, and does not use traditional EI methodology.^{9b}

Lien *et al.* used (d₉)-labeled trimethylsilyl trifluoroacetamide (MS-TFAm) for the derivatization of alcohol, amine, and carboxylic metabolites. In the same manner as Huang and Regnier, they encountered the chromatographic isotope effect.^{9c}

As with traditional GC-MS analyses, the isotope coding reagents utilize silicon-based derivatization reagents that help make polar metabolites more volatile and more thermally stable by generating silyl ethers. Silylating procedures also enhance GC separation by avoiding the tailing of the compounds in the column, and often produce more diagnostic fragments with more abundant ions to facilitate detection and identification of the derivative. Also, contrary to LC-MS reagents, these silylating reagents and their adducts are moisture sensitive and can hydrolyze rapidly if not handled properly.¹¹ However, the main challenge that remains for the GC-MS coding reagents presented in Table 4.2 is that the coded isotopologues do not co-elute in the GC column due to chromatographic isotope effect. Coded molecules that differ in numbers of heavy atoms (isotopes) can be partially or even completely resolved during liquid- and gas-chromatography, particularly in the case of deuterium labeling.¹² This drawback prevents the direct use of the mass spectrum for the quantitation of the samples, which then relies on the chromatogram peak area.

We present here the first isotope coding strategy that features EI-induced expulsion of a reporter tag for quantification using GC-MS (Figure 4.1). Specifically, we have developed a panel of isotopologous aminooxyethyl propionate (AEP) reagents for chemoselective derivatization of aldehydes and ketones.

Figure 4.1. Representation of a multiplexed GC-MS analysis using isotope-coded reagents designed for electron-induced expulsion of reporter mass spectral tags (MSTs). The derivatization reagents provide corresponding MSTs for relative quantification of substrates from separate sample mixtures.

The reagents are fitted with ^2H - and ^{13}C -isotope labels such that EI-induced fragmentation of the AEP-adducts results in identifiable reporter mass spectral tags (MSTs) for relative quantification of individual members of a multiplex set, as depicted in Figure 4.1. Derivatization of carbonyl substrates using the AEP isotopologues (Figure 1, step A) followed by pooling the samples (step B) generates a multiplex set for a single-

injection analysis by GC-MS. If the isotope labeling does not influence GC elution of the pooled adducts (i.e., a chromatographic effect), then identical substrates that have been labeled with different isotope tags will co-elute on GC separation (step C). Subsequent electron ionization of the eluted substrates induces fragmentation (step D) to yield characteristic fragment ions for substrate identification as well as release of coded isotopic tag ions for quantification. Although previously published GC-MS isotope coding approaches have used isotopically labeled derivatizing agents,⁹ as discussed above, none of these approaches exploit a *common* reporter tag that enables rapid quantification by direct mass spectral measurements (e.g., ion count comparisons).. Furthermore, earlier coding strategies have relied on binary sets of reagents, and this limitation, as well as noted chromatographic isotope effects leading to partial GC resolution of derivatized substrates, impede high throughput. A multiplex strategy that overcomes chromatographic separation of isotopically derivatized substrates would enable multiple samples to be labeled, mixed, and analyzed simultaneously to increase throughput and reduce technical variation.

4.B. Reagent Design and Synthesis

4.B.1. Development of POMS Reagents: First Generation

An important consideration to actuate such a coding strategy is the selection of an unambiguous reporter MST, one that can be observed free from obfuscation by other fragment ions. Deconvolution approaches that align and match derived fragment ions to resolve MSTs using pre-processing software for MS identification and quantification have been shown to be error prone.¹³ Ideally, direct ion count comparisons of MST isotopologues would be most practical. Thus, based on our previous study on the fragmentation of oxime substrates and the subsequent discovery of the *ZMI* range m/z 31-37 (Chapter 3), we surmised that the preparation of our reagents would require the presence of a propionyl ester functionality for the EI expulsion of an ethyl carbenium ion reporter MST ($C_2H_5^+$ m/z 29) via ester α -cleavage (Figure 4.2).¹⁴ As shown in Figure 4.2, after electron ionization, the radical cation rearranges to generate an acylium ion that spontaneously loses carbon monoxide to form an ethyl carbenium ion at m/z 29. Using different combinations of 2H - and ^{13}C -analogs of the ethyl carbenium ion would shift the mass range of the reporter tags to cover the range m/z 29-36 (Figure 4.3).

Ester α -cleavage

Figure 4.2. EI-induced α -cleavage of propionate ester generates acylium ion at m/z 57 and ethyl carbenium ion at m/z 29.

To demonstrate the viability of the propionate ester to deliver a reporter mass spectral tag in the form of an ethyl carbenium ion via the ester α -cleavage, we decided to synthesize a simple compound containing both silicon and propionate moieties. Scheme 4.1 shows the synthesis of such template, bis-propionyloxymethyldimethyl silane **4.1**. This one step synthesis is performed by heating bis(chloromethyl)dimethylsilane **4.0** with propionic acid and triethylamine to afford the bis-propionate **4.1** in 90% yield.

Scheme 4.1. Synthesis of bis-propionyloxymethyldimethyl silane **4.1**.

In the mass spectrum of **4.1** (Figure 4.3), we can easily observe the two fragments m/z 57 and 29 corresponding respectively to the acylium ion and the ethyl carbenium ion that we were expecting to arise from the α -cleavage of the propionate ester moiety. We can also identify other silicon-based fragments that provide structural information.

Figure 4.3. EI-MS (X-axis: m/z ; Y-axis: intensity) of bis-propionyloxymethyldimethyl silane **4.1**.

Figure 4.4. Ester α -cleavage of POMS derivatized metabolites delivers the labeled reporter tags in the in the range m/z 29-36 that overlaps with the *ZMI*.

As a first attempt, we explored the synthesis of a silicon-based reagent to readily silylate *N*- and *O*-nucleophiles. The reagent would be fitted with the stable isotope-labeled ester moiety. Thus, the reagent was conceived to feature a (propionyloxy)methylsilyl (POMS) group containing combinations of deuterium and ^{13}C at C(2) and C(3) of the ester. As illustrated in Figure 4.4, electron impact ionization of esters derived from reaction with POMS-Cl yields acylium ions via the facile α -cleavage of the POMS ester. The acylium ions spontaneously lose CO to give the corresponding reporter carbenium ions. The EI-induced α -cleavage of POMS from silylated metabolites will result in signals in the range m/z 29-36, corresponding to the degree of metabolite labeling (Figure 4.4). Initially, three unique combinations of isotope tags in POMS will give rise to three distinct reporter MST signals for simultaneous analyses of POMS-derivatized metabolite mixtures. Ultimately, up to eight isotopic reagents could be prepared using distinct combinations of ^2H and ^{13}C .

We aimed to perform POMS derivatization of metabolites (HY-mtb) using either the chlorosilane (POMS-Cl) or the well-established trifluoroacetamide methodology (POMS-TFAm), seen in Figure 4.4. As shown in Figure 4.5, once the metabolite is

derivatized and ionized on introduction to the MS ion beam, we expect that many different fragmentation pathways will compete. Mass spectroscopy of the POMS-metabolite adducts should furnish labeled acylium and labeled ethyl carbenium via the α -cleavage pathway (path **A**). Common to silyl-based derivatives is loss of a silyl group on EI ionization (path **S**) to give silyl ions. In the case of POMS-labeled substrates, loss of the (propionyloxy)methyl radical is expected to prevail over loss of methyl radical (Figure 4.5). Thus, the $[\text{Me}_2\text{Si-Y-mtb}]^+$ fragment should be a key analytical fragment of the present approach in addition to the expected $[\text{mtb}]^+$ fragment arising via loss of POMS-Y $\cdot$ (Path **M**), another common silyl-analog fragmentation. Both of these pathways should provide information for the identification of the metabolite such as the molecular weight and presence of functional groups.

Metabolite derivatization

Figure 4.5. POMS α -cleavage generates the reporter ethyl carbenium ion as well as silicon- and metabolite-specific ion fragments.

To demonstrate our idea and observe the propensity for the α -cleavage of our POMS reagent, we synthesized the unlabeled POMS derivative of benzyl alcohol (Scheme 4.2). Commercially available chloromethyl(dimethylsilylchloride) (**4.2**) was reacted with benzyl alcohol to afford the silylated benzyl alcohol **4.3**. The use of trimethylsilyl propionic acid in presence of cesium fluoride in dry dimethylformamide (DMF) was used to generate the cesium propionate anion *in situ*. As expected, reaction of compound **4.3** with cesium propionate smoothly generated POMS-OBn (Scheme 4.2).

Scheme 4.2. Synthesis of POMS-derivatized benzyl alcohol.

In the mass spectrum of POMS-OBn (Figure 4.6), we can easily observe the two fragments m/z 57 and 29 corresponding respectively to the acylium cation (**A₁**) and the ethyl carbenium ion (**A₂**) arising from the α -cleavage (pathway **A**) of the ester moiety of the POMS reagent. Pathways **M** and **S** are also observed with the fragments m/z 91 (tropylium ion) and m/z 165. These fragments provide information related to the structure of the silylated substrate (in this case benzyl alcohol) and thus can be used for identification of the parent substrate structure.

Figure 4.6. EI-MS (X-axis: m/z ; Y-axis: intensity) of POMS-derivatized benzyl alcohol. Shown are the fragmentation pathways predicted in Figure 4.4.

With the knowledge that the model POMS-Y-R adduct did in fact provide reporter ions at the *ZMI* border, as well as fragment ions for structure identification, we were encouraged to consider isotope labeling and to pursue a synthesis of POMS-Cl. The use of ^2H -, ^{13}C - or a combination of ^2H - and ^{13}C -labeled POMS reagent would increase the molecular weight of the carbenium ion and move it into the *ZMI* to facilitate the quantitative analysis and reduce the interference from metabolite fragments.

Our first attempted synthesis of the POMS reagent is shown in Scheme 4.3. Commercially available chloromethyldimethylsiloxane (**4.4**) was heated to reflux in the presence of propionic acid and triethylamine to afford siloxane **4.5** in excellent yield (93%).¹⁵ Unfortunately, the cleavage of the siloxane **4.4** to POMS-Cl could not be

performed under Korlyukov's¹⁶ or Sukeda's¹⁷ conditions. Instead of chlorinating the siloxane Si-O-Si bond, the reported conditions led to the displacement of the propionyl ester functionality and generated a mixture of starting chloromethyldimethylsiloxane (**4.4**) and mono-acylated siloxane.

Scheme 4.3. First attempted synthesis of POMS-Cl.

Shown in Figure 4.7 is the ¹H NMR spectrum of mono-acylated siloxane formed under Korlyukov's and Sukeda's conditions. Such conditions also formed the starting chloromethyldimethylsiloxane (**4.4**) shown in Figure 4.8.

Figure 4.7. ¹H NMR spectrum (CDCl₃) of mono-acylated siloxane.

Shown in Scheme 4.4, is a mechanistic explanation for the differences observed between Korlyukov's thionyl-chlorination of siloxanes and our failed attempt to chlorinate siloxanes in the presence of ester groups. Displacement of the thionyl-activated amide is more difficult than the displacement of thionyl-activated ester. We surmise that the difference in pK_a between the propionic acid (pK_a 5) and the amide (pK_a 10) could explain the selectivity reported by Korlyukov.

Scheme 4.4. Mechanistic explanation for chlorination of siloxanes.

Figure 4.8. shows the ^1H NMR spectra of compounds starting material **4.3** and propionyloxymethyldimethyl siloxane (**4.4**) in which can be noticed the downfield shift of the CH_2 protons after substitution of the chlorine by a propionate ester. Interestingly there is also a small upfield shift of the dimethyl signal attached to the silicon.

Figure 4.8. ^1H NMR spectra (CDCl_3) of compounds **4.3** and **4.4**.

The small upfield shift effect might be attributed to the formation of pentavalent silicon as shown in Figure 4.9. Based on the structure proposed by Korlyukov *et al.* the silicon center of our (bis)ester siloxane **4.4** could be imparted with a partial δ -negative charge that would shield the surrounding protons, leading to an upfield shift in the ^1H NMR spectrum.

Figure 4.9. Explanation for the observed upfield shift in ^1H NMR spectrum of **4.4**.

To develop an alternate synthesis of POMS-Cl we examined two alternative methods as shown in Schemes 4.5 and 4.6. Korlyukov *et al.* reported that trimethylsilylchloride (TMS-Cl) can catalyze the selective substitution of the chloromethyl group of silane **4.6** (Scheme 4.5) using *N*-trimethylsilyl-acetamide.¹⁶ Consequently we first attempted the same reaction, but instead of using *N*-trimethylsilyl-acetamide, we used *O*-silyl propionic acid. The key to this approach, when using the

appropriate 1:1 ratio of **4.6** to *N*-trimethylsilyl-acetamide, is to establish equilibrating conditions that would favor the displacement of chloride from carbon by the amidate ion generated *in situ*. Indeed, the poor stability of silylated amides generates an equilibrium while the displacement of chloride from terminates reactivity. If the reagents are in a 1:1 ratio, then the formation of the mono-acylated silane is favored. Unfortunately, no product was isolated after attempting the reaction using *O*-silyl propionic acid.

Scheme 4.5. Korlyukov alternative synthesis of POMS-Cl.

In a different approach, we aimed to prepare bis(ester) **4.7** using the conditions established by Yoder *et al.* for synthesis of analogous silyl esters (Scheme 4.6).¹⁸ Reaction of silyl ester **4.6** with *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide could have been expected to directly afford POMS-TFAM using a procedure developed by Baukov *et al.*¹⁹

Scheme 4.6. Yoder alternative synthesis for POMS-Cl and POMS-TFam

Unfortunately, the high moisture sensitivity of the intermediates, including the POMS reagents themselves, applied in small-scale reactions, led to hydrolysis of the desired products. Frustrated with the sensitivity issues involved in the preparation, isolation, and purification of silyl halides, we turned our attention to a less-reactive analog of POMS-Cl; namely, an aminoxy derivative. The next section describes our efforts to use the POMS scaffold to prepare chemoselective POMS-based reagents for carbonyl capture.

4.B.2. Development of POMS-ONH₂ Reagents: Second Generation

The second generation (propionyloxy)methylsilyl (POMS) reagents would be fitted with stable isotope-labeled ester moieties in the form of the POMS group, as the progenitor of EI-induced reporter tags. However, instead of using the trifluoroacetamide derivatizing technology, we opted for aminoxy technology to specifically target aldo- and keto-metabolites (Figure 4.10). One advantage of this change in functionality is the stability of the reagent in water or other protic solvents.

Metabolite derivatization

Figure 4.10. POMS-ONH₂ α-cleavage generates a reporter tag as an ethyl carbenium ion as well as oxime- and metabolite-specific fragments.

As shown in Figure 4.10, the POMS-ONH₂ chemoselectively derivatizes aldehyde and ketone metabolites. By analogy to the earlier observed fragmentations of POMS-OBn, several different MS fragmentation pathways can be expected. The presence of the propionyl ester functionality should still furnish labeled acylium and labeled ethyl carbenium via the α-cleavage pathway (path A). On the other hand, the

newly formed oxime ester group could generate, through EI (path **O**), nitrilium and McLafferty ions. These ions were clearly observed in our previous study in Chapter 3, but will be dependent on the nature of the metabolite and adduct (*E*) vs (*Z*) stereochemistry. Although not shown in Figure 4.10, silyl group fragmentations such as those observed for POMS-OBn and POMS-TFAm (e.g., path **S**, Figure 4.5) are also expected to give silenium ions either by loss of the (propionyloxy)methyl radical or by loss of methyl radical. Finally, metabolite specific cleavages (Path **M**) can also be expected and should provide information on the metabolite, such as the molecular weight or the presence of functional groups.

Our first attempt at synthesis of this reagent started with the reaction of commercially available bis(chloromethyl)dimethylsilane (**4.0**) with *N*-hydroxyphthalimide and triethylamine to obtain the mono-substituted phthalimide product **4.8** (Scheme 4.7). Then, using the same strategy we used to prepare the siloxane **4.1** (Scheme 4.1), we aimed to displace the chloride with the propionyl carboxylate anion to obtain propionyloxymethylsilyl methylphthalimide analogue **4.9**. Unfortunately, the harsh conditions necessary for chloride displacement by the carboxylate anion led primarily to the hydrolysis of the phthalimide moiety; no displacement product was isolated. The sensitivity of the phthalimide group towards nucleophiles led us to attempt the addition of the ester functionality first to obtain the mono-esterified product **4.11**,²⁰ followed by the S_N2 displacement of chlorine by *N*-hydroxyphthalimide to give compound **4.9**. However, this reaction also failed to provide the desired product, as the ester also was cleaved by the harsh conditions.

Scheme 4.7. Attempts to synthesize POMS-ONH₂ using an S_N2 displacement approach.

To overcome these problems, we developed a new synthesis pathway that could be performed under milder conditions (Scheme 4.8). We transformed the starting bis(chloromethyl)dimethylsilane **4.0** into diol **4.13** by heating with acetic acid and triethylamine to afford the bisacetate **4.12** in quantitative yields (99%).²¹ The diester was then reduced to give the bis(hydroxymethyl)dimethylsilane **4.13** using lithium hydroxide in excellent yield (89%). Diol **4.13** was subjected to mild Mitsunobu conditions²² to afford, in good yield (79%), mono-phthalimide **4.14**. Acylation of the remaining hydroxyl group was then performed using mild standard coupling conditions²³ to afford the propionyloxy product **4.9** in 93% yield. The phthalimide moiety was deprotected to afford **POMS-ONH₂** in excellent yields (92%) by hydrazinolysis (Scheme 4.8).

Scheme 4.8. Synthesis of POMS-ONH₂.

To demonstrate the reactivity of the POMS-ONH₂ reagent with carbonyl metabolites and to investigate the EI-induced fragmentation of POMS-ONH₂-derivatized metabolites, we derivatized acetone as our template metabolite by simple mixing of the two compounds. Figure 4.11 shows the EI-MS spectrum of the acetone adduct of POMS-ONH₂. As expected, we observe the two fragments m/z 57 and 29 corresponding respectively to the acylium cation and the ethyl carbenium ion arising from the α -cleavage (pathway **A**) of the ester moiety of the POMS reagent. In addition, pathway **O** generates the nitrilium ion m/z 56, and an oximium ion m/z 73. Pathway **S** is also observed with the major fragment m/z 145 arising from loss of the methyloxime ether radical ($\bullet\text{CH}_2\text{ON}=\text{CMe}_2$) and less prevalent m/z 144 loss of (propionyloxy)methyl radical ($\text{CH}_3\text{CH}_2\text{C(O)CH}_2\bullet$). It is unfortunate that the parent fragment (m/z 145) does not provide any structural information about the derivatized metabolite and dominates all the other silicon-based pathways (Figure 4.11). The *ZMI* remains in this example free from interfering ions, and the addition of ¹³C and ²H stable isotopes in the propionyl ester functionality should generate the corresponding ethyl carbenium ions within it.

Figure 4.11. EI-MS (X-axis: m/z ; Y-axis: intensity) spectrum of POMs-acetone adduct. Shown are the predicted fragment ions.

Encouraged by these results, we synthesized three isotopically labeled versions of the POMs-ONH₂ to attempt a proof of concept multiplex experiment. The three reagents were synthesized following the procedure described above but using differently labeled propionic acid at the acylation step. We synthesized *d*₅-POMs-ONH₂, *d*₂-POMs-ONH₂, and non-labeled POMs-ONH₂ using the corresponding commercially available propionic acids. For instance, to generate *d*₅-POMs-ONH₂ we used CD₃CD₂CO₂H. *d*₅-POMs-ONH₂ should generate a reporter MST in the form of a *d*₅-ethyl carbenium ion at m/z 34. *d*₂-POMs-ONH₂ was synthesized using CH₃CD₂CO₂H and should generate a reporter MST in the form of a *d*₂-ethyl carbenium ion at m/z 31. As already demonstrated (Figure 4.7), non-labeled POMs-ONH₂ generated a tag at m/z 29.

Figure 4.12. shows the ¹H NMR spectra of POMs-ONH₂, *d*₂-POMs-ONH₂, and *d*₅-POMs-ONH₂. Replacing the hydrogen atoms by deuterium isotopes in the propionate moiety is translated by the lack of proton signals in the ¹H NMR spectra.

Figure 4.12. ^1H NMR spectra (CDCl_3) of POMS-ONH₂, *d*₂-POMS-ONH₂, and *d*₅-POMS-ONH₂.

Having confirmed by NMR the structures of our labeled POMS-ONH₂ reagents, we reacted each one of them with acetone. Then, an equimolar solution of the three isotopologues was prepared and analyzed by GC-MS. Figure 4.13 shows the GC trace for the mixture as well as the *ZMI* range of the mass spectrum taken at different retention times for the peak corresponding to the adducts.

Figure 4.13. GC-MS chromatogram and spectra of a 1:1:1 mixture of POMS-acetone isotopologue adducts. The GC trace and the EI-MS taken at different retention times show a chromatographic isotope effect (i.e., the isotopic adducts do not co-elute).

The results obtained for the multiplex experiment depicted in Figure 4.13 came as a disappointment. Indeed, as can be observed by the shape of the GC peaks, the deuterium isotopologues of the POMS-acetone adduct do not co-elute. This result is also reflected in the mass spectrum of the adducts when measured at slightly different retention times. Thus, it appears through the presence of the reporter tag m/z 34 that the d_5 -POMS-acetone adduct elutes first at 6.07 min, followed by d_2 -POMS-acetone with the tag m/z 31 at 6.08 min, and finally POMS-acetone with the tag m/z 29 at 6.09 min. This chromatographic isotope effect has been described previously in the literature,¹² and represents a major drawback for the development of isotope coding strategies for direct

mass spectral quantitation. When the isotopologue adducts of a derivatized molecule do not co-elute, then all differently labeled mass spectral tags cannot be obtained in a single mass spectrum at a given elution time. This makes quantitation more difficult, in particular if the isotopologues are not entirely resolved, and limits throughput to a binary set of reagents. Quantitation would need to be achieved by GC peak area measurements assuming the peaks are well separated. In our case, having placed the heavy atoms near a polar functional group (propionate ester), we were hoping to minimize the chromatographic isotope effect by minimizing the interaction of the isotopes with the hydrophobic stationary phase of the GC column.^{12c} Indeed, separation in reversed-phase chromatography is generally explained by solvophobic theory.²⁴ According to this theory, a polar group would be expected to interact more weakly with the hydrophobic stationary phase of a column than with an aliphatic chain. However, based on our results, it seems that this method used in LC-MS to control deuterium chromatographic effect cannot be applied to GC-MS.

Another drawback observed for the POMS-ONH₂ reagent is the low propensity to generate fragments through the ester α -cleavage. Indeed, as seen in Figure 4.11, the parent fragment in the EI-MS spectrum of the acetone-POMS adduct is the silenium ion generated after the loss of methyloxime ether radical (m/z 145). As previously stated, this fragment does not provide any valuable information on the chemical structure of the derivatized metabolite. The presence of the dimethylsilyl group in the POMS reagent increases its molecular weight and reduces its volatility. It is essential to maintain small reagents that do not decrease the volatility of derivatized metabolites to a great extent.

To make the reagent more volatile, we attempted the synthesis of a reagent that would only contain the propionate ester group to generate the reporter MST, and the aminoxy moiety to chemoselectively derivatize carbonyl metabolites. Scheme 4.9 shows our synthetic approach to *O*-propionylhydroxylamine (**4.16**). Coupling propionic acid to *N*-hydroxyphthalimide using standard coupling conditions formed **4.15** in good yield (61%). However, deprotection of the phthalimide to give the *O*-propionylhydroxylamine did not afford the desired product; instead, the *N*-propionylhydroxylamine was obtained. Indeed, *O*-acylhydroxylamines are known to rearrange to the thermodynamically stable hydroxamic acids.²⁵ Isomerization can be minimized if a sufficiently bulky group is in the near vicinity of the carbonyl group,^{25a} but Marmer *et al.* reported that even at -78 °C *O*-pyvaloylhydroxylamine decomposes within one week.^{25b}

Scheme 4.9. Attempted synthesis of *O*-propionylhydroxylamine.

These results terminated our attempts to generate *O*-propionylhydroxylamine (**4.16**), since its high decomposition rate would make it impossible to use.

4.B.3. Development of AEP Reagents

To overcome the chromatographic isotope effect induced by the uneven presence of ^2H isotopes in our POMS- ONH_2 reagents, as well as to increase the volatility of the adducts, we opted to develop a set of ^{13}C isotopologues of the derivatizing reagent. We surmised that whether the chromatographic isotope effect is a consequence of the hydrogen bond interaction between the products and the GC column or the result of a change in the physical properties of the product (different boiling point), ^{13}C isotopologues of the reagent would greatly minimize these interactions. Also, to avoid competitive fragmentations that reduce the propensity of the ester α -cleavage as well as to make the reagent smaller and therefore more volatile, we redesigned the reagent by removing the dimethylsilyl group. Thus, we have developed a panel of isotopologous aminooxyethyl propionate (AEP) reagents for chemoselective derivatization of aldehydes and ketones (Figure 4.14).

Figure 4.14. Expected fragmentations from AEP-derivatized carbonyl metabolites.

Once again, we expect EI-induced ester α -cleavage as the mode for tag expulsion of AEP-labeled metabolites to give rise to acylium ion (**A**₁) followed by spontaneous loss of CO to generate the target m/z 32 carbenium ion MST (**A**₂). The target *ZMI* suggested modification of the ethyl carbenium ion, $C_2H_5^+$ m/z 29, to its 2H_3 -analog, $D_3CCH_2^+$ m/z 32. Corresponding ^{13}C -isotope insertions at the α - or α,β -positions of the AEP ester moiety yield isotopic MSTs having m/z 33 and 34, respectively. This limits throughput to a 3-plex experiment, but should afford highly accurate reading of the tags as they appear in the middle of the *ZMI*. Finally, oxime group cleavages (path **O**) should generate nitrilium and McLafferty ions, and path **M** should also generate metabolite specific fragments that provide information on the chemical structure of the derivatized metabolite.

Scheme 4.10. Synthesis of AEP reagents.

Synthesis of the AEP reagents (Scheme 4.10) proceeded by S_N2 displacement of bromide from 2-bromoethanol by *N*-hydroxyphthalimide to afford *N*-(2-hydroxyethoxy)phthalimide **4.17** in good yield (73%).²⁶ Carbodimide-mediated acylation of **4.17** with propionic acid, or 2H_3 -labeled propionic acid and corresponding $^{13}C_1$ - and

$^{13}\text{C}_2$ -isomers, afforded the differently labeled phthaloyl esters **4.18a-d** in good yield (76%-90%). Phthalimide deprotection to unmask the aminoxy group required careful optimization to avoid accompanying ester cleavage. Table 4.3 shows the different reaction conditions used during the optimization process. To improve the yields given by hydrazine, we opted to use methylhydrazine. Indeed, the methylated nitrogen of hydrazine preferably reacts with anhydrides while the non-methylated nitrogen preferably reacts with esters.²⁷ Although counter-intuitive with the reactivity-selectivity principle (the less reactive a reagent the more selective it is between substrates), the phenomenon has been elegantly explained by Condon.²⁸ Thus, we were gratified to find that treatment of the phthaloyl ester with a slight excess of methylhydrazine at 0 °C resulted in selective hydrazinolysis to give **AEP-29** to **AEP-34** (Scheme 4.6).

Table 4.3. Optimization of phthalimide deprotection.

Entry	Reagent	Equiv.	Temp. (°C)	Reaction Time (min)	Product Yield (%)
1	H ₂ NNH ₂ •H ₂ O	5	0 °C to rt	120	35
2	H ₂ NNH ₂ •H ₂ O	3	0 °C	60	52
3	MeHNNH ₂	2	0 °C	60	75
4	MeHNNH ₂	1.2	0 °C	45	89

Figure 4.15. shows the ^1H NMR spectra of the three reagents. The CH_2 signal in α -position of the ester is identical for **AEP-32** and **AEP-33**, but splits into 2 signals for **AEP-34** due to coupling with ^{13}C .

Figure 4.15. ^1H NMR spectra (CDCl_3) of AEP-32, AEP-33, and AEP-34.

Labeled propionic acid-**32** ($\text{D}_3^{12}\text{C}^{12}\text{CH}_2\text{CO}_2\text{H}$) is commercially available. Propionic acid-**33** ($\text{D}_3^{13}\text{C}^{12}\text{CH}_2\text{CO}_2\text{H}$) and propionic acid-**34** ($\text{D}_3^{13}\text{C}^{13}\text{CH}_2\text{CO}_2\text{H}$) were synthesized as shown in Scheme 4.11. Commercially available ^{12}C - or ^{13}C -acetic acid was acylated using standard coupling conditions to afford the 1-naphthalenemethylacetate esters (**4.19a-b**) in yields varying from 82% to 100%. Alkylation of the enolate esters using d_3 - and ^{13}C -labeled iodomethane and lithium hexamethyldisilazide (LHMDS) at -78°C to room temperature afforded the propionate

esters **4.20a-b** in moderate yields (47%). Saponification of the propionate esters under basic conditions gave the corresponding propionic acids. The product acids were immediately esterified without further purification after their formation by reaction with alcohol **4.17** using the standard dehydrative coupling conditions shown in Scheme 4.10.

Scheme 4.11. Synthesis of labeled propionic acids.

Figure 4.16 shows the ${}^{13}\text{C}$ NMR spectra of naphthalene propionate esters **4.20a** and **4.20b**. For both compounds, carbon 1 is ${}^{13}\text{C}$ enriched while carbon 2 is ${}^{13}\text{C}$ enriched only for ester **4.20b**.

Figure 4.16. ${}^{13}\text{C}$ NMR spectra (CDCl_3) of naphthalene propionate esters **4.20a** and **4.20b**.

Analysis on the fragmentations of an AEP-derivatized carbonyl adduct was again performed using acetone. The reaction of **AEP-32** with acetone, serving as a representative carbonyl metabolite, was quantitative. Figure 4.17 shows the EI-MS spectrum of the **AEP-32**-acetone adduct and its main fragments. As expected, the d_3 -labeled propionyl ester underwent α -cleavage to generate D_3 -labeled acylium ion at m/z 60 (**A₁**) and D_3 -labeled ethyl carbenium ion at m/z 32 (**A₂**). Oxime ether fragmentations generated the nitrilium ion at m/z 56 and a reverse charge McLafferty product at m/z 99. These two fragments provide structural information about the derivatized metabolite and could be used as finger print fragments for this adduct in a searchable mass spectral library. Finally, a reagent based fragment is also generated at m/z 104. In comparison to POMS-ONH₂ adducts studied in the previous section, it is clear that the AEP fragmentation patterns are not only more simple, but they also provide more structural information on the derivatized carbonyl as well as a more prominent ester α -cleavage for future quantification studies.

Figure 4.17. EI-MS (X-axis: m/z ; Y-axis: intensity) spectrum of the **AEP-32**-acetone adduct. Shown are some of the predicted fragmentation pathways.

To demonstrate the applicability of the AEP reagents for multiplexed mass spectral-based quantitation, we still needed to confirm that the three ^{13}C -isotopologues of AEP are not affected by the chromatographic isotope effect. We surmised that the smaller the AEP-derivatized carbonyl, the stronger would be the chromatographic isotope effect. Therefore, as previously done with POMS- ONH_2 reagents, we generated an equimolar solution of the acetone adducts of **AEP-32**, **AEP-33** and **AEP-34**. Without further treatment, the solution was injected into the GC-MS and analyzed. Figure 4.18 shows the GC trace and the mass spectrum generated at slightly different retention times for the peak corresponding to the labeled AEP-acetone adduct.

Figure 4.18. GC-MS chromatogram and spectra of a 1:1:1 mixture of labeled AEP-acetone isotopologue adducts. The GC trace and the EI-MS taken at different retention times show that the three products co-elute to generate a single mass spectrum with a reporter MST in the *ZMI*.

Conversely to the shape of the GC peak of the POMS-ONH₂ adduct, the GC peak of the isotopic AEP-acetone adducts is uniform, sharp, and narrow, which is a good indicator for co-elution of the isotopic products (Figure 4.18). More importantly, the spectra generated at different retention times within the GC peak are all identical. In particular, since the initial mixture was an equimolar solution of three isotopologues, we were gratified to find that the three reporter mass spectral tags (MSTs) had the same intensity regardless of which segment of the GC peak was sampled for MS measurement. Indeed, this implies that the reporter tags in the *ZMI* could provide the relative ratios of a compound in different solutions.

4.C. Results and Applications of AEP Reagent

4.C.1. Multiplex Experiment: Proof of Concept

To validate the concept that release of isotopic carbenium ions on GC-MS analysis of AEP-derived oxime ether adducts can be used to determine relative proportions of carbonyl substrates in different sample mixtures, we prepared three model carbonyl mixtures with known substrate ratios (Scheme 4.12).

Scheme 4.12. Formulation, labeling, and pooling of carbonyl sample mixtures for analysis. Depicted at bottom is the total ion chromatogram of the pooled sample mixture showing elution of the oxime ether adducts of the substrates indicated by name.

In this example, mixtures **A**, **B**, and **C** were formulated to contain equimolar quantities of hexanal, whereas the quantity of 2-heptanone in mixture **B** was tripled relative to 2-heptanone in mixtures **A** and **C** (Table entries 1-2, Scheme 4.12). Each mixture was derivatized with a single AEP isotopologue by simple mixing in dichloromethane at room temperature, and the resultant AEP-oxime ether mixtures **A**₃₂, **B**₃₃, and **C**₃₄ then were combined to yield the pooled sample that was analyzed by GC-MS.

Figure 4.19. ZMI plots (X-axis: m/z ; Y-axis: intensity) obtained from the eluted peaks of the pooled sample mixture of Scheme 3 showing the MST ion abundance (vertical) for the range m/z 32-34. Shown are the mean values of three separate experiments and the standard deviation from the mean. The starting **A**:**B**:**C** substrate ratio is given in brackets. Ion counts are normalized to 100 for the parent fragments (not shown).

Whereas the *Z*- and *E*-diastereomers derived from the asymmetrical substrates gave separate GC peaks, we were gratified to find that no GC separation occurred based

on stable isotope incorporation (GC trace, Scheme 4.12).²⁹ MS analysis of the eluted compounds revealed that each derivatized carbonyl substrate generated characteristic fragment ions that enabled its structural assignment and designation of either *E*- or *Z*-oxime ether stereochemistry.³⁰ The isotopic MSTs at *m/z* 32-34 were abundant and clearly measurable in the *ZMI* of each spectrum. Relative ratios for the carbonyl substrates that gave diastereomeric oxime ethers were determined readily using MST measurements from either the *Z*- or *E*-isomer. Importantly, direct MST ion count comparisons gave substrate ratios that were in close agreement with the actual formulation ratios of the starting compound mixtures (Figure 4.19). The average accuracy of the method obtained by comparison of the MST-derived ratio to the known carbonyl substrate compositions in mixtures **A-C** is $95.1\% \pm 0.3\%$.

4.C.2. Analysis of a Biologically Derived Sample

To demonstrate additional attributes of the *ZMI* tag approach, we examined samples of the root extract of the turmeric plant (*Curcuma longa*) to determine compositions of ketones and aldehydes. The complexity of such an extract³¹ makes it ideal for challenging the technology described above. Carbonyl metabolites from plants, in particular aliphatic methyl ketones, have a variety of important natural and commercial roles, including pheromonal and insecticidal roles in plants,³² as possible sources of biofuels,³³ and providing scents in essential oils as well as flavorings for cheese and other dairy products.³⁴ First, to profile only the carbonyl substrates in the extract, we sought to apply the unique *ZMI* reporter MSTs as selection tools to provide an extracted ion chromatogram (in this case, the profile of plant metabolites that had reacted with the AEP reagents). Second, we aimed to quantify the prominent methyl ketone substrates identified in the root extract.

Since AEP-derivatized substrates generate reporter MSTs in such a unique *m/z* region of a mass spectrum, we theorized that the appearance of these isotopic tag ions might be used to identify AEP-adducts in the total ion chromatogram (TIC), thus providing a rapid means for profiling the carbonyl metabolites. By using two isotopologues of AEP at a specified ratio in the derivatization step, we aimed to minimize the extraction of false-positive peaks from the TIC. Consequently, we derivatized the turmeric extract with both **AEP-33** and **AEP-34** in a 1:1 ratio. Subsequent profiling of the carbonyl adducts then was achieved by generating an extracted ion chromatogram (EIC) through the selection of peaks that exhibited the reporter MSTs at *m/z* 33 and 34 in a 1:1 ratio in their accompanying mass spectra (Figure 4.20).

Figure 4.20. **A.** GCxGC-TOF-MS total ion chromatogram (TIC) of turmeric root extract after derivatization using AEP reagents; **B.** Extracted ion chromatogram (EIC) for peaks that exhibit MS signals at m/z 33 and 34 (same field of view as in **A** with lower threshold); **C.** Expanded field of view of peaks within the highlighted rectangle of **B** where circled peaks 1-6 are, respectively, the AEP-adducts of 2-nonanone, 2-decanone, 2-undecanone, 2-dodecanone, 2-tridecanone and 2-octadecanone.

The GCxGC-TOF-MS total ion chromatogram of the derivatized turmeric extract (Figure 4.20A) clearly is populated with a multitude of compounds. By applying the ion selection tool for reporter ions at both m/z 33 and 34, an extracted ion chromatogram is obtained (Figure 4.20B) that profiles the carbonyl substrates (i.e., AEP-reactive). With the profile of carbonyls in hand, we next sought to identify the subset of aliphatic methyl ketones. Given that ketoxime ethers undergo characteristic EI-MS fragmentations (Figure 4.21), a targeted m/z mass spectral analysis of the EIC revealed the presence of

several AEP-methyl ketone adducts (Figure 4.20C and Table 4.4). The AEP-adducts were present as both *E*- and *Z*-isomers, and assignment of stereochemistry followed from analysis of the relative abundance of McLafferty fragment ion versus nitrilium ion formation.

Figure 4.21. Characteristic EI-MS fragmentations of ketoxime ethers.

Table 4.4. AEP-methyl ketone adducts extracted from the EIC (Figure 4.20B). Entry numbers correspond to GCxGC retention properties (Figure 4.20C). Substrates in entries 1-3 were confirmed by comparison to AEP-derivatized standards.

Entry	AEP-Methyl Ketone Adduct (Carbonyl Parent)	McLafferty Fragment Ions (<i>m/z</i>)	Nitrilium Ion (<i>m/z</i>)	Normalized GC Peak Area
1	2-nonanone	177, 178	140	66.0
2	2-decanone	177, 178	154	6.7
3	2-undecanone	177, 178	168	100
4	2-dodecanone	177, 178	182	9.4
5	2-tridecanone	177, 178	196	5.2
6	2-octadecanone	177, 178	266	20.4

Each AEP-methyl ketone adduct generated McLafferty fragment ions at m/z 177 and 178 (one from each AEP-adduct isotopologue), and these values thus functioned as a convenient searchable m/z for methyl ketone substrates. The nitrilium ion m/z is substrate specific and provides information on the constitution of the ketone aliphatic chain. These features are illustrated by the representative mass spectra of the *E*- and *Z*-2-nonanone adducts (Figure 4.22). The structural assignments for 2-nonanone, 2-decanone and 2-undecanone were confirmed by comparison to AEP-derivatized standards (e.g., library spectra), and the remaining suggested methyl ketones, while likely members of the same linear family, may be constitutionally isomeric at positions remote to the carbonyl. It is noteworthy that even when applying powerful separation conditions (i.e., GCxGC, 5 °C gradient) we did not observe chromatographic separation of AEP-adduct isotopologues, as confirmed by the observable 1:1 ratio of the reporter MSTs in the *ZMI* (Figure 4.22).

Figure 4.22. EI-Mass (X-axis: m/z ; Y-axis: intensity) spectra of AEP-2-nonanone adducts: (A) Z-isomer, (B) E-isomer. Adducts were obtained by derivatization of turmeric root extract using **AEP-33** and **AEP-34** in a 1:1 ratio.

Absolute quantification of the principal methyl ketone constituents in the root extract, 2-nonanone and 2-undecanone, followed from preparation of labeled standards. The standards were derivatized using **AEP-34**, while the turmeric extract was treated with **AEP-33**. Known concentrations of the derivatized standards then were added to the derivatized extract and the resulting spiked mixtures were analyzed by GCxGC-TOF-MS. Once again, the direct comparison of the ion intensities for the two reporter MSTs in the *ZMI* was key and provided the measures for determining the minimal concentrations of both 2-nonanone and 2-undecanone in the turmeric extract at $237 \pm 14 \mu\text{g/mL}$ and $315 \pm 32 \mu\text{g/mL}$, respectively.

4.D. Conclusions

4.D.1. General

In conclusion, we have demonstrated a new method for the quantification of substrates from multiple samples using a multiplexed, single injection GC-MS approach enabled by direct measurement of isotopic reporter mass spectral tags. Stable isotope-labeled AEP reagents have been designed for this application. We illustrated the method by reacting the AEP reagent panel with known ratios of sample aldehydes and ketones, and by applying the technology to profile turmeric extract. We engineered the AEP reagents to: 1) overcome the chromatographic isotope effect, typically observed when heavy isotopes (in particular deuterium) are incorporated in derivatizing reagents, by using ^{13}C stable isotopes; 2) generate an isotopic reporter MST in a zone of minimal interference (*ZMI*) by using the electron ionization-induced ester α -cleavage as an expulsion mechanism; and 3) derivatize chemoselectively carbonyl metabolites by functionalizing the reagent with an aminooxy moiety.

The identification of a *ZMI* at the m/z range 31-37 and the engineering of an expulsion mechanism to form labeled ethyl carbenium ions from AEP-adducts were both crucial for developing a means for unobstructed detection of mass spectral reporter ions. Of possibly even greater significance was the success in overcoming the chromatographic isotope effect to enable application of the technology to high throughput multiplexing. These elements facilitate rapid profiling and quantification of substrates that have been chemoselectively derivatized, as exemplified by our turmeric extract study. The results here provide a useful guideline for the development of isotopic carbenium ion- or other *ZMI* ion-producing reagents that can facilitate multiplexed GC-MS analyses.

4.D.2. Future Directions

As a logical expansion of the technology, we have begun development of new derivatizing reagents that use isotopic carbenium ions as reporter mass spectral tags. In preliminary experiments, we have examined chemoselectively derivatizing amine-containing metabolites, such as amino acids. To do so, we surmised that an MS-induced α -cleavage fragmentation could also be generalized to the amide functional group as a means to generate reporter ethyl carbenium ions (Figure 4.23). Thus, transforming primary or secondary amines into their corresponding isotopically labeled propionamides might be a viable way to derivatize amine metabolites as a *ZMI*-generating functional group.

Ester α -cleavage

Amide α -cleavage

Figure 4.23. Comparing ester α -cleavage and amide α -cleavage.

To demonstrate the viability of an approach that uses labeled propionamides as a means to generate *ZMI* reporter MSTs, we synthesized labeled *N*-hydroxysuccinimide propionate esters (NHS) reagents (Figure 4.24).

Metabolite derivatization

Figure 4.24. Expected fragmentations from NHS-derivatized amine metabolites.

We expect NHS reagents to react with primary or secondary amines to generate labeled propionamides that then would undergo EI-induced amide α -cleavage to give rise to acylium ion (A_1) followed by spontaneous loss of CO to generate the target m/z 32-34 carbenium ion MSTs (A_2). Path **M** should also generate metabolite specific fragments that provide information on the chemical structure of the derivatized metabolite.

Scheme 4.13. Synthesis of NHS reagents.

Differently labeled NHS reagents were synthesized in a single step using standard carbodiimide coupling conditions. *N*-hydroxysuccinimide reacted with the corresponding labeled propionic acids in good to excellent yields (61-90%).

Analysis on the fragmentations of an NHS-derivatized amine adduct was performed using amino acids glycine and proline as corresponding primary and secondary amine templates. The two amino acids were first derivatized in anhydrous pyridine with a 1:1:1 solution of **NHS-32**, **NHS-33**, and **NHS-34**, followed by a *tert*-butyldimethylsilyl trifluoroacetimide (TBS-TFA) derivatization. The second derivatizations with TBS-TFA are required to convert the carboxylic acid moieties to their non-polar (i.e., GC compatible) silyl esters. Figure 4.24 shows the EI-MS spectrum of the **NHS-TBS** adducts of glycine and proline and accompanying main fragments. As expected, the labeled propionamide underwent α -cleavage to generate labeled acylium ion at m/z 60-62 (**A₁**) and labeled ethyl carbenium ion at m/z 32-34 (**A₂**). As predicted, the ratio of the reporter MST in the *ZMI* correlated with the initial ratio of the NHS reagents. Loss of *tert*-butyl generates the silenium ion at m/z 191-193 for glycine and m/z 231-233 for proline. These fragments as well as metabolite specific fragments (path **M**) provide structural information about the derivatized metabolite and could be used as finger print fragments for this adduct in a searchable mass spectral library.

Figure 4.25. EI-Mass (X-axis: m/z ; Y-axis: intensity) spectra of NHS-TBS-glycine (A) and NHS-TBS-proline (B). Adducts were obtained by derivatization of glycine and proline using **NHS-32**, **NHS-33**, and **NHS-34** in a 1:1:1 ratio, followed by derivatization with TBS-TFA.

While these preliminary results are highly encouraging, further work needs to be done to demonstrate the viability of the amide α -cleavage as a means to generate ethyl carbenium ions in the *ZMI*. This study does show, however, that the NHS methodology is a good option for amine functional group targeting and that our labeled propionate approach can be readily adapted to such derivatization strategies.

CHAPTER 5

Aminoxy Linkers for Gold Nanoparticle Functionalization

5.A. Introduction

5.B. Synthesis of Heterobifunctional Diblock Linkers

5.B.1. Goal and Initial Synthetic Attempts

5.B.2. Revised Route to Heterobifunctional Diblock Linkers

5.B.3. Conclusion

5.C. Synthesis of Peptide Linkers and Cypate Functionalization

5.C.1. Synthesis of Peptide Linkers

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5.D. Nano-Entity Assembly

5.D.1. Solid Phase Assembly

5.D.2. Click Chemistry Assembly

5.D.3. Conclusion

5.A. Introduction

Breast cancer represents 29.0% of all cancers in women and caused ~39,510 deaths in the United States in 2012.¹ The screening for small and asymptomatic tumors in apparently healthy individuals to achieve an early diagnosis is based on the assumption that early detection will lead to more successful cancer therapy and improve survival rates.² Unfortunately, early detection of breast cancer represents a diagnostic challenge despite the current availability of imaging and localization methods.³ Therefore, the development of new biocompatible imaging probes is much needed, and fluorescent molecules that emit in the near-infrared (NIR) could play a major role in this setting. The use of strong NIR is particularly attractive since its absorption and scattering by endogenous biomolecules is minimal and, hence, it can propagate through tissues.⁴ In this instance, the use of a quenched, fluorescent imaging probe that can generate a strong near-infrared fluorescence signal after enzymatic activation (e.g., peptide cleavage by proteases endemic to malignant tumors) may be a viable option for cancer detection and diagnosis.⁵

As part of a collaboration with Dr. Kyung Kang (UoFL Chemical Engineering), we sought to prepare a gold-based fluorescing nano-entity using a click chemistry approach for cancer detection and diagnosis. The nano-entity was designed to contain a structurally modified fluorescent probe (cypate) attached to a peptide (short linker) that contains a recognition element for urokinase plasminogen activator (uPA), which is a serine protease causally involved in cancer invasion and metastasis.⁶ The probe also features a non-cleavable diblock (hydrophobic/hydrophilic) chain (long linker) (Figure 5.1). Our approach aims at enabling the formation of cypate nano-entities through simple

“click” mixing operations. To do so, we opted to use oximation technology as a simple and efficient way to ligate the elements of our gold-based fluorescing nano-entity. As shown in Figure 5.1, both the peptide linker (short linker) and the heterobifunctional diblock linker (long linker) have been functionalized with an aminooxy moiety for chemoselective ligation with the aldehyde moiety of cypate bis(aldehyde). The thiol functionality at the other end of the linker enables attachment onto gold nanoparticles. Thus, simple mixing of the elements should lead to the efficient assembly of the nano-entity.

Figure 5.1. Elements of the fluorescent nano-entity. Highlighted in red is the recognition sequence for uPA. The thiol functionality of each linker (green) enables loading onto gold nanoparticles (AuNPs), and the aminooxy and aldehyde functionalities (blue) allow chemoselective ligations of the fluorescent probe.

The dual-chain probe is designed so that its attachment onto gold nanoparticles (AuNPs) quenches fluorescent emission as long as cypate is tethered to the gold nanoparticle surface via the short linker (Figure 5.2A). The quenching of fluorescence by metals results primarily from non-radiative energy transfer from the fluorophore to the metal.⁷

Figure 5.2. Nanoparticle-fluorophore response to an encounter with uPA.

When the nano-entity encounters urokinase plasminogen activator (uPA), a serine protease enzyme synthesized and secreted by normal and cancer cells that has been linked to cancer invasion and metastasis,⁶ the resulting enzyme-mediated peptide-bond cleavage (short linker scission) would allow the constrained cypate to migrate from the gold nanoparticle surface to the length of the long linker, where cypate fluorescence is restored to serve as a signal (Fig. 5.2, C). Migration of the cypate away from the surface of the nanometal not only results in fluorescence emission dequenching but also may result in enhanced fluorescence if the distance of the long linker is appropriately controlled. The enhanced fluorescence appears to arise at a certain distance from the surface of the nanometal where the local electromagnetic field is highly concentrated.⁸

While the Kang laboratory focused on the various gold nanoparticle, fluorescence, and engineering elements of the project, our research objectives were to secure synthetic routes to peptide- and PEG-conjugates of cypate and to develop methods for their attachment to gold nanoparticles. In particular, our goal was to develop a new synthetic route to heterobifunctional linkers with flexible control over the length and the functionalization. We sought to prepare a panel of aminooxy functionalized spacers, an aminooxy functionalized peptide linker, and a new cypate bis(aldehyde) fluorescent probe for assembly of the nano-entity through “click” mixing operations.

5.B. Synthesis of Heterobifunctional Diblock Linkers

5.B.1. Goal and Initial Synthetic Attempts

Our goal was to develop a flexible synthetic route to heterobifunctional thiol- and aminooxy-terminated diblock (hydrophobic/hydrophilic) linkers (Figure 5.3). The linkers were functionalized with a) a thiol moiety for attachment to gold nanoparticles (AuNPs), and b) an aminooxy group that is presented at the surface of the AuNPs for chemoselective ligation to subsequently added aldehyde-functionalized cypate. Since AuNPs are coated with hydrophobic chains during their synthesis to avoid aggregation, we surmised that placing the thiol functionality at the terminal position of the hydrophobic domain would facilitate loading onto AuNPs. On the other hand, placing the aminooxy moiety proximal to the hydrophilic PEG-domain of the linker would facilitate ligation with our cypate aldehyde substrate in water.

Figure 5.3. Structure of the target heterobifunctional diblock linkers.

Our initial synthetic approach employed a convergent strategy whereby a long alkyl chain with a protected thiol would be added nucleophilically onto PEG reagents having protected aminooxy functionality (Scheme 5.1). Deprotections of the thiol and the aminooxy functionality would then be performed using standard methods to reveal the fully functionalized linker.

Scheme 5.1. Convergent synthesis for heterobifunctional diblock linkers.

Starting C(16)-bromocarboxylic acid **5.0** was reduced in high yields (93-100%) using borane in tetrahydrofuran (THF) to afford the corresponding bromoalcohol **5.1b**. C(12)- and C(16)-bromoalcohols (**5.1a**) and (**5.1b**) were transformed into trityl protected thiols **5.2a,b** by $\text{S}_{\text{N}}2$ displacement of bromine. This reaction was performed in high yields (*ca.* 98%) using the Hornillos *et al.* procedure.⁹ Reaction of **5.2** with iodo-PEG-ON(Boc)₂ reagent **5.3** to form the ether was met with great difficulty. Indeed, the competing addition of the derived alkoxide with the carbamate moiety of **5.3** led to a transferral of one Boc protecting group to give **5.4**. Virtually no desired product **5.5** was formed, as indicated by the ¹H NMR spectrum of the products. Instead, a prominent 9-proton singlet peak (red) at $\delta 1.5$ ppm in the product spectrum (Figure 5.4) showed that Boc transfer had occurred, thus terminating this approach.

Figure 5.4. ^1H NMR spectrum (CDCl_3) of byproduct **5.4**.

To circumvent the reaction between the alkoxide and bis-carbamate, we opted to use a dihalogenated PEG instead. The Williamson ether synthesis under these conditions (Scheme 5.2) still failed to afford the desired diblock chain product. Instead, E2 elimination prevailed and yielded the terminal alkenes **5.7**. Bis-chloro-PEG **5.6a** did afford the first full-length spacer, but the yield was prohibitively low (<5%).

Scheme 5.2. A dihalogenated PEG approach.

These results may be explained using a conformational argument. Figure 5.5 shows the favored Newman projection of a halogenated PEG. Since the S_N2 reaction requires nucleophilic attack via an *anti*-periplanar conformation, the proximal PEG oxygen possibly deters attack through electronic (and/or steric) repulsions. Thus, nucleophilic substitution by the alkoxide on the terminal carbon is discouraged and the elimination reaction becomes favorable.

Figure 5.5. Newman projection of halo-PEG compounds and $E2$ elimination mechanism.

Figure 5.6 shows a section of the ^1H NMR spectrum between 6.5 and 4 ppm of the terminal alkene **5.7**. The doublet of doublet signal at 6.42 ppm corresponds to the CH proton near the oxygen (red). The doublet signal at 4.10 ppm with a large J coupling corresponds to the terminal proton in *trans* (blue), and signal at 3.95 ppm corresponds to the terminal proton in *cis* (green).

Figure 5.6. ^1H NMR spectrum (CDCl_3) of alkene byproduct **5.7**.

5.B.2. Revised Route to Heterobifunctional Diblock Linkers

Since our strategy to perform an S_N2 reaction on an electrophilic PEG reagent did not afford the desired products, we further revised the strategy and opted to assign the electrophilic center to the aliphatic chain while making the PEG component the nucleophile. Examination of the literature for syntheses of alcohol or amino-terminated diblock compounds revealed that such a HO-PEG-OH alkylation was feasible, but the subsequent alkene-to-thiol transformation via radical methods was unwieldy (Figure 5.7).¹⁰ With compound **5.2** in hand, we opted instead to alkylate HO-PEG-OH with a trityl-protected alkyl halide to obtain the target linkers. Development of such a route would constitute a new, flexible path to this useful class of tethering compounds.

Figure 5.7. Comparison of approaches to heterobifunctional diblock linkers.

Scheme 5.3 shows the new synthetic strategy in which the initial steps to **5.1** and **5.2** have not changed. This approach gave the first quantifiable yields of non-functionalized spacer and showed promise for further optimization. In particular, this new route provides for control over linker length and functionalization. Conversion of primary alcohols **5.2** to either the alkyl iodide or the alkyl bromide **5.9** proceeded with good yields (78-96%), but the low solubility of the products in non-polar solvents made

purification difficult. Transformation of the alcohol into the alkyl iodide was performed using modified conditions developed by Skaanderup *et al.*¹¹ Transformation into bromide was first attempted, with discouraging results, following a procedure by Harrison *et al.* that requires PBr_3 .¹² Indeed, varying reaction conditions with PBr_3 never afforded the brominated product (**5.9a**) in more than 30% yield. The Appel reaction was then attempted using carbon tetrabromide and triphenylphosphine.¹³ Halogenation under optimized conditions generated the alkyl bromides **5.9a,b** in excellent yields.

Scheme 5.3. Synthetic route to heterobifunctional diblock linkers.

Results of all bromination reactions are shown in Table 5.1, with the optimized alkyl iodide-forming reaction as reference. As shown, the Appel reaction (entry 4) provided the best yields.

Table 5.1. Optimization of alkyl halide synthesis.

Entry	Reagents	Solvent	X	Temp (°C)	Yield (%)
1	I ₂ (1.5 eq), PPh ₃ (1.5 eq), Imidazole (2 eq)	THF	I	r.t.	78
2	PBr ₃ (1.1 eq)	CH ₂ Cl ₂	Br	r.t.	30
3	PBr ₃ (1.1 eq)	CH ₂ Cl ₂	Br	50	23
4	CBr ₄ (1.5 eq), PPh ₃ (1.5 eq), Imidazole (2 eq)	CH ₂ Cl ₂	Br	r.t.	96

Figure 5.8 shows the ¹H NMR spectra of hydroxythiol **5.2a** and bromothiol **5.9a** in which can be observed the upfield shift from 5.59 ppm to 3.35 ppm of the CH₂ signal (H1) adjacent to the hydroxyl group and the bromine groups, respectively.

Figure 5.8. ¹H NMR spectra (CDCl₃) of hydroxythiol **5.2a** and bromothiol **5.9a**.

The coupling reaction of the protected halothiols **5.9** with PEG units required considerable optimization to once again avoid E2 reactions. A variety of reaction temperatures, solvents, and reagent ratios were employed to optimize this low-yield step (Table 5.2).

Table 5.2. Optimization of S_N2 coupling reaction.

Entry	X	Solv	eq. PEG ₄	eq. NaH	Temp (°C)	Time (hr)	Yield (%)
1	I	DMF	3	15	50	12	21
2	I	DMSO	3	15	45→60	36	0
3	I	THF	3	15	45	12	<5
4	I	THF/HMPA	3	15	45	12	<5
5	I	DMF	6	30	35	72	24
6	I	DMF	6	12.2	50	12	33
7	Br	DMF	5	9.8	r.t.	24	10
8	Br	DMF	6	11.8	r.t.	72	41

Although Williamson ether syntheses generally require heating to initiate the S_N2 displacement of the halogen, in our case such conditions led to greater decomposition of the reactant. Yet, at low temperatures, the displacement reaction proceeded very slowly. The most promising yields were obtained when the reaction was performed at room temperature for a prolonged period (e.g., 48-72 hours). Using a large excess of PEG reagent only improved yields slightly. We postulated that any unreacted NaH might be exacerbating the E2 problem, so fewer equivalents NaH were used than actually required to fully deprotonate the alcohol groups of the PEG reactant. It is important to highlight that the PEG compounds are very hydrophilic and hygroscopic which also affects the S_N2

displacement yield. Indeed, Doi *et al.* have recently shown that one or two equivalents of water will effectively inhibit an S_N2 reaction.¹⁴ We examined whether better yields might be obtained using the alkyl bromide. As shown in Table 5.3, the bromide reaction afforded the best yield of the desired adduct, although still only in a modest 41% yield.

Imparting diblock linkers **5.10** with the aminooxy functionality (Scheme 5.3) was accomplished using a standard Mitsunobu reaction.¹⁵ Yields of **5.11** (Scheme 5.3) were encouragingly high (74-96%) after the difficulties of previous steps, and product purifications proceeded uneventfully. Deprotection of the phthalimide group to unmask the aminooxy was performed with hydrazine monohydrate in dichloromethane at room temperature. Flash column chromatography (SiO₂) of the crude material afforded products **5.12** in high yields (*ca.* 59-98%). Finally, deprotection of the trityl group to unmask the thiol functionality was performed using a 1:1 mixture of trifluoroacetic acid and CH₂Cl₂ under reducing conditions (5% triethylsilane). In this reaction, triethylsilane is used as a carbocation scavenger, which is known to increase yields and reduce reaction time.¹⁶ The reaction completed within one hour and afforded the final heterobifunctional diblock linker **5.13** in good yields (44-97%).

The aminooxy linkers were very reactive towards aldehydes as well as ketones. Indeed, we found that upon exposure to acetone vapors present in standard laboratory atmosphere, oxime ether formation took place. Also, over time, the thiol group forms disulfides on exposure to oxygen (Figure 5.9).

Figure 5.9. Comparison of the ^1H NMR spectra (CDCl_3) for the representative thiol-aminooxy diblock spacer **5.13a-PEG(4)** (black), its corresponding acetone adduct (red), and its disulfide dimer (blue).

We also explored synthesis of a primary amine-functionalized linker for use in solid phase synthesis. Scheme 5.4 shows how replacing *N*-hydroxyphthalimide by phthalimide in the Mitsunobu step of the synthesis afforded the amine-based linker **5.14** in 95% yield. Deprotection of the phthalimide followed the same hydrazine procedure and gave the primary amine **5.15** in 72% yield. We attempted to couple this product to cypate bis(carboxylic acid) using a solid phase synthesis procedure with little success as it will be shown in section 5.D of this chapter.

Scheme 5.4. Synthesis of an amine functionalized linker.

Computational studies of the final thiol-aminooxy linkers **5.13** and the thiol-amine linker **5.16** (never prepared) were conducted to estimate extended length of the synthesized diblock linker. Theoretical extended spacer lengths were calculated after energy minimization using HyperChem[®] software. The values are shown in Table 5.3.

Table 5.3. Theoretical linker length calculations.

Entry	Spacers	Length (nm)
5.13a-PEG(4)		3.2
5.13a-PEG(6)		3.9
5.13a-PEG(8)		4.6
5.13b-PEG(4)		3.7
5.13b-PEG(6)		4.4
5.16		5.0

5.B.3. Conclusion

We prepared a panel of heterobifunctional diblock (hydrophobic/hydrophilic) linkers to test the concept depicted in Figure 5.1. Design features in our linkers include a thiol functionality at one end of the linker (hydrophobic region) to improve loading onto gold nanoparticles and an aminooxy group at the other end (poly(ethylene glycol) (PEG) region) to enable facile oximation with aldehyde substrates, such as an aldehyde-modified cypate probe.

The present synthetic route to heterobifunctional thiol- and aminooxy-terminated diblock spacers proceeds with an improved overall yield as compared to previous diblock linker syntheses. In section 5.D of this Chapter, we will show how our aminooxy linkers enable formation of cypate nano-entities through simple “click” mixing operations.

5.C. Synthesis of Peptide Linkers and Cypate Functionalization

5.C.1. Synthesis of Peptide Linkers

As discussed in the previous sections, our goal was also to prepare peptide linkers containing a thiol moiety for attachment to the surface of the AuNP as well as aminooxy functionality for chemoselective ligation to a cypate bis(aldehyde). However, as presented in Figure 5.1, it is important that this short linker is of an appropriate length so that the AuNP quenches cypate fluorescence emission. Finally, the peptide must be cleaved by the enzyme urokinase plasminogen activator (uPA). In particular, uPA has been implicated in remodelling of the extracellular matrix, enhancing both cell proliferation and migration, and modulating cell adhesion.⁶ Consistent with its role in cancer progression, multiple groups have shown that high levels of uPA in primary breast cancers are independently associated with risks of recurrence.⁶ With these goals in mind, we selected **5.13** as our target peptide (Figure 5.10).

Figure 5.10. Aminooxy peptide spacer.

The specific amino acid sequence in this prototype follows from the literature that the Gly-Gly-Arg motif is selectively cleaved by uPA.¹⁷ Also, the length of the peptide chain (6 amino acids plus cysteamine, approximately 2.5nm) was chosen based on initial observations by our collaborator Achilefu *et al.* who showed that cypate fluorescence emission was quenched when cypate was attached to a NGP via an 8 amino acid spacer.¹⁸

Scheme 5.5 shows the solid phase synthesis strategy for the aminooxy peptide. Methoxy-activated, cysteamine-loaded polystyrene resin (Novabiochem) was reacted with the *N*-Fmoc-protected Gly-Gly amino acids depicted in Scheme 5.5 following the standard protocol of DIC-mediated coupling to obtain the resin-bound Fmoc-protected peptide **5.17**.

Scheme 5.5. Solid phase synthesis of aminooxy peptide.

Piperidine-mediated cleavage of the Fmoc group to unmask the primary amine allows for further elongation of the peptide by iterating standard protocol of DIC-mediated coupling with Fmoc-protected *N*-terminal arginine. Successive

deprotection/coupling cycles with the desired amino acids led to the resin-bound peptide aminoxy **5.18**. Releasing the peptide from the resin was achieved using standard trifluoroacetic acid (TFA) procedures in presence of triethylsilane in dichloromethane. The peptide **5.19** was confirmed by HRMS analysis (Figure 5.11). The help and knowledge of Dr. Archana Massey and Dr. Souvik Biswas was much appreciated in the initial synthetic efforts of these peptide linkers.

Figure 5.11. HRMS (X-axis: m/z ; Y-axis: intensity) of aminoxy peptide **5.19**.

In particular, we were gratified to find that overacylation of the aminoxy moiety did not occur during the coupling of the Fmoc-protected aminoxy glycine. Wahl^{19a} and Brask^{19b} were among the first to report this undesirable reaction in which mono-protected

aminoxy peptides do not satisfactorily prevent further reaction of the aminoxy with an activated amino acid, leading to *N*-overacylation (Scheme 5.6). Decostaire *et al.*²⁰ had shown that the α -effect of the oxygen affects the nucleophilic and the basic character of the amino-group by making it more acidic. Therefore, coupling conditions in the presence of strong base increase overacylation. To circumvent this side-reaction, Duléry *et al.*²¹ showed that ethoxyethylidene (Eei) could be used as a protecting group to prevent overacylation of aminoxy peptides. Eei can be cleaved to unmask the aminoxy using mild acidic conditions (1% TFA in CH₂Cl₂).

Scheme 5.6. Coupling conditions leading to overacylation, and Eei protected aminoxy peptides.

The aminoxy peptide **5.19** was provided to Kyung Kang and her graduate student Jianting Wang who confirmed cleavage of uPa sequence during uPa incubation studies.²²

3.C.2. Aldehyde Functionalization of Cypate

To enable the direct chemoselective ligation, under neutral conditions, of both the peptide aminooxy and the heterobifunctional diblock linkers with the fluorescent probe cypate, we needed to functionalize cypate with two aldehyde moieties. Shown in Figure 5.12 is our target cypate bis(aldehyde).

Figure 5.12. Cypate bis(aldehyde) target to enable oximation ligation.

Dr. Samuel Achilefu, one of our collaborators, provided the initial cypate bis(carboxylic) acid **5.14**. Cypate has excitation and emission wavelengths of 780 nm and 830 nm respectively, which places them in the near infrared (NIR), and therefore could be applied for cancer detection and diagnosis.^{4,5} More importantly, cypate is FDA approved.²³

Our synthetic strategy (as outlined in Scheme 5.7) aimed at modifying the carboxylic acid via a bis-amide formation using a reagent that contains a masked aldehyde in the form of an acetal. Reaction of **5.14** with 4-aminobutyraldehyde diethyl acetal via a carbodiimide mediated coupling reaction generated the desired bis(amide-acetal) cypate adduct **5.15** in good yields (68%). It is important to highlight that cypate and its analogs are extremely sensitive to light. Therefore, isolation and purification of **5.15** was performed in the dark using column chromatography. Treatment of **5.15** with

acetic acid in water at room temperature effected hydrolysis of the acetal moieties within 4 hours to give the corresponding bis-aldehyde analog **5.16** in 63% yield.

Scheme 5.7. Synthesis of cypate bis(aldehyde).

Although standard coupling syntheses generally require mild basic conditions, in our case, the synthesis of **5.15** gave the best yields in absence of base. Indeed, it is possible, as shown in Scheme 5.8, that base leads to decomposition of cypate via a β -

elimination reaction. This elimination process can occur either at the carboxylic state ($R = OH$), or after coupling, at the amide state ($R = NHR'$).

Scheme 5.8. Decomposition of cypate analogs via β -elimination.

5.D. Nano-Entity Assembly

5.D.1. Solid Phase Assembly

Previous work by Dr. Biswas and Dr. Massey aimed to prepare the peptide-cypate conjugate via solid phase synthesis. They first attempted the direct coupling of terminal amine peptide **5.17** with cypate (Schme 5.9).

Scheme 5.9. Solid phase coupling of cypate with terminal amine peptide.

The short spacer peptide was fitted with a terminal amine moiety by substituting the last glycine in Scheme 5.5 with Fmoc-protected amino-glycine, as shown in Scheme 5.9. Following the standard coupling and Fmoc-deprotection procedures, **5.17** was reacted with cypate and then cleaved from the resin to obtain the peptide-cypate conjugate **5.18**. In general, based on the theoretical concentration of resin reactive sites, conjugate **5.18** was obtained in 8-12% yield. This is due to the large excess of cypate (*ca.* 4-5 equiv.) required to prevent the reaction at both carboxylic acid groups of cypate and to insure that all the peptides have been coupled.

Following this work, we attempted to couple our trityl-protected amine spacer TrS-C16PEG8-NH₂ (Scheme 5.4, compound **5.9**) to the available –CO₂H group of the peptide-bound cypate (Scheme 5.9, **5.17**) using standard amide-forming coupling conditions. Unfortunately, cleavage of the material from the resin using the established conditions (TFA, TES, CH₂Cl₂) gave a mixture of products. The primary component, shown in Figure 5.13, was identified by HRMS as an elimination product (Scheme 5.8).

Figure 5.13. Major byproduct of the coupling of amine space **5.9** with peptide-cypate conjugate **5.17** using solid phase synthesis.

This preliminary work illustrates the difficulty associated with cypate functionalization and coupling, and hence the value of the neutral aminooxy ligation strategy.

5.C.2. Click Chemistry Assembly

Facing major decomposition of cypate and low synthetic yields through the solid phase synthesis strategy, we examined the reactivity of cypate bis(aldehyde) **5.16** with our thiol-aminoxy diblock linkers for ligation of the two elements. We anticipated that the neutral conditions required for the oximation reaction would minimize cypate decomposition.

Scheme 5.10. Ligation reaction between a diblock linker and cypate bis(aldehyde).

We tested the key oximation reaction independently of any gold nanoparticles. Cypate analog **5.16** was treated with spacer molecule HS-(CH₂)₁₂-PEG₈-ONH₂ (Scheme 5.3, compound **5.7a-3**) using an excess of cypate bis(aldehyde) to favor mono-oximation. Simple stirring of the solution resulted in oximation, and the mono-product **5.19** was isolated (52% yield unoptimized) after SiO₂ chromatography. Figure 5.14 shows the HRMS of the spacer-cypate conjugate **5.19**.

Figure 5.14. HRMS (X-axis: m/z ; Y-axis: intensity) of a spacer-cypate conjugate.

Gratified by this result we attempted the ligation of our aminooxy peptide bound to the polymer resin (Scheme 5.5, compound **5.12**) with cypate bis(aldehyde) **5.16** and one of our aminooxy diblock linkers. We started the synthesis of the fluorescent probe

by ligating cypate bis(aldehyde) **5.16** to the aminooxy peptide **5.12** in DMF at room temperature for 12 hours. Attachment of cypate in this manner was visually evident by the change in color of the resin after reaction with the bis(aldehyde) analog (beads changed from yellow to blue, analogous to the change in the amide-coupling approach). After washing the resin from unreacted cypate, we reacted the cypate-loaded resin with aminooxy spacer TrS-(CH₂)₁₂-PEG₈-ONH₂. Cleavage from the resin was conducted using TFA and Et₃SiH. These same conditions would also unmask the thiol group by deprotecting the trityl group, affording the final target conjugate **5.26**.

Figure 5.15. Structure of the target conjugate composed of a peptide short linker with a cancer specific enzyme recognition sequence, a fluorophore, and a diclock linker.

Unfortunately, MS analysis revealed a mixture of different products. First it seems that the N-O bond of the peptide chain was cleaved under these conditions (likely due to reduction by the Et₃SiH reagent). Indeed, the hydroxy peptide shown in Figure 5.16 indicates that the RO-N=C linkage (oxime ether) does not survive the cleavage conditions.

hydroxyl derivative formed on reduction of O-N linkage

Figure 5.16. Residual hydroxy peptide generated by the reduction of N-O bond.

From these observations, we attempted the direct formation of the final target conjugate **5.26** by mixing ligated cypate **5.25** with unbound peptide aminooxy **5.19**, under neutral and mild conditions, as shown in Scheme 5.11.

Scheme 5.11. Reaction for the formation of the final peptide-cypate-linker conjugate.

After reacting for 12 hours at room temperature, we used HRMS to identify the products formed. As we expected, the desired dual-chain product **5.26** was formed. Figure 5.17 shows the HRMS mass distribution of our dual-chain probe **5.26** formed in Scheme 5.11, versus the theoretical HRMS mass distribution. It can be seen that both figures match, confirming the formation of **5.26**.

Figure 5.17. Our dual chain probe **5.26** (top) vs theoretical (bottom) HRMS (X-axis: m/z ; Y-axis: intensity) of dual chain probe.

Gratified by these results, we provided the Kang group with both our heterobifunctional diblock linkers and cypate bis(aldehyde) for loading onto gold nanoparticles and subsequent fluorescent studies. Four batches of 10 nm citric-AuNP were reacted with varying ratios of our heterobifuntional diblock linker **5.13a-PEG(4)**

and a shorter alcohol/thiol linker **sPEG** (HS-(CH₂)₁₁-PEG₃-OH). After loading and purification by dialysis, the sizes of the nanoparticles were measured again using a 90Plus/BI-MAS particle size analyzer. Table 5.4 shows the different ratios by percentage of the **sPEG** to **5.13a-PEG(4)** linkers, as well as the measured size of the AuNP.

Table 5.4. Particle size measurements before and after spacer conjugation to AuNP.

	sPEG (% used in reaction)	5.13a-PEG(4) (% used in reaction)	Analyzed Size (nm)
Citric-AuNP	0	0	7.26 ± 2.90
Sample 1 (S1)	100	0	10.16 ± 2.18
Sample 2 (S2)	95	5	13.27 ± 3.46
Sample 3 (S3)	80	20	12.34 ± 2.27
Sample 4 (S4)	0	100	aggregated

It can be clearly seen that the size of the AuNP increased from 7.26 nm for simple citric-AuNPs to as high as 13.27 nm after the reaction with the linkers. This indicates that the citric AuNP did successfully bind to **sPEG** and **5.13a-PEG(4)**. The analyzed size of the particles conjugated with **4a** was larger than that of the particles with only **sPEG**, suggesting that **5.13a-PEG(4)** successfully coupled with the AuNP. However, large concentrations of **5.13a-PEG(4)** led to aggregation and precipitation of the gold nanoparticles. It is possible that the increased concentration of aminoxy groups at the surface of the AuNPs led to reactions with other gold particles, forming undesired and unreactive aggregates. Thus, the **sPEG** served as a stabilizer to keep the functionalized gold particles from aggregating and precipitating.

After proving the loading of our aminoxy linkers, samples **S1-S3** were reacted with cypate bis(aldehyde) **5.22** for 4 hrs. After purification by dialysis the particles were suspended in DI water for fluorescence studies. Figure 5.18 shows the absorption scan

for all three samples (**S1-S3**), as well as the absorption scan of the gold nanoparticles, and the cypate **5.20**.

Figure 5.18. Absorption scan of AuNP-sPEG/AuNP-**5.13a**-PEG(**4**)-cypate samples.

The scan showed that compared to bare AuNP and sample **S1**, which should not bind cypate bis(aldehyde) since it lacks aminoxy moieties, samples **S2** and **S3** have increased absorption at 780 nm indicating cypate bis(aldehyde) ligated via oximation reaction. However, as can be seen in Figure 5.19, the levels of fluorescence emissions of samples **S2** and **S3** that had been coupled with cypate bis(aldehyde) were 90% lower than free cypate bis(aldehyde). The conjugated cypate bis(aldehyde) emitted fluorescence at 830 nm after the samples were excited with light of 780 nm and 794 nm wavelengths.

Figure 5.19. Fluorescence of free cypate bis(aldehyde) (control) and samples **S2** and **S3** after conjugation with cypate bis(aldehyde).

Given the evidence shown previously for conjugation of the linker **5.13a-PEG(4)** to both the AuNP and the cypate fluorophore, it was difficult to explain the fluorescence quenching observed in these experiments. One possibility, as shown in Figure 5.20, was that the hydrophobic nature of the cypate fluorophore was causing it to migrate into the hydrophobic domain generated by the **sPEG** linkers, as well as the **5.13a-PEG(4)** linkers, and surrounding the gold nanoparticle. To verify this hypothesis, we provided the Kang group with aminooxy compound **5.27** (Figure 5.20), which contains an aminooxy group for chemoselective reaction with aldehydes, and an ammonium salt, which makes it very hydrophilic. We surmised that, if conjugated cypate was migrating to the hydrophobic domain, then reaction with **5.27** should increase its hydrophilicity, pulling cypate away for the hydrophobic quenching environment, and restore the fluorescence. As expected, making cypate bis(aldehyde) more hydrophilic by reaction with ammonium **5.27** restored the fluorescence of the nano-entity.²²

Figure 5.20. Fluorescence quenching and restoring experiments.

Figure 5.20A shows the loading of cypate bis(aldehyde) and **5.13a-PEG(4)** linkers in the presence of **sPEG** linkers (to avoid aggregation). However, as shown in Figure 5.20B, mono-conjugated cypate migrates to the hydrophobic domain surrounding the AuNP, and thus its fluorescence is quenched. However, when adding hydrophilic compound **5.27** to the system, fluorescence was restored. Indeed, aminoxy **5.27** reacts with the second available aldehyde in cypate and increases its hydrophilicity so that the fluorophore migrates out of the hydrophobic layer.

5.D.3. Conclusion

We demonstrated in this section the unquestionable advantages of aminooxy ligation for the assembly of the cypate dual-chain probe. Indeed, we showed that normal coupling conditions, which require the presence of base and multiple reagents, led to significant decomposition of the cypate bis(aldehyde). Conversely, the use of an oximation reaction to ligate heterobifunctional diblock linkers, cypate bis(aldehyde), and the aminooxy peptide generated the desired cypate dual-chain probe **5.26**.

Finally, the aminooxy reaction enabled by our heterobifunctional diblock linkers showed its versatility during the stepwise loading of cypate bis(aldehyde) onto the gold nanoparticles. First, we showed that the **5.13a-PEG(4)** linker could be loaded onto gold nanoparticles by mixing with gold citrate; then simple mixing of cypate bis(aldehyde) with the resulting **5.13a-PEG(4)**-activated AuNPs led to ligation of cypate with the nano-entity. Fluorescence studies showed that although cypate was properly bound to the gold surface, its fluorescence was partially quenched due to an unexpected migration of cypate bis(aldehyde) into the hydrophobic domain surrounding the gold nano-entity. To demonstrate this theory, we used again the oximation reaction to attach a highly hydrophilic aminooxy-ammonium salt **5.27** to cypate and restore its fluorescence within the nano-entity.

CHAPTER 6

Experimental Procedures

6.A. General Statement and Index of Experimental Procedures

6.B. Experimental Procedures of Chapter 2

6.C. Experimental Procedures of Chapter 3

6.D. Experimental Procedures of Chapter 4

6.E. Experimental Procedures of Chapter 5

6.A. General Statement

All solvents and reagents used in this thesis were reagent grade and were used as received unless otherwise indicated. Dry THF and CH₂Cl₂ were obtained from SPBT-101 Bench Top Solvent Purification System (LC Technology Solutions, Inc., USA). Anhydrous DMF was purchased from Sigma-Aldrich. Unless otherwise noted, all reagents were purchased from commercial suppliers and were used without further purification. The progress of reactions was monitored by thin-layer chromatography (TLC) using precoated silica plates (EMD Silica Gel 60 F254). Visualization was accomplished by staining the plates with iodine, PMA (3% phosphomolybdic acid/ethanol solution), PAA stain (2.5% *p*-anisaldehyde acid/ethanol solution). UV active compounds were visualized by UV light (254 nm). Silica gel 60 (230-400 mesh) was used for flash column chromatography. ¹H NMR spectra were recorded at 400, 500 or 700 MHz, and ¹³C spectra were recorded at 100, 125 or 175 MHz, respectively, in the indicated solvents. The chemical shifts are reported in ppm values relative to the solvent residual peak CDCl₃ (7.26 ppm for ¹H NMR and 77.23 ppm for ¹³C NMR). Coupling constants are reported in hertz (Hz). High resolution ESI-MS were obtained using a FTICR-MS system (LTQ FT, Thermo Electron Corp.) at the Center for Regulatory and Environmental Analytical Metabolomics (CREAM) Mass Spectrometry Facility, University of Louisville, Kentucky. Solid phase peptide synthesis was performed using methoxy-activated, cysteamine-loaded polystyrene resin (Novabiochem, cat. no. 01-64-0086). All amino acids (Novabiochem) and peptide coupling reagents (Aldrich Chemical Company) were used as received from vendors. Cypate was provided by our collaborator Dr. Samuel Achilefu (Washington University, St. Louis).

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6.B. Experimental Procedures of Chapter 2

Improved Preparation of O-(diphenylphosphinyl)hydroxylamine

O-(Diphenylphosphinyl)hydroxylamine (DPPH)

To a stirred solution (mechanical stirring) of hydroxylamine hydrochloride (6.46 g, 93.0 mmol) in water (20 mL) at -10 °C was added a solution of sodium hydroxide (3.55 g, 88.7 mmol) in water (20 mL). Diethyl ether (180 mL) was then added and the biphasic mixture was further cooled using an ice-methanol bath (*ca.* -15 °C). Diphenylphosphinic chloride (10.0 g, 42.3 mmol) was added rapidly via syringe, and the reaction mixture was stirred vigorously for 10 min, and then warmed to 0 °C and stirred an additional 15 min. The resultant white slurry was filtered and the retentate was washed successively with cold water (60 mL) and Et₂O (70 mL). The resultant amorphous paste was dried under vacuum to afford a white powder that then was treated with aq. NaOH (0.25 M, 120 mL) at 0 °C for 30 min. The slurry was filtered and the resultant amorphous solid was washed with cold water (40 mL), and was dried under vacuum 10 h to afford the title compound (7.68 g, 78%) with >97% purity by ³¹P nmr. Recrystallization of an analytical sample from methanol delivered DPPH in crystalline form, mp. 140–141 °C, lit.¹ (uncrystallized sample) 130 °C (dec); ¹H NMR (400 MHz, CDCl₃) δ 7.87–7.82 (4H, m), 7.57–7.53 (2H, m), 7.49–7.47 (4H, m), 5.84 (2H, br) ppm; ³¹P NMR (162 MHz, CDCl₃) δ 37.5 ppm; HRMS calculated for [DPPH+Na]⁺: 256.04979, found: 256.04965.

Experimental Procedures for a Chemoselective, One-Pot Transformation of Aldehydes to Nitriles

Method A: Typical Procedures for One-Pot Aldehyde to Nitrile Transformation.

Preparation of trans-cinnamonnitrile (Table 2.5, Entry 8)

To trans-cinnamaldehyde (0.13 g, 1.0 mmol) in toluene (5 mL) at room temperature was added DPPH (0.267 g, 1.15 mmol) in one portion. The resulting suspension was stirred at room temperature for 3 h and then gradually warmed over 45 min to 85 °C. The reaction mixture became clear as the temperature reached 80 °C. After being heated at 85 °C for 5 h, the reaction was allowed to cool to room temperature and then diluted by addition of Et₂O (20 mL) and saturated aq. NaHCO₃ to dissolve precipitated diphenylphosphinic acid. The layers were separated, and the organic layer was washed successively with saturated aq. NaHCO₃ and brine (2 x 10 mL). The aqueous layer was extracted with EtOAc (2x). All of the organic layers were combined and then dried (Na₂SO₄). The solvents were removed by rotary evaporation, and the crude residue was purified by column chromatography (SiO₂, hexane/ethyl acetate, 2:1 v/v, *R_f* = 0.62) to afford trans-cinnamonnitrile (105 mg, 81%) as a light yellow oil with spectroscopic data in agreement with published values.² ¹H NMR (400 MHz, CDCl₃) δ 7.46-7.38 (m, 6H), 5.90 (d, *J* = 16.8 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 150.5, 133.5, 131.2, 129.1, 127.33, 118.11, 96.3.

Method B: Typical Procedures for One-Pot Aldehyde to Nitrile Transformation.

Preparation of 4-hydroxybenzonitrile (Table 2.5, Entry 3)

To 4-hydroxybenzaldehyde (0.09 g, 0.78 mmol) in toluene (5 mL) at room temperature was added DPPH (0.366 g, 1.57 mmol) in one portion. The resulting suspension was stirred at room temperature for 3 h and then gradually warmed over 45 min to 95 °C. After heating at 95 °C for 12 h, the reaction was allowed to cool to room temperature and then diluted by addition of EtOAc (20 mL) and saturated aq. NaHCO₃ to dissolve the precipitated diphenylphosphinic acid. The layers were separated, and the organic layer was washed successively with saturated aq. NaHCO₃ and brine (2 x 10 mL). The aqueous layer was extracted with EtOAc (2x). The combined organic layer was dried (Na₂SO₄). The solvents were removed by rotary evaporation, and the crude residue was purified by column chromatography (SiO₂), eluting with hexane/ethyl acetate (2:1, v/v; R_f = 0.33) to afford 4-hydroxybenzonitrile (72 mg, 78% yield) as a yellow solid: mp 111-113 °C, having spectral characteristics in agreement with published data.³ ¹H NMR (CDCl₃, 700 MHz) δ 7.55 (d, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 5.78 (s, 1H); ¹³CNMR (CDCl₃, 100 MHz) δ 159.9, 134.3, 119.2, 116.4, 103.3.

Method C: Typical Procedures for One-Pot Aldehyde to Nitrile Transformation.

Preparation of 3-pyridinecarbonitrile (Table 2.5, Entry 11)

To 3-pyridinecarboxaldehyde (0.10 g, 0.93 mmol) in toluene (5 mL) at room temperature was added trifluoroacetic acid (72 μ L, 1 mmol). After the mixture was stirred for 5 min, DPPH (0.250 g, 1.07 mmol) was added in one portion. The resulting suspension was stirred at room temperature for 3 h and then gradually warmed over 45 min to 95 °C. After heating at 95 °C for 12 h, the reaction was allowed to cool to room temperature and then diluted by addition of EtOAc (15 mL) and saturated aq. NaHCO₃ to dissolve the precipitated diphenylphosphinic acid. The layers were separated, and the organic layer was washed successively with saturated aq. NaHCO₃ and brine (2 x 10 mL). The aqueous layer was extracted with EtOAc (2x) and DCM (2x). The combined organic layer was dried (Na₂SO₄). The solvents were removed by rotary evaporation, and the crude residue was purified by column chromatography (SiO₂), eluting with CH₂Cl₂/hexane/ethyl acetate (7:2:1, v/v; *R_f* = 0.34) to afford 3-pyridinecarbonitrile (60 mg, 64% yield) as a white solid, mp 49.1-50.2 °C, having spectral characteristics in agreement with published data.⁴ ¹H NMR (CDCl₃, 400MHz) δ 8.90 (s, 1H), 8.83 (d, *J* = 4.8 Hz, 1H), 7.98 (d, *J* = 8 Hz, 1H), 7.45 (dd, *J* = 4.8, 8.0 Hz, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 153.2, 152.7, 139.4, 123.7, 116.7, 110.3.

Polar Solvent Procedure: One-Pot Aldehyde to Nitrile Transformation.

Preparation of 3-phenylpropionitrile (Table 2.5, Entry 6)

To 3-phenylpropionaldehyde (0.70 g, 0.52 mmol) in water (8 mL) at room temperature was added DPPH (0.14 g, 0.60 mmol) in one portion. The resulting suspension was stirred at room temperature for 3 h and then gradually warmed over 45 min to 95 °C. After heating at 95 °C for 12 h, the reaction was cooled to room temperature and extracted with EtOAc (2 x 10 mL) and Et₂O (10 mL). The combined organic layer was washed with brine (10 mL) and then dried (Na₂SO₄). The solvents were removed by rotary evaporation, and the crude residue was purified by column chromatography (SiO₂), eluting with hexane/ethyl acetate (2:1, v/v; *R_f* = 0.55) to afford 3-phenylpropionitrile (50 mg, 73% yield) as a yellow oil, having spectral characteristics in agreement with published data.⁵ ¹H NMR (CDCl₃, 400 MHz) δ 7.34 (t, *J* = 7.2 Hz, 2H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.22 (d, *J* = 7.2 Hz, 3H), 2.96 (t, *J* = 7.6 Hz, 2H), 2.61 (t, *J* = 7.6 Hz, 2H); ¹³C NMR (CDCl₃, 100 MHz) δ 138.2, 129.0, 128.5, 127.4, 119.3, 31.7, 19.5.

Tabulation of spectroscopic data of all the nitrile products (entry numbers refer to position in Table 2.5)

Benzonitrile (entry 1)

^1H NMR (400 MHz): δ 7.67 (d, J = 7.6 Hz, 2H), 7.60 (m, 1H), 7.47 (m, 2H)

^{13}C NMR (100 MHz): δ 132.7, 132.1, 129.0, 118.8, 112.4

1-Naphthonitrile (entry 2)

^1H NMR (400 MHz): δ 8.26 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.93-7.91 (m, 2H), 7.71 (t, J = 7.6 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.53 (t, J = 7.6 Hz, 1H)

^{13}C NMR (100 MHz): δ 133.2, 132.9, 132.6, 132.3, 128.6, 128.5, 127.5, 125.1, 124.9, 117.7, 110.2

4-Hydroxybenzonitrile (entry 3)

^1H NMR (700 MHz): δ 7.55 (d, J = 8.4 Hz, 2H), 6.91 (d, J = 8.4 Hz, 2H), 5.78 (s, 1H)

^{13}C NMR (100 MHz): δ 159.9, 134.3, 119.2, 116.4, 103.3

***p*-Methoxybenzonitrile (entry 4)**

^1H NMR (400 MHz): δ 7.55 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2H), 3.82 (s, 3H)

^{13}C NMR (100 MHz): δ 162.8, 133.9, 119.2, 114.7, 103.8, 55.5

4-(Methylthio)benzonitrile (entry 5)

Spectral characteristics in agreement with published data.⁶

^1H NMR (400 MHz): δ 7.54 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 2.51 (s, 3H)

^{13}C NMR (100 MHz): δ 146.0, 132.1, 125.4, 118.9, 107.6, 14.6

3-Phenylpropionitrile (entry 6)

^1H NMR (400 MHz): δ 7.34 (t, J = 7.2 Hz, 2H), 7.27 (t, J = 7.2 Hz, 1H), 7.22 (d, J = 7.2 Hz, 3H), 2.96 (t, J = 7.6 Hz, 2H), 2.61 (t, J = 7.6 Hz, 2H)

^{13}C NMR (100 MHz): δ = 138.2, 129.0, 128.5, 127.4, 119.3, 31.7, 19.5

Myristonitrile (entry 7)

^1H NMR (400 MHz): δ 2.31 (t, J = 7.6 Hz, 2H), 1.64 (q, J = 7.6 Hz, 2H), 1.44-1.41 (m, 2H), 1.39-1.24 (m, 18H), 0.86 (t, J = 7.2 Hz, 3H)

^{13}C NMR (100 MHz): δ 119.8, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 28.7, 28.6, 25.3, 22.6, 17.0, 14.0

***trans*-Cinnamonitrile (entry 8)**

^1H NMR (400 MHz): δ 7.46–7.38 (m, 6H), 5.9 (d, J = 16.8 Hz, 1H)

^{13}C NMR (100 MHz): δ 150.5, 133.5, 131.2, 129.1, 127.3, 118.1, 96.3

3-Acetylbenzonitrile (entry 9)

Spectral characteristics in agreement with published data.⁷

^1H NMR (400 MHz): δ 8.23 (app. s, 1H), 8.18 (app. d, J = 8.0 Hz, 1H), 7.85 (app. d, J = 8.0 Hz, 1H), 7.61 (app. t, J = 8.0 Hz, 1H) 2.64 (s, 3H)

^{13}C NMR (100 MHz): δ 195.7, 137.7, 135.9, 132.1, 132.0, 129.6, 117.8, 113.1, 26.5

4-(Acetyloxy)butanenitrile (entry 10)

Spectral characteristics in agreement with published data.⁸

¹H NMR (700 MHz): δ 4.17 (t, J = 6.2 Hz, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.05 (s, 3H), 2.03-1.96 (m, 2H)

¹³C NMR (175 MHz): δ 170.9, 119.0, 62.3, 30.9, 24.9, 22.8, 20.9, 14.4

3-Pyridinecarbonitrile (entry 11)

Spectral characteristics in agreement with published data.⁹

¹H NMR (400 MHz): δ 8.90 (s, 1H), 8.83 (d, J = 4.8 Hz, 1H), 7.98 (d, J = 8 Hz, 1H), 7.45 (dd, J = 4.8 Hz, J = 8 Hz, 1H)

¹³C NMR (100 MHz): δ 153.2, 152.7, 139.4, 123.7, 116.7, 110.3

***N*-Boc-4-(cyanomethyl)piperidine (entry 12)**

Spectral characteristics in agreement with published data.¹⁰

¹H NMR (400 MHz): δ 4.15 (app. d, J = 12.8 Hz, 2H), 2.69 (app. t, J = 12.8 Hz, 2H), 2.30 (d, J = 6.4 Hz, 2H), 1.82-1.76 (m, 3 H), 1.44 (s, 9H), 1.27-1.23 (m, 2H)

¹³C NMR (100 MHz): δ 154.8, 118.3, 79.8, 43.5, 33.5, 31.4, 28.6, 24.2

***N*-Boc-4-cyanopiperidine (entry 13)**

Spectral characteristics in agreement with published data.¹¹

¹H NMR (500 MHz): δ 3.68-3.63 (m, 2H), 3.36-3.31 (m, 2H), 2.80-2.78 (m, 1H), 1.89-1.86 (m, 2H), 1.85-1.77 (m, 2H), 1.45 (s, 9H)

¹³C NMR (100 MHz): δ 154.5, 121.1, 80.1, 41.8, 28.5, 26.4

Ethyl 2-(3-cyano-1H-indol-1-yl)acetate (entry 14)

^1H NMR (400 MHz): δ 7.78 (d, J = 7.6 Hz, 1H), 7.62 (s, 1H), 7.34-7.30 (m, 3H), 4.87 (s, 1H), 4.25 (q, J = 7.2 Hz, 2H), 4.25 (q, J = 7.2 Hz, 3H)

^{13}C NMR (100 MHz): δ 167.2, 135.9, 135.6, 127.8, 124.5, 122.7, 120.3, 115.6, 110.2, 87.5, 62.5, 48.3, 14.2

IR (cm⁻¹): 2223, 1740

HRMS (ESI+, m/z) calcd for $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}_2$, $[\text{M} + \text{Na}]^+$ 251.0791, found 251.0791

Light yellow solid; Melting point: 96.5-98 °C

4-((*tert*-Butyldimethylsilyl)oxy)butanenitrile (entry 15)

Spectral characteristics in agreement with published data.¹²

^1H NMR (500 MHz): δ 3.70 (t, J = 5.5 Hz, 2 H), 2.44 (t, J = 7 Hz, 2 H), 1.83 (m, 2 H), 0.88 (s, 9 H), 0.05 (s, 6 H)

^{13}C NMR (175 MHz): δ 119.8, 60.6, 28.5, 25.9, 18.3, 13.8, -5.3

Phthalonitrile (entry 16)

^1H NMR (400 MHz): δ 7.85-7.82 (m, 2 H), 7.78-7.73 (m, 2H)

^{13}C NMR (175 MHz): δ 133.8, 133.4, 116.1, 115.5

Synthesis

1-Naphthaldehyde *O*-diphenylphosphoryl oxime (2.2)

To 1-naphthaldehyde (0.15 g, 1.0 mmol) in tetrahydrofuran (10 mL) at room temperature was added DPPH (0.267 g, 1.15 mmol) in one portion. The resulting suspension was stirred at room temperature for 30 h. Then, the reaction was concentrated by rotary evaporation, and the crude residue was purified by column chromatography (SiO₂), eluting with CH₂Cl₂/MeOH (95:5, v/v; *R_f* = 0.52) to afford 1-naphthaldehyde *O*-diphenylphosphoryl oxime (**2.2**) (50 mg, 79% yield) as a gum. ¹H NMR (CDCl₃, 400MHz) δ 9.04 (s, 1H), 8.34 (d, *J* = 8.8Hz, 1H), 8.00-7.95 (m, 4H), 7.91 (d, *J* = 8.0Hz, 1H), 7.84 (d, *J* = 7.6Hz, 1H), 7.71 (d, *J* = 7.2 Hz, 1 H), 7.59-7.43 (m, 9H); ¹³C NMR (CDCl₃, 100 MHz) δ 158.1, 158.0, 133.8, 132.6, 132.4, 132.3, 131.1, 130.6, 129.9, 129.8, 128.8, 128.7, 228.6, 127.8, 126.5, 126.3, 125.2, 125.0; ³¹P NMR (CDCl₃, 162 MHz) δ 35.60; FT-ICR-MS (ESI+, *m/z*) calcd for C₂₃H₁₈NO₂P, [M + Na]⁺ 394.0967, found 394.0976.

6.C. Experimental Procedures of Chapter 3

Synthesis

Hexanal oxime (**3.5**)

To hexanal (**3.1**) (1.5 g, 15 mmol) in methanol (5 mL) was added saturated aq sodium bicarbonate (2.5 mL) and hydroxylamine hydrochloride (1.14 g, 16.5 mmol). After stirring 16 h at room temperature, the reaction mixture was extracted with Et₂O (3x). The combined organic extract was washed with brine (3x) and then dried (Na₂SO₄). The ether was removed by rotary evaporation and the crude residue was purified by SiO₂ column chromatography (hexane:EtOAc, 1:1 v/v; *R_f* = 0.73) to afford **3.5** (0.48 g, 89% yield) as a 1.5:1 mixture of *E*:*Z* isomers. The spectral characterization of **3.5** was in agreement with published values.¹³ ¹H NMR (400 MHz, CDCl₃) (*E*)-isomer δ 7.42 (t, *J* = 6.0 Hz, 1H), 2.20 (dt, *J* = 7.6, 6.4 Hz, 2H), 1.48 (m, 2H), 1.31 (m, 4H), 0.93 (s, 9H), 0.90 (t, *J* = 2.4 Hz, 3H).

Hexanal *O*-(tert-butyldimethylsilyl) oxime (**3.9**)

To a solution of aldoxime **3.5** (0.20 g, 1.74 mmol) in dry CH₂Cl₂ (1.5 mL) at 0 °C was added TBS-Cl (0.314 g, 2.08 mmol), imidazole (0.238 g, 3.50 mmol), and 4-(*N,N*-dimethylamino)pyridine (*ca.* 5 mg). The reaction was warmed to room temperature and stirred 72 hours whereupon the solvent was removed by rotary evaporation. The residue was purified by SiO₂ column chromatography (hexane:EtOAc, 2:1, v/v; *R_f* = 0.69) to afford **3.9** (0.26 g, 65% yield) as a 1:1 mixture of *E:Z* isomers; ¹H NMR (400 MHz, CDCl₃) (*E*)-isomer δ 7.49 (t, *J* = 6.0 Hz, 1H), 2.19 (dt, *J* = 7.6, 6.8 Hz, 2H), 1.47 (m, 2H), 1.31 (m, 4H), 0.93 (s, 9H), 0.89 (t, *J* = 6.8 Hz, 3H), 0.16 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) isomer mixture δ 156.8, 155.9, 31.7, 31.4, 29.6, 26.4, 26.3, 26.2, 26.0, 25.5, 22.5, 18.4, 18.3, 14.1, −5.05, −5.09.

1-(2,2-Dimethoxyethoxy)propane (**3.13**)

To a solution of sodium hydroxide (2.98 g, 74.4 mmol) in *n*-propanol (9.84 g, 163.7 mmol) at room temperature was added commercial α -bromo-acetaldehyde dimethylacetal (5 g, 29.8 mmol). The reaction mixture was heated to reflux for 45 h. The reaction then was cooled to room temperature and extracted with ether (75 mL). The ether layer was washed successively with saturated aq. NaHCO₃ (2x15 mL), brine (2x15 mL) and then dried (Na₂SO₄). The solvents were removed by rotary evaporation and the residue was purified by SiO₂ column chromatography (hexane:EtOAc, 6:1, v/v; R_f = 0.45) to give the corresponding α -(*n*-propoxy)-acetaldehyde dimethylacetal (**3.13**) (1.51 g, 25% yield); ¹H NMR (400 MHz, CDCl₃) δ 4.50 (t, *J* = 5.2 Hz, 1H), 3.47 (d, *J* = 5.2 Hz, 2H), 3.43 (t, *J* = 7.2 Hz, 2H), 3.39 (s, 6H), 1.61 (m, 2H), 0.91 (t, *J* = 7.6 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 102.7, 73.3, 70.4, 53.8, 22.7, 10.3.

***n*-Propoxyaldehyde (3.3)**

To the propoxyacetal **3.13** (0.642 g, 4.33 mmol) in water (20 mL) was added *p*-toluene sulfonic acid (PTSA) (3.296 g, 17.32 mmol). The reaction was stirred 20 h at room temperature. The reaction mixture then was extracted with ether (4x70 mL) and the combined organic extract was washed with brine (2x) and dried (Na₂SO₄). The solvent was removed by rotary evaporation and the residue was purified by short path distillation (35 mbar, collect bp 42-45 °C), using an acetone - dry ice bath to cool the collection vessel, to give aldehyde **3** (0.36 g, 81% yield). The spectral characterization of **3** was in agreement with published values.¹⁴ ¹H NMR (400 MHz, CDCl₃) δ 9.73 (s, 1H), 4.05 (s, 2H), 3.50 (t, *J* = 6.8 Hz, 2H), 1.62 (m, 2H), 0.93 (t, *J* = 6.8 Hz, 3H).

2-Propoxyacetaldehyde oxime (**3.7**)

Using the procedure outlined for synthesis of aldoxime **3.5**, *n*-propoxyethanal **3.3** (0.300 g, 2.89 mmol) was transformed to aldoxime **3.7** (0.191 g, 56% yield). Purification by SiO₂ column chromatography (hexane:EtOAc, 6:1, v/v; *R_f* = 0.20) gave aldoxime **3.7** as a 1.2:1 mixture of *E*:*Z* isomers; ¹H NMR (400 MHz, CDCl₃) (*E*)-isomer: δ 7.50 (bs, 1H), 4.08 (d, *J* = 5.6 Hz, 2H), 3.42 (t, *J* = 6.8 Hz, 2H), 1.61 (m, 2H), 0.92 (t, *J* = 6.8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) (*E*)-isomer δ 148.4, 72.5, 67.3, 22.7, 10.4.

2-Propoxyacetaldehyde *O*-(tert-butyldimethylsilyl) oxime (**3.11**)

Using the procedure outlined for synthesis of silyl aldoxime **3.9**, *n*-propoxyethanaldoxime **3.7** (0.034 g, 0.29 mmol) was transformed to silyl aldoxime **3.11** (0.062 g, 62% yield). Purification by SiO₂ column chromatography (hexane:ether, 3:1, v/v; *R_f* = 0.75) gave silyl aldoxime **11** as a 1:1 mixture of *E*:*Z* isomers; ¹H NMR (400 MHz, CDCl₃) (*E*)-isomer δ 7.56 (t, *J* = 5.6 Hz, 1H), 4.08 (d, *J* = 5.6 Hz, 2H), 3.40 (t, *J* = 6.8 Hz, 2H), 1.60 (m, 2H), 0.93 (t, *J* = 9.2 Hz, 3H), 0.92 (s, 9H), 0.16 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) isomer mixture δ 155.6, 152.4, 73.3, 72.5, 67.7, 65.4, 26.2, 26.1, 23.0, 23.0, 18.3, 18.3, 10.72, 10.71, -5.0, -5.1.

2-Heptanone oxime (**3.6**)

Using the procedure outlined for synthesis of aldoxime **3.5**, 2-heptanone (**3.2**) (1.5 g, 13.1 mmol) was transformed to 2-heptaketoxime **3.6** (1.25g, 74%). Purification by SiO₂ column chromatography (hexane:EtOAc, 2:1, v/v; *R_f* = 0.42) gave ketoxime **3.6** as a 1:0.3 mixture of *E*:*Z* isomers. The spectral characterization of **6** was in agreement with published values.¹⁵ ¹H NMR (400 MHz, CDCl₃) (*E*)-isomer δ 2.17 (t, *J* = 8 Hz, 2H), 1.87 (s, 3H), 1.50 (quin, *J* = 7.6, 7.2 Hz, 2H), 1.30 (m, 4H), 0.90 (t, *J* = 6.4 Hz, 3H).

Heptan-2-one *O*-(tert-butyldimethylsilyl) oxime (**3.10**)

Using the procedure outlined for synthesis of silyl aldoxime **3.9**, ketoxime **3.6** (0.100 g, 0.77 mmol) was transformed to silyl ketoxime **3.10** (0.124 g, 66% yield). Purification by SiO₂ column chromatography (hexane:EtOAc, 2:1, v/v; *R_f* = 0.76) gave silyl ketoxime **3.10** as a 1:0.3 mixture of *E*:*Z* isomers. ¹H NMR (400 MHz, CDCl₃) (*E*)-isomer δ 2.17 (t, *J* = 7.6 Hz, 2H), 1.83 (s, 3H), 1.49 (m, 2H), 1.29 (m, 4H), 0.92 (s, 9H), 0.87 (t, *J* = 6.8 Hz, 3H), 0.14 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) isomer mixture δ 162.9, 162.3, 35.8, 32.0, 31.5, 29.2, 26.3, 26.3, 26.1, 25.5, 22.6, 20.1, 18.3, 14.1, 14.1, 14.0, -5.0.

2-Propoxyacetic acid (3.14)

To a solution of α -bromoacetic acid (3.0 g, 21.6 mmol) in *n*-propanol (7.14 g, 119 mmol) at 0 °C was added sodium hydroxide (1.29 g, 46.5 mmol). The reaction mixture was heated to reflux for 12 h. The reaction then was cooled to room temperature and basified using a small portion of aq. sodium hydroxide (1N) prior to removing the *n*-propanol by rotary evaporation. Water was added to the residue and the resultant mixture was washed with ether (3x), cooled to 0 °C and acidified with aq. HCl, and then extracted with EtOAc (3x). The combined EtOAc layer was dried (Na₂SO₄). Removal of the solvents by rotary evaporation gave the corresponding α -(*n*-propoxy)acetic acid (1.3 g, 51% yield); ¹H NMR (400 MHz, CDCl₃) δ 4.11 (s, 2H), 3.50 (t, *J* = 6.8 Hz, 2H), 1.64 (m, 2H), 0.93 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 175.4, 73.6, 67.6, 22.6, 10.2.

1-Propoxypropan-2-one (**3.4**)

To a solution of α -(*n*-propoxy)acetic acid (0.234 g, 1.98 mmol) in dry diethyl ether (2 mL) at $-78\text{ }^{\circ}\text{C}$ was added dropwise a solution of methyl lithium (1.6M in Et_2O , 3.1 mL, 4.94 mmol). The reaction mixture was stirred at $-78\text{ }^{\circ}\text{C}$ for 30 min and then warmed to room temperature. After 4 h, the reaction mixture was cooled to $0\text{ }^{\circ}\text{C}$ and quenched by addition of cold ($0\text{ }^{\circ}\text{C}$) saturated aq. NH_4Cl (3 mL). The quenched mixture was extracted with ether (3x) and the combined organic extract was dried (Na_2SO_4). The solvent was removed by rotary evaporation and the crude product was purified by short path distillation (35 mbar, collect bp $45\text{--}50\text{ }^{\circ}\text{C}$) to afford **3.4** (0.086 g, 37% yield). The spectral characterization of **3.4** was in agreement with published values.¹⁶ ^1H NMR (400 MHz, CDCl_3) δ 4.00 (s, 2H), 3.42 (t, $J = 6.8\text{ Hz}$, 2H), 2.15 (s, 3H), 1.64 (m, 2H), 0.93 (t, $J = 7.2\text{ Hz}$, 3H).

1-Propoxypropan-2-one oxime (**3.8**)

Using the procedure outlined for synthesis of aldoxime **3.5**, *n*-propoxyacetone (**3.4**) (0.086 g, 0.74 mmol) was transformed to ketoxime **3.8** (0.043 g, 44% yield). Purification by SiO₂ column chromatography (hexane:ether, 2:1, v/v; *R_f* = 0.37) gave ketoxime **3.8** as a 1:0.17 mixture of *E*:*Z* isomers. ¹H NMR (400 MHz, CDCl₃) (*E*)-isomer δ 3.98 (s, 2H), 3.35 (t, *J* = 6.8 Hz, 2H), 1.93 (s, 3H), 1.60 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 156.4, 73.1, 72.0, 71.8, 65.6, 22.7, 11.2, 10.5.

1-Propoxypropan-2-one *O*-(*tert*-butyldimethylsilyl) oxime (**3.12**)

Using the procedure outlined for synthesis of silyl aldoxime **3.9**, ketoxime **3.8** (0.051 g, 0.39 mmol) was transformed to silyl ketoxime **3.12** (0.065 g, 68% yield). Purification by SiO₂ column chromatography (hexane:ether, 2:1, v/v; *R_f* = 0.9) gave silyl ketoxime **3.12** as a 1:0.2 mixture of *E*:*Z* isomers. ¹H NMR (400 MHz, CDCl₃) (*E*)-isomer δ 3.99 (s, 2H), 3.33 (t, *J* = 6.4 Hz, 2H), 1.91 (s, 3H), 1.57 (m, 2H), 0.93 (s, 9H), 0.91 (t, *J* = 7.2 Hz, 3H), 0.15 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 159.9, 73.2, 72.2, 72.0, 66.5, 26.2, 26.2, 25.9, 23.0, 23.0, 18.3, 18.2, 16.7, 12.0, 10.7, -2.7, -5.03, -5.07.

Gas Chromatography-Mass Spectrometry Procedures

The GC/TOF-MS analyses were performed on a LECO Pegasus[®] 4D time-of-flight mass spectrometer (LECO Corporation, St. Joseph, MI) equipped with a Gerstel MPS2 auto-sampler (GERSTEL Inc, Linthicum, MD). The Pegasus 4D GC GC/TOF-MS instrument was equipped with an Agilent 6890 gas chromatograph featuring a LECO two-stage cryogenic modulator and a secondary oven. A 30 m x 0.25 mm ¹*d*_c x 0.25 μm ¹*d*_f, DB-225 GC capillary column [(50%-phenyl)-dimethylpolysiloxane] (Agilent Technologies J&W, Santa Clara, CA) was used as the primary column for the GCxGC/TOF-MS analysis. A second column of 2 m x 0.18 μm ²*d*_c x 0.25 μm ²*d*_f, DB-5MS [(5%-cyanopropylphenyl)dimethylpolysiloxane] (Agilent Technologies J&W, Santa Clara, CA) was placed inside the secondary oven after the thermal modulator. The helium carrier gas flow rate was set to 1.0 mL/min at a corrected constant flow via pressure ramps. A 2.0 μL liquid sample was injected into the liner using the split mode (25 : 1), with the injection port temperature set at 250 °C. The primary column temperature was programmed with an initial temperature of 60 °C for 0.5 min and then increased at a rate of 10 °C/ min to 210 °C. The secondary column temperature program was set to an initial temperature of 65 °C for 0.5min and then increased at the same temperature gradient employed in the first column up to a temperature of 215 °C accordingly. The thermal modulator was set to +20 °C relative to the primary oven, and a modulation time of 0s was used, which allowed the instrument to be operated in the GC/TOF-MS mode. The MS mass range was *m/z* = 25–500 with an acquisition rate of 10 spectra per second. The ion source chamber was set at 230 °C with the MS transfer line temperature set to 225 °C, the detector voltage was set at 1600 V, and the electron energy

for ionization was set at 70 eV. The ion acceleration voltage was turned on after a solvent delay of 168s. LECO's ChromaTOF software package (version 4.21) equipped with the National Institute of Standards and Technology (NIST) MS database (NIST MS Search 2.0, NIST/EPA/NIH Mass Spectral Library; NIST 2002) was used for instrument control, spectrum deconvolution and metabolite identification in all experiments. These parameters are baseline offset = 0.5; smoothing = auto; peak width = 1s; signal-to-noise ratio (S/N) = 4; mass threshold = 100; minimum forward similarity match before name is assigned = 600. The peak true spectrum was also exported as part of the information for each peak in absolute format of intensity values.

Quantum Chemical Calculations

Dean Tantillo and his graduate students Osvaldo Gutierrez and Jason Harrison performed all theoretical calculations.

All structures were optimized at the UB3LYP/6-31 G(d) level of theory¹⁷ with the guess = (mix,always) keyword as implemented in the *GAUSSIAN03* suite of programs,¹⁸ unless otherwise noted. Some selected structures were also optimized at the UB3LYP/6-311++G(2d,2p) level of theory to investigate the effect of a larger basis set. Although B3LYP has been used previously, with success, for studies of neutral and radical cation pericyclic reactions,¹⁹ selected structures were also optimized using UM06-2X/6-31 G(d),²⁰ UMP2/6-31 G(d),²¹ and SCS-UMP2/6-31G(d)²² as implemented in *GAUSSIAN09*.²³ All stationary points were characterized as minima or transition state structures by vibrational analysis, and intrinsic reaction coordinate calculations²⁴ were performed for selected transition state structures to confirm their connections to surrounding minima. Structural drawings were produced using Ball & Stick.²⁵

In order for the McLafferty rearrangement to take place, another structural feature, beside the characteristics described in Chapter 3, must be fulfilled: the γ -hydrogen must be properly aligned for the initial H-transfer. In other words, the conformation of the alkyl side-chain must be favorable. For all compounds two conformations are important: R1-*trans* and R2-*gauche* (see Scheme 6.1).

Scheme 6.1. The R1-*trans* and R2-*gauche* equilibrium.

It is clear that only the R2-*gauche* conformation can undergo the McLafferty rearrangement. The results of calculations to determine the relative energies of the twelve compounds investigated are shown in Table 6.1.

Table 6.1. Relative energies (R&UB3LYP/6-31G(d); 298.15 K; kcal/mol) for the species involved in the R1-*trans* and R2-*gauche* equilibrium.

Class	Entry	R	X	Y	Neutral		Odd-electron positive ion		EI (eV)	EI (kcal/mol)
					R1	R2	R1	R2		
Aldehyde/Ketone	1	H	O	CH ₂	0.0	0.4	4.4	0.0	9.1	209.1
	2	CH ₃	O	CH ₂	0.0	0.9	3.0	0.0	8.7	201.5
	3	H	O	O	0.9	0.0	N/A	0.0	8.7	201.6
	4	CH ₃	O	O	1.3	0.0	N/A	0.0	8.4	193.0
Oxime	5	H	NOH	CH ₂	0.0	0.9	2.0	0.0	8.8	201.9
	6	CH ₃	NOH	CH ₂	0.0	1.0	0.0	0.0	8.4	194.2
	7	H	NOH	O	0.6	0.0	0.0	0.6	8.6	198.9
	8	CH ₃	NOH	O	0.6	0.0	0.0	0.6	8.3	191.6
Silyl oxime	9	H	NOSiH ₃	CH ₂	0.0	0.9	0.0	0.0	8.5	197.1
	10	CH ₃	NOSiH ₃	CH ₂	0.0	1.0	0.0	0.4	8.3	190.3
	11	H	NOSiH ₃	O	0.5	0.0	0.0	1.0	8.5	195.4
	12	CH ₃	NOSiH ₃	O	0.6	0.0	0.0	1.5	8.2	188.5

It can be seen that except for compounds **3.1** and **3.2** in the odd-electron positive ions state, the relative energy differences are quite small and that there tends to be a preference for the R1-*trans* conformations in the neutral state and for the R2-*gauche* conformation in the ionized state. For the oxime and silyl oxime ether compounds the preference disappears or even reverses. The reversed R1-*trans* preference when Y = CH₂ can be understood if one takes into account the donation of the lone pair on oxygen to the σ^*_{C-C} bond. If the barrier height between the R1-*trans* and R2-*gauche* conformations is

not too high then at the calculated relative energies conversion between the conformations might take place. However, it is not clear if this will positively, or negatively, influence the extent to which the McLafferty rearrangement is taking place in the odd-electron positive ions of compounds.

inverse McL

carbonyl

oximes

silyl-oximes

B3LYP/6-31G(d) energies (with ZPE) and Gibbs free energies (298.15 K) are of separated products (i.e., neutral carbonyl and radical cation propene) with respect to radical cation substrate.

Figure 6.1. Overview of the McLafferty reverse charge rearrangement thermochemistry for the twelve model compounds **3.1-3.12**.

6.D. Experimental Procedures of Chapter 4

Synthesis

(Dimethylsilanediyl)bis(methylene) dipropionate (4.1)

To a solution of bis(chloromethyl)dimethylsilane **4.0** (1.04 g, 6.63 mmol) in propionic acid (2 mL, 26.7 mmol) at 0 °C was added dropwise triethylamine (5.6 mL, 39.8 mmol). The reaction mixture was stirred at 0 °C for 5 min and then heated to reflux. After 12 h, the reaction mixture was allowed to cool to room temperature. The solution was then diluted by addition of Et₂O (40 mL) and filtered to remove triethylamine salt. The filtrate was washed successively with saturated aq NaHCO₃ and brine (2 x 10 mL) and the organic layer was dried (Na₂SO₄). The solvent was removed by rotary evaporation, and the crude residue was purified by column chromatography (SiO₂, hexane/ethyl acetate, 6:2, v/v; R_f = 0.49) to afford **4.1** (1.39 g, 90%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 3.84 (s, 2H), 2.33 (q, *J* = 7.2 Hz, 2H), 1.14 (t, *J* = 7.2 Hz, 3H), 0.14 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 175.2, 55.4, 27.6, 9.5, -5.8.

(Benzyloxy)(chloromethyl)dimethylsilane (4.3)

To a solution of benzyl alcohol (1.96 g, 18.2 mmol) and imidazole (1.23 g, 18.21 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C, was added dropwise via syringe chloromethyl(dimethylsilylchloride) **4.2** (2.17 g, 15.2 mmol). The reaction was stirred and allowed to come to room temperature. After 2 h stirring at room temperature, the solution was concentrated by rotary evaporation. The residue was diluted in Et_2O (10 mL) and filtered. The filtrate was concentrated by rotary evaporation and the resulting crude was purified by column chromatography (SiO_2 , hexane/ethyl acetate/ Et_2O , 8:1:2, v/v) to afford the desired product **4.3** as clear oil (1.65 g, 51%). ^1H NMR (400 MHz, CDCl_3) δ 7.27-7.18 (m, 5H), 4.71 (s, 2H), 2.72 (s, 2H), 0.21 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 140.4, 128.5, 127.5, 126.7, 65.6, 29.7, -3.3.

((Benzyloxy)dimethylsilyl)methyl propionate (POMS-OBn)

To a solution of CsF (0.3g, 1.97 mmol) in anhydrous DMF (5 mL) under N₂ atmosphere was added via syringe *O*-trimethylsilylpropionic acid (0.315g, 2.15 mmol). The solution was stirred 20 min at room temperature and then chloromethylbenzyl silane **4.3** (0.366 g, 1.7 mmol) was added dropwise via syringe. The resulting solution was heated to 45 °C for 4h and then was allowed to cool to room temperature. The solvents were removed by rotary evaporation and the resulting crude was analyzed by GC-MS and ¹H NMR (400 MHz, CDCl₃) δ 7.35-7.23 (m, 5H), 4.73 (s, 2H), 3.79 (s, 2H), 2.30 (q, J = 8 Hz, 2H), 1.11 (t, J = 8 Hz, 3H), 0.18 (s, 6H).

(1,1,3,3-Tetramethyldisiloxane-1,3-diyl)bis(methylene) dipropionate (4.5)

To a solution of chloromethyldimethylsiloxane (**4.4**) (5.0 g, 21.6 mmol) in propionic acid (4.85 mL, 64.8 mmol) at 0 °C was added dropwise triethylamine (8.8 mL, 62.7 mmol). The reaction mixture was stirred at 0 °C for 5 min and then heated to 100 °C. After 20 h, the reaction mixture was allowed to cool to room temperature. The solution was then diluted by addition of Et₂O (20 mL) and filtered to remove triethylamine salt. The filtrate was washed successively with saturated aq NaHCO₃ and brine (2 x 15 mL) and the organic layer was dried (Na₂SO₄). The solvent was removed by rotary evaporation, and the crude residue was purified by column chromatography (SiO₂, hexane/ethyl acetate, 6:2, v/v) to afford **4.5** (6.16 g, 93% yield) as a clear liquid. ¹H NMR (400 MHz, CDCl₃) δ 3.71 (s, 2H), 2.34 (q, *J* = 7.6 Hz, 2H), 1.14 (t, *J* = 7.6 Hz, 3H), 0.15 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 175.1, 57.5, 27.5, 9.3, -1.0.

(Dimethylsilanediyl)bis(methylene) diacetate (4.12)

To a solution of bis(chloromethyl)dimethylsilane **4.0** (1.02 g, 6.5 mmol) in acetic acid (2.24 mL, 37.2 mmol) at 0 °C was added dropwise triethylamine (8.24 mL, 58.7 mmol). The reaction mixture was stirred at 0 °C for 5 min and then heated to reflux. After 19 h, the reaction mixture was allowed to cool to room temperature. The solution was then diluted by addition of Et₂O (40 mL) and filtered to remove triethylamine salt. The filtrate was washed successively with saturated aq NaHCO₃ and brine (2 x 10 mL) and the organic layer was dried (Na₂SO₄). The solvent was removed by rotary evaporation, and the crude residue was purified by column chromatography (SiO₂, hexane/ethyl acetate, 6:2, v/v; R_f = 0.47) to afford **4.12** (1.33 g, 100%) as a colorless oil, having spectral characteristics in agreement with published data.²⁶ ¹H NMR (400 MHz, CDCl₃) δ 3.84 (s, 2H), 2.04 (s, 3H), 0.144 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 171.9, 55.7, 20.9, -5.7.

(Dimethylsilanediyl)dimethanol (3.13)

To a solution of lithium hydroxide (0.66 g, 15.8 mmol) in MeOH/H₂O (8:3, v/v, 11 mL) at 0 °C was slowly added a solution of the bisacetate **4.12** (0.54 g, 2.6 mmol) in tetrahydrofuran (2 mL). The reaction mixture was stirred at 0 °C for 30 min and then warmed to room temperature. After 4 h, the reaction mixture was extracted with Et₂O (3x) and the combined organic extract was washed with brine (2x) and then dried (Na₂SO₄). The solvents were removed by rotary evaporation and the crude product was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH, 9:1, v/v; R_f = 0.35) to afford **4.13** (0.28 g, 89%) as a colorless oil having spectral characteristics in agreement with published data.²⁶ ¹H NMR (400 MHz, CDCl₃) δ 3.58 (s, 2H), 1.80 (bb, 2H), 0.121 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 55.4, -6.5.

2-(((Hydroxymethyl)dimethylsilyl)methoxy)isoindoline-1,3-dione (4.14)

To a solution of silanediol **4.13** (0.28 g, 2.33 mmol), N-hydroxyphthalimide (0.34 g, 2.1 mmol), and triphenylphosphine (0.55 g, 2.1 mmol) in dry THF (15 mL) under N₂ at 0°C was added dropwise diisopropyl azodicarboxylate (0.5 mL, 2.5 mmol) by syringe. The solution quickly turned a dark yellow-orange. The reaction flask was then allowed to return to room temperature. After 12h, the mixture then was concentrated by rotary evaporation, diluted with EtOAc (60 mL), transferred to a separatory funnel, and washed successively with saturated aq NaHCO₃ and brine (2 x 15 mL). The organic layer was then dried over Na₂SO₄, concentrated by rotary evaporation, and the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂ /ethyl acetate/Et₂O/hexane, 3:3:3:1, v/v; R_f = 0.48) to afford **4.14** (0.49 g, 79%). ¹H NMR (400 MHz, CDCl₃) δ 7.60 (m, 2H), 5.52 (m, 2H), 4.00 (s, 2H), 3.41 (s, 2H), 1.90 (bb, 1H), 0.00 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 163.6, 134.7, 129.17, 123.8, 73.06, 53.9, -6.3.

(((1,3-Dioxoisindolin-2-yl)oxy)methyl)dimethylsilyl)methyl propionate (4.9)

To mono-phthalimide alcohol **4.14** (0.19 g, 0.72 mmol) in dry dichloromethane (5 mL) at room temperature was added propionic acid (0.08 g, 1.1 mmol). After cooling the resulting solution at 0 °C were added *N,N'*-diisopropylcarbodiimide (0.54 mL, 3.5 mmol) and a pinch of DMAP. The reaction was stirred at 0 °C for 10 min, and then allowed to come to room temperature. After being stirred at room temperature for 1h the solution was filtered, and the filtrate was concentrated by rotary evaporation. The crude residue was purified by column chromatography (SiO₂, dichloromethane/hexane/ethyl acetate, 2:2:1, v/v; *R_f* = 0.66) to **4.9** (0.2 g, 90% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.82 (m, 2H), 7.73 (m, 2H), 4.16 (s, 2H), 3.95 (s, 2H), 2.35 (q, *J* = 8 Hz, 2H), 1.15 (t, *J* = 8 Hz, 3H), 0.28 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 175.4, 163.3, 134.5, 129.3, 123.6, 72.4, 55.2, 27.6, 9.5, -5.9.

POMS-ONH₂

(((Aminooxy)methyl)dimethylsilyl)methyl propionate (POMS-ONH₂)

To the propionyloxymethylphthalimide silane **4.9** (0.03 g, 0.1 mmol) in dichloromethane (5 mL) at 0 °C was added hydrazine (23 μ L, 0.5 mmol). After being stirred at 0 °C for 5 min. The reaction flask was then allowed to return to room temperature. After 1h at room temperature, the mixture was filtered and the filtrate was concentrated by rotary evaporation. The crude residue was purified by column chromatography (SiO₂, dichloromethane/methanol, 95:5, v/v to afford **POMS-ONH₂** (17 mg, 92%) as a clear liquid. ¹H NMR (400 MHz, CDCl₃) δ 5.48 (bb, 2H), 3.84 (s, 2H), 3.58 (s, 2H), 2.33 (q, *J* = 8 Hz, 2H), 1.14 (t, *J* = 8 Hz, 3H), 0.11 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ = 175.4, 69.3, 55.8, 27.7, 9.6, -5.7.

(((1,3-Dioxoisindolin-2-yl)oxy)methyl)dimethylsilyl)methyl 2,2-²H₂-propionate (*d*₂-4.9)

Using the same procedure outlined for the synthesis of **4.9**, mono-phthalimide alcohol **4.14** (0.17 g, 0.64 mmol) was reacted with 2,2-²H₃-propionic acid (0.076 g, 1.0 mmol), *N,N'*-diisopropylcarbodiimide (490 μ L, 0.63 mmol) and DMAP (cat.) to afford ***d*₂-4.9** (0.19g, 93% yield); ¹H NMR (400 MHz, CDCl₃) δ 7.82 (m, 2H), 7.73 (m, 2H), 4.16 (s, 2H), 3.95 (s, 2H), 1.13 (s, 3H), 0.28 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 175.5, 163.3, 134.6, 129.3, 123.6, 72.4, 55.2, 9.4, -5.9.

(((Aminooxy)methyl)dimethylsilyl)methyl 2,2-²H₂-propionate (*d*₂-POMS-ONH₂)

Using the same procedure for the synthesis of **POMS-ONH₂**, ester phthalimide ***d*₂-4.9** (0.03 g, 0.09 mmol) was reacted with hydrazine (19 μ L, 0.39 mmol) to afford ***d*₂-POMS-ONH₂** (13 mg, 91% yield); ¹H NMR (400 MHz, CDCl₃) δ 5.48 (bb, 2H), 3.84 (s, 2H), 3.58 (s, 2H), 1.13 (s, 3H), 0.12 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 175.4, 69.3, 55.7, 9.4, -5.7.

(((1,3-Dioxoisindolin-2-yl)oxy)methyl)dimethylsilyl)methyl 2,2,3,3,3-²H₂-propionate (*d*₅-4.9)

Using the same procedure outlined for the synthesis of **4.9**, mono-phthalimide alcohol **4.14** (0.18 g, 0.68 mmol) was reacted with 2,2,3,3,3-²H₃-propionic acid (85 mg, 1.07 mmol), *N,N'*-diisopropylcarbodiimide (516 μ L, 3.33 mmol) and DMAP (cat.) to afford ***d*₅-4.9** (0.19g, 89% yield); ¹H NMR (400 MHz, CDCl₃) δ 7.82 (m, 2H), 7.73 (m, 2H), 4.16 (s, 4H), 3.95 (s, 2H), 0.28 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 175.5, 163.3, 134.6, 129.3, 123.6, 72.4, 55.2, -5.9.

(((aminooxy)methyl)dimethylsilyl)methyl 2,2,3,3,3-²H₂-propionate (*d*₅-POMS-ONH₂)

Using the same procedure for the synthesis of **POMS-ONH₂**, ester phthalimide ***d*₅-4.9** (0.03 g, 0.09 mmol) was reacted with hydrazine (20 μ L, 0.41 mmol) to afford ***d*₅-POMS-ONH₂** (13 mg, 81% yield); ¹H NMR (400 MHz, CDCl₃) δ 5.47 (bb, 2H), 3.84 (s, 2H), 3.58 (s, 2H), 0.12 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 175.4, 69.3, 55.7, -5.7.

4.15

1,3-Dioxoisindolin-2-yl propionate (4.15)

To a solution of *N*-hydroxyphthalimide (0.363 g, 2.22mmol) in CH₂Cl₂ (7 mL), was added propionic acid (0.15 g, 2.0mmol), *N,N'*-Dicyclohexylcarbodiimide (1.25 g, 6.0 mmol) and a pinch of DMAP. After being stirred at room temperature for 1.5 h, the solution was diluted in Et₂O and filtered. The filtrate was concentrated by rotary evaporation and the resulting crude was purified by column chromatography (SiO₂, hexane/ethyl acetate, 6:4, v/v) to afford the desired phthalimide ester **4.15** (0.27 g, 61%).
¹H NMR (500 MHz, CDCl₃) δ 7.88 (m, 2H), 7.79 (m, 2H), 2.70 (q, *J* = 8 Hz, 2H), 1.31 (t, *J* = 8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 170.6, 162.2, 134.9, 129.2, 124.2, 24.7, 8.9.

4.19a

Naphthalen-1-ylmethyl acetate (**4.19a**)

To a solution of 1-naphthalenemethanol (2g, 12.64 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C, was added glacial acetic acid (0.91 g, 15.17 mmol), diisopropylcarbodiimide (2.9 mL, 18.96 mmol) and DMAP (cat.). After stirring for 10 min at 0 °C, the reaction was allowed to come to room temperature. After stirring at room temperature for 1.5h, the reaction was diluted in Et_2O and filtered. The filtrate was concentrated by rotary evaporation and the resulting crude was purified by column chromatography (SiO_2 , hexane:ethyl acetate, 7:2, v/v; R_f = 0.48) to afford ester **4.19a** (2.5 g, 82% yield) as a thick oil. ^1H NMR (400 MHz, CDCl_3) δ 8.02 (d, J = 8.4 Hz, 1H), 7.90-7.85 (m, 2H), 7.58-7.46 (m, 4H), 5.59 (s, 2H), 2.12 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 171.6, 133.9, 131.8, 131.6, 129.5, 128.9, 127.7, 126.7, 126.2, 125.5, 123.7, 64.8, 21.2.

4.19b

naphthalen-1-ylmethyl 2-¹³C-acetate (4.19b)

Using the same procedure outlined for the synthesis of naphthalene acetate **4.19a**, ¹³C-labeled acetic acid (1 g, 16.38 mmol), 1-naphthalenemethanol (3.1 g, 19.65 mmol), diisopropylcarbodiimide (3.8 mL, 24.57 mmol), and DMAP (cat.) were reacted to afford **4.19-b** (3.33 g, 100%). ¹H NMR (700 MHz, CDCl₃) δ 8.02 (d, *J* = 8.4 Hz, 1H), 7.89 (d, *J* = 7.7 Hz, 1H), 7.85 (d, *J* = 7.7 Hz, 1H), 7.58-7.52 (m, 3H), 7.44 (t, *J* = 7.0 Hz, 1H), 5.58 (s, 2H), 2.33 (d, 129.5 Hz, 2H); ¹³C NMR (175 MHz, CDCl₃) δ 133.9, 131.8, 131.6, 129.5, 128.9, 127.7, 126.7, 126.2, 125.5, 123.8, 64.8, 21.3.

4.20a

Naphthalen-1-ylmethyl 3-¹³C-3,3,3-²H-propionate (4.20a)

To a solution of naphthalene acetate **4.19a** (2.88 g, 14.38 mmol) in dry THF (15 mL) under N₂ atmosphere at -78 °C, was added dropwise a 1M solution of lithium bis(trimethylsilyl)amide in THF (13.7 mL, 13.7 mmol). After stirring at -78 °C for 15 minutes, ¹³CD₃-labeled iodomethane was added to the reaction via syringe. The reaction was left at -78 °C for 3h and then was allowed to come to room temperature. After stirring for 3h at room temperature, the reaction was cooled to 0 °C and the reaction was quenched by adding dropwise cold saturated aq. solution of NH₄Cl (15 mL). The resulting solution was then extracted with Et₂O (3 x 20 mL) and the combined organic layers were washed with brine (2 x 10 mL) and dried over Na₂SO₄. The solvents were removed by rotary evaporation and the resulting crude was purified by column chromatography (SiO₂, hexane:ethyl acetate, 9:1, v/v; *R_f*= 0.36) to afford naphthalene propionate ester **4.20-a** (1.4 g, 47%). ¹H NMR (400 MHz, CDCl₃) δ 8.02 (d, *J* = 8.4 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.85 (d, *J* = 7.6 Hz, 1H), 7.58-7.52 (m, 3H), 7.44 (t, *J* = 7.2 Hz, 1H), 5.58 (s, 2H), 2.36 (bs, 2H); ¹³C NMR (175 MHz, CDCl₃) δ 174.3, 133.9, 131.84, 131.81, 129.4, 128.9, 127.6, 126.7, 126.6, 126.1, 125.5, 123.8, 64.7, 27.7-27.5 (m), 8.9-8.3 (m).

4.20b

Naphthalen-1-ylmethyl 2,3-¹³C₂-3,3,3-²H-propionate (4.20b)

Using the same procedure outlined for the synthesis of labeled naphthalene propionate **4.20a**, ¹³C-labeled naphthalene acetate **4.19b** (2.9 g, 14.41 mmol), lithium bis(trimethylsilyl)amide (13.7 mL, 13.7 mmol), and ¹³CD₃-labeled iodomethane were reacted to give the desired labeled naphthalene propionate ester **4.20b** (1.245g, 41%). ¹H NMR (400 MHz, CDCl₃) δ 8.02 (d, *J* = 8.4 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.85 (d, *J* = 7.6 Hz, 1H), 7.58-7.52 (m, 3H), 7.44 (t, *J* = 7.2 Hz, 1H), 5.58 (s, 2H), 2.36 (dd, *J* = 4.2, 128.4 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 133.9, 131.8, 129.4, 128.9, 127.6, 126.7, 126.1, 125.5, 123.78, 64.7, 27.7-27.4 (m), 9.1-7.9 (m).

Propionic acid-33

3-¹³C-3,3,3-²H-propionic acid (Propionic acid-33)

To propionate naphthalene ester **4.20a** (1.4 g, 6.45 mmol) in THF (4 mL), was added a solution of NaOH (1.03 g, 25 mmol) in water/MeOH (2:1, 12 mL). After stirring the reaction for 4h at room temperature, the resulting solution was extracted with Et₂O (4 x 15 mL). The aqueous layer was the concentrated by rotary evaporation to remove all traces of MeOH. The resulting solution was the acidified using concentrated HCl until pH 2, and NaCl was added until saturation. The resulting aqueous solution was then extracted with Et₂O (4 x 15 mL), the organic layers were combined and dried over Na₂SO₄, and the solvent was carefully removed by rotary evaporation. The resulting concentrate containing **Propionic acid-33** was directly used for the synthesis of **4.18-c**.

Propionic acid-34

2,3-¹³C₂-3,3,3-²H-propionic acid (Propionic acid-34)

Using the same procedure outlined for the synthesis of **Propionic acid-33**, labeled naphthalene propionate **4.20b** (1.24 g, 5.67 mmol) in THF (4 mL) and NaOH (0.9 g, 22.7 mmol) in water/MeOH (2:1, 12 mL) were reacted to give labeled **Propionic acid-44**. The product was directly used for the the synthesis of **4.18-d**.

***N*-(2-hydroxyethoxy)phthalimide (4.17).**

To 2-bromoethanol (3.00 g, 24.00 mmol) in DMF (15 mL) at room temperature were added *N*-hydroxyphthalimide (4.30 g, 26.35 mmol) and triethylamine (3.71 mL, 26.40 mmol). The reaction mixture then was stirred at 75 °C for 24 h whereupon the reaction was allowed to cool to room temperature and then diluted by addition of Et₂O (20 mL). The resulting solution was filtered to remove the precipitated triethylamine salt, and the filtrate was concentrated by rotary evaporation. The resulting paste was dissolved in EtOAc (70 mL) and washed successively with saturated aq. NaHCO₃ until the aqueous layer remained clear, and then brine (2 X 10 mL). The organic layer was dried (Na₂SO₄) and the solvent was removed by rotary evaporation to afford phthalimide **4.17** (3.65 g, 73%) as a pale yellow solid, mp. 74-76 °C, (lit. 82-84 °C) having spectral characteristics in agreement with published data.²⁷ ¹H NMR (400 MHz, CDCl₃) δ 7.84 (m, 2H), 7.76 (m, 2H), 4.28 (t, *J* = 4.4 Hz, 2H), 3.79 (t, *J* = 4.4 Hz, 2H), 3.51 (br s, 1H); ¹³C NMR (100 MHz) δ 164.6, 135.0, 128.9, 124.0, 80.1, 59.7.

2-((1,3-Dioxoisindolin-2-yl)oxy)ethyl 3,3,3-²H₃-propionate (4.18-b).

To phthalimide **4.17** (1.48 g, 7.13 mmol) in dichloromethane (8 mL) at room temperature was added 3,3,3-²H₃-propionic acid (0.5 g, 6.48 mmol). After cooling the resulting solution to 0 °C, *N,N'*-diisopropylcarbodiimide (1.5 mL, 9.72 mmol) and DMAP (cat.) were added. The reaction mixture was stirred at 0 °C for 5 min, and then allowed to warm to room temperature. After stirring at room temperature for 1h the reaction mixture was filtered and the filtrate was concentrated by rotary evaporation. The crude residue was purified by column chromatography (SiO₂, dichloromethane:hexane:ethyl acetate, 3:1:1, v/v; *R_f*= 0.62) to afford ester **4.18-b** (1.30 g, 76% yield) as a white solid, mp. 56.5-58.5 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.84 (m, 2H), 7.75 (m, 2H), 4.42 (s, 4H), 2.33 (s, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 174.5, 163.5, 134.8, 129.0, 123.8, 76.0, 61.9, 27.3; FT-ICR-MS (ESI⁺, *m/z*) calcd for C₁₃H₁₁D₃NO₅, [M + H]⁺ 267.1055, found 267.1058.

AEP-32

2-(Aminooxy)ethyl 3,3,3-²H₃-propionate (AEP-32).

To ester **4.18-b** (1.30 g, 4.90 mmol) in dichloromethane (10 mL) at 0 °C was added methylhydrazine (284 mL, 5.38 mmol). After stirring at 0 °C for 45 min, the reaction mixture was filtered and the filtrate was concentrated by rotary evaporation. The crude residue was passed through a short column of SiO₂, eluting with dichloromethane:methanol, 95:5, v/v (*R_f* = 0.47), to afford a light yellow liquid. Kugelrohr distillation of the product (5 mmHg, collect at 80 °C) afforded **AEP-32** (0.56 g, 84% yield) as a clear liquid; ¹H NMR (400 MHz, CDCl₃) δ 5.29 (br. s, 2H), 4.28 (t, *J* = 4.4 Hz, 2H), 3.83 (t, *J* = 4.4 Hz, 2H), 2.33 (s, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 174.8, 73.7, 62.1, 27.4; IR (neat) 3324, 1729, 1590, 1461, 1180, 1080, 1046 cm⁻¹; FT-ICR-MS (ESI⁺, *m/z*) calcd for C₅H₉D₃NO₃, [M + H]⁺ 137.1000, found 137.1000.

2-((1,3-Dioxoisindolin-2-yl)oxy)ethyl 3-¹³C₁-3,3,3-²H₃-propionate (5b).

Using the general procedure outlined for the synthesis of ester **4.18-b**, phthalimide **4.17** (1.07 g, 5.16 mmol) was reacted with 3-¹³C₁-3,3,3-²H₃-propionic acid (0.366 g, 4.69 mmol), *N,N'*-diisopropylcarbodiimide (1.09 mL, 7.03 mmol) and DMAP (cat.) to afford ester **4.18-c** (1.13g, 90% yield); ¹H NMR (400 MHz, CDCl₃) δ 7.83 (m, 2H), 7.75 (m, 2H), 4.42 (s, 4H), 2.33 (d, *J* = 3.6 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 174.5, 163.5, 134.8, 129.0, 123.8, 76.9, 76.1, 61.9, 27.5, 27.1, 9.0-7.8 (m); FT-ICR-MS (ESI⁺, *m/z*) calcd for C₁₂¹³CH₁₁D₃NO₅, [M + H]⁺ 268.1088, found 268.1095.

2-(Aminooxy)ethyl 3-¹³C₁-3,3,3-²H₃-propionate (AEP-33).

Using the general procedure for the synthesis of **AEP-32**, ester **4.18-c** (1.29 g, 4.84 mmol) was reacted with methylhydrazine (280 mL, 5.33 mmol) to afford **AEP-33** (0.514 g, 77% yield); ¹H NMR (400 MHz, CDCl₃) δ 5.52 (br. s, 2H), 4.30 (t, *J* = 4.4 Hz, 2H), 3.84 (t, *J* = 4.4 Hz, 2H), 2.35 (d, *J* = 3.6 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 174.8, 73.7, 62.1, 27.6, 27.3, 9.1-7.9 (m); FT-ICR-MS (ESI⁺, *m/z*) calcd for C₄¹³CH₉D₃NO₃, [M + H]⁺ 138.1034, found 138.1033.

2-((1,3-Dioxoisindolin-2-yl)oxy)ethyl 2,3-¹³C₂-3,3,3-²H₃-propionate (5c).

Using the general procedure outlined for the synthesis of ester **4.18-b**, phthalimide **4.17** (1.03 g, 4.99 mmol) was reacted with 2,3-¹³C₂-3,3,3-²H₃-propionic acid (0.33 g, 4.15 mmol), *N,N'*-diisopropylcarbodiimide (0.96 mL, 6.23 mmol) and DMAP (cat.) to afford ester **4.18-d** (0.96 g, 87% yield); ¹H NMR (400 MHz, CDCl₃) δ 7.83 (m, 2H), 7.75 (m, 2H), 4.42 (s, 4H), 2.33 (dd, *J* = 4.2, 128.4 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 163.5, 134.8, 129.0, 123.8, 76.1, 61.9, 27.8-27.1 (m), 9.1-7.6 (m); FT-ICR-MS (ESI⁺, *m/z*) calcd for C₁₁¹³C₂H₁₀D₃NNaO₅, [M + Na]⁺ 291.0941, found 291.0959.

2-(Aminooxy)ethyl 2,3-¹³C₂-3,3,3-²H₃-propionate (AEP-34).

Using the general procedure for the synthesis of **AEP-32**, ester **4.18-d** (0.95 g, 3.55 mmol) was reacted with methylhydrazine (205 mL, 3.90 mmol) to afford **AEP-34** (0.404 g, 83% yield); ¹H NMR (400 MHz, CDCl₃) δ 5.52 (br. s, 2H), 4.29 (t, *J* = 4.4 Hz, 2H), 3.83 (t, *J* = 4.4 Hz, 2H), 2.35 (dd, *J* = 3.6, 128.4 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 73.7, 62.1, 27.6- 27.3 (m), 9.1-7.9 (m); FT-ICR-MS (ESI⁺, *m/z*) calcd for C₃¹³C₂H₉D₃NO₃, [M + H]⁺ 139.1067, found 139.1069.

2,5-Dioxopyrrolidin-1-yl 3,3,3-²H₃-propionate (NHS-32)

To *N*-hydroxysuccinimide (0.360 g, 3.12 mmol) in dry dichloromethane (8 mL) at room temperature was added 3,3,3-²H₃-propionic acid (0.2 g, 2.26 mmol). After cooling the resulting solution to 0 °C, *N,N'*-diisopropylcarbodiimide (0.490 mL, 3.12 mmol) and DMAP (cat.) were added. The reaction mixture was stirred at 0 °C for 5 min, and then allowed to warm to room temperature. After stirring at room temperature for 1h the reaction mixture was filtered and the filtrate was concentrated by rotary evaporation. The crude residue was purified by column chromatography (SiO₂, dichloromethane:hexane:ethyl acetate, 5:3:2, v/v; *R_f*= 0.47) to afford **NHS-32** (0.408 g, 90% yield) as a white solid, mp. 47.0-47.6 °C; ¹H NMR (400 MHz, CDCl₃) δ 2.83 (bs, 4H), 2.63 (s, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 169.6, 169.4, 25.8, 24.5; IR (neat) 2951, 1817, 1781, 1718, 1381, 1210, 1066, 917, 805, 652 cm⁻¹ FT-ICR-MS (ESI⁺, *m/z*) calcd for C₈H₁₀D₃NNaO₅, [M + MeOH + Na]⁺ 229.0874, found 229.0874.

2,5-Dioxopyrrolidin-1-yl 3-¹³C-3,3,3-²H₃-propionate (NHS-33)

Using the general procedure for the synthesis of **NHS-32**, *N*-hydroxysuccinimide (0.234 g, 2.04 mmol) was reacted with 3-¹³C₁-3,3,3-²H₃-propionic acid (0.1 g, 1.36 mmol) to afford **NHS-33** (0.156 g, 70% yield over 2 steps; saponification of ester **4.20a** followed by coupling of corresponding propionic acid with *N*-hydroxysuccinimide); ¹H NMR (400 MHz, CDCl₃) δ 2.83 (bs, 4H), 2.63 (d, *J* = 4.4 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 169.6, 169.4, 25.8, 24.6, 24.3, 8.7-7.5 (m); FT-ICR-MS (ESI⁺, *m/z*) calcd for C₇¹³CH₁₀D₃NNaO₅, [M + MeOH + Na]⁺ 230.0908, found 230.0908.

2,5-Dioxopyrrolidin-1-yl 2,3-¹³C₂-3,3,3-²H₃-propionate (NHS-34)

Using the general procedure for the synthesis of **NHS-32**, *N*-hydroxysuccinimide (0.234 g, 2.04 mmol) was reacted with 2,3-¹³C₁-3,3,3-²H₃-propionic acid (0.1 g, 1.36 mmol) to afford **NHS-34** (0.137 g, 61% yield over 2 steps; saponification of ester **4.20b** followed by coupling of corresponding propionic acid with *N*-hydroxysuccinimide); ¹H NMR (400 MHz, CDCl₃) δ 2.83 (bs, 4H), 2.63 (dd, *J* = 130 Hz, *J* = 4.4 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 169.6, 169.4, 25.8, 24.6-24.0 (m), 8.7-7.5 (m); FT-ICR-MS (ESI⁺, *m/z*) calcd for C₆¹³C₂H₁₀D₃NNaO, [M + MeOH + Na]⁺ 231.0941, found 231.0941.

Gas Chromatography-Mass Spectrometry Procedures

A. Multiplex Experiment: Proof of Concept

A.1. General Procedure for Sample Mixture Derivatization.

The sample mixtures A, B and C were prepared (see below) and derivatized by treatment with an excess (~4 equiv) of reagents AEP-32, AEP-33, and AEP-34, respectively, to afford the derivatized mixtures A₃₂, B₃₃, and C₃₄. The resultant mixtures A₃₂, B₃₃ and C₃₄ were combined and an aliquot (50 μ L) from the combined mixture was directly injected into the GC-MS instrument for analysis. All GC-MS analyses were performed using an Agilent Technologies GC-MS instrument (Agilent Technologies, Palo Alto, CA); GC component: Agilent 7820A gas chromatograph, MS component: Agilent 5975 Series MSD. The GC-MS instrument was fitted with a HP-5MS chromatographic column 30 m long with an internal diameter of 250 μ m and a stationary-phase film thickness of 0.25 μ m. High-purity helium was used as the carrier gas at a flow rate of 1.0 mL/min. The starting column oven temperature was 60 °C with a ramp of 20 °C/min to a maximum temperature of 315 °C and then held at this temperature for 2 min. The combined mixture of derivatized carbonyl substrates was examined using a 120-s solvent delay. The 1 μ L of sample solution was injected in the split mode of injection at a ratio of 10:1. The inlet temperature was set at 275 °C and the transfer line at 250 °C. The ion MS source was held at 230 °C and the MS Quad at 150 °C. The detector voltage was set at 1200 V, and the electron energy for ionization was set at 70 eV. Mass spectra were collected from 25 to 400 *m/z*. The A-C sample mixtures were prepared, derivatized and then analyzed by GC-MS a total of three times.

A.2. Sample Mixture Preparation

Sample Mixture A. To a solution mixture consisting of hexanal (100 μL of a 0.66 M solution), 1-naphthaldehyde (300 μL of a 0.60 M solution), 2-heptanone (100 μL of a 0.84 M solution), 1-methyl-4-piperidone (500 μL of a 0.60 M solution), tetrahydro-4H-pyran-4-one (200 μL of a 0.58 M solution), and 2-indanone (500 μL of a 0.40 M solution), total volume 1.7 mL, was added dichloromethane (300 μL) to generate a sample mixture A (2.0 mL).

Derivatized Mixture A₃₂. To a 100 μL aliquot of sample mixture A at room temperature was added AEP-32 (23 μL). After incubation at room temperature 48h, the dichloromethane was removed by rotary evaporation and the resultant mixture of oxime ethers was redissolved in dichloromethane (1.0 mL) to generate derivatized mixture A₃₂.

Sample Mixture B. To a solution mixture consisting of hexanal (100 μL of a 0.66 M solution), 1-naphthaldehyde (100 μL of a 0.60 M solution), 2-heptanone (300 μL of a 0.84 M solution), 1-methyl-4-piperidone (500 μL of a 0.60 M solution), tetrahydro-4H-pyran-4-one (100 μL of a 0.58 M solution), and 2-indanone (300 μL of a 0.40 M solution), total volume 1.4 mL, was added dichloromethane (600 μL) to generate a sample mixture B (2.0 mL).

Derivatized Mixture B₃₃. To a 100 μL aliquot of sample mixture B at room temperature was added AEP-33 (21 μL). After incubation at room temperature 48h, the

dichloromethane was removed by rotary evaporation and the resultant mixture of oxime ethers was redissolved in dichloromethane (1.0 mL) to generate derivatized mixture B₃₃.

Sample Mixture C. To a solution mixture consisting of hexanal (100 μ L of a 0.66 M solution), 1-naphthaldehyde (100 μ L of a 0.60 M solution), 2-heptanone (100 μ L of a 0.84 M solution), 1-methyl-4-piperidone (100 μ L of a 0.60 M solution), tetrahydro-4H-pyran-4-one (200 μ L of a 0.58 M solution), and 2-indanone (100 μ L of a 0.40 M solution), total volume 0.7 mL, was added dichloromethane (1.3 mL) to generate a sample mixture C (2.0 mL).

Derivatized Mixture C₃₄. To a 100 μ L aliquot of sample mixture C at room temperature was added AEP-34 (11 μ L). After incubation at room temperature 48h, the dichloromethane was removed by rotary evaporation and the resultant mixture of oxime ethers was redissolved in dichloromethane (1.0 mL) to generate derivatized mixture C₃₄.

A.3. Ion Count Averages and Accuracy Determination

Table 6.2 summarizes the averages, calculated from three separate experiments, for the MST ion counts, accuracy (% error) and the standard deviation. Total average error was calculated at $4.9\% \pm 0.3\%$.

Table 6.2. Summary of the calculated accuracy (% error) and the standard deviation for the MSTs.

Carbonyl Substrate	MST (<i>m/z</i>)	Actual A,B,C Ratio	Accuracy (% error)		Normalized average ion count		Standard deviation	
			<i>Z</i>	<i>E</i>	<i>Z</i>	<i>E</i>	<i>Z</i>	<i>E</i>
hexanal	32	1	4.16	2.47	10.85	9.34	0.55	0.45
	33	1	3.52	5.23	10.05	8.65	0.14	0.13
	34	1	1.18	2.96	10.35	9.4	0.35	0.31
1-naphth- aldehyde	32	3	3.82	4.46	10.74	8.16	0.47	0.26
	33	1	4.42	4	3.83	2.95	0.25	0.11
	34	1	8.54	9.72	4.04	3.12	0.21	0.15
2-heptanone	32	1	6.24	4.82	3.05	2.44	0.45	0.43
	33	3	4.05	3.7	8.24	6.69	0.65	0.74
	34	1	5.9	6.29	3.04	2.47	0.39	0.41
tetrahydro-4H- pyran-4-one	32	2	3		13.09		0.98	
	33	1	4.3		6.87		0.18	
	34	2	3.45		12.96		0.15	
1-methyl-4- piperidone	32	5	3.16		11.91		0.28	
	33	5	5.21		10.98		0.34	
	34	1	11.95		2.59		0.1	
2-indanone	32	5	2.34		5.47		0.21	
	33	3	2.89		3.26		0.1	
	34	1	9.67		1.21		0.05	

A.4. Representative calculation of accuracy for 2-indanone, run 1.

The ratio of AEP–2-indanone in the combined mixture A₃₂:B₃₃:C₃₄ (run 1) was 5:3:1. Thus, the expected MST ratio at *m/z* 32, 33, and 34 is 5:3:1, respectively. The 32-34 MST intensities were measured from the mass spectrum generated by the integration of the GC signal for the AEP–2-indanone adduct and found to be 5.66, 3.17, and 1.17 ion

counts (parent fragment ion normalized to 100), respectively. Summing the total ion counts for the three MSTs gives 10.00, and dividing this value by 9 (sum of the total 2-indanone adduct ratio, 5:3:1) gives 1.11, which represents the unit ion count of one MST for 2-indanone. From this we can calculate the full, expected intensities for each MST: 5 = 5.55: 3 = 3.33: 1 = 1.11. Comparing the measured intensities to the expected intensities gives the error ($|\text{measured} - \text{expected}| / \text{expected}$): MST m/z 32 gave 5.66 ion counts = 1.98% error; MST m/z 33 gave 3.17 ion counts = 4.80% error; MST m/z 34 gave 1.17 ion counts = 5.40% error. This procedure was repeated for the second and third runs, and the three values obtained for each MST then were averaged to provide the value given in the table above.

B. Turmeric Extract Profiling and Absolute Quantification

B.1. Derivatization Procedure

Dr. Xiang Zhang and his graduate student Xue Shi performed the sample preparation and the GCxGC-MS injections in this section.

Turmeric extract (steam distilled curcuma essential oil) from the roots of *Curcuma Longa* (India) was purchased from New Directions Aromatics Inc. (San Ramon, CA). Curcuma oil (10 μ L) was dissolved in acetonitrile (90 μ L). Equimolar quantities of AEP-33 reagent and AEP-34 reagent (2 mgs each) were dissolved in acetonitrile (100 μ L), and the mixture then was added to the curcuma oil solution. The resultant AEP reagent – curcuma oil mixture was stirred at 40 °C for 1 hour.

Analyses of the derivatized mixture were performed using a Pegasus 4D GCxGC/TOF-MS instrument (LECO Corporation, St. Joseph, MI) equipped with an Agilent 6890 gas chromatograph featuring a two-stage cryogenic modulator and secondary oven. A 60 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m (film thicknesses) DB-5ms GC capillary column ((5%-phenyl)-dimethylpolysiloxane, Agilent Technologies J&W) was used as the primary column for the GCxGC/TOF-MS analysis. A second GC column of 1 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness, DB-17ms ((50%-Phenyl)-methylpolysiloxane, Agilent Technologies J&W) was placed inside the secondary GC oven after the thermal modulator. The helium carrier gas flow rate was set to 2 mL/min at a corrected constant flow via pressure ramps. The inlet temperature was set at 280 °C. The primary column temperature was programmed with an initial temperature of 60 °C for 0.5 min and then ramped at 5 °C/min to 270 °C to keep 12 min. The secondary column temperature program was set to an initial temperature of 80 °C for 0.5 min and

then also ramped at the same temperature gradient employed in the first column to 290 °C accordingly. The thermal modulator was set to +20 °C relative to the primary oven, and a modulation time of 2 s was used. The hot pulse time is 0.4 s. The mass range was 25–800 m/z with an acquisition rate of 200 spectra per second. The ion source chamber was set at 230 °C with the MS transfer line temperature set to 280 °C, and the detector voltage was 1450 V with electron energy of 70 eV. The acceleration voltage was turned on after a solvent delay of 325 s. 2 μ L samples were injected into the system with a split ratio of 50:1. The GC $\times$ GC–TOF MS data were processed using LECO's instrument control software ChromaTOF for peak picking.

B.2. Absolute Quantification

Turmeric extract (steam distilled curcuma essential oil) from the roots of *Curcuma Longa* (India) was purchased from New Directions Aromatics Inc. (San Ramon, CA). Curcuma oil (10 μ L) was dissolved in acetonitrile (90 μ L). 2 mg of AEP-33 reagent were dissolved in 100 μ L of acetonitrile and added to the dissolved curcuma oil. Derivatization was carried out at 40 °C for 1 hour and followed by storing at 5 °C for 72 hours.

AEP-34 adduct of 2-nonanone (*Z*-isomer) and AEP-34 adduct of 2-undecanone (*Z*-isomer) were used as standards for the quantification experiments. Solutions of these two standards were formulated in acetonitrile at 40.6 μ g/mL (155 nmol/mL) and 16 μ g/mL (55 nmol/mL), respectively. The derivatized curcuma oil (30 μ L) was mixed with the AEP-34 adduct of 2-nonanone (*Z*-isomer, 7.5 μ L of a 40.6 μ g/mL (155 nmol/mL) solution) and the AEP-34 adduct of 2-undecanone (*Z*-isomer, 7.5 μ L of a 16 μ g/mL (55 nmol/mL) solution). Three separate ‘spike-in’ experiments were performed, and then each was analyzed by GCxGC-TOF-MS.

Table 6.3 summarizes the averages, calculated from three separate experiments, for the ratios of the reporter MSTs, the average concentration for each isomer of the carbonyl substrates and the total concentration of the carbonyl substrates in the sample. The table shows the chromatogram peak area ratio of the *Z*- and *E*-isomers in the turmeric extract derivatized with AEP-33 before spike-in with the AEP-34 modified *Z*-isomers.

The total concentration of the AEP-33 adducts in the extract were found to be $436.0 \pm 26 \mu\text{g/mL}$ ($1.67 \pm 0.1 \mu\text{mol/mL}$) and $536 \pm 54 \mu\text{g/mL}$ ($1.85 \pm 0.2 \mu\text{mol/mL}$) respectively. These values correspond to $237 \pm 14 \mu\text{g/mL}$ of 2-nonanone in the extract and $315 \pm 32 \mu\text{g/mL}$, respectively.

Table 6.3. Summary of the calculated concentrations of the AEP-adducts of 2-nonanone and 2-undecanone in the turmeric extract.

Carbonyl substrate AEP-adduct	Chromatogram isomer ratio before spike-in		Average MST ratio in spike-in (<i>Z-isomer</i>)		Normalized average ion counts		Standard deviation		Determined injection concentration ($\mu\text{g/mL}$)		Determined injection concentration ($\mu\text{g/mL}$)	Total concentration in the extract ($\mu\text{g/mL}$)
	<i>Z</i>	<i>E</i>	33	34	33	34	33	34	<i>Z</i>	<i>E</i>		
2-nonanone	1	2.07	1.05	1	18.4	17.5	1.3	0.9	7.1 (± 0.4)	14.7 (± 0.9)	21.8 (± 1.3)	436.0 (± 26)
2-undecanone	1	1.63	3.55	1	13.5	3.8	0.5	1.3	10.2 (± 1)	16.6 (± 1.7)	26.8 (± 2.7)	536.0 (± 54)

6.E. Experimental Procedures of Chapter 5

Synthesis

14-Bromo-tetradecan-1-ol (5.1b)

To a solution of 14-bromotetradecanoic acid (1 g, 2.98 mmol) in dry THF (20 mL) at -15 °C under N₂, a 1M solution of BH₃ in THF (7.75 mL) was added via syringe. The reaction was stirred at 0 °C for 2 hours, and then at room temperature for 4 hours. The crude was slowly poured in a cooled (0 °C) saturated aq NaHCO₃ solution (10 mL) and stirred for 15 min. The resulting solution was then extracted with Et₂O (5 x 20 mL). The organic layers were combined and washed with brine (2 x 10 mL) and then dried over Na₂SO₄ and concentrated by rotary evaporation. The crude residue was purified by column chromatography (SiO₂, CH₂Cl₂) to afford the desired product as a clear oil (0.9 g, 95% yield) having spectral characteristics in agreement with published data.²⁸ ¹H NMR (500 MHz, CDCl₃) δ 3.63 (q, *J* = 6.5 Hz, 2H), 3.40 (t, *J* = 7 Hz, 2H), 1.86 (qn, *J* = 7.5 Hz, 2H), 1.55 (qn, *J* = 7 Hz, 2H), 1.44-1.20 (m, 24H).

12-Tritylsulfanyl-dodecan-1-ol (**5.2a**)

To a solution of 12-bromo-dodecan-1-ol (**5.1a**) (1.0 g, 3.78 mmol) in MeOH (40 mL) at room temperature was added potassium carbonate (1.04 g, 7.56 mmol). After stirring 5 minutes, triphenylmethanethiol (1.36 g, 4.90 mmol) was added, and the reaction was stirred at room temperature for 16 hours. The reaction solution was then diluted with diethyl ether (100 mL), transferred to a separatory funnel, and neutralized with a Na₂HPO₄/NaH₂PO₄ buffer (10 mL). The buffer was removed, and the organic layer was then washed with NaCl brine (3 × 75 mL). The aqueous layers were combined and extracted with diethyl ether (25 mL), and the organic layers were combined, dried over Na₂SO₄ and concentrated by rotary evaporation. The crude residue was purified by flash chromatography (12 g SiO₂; CH₂Cl₂:MeOH (v:v) 100:0 → 95:5) to afford the desired product **5.2a** (1.71 g, 99% yield) as a white solid having spectral characteristics in agreement with published data.²⁹ ¹H NMR (500 MHz, CDCl₃) δ 7.34 (d, *J* = 7.5 Hz, 6H), 7.20 (t, *J* = 7.5 Hz, 6H), 7.13 (t, *J* = 7.4 Hz, 3H), 3.56 (t, *J* = 4.5 Hz, 2H), 2.06 (t, *J* = 7.5 Hz, 2H), 1.49 (qn, *J* = 7 Hz, 2H), 1.02-1.35 (m, 18H).

1-Bromo-12-tritylsulfanyl-dodecane (**5.9a**)

To an evacuated and N₂ purged flask containing **5.2a** (0.900 g, 1.95 mmol), sodium bicarbonate (0.164 g, 1.95 mmol), and a stir bar, dry CH₂Cl₂ (30 mL) was added by syringe. In separate flasks, carbon tetrabromide (1.618 g, 4.88 mmol) and triphenylphosphine (1.330 g, 5.07 mmol) were each purged with N₂, and minimal dry CH₂Cl₂ was added by syringe to dissolve each solid. Both the carbon tetrabromide and triphenylphosphine were cannulated into the reaction flask, and the solution was stirred for 12 hours. The resulting dark red solution was diluted with Et₂O and washed with NaCl brine (3 x 50 mL). The aqueous washes were combined and extracted with Et₂O (50 mL), and all organic extracts were combined and washed once more with NaCl brine (100 mL). The organic solution was dried over Na₂SO₄, concentrated by rotary evaporation, and purified by flash column chromatography (12 g SiO₂; CH₂Cl₂, 100 mL) to give the desired product **5.9a** (0.989 g, 96% yield) as a white solid having spectral characteristics in agreement with published data.³⁰ ¹H NMR (500 MHz, CDCl₃) δ 7.34 (d, *J* = 7.5 Hz, 6H), 7.20 (t, *J* = 7.5 Hz, 6H), 7.13 (t, *J* = 7.4 Hz, 3H), 3.33 (t, *J* = 7 Hz, 2H), 2.06 (t, *J* = 7 Hz, 2H), 1.78 (qn, *J* = 7.5 Hz, 2H), 1.27-1.38 (m, 4H), 1.02-1.26 (m, 14H).

14-Tritylsulfanyl-tetradecan-1-ol (5.2b).

Using the same procedure outlined for the synthesis of **5.2a**, 14-bromo-dodecan-1-ol (**5.1b**) (0.9 g, 2.82 mmol), potassium carbonate (1.17 g, 8.45 mmol), and triphenylmethanethiol (1.32 g, 4.79 mmol) were reacted to afford the desired product **5.2b** as a clear oil (1.3 g, 89.7% yield) having spectral characteristics in agreement with published data.³¹ ¹H NMR (500 MHz, CDCl₃) δ 7.41 (d, J = 7.5 Hz, 6H), 7.25 (t, J = 7.5 Hz, 6H), 7.20 (t, J = 7.4 Hz, 3H), 3.62 (q, J = 6.5 Hz, 2H), 2.12 (t, J = 7 Hz, 2H), 1.54 (qn, J = 7 Hz, 2H), 1.41-1.14 (m, 26H).

1-iodo-12-tritylsulfanyl-tetradecane (**5.9b'**)

To an evacuated and N₂ purged flask containing triphenylphosphine (0.22 g, 0.84 mmol), I₂ (0.213 g, 0.84 mmol), and a stir bar, dry tetrahydrofuran (20 mL) was added by syringe. To the resulting solution was added imidazole (0.076 g, 1.12 mmol) in dry tetrahydrofuran (3 mL) and stirred for 5 min. A solution of alcohol **5.2b** (0.29 g, 0.56 mmol) in minimal tetrahydrofuran was finally added via cannula. After 12 h at room temperature, the solution was diluted with Et₂O (100 mL) and washed with saturated aq. NaHCO₃ and brine (3 x 10 mL). The organic solution was dried over Na₂SO₄, concentrated by rotary evaporation, and purified by flash column chromatography (12 g SiO₂; CH₂Cl₂, 100 mL) to give the desired product **5.9b'** (0.31 g, 88% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ 7.35 (d, *J* = 7.5 Hz, 6H), 7.22 (t, *J* = 7.5 Hz, 6H), 7.14 (t, *J* = 7.4 Hz, 3H), 3.13 (t, *J* = 7.5 Hz, 2H), 2.07 (t, *J* = 7.5 Hz, 2H), 1.77 (qn, *J* = 7.5 Hz, 2H), 1.33-1.28 (m, 4H), 1.28-1.0 (m, 24); ¹³C NMR (125 MHz, CDCl₃) δ 145.4, 129.9, 128.2, 128.1, 126.8, 66.6, 33.9, 32.3, 30.8, 29.95, 29.9, 29.8, 29.7, 29.5, 29.3, 28.9, 28.8, 7.6.

1,1,1-triphenyl-15,18,21,24-tetraoxa-2-thiahexacosan-26-ol (5.10a-PEG(4))

To a suspension of sodium hydride (0.292 g, 12.17 mmol) in DMF (10 mL) at 0°C under N₂, a solution of vacuum-dried tetra(ethylene glycol) (1.201 g, 6.18 mmol) in anhydrous DMF (20 mL) under N₂ was transferred by cannula, and the reaction flask was purged with N₂. After 5 minutes, the solution turned a pale yellow color. Then **5.9a** (0.54 g, 1.03 mmol) in anhydrous DMF (25 mL) under N₂ was transferred to the reaction flask in the same manner. The reaction was allowed to come to room temperature, kept under N₂ atmosphere, and stirred for 72 hours. The resulting dark yellow solution was then dissolved in Et₂O (75 mL), transferred to a separatory funnel, and washed with NaCl brine (75 mL) mixed with RO water (5 mL). Aqueous layers were combined and extracted with Et₂O (50 mL), and then organic layers were combined and washed once more with NaCl brine (50 mL). The final organic extract was then dried over Na₂SO₄ and concentrated by rotary evaporation. The crude residue was purified by flash column chromatography (12 g SiO₂; CH₂Cl₂:MeOH (v:v) 100:0 → 90:10) to give the desired product as a yellow oil (0.268g, 41% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.40 (d, *J* = 7.5 Hz, 6H), 7.27 (t, *J* = 7.5 Hz, 6H), 7.19 (t, *J* = 7.4 Hz, 3H), 3.72 (t, *J* = 3 Hz, 2H), 3.56-3.69 (m, 12H), 3.44 (t, *J* = 7 Hz, 2H), 2.13 (t, *J* = 7.5 Hz, 2H), 1.57 (qn, *J* = 7 Hz, 2H), 1.38 (qn, *J* = 7.5 Hz, 2H), 1.10-1.33 (m, 18H); ¹³C NMR (125 MHz, CDCl₃) δ 145.33, 129.85, 128.02, 126.72, 72.73, 71.780, 70.88, 70.85, 70.631, 70.30, 62.03, 32.28, 29.87, 29.83, 29.81, 29.78, 29.73, 29.65, 29.43, 29.26, 28.84, 26.33, 21.37, 21.32; FTMS calcd for C₃₉H₅₆O₅S [M + Na] 659.3746, found 659.3744.

5.10a-PEG(6)

1,1,1-triphenyl-15,18,21,24,27,30-hexaoxa-2-thiadotriacontan-32-ol (5.10a-PEG(6))

Using the same procedure outlined for the synthesis of **5.10a-PEG(4)**, sodium hydride (0.178 g, 7.43 mmol), hexa(ethylene glycol) (1.07 g, 3.81 mmol), and **5.9a** (0.330 g, 0.632 mmol) were reacted in to give the desired product **5.10a** as a yellow oil (0.184g, 46% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.34 (d, $J = 7.5$ Hz, 6H), 7.20 (t, $J = 7.5$ Hz, 6H), 7.13 (t, $J = 7.4$ Hz, 3H), 3.72 (t, $J = 4$ Hz, 2H), 3.56-3.69 (m, 18H), 3.44 (t, $J = 7$ Hz, 2H), 2.13 (t, $J = 7.5$ Hz, 2H), 1.57 (qn, $J = 7$ Hz, 2H), 1.38 (qn, $J = 7.5$ Hz, 2H), 1.11-1.34 (m, 18H); ^{13}C NMR (125 MHz, CDCl_3) δ 145.34, 129.85, 128.02, 126.72, 72.73, 71.80, 70.88, 70.84, 70.63, 70.30, 62.02, 32.27, 29.89, 29.82, 29.81, 29.78, 29.74, 29.66, 29.43, 29.26, 28.84, 26.34, 21.43; FTMS calcd for $\text{C}_{43}\text{H}_{64}\text{O}_7\text{S}$ [$\text{M} + \text{Na}$] 747.4271, found 747.4263.

5.10a-PEG(8)

1,1,1-triphenyl-15,18,21,24,27,30,33,36-octaoxa-2-thiaoctatriacontan-38-ol

(5.10a-PEG(8))

Using the same procedure outlined for the synthesis of **5.10a-PEG(4)**, sodium hydride (0.14 g, 5.88 mmol), octa(ethylene glycol) (1.12 g, 3.02 mmol), and **5.9a** (0.42 g, 0.8 mmol) were reacted in to give the desired product **5.10a-PEG(8)** as a yellow oil (0.31g, 48% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.43 (d, $J = 7.5$ Hz, 6H), 7.30 (t, $J = 7.5$ Hz, 6H), 7.23 (t, $J = 7.4$ Hz, 3H), 3.73 (t, 2H), 3.64-3.58 (m, 30H), 3.47 (t, 2H), 2.16 (t, 2H), 1.58 (qn, 2H), 1.41 (qn, 2H), 1.27-1.22 (m, 16H); ^{13}C NMR (125 MHz, CDCl_3) δ 145.1, 129.6, 127.8, 126.5, 72.5, 71.5, 70.65, 70.6, 70.3, 70.0, 66.3, 61.7, 32.0, 29.65, 29.6, 29.5, 29.4, 29.2, 29.0, 28.6, 26.1; FTMS calculated for $\text{C}_{47}\text{H}_{72}\text{NaO}_9\text{S}$ $[\text{M} + \text{Na}^+]$ 835.4789, found 835.4774.

1,1,1-triphenyl-19,22,25,28-tetraoxa-2-thiatriacontan-30-ol (5.10b-PEG(4))

Using the same procedure outlined for the synthesis of **5.10a-PEG(4)**, sodium hydride (0.38 g, 15.7 mmol), tetra(ethylene glycol) (1.6 g, 8.22 mmol), and **5.9b'** (0.330 g, 0.632 mmol) were reacted in to give the desired product **5.10b-PEG(4)** as a yellow oil (0.212 g, 24%). ^1H NMR (500 MHz, CDCl_3) δ 7.43 (d, $J = 7.5$ Hz, 6H), 7.30 (t, $J = 7.5$ Hz, 6H), 7.23 (t, $J = 7.4$ Hz, 3H), 3.75 (t, 2H), 3.68-3.59 (m, 14H), 3.47 (t, $J = 6.5$ Hz, 2H), 2.16 (t, $J = 7.5$ Hz, 2H), 1.59 (qn, $J = 7$ Hz, 2H), 1.41 (qn, 2H), 1.38-1.10 (m, 26H).

1,1,1-triphenyl-19,22,25,28,31,34-hexaoxa-2-thiahexatriacontan-36-ol

(5.10b-PEG(6))

Using the same procedure outlined for the synthesis of **5.10a-PEG(4)**, sodium hydride (78 mg, 3.27 mmol), hexa(ethylene glycol) (0.52 g, 1.8 mmol), and **5.9b** (0.2 g, 0.35 mmol) were reacted in to give the desired product **5.10b-PEG(6)** as a yellow oil (0.123 g, 38%). ^1H NMR (500 MHz, CDCl_3) δ 7.41 (d, $J = 7.5$ Hz, 6H), 7.28 (t, $J = 7.5$ Hz, 6H), 7.21 (t, $J = 7.4$ Hz, 3H), 3.71 (t, $J = 4$ Hz, 2H), 3.65-3.57 (m, 22H), 3.45 (t, $J = 7$ Hz, 2H), 2.14 (t, $J = 7.5$ Hz, 2H), 1.56 (qn, $J = 7$ Hz, 2H), 1.37 (m, 2H), 1.37-1.10 (m, 26H); ^{13}C NMR (125 MHz, CDCl_3): 145.1, 129.6, 127.8, 126.5, 72.5, 71.5, 70.6, 70.5, 70.0, 61.7, 32.0, 29.65, 29.6, 29.55, 29.5, 29.4, 29.2, 29.0, 28.6, 26.1.

1,1,1-triphenyl-19,22,25,28,31,34,37,40-octaoxa-2-thiadotetracontan-42-ol

(5.10b-PEG(8))

Using the same procedure outlined for the synthesis of **5.10a-PEG(4)**, sodium hydride (0.13 g, 5.52 mmol), octa(ethylene glycol) (1.02 g, 2.76 mmol), and **5.9b** (0.4 g, 0.69 mmol) were reacted in to give the desired product **5.10b-PEG(8)** as a yellow oil (0.29 g, 48%). ^1H NMR (400 MHz, CDCl_3) δ 7.40 (d, $J = 8.0$ Hz, 5H), 7.28-7.24 (m, 7H), 7.13 (t, $J = 7.4$ Hz, 3H), 3.72 (t, $J = 4$ Hz, 2H), 3.64-3.54 (m, 30H), 3.44 (t, $J = 7$ Hz, 2H), 2.13 (t, $J = 7.5$ Hz, 2H), 1.57 (qn, $J = 7$ Hz, 2H), 1.39 (qn, $J = 7.5$ Hz, 2H), 1.33-1.10 (m, 24H); ^{13}C NMR (100 MHz, CDCl_3): 145.1, 129.6, 127.8, 127.5, 72.55, 71.5, 70.65, 70.6, 70.3, 70.0, 66.3, 61.7, 32.0, 29.7, 29.6, 29.55, 29.5, 29.4, 29.2, 29.0, 28.6, 26.1; FTMS calculated for $\text{C}_{51}\text{H}_{80}\text{NaO}_9\text{S}$ [$\text{M} + \text{Na}^+$] 891.5415, found 891.5447.

2-[2-(2-{2-[2-(12-Tritylsulfanyl-dodecyloxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethoxy]-isoindole-1,3-dione (5.11a-PEG(4)).

To a solution of product **5.10a-PEG(4)** (0.220 g, 0.345 mmol) in dry THF (5 mL) under N₂ at 0°C were added N-hydroxyphthalimide (0.068 g, 0.414 mmol) and triphenylphosphine (0.109 g, 0.414 mmol). Over the next 5-10 minutes, diisopropyl azodicarboxylate (0.082 mL, 0.414 mmol) was added dropwise by syringe. The solution quickly turned a dark yellow-orange. The reaction flask was then allowed to return to room temperature and stirred overnight to yield an opaque golden solution. The mixture then was concentrated by rotary evaporation, diluted with EtOAc (100 mL), transferred to a separatory funnel, and washed with NaCl brine (3 × 75 mL). The organic layer was then dried over Na₂SO₄, concentrated by rotary evaporation, then concentrated further by vacuum pump. The crude residue was purified by flash column chromatography (12 g SiO₂; Hexane:EtOAc (v:v) 90:10 → 0:100) to give the desired product **5.11a-PEG(4)** as a bright yellow oil (0.241 g, 74% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.84 (dd, *J* = 5.5, 3 Hz, 2H), 7.74 (dd, *J* = 5.5, 3 Hz, 2H), 7.40 (d, *J* = 7.5 Hz, 6H), 7.27 (t, *J* = 7.5 Hz, 6H), 7.20 (t, *J* = 7.4 Hz, 3H), 4.38 (t, *J* = 4.5 Hz, 2H), 3.87 (t, *J* = 4.5 Hz, 2H), 3.67 (t, *J* = 4.5 Hz, 2H), 3.55-3.65 (m, 10H), 3.43 (t, *J* = 7 Hz, 2H), 2.13 (t, *J* = 7.5 Hz, 2H), 1.56 (qn, *J* = 7 Hz, 2H), 1.38 (qn, *J* = 7 Hz, 2H), 1.10-1.35 (m, 16H); ¹³C NMR (125 MHz, CDCl₃) δ 171.10, 134.64, 129.84, 128.01, 126.72, 123.72, 109.99, 71.79, 71.02, 70.837, 70.79, 70.76, 70.73, 70.27, 69.54, 65.48, 29.77, 29.65, 29.25, 28.83, 23.14, 23.11, 23.09.

2-((1,1,1-triphenyl-15,18,21,24,27,30-hexaoxa-2-thiadotriacontan-32-yl)oxy)isoindoline-1,3-dione (5.11a-PEG(6)).

Using the same procedure outlined for the synthesis of **5.11a-PEG(4)**, thiol-PEG diblock chain **5.10a-PEG(6)** (0.100 g, 0.138 mmol), N-hydroxyphthalimide (0.027 g, 0.166 mmol) and triphenylphosphine (0.044 g, 0.166 mmol) were reacted in presence of diisopropyl azodicarboxylate (0.032 mL, 0.166 mmol) to give the desired product **5.11a-PEG(6)** as a bright yellow oil (0.091g, 75% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.84 (dd, $J = 5.5$, 3 Hz, 2H), 7.75 (dd, $J = 5.5$, 3 Hz, 2H), 7.40 (d, $J = 7.5$ Hz, 6H), 7.27 (t, $J = 7.5$ Hz, 6H), 7.20 (t, $J = 7.4$ Hz, 3H), 4.38 (t, $J = 4.5$ Hz, 2H), 3.86 (t, $J = 4.5$ Hz, 2H), 3.55-3.68 (m, 20H (plus H_2O impurities)), 3.44 (t, $J = 7$ Hz, 2H), 2.38 (t, $J = 7.5$ Hz, 2H), 2.13 (t, $J = 7.5$ Hz, 2H), 1.56 (qn, $J = 7$ Hz, 2H), 1.38 (qn, $J = 7$ Hz, 2H), 1.10-1.35 (m, 14H); ^{13}C NMR (125 MHz, CDCl_3) δ 154.49, 134.68, 129.95, 129.92, 129.84, 128.23, 128.213, 128.18, 128.11, 128.02, 126.72, 71.17, 70.93, 70.86, 70.83, 64.76, 64.68, 29.89, 29.837, 29.81, 29.65, 22.39, 22.31.

2-((1,1,1-triphenyl-15,18,21,24,27,30,33,36-octaoxa-2-thiaoctatriacontan-38-yl)oxy)isoindoline-1,3-dione (5.11a-PEG(8))

Using the same procedure outlined for the synthesis of **5.11a-PEG(4)**, thiol-PEG diblock chain **5.10a-PEG(8)** (0.29 g, 0.36 mmol), N-hydroxyphthalimide (0.077 g, 0.47 mmol) and triphenylphosphine (0.116 g, 0.44 mmol) were reacted in presence of diisopropyl azodicarboxylate (0.1 mL, 0.5 mmol) to give the desired product **5.11a-PEG(8)** as a bright yellow oil (0.33g, 96% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.79 (dd, 2H), 7.72 (dd, 2H), 7.38 (d, $J = 7.5$ Hz, 5H), 7.26 (t, $J = 7.5$ Hz, 7H), 7.18 (t, 3H), 3.84 (t, $J = 4.5$ Hz, 2H), 3.64-3.52 (m, 30H), 3.42 (t, $J = 7$ Hz, 2H), 2.11 (t, $J = 7.2$ Hz, 2H) 1.55 (m, 2H), 1.36 (m, 2H), 1.33-1.12 (m, 18H); ^{13}C NMR (125 MHz, CDCl_3) δ 163.4, 145.1, 134.4, 129.6, 129.0, 127.8, 126.5, 123.5, 71.5, 70.8, 70.6, 70.0, 69.3, 32.0 29.6, 29.55, 29.5, 29.4, 29.2, 29.0, 28.6, 26.1; FTMS calculated for $\text{C}_{55}\text{H}_{75}\text{KNO}_{11}\text{S}$ [$\text{M} + \text{K}^+$] 996.4692, found 996.4712.

2-((1,1,1-triphenyl-19,22,25,28-tetraoxa-2-thiatriacontan-30-yl)oxy)isoindoline-1,3-dione (5.11b-PEG(4))

Using the same procedure outlined for the synthesis of **5.11a-PEG(4)**, thiol-PEG diblock chain **5.10b-PEG(4)** (54 mg, 0.078 mmol), N-hydroxyphthalimide (15 mg, 0.093 mmol) and triphenylphosphine (24 mg, 0.093 mmol) were reacted in presence of diisopropyl azodicarboxylate (20 μ L, 0.1 mmol) to give the desired product **5.11b-PEG(4)** as a bright yellow oil (52 mg, 80% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.86 (dd, $J = 5.5$, 3 Hz, 2H), 7.77 (dd, $J = 5.5$, 3 Hz, 2H), 7.43 (d, $J = 7.5$ Hz, 6H), 7.30 (t, $J = 7.5$ Hz, 6H), 7.23 (t, $J = 7.4$ Hz, 3H), 4.40 (t, $J = 4.5$ Hz, 2H), 3.89 (t, $J = 4.5$ Hz, 2H), 3.6 (t, 2H), 3.64-3.57 (m, 10H), 3.46 (t, $J = 7$ Hz, 2H), 2.16 (t, $J = 7.5$ Hz, 2H), 1.59 (m, 4H), 1.41 (m, 2H), 1.38-1.10 (m, 24H).

2-((1,1,1-triphenyl-19,22,25,28,31,34-hexaoxa-2-thiahexatriacontan-36-yl)oxy)isoindoline-1,3-dione (5.11b-PEG(6))

Using the same procedure outlined for the synthesis of **5.11a-PEG(4)**, thiol-PEG diblock chain **5.10b-PEG(6)** (0.1 g, 0.13 mmol), N-hydroxyphthalimide (30 mg, 0.18 mmol) and triphenylphosphine (48 mg, 0.18 mmol) were reacted in presence of diisopropyl azodicarboxylate (47 μ L, 0.23 mmol) to give the desired product **5.11b-PEG(6)** as a bright yellow oil (96 mg, 80% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.84 (m, 2H), 7.60 (m, 2H), 7.43 (m, 6H), 7.28 (t, $J = 7.5$ Hz, 6H), 7.22 (t, $J = 7.4$ Hz, 3H), 4.39 (t, $J = 4.5$ Hz, 2H), 3.88 (t, $J = 4.5$ Hz, 2H), 3.66-3.59 (m, 20H), 3.45 (t, $J = 7$ Hz, 2H), 2.14 (t, $J = 7.5$ Hz, 2H), 1.59 (m, 2H), 1.39 (m, 2H), 1.38-1.10 (m, 24H).

2-(1,1,1-triphenyl-19,22,25,28,31,34,37,40-octaoxa-2-thiadotetracontan-42-yl)isoindoline-1,3-dione (5.14)

Using the same procedure outlined for the synthesis of **5.11a-PEG(4)**, thiol-PEG diblock chain **5.10b-PEG(8)** (0.125 g, 0.14 mmol), phthalimide (25 mg, 0.17 mmol) and triphenylphosphine (44 mg, 0.17 mmol) were reacted in presence of diisopropyl azodicarboxylate (50 μ L, 0.25 mmol) to give the desired product **5.14** as a bright yellow oil (134 mg, 95% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.84 (dd, 2H), 7.70 (dd, 2H), 7.40 (d, 6H), 7.27 (t, 6H), 7.20 (t, 3H), 3.90 (t, 2H), 3.73 (t, 2H), 3.55-3.64 (m, 28H), 3.44 (t, 2H), 2.13 (t, 2H), 1.56 (qn, 2H), 1.37 (qn, 2H), 1.10-1.35 (m, 24H); ^{13}C NMR (100 MHz, CDCl_3) δ 168.21, 145.069, 133.88, 132.13, 129.58, 127.75, 126.45, 123.20, 70.57, 70.55, 70.52, 70.06, 70.02, 67.95, 67.88, 37.23, 32.23, 30.29, 29.65, 29.61, 29.54, 29.48, 29.40, 29.16, 28.99, 28.56, 26.07, 25.58; HRMS: $\text{C}_{59}\text{H}_{83}\text{NO}_{10}\text{S}$ $[\text{M}+\text{Na}^+]$, calculated 1020.5635, found 1020.5651.

***O*-(1,1,1-triphenyl-15,18,21,24-tetraoxa-2-thiahexacosan-26-yl)hydroxylamine
(5.12a-PEG(4)).**

To a solution of **5.11a-PEG(4)** (0.131 g, 0.168 mmol) in CH₂Cl₂ (5 mL), hydrazine monohydrate (0.163 mL, 3.35 mmol) was added at room temperature. Within 10 seconds, the solution changed from transparent to dark red, and back to transparent. The reaction was stirred for 3 hours, and the solution changed color once again to an opaque white. The CH₂Cl₂ was then removed by rotary evaporation, and the resulting white solid was purified by flash chromatography (8 g SiO₂; CH₂Cl₂:MeOH (v:v) 99:1 → 90:10) to afford the desired product as a pale yellow oil (0.106 g, 98% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.41 (d, *J* = 7.5 Hz, 6H), 7.27 (t, *J* = 7.5 Hz, 6H), 7.20 (t, *J* = 7.4 Hz, 3H), 5.52 (s, br), 3.84 (t, *J* = 4.5 Hz, 2H), 3.62-3.70 (m, 12H), 3.57 (t, *J* = 3 Hz, 2H), 3.44 (t, *J* = 6.5 Hz, 2H), 2.13 (t, *J* = 7.5 Hz, 2H), 1.57 (qn, *J* = 7 Hz, 2H), 1.38 (qn, *J* = 7.5 Hz, 2H), 1.10-1.32 (m, 16H); ¹³C NMR (125 MHz, CDCl₃) δ 145.33, 129.84, 128.01, 126.72, 75.03, 71.79, 70.85, 70.29, 69.85, 32.26, 29.77, 29.72, 29.64, 29.42, 29.25, 26.32, 23.69, 23.65; FTMS calcd for C₃₉H₅₇NO₅S [M + Na] 674.3855, found 674.3857.

***O*-(1,1,1-triphenyl-15,18,21,24,27,30-hexaoxa-2-thiadotriacontan-32-yl)hydroxylamine (5.12a-PEG(6)).**

Using the same procedure outlined for the synthesis of **5.12a-PEG(4)**, a solution of **5.11a-PEG(6)** (0.080 g, 0.090 mmol) in CH₂Cl₂ (5 mL) and hydrazine monohydrate (0.069 mL, 1.31 mmol), afforded the desired aminooxy product **5.12-PEG(6)** as a pale yellow oil (0.066 g, 98% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.34 (d, *J* = 7.5 Hz, 6H), 7.20 (t, *J* = 7.5 Hz, 6H), 7.13 (t, *J* = 7.4 Hz, 3H), 5.54 (s, br), 3.84 (t, *J* = 4.5 Hz, 2H), 3.62-3.70 (m, 20H), 3.57 (t, *J* = 3 Hz, 2H), 3.44 (t, *J* = 6.5 Hz, 2H), 2.13 (t, *J* = 7.5 Hz, 2H), 1.57 (qn, *J* = 7 Hz, 2H), 1.38 (qn, *J* = 7.5 Hz, 2H), 1.12-1.30 (m, 16H); ¹³C NMR (125 MHz, CDCl₃) δ 145.33, 129.84, 128.01, 126.71, 75.03, 71.79, 70.84, 70.29, 69.86, 64.61, 32.27, 29.88, 29.78, 29.73, 29.65, 29.42, 29.26, 28.84, 26.33, 26.31, 22.28, 21.84; FTMS calcd for C₄₃H₆₅NO₇S [M + Na] 762.4380, found 762.4381.

***O*-(1,1,1-triphenyl-15,18,21,24,27,30,33,36-octaoxa-2-thiaoctatriacontan-38-yl)hydroxylamine (5.12a-PEG(8))**

Using the same procedure outlined for the synthesis of **5.12a-PEG(4)**, a solution of **5.11a-PEG(8)** (0.13 g, 0.135 mmol) in CH₂Cl₂ (5 mL) and hydrazine monohydrate (0.040 mL, 0.82 mmol), afforded the desired aminooxy product **5.12a-PEG(8)** as a pale yellow oil (0.065 g, 59% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.40 (m, 6H), 7.28 (m, 6H), 7.2 (m, 3H), 5.52 (bb, 2H), 3.83 (t, *J* = 4.5 Hz, 2H), 3.67-3.61 (m, 28H), 3.56 (t, *J* = 3 Hz, 2H), 3.44 (t, *J* = 6.5 Hz, 2H), 2.13 (t, *J* = 7.5 Hz, 2H), 1.58 (m, 2H), 1.38 (m, 2H), 1.38-1.13 (m, 16H); ¹³C NMR (125 MHz, CDCl₃) δ 145.1, 129.6, 127.8, 126.5, 74.8, 71.5, 70.6, 70.0, 69.6, 32.0, 29.65, 29.6, 29.55, 29.5, 29.5, 29.4, 29.2, 29.0, 28.6, 26.1; FTMS calculated for C₄₇H₇₃NNaO₉S [M + Na⁺] 850.4898, found 850.4880.

5.12b-PEG(4)

***O*-(1,1,1-triphenyl-19,22,25,28-tetraoxa-2-thiatriacontan-30-yl)hydroxylamine**

(5.12b-PEG(4))

Using the same procedure outlined for the synthesis of **5.12a-PEG(4)**, a solution of **5.11b-PEG(4)** (57 mg, 0.068 mmol) in CH₂Cl₂ (5 mL) and hydrazine monohydrate (66 μ L, 1.36 mmol), afforded the desired aminooxy product **5.12b-PEG(4)** as a pale yellow oil (38 mg, 79% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.43 (m 5H), 7.30 (t, 7H), 7.23 (m, 3H), 5.54 (bb, 2H), 3.86 (t, *J* = 4.5 Hz, 2H), 3.71-3.64 (m, 12H), 3.60 (t, *J* = 3 Hz, 2H), 3.47 (t, *J* = 6.5 Hz, 2H), 2.16 (t, *J* = 7.5 Hz, 2H), 1.59 (m, 4H), 1.41 (qn, *J* = 7.5 Hz, 2H), 1.38-1.15 (m, 24H); ¹³C NMR (125 MHz, CDCl₃) δ 145.3, 129.9, 128.0, 126.7, 75.0, 71.8, 70.9, 70.3, 69.9, 32.3, 29.9, 29.75, 29.7, 29.4, 29.3, 28.8, 26.3, 22.2, 22.15, 22.1.

5.12b-PEG(6)

***O*-(1,1,1-triphenyl-19,22,25,28,31,34-hexaoxa-2-thiahexatriacontan-36-yl)hydroxylamine (5.12bPEG(6))**

Using the same procedure outlined for the synthesis of **5.12a-PEG(4)**, a solution of **5.11b-PEG(6)** (62 mg, 0.067 mmol) in CH₂Cl₂ (5 mL) and hydrazine monohydrate (64 μ L, 1.33 mmol), afforded the desired aminooxy product **5.12b-PEG(6)** as a pale yellow oil (37 mg, 70% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.43 (m 5H), 7.28 (t, 7H), 7.27 (m 3H), 5.54 (s, br), 5.58 (bb, 2H), 3.85 (t, *J* = 4.5 Hz, 2H), 3.62-3.70 (m, 12H), 3.57 (t, *J* = 3 Hz, 2H), 3.44 (t, *J* = 6.5 Hz, 2H), 2.15 (t, *J* = 7.5 Hz, 2H), 1.58 (qn, *J* = 7 Hz, 2H), 1.38 (qn, *J* = 7.5 Hz, 2H), 1.12-1.30 (m, 24H); ¹³C NMR (125 MHz, CDCl₃) δ 145.1, 129.6, 127.8, 126.4, 71.5, 70.6, 70.5, 70.0, 61.7, 29.7, 29.6, 29.55, 29.5, 29.4, 29.0, 26.1.

1,1,1-triphenyl-19,22,25,28,31,34,37,40-octaoxa-2-thiadotetracontan-42-amine (5.15)

Using the same procedure outlined for the synthesis of **5.12a-PEG(4)**, a solution of **5.14** (70 mg, 0.07 mmol) in CH₂Cl₂ (5 mL) and hydrazine monohydrate (30 μ L, 0.62 mmol), afforded the desired aminoxy product **5.15** as a pale yellow oil (44 mg, 72% yield). ¹H NMR (500 MHz, CDCl₃) δ 7.40 (d, 6H), 7.27 (t, 6H), 7.20 (t, 3H), 3.92 (t, 2H), 3.75 (t, 2H), 3.55-3.67 (m, 24H), 3.57 (t, 2H), 3.44 (t, 2H), 3.17(t, 2H), 2.13 (t, 2H), 1.56 (qn, 2H), 1.37 (qn, 2H), 1.10-1.35 (m, 24H); HRMS: C₅₁H₈₂NO₈S, [M+H⁺] calculated 868.5761, found 868.5773.

5.13a-PEG(4)

1-(aminooxy)-3,6,9,12-tetraoxatetracosane-24-thiol (5.13a-PEG(4))

To a N₂ purged solution of **5.12a-PEG(4)** (0.080 g, 0.123 mmol) in CH₂Cl₂ (0.5 mL), trifluoroacetic acid (0.5 mL, excess), and triethylsilane (0.085 mL, 0.073 mmol) were added at room temperature. The solution immediately turned dark yellow, then clear. The reaction was kept under N₂ atmosphere and stirred for 3 hours. The volatiles were then removed by rotary evaporation and subsequent vacuum pumping, and the resulting white solid was purified by flash chromatography (5 g SiO₂; CH₂Cl₂:MeOH, 9:1, v/v) to afford the desired aminooxy-thiol **5.12a-PEG(4)** as a pale yellow oil (0.035 g, 70% yield). ¹H NMR (500 MHz, CDCl₃) δ 5.56 (s, br), 3.84 (t, *J* = 4.5 Hz, 2H), 3.62-3.70 (m, 12H (plus H₂O impurities)), 3.58 (t, *J* = 3 Hz, 2H), 3.44 (t, *J* = 6.5 Hz, 2H), 2.52 (q, *J* = 7.5 Hz, 2H), 1.57 (m, *J* = 7 Hz, 2H), 1.23-1.41 (m, 18H); ¹³C NMR (125 MHz, CDCl₃) δ 71.79, 70.85, 70.81, 70.78, 70.30, 69.86, 29.79, 29.72, 29.30, 28.61, 23.14, 23.08; FTMS calcd for C₂₀H₄₃NO₅S [M + Na] 432.2760, found 432.2760.

5.13a-PEG(6)

1-(aminooxy)-3,6,9,12,15,18-hexaoxatriacontane-30-thiol (5.13a-PEG(6)).

Using the same procedure outlined for the synthesis of **5.13a-PEG(4)**, trityl deprotection of **5.12a-PEG(6)** (0.045 g, 0.061 mmol) was performed using a solution of CH_2Cl_2 /trifluoroacetic acid (1:1 ratio), and triethylsilane (5% by volume) to afford the desired aminooxy-thiol **5.13a-PEG(6)** as a pale yellow oil (0.024 g, 79% yield). ^1H NMR (500 MHz, CDCl_3) δ 5.55 (s, br), 3.84 (t, $J = 4.5$ Hz, 2H), 3.62-3.70 (m, 18H (plus H_2O impurities)), 3.58 (t, $J = 3$ Hz, 2H), 3.44 (t, $J = 6.5$ Hz, 2H), 2.52 (q, $J = 7.5$ Hz, 2H), 1.67 (qn, $J = 7$ Hz, 2H), 1.59 (m, $J = 7$ Hz, 2H), 1.23-1.41 (m, 16H); ^{13}C NMR (125 MHz, CDCl_3) δ 71.55, 71.25, 70.61, 70.56, 70.54, 70.05, 69.61, 34.04, 29.55, 29.48, 29.06, 28.37, 22.90, 22.84; FTMS calcd for $\text{C}_{24}\text{H}_{51}\text{NO}_7\text{S}$ [$\text{M} + \text{Na}$] 520.3284, found 520.3280.

1-(Aminooxy)-3,6,9,12,15,18,21,24-octaoxahexatriacontane-36-thiol (5.13a-PEG(8))

Using the same procedure outlined for the synthesis of **5.13a-PEG(4)**, trityl deprotection of **5.12a-PEG(8)** (32 mg, 0.038 mmol) was performed using a solution of CH_2Cl_2 /trifluoroacetic acid (1:1 ratio), and triethylsilane (5% by volume) to afford the desired aminooxy-thiol **5.13a-PEG(8)** as a pale yellow oil (22 mg, 97% yield). ^1H NMR (400 MHz, CDCl_3) δ 5.55 (bb, 2H), 3.85 (t, $J = 4.8$ Hz, 2H), 3.69-3.63 (m, 26H), 3.58 (t, 2H), 3.46 (t, $J = 7.2$ Hz, 2H), 2.55 (q, $J = 7.6$ Hz, 2H), 1.64-1.55 (m, 2H), 1.37-1.26 (m, 16H); ^{13}C NMR (125 MHz, CDCl_3) δ 74.8, 71.53, 70.6, 70.5, 70.0, 69.6, 34.0, 29.6, 29.55, 29.5, 29.1, 28.4, 26.1, 24.6; FTMS calculated for $\text{C}_{28}\text{H}_{59}\text{NNaO}_9\text{S}$ [$\text{M} + \text{Na}^+$] 608.3803, found 608.3798.

1-(Aminooxy)-3,6,9,12-tetraoxaoctacosane-28-thiol (5.13b-PEG(4))

Using the same procedure outlined for the synthesis of **5.13a-PEG(4)**, trityl deprotection of **5.12b-PEG(4)** (28 mg, 0.039 mmol) was performed using a solution of CH_2Cl_2 /trifluoroacetic acid (1:1 ratio), and triethylsilane (5% by volume) to afford the desired aminooxy-thiol **5.13b-PEG(4)** as a pale yellow oil (8 mg, 44% yield). ^1H NMR (500 MHz, CDCl_3) δ 5.55 (bb, 2H), 3.86 (t, $J = 5$ Hz, 2H), 3.70-3.64 (m, 12H), 3.59 (t, $J = 3$ Hz, 2H), 3.47 (t, $J = 6.5$ Hz, 2H), 2.54 (q, $J = 7.5$ Hz, 2H), 1.63-1.57 (m 4H), 1.40-1.21 (m, 26H); ^{13}C NMR (125 MHz, CDCl_3) δ 75.0, 71.8, 70.9, 70.85, 70.8, 70.3, 69.9, 64.5, 34.3, 29.9, 29.8, 29.7, 29.3, 28.6, 26.3, 24.9, 22.15, 22.1.

1-(Aminooxy)-3,6,9,12,15,18-hexaoxatetratriciacontane-34-thiol (5.13b-PEG(6))

Using the same procedure outlined for the synthesis of **5.13a-PEG(4)**, trityl deprotection of **5.12b-PEG(6)** (27 mg, 0.034 mmol) was performed using a solution of CH_2Cl_2 /trifluoroacetic acid (1:1 ratio), and triethylsilane (5% by volume) to afford the desired aminooxy-thiol **5.13b-PEG(6)** as a pale yellow oil (17 mg, 90% yield). ^1H NMR (400 MHz, CDCl_3) δ 5.46 (bb, 2H), 3.78 (t, $J = 4.5$ Hz, 2H), 3.62-3.58 (m, 20H), 3.51 (t, $J = 3$ Hz, 2H), 3.39 (t, $J = 6.5$ Hz, 2H), 2.48 (q, $J = 7.5$ Hz, 2H), 1.55-1.48 (m, 4H), 1.59 1.30-1.18 (m, 28H); ^{13}C NMR (125 MHz, CDCl_3) δ 74.7, 71.5, 70.6, 70.5, 70.05, 69.6, 34.0, 29.65, 29.63, 29.60, 29.57, 29.5 29.1, 28.4, 26.6 24.6.

Scheme 6.2. Synthesis of aminooxy-peptide **5.19**.

Solid Phase Synthesis: Aminooxy-peptide **5.19**

Steps 1&2. Cysteamine-loaded polystyrene resin (200 mg, 0.14 mmol active amine) was treated with Fmoc-protected diglycine (Novabiochem, 150 mg, 0.42 mmol) in the presence of diisopropylcarbodiimide (DIC, 0.064 mL, 0.42 mmol), *N*-hydroxybenzotriazole (HOBt, 56 mg, 0.42 mmol) and *N,N*-diisopropylethylamine (DIPEA, 0.12 mL, 0.84 mmol) in DMF (3 mL). After shaking overnight, the resin was filtered and thoroughly washed with CH₂Cl₂ (7x). The resin then was dried under vacuum, swelled using DMF, filtered, and treated with 20% piperidine in DMF (3 mL) at room temperature for 40 mins. The piperidine washing process was repeated once. The

resin was filtered and washed successively with DMF (3x) and CH₂Cl₂ (5x), and then dried under vacuum.

Steps 3&4. The diglycine loaded resin (®-G-G-NH₂) was swelled using DMF over 30 mins, filtered, and then treated with Fmoc-protected arginine-Boc₂ (Novabiochem, 250 mg, 0.42 mmol), DIC (0.064 mL, 0.42 mmol), HOBt (56 mg, 0.42 mmol) and DIPEA (0.12 mL, 0.84 mmol) in DMF (3 mL). After shaking overnight, the resin was filtered and thoroughly washed with CH₂Cl₂ (7x). The resin was dried under vacuum, swelled using DMF, filtered, and then treated with 20% piperidine in DMF (3 mL) at room temperature for 40 mins. The piperidine washing process was repeated once. The resin was filtered and washed successively with DMF (3x) and CH₂Cl₂ (5x), and then dried under vacuum.

Steps 5&6. The ®-G-G-R-NH₂ resin was swelled using DMF over 30 mins, filtered, and then treated with Fmoc-protected diglycine (Novabiochem, 250 mg, 0.70 mmol), DIC (0.108 mL, 0.70 mmol), HOBt (90 mg, 0.70 mmol) and DIPEA (0.25 mL, 1.4 mmol) in DMF (3 mL). After shaking overnight, the resin was filtered and thoroughly washed with CH₂Cl₂ (7x). The resin was dried under vacuum, swelled using DMF, filtered, and then treated with 20% piperidine in DMF (3 mL) at room temperature for 40 mins. The piperidine washing process was repeated once. The resin was filtered and washed successively with DMF (3X) and CH₂Cl₂ (5X), and then dried under vacuum.

Steps 7&8. The ®-G-G-R-G-G-NH₂ resin (100 mg) was swelled using DMF over 30 mins, filtered, and then treated with Fmoc-protected aminooxyglycine (Polypeptide, 75 mg, 0.21 mmol), DIC (0.033 mL, 0.21 mmol), HOBt (28 mg, 0.21 mmol) and DIPEA (0.075 mL, 0.42 mmol) in DMF (2 mL). After shaking overnight, the

resin was filtered and thoroughly washed with CH_2Cl_2 (7x). The resin was dried under vacuum, swelled using DMF, filtered, and then treated with 20% piperidine in DMF (3 mL) at room temperature for 40 mins. The piperidine washing process was repeated once. The resin was filtered and washed successively with DMF (3x) and CH_2Cl_2 (5x), and then dried under vacuum.

To confirm the integrity of the aminooxy peptide linker, a sample of the resin was treated with TFA (50%), triethylsilane (5%) in CH_2Cl_2 at room temperature to cleave the peptide from the resin as well as to deprotect the arginine. Analysis by HRMS confirmed the title compound; HRMS, $\text{C}_{18}\text{H}_{35}\text{N}_{10}\text{O}_7\text{S}$, $[\text{M}+\text{H}^+]$, calculated 535.2405, found 535.2411.

Cypate bis(acetal) **5.21**

To a solution of cypate (**5.20**) (75 mg, 0.12 mmol) in dimethylformamide (5 mL) at 0 °C, were added consecutively hydroxybenzotriazole (64 mg, 0.48 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) (92 mg, 0.48 mmol). After stirring for 15 min, 4,4-diethoxybutan-1-amine (85 μ L, 0.48 mmol) was added in one portion. The resulting solution was allowed to come to room temperature and was stirred in the dark. After 12 h, the solution was concentrated by rotary evaporation, and the resulting crude was dissolved in CH₂Cl₂ (40 mL) and washed with brine (3x 10 mL). The organic layer was dried over Na₂SO₄ and the solvents were removed by rotary evaporation. The resulting dark green paste was purified by chromatography (SiO₂; CH₂Cl₂/MeOH, 9:1, v/v; R_f = 0.3) to afford the desired cypate bis(acetal) (74 mg, 64%). HRMS, C₅₇H₇₅N₄O₆⁺, [M⁺], calculated 911.5681, found 911.5665.

Cypate bis(aldehyde) 5.22

A solution of cypate bis(acetal) **2.21** (42 mg, 0.046 mmol) in water (4 mL) and acetic acid (2 mL) was stirred at room temperature for 4h. Then the solvents were removed by rotary evaporation and the resulting crude was further dried in a vacuum pump in the dark for 8h. The dark green solid was purified by column chromatography (5 g SiO₂; CH₂Cl₂:MeOH, 9:1, v/v, R_f = 0.25) to afford the desired cypate bis(aldehyde) (22mg, 63%). HRMS, C₄₉H₅₅N₄O₄⁺ [M⁺], calculated 763.4218, found 763.4223.

5.25

Cypate mono-aldehyde linker **5.25**

To a solution of cypate bis(aldehyde) **5.22** (10 mg, 0.013 mmol) in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (9:1, 2 mL) was added dropwise a first portion of linker **5.13a-PEG(8)** (3 mg, 0.005 mmol) dissolved in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1 mL). After 2 h, a second portion of linker **5.13a-PEG(8)** (3 mg, 0.005 mmol) dissolved in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1 mL) was added. The solution was left at room temperature in the dark for 12h. The solution was then concentrate by rotary evaporation and the resulting crude was purified by column chromatography (SiO_2 ; $\text{CH}_2\text{Cl}_2:\text{MeOH}$, 9:1, v/v, $R_f = 0.37$) to obtain the desired cypate mono-aldehyde linker **5.25** (7 mg, 52%). HRMS, $\text{C}_{77}\text{H}_{112}\text{N}_5\text{O}_{12}\text{S}^+$, $[\text{M}^+]$ calculated 1330.8023, found 1330.8015.

Cypate linker/peptide **5.26**

To a solution of cypate monoaldehyde spacer **5.25** (7 mg, 0.0052 mmol) in CH_2Cl_2 (3 mL) was added peptide aminooxy **5.19** (3 mg, 0.006 mmol) dissolved in MeOH (1 mL). The solution was left at room temperature in the dark for 12h. The solution was then concentrate by rotary evaporation to afford the desired product **5.26**. HRMS, $\text{C}_{95}\text{H}_{145}\text{N}_{15}\text{O}_{18}\text{S}_2^+$ $[\text{M}^+ + \text{H}^+]/2$ calcd 924.0161, found 924.0185 (m/z where z = 2).

REFERENCES

R.1. Chapter 1 References

R.2. Chapter 2 References

R.3. Chapter 3 References

R.4. Chapter 4 References

R.5. Chapter 5 References

R.6. Chapter 6 References

R.1. Chapter 1 References

1. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.
2. Evans, R. A. *Australian Journal of Chemistry* **2007**, *60*, 384-395.
3. Amantini, D.; Fringuelli, F.; Piermatti, O.; Tortoioli, S.; Vaccaro, L. *ARKIVOC* **2002**, *xi*, 293-311.
4. Hoyle, C. E.; Bowman, C. N. *Angew. Chem. Int. Ed.* **2010**, *49*, 1540-1573.
5. (a) Huisgen, R. *Proc. Chem. Soc.* **1961**, 357-396; (b) Huisgen, R. *Angew. Chem. Int. Ed.* **1963**, *2*, 565-598.
6. Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210-216.
7. Boren, B. C.; Narayan, S.; Rasmussen, L. K.; Zhang, L.; Zhao, H.; Lin, Z.; Jia, G.; Fokin, V. V. *J. Am. Chem. Soc.* **2008**, *130*, 8923-8930.
8. Worrell, B. T.; Malik, J. A.; Fokin, V. V. *Science* **4 April 2013**, [DOI: 10.1126/science.1229506].
9. Grekov, A. P.; Veselov, V. Y. *Rus. Chem. Rev.* **1978**, *47*, 1200-1230.
10. Edwards, J. O. ; Pearson, R. G. *J. Am. Chem. Soc.* **1962**, *84*, 16-24.
11. Khomutov A. R.; Vepsäläinen, J. J.; Shevetsov, A. S.; Hyvönen, T.; Keinänen, T. A.; Pustobaev, V. N.; Eloranta, T. O.; Khomutov, R. M. *Tetrahedron* **1996**, *52*, 13751-13766.
12. Kalia, J.; Raines, R. T. *Angew. Chem. Int. Ed.* **2008**, *47*, 7523-7526.
13. Winberg, K. B. ; Glaser, R. *J. Am. Chem. Soc.* **1992**, *114*, 841-850.
14. More O'Ferrall, R. A.; O'Brien, D. M.; Murphy, D. G. *Can. J. Chem.* **2000**, *78*, 1594-1612.
15. Heredia, K. L.; Tolstyka, Z. P.; Maynard, H. D. *Macromolecules* **2007**, *40*, 4772.
16. Zeng, Y.; Ramya, T. N. C.; Dirksen, A.; Dawson, P. E.; Paulson, J. C. *Nat Methods.* **2009**, *6*, 207-209.
17. Dirksen, A.; Dawson, P. E. *Bioconjugate Chem.* **2008**, *19*, 2543-2548.

18. Thyngensen, M.; Munch, H.; Sauer, J.; Clo, E.; Jorgensen, M. R.; Hindsgaul, O.; Jensen, K. J. *J. Org. Chem.* **2010**, *75*, 1752-1755.
19. (a) Crisalli, P.; Kool, E. T. *J. Org. Chem.* **2013**, *78*, 1184-1189, (b) Rashidian, M.; Mahmoodi, M. M.; Shah, R.; Dozier, J. K.; Wagner, C. R.; Distefano, M. D. *Bioconjugate Chem.* **2013**, *24*, 333-342;
20. (a) Glover, S. A.; Mo, G. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1728; (b) Flipo, M.; Florent, I.; Grellier, P.; Sergheraert, C.; Deprez-Poulain, R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2659; (c) Janza, B.; Studer, A. *J. Org. Chem.* **2005**, *70*, 6991-6994.
21. (a) Apfel, C.; Banner, D. W.; Bur, D.; Dietz, M.; Hirata, T.; Hubschwerlen, C.; Malcolm, H. L.; Page, M. G. P.; Pirson, W.; Specklin, J.-L. *J. Med. Chem.* **2000**, *43*, 2324-2331; (b) Conejo-Garcia, A.; Schofield, C. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4004-4009.
22. (a) Langlois, N.; Moro, A. *Eur. J. Org. Chem.* **1999**, 3483-3488; (b) Dongol, K. G.; Tay, B. Y.; Xiang, K. *Synthetic Communications* **2006**, *36*, 1247-1257; (c) Pinto, L. F. V.; Gloria, P. M. C.; Gomes, M. J. S.; Rzepa, H. S.; Prabhkar, S.; Lobo, A. M. *Tetrahedron Lett.* **2009**, *50*, 3446-3449; (d) Hanessian, S.; Johnstone, S. *J. Org. Chem.* **1999**, *64*, 5896-5903
23. Duan, J. J.-W.; Lu, Z.; Xue, C.-B.; He, X.; Seng, J. L.; Roderick, J. J.; Wasserman, Z. R.; Liu, R.-Q.; Covington, M. B.; Magolda, R. L.; Newton, R. C.; Trzaskos, J. M.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2035-2040.
24. (a) Yamaguchi, T.; Harada, N.; Ozaki, K.; Hayashi, M.; Arakawa, H.; Hashiyama, T. *Tetrahedron* **1999**, *55*, 1005-1016. (b) Wroblewski, A. E.; Halajewska-Wosik, A. *Tetrahedron: Asymmetry* **2004**, *15*, 3201-3205.
25. (a) Ishikawa, T.; Kawakami, M.; Fukui, M.; Yamashita, A.; Urano, J.; Saito, S. *J. Am. Chem. Soc.* **2001**, *123*, 7734-7735 ; (b) Nicolaou, K. C.; Mathison, C. J. N.; Montagnon, T. *J. Am. Chem. Soc.* **2004**, *126*, 5192-5201.
26. (a) Gribble, G. W.; Leiby R. W.; Sheehan, M. N. *Synthesis*, 1977, 856; (b) Beak, P.; Selling, G. W. *J. Org. Chem.* **1989**, *54*, 5574-5580; (c) Daher, R.; Coinçon, M.; Fonville, M.; Gest, P. M.; Guerin, M. E.; Jackson, M.; Sygusch, J.; Therisod, M. *J. Med. Chem.* **2010**, *53*, 7836-7842; (d) Gantt, R. W.; Peltier-Pain, P.; Cournoyer, W. J.; Thorson, J. S. *Nature Chemical Biology* **2011**, *7*, 685-691.
27. (a) Beckmann, E. *Berichte der Deutschen Chemischen Gesellschaft* **1886**, *19*, 988-993; (b) Gawley, R. E. *Org. React.* **1988**, *35*, 14-24; (c) Ganguly, N. C.; Mondal, P. *Synthesis* **2010**, 3705-3709.
28. (a) Neber, P. W.; Friedolsheim, A. V. *Justus Liebigs Annalen der Chemie* **1926**, *449*, 109-134; (b) House, H. O.; Berkowitz, W. F. *J. Org. Chem.* **1963**, *28*, 307; (c) Ooi, T.; Takahashi, M.; Doda, K.; Maruoka, K. *J. Am. Chem. Soc.* **2002**, *124*, 7640.

29. (a) Lossen, W. *Ann.* **1872**, *161*, 347; (b) Bauer, L.; Exner, O. *Angew. Chem. Int. Ed.* **1974**, *13*, 376; (c) Dubé, P.; Nathel, N. F. F.; Vetelino, M.; Couturier, M.; Abossafy, C. L.; Pichette, S.; Jorgensen, M. L.; Hardink, M. *Org. Lett.* **2009**, *11*, 5622-5625.
30. (a) Bamberger, E. *Chemische Berichte* **1894**, *27*, 1347-1350; (b) Kohnstam, G.; Petch, W. A.; Williams, D. L. H. *J. Chem. Soc., Perkin Trans. 2* **1984**, 423-427.
31. Nauman, D. A.; Bertozzi, C. R. *Biochimica et Biophysica Acta* **2001**, *1568*, 147-154.
32. Canne, L. E.; Ferré-D'Amaré, A. R.; Burley, S. K.; Kent, S. B. H. *J. Am. Chem. Soc.* **1995**, *117*, 2998-3007.
33. (a) Kunz, H. *Angew. Chem. Int. Ed* **1987**, *26*, 294-308; (b) Hoffmann, F. M.; Kessler, H. *Angew. Chem. Int. Ed.* **1995**, *34*, 2026-2028.
34. Cervigni, S. E.; Dumy, P. and Mutter, M. *Angew. Chem., Int. Ed.* **1996**, *35*, 1230.
35. Lees, A.; Sen, G.; LopezAcosta, A. *Vaccine* **2006**, *24*, 716-729.
36. Thygesen, M. B.; Sauer, J.; Jensen, K. J. *Chem. Eur. J.* **2009**, *15*, 1649-1660.
37. Chan, E. W. L.; Yu, L. *Langmuir* **2002**, *18*, 311-313.
38. (a) Lelacheur, R. M.; Sonnenberg, L. B.; Singer, P. C.; Christman, R. F.; Charles, M. J. *Environ. Sci. Technol.* **1993**, *27*, 2745-2753; (b) Yu, J. Z.; Flagan, R. C.; Seinfeld, J. H. *Environ. Sci. Technol.* **1998**, *32*, 2357-2370; (c) Jaoui, M.; Kleindienst, T. E.; Lewandowski, M.; Edney, E. O. *Anal. Chem.* **2004**, *76*, 4765-4778; (d) Jakober, C. A.; Charles, M. J.; Kleeman, M. J.; Green, P. G. *Anal. Chem.* **2006**, *78*, 5086-5093; (e) Mattingly, S. J.; Xu, T.; Nantz, M. H.; Higashi, R. M.; Fan, T. W.-M. *Metabolomics* **2012**, *8*, 989-996; (f) Li, M.; Biswas, S.; Nantz, M. H.; Higashi, R. M.; Fu, X.-A. *Sensors and Actuators B: Chemical* **2013**, *180*, 130-136.
39. Hovinen, J. *Chemistry & Biodiversity* **2006**, *3*, 296-303.
40. Bahta, M.; Liu, F.; Kim, S.-E.; Stephen, A. G.; Fisher, R. J.; Burke, T. R. Jr. *Nature Protocols* **2012**, *12*, 686-702.
41. Mitsunobu, O.; Yamada, Y. *Bulletin of the Chemical Society of Japan* **1967**, *40*, 2380-2382.
42. Grochowski, E.; Jurczak, J. *Synthesis* **1976**, 682-684.
43. (a) Grochowski, E.; Hilton, B. D.; Kupper, R. J.; Michejda, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 6876-6877; (b) Camp, D.; Jenkins, I. D. *J. Org. Chem.* **1989**, *54*, 3045-3049; (c) Camp, D.; Jenkins, I. D. *J. Org. Chem.* **1989**, *54*, 3049-3054.

44. Hughes, D. L.; Reamer, R. A.; Bergan, J. J.; Grabowski, E. J. J. *J. Am. Chem. Soc.* **1988**, *110*, 6487-6491.
45. Nicolaou, K. C.; Groneberg, R. D. *J. Am. Chem. Soc.* **1990**, *112*, 4085-4086.
46. Karskela, M.; Helkearo, M.; Virta, P.; Lonnberg, H. *Bioconjugate Chem.* **2010**, *21*, 748-755.
47. Su, S.; Giguere, J. R.; Schaus, S. E.; Porco, J. A. Jr. *Tetrahedron* **2004**, *60*, 8645.
48. Palandoken, H.; Bocian, C. M.; McCombs, M. R.; Nantz, M. H. *Tetrahedron Letters* **2005**, *46*, 6667-6669.
49. Choong, I. C.; Ellman, J. C. *J. Org. Chem.* **1999**, *64*, 6528-6529.
50. Jones, D. S.; Hammaker, J. R.; Tedder, M. E. *Tetrahedron Lett.* **2000**, *41*, 1531.
51. Carrasco, M. R.; Alvarado, C. I.; Dashner, S. T.; Wong, A. J.; Wong, M. A. *J. Org. Chem.* **2010**, *75*, 5757-5759.
52. Lemieux, G. A.; Yarema, K. J.; Jacobs, C. L.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1999**, *121*, 4278-4279.
53. Marcaurelle, L. A.; Shin, Y.; Goon, S.; Bertozzi, C. R. *Org. Lett.* **2001**, *3*, 3691.
54. Kim, J. N.; Kim, K. M.; Ryu, E. K. *Synth. Commun.* **1992**, *22*, 1427-1432.
55. Mikola, H.; Hanninen, E. *Bioconjugate Chem.* **1992**, *3*, 182-186.
56. Laulhé, S.; Nantz, M. H. *Org. Prep. Proc. Int.* **2011**, *43*, 475-476.
57. Laulhé, S.; Gori, S. S.; Nantz, M. H. *J. Org. Chem.* **2012**, *77*, 9394-9337.
58. Laulhé, S.; Bogdanov, B.; Johannes, L. M.; Gutierrez, O.; Harrison J. G.; Tantillo, D. J.; Zhang, X.; Nantz, M. H. *J. Mass. Spectrom.* **2012**, *47*, 676-686.
59. (a) Kang, K. A.; Wang, J.; O'Toole, M. G.; Nantz, M. H.; Moore, J. D.; Laulhé, S.; Achilefu, S. *Advances in Experimental Medicine and Biology* **2012**, *737*, 285-291;
 (b) Wang, J.; Moore, J. D.; Laulhé, S.; Nantz, M. H.; Achilefu, S.; Kang, K. A. *Nanotechnology* **2012**, *23*, 095501 (13pp).

R.2. Chapter 2 References

1. Shen, Y.; Friestad, G. K. *J. Org. Chem.* **2002**, *67*, 6236-6239.
2. Sosnovsky, G.; Purgstaller, K. *Z. Naturforsch. B Chem. Sci.* **1989**, *44b*, 582-586.
3. Keller, R. N.; Smith, P. A. S. *J. Am. Chem. Soc.* **1946**, *68*, 899-902.
4. Boche, G. B., M.; Schrott, W. *Tetrahedron Letters* **1982**, *23*, 5399.
5. Smulik, J. A.; Vedejs, E. *Org. Lett.* **2003**, *5*, 4187-4190.
6. (a) Macaulay, J. B.; Fallis, A. G. *J. Am. Chem. Soc.* **1990**, *112*, 1136-1144. (b) Schmidhammer, H.; Obendorf, D.; Pirkner, G.-F.; Sams, T. *J. Org. Chem.* **1991**, *56*, 3457-3459. (c) Elliot, E. L.; Bushell, S. M.; Cavero, M.; Tolan, B.; Kelly, T. R. *Org. Lett.* **2005**, *7*, 2449-2451. (d) Cook, G. R.; Kargbo, R.; Maity, B. *Org. Lett.* **2005**, *7*, 2767-2770.
7. (a) Baldwin, J. E.; Adlington, R. M.; Jones, R. H.; Schofield, C. J.; Zaracostas, C.; Greengrass, C. W. *Tetrahedron* **1986**, *42*, 4879-4888. (b) Smulik, J. A.; Vedejs, E. *Org. Lett.* **2003**, *5*, 4187-4190.
8. Heim-Riether A.; Healy J. *J. Org. Chem.* **2005**, *70*, 7331-7337
9. Erdik, E. A.; Ay, M. *Chem. Rev.* **1989**, *89*, 1947-1980.
10. (a) Armstrong, A.; Baxter, C. A.; Lamont, S. G.; Pape, A. R.; Wincewicz, R. *Org. Lett.* **2007**, *9* (2), 351-353; (b) Armstrong, A.; Pullin, R. D.; Jenner, C. R.; Scutt, J. N. *J. Org. Chem.* **2010**, *75* (10), 3499-502.
11. (a) Harger, M. J. P. *J. Chem. Soc., Chem. Commun.* **1979**, *17*, 768-769; (b) Harger, M. J. P. *J. Chem. Soc., Chem. Commun.* **1981**, *19*, 3284-3288.
12. Kreutzkamp, N.; Schindler, H. *Arch. Pharm.* **1960**, *293*, 296.
13. (a) Jencks, W.P. *J. Am. Chem. Soc.* **1958**, *80*, 4581 ; (b) Jencks, W.P. *J. Am. Chem. Soc.* **1958**, *80*, 4585 ; (c) Jencks, W.P.; Carriuolo, J. *J. Am. Chem. Soc.* **1960**, *82*, 1778 ; (d) Bruice, T.C.; Fedor, L.R. *J. Am. Chem. Soc.* **1964**, *86*, 738-739.
14. Jandorf, B. J. *J. Am. Chem. Soc.* **1956**, *78*, 3686.
15. Colvin, E. W.; Kirby, G. W.; Wilson, A. C. *Tetrahedron Letters* **1982**, *23*, 3835-3836.
16. Cook, G. R.; Kargbo, R.; Maity, B. *Org. Lett.* **2005**, *7*, 2767.

17. Klötzer, W.; Stadlwieser, J.; Raneburger, J. *Org. Synth.* **1986**, *64*, 96-100.
18. Lecher, H.; Hofmann, J. *Chem. Ber.* **1922**, *55*, 912.
19. (a) Iwata, Y.; Koseki, H. *Process Safety Progress* **2002**, *21*, 136-141 ; (b) Cisneros, L. O.; Wu, X.; Rogers, W. J.; Mannan, M. S.; Park, J.; North, S. W. *Process. Saf. Environ. Prot.* **2003**, *81*, 121-124.
20. Higgins, W.A. ; Vogel, P.W. ; Craig, W.G. *J. Am. Chem. Soc.* **1955**, *77*, 1864-1866.
21. Bell, T.W.; Anugu, S.; Bailey, P.; Catalano, V.J.; Dey, K.; Drew, M.G.B.; Duffy, N.H.; Jin, Q.; Samala, M.F.; Sodoma, A.; Welch, W. H.; Schols D.; Vermeire, K. *J. Med. Chem.* **2006**, *49*, 1291.
22. Laulhé, S.; Nantz, M. H. *Org. Prep. Proc. Int.* **2011**, *43*, 475-476.
23. Laulhé, S.; Gori, S. S.; Nantz, M. H. *J. Org. Chem.* **2012**, *77*, 9394-9337.
24. (a) Larock, R. C. *Comprehensive Organic Transformations*, 2nd ed.; Wiley-VCH: New York, 1999; pp 1949-1950, 1955, 1983-1985. (b) Fleming, F. F. *Nat. Prod. Rep.* **1999**, *16*, 597-606. (c) Fleming, F. F.; Yaot, L.; Ravikumart, P. C.; Funk, L.; Shook, B. C. *J. Med. Chem.* **2010**, *53*, 7902-7917.
25. (a) Nakajima, N.; Saito, M.; Ubukata, M. *Tetrahedron* **2002**, *58*, 3561-3577. (b) Kuo, C. W.; Zhu, J. L.; Wu, J. D.; Chu, C. M.; Yao, C. F.; Shia, K. J. *Chem. Commun.* **2007**, *3*, 301-303. (c) Vaccari, D.; Davoli, P.; Ori, C.; Spaggiari, A.; Prati, F. *Synlett* **2008**, 1317-1320. (d) Hada, K.; Suda, A.; Asoh, K.; Tsukuda, T.; Hasegawa, M.; Sato, Y.; Ogawa, K.; Kuramoto, S.; Aoki, Y.; Shimma, N.; Ishikawa, T.; Koyano, H. *Bioorg. Med. Chem.* **2012**, *20*, 1442-1460.
26. (a) Zhou, S.; Junge, K.; Addis, D.; Das, S.; Beller, M. *Org. Lett.* **2009**, *11*, 2461-2464. (b) Sueoka, S.; Mitsudome, T.; Mizugaki, T.; Jitsu-kawa, K.; Kaneda, K. *Chem. Commun.* **2010**, *46*, 8243-8245. (c) Junge, K.; Schröder, K.; Beller, M. *Chem. Commun.* **2011**, *47*, 4849-4859. (d) Enthaler, S.; Weidauer, M. *Catal. Lett.* **2011**, *141*, 1079-1085.
27. (a) Rogick, M. M.; Van Peppen, J. F.; Kline, K. P.; Demmin, T. R. *J. Org. Chem.* **1974**, *39*, 3424-3426. (b) Shinozaki, H.; Imaizumi, M.; Tajima, M. *Chem. Lett.* **1983**, 929-931. (c) Meshram, H. M. *Synthesis* **1992**, 943-944. (d) Katritzky, A. R.; Zhang, G. F.; Fan, W. Q. *Org. Prep. Proc. Int.* **1993**, *25*, 315-319. (e) Hegedus, A.; Cwik, A.; Hell, Z.; Horvath, Z.; Esek, A.; Uzsoki, M. *Green Chem.* **2002**, *4*, 618-620. (f) Denton, R. M.; Nan, J.; Lindovska, P.; Lewis, W. *Tetrahedron* **2012**, *68*, 2899-2905.

28. (a) Brackman, W.; Smith, P. *Recl. Trav. Chem.* **1963**, 82, 757-762. (b) Parameswaran, K. N.; Friedman, O. M. *Chem. Ind* **1965**, 988-989. (c) Dauzonne, D.; Demerseman, P.; Royer, R. *Synthesis* 1981, 739-740. (d) Capdevielle, P.; Lavigne, A.; Maumy, M. *Synthesis* **1989**, 451-452. (e) Bose, D. S.; Narsaiah, A. V. *Tetrahedron Lett.* **1998**, 39, 6533-6534. (f) Chakraborti, A. K.; Kaur, G. *Tetrahedron* **1999**, 55, 13265-13268. (g) Kumar, H. M. S.; Reddy, B. V. S.; Reddy, P. T.; Yadav, J. S. *Synthesis* **1999**, 586-587. (h) Erman, M. B.; Snow, J. W.; Williams, M. J. *Tetrahedron Lett.* **2000**, 41, 6749-6752. (i) Talukdar, S.; Hsu, J.-L.; Chou, T.-C.; Fang, J.-M. *Tetrahedron Lett.* **2001**, 42, 1103-1105. (j) Bandgar, B. P.; Makone, S. S. *Synth. Commun.* **2006**, 36, 1347-1352. (k) Arote, N. D.; Bhalerao, D. S.; Akamanchi, K. G. *Tetrahedron Lett.* **2007**, 48, 3651-3653. (l) Telvekar, V. N.; Patel, K. N.; Kundiakar, H. S.; Chaudhari, H. K. *Tetrahedron Lett.* **2008**, 49, 2213-2215. (m) Augustine, J. K.; Bombrun, A.; Atta, R. N. *Synlett* **2011**, 15, 2223-2227.
29. (a) Hecker, J. G.; Berger, G. O.; Scarfo, K. A.; Zou, S.; Nantz, M. H. *ChemMedChem* **2008**, 3, 1356-1361. (b) Biswas, S.; Huang, X.; Badger, W. R.; Nantz, M. H. *Tetrahedron Lett.* **2010**, 51, 1727-1729.
30. Sharghi, H.; Sarvari, M. H. *Tetrahedron* **2002**, 58, 10323-10328.
31. Miyatake, K.; Hay, A. S. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, 39, 1854-1859.

R.3. Chapter 3 References

1. Ong S.E.; Mann, M. *Nature Chemical Biology* **2005**, *5*, 252–262.
2. Smith, C. A.; O'Maille, G.; Want, E. J.; Qin, C.; Trauger, S. A.; Brandon, T. R.; Custodio, D. E.; Abagyan, R.; Siuzdak, G. *Ther. Drug Monit.* **2005**, *27*, 747–751.
3. Jordan, K. W.; Nordenstam, J.; Lauwers, G. Y.; Rothenberger, D.A.; Alavi, K.; Garwood, M.; Cheng, L. L. *Diseases of the Colon & Rectum* **2009**, *52*, 520–525.
4. (a) Yang, W.-C.; Mirzaei, H.; Liu, X.; Regnier, F. E. *Anal. Chem.* **2006**, *78*, 4702–4708; (b) Shortreed, M. R.; Lamos, S. M.; Frey, B. L.; Phillips, M. F.; Patel, M.; Belshaw, P. J.; Smith, L. M. *Anal. Chem.* **2006**, *78*, 6398–6403; (c) Tsukamoto, Y.; Santa, T.; Yoshida, H.; Miyano, H.; Fukushima, T.; Hirayama, K.; Imai, K.; Funatsu, T. *Biomed. Chromatogr.* **2006**, *20*, 358–364; (d) Tsukamoto, Y.; Santa, T.; Yoshida, H.; Miyano, H.; Fukushima, T.; Hirayama, K.; Imai, K.; Funatsu, T. *Biomed. Chromatogr.* **2006**, *20*, 1049–1055; (e) Lamos, S. M.; Shortreed, M. R.; Frey, B. L.; Belshaw, P. J.; Smith, L. M. *Anal. Chem.* **2007**, *79*, 5143–5149; (f) Yang, W.-C.; Adamec, J.; Regnier, F. E. *Anal. Chem.* **2007**, *79*, 5150–5157.
5. (a) Bowen, R. D.; Maccoll, A. *Int. J. Mass Spectrom. Ion. Proc.* **1992**, *122*, 337. (b) Horváth, K.; Molnar-Perl, I. *Chromatographia* **1997**, *45*, 321. (c) Molnar-Perl, I.; Horváth, K. *Chromatographia* **1997**, *45*, 328. (d) Katona, Z. F.; Sass, P.; Molnár-Perl, I. *J. Chromatogr. A* **1999**, *847*(1–2), 91. (e) Nazarpak-Kandlousy, N.; Nelen, M. I.; Goral, V.; Eliseev, A. V. *J. Org. Chem.* **2002**, *67*, 59. (f) Seböka, Á.; Sezer, K.; Vasanits-Zsigrai, A.; Helenkára, A.; Záraya, G.; Molnár-Perl, I. *J. Chromatogr. A* **2008**, *1211*(1–2), 104. (g) Füzfa, Z.; Boldizsar, I.; Molnar-Perl, I. *J. Chromatogr. A* **2008**, *1177*, 183. (h) Brokl, M.; Soriab, A. Martínez-Castroa, C. I.; Sanza, M. L.; Ruiz-Matute, A. I. *J. Chromatogr. A* **2009**, *1216*, 4689. (i) Iglesias, J.; Gallardo, J. M.; Medina, I. *Food Chem.* **2010**, *123*, 771. (j) András, N.; Helenkár, A.; Záray, G.; Vasanits, A.; Molnár-Perl, I. *J. Chromatogr. A* **2011**, *1218*, 1878. (k) Mattingly, S. J.; Xu, T.; Nantz, M. H.; Higashi, R. M.; Fan, T. W.-M. *Metabolomics* **2012**, *8*, 989–996.
6. (a) Laffel, L. *Diabetes Metab. Res. Rev.* **1999**, *15*, 412. (b) O'Brien, P. J.; Siraki, A. G.; Shangari, N. *Crit. Rev. Toxicol.* **2005**, *35*, 609. (c) Campbell, M. K.; Farrell, S. O. *Biochemistry* (5th edn). Cengage Learning: Independence, KY, **2006**, 579, ISBN 0534405215.
7. Negre-Salvayre, A.; Auge, N.; Ayala, V.; Basaga, H.; Boada, J.; Brenke, R.; Chapple, S.; Cohen, G.; Feher, J.; Grune, T.; Lengyel, G.; Mann, G. E.; Pamplona, R.; Poli, G.; Portero-otín, M.; Riahi, Y.; Salvayre, R.; Sasson, S.; Serrano, J.; Sham, O.; Siems, W.; Siow, R. C. M.; Wiswedel, I.; Zarkovic, K.; Zarkovic, N. Pathological aspects of lipid peroxidation. *Free Radic. Res.* **2010**, *44*, 1125–1171.

8. Zajdel, A.; Wilczok, A.; Slowinski, J.; Orchel, J.; Mazurek, U. *J. Neurooncol.* **2007**, *84*, 167.
9. Spiteller, G. *Chemistry and Physics of Lipids* **1998**, *95*, 105-162.
10. Kitabchi, A. E.; Umpierrez, G. E.; Murphy, M. B.; Kreisberg, R. A. *Diabetes Care*, **2006**, *29*, 2739.
11. Rochfort, S. *J. Nat. Prod.* **2005**, *68*, 1813.
12. Mazzio, E. A.; Smith, B.; Soliman, K. F. A. *Cell Biol. Toxicol.* **2010**, *26*, 177.
13. Barderas, M. G.; Laborde, C. M.; Posada, M.; de la Cuesta, F.; Zubiri, I.; Vivanco, F.; Alvarez-Llamas, G. *J. Biomed. Biotech.* **2011**, *2011*, 790132.
14. McLafferty, F. W. *Anal. Chem.* **1959**, *31*, 82-87.
15. Turecek, F. McLafferty and related rearrangements Encyclopedia of Mass Spectrometry, Volume 4, Fundamentals of and applications to organic (and organometallic) compounds, N. M. M. Nibbering (Ed). Elsevier: Amsterdam, **2005**, Chapter 4, 396.
16. Bouchoux, Hoppilliard, G. Longevialle, Y. P. *Rapid Commun. Mass Spectrom.* **1987**, *1*, 94-96
17. (a) Oh, S. T.; Choe, J. C.; Kim, M. S. *J. Phys. Chem.* **1996**, *100*, 13367; (b) Stringer, M. B.; Underwood, D. J.; Bowie, J. H.; Allison, C. E.; Donchi, K. F.; Derrick, P. J. *Org. Mass Spectrom.* **1992**, *27*, 270; (c) Osterheld, T. H.; Brauman, J. I. *J. Am. Chem. Soc.* **1990**, *112*, 2014; (d) Breijer, A. J.; Buurmans, H. M. A.; Van De Graaf, B.; Schuijl, P. J. W. Kieboom, A. P. G. *Tetrahedron* **1974**, *30*, 2797; (e) Boer, F. P.; Shannon, T. W.; McLafferty, F. W. *J. Am. Chem. Soc.* **1968**, *90*, 7239.
18. (a) Hudson, C.E.; Griffin, L.L.; McAdoo, D.J. *Org. Mass Spectrom.* **1989**, *24*, 866; (b) Ha, T.-K.; Radloff, C.; Nguyen, M. T. *J. Phys. Chem.* **1986**, *90*, 2991; (c) Liu, R. F.; Pulay, P. *J. Comput. Chem.* **1992**, *13*, 183; (d) Trigueros, P. P.; Casanovas, J.; Aleman, C.; Vega, M. C. *Theochem -J. Mol. Struct.* **1992**, *277*, 117; (e) Norberg, D.; Salhi-Benachenhou, N. *J. Comput. Chem.* **2008**, *29*, 392.
19. Rice, O. K.; Ramsperger, H. C. *J. Am. Chem. Soc.* **1927**, *49*(7), 1617.
20. (a) Nicholson, A. J. C. *Trans. Faraday Soc.* **1954**, *50*, 1067; (b) F. W. McLafferty. *Anal. Chem.* **1956**, *28*, 306.

21. (a) Diekman, J.; MacLeod, J. K.; Djerassi, C.; Baldeschwieler, J. D. *J. Am. Chem. Soc.* **1969**, *91*, 2069. (b) Eadon, G. *J. Am. Chem. Soc.* **1972**, *94*, 8938. (c) Kingston, D. G. I.; Bursey, J. T.; Bursey, M. M. *Chem. Rev.* **1974**, *74*, 215. (d) Levsen, K.; Schwarz, H. *J. Chem. Soc. Perkin Trans. II*, **1976**, *11*, 1231. (e) Litton, J. F.; Kruger, T. L.; Cooks, R. G. *J. Am. Chem. Soc.* **1976**, *98*, 2011. (f) Turecek, F.; Hanus, V. *Org. Mass Spectrom.* **1980**, *15*, 8. (g) Zollinger, M.; Seibl, J. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *47*, 363. (h) Van Baar, B. L. M.; Terlouw, J. K.; Akkk, S.; Zummack, W.; Schwarz, H. *Int. J. Mass Spectrom. Ion Proc.* **1987**, *81*, 217. (i) Bernard, D. M.; Stace, A. J. *Int. J. Mass Spectrom. Ion Proc.* **1988**, *84*(1–2), 215. (j) Bouchoux, G.; Hoppilliard, Y.; Tortajada, J. *Int. J. Mass Spectrom. Ion Proc.* **1989**, *90*, 197. (k) Turecek, F.; Drinkwater, D.E.; McLafferty, F.W. *J. Am. Chem. Soc.* **1990**, *112*, 993. (l) Zha, Q. M.; Hayes, R. N.; Nishimura, T.; Meisels, G. G.; Gross, M. L. *J. Phys. Chem.* **1990**, *94*, 1286. (m) Budzikiewics, H.; Bold, P. *Org. Mass Spectrom.* **1991**, *26*, 709. (n) Kenny, D. V.; Olesik, S. V. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 544. (o) Dohmeierfischer, S.; Kramer, N.; Grutzmacher, H. F. *Eur. Mass Spectrom.* **1995**, *1*, 3. (p) Takayama, M. *Int. J. Mass Spectrom. Ion Proc.* **1995**, *144*, 199. (q) Anbalagan, V.; Patel, J. N.; Niyakorn, G.; Van Stipdonk, M. J. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 291. (r) van der Rest, G.; Jensen, L. B.; Azeim, S. A.; Mourgues, P.; Audier, H. E. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 966. (s) Olah, G. A.; Mathew, T.; Goepfert, A.; Rasul, G.; Prakash, G. K. S.; Esteves, P. M. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 959. (t) Loos, J.; Schrder, D.; Schwarz, H.; Thissen, R.; Dutuit, O. *Int. J. Mass Spectrom.* **2005**, *240*, 121. (u) Loos, J.; Schrder, D.; Schwarz, H. *J. Org. Chem.* **2005**, *70*, 1073. (v) Klyba, L. V.; Nedolya, N. A.; Shlyakhtina, N. I.; Zhanchipova, E. R. *Rus. J. Org. Chem.* **2005**, *41*, 1544. (w) Grossert, J. S.; Cook, M. C.; White, R. L. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1511. (x) Van Stipdonk, M. J.; Kerstetter, D. R.; Leavitt, C. M.; Groenewold, G. S.; Steill, J.; Oomens, J. *Phys. Chem. Chem. Phys.* **2008**, *10*, 3209. (y) Yang, S.-Z.; Liu, X.-Y.; Mu, B.-Z. *J. Mass Spectrom.* **2008**, *43*, 1673. (z) Ramesh, V.; Srinivas, R.; Kumaraswamy, G.; Markondaiah, B. *J. Mass Spectrom.* **2009**, *44*, 285. (aa) Schilderout, S. M.; Masnovi, J. *J. Mass Spectrom.* **2010**, *46*, 572. (bb) Jeilani, Y. A.; Cardelino, B. H.; Ibeanusi, V. M. *J. Mass Spectrom.* **2010**, *45*, 678. (cc) Jobst, K. J.; Bowen, R. D.; Terlouw, J. K. *Int. J. Mass Spectrom.* **2011**, *306*, 9.
22. Mokhtari, J.; Naimi-Jamal, M. R.; Hamzeali, H.; Dekamin, M. G.; Kaupp, G. *ChemSusChem* **2009**, *2*, 248-254.
23. Ortiz-Marciales, M.; Rivera, L. D.; De Jesus, M.; Espinosa, S.; Benjamin, J. A.; Casanova, O. E.; Figueroa, I. G.; Rodriguez, S.; Correa, W. *J. Org. Chem.* **2005**, *70*, 10132-10134.
24. Cisneros, L. O.; Wu, X.; Rogers, W. J.; Mannan, M. S.; Park, J.; North, S. W. *Process. Saf. Environ. Prot.* **2003**, *81*, 121-124.
25. Karabatsos, G. J.; Taller, R. A. *Tetrahedron* **1968**, *24*, 3347-3360.

26. Laulhé, S.; Bogdanov, B.; Johannes, L. M.; Gutierrez, O.; Harrison J. G.; Tantillo, D. J.; Zhang, X.; Nantz, M. H. *J. Mass. Spectrom.* **2012**, *47*, 676-686.
27. Lind, J.; Merenyi, G. *J. Phys. Chem. A* **2006**, *110*, 192.
28. (a) Morton, T. H.; Collisional Activation and Dissociation: Via Ion- Neutral Complexes Encyclopedia of Mass Spectrometry, Volume 1, Theory and Ion Chemistry, P. B. Armentrout (Ed.) Elsevier: Amsterdam, **2003**, 467; (b) Morton, T. H.; Theoretical Models for Ion-Neutral Complexes in Unimolecular Ion Decompositions Encyclopedia of Mass Spectrometry, Volume 4, Fundamentals of and applications to organic (and organometallic) compounds, N. M. M. Nibbering (Ed.) Elsevier: Amsterdam, **2005**, 165.
29. Nsikabaka, S.; Harb, W.; Ruiz-López, M. F. *J. Mol. Struct. (THEOCHEM)* **2006**, *764*(1-3), 161.
30. Felgel, M. *J. Phys. Chem.* **1983**, *87*, 3054.
31. LeMaster, C. B.; True, N. S. *J. Phys. Chem.* **1989**, *93*, 1307.
32. Rusinska-Roszak, D.; Lozynski, M. *J. Prakt. Chem.* **1990**, *332*, 300.
33. Cooks, R. G.; Varvoglis, A. G. *Org. Mass Spectrom.* **1971**, *5*, 687.
34. Zaitsev, K.; Katagi, M.; Kamata, H. T.; Miki, A.; Tsuchihashi, H. *Forensic Toxicol.* **2008**, *26*, 45.

R.4. Chapter 4 References

1. Almstetter, M. F.; Oefner, P. J.; Dettmer, K. *Anal. Bioanal. Chem.* **2012**, *402*, 1993-2013.
2. Wei, R.; Li, G.; Seymour, A. B. *Anal. Chem.* **2010**, *82*, 5527-5533.
3. Koulman, A.; Lane, G. A.; Harrison, S. J.; Volmer, D. A. *Anal. Bioanal. Chem.* **2009**, *394*, 663-670.
4. (a) Dettmer, K.; Aronov, P. A.; Hammock, B. D. *Mass Spectrom. Rev.* **2007**, *26*, 51-78; (b) Theodoridis, G.; Gika, H. G.; Wilson, I. D. *Mass Spectrom. Rev.* **2011**, *30*, 884-906.
5. (a) Courant, F.; Pinel, G.; Bichon, E.; Monteau, F.; Antignac, J. P.; Le Bizec, B. *Analyst* **2009**, *134*, 1637-1646; (b) Lv, H.; Palacios, G.; Hartil, K.; Kurland, I. J. *J. Proteome Res.* **2011**, *10*, 2104-2112; (c) Aoki, M.; Konya, Y.; Takagaki, T.; Umemura, K.; Sogame, Y.; Katsumata T.; Komuro, S. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 1847-1852; (d) Spagou, K.; Wilson, I. D.; Masson, P.; Theodoridis, G.; Raikos, N.; Coen, M.; Holmes, E.; Lindon, J. C.; Plumb, R. S.; Nicholson, J. K.; Want, E. J. *Anal. Chem.* **2011**, *83*, 382-390. (e) Xiao, J. F.; Zhou, B.; Ransom, H. W. *Trends Anal. Chem.* **2012**, *32*, 1-14.
6. (a) Kuhara, T.; Ohse, M.; Inoue, Y.; Cooper, A. J. *Anal. Bioanal. Chem.* **2011**, *400*, 1881-1894; (b) Tsugawa, H.; Bamba, T.; Shinohara, M.; Nishiumi, S.; Yoshida M.; Fukusaki, E. *J. Biosci. Bioeng.* **2011**, *112*, 292-298.
7. Koek, M. M.; Jellema, R. H.; Van der Greef, J.; Tas, A. C.; Hankemeier, T. *Metabolomics* **2011**, *7*, 307-328.
8. (a) Tsukamoto, Y.; Santa, T.; Yoshida, H.; Miyano, H.; Fukushima, T.; Hirayama, K.; Imai, K.; Funatsu, T. *Biomed. Chromatogr.* **2006**, *20*, 358-364; (b) Yang, W.-C.; Mirzaei, H.; Liu, X.; Regnier, F. E. *Anal. Chem.* **2006**, *78*, 4702-4708; (c) Shortreed, M. R.; Lamos, S. M.; Frey, B. L.; Phillips, M. F.; Patel, M.; Belshaw, P. J.; Smith, L. M. *Anal. Chem.* **2006**, *78*, 6398-6403; (d) Lamos, S. M.; Shortreed, M. R.; Frey, B. L.; Belshaw, P. J.; Smith, L. M. *Anal. Chem.* **2007**, *79*, 5143-5149; (e) Yang, W.-C.; Adamec, J.; Regnier, F. E. *Anal. Chem.* **2007**, *79*, 5150-5157.
9. (a) Huang, X.; Regnier, F. E. *Anal. Chem.* **2008**, *80*, 107-114; (b) Kvitvang, H. F. N.; Andreassen, T.; Adam, T.; Villas-Boas, S. G.; Bruheim, P. *Anal. Chem.* **2011**, *83*, 2705-2711; (c) Lien, S. K.; Kvitvang, H. F. N.; Bruheim, P. *J. Chromatogr. A* **2012**, *1247*, 118-124.
10. Fiehn, O. *Trends Anal. Chem.* **2008**, *27*, 261-269.

11. Sobolevsky, T. G.; Revelsky, A. I.; Miller, B.; Oriedo, V.; Chernetsova, E. S.; Revelsky, I. A. *J. Sep. Sci.* **2003**, *26*, 1474-1478.
12. (a) Liberti, A.; Cartoni, G. P.; Bruner, F. *J Chromatogr. A* **1963**, *12*, 8-14; (b) VAN HOOK, A. W. *Advances in Chemistry*, **1969**, *89*, Chapter 7, 99-118; (c) Zhang, R.; Sioma, C. S.; Thompson, R. A.; Xiong, L.; Regnier, F. E. *Anal. Chem.* **2002**, *74*, 3662-3669.
13. Luedemann, A.; Strassburg, K.; Erban, A.; Kopka, J. *Bioinformatics* **2008**, *24*, 732-737.
14. Beynon, J. H.; Saunders, R. A.; Williams, A. E. *Anal. Chem.* **1961**, *33*, 221-225.
15. Merker, R. L.; Scott, M. J. *J. Org. Chem.* **1961**, *26*, 5180-5182.
16. Korlyukov, A. A.; Pogozhikh, S. A.; Ovchinnikov, Y. E.; Lyssenko, K. A.; Antipin, M. Y.; Shipov, A. G. Zamyshlyeva, O. A.; Kramarova, E. P.; Negrebetsky, V. V.; Yakovlev, I. P.; Baukov, Y. I. *J. Organomet. Chem.* **2006**, *691*, 3962-3975.
17. Sakeda, M.; Matsuda A.; Shuto, S. *Tetrahedron* **2005**, *61*, 7865-7873.
18. Yoder, C. H.; Tesno, S. L.; Heaney, S. M.; Bohan, C. *Synthesis and reactivity in inorganic and metal-organic chemistry*, **1985**, *15*, 321-326.
19. Baukov, Y. I. *Zhurnal Obshchei Khimii*, **1989**, *59*, 127-145.
20. Troegel, D.; Lippert, W. P.; Möller, F.; Burschka, C.; Tacke, R. *J. Organomet. Chem.* **2010**, *695*, 1700-1707
21. Evangelisti, C.; Klapötke, T. M.; Krumm, B.; Nieder, A.; Berger, R. J. F.; Hayes, S. A.; Mitzel, N. W.; Troegel, D.; Tacke, R. *Inorg. Chem.* **2010**, *49*, 4865-4880.
22. Grochowski, E.; Jurczak, J. *Synthesis* **1976**, 682-684.
23. Stayshich, R. M.; Meyer, T. Y. *J. Am. Chem. Soc.* **2010**, *132*, 10920-10934.
24. Vailaya, A.; Horvath, C. *J. Chromatogr. A* **1998**, *829*, 1-27.
25. (a) Carpino, L. A. *J. Am. Chem. Soc.* **1960**, *82*, 3133; (b) Marmer, W. N.; Maerker, G. *J. Org. Chem.* **1972**, *37*, 3520-3523
26. Xu, Y.; Suzuki, Y.; Lönnberg, T. ; Komiyama, M. *J. Am. Chem. Soc.* **2009**, *131*, 2871-2874.
27. (a) Hinman, R. L.; Fulton, D. *J. Am. Chem. Soc.* **1958**, *80*, 1895; (b) Theuer, W. J.; Moore, J. A. *J. Org. Chem.* **1964**, *29*, 3734.

28. Condon, F. E. *J. Org. Chem.* **1972**, *37*, 3608-3615.
29. Chromatographic separation of derivative isotopologues has been noted previously; see: Zhang, R.; Regnier, F. E. *J. Proteome Res.* **2002**, *1*, 139-147.
30. Laulhé, S.; Bogdanov, B.; Johannes, L. M.; Gutierrez, O.; Harrison, G. J.; Tantillo, D. J.; Zhang, X.; Nantz, M. H. *J. Mass Spec.* **2012**, *47*, 676-686.
31. (a) Richmond, R.; Pombo-Villar, E. *J. Chromatogr. A* **1997**, *760*, 303-308; (b) Raina, V. K.; Srivastava, S. K.; Jain, N.; Ahmad, A.; Syamasundar, K. V.; Aggarwal, K. K. *Flavour Fragr. J.* **2002**, *17*, 99-102.
32. Antonious, G. F.; Dahlman, D. L.; Hawkins, L. M. *Bull. Environ. Contam. Toxicol.* **2003**, *71*, 400-407.
33. Goh, E.-B.; Baidoo, E. E. K.; Keasling, J. D.; Beller, H. R. *Appl. Environ. Microbiol.* **2012**, *78*, 70-80.
34. Forney, F. W.; Markovetz, A. J. *J. Lipid Res.* **1971**, *12*, 383-395.

R.5. Chapter 5 References

1. “Cancer Facts & Figures 2012“, *American Cancer Society* **2012**.
2. (a) Shapiro S.; Strax P.; Venet L. *Arch. Environ. Health* **1967**, *15*, 547-553; (b) Kopans, D.B. *Surg. Oncol. Clin. N. Am.* **1997**, *6*, 233-263; (c) Devita, V.; Hellman, S.; Rosenberg, S. *Cancer: Principles and Practice of Oncology* (Lippincott, Philadelphia, PA, 1997); (d) Stocchi, L.; Nelson, H. *Dis. Colon Rectum* **1998**, *41*, 232–250.
3. (a) Teates, C.D.; Parekh, J.S. *Curr. Probl. Diagn. Radiol.*, **1993**, *22*, 229–266; (b) Dessureault, S.; Koven, I.; Reilly, R. M.; Couture, J.; Schmocker, B.; Damani, M.; Kirsh, J.; Ichise, M.; Sidlofsky, S.; McEwan, A. J.; Boniface, G.; Stern, H.; Gallinger, S. *Breast Cancer Res. Treat.* **1997**, *45*, 29-37; (c) Pasqualini, R.; Koivunen, E.; Ruoslahti, E. *Nat. Biotechnol.* **1997**, *15*, 542-546.
4. (a) Hawrysz, D. J.; Sevcik-Muraca, E. M. *Neoplasia* **2000**, *2*, 388. (b) Ntziachristos, V.; Tung, C. H.; Bremer, C.; Weissleder, R. *Nat. Med.* **2002**, *8*, 757. (c) Ye, Y.; Li, W. P.; Anderson, C. J.; Kao, J.; Nikiforovich, G. V.; Achilefu, S. *J. Am. Chem. Soc.* **2003**, *125*, 7766-7767.
5. (a) Kim, J.; Yu, W.; Kovalski, K.; Ossowski, L. *Cell*, **1998**, *94*, 353–362; (b) Ye, Y.; Bloch, S.; Xu, B.; Achilefu, S. *J. Med. Chem.* **2006**, *49*, 2268-2275.
6. a) Miseljic, S.; Galandiuk, S.; Myers, S. D.; Wittliff, J. L. *J. Clin. Lab. Anal.* **1995**, *9*, 413-417; (b) Schmitt, M.; Harbeck, N.; Thomssen, C.; Wilhelm, O.; Magdolen, V.; Reuning, U.; Ulm, K.; Hoefler, H.; Jaenicke, F.; Graeff, H. *Thromb. Haemost.* **1997**, *78*, 285-296. (c) Duffy, M. J.; Maguire, T. M.; McDermott, E. W.; O’Higgins, N. *J. Surg. Oncol.* **1999**, *71*, 130-135; (d) Tecimer, C.; Doering, D. L.; Goldsmith, L. J.; Meyer, J. S.; Abdulhay, G.; Wittliff, J. L. *Int. J. Gynecol. Cancer* **2000**, *10*, 372-381; (e) Tecimer, C.; Doering, D. L.; Goldsmith, L. J.; Meyer, J. S.; Abdulhay, G.; Wittliff, J. L. *Gynecol. Oncol.* **2001**, *80*, 48-55; (f) Duffy, M. J. *Clin. Chem.* **2002**, *48*, 1194-1197; (g) Andres, S. A.; Edwards, A. B.; Wittliff, J. L. *J. Clin. Lab. Anal.* **2012**, *26*, 93-103.
7. (a) Chance, R. R.; Prock, A.; Silbey, R. *Adv. Chem. Phys.* **1978**, *37*, 1–65; (b) Dubertret, B.; Calame, M.; Libchaber, A. J. *Nature Biotechnol.* **2001**, *19*, 365–370.
8. (a) Schneider, G.; Decher, G.; Nerambourg, N.; Praho, R.; Werts, M. H. V.; Blanchard-Desce, M. *Nano Lett.* **2006**, *6*, 530–536; (b) Eustis, S.; El-Sayed, M. A.; *Chem. Soc. Rev.* **2006**, *35*, 209-217.
9. Hornillos, V.; Saugar, J. M.; de la Torre, B. G.; Andreu, D.; Rivas, L.; Acuna, A. U.; Amat-Guerri, F. *Bioorganic & Medicinal Chemistry Letters* **2006**, *16*, 5190-5193.

10. Nelson, K. E.; Gamble, L.; Jung, L. S.; Boeckl, M. S.; Naeemi, E.; Golledge, S.; Sasaki, T.; Castner, D. G.; Campbell, C. T.; Stayton, P. S. *Langmuir* **2001**, *17*, 2807-2816.
11. Skaanderup, P. R.; Poulsen, C. S.; Hyldtoft, L.; Jørgensen, M. R.; Madsen, R. *Synthesis* **2002**, 1721-1727.
12. Harrison, G. C.; Diehl, H. *Org. Synth.* **1943**, *23*, 32.
13. Appel, R. *Angew. Chem. Int. Ed.* **1975**, *14*, 801-811.
14. Doi, K.; Togano, E.; Xantheas, S. S.; Nakanishi, R.; Nagata, T.; Ebata, T.; Inokuchi, Y. *Angew. Chem. Int. Ed.* **2013**, *52*, 1-5.
15. Grochowski, E.; Jurczak, J. *Synthesis* **1976**, *10*, 684-684.
16. Mehta, A.; Jaouhari, R.; Benson, T. J.; Douglas, K. T. *Tetrahedron* **1992**, *33*, 5441-5444.
17. (a) Zimmerman, M.; Quigley, J. P.; Ashe, B.; Dorn, C.; Goldfarb, R.; Troll, W. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 750-753; (b) Chung, D.-E.; Kratz, F. *Biorg. & Med. Chem. Lett.* **2006**, *16*, 5157-5163.
18. Achilefu, S.; Ye, Y.; Zhang, Z.; Lee, H.; Fan, J.; Liang, K.; Berezin, M.; Cheney, P.; Edwards, W. *Abstracts of Papers, 236th ACS National Meeting*, Philadelphia, PA, August 17-21, **2008**.
19. (a) Wahl, F.; Mutter, M. *Tetrahedron Lett.* **1996**, *37*, 6861-6864; (b) Brask, J.; Jensen, K. J. *J. Pept. Sci.* **2000**, *6*, 290-299.
20. Decostaire, I. P.; Lelievre, D.; Zhang, H.; Delmas, A. F. *Tetrahedron Lett.* **2006**, *47*, 7057-7060.
21. Duléry, V.; Renaudet, O.; Dumy, P. *Tetrahedron* **2007**, *63*, 11952-11958.
22. Wang, J. Theoretical and Experimental Studies on Manipulation of Fluorescence by Gold Nanoparticles: Application for Molecular Imaging. *University of Louisville Electronic Theses & Dissertations*, **2011**.
<http://digital.library.louisville.edu/cdm/ref/collection/etd/id/2309>
23. Liu, Y.; Bauer, A. Q.; Akers, W.; Sudlow, G.; Liang, K.; Shen, D.; Berezin, M.; Culver, J. P.; Achilefu, S. *Surgery* **2011**, *149*, 689-698.

R.6. Chapter 6 References

1. (a) Harger, M. J. P. *J. Chem. Soc., Chem. Commun.* **1981**, 19, 3284-3288; (b) Armstrong, A.; Pullin, R. D.; Jenner, C. R.; Scutt, J. N. *J. Org. Chem.* **2010**, 75 (10), 3499-502.
2. Li, Y.-T.; Liao, B.-S.; Chen, H.-P.; Liu, S.-T. *Synthesis* **2011**, 16, 2639-2643.
3. Molander, G. A.; Cavalcanti, L. N. *J. Org. Chem.* **2011**, 76, 623-630.
4. Yu, H.; Richey, R. N.; Miller, W. D.; Xu, J.; May, S. A. *J. Org. Chem.* **2011**, 76, 665-668.
5. Suzuki, Y.; Yoshino, T.; Moriyama, K.; Togo, H. *Tetrahedron* **2011**, 67, 3809-3814.
6. Zhou, W.; Xu, J.; Zhang, L.; Jiao, N. *Org. Lett.* **2010**, 12, 2888-2891.
7. Mo, J.; Xu, L.; Xiao, J. *J. Am. Chem. Soc.* **2005**, 127, 751-760.
8. Benati, L.; Leardini, R.; Minozzi, M.; Nanni, D.; Scialpi, R.; Spagnolo, P.; Strazzari, S.; Zanardi, G. *Angew. Chem. Int. Ed.* **2004**, 43, 3598-3601.
9. Yu, H.; Richey, R. N.; Miller, W. D.; Xu, J.; May, S. A. *J. Org. Chem.* **2011**, 76, 665-668.
10. Goble, S. D.; Mills, S. G.; Yang, L.; Pasternak, A. Bonnefous, C.; Kamenecka, T. M.; Vernier, J.-M.; Hutchinson, J. H.; Hu, E.; Govek, S. Benzoxazinyl-amidocyclopentyl-heterocyclic modulators of chemokine receptors. U.S. Patent 7,700,591, April, 20, 2010.
11. Augustine, J. K.; Bombrun, A.; Atta, R. N. *Synlett* **2011**, 15, 2223-2227.
12. Nakao, Y.; Yada, A.; Hiyama, T. *J. Am. Chem. Soc.* **2010**, 132, 10024-10026.
13. Kidwai, M.; Bhardwaj, S. *Synthetic Communications* **2011**, 41, 2655.
14. Parsons, S. R.; Hooper, J. F.; Willis, M. C. *Organic Letters* **2011**, 13, 998.
15. Kofron, W. G.; Yeh, M.-K. *J. Org. Chem.* **1976**, 41, 439.
16. Bailey, P. S.; Lerdal, D. A. *J. Am. Chem. Soc.* **1978**, 100, 5820.
17. a) Becke, A. D. *J. Chem. Phys.* **1993**, 98, 5648; (b) Becke, A. D. *J. Chem. Phys.* **1993**, 98, 1372; (c) Lee, C.; Yang, W. *Phys. Rev. B* **1988**, 37, 785; (d) Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, 98, 11623.

18. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Montgomery, J. A.; Vreven, Jr. T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, J.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. GAUSSIAN03, Revision D.01, Gaussian, Inc., Wallingford CT, **2004**.
19. For leading references, see: (a) Wiest, O.; Montiel, D. C.; Houk, K. N. *J. Phys. Chem. A* **1997**, *101*, 8378; (b) Guner, V. A.; Khuong, K. S.; Houk, K. N.; Chuma, A.; Pulay, P. *J. Phys. Chem.* **2004**, *108*, 2959; (c) Saettel, N.; Oxgaard, J.; Wiest, O. *Eur. J. Org. Chem.* **2001**, *8*, 1429; (d) Wiest, O.; Oxgaard, J.; Saettel, N. J. *Adv. Phys. Org. Chem.* **2003**, *38*, 87.
20. Zhao, Y.; Truhlar, D. G. *Theor. Chem. Acc.* **2008**, *120*, 215.
21. Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618.
22. Gerenkamp, M.; Grimme, S. *Chem. Phys. Lett.* **2004**, *392*, 229.
23. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, Jr., J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. GAUSSIAN09, Revision B.01, Gaussian, Inc.: Wallingford CT, **2010**.
24. (a) Gonzalez, C.; Schlegel, H. B. *J. Phys. Chem.* **1990**, *94*, 5523; (b) Fukui, K. *Acc. Chem. Res.* **1981**, *14*, 363.

25. Müller, N.; Falk, A.; Gsaller, G. Ball & Stick V.4.0a12, molecular graphics application for MacOS computers. Johannes Kepler University: Linz, **2004**.
26. Evangelisti, C.; Klapötke, T. M.; Krumm, B.; Nieder, A.; Berger, R. J. F.; Hayes, S. A.; Mitzel, N. W.; Troegel, D.; Tacke, R. *Inorg. Chem.* **2010**, *49*, 4865–4880.
27. Xu, Y.; Suzuki, Y.; Lönnberg, T.; Komiyama, M. *J. Am. Chem. Soc.* **2009**, *131*, 2871–2874.
28. Banaszak, E.; Xu, L.-W.; Bardeau, J.-F.; Castanet, A.-S.; Mortier, J. *Tetrahedron* **2009**, *65*, 3961–3966.
29. (a) Oretskaya, T. S.; Romanova, E. A.; Andreev, S. Y.; Antsyovich, S. I.; Toth, C.; Gajdos, V.; Hianikb, T. *Bioelectrochemistry* **2002**, *56*, 47–51; (b) Tanaka, M.; Sawaguchi, T.; Sato, Y.; Yoshioka, K.; Niwa, O. *Tetrahedron Letters* **2009**, *50*, 4092–4095;
30. Hahn, C. D.; Tinazli, A.; Hölzl, M.; Leitner, C.; Frederix, F.; Lackner, B.; Müller, N.; Klampfl, C.; Tampé, R.; Gruber, H. J. *Monatshefte für Chemie* **2008**, *138*, 245–252.
31. Hornillos, V.; Saugar, J. M.; de la Torre, B. G.; Andreu, D.; Rivas, L.; Acuna, A. U.; Amat-Guerri, F. *Bioorganic & Medicinal Chemistry Letters* **2006**, *16*, 5190–5193.

APPENDIX A

Spectra

A.1. Index of NMR Spectra and Other Spectroscopic Data

A.2. Selected NMR Spectra and Spectroscopic Data from Chapter 2

A.3. Selected NMR Spectra and Spectroscopic Data from Chapter 3

A.4. Selected NMR Spectra and Spectroscopic Data from Chapter 4

A.5. Selected NMR Spectra and Spectroscopic Data from Chapter 5

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<hr/>		

DPPH: ^{31}P NMR (162 MHz) CDCl_3

37.485

Benzonitrile (Entry 1): ^1H NMR (400 MHz) CDCl_3

Benzonitrile (Entry 1): ^{13}C NMR (100 MHz) CDCl_3

3-Phenylpropionitrile (entry 6): ^1H NMR (400 MHz) CDCl_3

3-Phenylpropionitrile (entry 6): ^{13}C NMR (100 MHz) CDCl_3

4-(Acetyloxy)butanenitrile (entry 10): ^1H NMR (700 MHz) CDCl_3

4-(Acetyloxy)butanenitrile (entry 10): ^{13}C NMR (176 MHz) CDCl_3

***N*-Boc-4-(cyanomethyl)piperidine (entry 12): ^1H NMR (400 MHz) CDCl_3**

***N*-Boc-4-(cyanomethyl)piperidine (entry 12): ^{13}C NMR (100 MHz) CDCl_3**

Ethyl 2-(3-cyano-1H-indol-1-yl)acetate (entry 14): ^1H NMR (400 MHz) CDCl_3

Ethyl 2-(3-cyano-1H-indol-1-yl)acetate (entry 14): ^{13}C NMR (100 MHz) CDCl_3

1-Naphthaldehyde *O*-diphenylphosphoryl oxime (2.2): ^{13}C NMR (100 MHz) CDCl_3

1-Naphthaldehyde *O*-diphenylphosphoryl oxime (2.2): ^{31}P NMR (162 MHz) CDCl_3

Hexanal *O*-(tert-butyldimethylsilyl) oxime (3.9): ^1H NMR (400 MHz) CDCl_3

Hexanal *O*-(tert-butyl(dimethylsilyl) oxime (3.9): ^{13}C NMR (100 MHz) CDCl_3

2-Propoxyacetaldehyde *O*-(tert-butyltrimethylsilyl) oxime (3.11): ^1H NMR (400 MHz) CDCl_3

2-(Aminoxy)ethyl 3,3,3-²H₃-propionate (AEP-32): ¹H NMR (400 MHz) CDCl₃

2-(Aminooxy)ethyl 3,3,3-²H₃-propionate (AEP-32): ¹³C NMR (100 MHz) CDCl₃

2-(Aminooxy)ethyl 3-¹³C-3,3,3-²H₃-propionate (AEP-33): ¹H NMR (400 MHz) CDCl₃

2-(Aminooxy)ethyl 3-¹³C-3,3,3-²H₃-propionate (AEP-33): ¹³C NMR (100 MHz) CDCl₃

2-(Aminooxy)ethyl 2,3-¹³C₂-3,3,3-²H₃-propionate (AEP-34): ¹H NMR (400 MHz) CDCl₃

2-(Aminoxy)ethyl 2,3-¹³C₂-3,3,3-²H₃-propionate (AEP-34): ¹³C NMR (100 MHz) CDCl₃

2,5-Dioxopyrrolidin-1-yl 3,3,3-²H₃-propionate (NHS-32): ¹H NMR (400 MHz) CDCl₃

2,5-Dioxopyrrolidin-1-yl 3,3,3-²H₃-propionate (NHS-32): ¹³C NMR (100 MHz) CDCl₃

2,5-Dioxopyrrolidin-1-yl 3-¹³C-3,3,3-²H₃-propionate (NHS-33): ¹H NMR (400 MHz) CDCl₃

2,5-Dioxopyrrolidin-1-yl 3-¹³C-3,3,3-²H₃-propionate (NHS-33): ¹³C NMR (100 MHz) CDCl₃

2,5-Dioxopyrrolidin-1-yl 2,3-¹³C₂-3,3,3-²H₃-propionate (NHS-34): ¹H NMR (400 MHz) CDCl₃

2,5-Dioxopyrrolidin-1-yl 2,3-¹³C₂-3,3,3-²H₃-propionate (NHS-34): ¹³C NMR (100 MHz) CDCl₃

1-(Aminoxy)-3,6,9,12,15,18,21,24-octaohexatriacontane-36-thiol (5.13a-PEG(8)):
¹H NMR (400 MHz) CDCl₃

5.13a-PEG(8)

1-(Aminooxy)-3,6,9,12-tetraoxaoctacosane-28-thiol (5.13b-PEG(4)): ^1H NMR (500 MHz) CDCl_3

1-(Aminooxy)-3,6,9,12,15,18-hexaoxatetracontane-34-thiol (5.13b-PEG(6)):
¹H NMR (500 MHz) CDCl₃

Mass spectrum of the peptide amide 542.58689. The x-axis represents the mass-to-charge ratio (m/z) from 480 to 660, and the y-axis represents the relative intensity from 0 to 100. The base peak is at m/z 535.24119. Other labeled peaks include 480.26590, 501.72739, 528.92373, 542.58689, 555.05337, 577.30237, 603.20200, 630.47748, and 657.97908.

338

HRMS of cypate bis(aldehyde) 5.22

$C_{49}H_{55}N_4O_4^+$ $[M^+]$, calculated 763.4218, found 763.4223

HRMS of cypate mono-aldehyde linker 5.25

C₇₇H₁₁₂N₅O₁₂S⁺, [M⁺] calculated 1330.8023, found 1330.8015.

NL:
2.28E5
liquid final isolate 924#1-26
RT: 0.02-1.96 AV: 26 T: FTMS
+ p ESI Full ms2
924.00@cid0.00
[250.00-2000.00]

NL:
7.57E3
C₉₅ H₁₄₃ N₁₅ O₁₈ S₂ +H:
C₉₅ H₁₄₅ N₁₅ O₁₈ S₂
p (gss, s /p:40) Chrg 2
R: 50000 Res .Pwr . @FWHM

C₉₅H₁₄₅N₁₅O₁₈S₂⁺ [M⁺ + H⁺]/2 calculated 924.0161, found 924.0185 (m/z where z = 2).

APPENDIX B

List of Publications

B.1. List of Publications

B.2. Copyright Permissions

B.3. Manuscripts Title Page

B.1. List of Publications

The work presented in this thesis was published in various peer-reviewed journals. The following list summarizes the publications that arose from my research, and the chapters in which the work is described.

Publications:

1. Laulhé, S.; Nantz, M. H. An Improved Preparation of *O*-Diphenylphosphinylhydroxylamine. *Organic Preparations & Procedures International* **2011**, 43, 475-476. [Chapter 2]
2. Laulhé, S.; Gori, S. S.; Nantz, M. H. A Chemoselective, One-Pot Transformation of Aldehydes to Nitriles. *Journal of Organic Chemistry* **2012**, 77, 9394-9337. [Chapter 2]
3. Laulhé, S.; Bogdanov, B.; Johannes, L. M.; Gutierrez, O.; Harrison J. G.; Tantillo, D. J.; Zhang, X.; Nantz, M. H. Fragmentation of Oxime and Silyl-Oxime Ether Odd-Electron Positive Ions by the McLafferty Rearrangement: New Insights on Structural Factors that Promote α,β -Fragmentation. *Journal of Mass Spectrometry* **2012**, 47, 676-686. [Chapter 3]
4. Laulhé, S.; Geers, T. E.; Shi, X.; Zhang, X.; Nantz, M. H. Electron Ionization–Induced Release of Coded Isotopic Reporter Ions in an *m/z* Zone of Minimal Interference for Quantifiable, Multiplexed GC-MS Analyses. Submitted to *Analyst*. [Chapter 4]
5. Laulhé, S.; Shi, X.; Zhang, X.; Nantz, M. H. Coded Isotopic Reporter Ions for Analysis of Amine Metabolites Using Chemoselective Labeling Reagent for Quantifiable Multiplexed GC-MS. *Manuscript in preparation (to be submitted to Analytical Chemistry)*. [Chapter 4]
6. a) Kang, K. A.; Wang, J.; O'Toole, M. G.; Nantz, M. H.; Moore, J. D.; Laulhé, S.; Achilefu, S. Sensitivity Enhancement of NIR Fluorescence Contrast Agent Utilizing Gold Nanoparticles. *Advances in Experimental Medicine and Biology* **2012**, 737, 285-291. [Chapter 5]
7. Wang, J.; Moore, J. D.; Laulhé, S.; Nantz, M. H.; Achilefu, S.; Kang, K. A. Fluorophore-Gold Nanoparticle Complex for Sensitive Optical Biosensing and Imaging. *Nanotechnology* **2012**, 23, 095501 (13pp). [Chapter 5]

B.2. Copyright Permissions

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Title: An Improved Preparation of O-(Diphenylphosphinyl)hydroxylamine
Author: Sébastien Laulhé, Michael H. Nantz
Publication: Organic Preparations and Procedures International: The New Journal for Organic Synthesis
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Title: A Chemoselective, One-Pot Transformation of Aldehydes to Nitriles
Author: Sébastien Laulhé, Sadakatali S. Gori, and Michael H. Nantz
Publication: The Journal of Organic Chemistry
Publisher: American Chemical Society
Date: Oct 1, 2012
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Title: Fragmentation of oxime and silyl oxime ether odd-electron positive ions by the McLafferty rearrangement: new insights on structural factors that promote α,β fragmentation

Author: Sébastien Laulhé, Bogdan Bogdanov, Leah M. Johannes, Osvaldo Gutierrez, Jason G. Harrison, Dean J. Tantillo, Xiang Zhang, Michael H. Nantz

Publication: Journal of Mass Spectrometry

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B.3. Manuscripts

Organic Preparations and Procedures International, 43:475–476, 2011
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OPPI BRIEFS

An Improved Preparation of *O*-(Diphenylphosphinyl)hydroxylamine

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O-(Diphenylphosphinyl)hydroxylamine (DPPH) is a versatile electrophilic amination reagent that has been used widely for the amination of stable carbanions, Grignard reagents and π -electron-rich nitrogen heterocycles.^{1–4} More recently, Armstrong *et al.* have used DPPH for the aziridination of enones.⁵ Compared to other hydroxylamine derivatives that typically are stored as hydrochloride salts, this activated phosphinyl reagent exhibits good stability and can be stored indefinitely at 0°C.

DPPH is prepared in a single step from hydroxylamine hydrochloride and diphenylphosphinic chloride (**1**).⁶ However, the general and most used procedure, developed by Colvin *et al.*,¹ is not reliable. Yields ranging from 42% to a maximum of 70% have been reported using this method.^{7,8} In our hands, we found Colvin's method to be sensitive to reaction scale, with multigram attempts generally delivering DPPH in *ca.* 30–60% yield. Our interest in aminooxy chemistry⁹ led us to develop a reproducible, higher yield synthesis of DPPH. We report herein a convenient procedure for the preparation of DPPH on multigram scale.

Adaptation of Schotten-Baumann conditions¹⁰ using a two-phase solvent system helped minimize hydrolysis of starting chloride **1** prior to its reaction with hydroxylamine and afforded DPPH in 78% yield on a 10-gram scale. The yield of DPPH formed using these conditions did not vary significantly when the reaction scale was decreased (*e. g.*, 4-gram scale gave an 84% yield of DPPH). Product purity, generally compromised by the presence of $\text{Ph}_2\text{P}(\text{O})\text{OH}$ prior to base treatment, was readily assessed using ³¹P NMR (CDCl_3): DPPH, δ 37.5; $\text{Ph}_2\text{P}(\text{O})\text{OH}$ δ 28.3.

Experimental Section

To a stirred solution (mechanical stirring) of hydroxylamine hydrochloride (6.46 g, 93.0 mmol) in water (20 mL) at –10°C was added a solution of sodium hydroxide (3.55 g,

Submitted May 20, 2011.

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A Chemoselective, One-Pot Transformation of Aldehydes to Nitriles

Sébastien Laulhé, Sadakatali S. Gori, and Michael H. Nantz*

Department of Chemistry, University of Louisville, Louisville, Kentucky 40292, United States

Supporting Information

ABSTRACT: This paper describes a procedure for direct conversion of aldehydes to nitriles using *O*-(diphenylphosphinyl)hydroxylamine (DPPH). Aldehydes are smoothly transformed to their corresponding nitriles by heating with DPPH in toluene. The reaction can be accomplished in the presence of alcohol, ketone, ester, or amine functionality.

The preparation of nitriles by transformation of carbon-equivalent functional groups is an important synthetic route to these versatile intermediates and valued target pharmacophores.¹ Among the most popular approaches is the oxidation-state neutral conversion of carboxylic acids to nitriles via dehydration of intermediate primary amides (Figure 1) either

Figure 1. Common functional group interconversions to prepare nitriles.

using traditional dehydrating reagents² or recently developed metal-mediated procedures.³ An efficient complement to amide dehydration is the oxidative transformation of aldehydes to nitriles. Isolation of aldoxime intermediates (Figure 1) generally is followed by activation of the oxime hydroxyl group (e.g., as a sulfonate ester derivative) and then its elimination to afford the nitrile.⁴ The appeal of this approach has led to several one-pot methods for direct synthesis of nitriles from aldehydes using either hydroxylamine or ammonia in combination with a variety of activating reagents.⁵

Unfortunately, the accompanying reagents for these one-pot approaches, such as $\text{CuCl}_2/\text{NaOMe}/\text{O}_2$,^{5a} $\text{Pb}(\text{OAc})_4$,^{5b} Oxone,^{5c} H_2O_2 ,^{5d} I_2 ,^{5e} NBS,^{5f} IBX,^{5g} and NaClO_2 ,^{5h} often are not tolerant of other functional groups or require somewhat harsh conditions to effect the transformation. Ideally, an aldehyde-selective reagent that would facilitate the conversion to the nitrile under neutral conditions would greatly expand the utility of this direct approach. We report here the use of *O*-(diphenylphosphinyl)hydroxylamine (DPPH) as such a reagent.

Our interest in aminoxy chemistry⁶ led us to consider the use of DPPH ($\text{Ph}_2\text{P}(\text{O})\text{ONH}_2$) as a possible chemoselective alternative to hydroxylamine or ammonia for introduction of nitrogen onto the carbonyl carbon of aldehydes. Since the reaction of this reagent with an aldehyde would directly form an

activated oxime ester as an intermediate (e.g., **1**, Scheme 1), we reasoned that it should be possible to thermally induce an

Scheme 1

electrocyclic rearrangement resulting in the elimination of diphenylphosphinic acid. A similar mechanism involving elimination of methanesulfonic acid has been proposed for formation of nitriles from intermediate sulfonylated aldoximes.⁷ Although DPPH is well appreciated as an electrophilic reagent for the amination of a variety of nucleophiles,⁸ including Grignard reagents,⁹ enols,¹⁰ or for amination of tertiary amines in syntheses of aziridines from α,β -unsaturated carbonyl substrates,¹¹ its use as a nucleophilic counterpart, especially in chemoselective “click” transformations, has received limited attention.¹² To test the action of DPPH as a suitable reagent for oxime ester formation as well as the subsequent elimination to the nitrile, we examined the reaction between DPPH and α -naphthaldehyde (Scheme 1).

While presently not commercially available, DPPH can be readily prepared in good yield in one step from commercially available diphenylphosphinic chloride and hydroxylamine hydrochloride.¹³ Reaction of DPPH with naphthaldehyde in THF gave oxime ester **1** in 79% yield. Subsequent heating in toluene revealed that the elimination of diphenylphosphinic acid from **1** required warming to above 80 °C to achieve a significant rate of formation of naphthonitrile (**2**). Of particular note is that no

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Fragmentation of oxime and silyl oxime ether odd-electron positive ions by the McLafferty rearrangement: new insights on structural factors that promote α,β fragmentation

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The McLafferty rearrangement is an extensively studied fragmentation reaction for the odd-electron positive ions from a diverse range of functional groups and molecules. Here, we present experimental and theoretical results of 12 model compounds that were synthesized and investigated by GC-TOF MS and density functional theory calculations. These compounds consisted of three main groups: carbonyls, oximes and silyl oxime ethers. In all electron ionization mass spectra, the fragment ions that could be attributed to the occurrence of a McLafferty rearrangement were observed. For *t*-butyldimethylsilyl oxime ethers with oxygen in a β -position, the McLafferty rearrangement was accompanied by loss of the *t*-butyl radical. The various mass spectra showed that the McLafferty rearrangement is relatively enhanced compared with other primary fragmentation reactions by the following factors: oxime *versus* carbonyl, oxygen *versus* methylene at the β -position and ketone *versus* aldehyde. Calculations predict that the stepwise mechanism is favored over the concerted mechanism for all but one compound. For carbonyl compounds, C–C bond breaking was the rate-determining step. However, for both the oximes and *t*-butyldimethylsilyl oxime ethers with oxygen at the β -position, the hydrogen transfer step was rate limiting, whereas with a CH₂ group at the β -position, the C–C bond breaking was again rate determining. *n*-Propoxy-acetaldehyde, bearing an oxygen atom at the β -position, is the only case that was predicted to proceed through a concerted mechanism. The synthesized oximes exist as both the (*E*)- and (*Z*)-isomers, and these were separable by GC. In the mass spectra of the two isomers, fragment ions that were generated by the McLafferty rearrangement were observed. Finally, fragment ions corresponding to the McLafferty reverse charge rearrangement were observed for all compounds at varying relative ion intensities compared with the conventional McLafferty rearrangement. Copyright © 2012 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: silyl oxime ethers; GC-TOF MS; concerted; stepwise; computational study

INTRODUCTION

The analysis of cellular aldehyde and ketone species is of considerable importance for the field of metabolomics.^[1–3] Aldehydes and ketones can be formed endogenously by various biochemical pathways. For example, aldehydes can be formed by lipid peroxidation, carbohydrate metabolism and ascorbate autooxidation as well as by various enzymatic processes, such as those involving amine oxidase, cytochrome P-450 or myeloperoxidase.^[4] Endogenous ketones, such as acetone and acetoacetic acid, are molecules produced as by-products when fatty acids are broken down for energy in the liver and kidney.^[5] Other ketones, such as β -ketopentanoate, may be created as a result of the metabolism of synthetic triglycerides.^[6] Furthermore, ketone bodies, such as acetone, are produced from acetyl-CoA mainly in the mitochondrial matrix of hepatocytes when carbohydrates are so scarce that energy must be obtained from breaking down fatty acids.^[7] Acetone is responsible for the characteristic “fruity” odor of the breath of persons with ketoacidosis.^[8]

To isolate these carbonyl metabolites for analysis by mass spectrometry, their selective conversion into labeled oximes or oxime ether analogs by water-based “click chemistry” (i.e. oximation) has become an elegant and highly chemo-specific approach.^[9,10] Oxime chemistry and subsequent silylations to form silyl oxime ethers have been used to detect and analyze natural and synthetic steroids,^[11] trisaccharides^[12–15] and other classes of compounds^[16,17] by gas chromatography–mass spectrometry (GC-MS). Oximation proceeds rapidly under

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Chapter 42

Sensitivity Enhancement of NIR Fluorescence Contrast Agent Utilizing Gold Nanoparticles

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1 Introduction

Indocyanine green (ICG) is one of a few, if not the only, near infrared (NIR) contrast agents that can be used for humans. The quantum yield of this fluorophore is only 0.0028 and 0.012, in water and plasma [1], respectively, and, therefore, artificially enhancing its quantum yield is highly desired. Since fluorescence of a fluorophore is initiated by optically exciting its electrons, one way of changing the fluorescence level is by placing it near an electric field. Gold nanoparticles (GNPs) generate a strong electric (plasmon) field on and around them upon receiving light [2, 3]. Therefore, they can be good candidates for influencing the electron status of a fluorophore [3]. Gold has been used extensively in bio-applications because of its chemical inertness.

The strength of the plasmon field generated by a GNP is the highest on its surface and decays rapidly with the distance from the GNP surface [4, 5]. If the field strength that increases the fluorescence of a fluorophore is known, then one can achieve a higher fluorescence by placing the fluorophore at the distance from a GNP with this particular field strength.

Here, we present the relationship between the plasmon field strength on/around a GNP and fluorescence of Cypate [6] (an ICG-based NIR fluorophore), which may be helpful for developing more effective and safe NIR optical contrast agents.

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Fluorophore–gold nanoparticle complex for sensitive optical biosensing and imaging

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Abstract

Fluorophores have been extensively used as the signal mediator in biosensing and bioimaging for a long time. Enhancement of fluorescence can amplify the signal, thus improving the sensitivity, enabling earlier and accurate disease detection and diagnosis. Some metal nanoparticles, such as gold and silver, can generate a strong electromagnetic field on their surface (surface plasmon field) upon receiving photonic energy. When a fluorophore is placed in the field, the field can affect the fluorophore electrons participating in fluorescence emission and change the fluorescence output. The change can be from complete quenching to significant enhancement, depending on the metal type, particle size and shape, excitation/emission wavelengths and quantum yield of the fluorophore, and the distance between the fluorophore and the particle surface. In this study, the effects of these parameters on the fluorescence enhancement of commonly used fluorophores by gold nanoparticles (GNPs) are theoretically analyzed. Experimentally, an NIR contrast agent with enhanced fluorescence was developed by carefully tailoring the distance between Cypate (ICG based fluorophore) and a GNP, via biocompatible spacer constructs. The effect of the GNP size (3.7–16.4 nm) and spacer length (3.2–4.6 nm) on fluorescence enhancement was studied, and the spacer length that provided the significant enhancement was determined. The spacer of 3.9 nm with 16.4 nm GNP provided the fluorescence of 360% of the control. The experimental data qualitatively agreed with the theoretical results and, thus, the theoretical analysis can be used as a guide for significantly improving the sensitivity of existing fluorescent contrast agents by properly utilizing GNPs and spacers.

1. Introduction

In fluorophore-mediated biosensing/imaging, fluorophores generating higher fluorescence per fluorophore molecule are highly desirable because they can provide better sensitivity with higher signal-to-noise ratio. Since fluorescence is generated by the electrons excited by photonic energy, a localized entity affecting the electron state of the fluorophore can change the fluorescence output [1, 2]. Nanosized metal particles forming a strong electromagnetic (plasmon) field can be, therefore, good candidates for this purpose [1, 3]. Noble

metal nanoparticles are known to form a strong plasmon field around them upon receiving light in the visible range [4–7]. Gold nanoparticles (GNPs) are appropriate for the biomedical application because they form a strong plasmon field and are chemically stable and non-toxic. Also its surface modification chemistry is well established [8–10].

Utilizing GNPs for enhancing the fluorescence of fluorophores has been previously explored by several researchers. Nakamura *et al* [11] studied the level of enhancement for the fluorophore Rose Bengal with respect to the GNP size; Kuhn *et al* [12] and Anger *et al* [13]

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EDUCATION

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Ph.D., Chemistry (May 2013)
<i>Thesis:</i> Aminooxy Reagents for Synthesis and Analysis: Expanding the Role of Oximation
2006-2009	ENSCM (National Engineering Graduate School of Chemistry), Montpellier, France
M.S., Chemical Engineering
2003-2007	ENSCM , Montpellier, France
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FELLOWSHIPS and AWARDS

- 2013 Recipient, The John Richard Binford Memorial Award for excellence in scholarship and leadership, *University of Louisville, School of Interdisciplinary Graduate Studies (SIGS)*
- 2013 The Graduate Dean's Citation, *University of Louisville, SIGS*
- 2013 Arno Spatola Endowment Graduate Research Fellowship, *University of Louisville, Institute for Molecular Diversity and Drug Design (IMD³)*
- 2012 1st Place, Graduate Student Presentation, *14th Annual IMD³ Symposium*
- 2011 Recipient, Celebration Award, *University of Louisville, Cultural Center*
- 2010 Recipient, International Student Award, *University of Louisville, International Center*
- 2008 Graduate Research Fellow, *University of Louisville, Department of Chemistry*

RESEARCH EXPERIENCE

University of Louisville, Department of Chemistry (2008-Present)

Advisor: Dr. Michael H. Nantz

- Developed a new strategy for quantitative high throughput GC-MS analysis of biological samples using a ‘click-chemistry’ approach for the derivatization of carbonyl metabolites. Achieved the first electron impact-induced isotopic tag delivery in a zone of minimal interference for relative quantification using GC-MS.
- Developed a flexible synthetic route to heterobifunctional thiol- and aminooxy-terminated diblock linkers for attachment of substrates to gold nanoparticles. The route provides for control over linker length and terminal functionality.
- Optimized the use of *O*-(diphenylphosphinyl)hydroxylamine as a chemoselective reagent for the one-pot transformation of aldehydes to nitriles.

Bel Brands, France (2008, 3 month summer research internship)

- Performed a study on the biosynthesis of aromatic molecules for cheese-based products.

ENSCM, Institut Charles Gerhardt (2007, 3 month summer research internship)

- Worked on the synthesis of hybrid silica materials with molecular recognition properties for melamine.

TEACHING EXPERIENCE

<i>Instructor:</i>	Undergraduate Organic Chemistry (CHEM 341), University of Louisville, Summer 2013. Undergraduate Organic Chemistry Lab (CHEM 343), University of Louisville, Summer 2013.
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PUBLICATIONS

S Laulhé, X Shi, TE Geers, X Zhang and MH Nantz. Coded Isotopic Reporter Ions for Analysis of Amine Metabolites Using Chemoselective Labeling Reagent for Quantifiable Multiplexed GC-MS. *Manuscript in preparation (to be submitted to Analytical Chemistry)*.

AN Lane, S Arumugam, P Lorkiewicz, R Higashi, **S Laulhé**, MH Nantz, HNB Moseley, TWM Fan. Chemoselective Detection of Carbonyl Compounds in Metabolite Mixtures by NMR. *Manuscript in preparation (to be submitted to Journal of the American Chemical Society)*.

S Laulhé, TE Geers, X Shi, X Zhang and MH Nantz. Electron Ionization–Induced Release of Coded Isotopic Reporter Ions in an *m/z* Zone of Minimal Interference for Quantifiable, Multiplexed GC-MS Analyses. Submitted to *Analyst*.

S Laulhé, SS Gori and MH Nantz. A Chemoselective, One-Pot Transformation of Aldehydes to Nitriles. *Journal of Organic Chemistry* **2012**, 77, 9334-9337.

S Laulhé, B Bogdanov, LM Johannes, O Gutierrez, GJ Harrison, DJ Tantillo, X Zhang and MH Nantz. Fragmentation of Oxime and Silyl-Oxime Ether Odd-Electron Positive Ions by the McLafferty Rearrangement: New Insights on Structural Factors that Promote α,β –Fragmentation. *Journal of Mass Spectrometry* **2012**, 47, 676-686.

KA Kang, J Wang, MG O'Toole, MH Nantz, JD Moore, **S Laulhé** and S Achilefu. Sensitivity Enhancement of NIR Fluorescence Contrast Agent Utilizing Gold Nanoparticles. *Advances in Experimental Medicine and Biology* **2012**, 737, 285-291.

J Wang, JD Moore, **S Laulhé**, MH Nantz, S Achilefu and KA Kang. Fluorophore-Gold Nanoparticle Complex for Sensitive Optical Biosensing and Imaging. *Nanotechnology* **2012**, 23, 095501 (13pp).

S Laulhé and MH Nantz. An Improved Preparation of *O*-Diphenylphosphinylhydroxylamine. *Organic Preparations & Procedures, Int.* **2011**, 43, 475-476.

CONFERENCES and PRESENTATIONS

S Laulhé, X Shi, TE Geers, X Zhang and MH Nantz. Quantifiable Multiplexed GC-MS Analysis of Carbonyl and Amine Metabolites Using the Electron-Induced Cleavage of an Isotopic Reporter Tag. Poster No. 13, 15th Annual IMD³ Symposium, Louisville, KY; March 12, 2013.

AN Lane, S Arumugam, P Lorkiewicz, R Higashi, **S Laulhé**, MH Nantz, HNB Moseley, TWM Fan. Chemoselective Detection of Carbonyl Compounds in Metabolite Mixtures by NMR. Poster, Keystone Symposia: Frontiers of NMR in Biology, Snowbird, UT; January 15, 2013.

S Laulhé, TE Geers, X Shi, X Zhang and MH Nantz. Multiplexed Metabolite Quantitation Using an Isotope Coding Strategy for GC-MS Analysis. Oral, SERMACS, Raleigh, NC; November 16, 2012.

S Laulhé. Aminooxy Applications in Synthesis and Multiplexed Mass Spectrometry. Oral, Columbus American Chemical Society, Columbus, OH; September 20, 2012.

S Laulhé, LM Johannes, TE Geers, Y Zhang, X Shi, X Zhang and MH Nantz. Strategy for Relative Quantification of Metabolites via Multiplexed GC-MS Analysis. Poster No. 110, 244th ACS National Meeting and Exposition, Philadelphia, PA; August 19, 2012.

MH Nantz, SJ Mattingly, **S Laulhé** and S Biswas. Oximation Applications for Analysis of Carbonyl Metabolites. Presented at the Sino-US Forum on Chemical Biology, 28th Chinese Chemical Society Congress, Chengdu, China; April 13, 2012.

S Laulhé, L Johannes, T Geers, Y Zhang, X Zhang and MH Nantz. A Strategy for Relative Quantification of Metabolites via Multiplexed GC-MS Analysis. Poster No. 7, 14th Annual IMD³ Symposium, Louisville, KY; March 13, 2012. **1st Place Award Winner**

S Laulhé, L Johannes, T Geers, Y Zhang, X Zhang and MH Nantz. A 'Click-Chemistry' Reagent for High Throughput Carbonyl Profiling. Poster No. 49, Research! Louisville, Louisville, KY; October 11, 2011. **Finalist**

S Laulhé, JD Moore, S Biswas and MH Nantz. A Click-Chemistry Modification of the Fluorescent Probe Cypate for Attachment to Nanogold Particles. Poster No. 31-22, DOD Breast Cancer Research Program – Era of Hope Conference, Orlando, FL; August 3, 2011.

J Wang, S Biswas, JD Moore, S Laulhé, MH Nantz, S Achilefu and KA Kang. Enzyme Triggered, Nanoparticle Controlled Fluorescence Emission for Sensitive and Specific Breast Cancer Detection. AIChE Annual Meeting, Salt Lake City, UT; November 11, 2010.

LM Johannes, S Laulhé, X Shi, X Zhang and MH Nantz. McLafferty Rearrangement: Structural Factors that Promote α,β -Fragmentation. Poster, Summer Research Opportunities for Undergraduates, University of Louisville, Louisville, KY; August 4, 2010.

EXTRACURRICULAR ACTIVITIES

- **President**, Chemistry Graduate Student Association, UofL (2010-12); **Public Relations** (2009-10)
- **President**, American International Relations Club, UofL (2010-12); **Vice President** (2009-10); **Treasurer** (2008-09)
- **Graduate Student Council Representative**, Department of Chemistry, UofL (2009-11)
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