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Rapid and mild silylation of β-amino alcohols at room temperature mediated by *N*-methylimidazole for enhanced detectability by gas chromatography/electron ionization mass spectrometry

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RATIONALE: In this work, we expand the use of *in situ* activation of chloro(dimethyl)phenylsilane using *N*-methylimidazole (NMI) for the effective derivatization of β-aminoethyl alcohols. Due to its enhanced nucleophilic character, NMI is expected to act as an efficient activator in these reactions.
METHODS: The derivatization of a panel of β-aminoethyl alcohols was accomplished by reacting the analyte with chloro(dimethyl)phenylsilane in the presence of either NMI or pyridine. After the addition of chloro(dimethyl)phenylsilane, the vials were gently tumbled for 1 h at ambient temperature. The phenyldimethylsilyl derivatives were identified using gas chromatography/electron ionization mass spectrometry (GC/EI-MS).
RESULTS: A total of ten β-aminoethyl alcohols were successfully derivatized via *in situ* activation of chloro(dimethyl)-phenylsilane with NMI. Derivatization with NMI was significantly more efficient than with pyridine by a factor of 3–6 for the studied alcohols. The derivatizations in the presence of NMI were found to occur in just 1 h and were conveniently executed at ambient temperature.
CONCLUSIONS: The use of the nitrogenous base NMI in order to activate chloro(dimethyl)phenylsilane for the efficient silylation of a panel of β-aminoethyl alcohols has been demonstrated. The present work shows that NMI is an efficient base for the smooth derivatization of these types of alcohols. Furthermore, the installation of the bulky PDMS group onto these alcohols adds to the certainty that this is a viable approach for the installation of the more commonly employed, trimethylsilyl group. Published in 2014. This article is a U.S. Government work and is in the public domain in the USA.

Derivatization to increase the volatility of analytes by transforming them into suitable species for analysis by gas chromatography/mass spectrometry (GC/MS) is a commonly employed tactic in analytical chemistry. One of the most widely employed derivatization techniques is silylation where a trisubstituted silicon atom is directly appended onto the heteroatom of an amine or alcohol.[1–3] Due to the wide impact in the field of GC/MS analysis brought upon by this type of derivatization, it is not surprising that several reagents have been cleverly designed for this procedure. Two of the most commonly used silylating reagents are bis(trimethylsilyl)-trifluoroacetamide (BSTFA) (1) and *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) (2) (Fig. 1).[4–7] Despite the fact that these reagents have found widespread use, there remain certain aspects of their experimental manipulation that can be further improved upon. One such aspect is the inherent limitation of introducing only the trimethylsilyl (TMS) group that, in the full spectrum of silicon-based protective groups, ranks last in acid and basic stability.[8–11] However, a more serious drawback lies in their manipulation that implicates heating for several hours (>2 h) often to temperatures over 80 °C in order to obtain an efficient derivatization, effectively thwarting their use in the rapid analysis of temperature-sensitive substrates.

An alternative method that has found extensive application in the field of natural product synthesis but thus far has found limited applicability in the field of sample derivatization is the *in situ* silyl chloride activation. The method involves the reaction of a silyl chloride reagent with a nitrogenous base (typically imidazole) to initially generate a silyl-imidazolium species that then reacts with hydroxyl and amine groups.[12] The efficiency of the *in situ* derivatization stems from the high reactivity of the silyl-imidazolium intermediate formed from the reaction of the silyl chloride with the base (Scheme 1). Two characteristics that make this approach superior over using BSTFA or MSTFA is the ability to introduce any silyl group in the analytes under study. The second one, and perhaps the most attractive, is the exclusion of elevated temperatures for its execution as the derivatization takes place readily at ambient temperature and in only 1 h. Recent work from our laboratory, using *N*-methylimidazole (NMI) (**3**) rather than imidazole, has demonstrated that the approach is an effective one for the silylation of alcohols for detection by GC/MS in electron ionization (EI) under mild conditions and in a rapid fashion.[13] Two reasons for choosing NMI over imidazole in our original work are that NMI is a liquid at ambient temperature in contrast to

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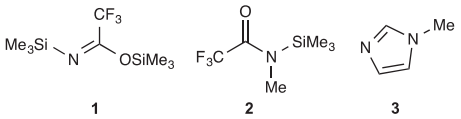


Figure 1. Common silylating agents BSTFA (**1**), MSTFA (**2**) and the highly nucleophilic nitrogenous base *N*-methylimidazole (NMI, **3**).

imidazole which is a solid thus avoiding the need for stock solution preparations; and that it is a more nucleophilic base than imidazole ($pK_a = 7.05$ vs imidazole $pK_a = 6.95$) resulting in its more efficient activation of the silyl chloride.

Given our interest and need for reliable, mild and rapid derivatization methodologies in the areas of chemical warfare agent (CWA) analysis, we focused on expanding the application of the *in situ* activation protocol for the silylation to *β*-aminoethyl alcohols. This structural motif can be found in a number of biologically important small molecules such as neurotransmitters (adrenaline, **4**), *β*-blocker drugs (propanolol, **5**) and, most relevant for us, it is present in the products arising from the hydrolysis of the blistering nitrogen mustard agents HN1 (**6**) and HN2 (**7**) (Fig. 2).[14,15] In addition to their occurrence in this class of agents, some of these alcohols (e.g. diisopropylaminoethanol and diethylaminoethanol) are used as starting materials in the manufacture and are themselves degradation products of the highly toxic organophosphorus-based nerve agents VX (**8**) and VR (**9**) (Fig. 2). Therefore, their detection (either direct or via derivatization) in a matrix may signal the underlying presence of one of these substances[16–18] and in addition provide critical chemical forensics information. The panel of *β*-aminoethyl alcohols (**10–19**) chosen for our studies is provided in Fig. 3.

With regards to the silyl group that was chosen for our derivatizations, we opted to employ the phenyldimethylsilyl (PDMS) protecting group for two reasons. The first one is its superior stability towards acid/base hydrolysis in contrast to the more commonly employed TMS counterpart. A second attribute is that the success of PDMS installation in our analytes by this method would strongly support the eventual success of the protocol in the installation of less sterically demanding silyl groups such as TMS and triethylsilyl (TES), as well as other bulky groups such as the *tert*-butyldimethylsilyl (TBDMS) moiety.[19,20]

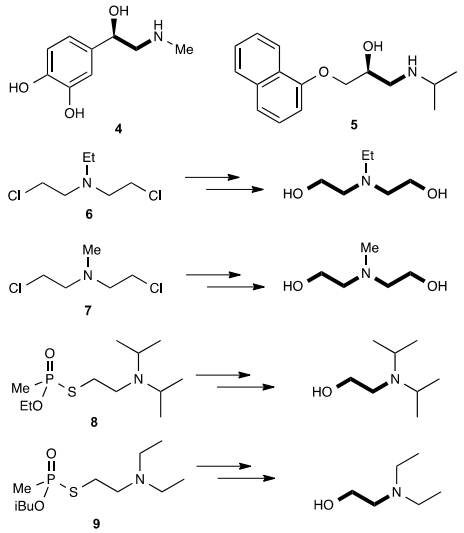


Figure 2. Prevalence of the *β*-aminoethyl alcohol motif in various compounds.

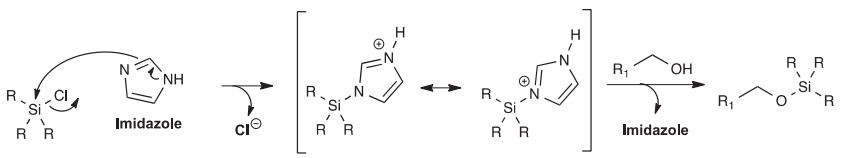
EXPERIMENTAL

Derivatization of the *β*-aminoethyl alcohols

Stock solutions were prepared by dissolving the *β*-aminoethyl alcohol (100 µL) in 1.5 mL anhydrous methylene chloride. For the derivatization, 100 µL of each *β*-aminoethyl alcohol stock solution was placed in a 2 mL glass autosampler vial and treated sequentially with the nitrogenous base (pyridine or NMI, 50 µL) followed by chloro(dimethyl)phenylsilane (20 µL). The resulting mixture was shaken for 1 h at ambient temperature (24 °C), after which time 15 µL were transferred to another autosampler vial and diluted further to 1.5 mL with anhydrous methylene chloride. The sample was then analyzed by gas chromatography/electron ionization mass spectrometry (GC/EI-MS). A volume of 5 µL of the final, diluted sample was injected into the GC/MS system for analysis to ensure that the analyte signal would not saturate the GC/MS detector.

Mass spectrometric analysis

A 6890 Agilent gas chromatograph with a 5975 MS detector equipped with a split/splitless injector was used for the analysis. The GC column used for the analysis was an Agilent DB-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm i.f.). Ultra-high-purity helium was used as the carrier gas at 0.8 mL/min. The injector temperature was 250 °C, and the injection volume was 1 µL. The oven temperature program was as follows: 40 °C, held for 3 min, increased at 8 °C/min to 300 °C, held for 3 min. The MS ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. Electron ionization was used with ionization energy of 70 eV. The mass spectrometer was operated to scan from *m/z* 29 to 600 in 0.4 s.



Scheme 1. Proposed activation of silyl chloride by imidazole-based nucleophiles and subsequent alcohol silylation.

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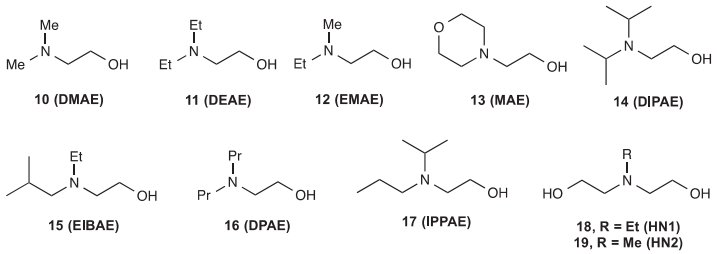


Figure 3. Panel of amino alcohols employed in this study. Synthetic protocols and NMR spectra for compounds **15–17** are given in the Supporting Information.

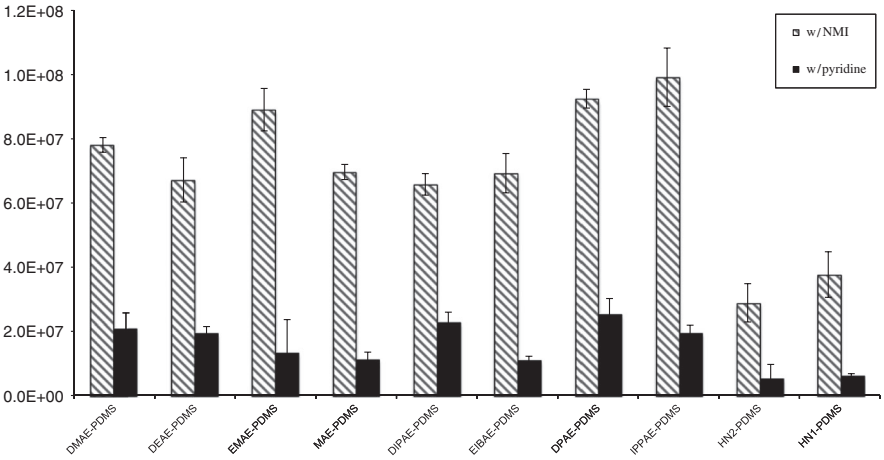
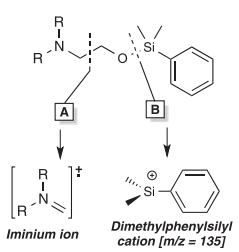


Figure 4. Average (n = 3) peak areas (±the standard deviation) for the ten β-aminoethyl alcohol-PDMS derivatives in this study in the presence of NMI or pyridine.

Table 1. Retention index (RI) values for PDMS derivatives of β-aminoethyl alcohols using the DB-5MS column

β-Aminoethyl alcohol-PDMS derivative	RI
N,*N*-dimethylaminoethyl alcohol-PDMS (**20**)	1424
N,*N*-diethylaminoethyl alcohol-PDMS (**21**)	1569
N-methyl-*N*-isopropylaminoethyl alcohol-PDMS (**22**)	1585
N-2-hydroxyethylmorpholine PDMS (**23**)	1799
N,*N*-diisopropylaminoethyl alcohol-PDMS (**24**)	1835
N-ethyl-*N*-isobutyl alcohol-PDMS (**25**)	1834
N,*N*-dipropylaminoethyl alcohol-PDMS (**26**)	1858
N-propyl-*N*-isopropylaminoethyl alcohol-PDMS (**27**)	1843
N-ethyldiethanolamine-PDMS (**28**)	2221
N-methyldiethanolamine-PDMS (**29**)	2223



Scheme 2. Common fragmentation patterns observed for PDMS-derivatized β-amino alcohols.

RESULTS AND DISCUSSION

Chloro(dimethyl)phenylsilane, in the presence of either pyridine or NMI, successfully derivatized all ten β-aminoethyl alcohols examined. The PDMS derivatives of the alcohols were well resolved from each other using the chosen chromatographic conditions for the analysis and eluted significantly later from the GC column (retention times of 17–24 min) than did the non-derivatized β-aminoethyl alcohols that in all cases exhibited a broad signal under the conditions used for the analyses (retention times of 7–13 min). Derivatization with NMI was significantly more efficient than with pyridine (paired Student's *t* test, significance level $\alpha = 0.05$), by a factor of 3–6 for the studied alcohols. Figure 4 shows the average (n = 3) relative concentrations (as measured by integrated area counts of the resulting GC/MS peaks in the total ion chromatograms) of the PDMS derivatives formed when either NMI or pyridine mediated the silylation reaction. The increased response of the derivatization procedure utilizing NMI is partially due to increased basicity of NMI over pyridine (NMI $pK_a = 7.05$ vs pyridine $pK_a = 5.25$). Both pyridine and NMI are nitrogenous bases that scavenge the hydrochloric acid produced by the derivatization. The neutralization efficiency of the added base in this reaction drives the derivatization of the alcohol with the silyl chloride by consuming the residual acid product. In addition to its role as a proton scavenger, NMI acts as an activating agent in the derivatization by initially reacting with chloro(dimethyl)phenylsilane to generate a more powerful and efficient imidazole-based silylating species (Scheme 1).

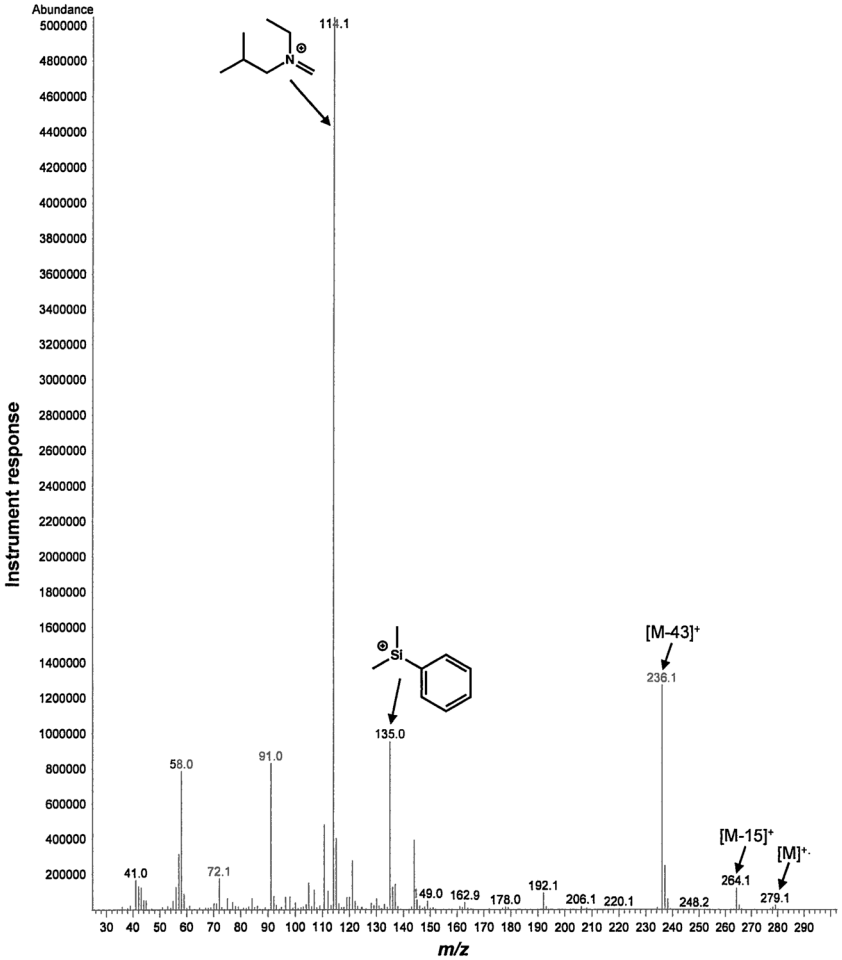


Figure 5. Ion chromatogram of PDMS derivative of *N*-isobutyl-*N*-ethyl-*β*-aminoethyl alcohol.

In addition to observing their retentions, their RI values were calculated in order to provide their retention times relative to straight-chain hydrocarbons (Table 1).

Although these alcohols display a diverse array of structural complexity, two main fragmentation patterns were observed for the derivatives that yielded predominant signals by GC/MS (Scheme 2). A consistently observed fragmentation is indicated by the formation of a *N*,*N*-substituted methyleneiminium species that originates from the heterolytic cleavage of the *α*–*β* bond of the *β*-amino alcohol (pathway A). The second pathway involves the heterolytic cleavage of the O–Si bond to furnish a phenyldimethylsilyl cationic species that is readily visible in the spectrum (*m/z* = 135) (pathway B). Additional fragments that are common among the derivatized *β*-aminoethyl alcohols arise from the loss of a methyl group ([M–15]$^+$) from the silyl group, and loss of an alkyl group from the methyleneiminium ion initially formed via pathway A.

For all the derivatized *β*-aminoethyl alcohols analyzed in our study, it was found that the base peak for the spectra belonged to the methyliminium ion species originating from fragmentation pathway A. It is important to notice that this is a common fragmentation pattern observed for plain, underivatized *β*-aminoethyl alcohols[21] as well as other derivatives of these such as their carbamates.[22] The molecular ion peak was observed for all derivatives as well, albeit present in very low abundance. Thus, the base peaks (given in parentheses) for PDMS derivatives were, for alcohol **20** (*m/z* 58), for isomeric derivatives **21** and **22** (*m/z* 86), for morpholine containing alcohol **23** (*m/z* 100), for isomeric derivatives **24–27** (*m/z* 114), for *N*-ethyldiethanolamine derivative **28** (*m/z* 236) and for *N*-methyldiethanolamine derivative **29** (*m/z* 222). Another common fragmentation pattern was the one leading to the dimethylphenylsilyl cation (*m/z* 135) arising from pathway B and this was observed in all PDMS derivatives. Other observed fragmentation patterns were the loss of a methyl group (M–15) as in the case of derivatives **20** (*m/z* 208), **21** and **22** (*m/z* 236), **23** (*m/z* 250), **24–27** (*m/z* 264), **28** (*m/z* 386) and **29** (*m/z* 378). A fragment arising from the loss of an ethyl group was also observed for PDMS derivatives **25** and **26** (*m/z* 250). A unique fragment arising from the loss of a propyl group was observed solely for PDMS derivative **27** (*m/z* 236) (Fig. 5). Mass spectra for all PDMS-derivatized alcohols (**20–29**) are provided in the Supporting Information.

CONCLUSIONS

The effect on the silylation of a panel of ten *β*-amino alcohols using chloro(dimethyl)phenylsilane in the presence of NMI and pyridine was investigated. We found that the *in situ* activation approach was successful in producing PDMS derivatives of the

studied *β*-amino alcohols. With regards to the bases studied, NMI was found to be superior in all derivatizations over pyridine by a factor of 3–6. These derivatives exhibited unique retention times within similarly structured analogs (retention times of 17–24 min) and very distinct from the parent, underivatized alcohol that in all cases exhibited a broad signal under the conditions used for the analyses (retention times ~7–13 min). The use of NMI as an activating base for the protocol allows for the silylation to be conveniently carried out at ambient temperature and in 1 h. In addition to providing strong, common fragment ions such as the methyleneiminium ion arising from lysis of the *α–β* bond and the phenyldimethylsilyl cation (*m/z* 135), the silylated *β*-amino alcohols also yielded signature fragmentation patterns directly arising from their different *N*-alkyl groups. The NMI *in situ* silyl chloride activation method should find widespread use as an alternative approach to other commonly employed techniques for the derivatization of compounds for GC/EI-MS analysis.

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