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Annals of Chromatography and Separation Techniques

Research Article

Liquid Chromatography Photodiode Array Detection of Hydrogen Sulfide and Methanethiol as Thioacrylates following Nucleophilic Addition to Ethyl Propiolate

Silvio Uhlig[1,2*], Kari K Heldal[1], Frode Rise[3], Mariell Negård[1] and Wijnand Eduard[1]

[1]*Department of the Chemical and Biological Working Environment, National Institute of Occupational Health, Norway*

[2]*Section for Chemistry, Norwegian Veterinary Institute, Norway*

[3]*Department of Chemistry, University of Oslo, Norway*

Article Information

Received date: Nov 24, 2017
Accepted date: Dec 28, 2017
Published date: Dec 30, 2017

***Corresponding author**

Silvio Uhlig, Department of the Chemical and Biological Working Environment, National Institute of Occupational Health, P.O.Box: 8149 Dep, 0033 Oslo, Section for Chemistry, Norwegian Veterinary Institute, P.O.Box: 750 Sentrum, 0106 Oslo, Norway, Tel: +47-23216264; Email: silvio.uhlig@vetinst.no

Keywords Liquid chromatography; Photodiode array; Methanethiol; Thioacrylates; Mercaptoethanol; Ethyl Propiolate; NMR Spectroscopy

Abstract

In this work, we investigate the "click" nature of the reaction of hydrogen sulfide and methanethiol with ethyl propiolate, with the aim to investigate its potential for sampling of volatile thiols. The principal reaction products were stable Z-thioacrylates, while the reaction with methanethiol also gave a minor E-configured product, and the reaction with hydrogen sulfide Z,E- and Z,Z- dimers. The thioacrylates were separated using a narrow-bore C18-amide column and detected using a photodiode array detector at wavelengths around 300nm. When the sulphides were produced from their sodium-thiolate salts and flushed through a series of impingers, nearly 100% of the generated hydrogen sulfide reacted and was trapped in the first impinger, while approximately 70% of methanethiol was absorbed by reaction with ethyl-propiolate. Our data show that the reaction of thiols with propiolic acid derivatives has the potential for application in a device for sampling of airborne thiols.

Introduction

Volatile thiols ("mercaptans") are of importance for global atmosphere chemistry as well as for certain occupational health problems due to their toxicity as well as offensive smell and low odour threshold [1,2]. Owing to their high reactivity and often low concentrations in air these compounds remain a challenge for unbiased sampling and measurement [2]. They are sampled either by physical methods, such as sorption on solid sorbents, cryogenic trapping or solid-phase micro-extraction, or by chemical reaction with mercury or mercury-containing probes [2-4]. The latter approach is today of less interest because of the hazards connected with the handling and waste-management of mercury. Rather few studies investigated the use of organic molecules other than those containing mercury for fast derivatisation, and thereby trapping, of volatile thiols. These include the reaction with 5,5′-dithiobis (2-nitrobenzoic acid) and o-phtaldialdehyde [5,6].

The nucleophilic addition of thiols to conjugated alkynes is very similar to the more often studied hydrothiolation of α,β-unsaturated carbonyls (thiol-ene addition, thiol-Michael addition) [7]. This type of reaction may either proceed via a base-catalysed reaction pathway or a nucleophile-catalysed reaction pathway and has been exploited extensively in the development of polymers [8,9]. The nucleophilic "click" addition of thiols to propiolic acid esters has thus found its application in polymer-polymer coupling [7]. To a limited extent, propiolic acid esters have been applied in analytical chemistry for the tagging of thiols, e.g. in flow injection analysis [10,11]. The aim of the present study was to establish reaction conditions under which the reaction of a propiolate, in this case ethyl propiolate, can be applied for the derivatisation and trapping of airborne thiols by 1) Characterising the products from the reaction with the environmentally important thiols hydrogen sulfide and Methanethiol, 2) Optimise reaction conditions using 2-mercaptoethanol, 3) Prepare standards for instrument calibration and 4) Apply the reaction in an impinger model.

Experimental

Chemicals

The following chemicals were from Sigma-Aldrich (St. Louis, MO, USA): 2-mercaptoethanol (≥99%). disodium sulfide (≥97%), ethyl propiolate (99%), sodium methanethiolate (≥90%), ammonium formate (97%) and formic acid (98%, pro analysis grade). Ammonium carbonate (for HPLC, ≥30% ammonia) and 2M hydrochloric acid were from Fluka (Buchs, Switzerland).

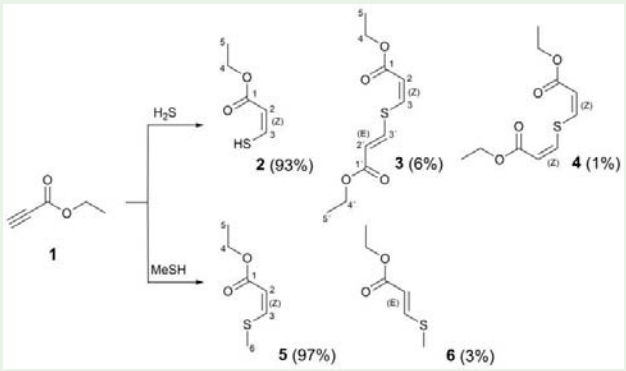

Figure 1: Products from reaction of hydrogen sulfide or methanethiol with ethyl propiolate.

Dichloromethane was of HiPerSolv quality and from VWR International (Radnor, PA, USA) and ethanol from Kemetyl AS (Vestby, Norway). Water was purified and deionised using a Millipore Elix 5/Milli-Q Aca-demic water purification system (Merck Millipore, Merck KGaA, Darmstadt, Germany).

Synthesis of Thioacrylates

Individual thioacrylates (Figure 1) were prepared for structural characterisation and calibration of instrumentation by dissolving 44.4µ mole of sodium thiolate (e.g: 3.1mg of sodium methanethiolate) in 0.9mL of 0.2M ammonium carbonate (pH 7.8). To this solution, 0.1mL of a 444mM solution of ethyl propiolate in ethanol (corresponding to 44.4µmole of the propiolate) was added and mixed by vortexing. A white precipitate formed immediately indicating the formation of the corresponding thioacrylate. The mixture was left to stand at room temperature for approximately ten minutes, and was then extracted four times with 0.2mL of dichloromethane. The dichloromethane phases were pooled and evaporated to dryness. The products were either dissolved in 0.5mL of deuterated chloroform for NMR or weighed on a Sartorius MC5 microbalance (Göttingen, Germany) and then dissolved in acetone/water (4:1, v/v) for calibration stock solutions.

NMR Spectroscopy

NMR spectra of thioacrylates were obtained from solutions (0.5mL) in deuterated chloroform (CDC_{l3}, 99.96 atom % D; Sigma-Aldrich) using 5mm o.d. Wilmad tubes (Sigma-Aldrich) (Table 1). The spectra were acquired on an Avance AVII 600 MHz NMR spectrometer (BrukerBioSpin, Rheinstetten, Germany) equipped with a 5mm CP-TCI (1H/13C, 15N–2H) triple-resonance inverse cryoprobe with a Z-gradient coil. NMR assignments were obtained from the examination of 1H, 13C, JMOD, COSY, g-HSQC, g-HMBC, and NOESY NMR spectra. The data were processed using Bruker TOPSPIN (version 2.1 pl4) software.

Optimization of Reaction Conditions using Mercaptoethanol

The nucleophilic addition of mercaptans to ethyl propiolate was optimized with regard to pH and ratio between thiol/propiolate. Mercaptoethanol was used in these initial trials. The reaction was performed directly in 1.5mL chromatography vials and followed by HPLC-PDA (Figure 2). The general protocol for the mercaptoethanol trials was identical for all tested reaction conditions. A stock solution of 17mM of mercaptoethanol in aqueous buffer was made by dissolving 12µL of the thiol in 10mL of the buffer. 0.1 mL aliquot of a 1:10 dilution of the stock solution (i.e. containing 1.7mM of mercaptoethanol) was then transferred to a chromatography vial and diluted with 0.8mL of the buffer and shaken. Ethyl propiolate in ethanol (0.1mL) was added to the mercaptoethanol solution and the mixture shaken and placed in the HPLC auto-sampler, which was thermostatted to 20°C. The following buffer or salt solutions were used: 0.0064M phosphate buffered saline (pH 7.38), 0.2M ammonium carbonate (pH 7.8) and 0.2M bicarbonate/carbonate buffer (pH 8.9). The tested concentrations of ethyl propiolate in ethanol were 9.9mM, 99mM and 444mM resulting in mercaptoethanol: Ethyl propiolate molar ratios of approximately 1:6, 1:60 and 1:260.

Gas Sampling Procedure

A series of three 25mL impinger flasks each containing 22.5mL of 0.2M ammonium carbonate and 2.5mL of 444mM of ethyl propiolate in ethanol (0.5mL in 10mL of ethanol) were connected to a 250mL

Table 1: ^{1}H and ^{13}C NMR data (CDC_{l3}) for thioacrylates from reaction of hydrogen sulfide and methanethiol with ethyl propiolate. Correlation constants J_{H-H} are shown in brackets. Chemical shifts, determined at 25°C, are reported relative to internal CHC_{l3} (7.26ppm) and CDC_{l3} (77.36ppm).

	2		3		5		6	
Atom nr.	$\delta^{13}C$ (ppm)	$\delta^{1}H$ (ppm)	$\delta^{13}C$ (ppm)	$\delta^{1}H$ (ppm)	$\delta^{13}C$ (ppm)	$\delta^{1}H$ (ppm)	$\delta^{13}C$ (ppm)	$\delta^{1}H$ (ppm)
1	166.1	-	166.4	-	166.9	-	165.5	-
2	116.6	5.97 (d, 10.3)	116.5	6.03 (d, 10.0)	113.4	5.83 (d, 10.1)	113.5	5.65 (d, 14.9)
3	147.7	7.09 (d, 10.3)	143.9	7.20 (d, 10.0)	152	7.04 (d, 10.1)	147.1	7.74 (d, 14.9)
4	70	4.24 (q, 7.1)	61.1	4.21 (q, 7.2)	60.3	4.20 (q, 7.1)	60.4	4.19 (q, 7.1)
5	14.7	1.32 (t, 7.1)	16.5	1.30 (t, 7.2)	14.6	1.29 (t, 7.1)	14.6	1.29 (t, 7.1)
6					19.4	2.39 (s)	19.4	2.33 (s)
1'			165.2	-				
2'			118.2	6.08 (d, 15.5)				
3'			146.1	7.70 (d, 15.5)				
4'			61.1	4.21 (q, 7.2)				
5'			16.5	1.30 (t, 7.2)				

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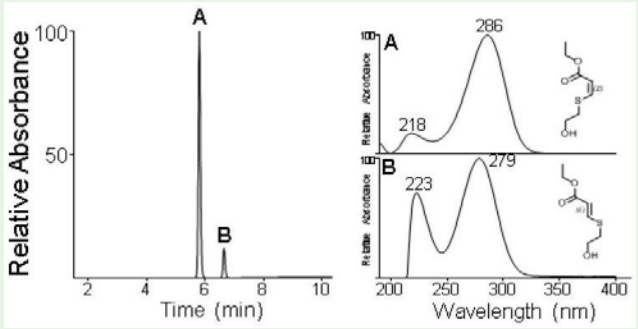

Figure 2: HPLC-PDA chromatogram (λ=280nm) and UV-spectra of the products from reaction of mercaptoethanol with ethyl propiolate. Structures are tentative.

three-neck round-bottom flask at ambient temperature (Figure 3). The flask was filled with 50mL of water, and 0.1mL of 0.1M HCl added. The apparatus was purged with nitrogen (150 mL min^{-1}) for approximately 10min and the contents of the flask blended by magnetic stirring. A stock solution of either disodium sulfide (2.1mg) or sodium methanethiolate (1.8mg) was prepared in 1mL of water and diluted with water such that an aliquot of 0.1mL would yield the desired quantity of hydrogen sulfide or methanethiol, respectively, when added to the diluted acid in the flask. After addition of the 0.1mL aliquot, the apparatus was purged with nitrogen (150mL/min) for one hour. To each impinger flask 0.1mL of formic acid was then added and the apparatus purged with nitrogen for another 5min. The acidified trapping solutions could be left at room temperature at least for one day without apparent degradation of the reaction products. Aliquots from each impinger flask were transferred to 1.5mL chromatography vials and analyzed by HPLC-PDA.

Liquid Chromatography Photodiode Array Detection (HPLC-PDA)

The HPLC system used was a Dionex UltiMate 3000 quaternary pump with auto-sampler and UltiMate 3000 Rapid Separation Photodiode Array Detector (DAD-3000RS) (Thermo Scientific Dionex, Sunnyvale, CA, USA). Separation was achieved on an ACE 3 150 × 1.0 mm i.d. C_{18}-Amide column with precolumn filter (Advanced Chromatography Technologies Ltd, Aberdeen, Scotland). Mobile phase A was 5mM ammonium formate/0.1% formic acid in water, and mobile phase B was 5mM ammonium formate/0.1% formic acid in acetonitrile/water (19:1, v/v). Two different linear gradients were used at a flow rate of 0.25 mL min^{-1}. Gradient-I was used during optimization using mercaptoethanol and started at 2% B after isocratic elution for 1min and was then raised to 30% B over 9min. The column was flushed with 100% B for 2min, returned to 2% B and equilibrated for 2.5min. Gradient-II was used for quantitation of the thioacrylates obtained from reaction of hydrogen sulfide and methanethiol, and started at 10% B after isocratic elution for 1min and was then raised to 50% B over 10min. The column was flushed with 100% B for 2min, returned to 10% B and equilibrated for 3.5min. The PDA was scanned in the wavelength range 190-400 nm at a sampling rate of 10Hz. External calibration plots for quantification were based on extracted wavelength chromatograms at 303nm for 2 and 289nm for 5, respectively (Figure 4).

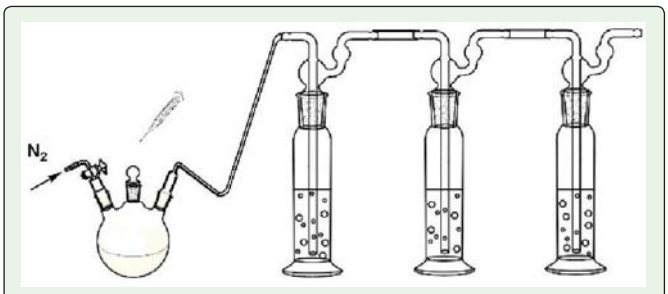

Figure 3: Schematic drawing of the impinger model. Reagents and conditions: thiol generation from thiolate salt in 0.2mM HCl, impinger flasks contain 25mL of 44mM of ethyl propiolate in ammonium carbonate/EtOH (9:1, v/v), 20°C.

Results and Discussion

Reaction Conditions and Reaction Products

By definition, click reactions occur in one pot, generate minimal byproducts, and are characterized by a high thermodynamic driving force that drives it quickly and irreversibly to high yield of a single or major reaction product [12]. The application of esters of propiolic acid for "click" reaction with thiols has been shown in a number of studies [10,13,14]. The reaction gives a mixture of E/Z diastereomers, where the ratio depends on the reaction conditions, especially the solvent and catalyst [7,15]. Thus, a protic solvent favors in general a Z–stereochemistry, while an aprotic solvent favors formation of an E-thioacrylate [15]. The reaction itself has been known for decades, but it has only rather recently been applied in a few analytical approaches for thiol derivatisation and in polymer chemistry. Examples for the former are the application of ethyl propiolate (1) for derivatization of thiols in wine or as a reagent for HPLC post-column derivatization in order to facilitate sensitive UV-detection of thiols [11,13,14]. Half-life's of biologically relevant thiols, such as cysteine, glutathione and cysteamine, for the reaction with ethyl propiolate have been reported in the range of a few seconds to minutes, even at neutral pH [10]. We used mercaptoethanol as a model thiol in order to optimise the conditions for "click" reaction with ethyl propiolate because it is easier to handle as the gaseous thiols that were the actual target of our work. Also, its reaction with 1 has been shown before [7,10]. The concentration of mercaptoethanol in these initial trials was 0.2mM

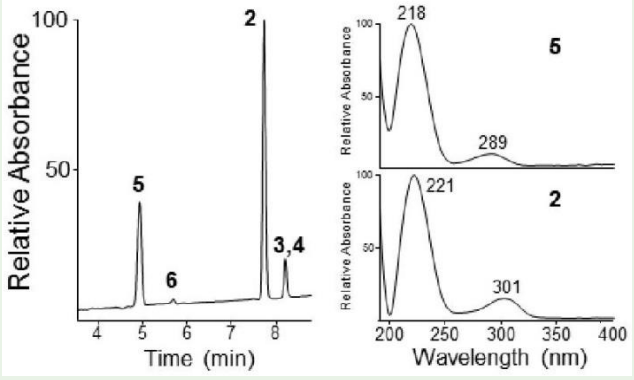

Figure 4: Extracted wavelength maximum chromatogram from HPLC with PDA detection (λ=289 + 301 nm) of a mixture of (Z)-ethyl 3-mercaptoacrylate (2), diethyl 3,3'-thiodiacrylates (3,4) and ethyl 3-(methylthio) acrylates (5, 6) together with UV absorption spectra of the major (Z)-isomers of 2 and 5.

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in water/ethanol (9:1, v/v) at neutral or moderately basic pH, and the reaction was followed by HPLC with photodiode-array (PDA) detection (Figure 2). The reaction of mercaptoethanol with 1 gave two products exhibiting UV-absorbance spectra with absorption maxima around 280nm (Figure 2). With reference to literature data, the putative major product (approximately 90%, based on relative HPLC-PDA peak areas) was Z-ethyl 3-((2-hydroxyethyl) thio) acrylate, and the minor product (approximately 10%) the corresponding E-diastereoisomer. This was supported by a UV absorption maximum of the putative Z isomer at slightly higher wavelength compared to the putative E isomer. The reaction rate was strongly dependent on the pH value and the concentration of propiolate, the former being a result of a higher concentration of thiolate ions (RS−) at higher pH (Figure 5). However, basic pH values may result in hydrolysis of the propiolate ester, and the peak area of 1in HPLC-PDA chromatograms was reduced by 25% within five hours at pH 8.9, while the ester was stable at pH 7.8 over the same time period (data not shown). As a result, we performed all experiments involving hydrogen sulfide and methanethiol as well as their thiolate salts in a solution of 0.2M ammonium carbonate (pH 7.8)/ethanol (9:1, v/v).

The thioacrylates obtained from reaction of HS− and CH_3S− with 1 were synthesised from solutions of their sodium salts using a simple method that could be performed within 30minutes. HPLC-PDA of the dichloromethane-extracted reaction mixture indicated the presence of a major and a minor reaction product both when disodium sulfide and sodium methanethiolate was reacted with 1 (Figure 4). The product mixtures were characterised using 1H and 13C NMR spectroscopy showing that the major reaction products were ethyl (Z)-3-mercaptoacrylate (2) and ethyl (Z)-3-(methylthio) acrylate (5) due to monoaddition of thiolate according to Figure 1 (Table 1).

The acrylate double bond in 2 and 5 was Z-configured as was shown by a 3JH-H coupling constant of 10.3 Hz in both compounds (Table 1). The 1H-NMR spectra of the product mixture from reaction of HS− with 1 showed the presence of two minor reaction products (Supplementary material). Compound 3 (6%) was a Z,E- dimer, as was shown by the presence of two pairs of olefinic protons(δ 6.03/7.20 and 6.08/7.70 ppm) that were 3Jcorrelated with coupling constants of 10.0 and 15.5 Hz, respectively (Figure 1) (Table 1). The HMBC spectra did not show any correlations from one acrylate monomer over the sulfur atom to the second. However, a correlation between H-3 and H-3′ in the NOESY spectra connected the two acrylate moieties in 3.

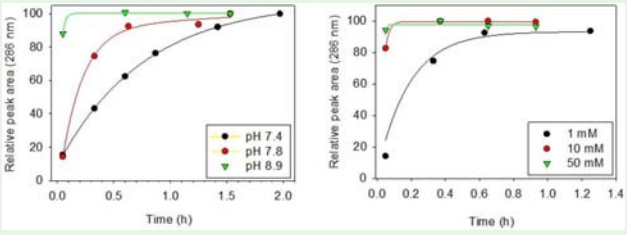

Figure 5: Change in the concentration of putative ethyl 3-((2-hydroxyethyl) thio) acrylate (measured by HPLC-PDA) from reaction of 2-mercaptoethanol (0.2mM) with ethyl propiolate (1mM) over time at 20°C at selected pH values (left) and effect of ethyl propiolate concentration on the rate of reaction of mercaptoethanol (0.2mM) with ethyl propiolate (right). Lines show exponential rise curves fitted to the data.

Compound 4 was only of 1% relative abundance (based on 1H-NMR peak areas) in the reaction mixture and could not be completely assigned as several of 1H-signals overlapped in the 1H-NMR spectra, and because of poor signal/noise of the corresponding resonances in the 2D-spectra. However, two pairs of 3J coupled olefinic protons (δ 5.83/7.09 and 6.37/6.95 ppm) of equal intensity and with coupling constants of 10.2 and 10.3 Hz, respectively, suggest that 4was a Z,Z-thioacrylate dimer (Figure 1). The free sulfhydryl group in 2 appeared thus to be of relatively low reactivity as the proportion of 2 in the mixture was as high as 93%.The reaction of methanethiolate with 1 under the chosen reaction conditions gave nearly exclusively ethyl (Z)-3-(methylthio)acrylate (5), while the relative amount of the corresponding E-isomer was only 3% (based on relative 1H-NMR peak areas, Supplementary material) (Figure 1). There was no evidence of further addition of thiolate to the acrylate double bond.

Dried reaction mixtures were dissolved in acetone/water (4:1, v/v) and used as analytical standards for HPLC with PDA detection. Standard solutions were kept at -24°C and were stable for at least ten months. Trials to ionise the compounds in a TSQ Vantage tandem quadrupole mass spectrometer or Q-Exactive high-resolution mass spectrometer failed when a stock solution was infused into a mobile phase consisting of acetonitrile/water (1:1, v/v), containing 5mM ammonium formate and 0.1% formic acid, and directed into the instrument via an electrospray ionisation or atmospheric pressure chemical ionisation interface. Thus, if the aim is the LC-MS based detection of hydrogen sulphide, methanethiol and similar volatile thiols via "click" reaction with propiolates, the propiolate moiety needs to be designed such that it favours ionisation in common LC-MS interfaces.

The Impinger Model

In order to test the potential of "click" addition of gaseous hydrogen sulfide and methanethiol to propiolate for the purpose of environmental sampling we generated the sulfides in a closed apparatus from their thiolate salts and flushed them through a series of impinger flasks, each containing a solution of 1 in aqueous ammonium carbonate/ethanol, using a flow of nitrogen (Figure 3).

Fresh solutions and appropriate dilutions of the thiolates were prepared in water and 100µL aliquots added to dilute hydrochloric acid (Figure 3). The flask and impingers were flushed with nitrogen for one hour, after which the contents of impinger flasks was acidified and left at room temperature for up to 12 hours before analysis using HPLC-PDA (Figure 4). Instrument calibration and quantification of thioacrylates was based on the peak area of the Z-diastereoisomers 2(λ=301nm) and 5(λ=289nm). Nearly all hydrogen sulfide was trapped as thioacrylate in the first impinger, while ca. 70% of the gaseous methanethiol was retained as thioacrylate by reaction with 1 in the first impinger (Table 2).

Faster reaction kinetics are expected for hydrogen sulfide compared to methanethiol because it is significantly more acidic than the latter (pKa 7.04 *vs.* 10.3), and, as a general rule, the relative reactivity of thiols is a function of their dissociation constants [16-18]. The total recoveries for hydrogen sulfide and methanethiol as thioacrylate derivatives were promising for the preliminary model (Table 2). Only minor amounts of the major reaction products 2 and 5 could be detected in the second or third impinger flask, respectively.

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Table 2: Results from recovery trials using the impinger model.

Thiol	Calculated amount of generated thiol (µg)	Mean total recovery of generated thiol as thioacrylate (%, n=3)	RSD (%)	Mean recovery of generated thiol as thioacrylate in first impinger (%, n=3)
Hydrogen sulfide	9.08	116	6.3	99
	91	81	23	96
Methanethiol	12.2	109	6.7	72
	122	128	3	68

The relatively high apparent total recoveries (up to 128%, Table 2) could be the result of a slightly different composition of major vs. minor reaction products when the reaction was carried out in a vial, or when small amounts of the gaseous thiols were flushed through the impingers.

Conclusions

In summary, we have synthesized thioacrylates of hydrogen sulfide and methanethiol by reaction with ethyl-propiolate and have shown the potential of the "click" nature of this type of reaction for potential application in a mercaptan sampler. Future research must focus on the development of such a sampler including reactant and carrier design, as well as testing of other volatile thiols.

Electronic Supplementary Information

1H NMR spectra of compounds 2, 3, 4, 5 and 6; 13C NMR spectra of compounds 2, 3, 5 and 6.

Acknowledgment

This work was supported by the Confederation of Norwegian Enterprise under grant S-2910.

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