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# Preparation of N-Alkyl Substituted Cysteine Derivatives by Reductive Amination

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**KEYWORDS**

Cysteine Derivatives;

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N-Alkyl Substitute;

**Abstract:** *The overall poor yield can be explained by attempting to condensate the aldehyde with cysteine in its zwitter ionic form. Due to the protonation of cysteines' amine functionality in this state, its nucleophilicity will be non existing unless a base can abstract the proton and leave the free amine to react with the aldehyde. If there is equilibrium for the zwitter ion form, it has to be strongly shifted to the charged form where its reactivity is lowest, which would be in line with the isolated yields. To avoid this protonation problem, two alternatives are possible; a methyl ester of cysteine could be utilized, or running the reaction in the presence of a base to deprotonate the charged amine. These compounds could not be purified by silica chromatography because of the low solubility in any tested solvents, eg H2O, MeOH, CHCl3, dioxane, MeOH/H2O, THF, acetonitrile, acetonitril+TFA, DMSO, DMF, Et2O, pyridine, and benzene. Several means of reducing reaction time and increasing yield are reported in literature, such as the use of molecular sieves, azeotropic removal of water and addition of acid22. We investigated the use of a dehydrating agent Na2SO4, and catalytic addition of acid, HCl, in the synthesis of 1c. Due to the low solubility of 1c, the dehydrating agent, Na2SO4, could not be separated from target compound. .*

## 1. INTRODUCTION

The synthesis of compounds 1a-c has been described in literature. The described procedure by Park et al17d (2002) reported the reductive amination of cysteine with NaBH3CN. The procedure had relatively low reported yield, about 30 %. According to the procedure, cysteine and NaBH3CN were mixed in methanol, before adding the aldehyde. In our hands, this gave only 5 % yield for 2-(benzylamino)-3-mercaptopropanoic acid (1a), and even less for 3-mercapto-2- (phenethylamino)propanoic acid (1b) and 3-mercapto-2-(3-phenylpropylamino)propanoic acid (1c). When the procedure was scaled up 4 times for 1b the yield was raised to 11 %. 1a and 1b gave relatively pure products, but 1c contained approximately 80 % unreacted cysteine. This indicated a need for longer reaction time than the two other compounds, 1a and 1c. The yield did not however improve when the reaction time was increased from 26 hours to 50 hours.

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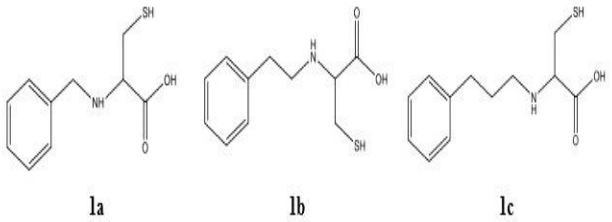


Figure 1 Chemical structures of the produced amines.

The procedure reported in literature49 for compound 1a-1c has reported that the 1H NMR and 13C NMR analysis were run in D2O.

It was impossible to solve the target compounds 1a-1c in this solvent. Therefore the use of NaOH was utilized, and the 1H NMR and 13C NMR analysis were run with NaOH in D2O. The spectra from literature and the ones obtained can therefore not be compared. 1H NMR and 13C NMR reference spectra with cysteine in NaOH/D2O were prepared for comparison for the contamination in the spectra for 1b and 1c.

The 1H NMR spectrum of target compound 1a is shown in Figure 9. The numbering in the following discussion is used as assigned. The aromatic protons (position 1-4 and 6) are in the expected region at 7.19-7.26 ppm, and integrated to five. The two doublets at 3.47 ppm and 3.62 ppm, integrated to one each, are the two protons in the benzylic position, 7a and 7b. The appearing double doublet at 2.91 ppm belong to the α-proton (position 9). The two double doublets at 2.64 ppm and 2.44 ppm, are the two diastereotopic protons, 11a and 11b. The 13C NMR spectrum validates the 1H NMR results. The peak at 181.66 ppm, correspond to the carbonyl carbon (C10), number ten. There are four signals, 158.55 ppm, 128.59 ppm, 127.12 ppm and 125.79 ppm, in the aromatic region as expected due to symmetry. The signal at 51.24 ppm arises from the carbon in the benzylic position (C7), while the two other peaks at 67.78 ppm and 28.37 ppm arises from C9 and C11, respectively.

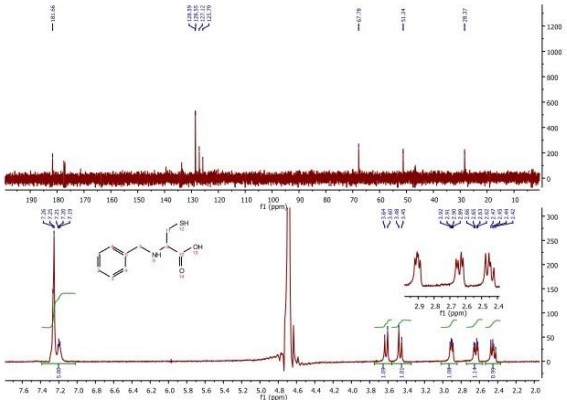

Figure 2 1H NMR and 13C NMR spectra of compound 1a.

The 1H NMR spectrum (Figure 10) for target compound 1b shows a small amount of unreacted starting material and the target compound. The aromatic protons resonate at 7.12-7.26 ppm, and are integrated to five. The appearing double doublet at 2.86 ppm belongs to the α-proton at position five, and is integrated to one. The mutiplet at 2.64-2.71 ppm is integrated to three, belonging to three of four protons in the aliphatic sidechain (position 1 and 3). The multiplet in the range from 2.55-2.60 ppm is the overlapping signals from one of the protons from the aliphatic side chain and one of the diasterotopic protons in position 7. The last signal is a double doublet integrated to one, and is the second proton at position 7. The 13C NMR spectrum has one signal at 181.84 ppm which belongs to the carbonyl carbon (C6). The aromatic protons are resonates at 140.31 ppm, 128.81 ppm, 128.63 ppm and 126.24 ppm. The peaks at 68.60 ppm and 28.19 belong to carbon 5 and 7, respectively. The last two peaks at 48.87 ppm and 35.11 ppm, belong to the two aliphatic carbons C3 and C1, in that order.

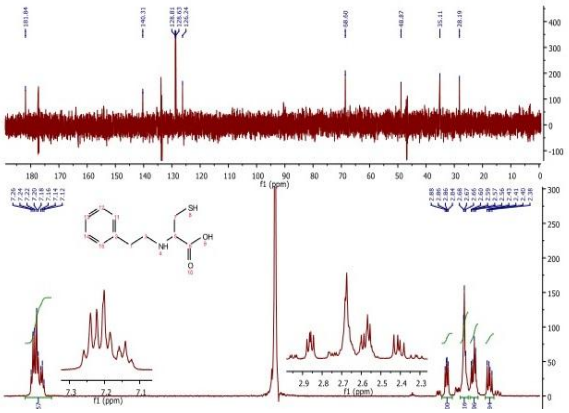

Figure 3 1H NMR and 13C NMR spectra of compound 1b.

The 1H NMR spectra (Figure 11) after reductive amination of 3-phenylpropionaldehyde shows two compounds, unreacted starting material and the target compound 1c. The aromatic protons are as expected at 7.12-7.26 ppm as a multiplet integrated to five. The appearing double doublet at 2.97 ppm and the two double doublets at 2.76 ppm and 2.34 ppm are cysteine (see reference spectra of cysteine in the appendix). These three peaks are in a 1:1:1 relationship. The appearing double doublet at 2.85 ppm has arisen from the α-proton (position 11). The double doublet at

2.62 ppm belongs to one of the diastereotopic protons (position 13a). The other diastereotopic proton in position 13b, is in overlap with the two protons in position 9, in a multiplet from 2.44- 2.48 ppm. The triplet integrated to two at 2.55 pmm is the benzylic protons at position 7, and the multiplet at 1.64-1.72 ppm is the two protons in position 8. The 13C NMR spectrum underlines the fact that it is two different compounds. The cysteine peaks are found at 182.28 ppm, 60.29 ppm and 31.92 ppm. Due to 1c poor solubility even in basic water, the sample was too weak for a clear view of the aromatic protons. Therefore, these are not all accounted for in a precise manner, but there are two clear signals at 128.56 ppm and 128.51 ppm. The peak at 177.15 ppm is from the carbonyl carbon (C12). The peak at 68.53 ppm arises from C11 and the peak at 46.90 from C9. The three left at 32.87 ppm, 30.71 ppm and 28.27 ppm arises from C7, C8 and C13, but can not be correctly assigned without further analysis.

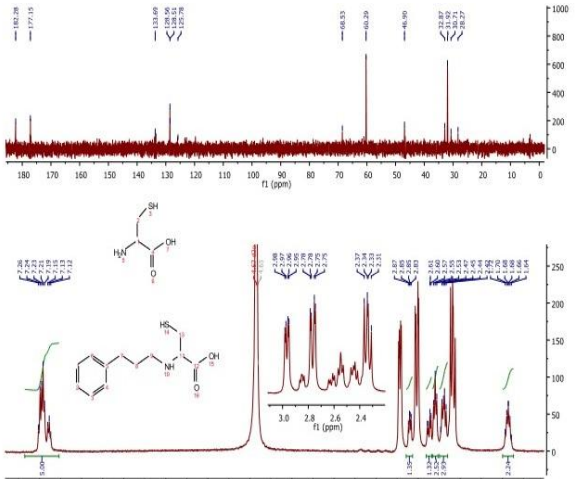

Figure 4 1H NMR and 13C NMR spectra of compound 1c.

Since attempts to improve the yield using NABH3CN failed, the use of Na(OAc)3BH was explored. This reducing agent did not provide the desired product. MS analysis indicated dialkylation of the amine had occurred. In literature, the occurrence of dialkylation products is reported, especially with aldehydes and primary amines18. In comparison to the primary amine, the secondary amine holds a higher degree of reactivity, which can result in overalkylation. This challenge can be suppressed by the addition of up to 5 % molar excess of the primary amine, or by a stepwise reductive amination with preformed imines. Normally the formation of overalkylated products can be monitored by TLC, but since the compounds 1a-1c had poor solubility TLC analysis could not be used.

**2. Preparation of N-sulfonyl substituted cysteine derivatives**

The synthesis of sulfonation of unprotected amino acids has been reported in literature23. However, for unprotected cysteine only sulfonylation with dansyl chloride has been described.46 Thedansylation of cysteine differed from the common procedure for sulfonation of unprotected amino acids in the use of a carbonate buffer instead of base.

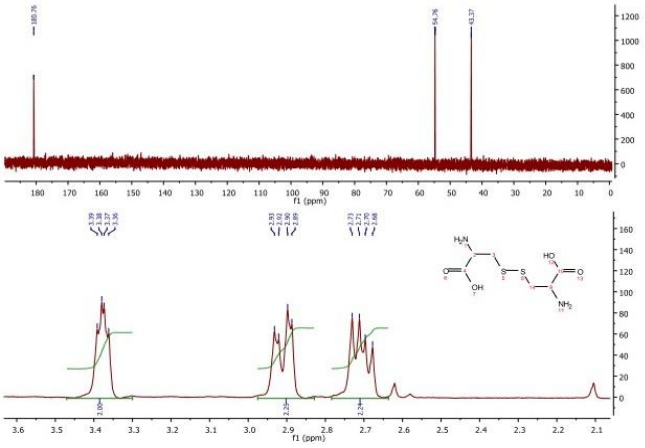

**Figure 5 1H NMR and 13C NMR spectra of cystine.**

The use of the dansylation procedure with sulfonyl chloride and unprotected cysteine gave an unsolvable product. Due to the low solubility, it was not possible to make any analysis, and it is therefore not known what the product was. Using the general procedure for sulfonation of amino acids for the sulfonation of cysteine did not generate the target compound 2a. The 1H NMR spectrum (Figure 12) was somewhat similar to cysteine, but the 13C NMR spectrum was different; the peak at 43.37 ppm had shifted from 31.85 ppm (cysteine reference spectrum), which indicates that the dimer of cysteine had formed47. MS analysis was not suited for this compound. Further tests are necessary before it is verified that this is a dimerization-

reaction of cysteine. To the best of our knowledge, a new way of synthesizing cystine with sulfonyl chloride in water has been discovered.

The general sulfonation procedure was applied to serine in order to test our hypothesis that the free thiol was not compatible with the reaction conditions. This reaction produced the expected sulfonamide, 2b. The 1H NMR and 13C NMR spectra demonstrate that the target compound 2b was achieved. The 1H NMR spectrum shows a doublet at 7.90 ppm arising from the sulfonamide proton (position nine). The aromatic protons appeared as two doublets at 7.65 ppm and 7.35 ppm. The multiplet at 3.42-3.50 ppm could be the α-proton and the two diastereotopic protons (position 10 and 12). The singlet integrated to three at 2.34 ppm is the methyl-group at the benzene ring (position 7). The 13C NMR spectra of 2b show a peak at 171.76 ppm, belonging to the carbonyl carbon (C11). The aromatic carbons are located at 140.00 ppm, 138.66 ppm, 129.83 ppm, and 126.98 ppm. The two peaks at 62.48 ppm and 58.41 ppm belong to C10 and C12, respectively. The last peak at 21.41 ppm has arisen from C7. MS analysis verified that compound 2b was prepared (see appendix)

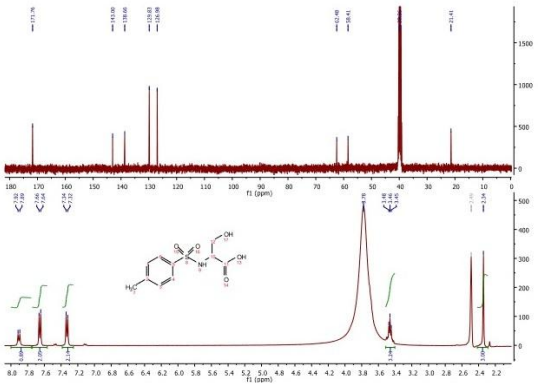

Figure 6: The 1H NMR and 13C NMR spectra of 2b.

The successful sulfonation of serine indicated the need for protection of the thiol group. An acetamidomethyl-protected cysteine was reacted under the same conditions as serine to give the S-protected derivative of 2a. The reaction conditions were the same as in the synthesis of 2b, but afforded a lower yield of 30 %. The 1H NMR and 13C NMR spectra (Figure 14) prove that the target compound was achieved. The 1H NMR spectrum has two appearing doublets at 7.75 ppm and 7.34 ppm, which belong to the protons on the aromatic ring. The singlet at 4.25 ppm, belong to the two protons at position 15. The α-proton at position 9 appeared as a triplet. The two diastereotopic protons at position 13 appear as two double doublets at 2.96 ppm and 2.85 ppm. The two singlets at 2.41 ppm and 1.95 ppm has arisen from the two methyl-groups in position 22 and 18. The 13C NMR spectra of protected 2a show two peaks at 171.63 ppm and 171.62 ppm, which belong to the two carbonyl carbons C12 and C17. Further, the aromatic carbons are located at 143.30 ppm, 137.74 ppm, 129.13 ppm, and 126.85 ppm. The three peaks at 55.98 ppm, 40.74 ppm, and 33.53 ppm can not be as easily assigned without further analysis, but they belong to C9, C13 and C15. The two peaks at 21.20 ppm and 20.05 ppm belong to C18 and C22, but can not be differentiated only by using this spectrum.

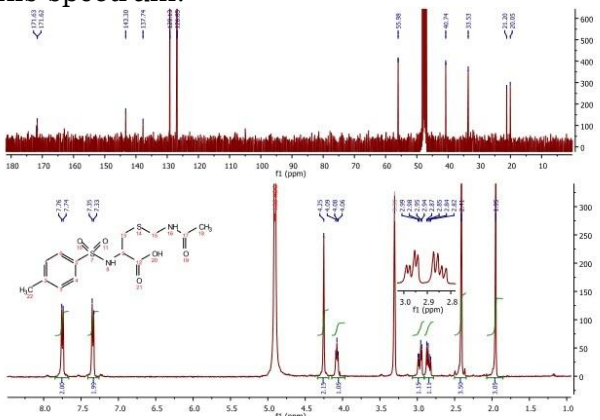

Figure 7 1H NMR and 13C NMR spectra of 2a.

## 3. Attempts towards 2-alkoxy derivatives of cysteine by ring opening of epoxide with sulfur nucleophiles

### *3.1 Ring opening with thioacetate nucleophiles*

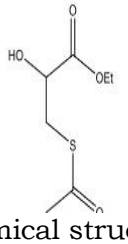

Figure 8 Chemical structure of ethyl 3-(acetylthio)-2-hydroxypropanoate, 3a.

Using KSAc as a nuclophile for ring opening of an epoxide has not been reported for structures similar to ethyl glycidate in literature. This particular protection group for the thiol is of special 31 interest because of its ability to be hydrolyzed under the same conditions as the ester. Many different approaches were tried. The first reaction was conducted by mixing KSAc in DMF before adding the solution slowly to a mixture of ethyl glycidate in DMF. The reaction mixture was left in room temperature with stirring for two hours. This was not successful, and variations in the reaction conditions with factors such as temperature, amount of Lewis acid, addition of crown ether, and the addition speed of KSAc (in DMF) to the reaction mixture, was applied. BF3*Et2O and 18-crown-6 was used to catalyze the reaction, and DMF was used as solvent. This lead to a successful reaction with achievement of the target compound 3a. In this reaction the ethyl glycidate, BF3*Et2O and 18-crown-6 was solved in DMF before the KSAc in DMF was added slowly and left at room temperature with stirring for two hours. A fractional factorial design was employed to get a feeling for the reaction. It underlined the successful reaction with indications it was important to carry out the reaction at 0° C, with stociometric amount BF3*Et2O, and slow addition of the KSAc solution. During work-up, the product, 3a, decomposed rapidly and was impossible to isolate and store, even at low temperature, as a pure compound. Tanabe et al32 have reported that acetylthiohydrin is unstable due to the weakness of the acetyl-sulfur bond adjacent to the hydroxy group.

An attempt to trap the β-hydroxythioacetate with an acid chloride/ benzyl bromide before work- up was unsuccessful. MS and NMR analysis did not indicate that the target compound had been formed. In literature, trapping of an acetylthiohydrin has not been reported, only trapping of a β- hydroxythiocyanate has been reported by Łukowska-Chojnackaet al48 (2011).

HSAc was also tested as nucleophile. The 1H NMR and 13C NMR spectra showed only unreacted start material, which was not surprising considering its poor nucleophilic abilities.

### 3.2 Ring opening with thiocyanate nucleophiles

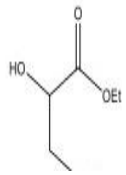


Figure 9 Chemical structure of ethyl 2-hydroxy-3-thiocyanatopropanoate, 3b.

Tanabe et al32 had reported a mild, effective and regioselective ring opening of oxiranes using TBAF as catalyst. The article gave a general procedure for ring opening suggesting that the TBAF and TMSNCS should be added successively to a stirred solution of the epoxide in DMF at room temperature, before heating the reaction mixture to 50°C for three hours. Additionally, for methyl 3-methyloxirane-2-carboxylate as substrate more specific reaction conditions were reported asking for benzene as solvent and increased reaction time (24 hours). Since ethyl glycidate is very similar to methyl 3-methyloxirane-2-carboxylate both approaches were tested. The application of TMSNCS was successful. Both reactions gave a mixture of two isomers (see Figure 17), but the general procedure gave the best result with respect to the purity and isomer ratio. The ethyl 2-hydroxy-3-thiocyanatopropanoate was highly unstable, and due to these stability difficulties the yield was not obtainable. The ethyl 2-hydroxy-3-thiocyanatopropanoate could not be stored in room temperature or over a period of time because of its degradation to its corresponding thiirane (see Figure 18).

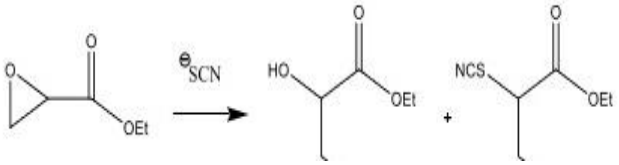


Figure 10 Chemical structure of the two isomers formed during ring opening of epoxide with TMSNCS.

After a silica gel flash chromatography had been employed the desired region-isomer was less contaminated with the unwanted region-isomer, but it was impossible to fully separate the isomers.

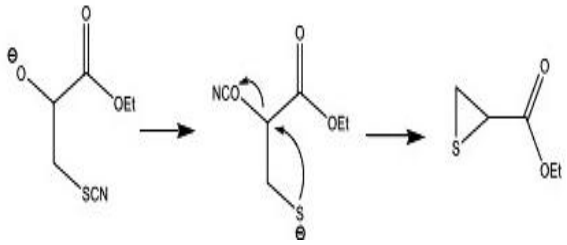


Figure 11 Reaction mechanism for the formation of thiirane.

1H NMR and 13C NMR analysis indicates the presence of two isomers. The following interpretation of the 1H NMR spectrum will be given for the compound 3b, and not the isomer ethyl 3-hydroxy-2-thiocyanatopropanoate. The appearing triplet at 4.24 belongs to the proton in position 1. The multiplet at 3.97-4.06 ppm is integrated to two, and belongs to the protons at position 7. The two double doublets at 3.13 ppm and 3.00 ppm belong to the two protons in position 4. The triplet at 1.03 ppm arises from the three protons in position 9. The 13C NMR spectrum can not as easily be interpreted. Using a spectrum with a larger isomer ratio, the peaks most that most likely belong to 3b are the following: 171.40 ppm (C4), 111.83 ppm (C10), 69.00 ppm (C1), 62.04 ppm (C7), 37.92 ppm (C2), 14.04 ppm (C8).

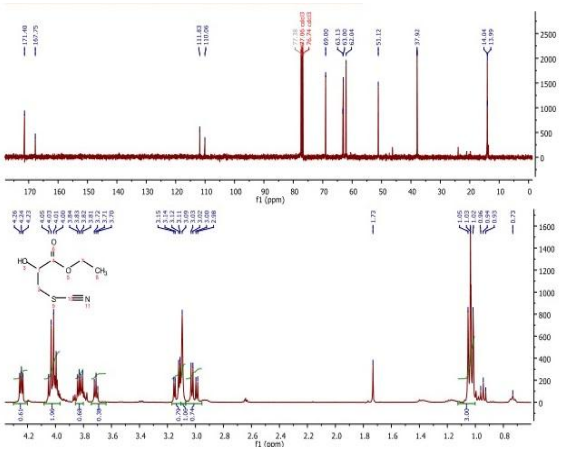

Figure 12 1H NMR and 13C NMR spectra for the ring opening of ethyl glycidate with TMSNCS.

Two different approaches of the nuclophile were possible: -NCS or –SCN. To determine which of these two nucleophiles had reacted, the 13C NMR spectrum was crucial. S-CN gives a signal at approximately 112 ppm, while N=C=S gives signal a broad signal at 130 ppm49. The signals from the 13C NMR spectrum for the ring opening of ethyl glycidate with TMSNCS are at 111.83 ppm, 110.06 ppm. This indicates clearly that the wanted S-CN product was obtained.

### 3.3 Different strategy

A different strategy that was utilized was to make the ethyl ester of glycolic acid and then make the ether with benzyl bromide. However, the Williamson ether synthesis of the glycolic ester and benzyl bromide yielded benzyl ethyl ether, and the strategy was therefore not pursued further.

### 4. Starting material; ethyl glycidate

The starting material ethyl glycidate was made by reacting serine with KBr/HBr and $NaNO_2$ using water as solvent. The crude oil from the first step was reacted with base in absolute ethanol giving the epoxide salt. The epoxide salt was reacted with EtBr in DCM to give the ethyl glycidate. First time the reaction was run the results were according to literature. Second time around however, the end result was contaminated with a byproduct, in a 2:1 relationship, and suspected formation of polymers since the yield was very low. The distillation gave no more than approx. 4 grams, and it should have yielded 18 grams. The reason for the differences in yield and purity has no obvious explanation.

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