Synthesis of Carbon Monoxide Releasing Organic Molecules for Pharmaceutical Applications

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Abstract

Carbon Monoxide has always been known as a toxic and noxious molecule. However, about 60 years ago, it was found that this molecule was produced endogenously in man under normal conditions and its production is increased under certain pathological conditions. Since then, many investigations have proved its beneficial effects and these findings encourage its use as a drug.

Because CO administration as a gas has many limitations, the development of compounds that incorporate and release CO under physiological conditions has started. These molecules are known as CO releasing molecules (CORM's).

Tertiary aldehydes are a class of CORM's that release CO. In this work several types of tertiary aldehydes were prepared and tested as CO releasing molecules. Their stability and reactivity in biological media was also investigated.

Aldehydes can react with some biomolecules and are rapidly metabolized in biological systems. Therefore, several types of prodrugs were developed to protect the aldehyde function.

Keywords: Carbon Monoxide, tertiary aldehydes, prodrugs, radicals.

1. Introduction

CO is a molecule that had an important feature in the beginning of life, along with nitrogen and oxygen. It was found in 18th century and is a colourless, odourless, non corrosive but toxic and pollutant molecule. Its principal source comes from the combustion of the organic matter.

CO has an affinity for the haemoglobin centre 245 times higher than oxygen, and death by poisoning occurs at CO-Hb levels higher then 70%.[1]

Symptoms of CO poisoning start to appear at 20% CO-Hb, and include dizziness, shortness of breath and headache. The antidote for CO poisoning is the removal of the CO source in favour of oxygen inspiration.[2,3]

However, in the early 1949 Sjöstrand[4] showed that CO is endogenously produced in small quantities. Its production is increased under certain pathophysiological conditions through haemoglobin degradation which is mediated by heame-oxygenase (HO). This enzyme has 3 isoforms: HO-1, is inducible in stress situations, HO-2, is a constitutively expressed isoform, present in vascular and nervous systems, and HO-3, a constitutively isoform that is not catalytically active but works in oxygen sensing.

The haemoglobin HO catalysed degradation produces one mole of ferrous iron and one mole of the linear tetrapyrrole biliverdin-IXa per mole of CO formed.

The first step of haemoglobin degradation involves NADPH, O_2 and Cytochrome P450 reductase to produce biliverdin-IXa, which is then reduced to bilirubin-IXa by the biliverdin reductase (Fig.1), present in the endoplasmatic reticulum.

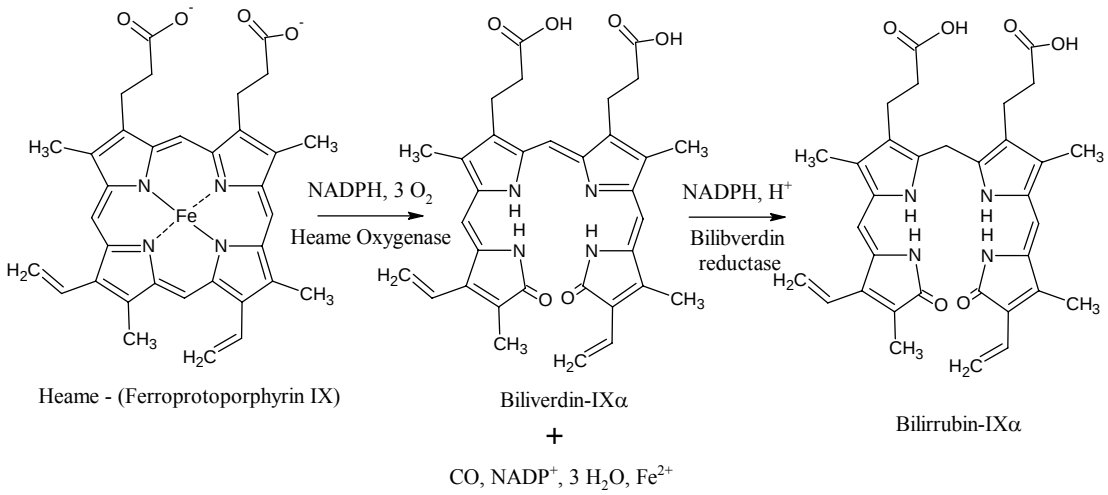

Figure 1: Heame oxygenase catalised heame degradation

This heame degradation is the major source of CO in the human body and it accounts for about 86% of the endogenously produced CO. The remaining 14% are due to lipid peroxidation, photo-oxidation, xenobiotic sources and bacteria. Under homeostatic deregulation conditions like haemolytic diseases, asthma, cystic fibrosis and diabetes, the expression of the inducible isoform HO-1 and the CO production are increased. This is how the body acts as a response to aggressions.[5,6]

In fact, it was observed that in several disease models like systemic and pulmonary hypertension, cardiac, renal and small bowel graft rejection, preservation of organs for transplantation and others, the induction of HO-1 had good results. So it became of great interest the development of a way to deliver CO at specific sites with controllable and tuneable rate.

CO is a very stable molecule which does not readily and reversibly react with substrates.

There are already some known CO releasing molecules – CORM's: the organometallic compounds with carbonyl ligands and dichloromethane. Both have been already patented.[7,8,9] Recently another type of CO release compounds was discovered: the tertiary aldehydes.

Aldehydes are organic compounds containing a terminal carbonyl functional group with at least one hydrogen atom attached. This hydrogen increases the reactivity of that carbonyl group and the carbon develops a partial positive charge leading to an increase in the electron density in the oxygen atom. Due to the difference of electronic density this type of compounds could react with biological substrates, e.g. water, alcohols, amines, thioalcohols, etc.

To develop aldehydes as CORM's it would be necessary that these compounds would be able to release CO under biological conditions. Aldehyde decarbonylation occurs under certain aggressive conditions like extremely acidic or basic conditions, catalysis by organometallic compounds, high temperatures, light or oxidative conditions,[10] where tertiary aldehydes can lose a CO molecule and create a radical localized in a tertiary carbon (Fig.2).

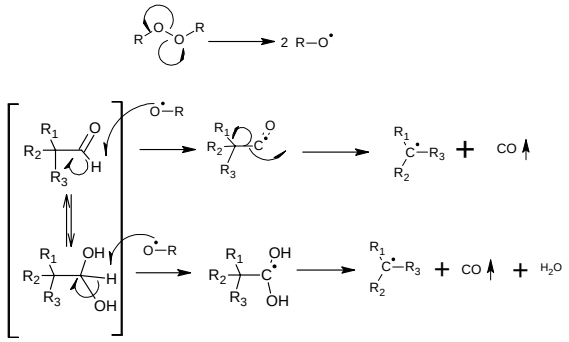

Figure 2: Oxidative decarbonylation of tertiary aldehydes.

Radicals are very reactive species with at least one unpaired electron and a short lifetime which depends on radical stabilization that can occur in two ways: hyperconjugation or conjugation.

The decarbonylation of tertiary aldehydes produces a radical that have a maximum stabilization by hyperconjugation and can be additionally stabilized by conjugation, depending on the substitutents.

With these findings, tertiary aldehydes became potential prodrugs and several types of molecules were developed.

Aldehydes are reactive molecules that can react with some biomolecules. The preparation of aldehydes` prodrugs could be a useful strategy to protect the aldehyde functionality while maintaining the CO release capacity.

Prodrugs are biologically inactive compounds that liberate the active metabolite after metabolization.[11] There are several types of aldehydes` prodrugs. They can be cyclic or non cyclic, hydrolytic or enzymatic.

The cyclic thiazolidine prodrugs were investigated.

Thiazolidines are cyclic Mannich bases with a S-C-N fragment which is prepared by the condensation of an aldehyde with the desired β-aminothiol (Fig.3).

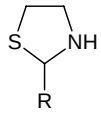

Figure 3: Thiazolidine structure

There are several examples of thiazolidines in the literature. They can be used to deliver cysteine and cysteamine avoiding their toxicity effects. Here, the thiazolidine prodrug strategy was adopted with success.[12] The thiazolidine is hydrolysed to release the aminothiol and the aldehyde (Fig.4).

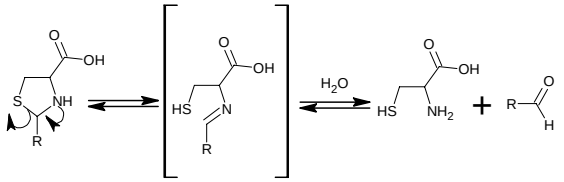

Figure 4: Thiazolidine ring hydrolysis.

2. Results and Discussion

The aldehydes` CO release was measured and is shown in the graph below (Fig. 5):

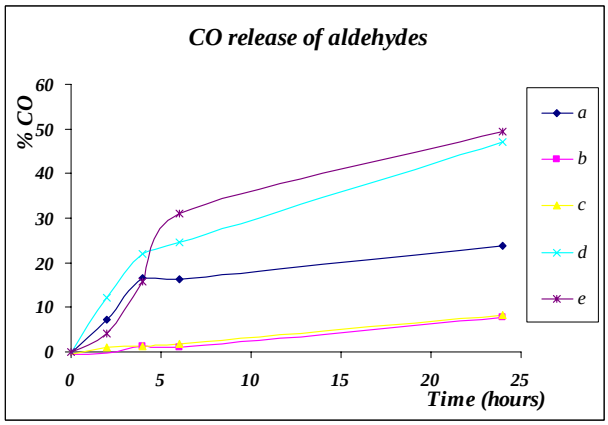

Figure 5: Aldehydes` CO release kinetics

Compound ***b*** and ***c*** show a similar behavior: Compound ***b*** releases 7.7% of CO at 24 hours and Compound ***c*** releases 8.2% of CO. These two compounds only differ in a substituents; the similar structure explains the similar behavior of CO release in these conditions.

The two aldehydes ***a*** and ***e*** have a similar group but they have distinct CO release behaviour. Compound ***a*** releases 24% of CO within 24 hours whereas compound ***e*** releases 49% of CO. This behaviour is due to a higher stabilization by resonance through ring aromaticity.

In the case of compound ***e*** the radical intermediate species can be stabilized by hyperconjugation and eventually by an intramolecular neighbouring group effect.

Compound ***d*** is one of the aldehydes that releases more CO. This could be due to the carboxylic acid group that protects the aldehyde and avoids interactions with biological molecules.

In this work several pivaldehyde thiazolidine prodrugs were synthesized and their CO release behavior was studied (Fig. 6).

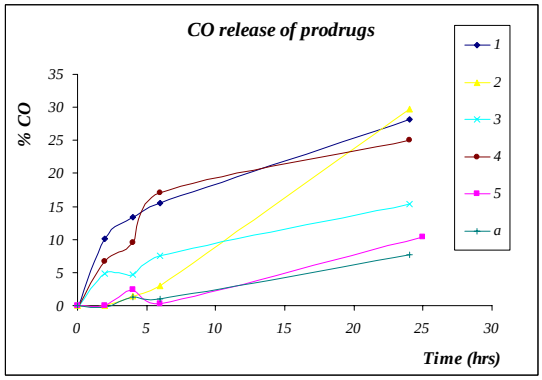

Figure 6: Prodrugs CO release kinetics

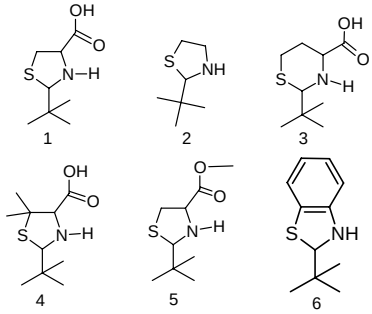

Figure 7: (1) 2-tert-butyl-thiazolidine-4-carboxylic acid, (2) 2-tert-butyl-thiazolidine, (3) 2-tert-butyl-1,3-thiazinane-4-carboxylic, (4) 2-tert-butyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid,(5) 2-tert-butyl-thiazolidine-4-carboxylic acid methyl ester, (6) 2-tert-butyl-2,3-dihydro-1,3-Benzothiazole

Pivaldehyde releases less CO when compared with the prodrugs, with the exception of 2-tert-butyl-2,3-dihydro-1,3-Benzothiazole (Fig. 7-6). This difference may arise from the enhanced solubility of the aldehyde when it is in the thiazolidine form.

2-tert-butyl-thiazolidine-4-carboxylic acid (Fig. 7-1) and 2-tert-butyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (Fig. 7-4) are the prodrugs that release more CO. The first releases about 28% of CO and the second releases 25%. These compounds differ only in the two methyl groups and this difference apparently was not very important for CO release. The compound 2-tert-butyl-thiazolidine (Fig. 7-2) has an initial slow release but at 24 hours releases (30 %) about the same as 2-tert-butyl-thiazolidine-4-carboxylic acid and 2-tert-butyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid. Initially the compound had low solubility but after hydrolysis the solubility and the amount of CO released increased (Fig.8).

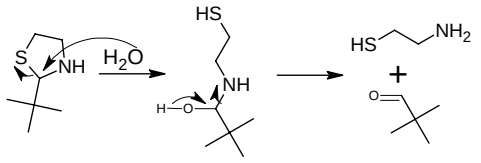

Figure 8: 2-tert-butyl-thiazolidine ring opening mechanism

The compound 2-tert-Butyl-1,3-thiazinane-4-carboxylic acid (Fig. 7-3) releases 15% of CO at 24 hours. This compound is a 6 membered ring and has a slower release when compared to the 5 membered rings. According to the Baldwin rules the 6-endo-trig ring closure is a favoured reaction, but the 5-endo-trig ring closure is disfavoured which could explain the different CO release behaviour.

The thiazolidine 2-tert-butyl-thiazolidine-4-carboxylic acid methyl ester (Fig. 7-5) differs from 2-tert-butyl-thiazolidine-4-carboxylic acid on a methyl group. This additional methyl group confers low solubility to the compound and the CO release was slower. This compound releases 10% of CO at 24 hours.

It was observed that 2-tert-butyl-2,3-dihydro-1,3-benzothiazole didn't release CO at all, and this fact could be due to the ring resonance stabilization (Fig. 9).

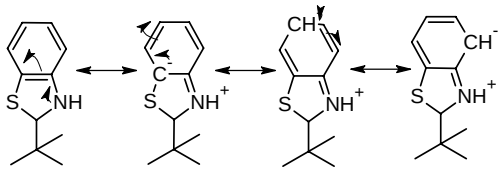

Figure 9: Ring resonance stabilization.

All the other prodrugs are stable with the exception of 2-tert-butyl-thiazolidine (Fig. 7-2). This compound is the only one that doesn't have a carboxylate group attached to the carbon alpha to the nitrogen atom. The carboxylate group stabilizes the molecule through hydrogen bonds (Fig. 10).

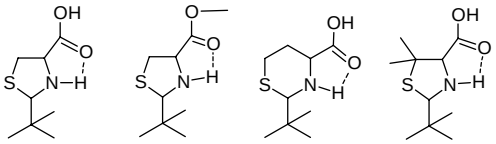

Figure 10: Ring stabilization by hydrogen bonds.

3. Conclusion

Compounds ***d*** and ***e*** are aldehydes that release higher amounts of CO under oxidative conditions. It was observed that pivaldehyde in the thiazolidine form is a better CO releaser than in the free form. The results obtained indicate that tertiary aldehydes are good CO releasers in similar physiological conditions and therefore potential CORM´s.

4. Experimental Section

Synthesis

All reagents were purchased from Sigma-Aldrich or Merck and used as received.

For some reactions were used a vaccum line, a nitrogen line and previously dried solvents[13]: THF was pre-dried with KOH and was dried with sodium wire, using benzophenone as indicator; dichloromethane was pre-dried and dried with CaH_2 and chloroform was dried with Na_2SO_4.

The purification of the compounds was made by distillation in a Kugelrohr 230 V apparatus from Aldrich or by preparative chromatography using Silica gel GF UV254, Uniplate Techware Z513040 from Analtech. The reaction evolution was followed by TLC using silica Merck Kieselgel 60 F_{254} plates, and revealed by ultraviolet light at 254 nm or indicators solutions of phosphomolybdenum acid in ethanol or ninhydrin in acetone.

The compounds were characterized by NMR, in a Bruker Avance^{II} 400 Ultrashield Plus or in a Bruker AMX 300, by elemental analysis, in an elemental analyser CHNS Carlo Erba, EA1108 model, and by infrared spectroscopy in a Unican Mattson 7000 FTIR spectrometer, using KBr platelets for solid compounds and NaCl platelets for liquid compounds.

Compound a: To a solution of starting material (10 mL, 0.075 mol) and aqueous 50% KOH (0,5 mL, 0.0044 mol) was added dropwise starting material 2 (5.5 mL, 1.1 eq.) at 60ºC. After all had been added the mixture was stirred for two hours at 60ºC to complete the reaction. The mixture was initially transparent and at the end is orange. The product was acidified to pH=1 with concentrated hydrochloric acid. The resulting solution was washed with water, extracted with Et_2O and dried under vacuum. The resulting yellow oil was distilled in a Kugelrohr apparatus and the desired product collected at 120ºC.

Elemental analysis: Exp (Cal.) % N – 9.22 (9.14), % C – 70.53 (70.55), % H – 10.28 (10.44);

IR ν (cm^{-1}): 3565, 2976, 2253,1733, 1469, 1199;

Compound c: To a THF (95 ml) suspension of sodium hydride cooled in an ice bath was added dropwise starting material (10 ml, 0.072 mol) during 20 minutes. Some effervescence was observed and the mixture turned slightly yellow. After effervescence ends starting material 2 (5 ml, 1.1 eq.) in THF (5 ml) was added dropwise and the solution became progressively yellow, then it changed to limpid yellow, then to colorless and in the end it was white milky. The solution was allowed to stir overnight at room temperature. In the morning 15 mL of water was added and the solution changes to colorless again. It was extracted 3 times with Et_2O, dried with Na_2SO_4, filtered and the solvents were removed under vacuum to yield a slightly yellow milky oil. This

oil was distilled twice in the Kugelrohr apparatus (70ºC) giving the desired and pure compound as a colorless oil.

Compound e: To a solution of starting material (0.88 mL, 0.0049 mol) and aqueous 50% KOH (0,27 mL, 0.002 mol) at 55ºC, was added dropwise starting material 2 (0.36 mL, 1.1 eq.). After all had been added, the mixture was stirred for two hours at 60ºC to complete the reaction. The mixture was initially transparent and at the end was slightly orange. The product was acidified to pH=1 with concentrated hydrochloric acid. It was washed twice with water, extracted with Et_2O, and dried under vacuum to yield slightly yellow oil. The residual oil was purified by preparative chromatography (10% AcOEt:90% n-pentane) giving the pure product as a transparent oil (R_f=0.3).
Elemental analysis: Exp (Cal.) % C: 81.71 (81,90); % H: 6.67 (6.06); % N: 5.09 (5.62);

Compound d: To 20 mL of water and KOH (1,605 g, 0.0286 mol) was added starting material (4.01g, 0.026 mol). The resulting solution was refluxed for 7 hours, and allowed to stir at r.t. for 2 days. Then the mixture was washed with water and the heavy layer was collected and dried under vacuum. The resulting slightly yellow oil was distilled in the Kugelrohr apparatus at 120ºC to give the desired product as a colorless and viscous oil.
Elemental analysis: Exp. (Calc.) %C: 62.25 (62.77), %O: (27.87), %H: 8.74 (9.36)
IR ν(cm^{-1}): 3228, 2982, 1728, 1464, 1232;

2-tert-butyl-thiazolidine-4-carboxylic acid[14,15,16,17]: L-cysteine (3.22 g, 0.026 mol) and pivaldehyde (3.6 ml, 1.2 eq) are mixed in 40 ml of methanol. Initially the solution is a suspension but the next day the solution is turve. The solvent was evaporated and the obtained white solid was dried under vacuum.
Elemental analysis Exp (Calc) %C: 50.89 (50.76); %H: 7.96 (7.99); %N 7.46 (7.40); %S 17.33 (16.94)
IR ν (cm^{-1}): 3455, 3065, 2966, 1644, 1481, 1360, 1305, 1202, 859, 619-1
^{1}H NMR (D_2O, 300 MHz) δ: 4.71(d, J = 1.2 Hz, 0.4H, S-CH-NH), 4.64 (d, J =0.6 Hz, 0.6H, S-CH-NH), 4.58-4.57 (m, 0.4H, CH_2-CH-NH), 4.3-4.29 (m, 0.6H, CH_2-CH-NH), 3.30 (m, 2H, CH_2), 1.0 (s, 0.6x(9H), $(CH_3)_3$), and 0.98 (s, 0.4x(9H), $(CH_3)_3$)

2-tert-butyl-thiazolidine[18]***:*** Cysteamine (2.02g, 0.025mol) and pivaldehyde (2.1 ml, 1.1 eq.) are mixed in 20 mL of methanol at room temperature. The reaction was exothermic and was followed by TLC (20%AcOEt in hexanes. After 19 hours another portion of pivaldehyde (0.5 mL) was added and the mixture stirred for a further 24h; Methanol was removed under vacuum to give the expected product as a transparent oil. This compound was unstable under nitrogen at 4ºC.

Elemental analysis Exp (Calc) %C 53.30 (57.88); %H 8.98 (10.41); %N 9.65 (9.64); %S 24.59 (22.07);
IR ν (cm^{-1}): 3321, 2971, 1673, 1482, 1371, 1050, 927, 839;
^{1}H NMR ($CDCl_3$, 300 MHz)δ: 4.36 (s, 1H, -S-C<u>H</u>-N-); 3.60-3.54, 3.00-2.81, 2.75-2.61 (m, rotamers, 1H+2H+1H, -S-C<u>H</u>$_2$-C<u>H</u>$_2$-N-), 1.53 (s, 1H, -N<u>H</u>) and 1.02 (s, 9H, (C<u>H</u>$_3$)$_3$).

2-tert-Butyl-[1,3]thiazinane-4-carboxylic acid[19]***:*** DL-Homocysteine (1 g, 0.007 mol) was dissolved in 10 ml of MeOH and 2 ml of distilled water and pivaldehyde (0.93 mL, 1.2 eq) was added. The solution was stirred at room temperature for 16 hours and the solvent was evaporated yielding a white powder.
Elemental analysis Exp (Calc) %C: 52.67 (53.17); %H: 7.93 (8.43); %N: 6.99 (6.89); %S: 16.26 (15.77);
IR ν (cm^{-1}): 3461, 2936, 2858, 1725, 1455, 1263, 1190, 1023, 908, 828;
^{1}H-NMR (D_2O, 300 MHz) δ: 4.28, (s, 0.4x1H, S-CH-NH), 4.17 (s, 0.6x 1H, S-CH-NH), 4.02-4.03 (m, 0.4xH, CH_2-CH-NH), 3.7-3.4 (m, 0.6x1H, CH_2-CH-NH), 2.93-2.55 (m, 2H, S-CH_2), 2.49-1.76 (m, 2H, CH_2-CH-NH), 0.98 (s, 0.4x(9H), $(CH_3)_3$), and 0.96 (s, 0.6x(9H), $(CH_3)_3$);

2-tert-butyl-2,3-dihydro-1,3-Benzothiazole: 2-aminothiophenol (0,9 mL, 0,008 mol) and pivaldehyde (1,0 mL, 1,1 eq) were mixed in 10 mL of methanol and stirred overnight. The reaction was followed by TLC (30% AcOEt/ 70% Heptane). After a few hours of stirring more pivaldehyde (0.3 ml) was added. When TLC showed completeness of reaction, the solvent was removed under vacuum, to yield a yellow solid which was purified by crystallization (ethanol/water) to yield white needles crystals.
Elemental analysis: Exp. (Calc.): %C 68.87 (68.35); %H 8.00 (7.82); %N 7.20 (7.25); %S 16.65 (16.59);
IR ν (cm^{-1}): 3375, 3071, 2968, 2871, 2426, 1580, 1476, 1075, 806, 741, 485;
^{1}H-NMR (MeOD, 300MHz) δ: 6,65-6,25 (m, 4H, Ar), 4.90 (s, 1H, S-CH-NH), and 0.71 (s, 9H, $(CH_3)_3$);

2-tert-butyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid: DL-Penicillamine (0,31 g, 0,002 mol) and pivaldehyde (0,25 mL, 1,1 eq) were mixed in 10 mL of methanol, at room temperature, and stirred overnight. The reaction was followed by TLC (30% AcOEt/70% n-pentane). When TLC showed completeness of the reaction the solvent was removed under vacuum to yield a white solid.
Elemental analysis: Exp (Calc.): %C 55.86 (55.27), %H 8.64 (8.81), %N 6.46 (6.44), %O (14.72), %S 14.25 (14.75);
IR ν (cm^{-1}): 3497, 3298, 2976, 2742, 2522, 2013, 1734, 1623, 1480, 1370, 1260;
^{1}H-NMR (MeOD) δ: 4,49 (s, 0,5x1H, N-CH-COOH), 4,33 (s, 0,5x1H, N-CH-COOH), 3,52 (s, 0,5x1H, S-CH-NH), 3,40 (s, 0,5x1H, S-CH-NH), 1,46 (s, 0,5x3H,CH_3-C-S), 1,41 (s, 0,5x3H,CH_3-C-S), 1,15 (s, 0,5x3H,CH_3-C-

S), 1,01 (s, 0,5x3H,CH_3-C-S), and 0.81 (s, 0,5x9H, $(CH_3)_3$), 0,79 (s,0,5x 9H, $(CH_3)_3$).

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