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CYSTEINOLS: IMPROVED SYNTHESIS OF 2-AMINO-2-(MERCAPTOMETHYL)PROPANE-1,3-DIOL HYDROCHLORIDE

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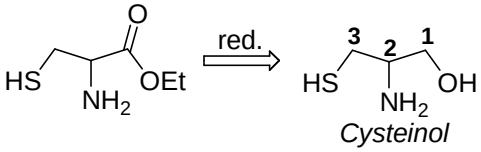
ABSTRACT. An enhanced know-how preparation of the title compound is described starting from TRIS® [2-amino-2-(hydroxymethyl)propane-1,3-diol, *2-(hydroxymethyl)serinol*]

Keywords: *cysteinols, 1,3-oxazolines, thionation, TRIS®*

INTRODUCTION

Cysteinol, 2-amino-3-mercaptopropanol, was seen as the reduced form of cysteine and entitled as such by Broadbent in 1976 [1a], inspired from the pioneering work of Enz and Cecchinato as early as 1961 [1b] *("cysteine synthetic approach"*, Scheme 1).



Scheme 1

With minor modifications, *"cysteine synthetic approach"* remained of interest in the next period [2] for the preparation of functionalised heterocyclic saturated systems, 1,3-thiazolidines, 1,3-oxazolidines and fused systems thereof *e.g.* as key intermediates in the total synthesis of *Biotin*,[2b] *Micacocidine* [2c] and *Farnesyl Transferase*.[2e] Regardless the context, the isolation of *cysteinol* as hydrochloride or the trapping *in situ* of its free base with >C=O electrophiles were throughout preferred.

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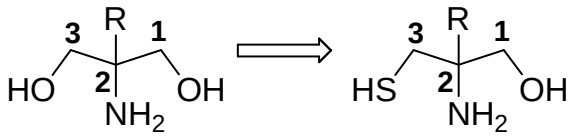
Therefore, our expertise in *C*-substituted-2-aminopropane-1,3-diols' chemistry (*serinols*),[3] seen *mutatis-mutandis* as reduced forms of *C*-substituted serines, prompted us to investigate their conversion into mercapto analogues, for example the well known TRIS®, 2-amino-2-(hydroxymethyl)propane-1,3-diol **Ia** [*2-(hydroxymethyl)serinol*] into 2-amino-2-(mercaptomethyl)propane-1,3-diol **IIa** [*2-(hydroxymethyl)cysteinol*, Sche-me 2].



R = H

serinol *cysteinol*

R = CH_2OH (**a**), Me (**b**), Et (**c**)

Ia *2-(hydroxymethyl)serinol* → **IIa** *2-(hydroxymethyl)cysteinol*
Ib *2-methylserinol* → **IIb** *2-methylcysteinol*
Ic *2-ethylserinol* → **IIc** *2-ethylcysteinol*

Scheme 2

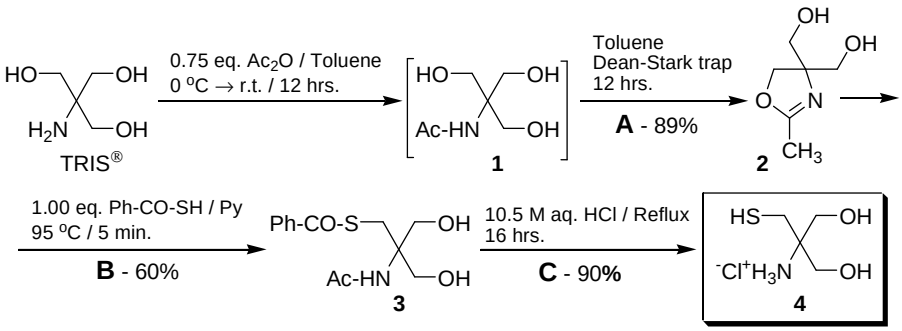
That is, we explored a *"serinolic synthetic approach"* directed to *cysteinols*.

To the best of our knowledge, there are only two old patents [5] from which one dedicated completely to the preparation of *cysteinols* **IIa-c** (Scheme 2), **IIb** and **IIc** being by far much better documented with respect to **IIa**. However, careful examination of the cited patents inferred us about the instability, as free bases, of all **IIa-c**. They were claimed to be useful in protection of mammalians against radiation (free bases or hydrochlorides). Except elemental analysis, no spectroscopic data of **IIa-c** were reported so far.

Hence, the aim of the present preliminary report consists of the essentially improved synthesis of *cysteinol* **IIa** starting from TRIS® **Ia** (Scheme 2). Indeed, **IIa** appeared to us more attractive due to its additional functionality with respect to **IIb** and **IIc**.

RESULTS AND DISCUSSION

Targeting *cysteinol* **IIa** (Scheme 2), the chemistry we applied, based orientatively on the above two cited patents [5], is resumed in Scheme 3.



Scheme 3

Each step made the object of serious improvements.

In step **A**, one of the three homotopic methylenes of TRIS® was activated by including it into a 1,3-oxazoline ring, **2**. Differently than previously reported,[5] rather than operating in boiling acetic acid with azeotropic removal of water from the reaction mixture followed by fractional distillation of crude **2** under reduced pressure, then recrystallisation from THF (52% yield [5b]), we opted for a more reactive electrophile, acetic anhydride in toluene, according to the below stoichiometry (Eq. 1, 2).

$$H_2NC(CH_2OH)_3 + Ac_2O \longrightarrow \mathbf{2} + AcOH + H_2O \quad (1)$$

$$H_2NC(CH_2OH)_3 + AcOH \longrightarrow \mathbf{2} + 2\,H_2O \quad (2)$$

$$2\,H_2NC(CH_2OH)_3 + Ac_2O \longrightarrow 2 \times (\mathbf{2}) + 3\,H_2O \quad (1 + 2)$$

Thus, although 0.5 molar equivalents of acetic anhydride theoretically ensured that all acetyl groups were incorporated in the 1,3-oxazoline **2** with no statistically favoured side *O*-acylation of TRIS®, we anyhow used 0.75 eq. of Ac_2O and removed the resulting water with the use of a Dean-Stark trap.[♣] To our surprise, while the yield of crude freshly isolated **2** was 93% (96 % ^{1}H NMR purity and 4% amide **1**), in our hands, it was an unstable compound. Indeed, in the crude **2**, the content of TRIS' amide **1** increased to 15% within drying 24 hrs. at room temperature to reach 40% after one week of storage.[♥] Fortunately, in this case, the mixture **1** + **2** could be resubmitted to the dehydrating cyclisation conditions (**1** → **2** + H_2O, Scheme 3) affording quantitatively, again, the above crude **2** (96% ^{1}H NMR purity, 4% **1**).

[♣] In both Patents,[5] the use of 1.5 eq. AcOH is recommended for the synthesis of **2**.

[♥] It appeared to us that **2** was sensitive to moisture, especially if residual AcOH was present in the crude material.

So, we used oxazoline **2** in the next step of the synthesis (**B**, Scheme 3) with no other purification than a rapid triturating with dry ether, intended to eliminate the residual amounts of acetic acid.

In the key step **B**, we obtained the *S*-, *N*-protected form **3** of the *cysteinol* **IIa** in 60% yield (previously reported, 57% [6a]). Despite the excellent NMR appearance of crude **3** (71% yield), it required two purifications from EtOH, in order to reach the analytical purity.

Both ^{1}H and ^{13}C NMR spectra of crude compound **3** exhibited just a single pair of enantiotopic CH_2O groups located at 60.3 ppm ppm [$\delta(CH_2S)$ = 30.3 ppm] and just one clear AX geminal coupling pattern between their diastereotopic protons [$\Delta\delta$(H-a/H-b) = 0.03 – 0.05 ppm, $^{2}J_{a\text{-}b}$ = 10.3 – 10.6 Hz]. Accordingly, we concluded that thionation of **2** (Scheme 3) was completely chemioselective.

Finally, in step **C**, we *N*-, *S*-deprotected the amidoester **3** in boiling 10.5 M aq. HCl in 90% yield (lit. 68% [5b]) and isolated the *cysteinol* **IIa** as hydrochloride **4** following one of our previous patented protocols [6] (see EXPERIMENTAL SECTION and Figure1).

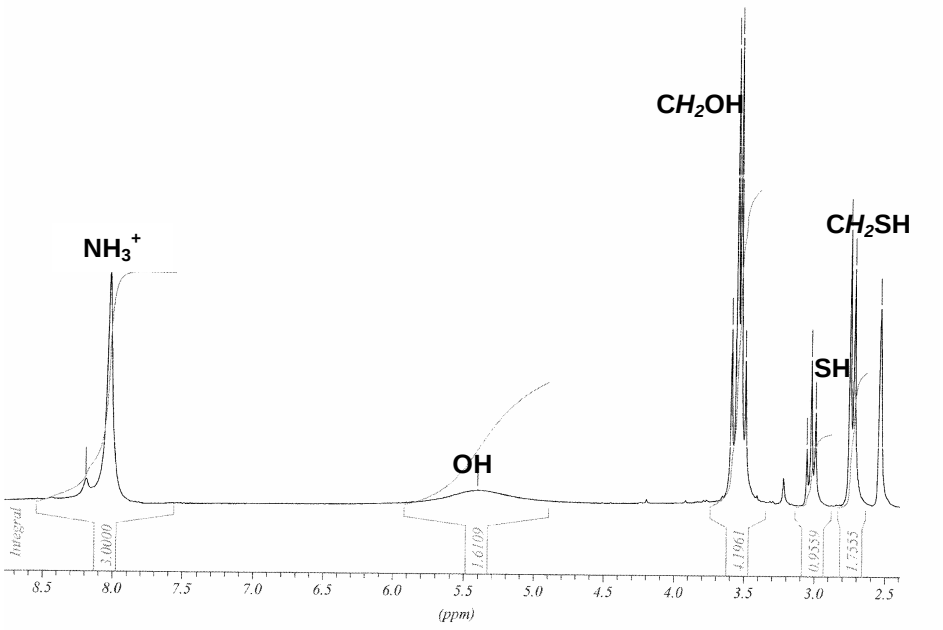


Figure 1. ^{1}H NMR of compound **4** on 300 MHz timescale ([D_6]DMSO)

The amount of the resulting side product, benzoic acid (90% yield), was indicative for the very clean reaction observed. Compound **4** was stable on storage indefinitely.

CONCLUSIONS

Starting from TRIS®, an essentially improved three steps synthesis of 2-amino-2-(mercaptomethyl)propane-1,3-diol, [*2-(hydroxymethyl)cystei-nol*], hydrochloride, occurring in 48 % overall yield, was described (lit. 20% [5b]).

EXPERIMENTAL SECTION

General. Melting points are uncorrected; they were carried out on ELECTROTHERMAL® instrument. NMR spectra were recorded on a Brucker® AM 300 instrument operating at 300 and 75 MHz for ^{1}H and ^{13}C nuclei respectively. All NMR spectra were measured in anhydrous commercially available deuteriated solvents. No $SiMe_4$ was added; chemical shifts were measured against the solvent peak. All chemical shifts (δ values) are given throughout in ppm; all coupling patterns ($^{n}J_{H,H}$ values) are given throughout in Hz.TLC was performed by using aluminium sheets with silica gel 60 F_{254} (Merck®); flash column chromatography was conducted on Silica gel Si 60 (40–63 μm, Merck®). IR spectra were performed on a Perkin-Elmer® Paragom FT-IR spectrometer. Only relevant absorptions are listed [throughout in cm^{-1}: weak (w), medium (m) or (s) strong]. Microanalyses were performed on a Carlo Erba® CHNOS 1160 apparatus. Mass spectra (MS) were recorded on a Brucker® Esquire Instrument.

Preparation of 4,4-bis(hydroxymethyl)-2-methyl-1,3-oxazoline ***(2)*****.**

To TRIS® (8.48 g, 70 mmol) suspended in cooled (0 ^{o}C) dry toluene (125 mL), acetic anhydride (5.00 mL, 5.36 g, 52.5 mmol) was rapidly injected under vigorous stirring. The resulted suspension was stirred and let to reach room temperature overnight (12 hrs.) then heated at reflux for additional 12 hrs. with continuous removal of water in a Dean-Stark trap (TLC monitoring, eluent toluene : ethanol 3/1 v/v, visualisation in a I_2 bath). The reaction was stopped when no more water was separated and no more evolution was observed on TLC. At this step, starting from refluxing toluene, the reaction mixture, as a fine colourless emulsion, was let very gently to reach room temperature under vigorous stirring in order to ensure successfully the obtention of a fine white suspension. After filtering, well washing with dry ether ($\times$ 25 mL) and drying at room temperature within one hour, 9.45 g of the crude title compound **2** were obtained (yield 89% with respect to TRIS®; ^{1}H NMR purity 96%; 4% compound **1**).

2-Acetamido-2-(hydroxymethyl)propane-1,3-diol (1): ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.73 (s, 3 H, CH_3), 3.50 (s, 6 H, C*H*$_2$OH), 4.62 (bs, 3 H, CH_2O*H*), 7.22 (bs, 1 H, NH) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 24.0 (1 C, CH_3), 60.0 (1 C, Cq., C-2), 60.8 (3 C, 3 × CH_2OH), 175.2 (1 C, >C=O) ppm.

4,4-Bis(hydroxymethyl)-2-methyl-1,3-oxazoline (2): white crystalline powder, m.p. 82–84 °C (Et_2O) [lit.[5a] 95 – 97 °C ($CHCl_3$/Et_2O/AcOEt); lit. [5b] 88 – 89 °C (THF)]. R_f (75% toluene/EtOH) = 0.45. IR (KBr): ν = 3360 (s), 3271 (s), 3108 (s), 2982 (s), 2941 (s), 2872 (s), 1670 (s), 1629 (m), 1572 (m), 1459 (m), 1384 (s), 1270 (s), 1229 (w), 1187 (w), 1143 (w), 1029 (s), 994 (s), 972 (w), 942 (w), 882 (w), 843 (w), 691 (w), 649 (w), 625 (w), 588 (w), 525 (w) cm^{-1}. ^{1}H NMR (300 MHz, [D$_6$]DMSO): *δ* = 1.84 (s, 3 H, CH_3), 3.29 (d, 2 H, $^{2}J_{H,H}$ = 11.0 Hz, C*H*$_2$OH), 3.36 (d, 2 H, $^{2}J_{H,H}$ = 11.0 Hz, C*H*$_2$OH), 4.02 (s, 2 H, H-5), 4.69 (bs, 2 H, CH_2O*H*) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): *δ* = 13.6 (1 C, CH_3), 63.9 (2 C, CH_2OH), 70.3 (1 C, C-4), 76.4 (1 C, C-5), 163.4 (1 C, C-2) ppm. MS (positive CI, isobutane, 200 eV): *m/z* (%) = 202 (17) [M+*i*-BuH-2]$^+$, 188 (7) [M+42]$^+$, 164 (15) [M+18]$^+$, 146 (100) [M+1]$^+$, 114 (6), 73 (< 5); $C_6H_{11}NO_3$ (145.07).

*Preparation of 2-acetamido-2-(benzoylthiomethyl)propane-1,3-diol **(3)***

In a vigorously stirred solution prepared from 4,4-bis(hydroxymethyl)-2-methyl-1,3-oxazoline **(2)** (3.68 g, 25.35 mmol) dissolved in dry pyridine (10.00 mL, 10.50 g, 133 mmol), thiobenzoic acid (3.30 mL, 3.50 g 100%, 25.35 mmol) was rapidly injected at room temperature. The resulted yellow solution was heated at 95 °C and kept at this temperature for 5 min. A clear orange-reddish solution was obtained which was cooled at 0 °C for 30 min. then poured on aq. HCl (45.00 mL, 47.70 g, 4 M aq. HCl, 179 mmol). Crude **3** crystallised as a yellow mass (pH = 0.5 - 1) which was cooled for additional 12 hrs. at 0 °C. After filtering, washing with cooled water (3 × 15 mL) and drying at r.t., 6.90 g crude **3** were obtained as a yellow amorphous powder. This was twice recrystallised from boiling ethanol (15 mL) to yield 4.30 g pure **3** as a white crystalline powder (60% yield with respect to **2**).

2-Acetamido-2-(benzoylthiomethyl)propane-1,3-diol (3):white crystalline powder, m.p. 143–145 °C (EtOH) [lit. [5a] 147 – 148 °C (-); lit. [5b] 146 – 147 °C (EtOH)]. $C_{13}H_{17}NO_4S$ (283.09): calcd. C 55.11, H 6.05, N 4.94; found C 54.88, H 5.88, N 5.29. R_f (75% toluene/EtOH) = 0.75. IR (KBr): ν = 3380 (s), 3344 (m), 3153 (m), 2942 (m), 2895 (m), 2837 (m), 1662 (s), 1648 (s), 1557 (s), 1450 (m), 1372 (m), 1323 (w), 1205 (s), 1176 (m), 1079 (m), 1064 (s), 969 (w), 913 (s), 774 (m), 690 (m), 645 (m), 596 (w), 554 (m), 533 (w) cm^{-1}. ^{1}H NMR (300 MHz, [D$_6$]DMSO): *δ* = 1.86 (s, 3 H, CH_3), 3.62 (dd, 2 H, $^{2}J_{H,H}$ = 10.3 Hz, $^{3}J_{H,H}$ = 5.7 Hz, C*H*$_2$OH), 3.61 (dd, 2 H, $^{2}J_{H,H}$ = 10.3 Hz, $^{3}J_{H,H}$ = 4.6 Hz, C*H*$_2$OH), 4.99 (dd as t, $^{3}J_{H,H}$ = 5.7 Hz, CH_2O*H*), 7.39 (s, 1 H, NH), 7.59 (t, 2 H,

$^3J_{H,H}$ = 7.5 Hz, H-3, Ph), 7.72 (t, 1 H, $^3J_{H,H}$ = 7.4 Hz, H-4, Ph), 7.95 (d, 2 H, $^3J_{H,H}$ = 7.5 Hz, H-2, Ph) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 23.4 (1 C, CH_3), 30.3 (1 C, CH_2S), 60.3 (2 C, CH_2OH), 61.2 (1 C, C-2), 126.8 (2 C, C-2, -6, Ph), 129.0 (2 C, C-3, -5, Ph), 133.7 (1 C, C-4, Ph), 136.5 (1 C, C-1., Ph), 170.3 [1 C, >C(=O)-NH-), 191.1 [1 C, >C(=O)-S-] ppm. MS (positive CI, isobutane, 200 eV): *m/z* (%) = 340 (< 5) [M+*i*-BuH-1]$^+$, 284 [M+1]$^+$ (48), 266 (8), 252(< 5), 236 (5), 218 (7), 180 (100), 162 (10), 148 (8), 130 (< 5), 123 (10), 116 (< 5), 73 (< 5).

*Preparation of 2-amino-2-(mercaptomethyl)propane-1,3-diol hydrochloride **(4)**.* 2-Acetamido-2-(benzoylthiomethyl)propane-1,3-diol **(3)** (2.41 g, 8.50 mmol) was suspended in aq. HCl (7.40 mL, 8.57 g, 10.5 M aq. HCl, 77.7 mmol) and the reaction mixture was heated at reflux with stirring for 16 hrs. After cooling at room temperature, benzoic acid crystallised abundantly and was filtered off, washed with aq. HCl (3 × 5 mL, 10.5 M aq. HCl) to yield 0.93g pure by product (90% with respect to the theoretical amount). The combined aqueous filtrate was added to benzene (100 mL) and the resulted mixture was heated with stirring at reflux with azeotropic removal of water (Dean-Stark trap). During anhydrification, compound **4** separated as an oily mass. When no more water separated, the mixture was kept at reflux for additional 2 hrs. in order to eliminate thes excess of hydrochloric acid. After cooling at room temperature, benzene was decanted and crude oily **4** was crystallised at 0 ^{o}C from 10 mL 1:1 v/v isopropanol : ether to yield 1.32 g pure **4** (90% yield).

2-Amino-2-(mercaptomethyl)propane-1,3-diol hydrochloride (4): white crystalline powder, m.p. 102 - 104 ^{o}C (*i*-PrOH/Et_2O 1:1 v/v) [lit. [5b] 104 – 105 ^{o}C (*i*-PrOH)]. $C_4H_{12}ClNO_2S$ (173.03): calcd. C 27.66, H 6.96, N 8.07; found C 28.02, H 6.88, N 7.99. IR (KBr): ν = 3344 (s), 3265 (s), 3008 (s), 2554 (m), 1602 (m), 1542 (m), 1514 (s), 1458 (m), 1399 (m), 1277 (m), 1247 (m), 1112 (m), 1062 (s), 918 (m), 671 (m), 546 9w) cm^{-1}. ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 2.74 (d, 2 H, $^3J_{H,H}$ = 9.1 Hz, C*H*$_2$SH), 3.03 (d, 1 H, $^3J_{H,H}$ = 9.1 Hz, CH_2SH), 3.52 (d, 2 H, $^2J_{H,H}$ = 11.6 Hz, C*H*$_2$OH), 3.58 (d, 2 H, $^2J_{H,H}$ = 11.6 Hz, C*H*$_2$OH), 5.40 (bs, 2 H, CH_2OH), 8.03 (s, 3 H, NH_3^+) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 24.5 (1 C, CH_2S), 59.7 (1 C, C-2), 61.0 (2 C, CH_2OH) ppm. MS (positive CI, isobutane, 200 eV): *m/z* (%) = 176 [M+3]$^+$, 152 (7), 138 (100), 103 (9), 90 (6).

ACKNOWLEDGEMENTS

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