Supplementary Information

Characterization of the promiscuous *N*-acyl CoA transferase, LgoC, in the legonoxamine biosynthesis

Fleurdeliz Maglangit, Saad Alrashdi, Justine Renault, Laurent Trembleau, Catherine Victoria, Ming Him Tong, Shan Wang, Kwaku Kyeremeh, Hai Deng

Contents

1. Experimental

1.1 General Experimental Procedures

Synthesis reactions were monitored by Thin Layer Chromatography (TLC) using TLC Silica Gel F254 (Germany). TLC plates were visualized by exposure to ultraviolet light (UV). Column chromatography purification for the acyl-SNACs were carried out using 50:50 mixture of ethyl acetate and diethyl-ether on a silica gel 60 (Acros Organics™ ultra-pure 60A 40-63u). Organic solutions were concentrated by rotary evaporation (Buchi Rotavapor R200, UK).

1.2 Materials

All the chemicals and reagents employed were purchased at highest commercial quality from Fisher Scientific and/or from Sigma Aldrich and used without further purification.

1.3 Instrumentation

Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) were recorded on Bruker AVANCE III HD 400 MHz (Ascend™ 9.4 Tesla) or Bruker AVANCE III HD 600 MHz (Ascend™ 14.1 Tesla) with Prodigy TCI™ cryoprobe. Chemical shifts for protons are reported in parts per million scale (δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvents, CD_3OD δ3.31 and $CDCl_3$ δ7.26 (Goss Scientific). Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), integration, coupling constant in Hz. High-Resolution Electrospray Ionisation Mass Spectrometry (HR-ESIMS) was measured by LTQ Orbitrap Thermo Scientific MS system coupled to a Thermo Instrument HPLC system (Accela PDA detector, Accela PDA autosampler and Accela Pump). The absorbance readings were measured using UV-vis spectrophotometer (Jenway 6300/05/20D).

2. Cloning and overexpression of LgoB and LgoC in *E. coli*

LgoB from *Streptomyces* sp. MA37 was amplified by PCR using the genomic DNA as a template. The PCR mixture (25uL) contained 50ng of genomic DNA, 1.0 µM primer (forward: 5'-AAA GAA TTC ATG TCC ACC CAT GAC TTC ATC GCG ATC GGAC -3' and reverse 5'-AAA AAG CTT TCA TGC CAG GTC CCC CTG CGG TGC GCC GAAC -3')(restriction sites added for cloning are underlined), 0.1 mM dNTP (Novagen®), 0.1 mM $MgSO_4$, 4% DMSO, 10x KOD buffer, and 0.5 µL KOD hot start DNA polymerase enzyme (Sigma-Aldrich). Reaction conditions consisted of an initial denaturation step of 95^{0}C for 5 min followed by 30 cycles of 95^{0}C for 45sec, annealing at 65^{0}C for 45 sec and a final extension step at 72^{0}C for a minute.

The PCR products were digested by restriction enzymes and cloned into pET28a vector. The obtained constructs were transformed into *E. coli* BL21(DE3) strain. Cells were grown at 37^{0}C, 220 rpm in Luria-Bertani broth (1% tryptone, 0.5% yeast extract, 1% sodium chloride) supplemented with kanamycin (50 µg/mL, Sigma) to OD_{600} 0.6-0.9. The cultures were chilled on ice for 20min, followed by induction of protein expression with 1mM of isopropyl β-D-1-thiogalactopyranoside (IPTG), and overnight incubation at 16^{0}C, 220 rpm.

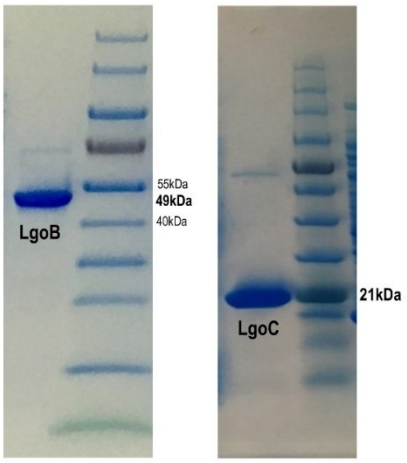
The cloning and overexpression of LgoC were performed as described above using 1.0 µM primer (forward: 5'-AAA GGA TCC ATG ACC GCG CTC ACC GGC TTC TCC CGC ACC G-3' and reverse 5'-AAA AAG CTT TCA CTG CGC GGC TCC TCG GGT CAC TGC GGC C-3').

3. Purification of His_6-LgoB and His_6-LgoC

The purification of His_6-LgoB and His_6-LgoC were as follows. The cells were harvested by centrifugation (3200g for 15min at 4^0C) and washed with 1x Phosphate Buffered Saline (PBS) buffer pH 7.4 (Fisher Scientific). The resulting pellets were resuspended in PBS buffer and the cells were lysed by sonication in an ice bath (5 min total pulse time, 2 seconds/pulse, amplitude 30-34%). The crude cell lysate was centrifuged at 4600rpm (Heraeus Multifuge 3 S-R bucket number 6441), for 20min at 4^0C, and the supernatant was filtered using a 0.45µm filter.

The filtered supernatant was collected and purified using a Ni column, equilibrated with washing buffer (1x PBS pH 7.4 with 10mM imidazole). The Ni exhibits high affinity and selectivity for 6xHis-tagged recombinant fusion proteins. Unbound proteins were removed by washing buffer (10mL) and the His_6-*LgoB* fusion proteins were eluted with 1CV of elution buffer (500mM imidazole). The proteins were concentrated using a centrifugal filter unit (Millipore) and stored at -80^0C after addition of equal amount of preservation buffer (10mM PBS buffer pH7.4, 50mM NaCl, 20% v/v glycerol).

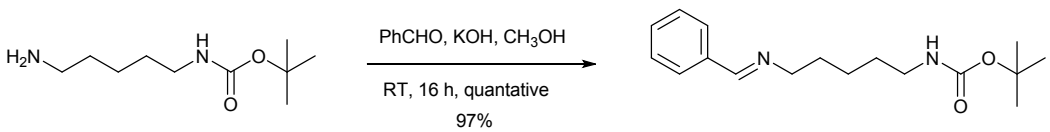
The purity and size of the proteins were verified by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Protein concentrations were measured using the Colibri microvolume spectrometer - Titertek Berthold.



SDS-PAGE Analysis of overexpressed enzymes, LgoB and LgoC

4. Synthesis of *N*-hydroxy-cadaverine 9

4.1 Synthesis of N1-Boc-N6-(3-phenyl-imine)-cadaverine



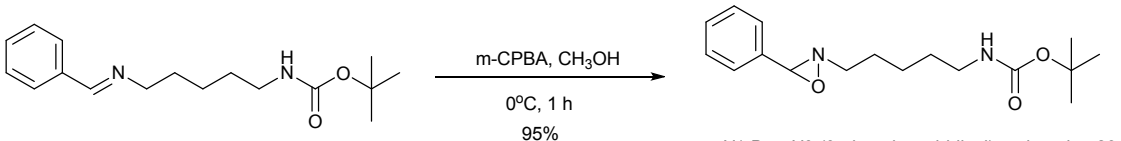
*N*1-Boc-cadaverine **12** *N*1-Boc-*N*6-(3-phenyl-imine)-cadaverine **22**

To a solution of potassium hydroxide, KOH (153 mg, 2.7 mmol, 1.1 eq) in methanol, CH_3OH (6.7 mL) at room temperature was added *N*-Boc-cadaverine **12** (500 mg, 2.5 mmol, 1

eq.), followed by benzaldehyde (275 µL, 2.7 mmol, 1.1 eq.) and 3 Å molecular sieves. The reaction mixture was stirred for 16h at room temperature. The molecular sieves were filtered off and washed with CH_3OH. The filtrate was concentrated under vacuo to yield 698 mg (97%) of imine **22** as an orange oil which was used without further purification.

^{1}H NMR (400 MHz, CD_3OD) δ 8.34 (s, 1H), 7.74-7.72 (m, 2H), 7.45-7.43 (m, 3H), 3.62 (tr, 2H), 3.04 (tr, 2H), 1.72 (m, 2H), 1.52 (m, 2H), 1.41 (s, 9H), 1.37 (m, 2H); ^{13}C-NMR (400 MHz, CD_3OD) δ 164.3, 158.8, 137.1, 137.1, 129.8, 129.3, 79.8, 62.1, 41.2, 31.4, 30.8, 28.8, 25.5; IR ν_{max} / cm^{-1} 2938, 1691, 1648, 1518; HRESIMS calculated for $C_{17}H_{27}N_2O_2^+$ $[M+H]^+$ 291.2067, found 291.2051.

4.2 Synthesis of N1-Boc-N6-(3-phenyloxaziridinyl)-cadaverine



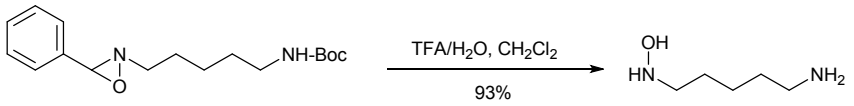
*N*1-Boc-*N*6-(3-phenyl-imine)-cadaverine **22**

*N*1-Boc-*N*6-(3-phenyloxaziridinyl)-cadaverine **23**

To a solution of imine **22** (698 mg, 2.4 mmol, 1 eq.) in CH_3OH (3.5 mL) at 0°C was added a solution of *m*-CPBA (622 mg, 3.61 mmol, 1.5 eq.) in CH_3OH (13mL) over 1hr. The reaction was stirred at 0°C for an additional hour. The resulting precipitate was filtered off and washed with CH_3OH (2x20 mL). The filtrate was concentrated at room temperature to give a white solid, which was partitioned between H_2O (15 mL) and ethyl acetate, EtOAc (15 mL). The pH of the mixture was adjusted to 2 with 2N HCl (approximatively 3.75mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3x30 mL). The combined extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated to give crude oxaziridine **23** as a white solid, which was used without further purification.

^{1}H NMR (400 MHz, CD_3OD) δ 7.74-7.72 (m, 2H), 7.45-7.43 (m, 3H), 4.64 (s, 1H), 3.62 (tr, 2H), 3.04 (tr, 2H), 1.72 (m, 2H), 1.52 (m, 2H), 1.41 (s, 9H), 1.37 (m, 2H); ^{13}C-NMR (400 MHz, CD_3OD) δ 158.8, 137.1 (2C), 129.8, 129.3, 79.8, 62.1, 41.2, 31.4, 30.8, 28.8, 25.5; HRESIMS calculated for $C_{17}H_{27}N_2O_3^+$ $[M+H]^+$ 307.2016, found 307.2020.

4.3 Synthesis of N-hydroxy-cadaverine



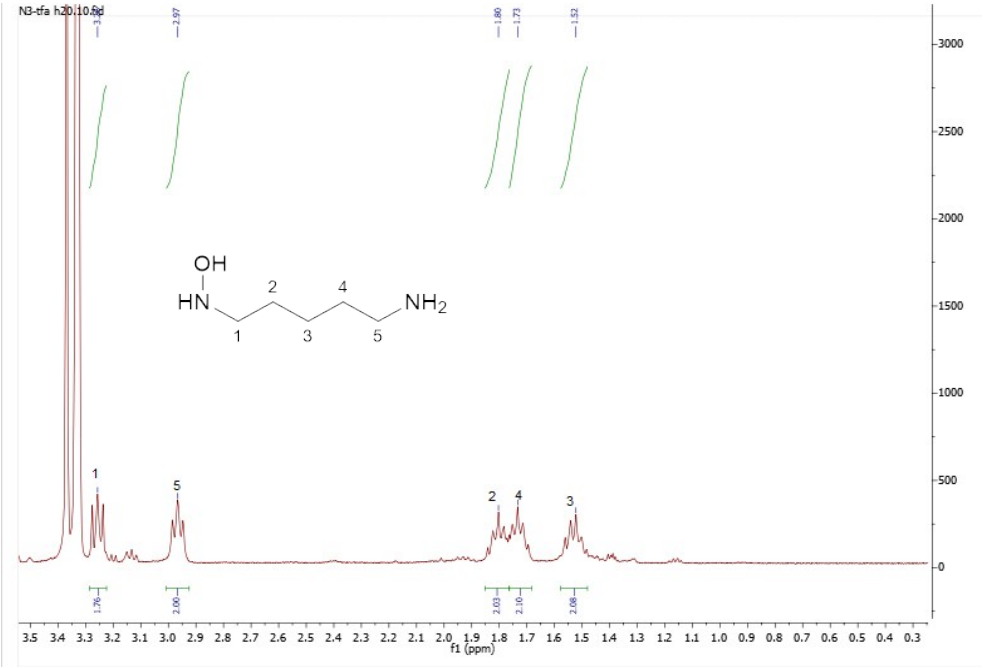
*N*1-Boc-*N*6-(3-phenyloxaziridinyl)-cadaverine **23**

N-hydroxy-cadaverine **9**

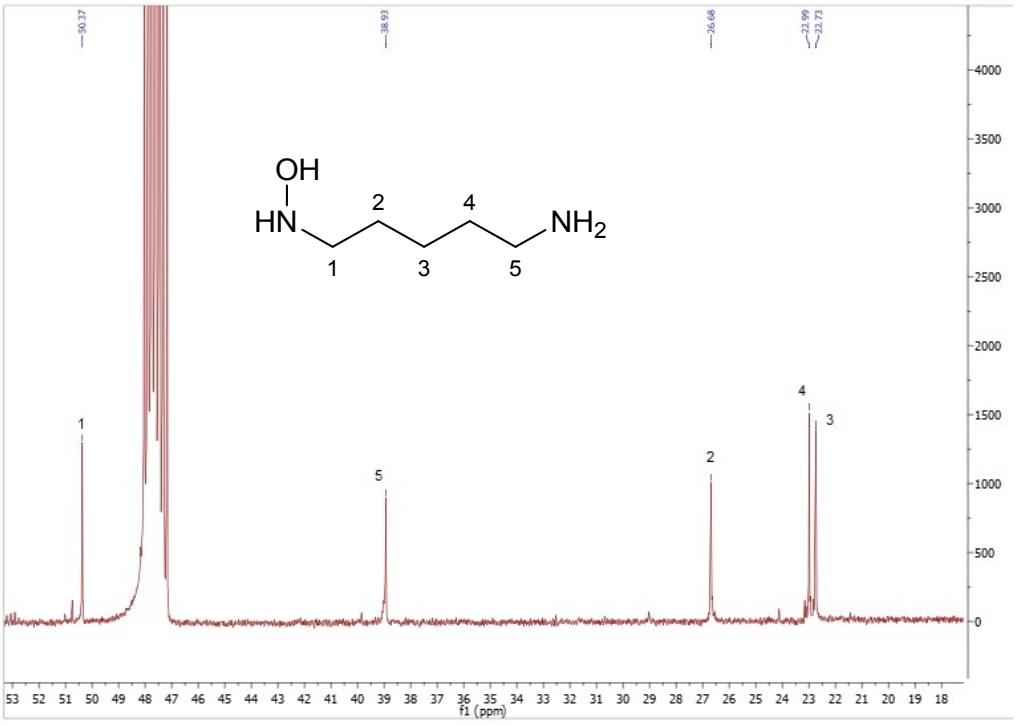
A solution of oxaziridine **23** (20 mg, 0.065 mmol) in CH_2Cl_2 (1 mL) was treated with TFA/H_2O (0.34 mL, v/v, 4/1) at room temperature. The reaction mixture was stirred for 4h, H_2O (5 mL) was added, and the layers were separated. The aqueous layer was washed with CH_2Cl_2 (2x3 mL) and concentrated to yield 7.25 mg (93%) of *N*-hydroxy-cadaverine **9**.

^{1}H-NMR (600MHz, CD_3OD, Me_4Si) δ 3.26 (tr, 2H), 2.97 (tr, 2H), 1.80 (m, 2H), 1.73 (m, 2H), 1.52 (m, 2H) ^{13}C-NMR (600MHz, CD_3OD, Me_4Si) δ 51.7, 40.3, 28.1, 24.4, 24.2. HRESIMS calculated for $C_5H_{15}N_2O^+$ $[M+H]^+$ 119.1179, found 119.1183; Δ = 1.514ppm.

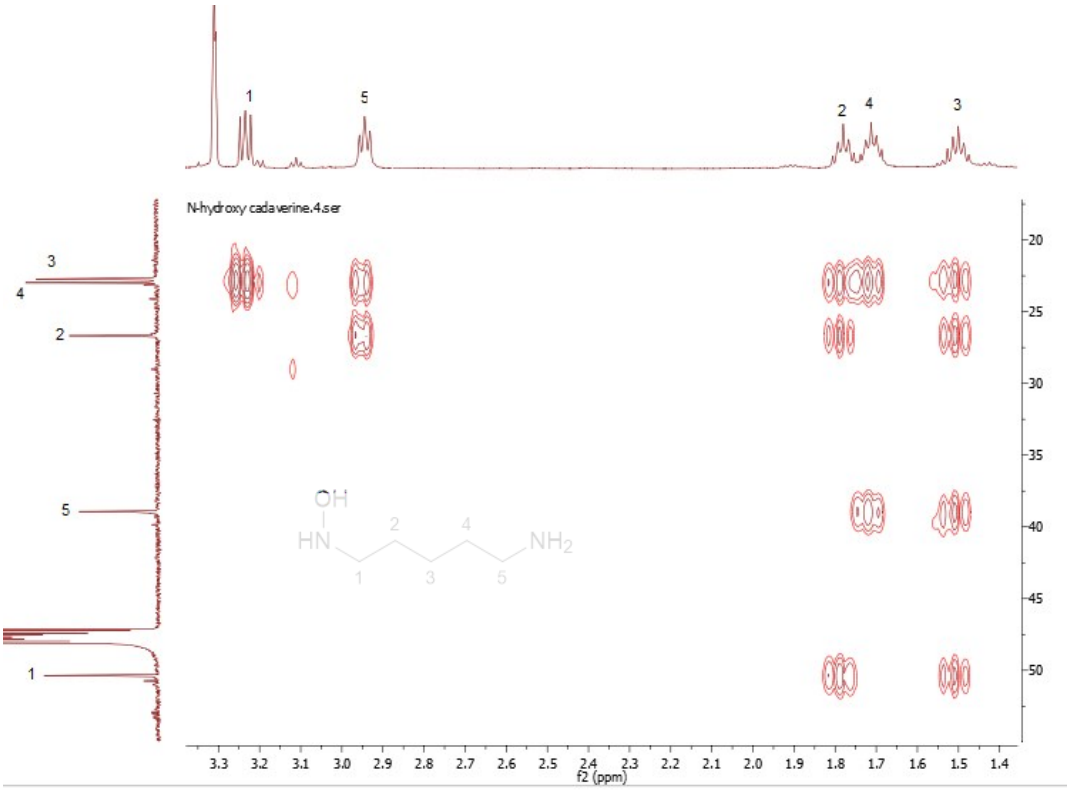
The spectral data (^{1}H NMR, ^{13}C NMR, HMBC, HR ESIMS) were in agreement with those reported in literature.[1]



^{1}H-NMR of *N*-hydroxy-cadaverine **9** (600MHz, CD_3OD)



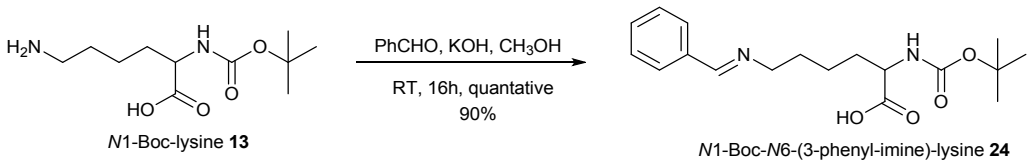
^{13}C-NMR of *N*-hydroxy-cadaverine **9** (600MHz, CD_3OD)



HMBC-NMR of *N*-hydroxy-cadaverine **9** (600MHz, CD_3OD)

5. Synthesis of *N*-hydroxy-lysine 11

5.1 Synthesis of N1-Boc-N6-(3-phenyl-imine)-lysine



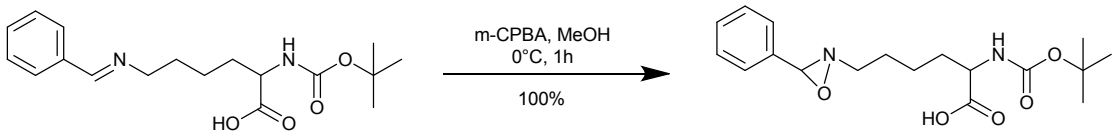
*N*1-Boc-lysine **13**

*N*1-Boc-*N*6-(3-phenyl-imine)-lysine **24**

To a solution of KOH (252 mg, 4.5 mmol, 1.1 eq) in methanol (11 mL) at room temperature was added *N*-Boc-lysine **13** (1 g, 4.1 mmol, 1 eq.), followed by benzaldehyde (452 µL, 4.51 mmol, 1.1 eq.) and 3 Å molecular sieves. The reaction mixture was stirred at room temperature for 17h. The molecular sieves were filtered off and washed with methanol. The filtrate was concentrated to give 1.22 g (90%) of imine **24** as orange foam which was used without further purification.

^{1}H NMR (400 MHz, CD_3OD) δ 8.84 (s, 1H), 7.72 (m, 2H), 7.44 (m, 3H), 3.98 (m, 1H), 3.62 (t, 2H), 1.84 (m, 2H), 1.72 (m, 2H), 1.70 (m, 2H), 1.39 (s, 9H); ^{13}C-NMR (400 MHz, CD_3OD) δ 180.3, 168.9, 155.9, 132.2, 130, 129.6, 82.1, 62.2, 55.0, 31.8, 31.1, 29, 21.0; HRESIMS calculated for $C_{18}H_{27}N_2O_4^+$ $[M+H]^+$ 335.1965, found 335.1985.

5.2 Synthesis of N1-Boc-N6-(3-phenyloxaziridinyl)-lysine



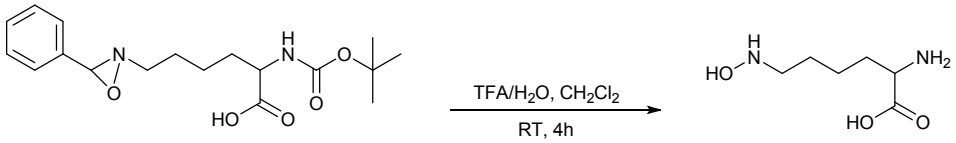
*N*1-Boc-*N*6-(3-phenyl-imine)-lysine **24**

*N*1-Boc-*N*6-(3-phenyloxaziridinyl)-lysine **25**

To a solution of imine **24** (1.36 mg, 4.1 mmol, 1 eq.) in CH_3OH (14 mL) at 0°C was added a solution of m-CPBA (1.05 mg, 6.1 mmol, 1.5 eq.) in CH_3OH (21 mL) over 1hr. The reaction was stirred at 0°C for an additional hour. The resulting precipitate was filtered off and washed with CH_3OH. The filtrate was concentrated at room temperature to give a white solid, which was partitioned between H_2O (25 mL) and EtOAc (25 mL). The pH of the mixture was adjusted to 2 with 2 N HCl (3.9 mL). The layers were separated, and the aqueous layer was extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated to give crude oxaziridine **25** (1.25g, 100%) as a pale yellow solid, which was used without further purification.

^{1}H NMR (400 MHz, CD_3OD) δ 7.90-7.46 (m, 5H), 4.64 (s, 1H), 4.06 (m, 1H), 3.02 (m, 1H), 2.75 (m, 1H), 1.99 (m, 2H), 1.85 (m, 2H), 1.72 (m, 2H), 1.44 (s, 9H); ^{13}C-NMR (400 MHz, CD_3OD) δ 172.3, 151.1, 133.7, 129.4, 129.0, 128.6, 81.4, 77.7, 56.7, 54.9, 32.7, 28.7, 24.8, 23.5; HRESIMS calculated for $C_{18}H_{27}N_2O_5^+$ $[M+H]^+$ 351.1914, found 351.1920.

5.3 Synthesis of N-hydroxy-lysine

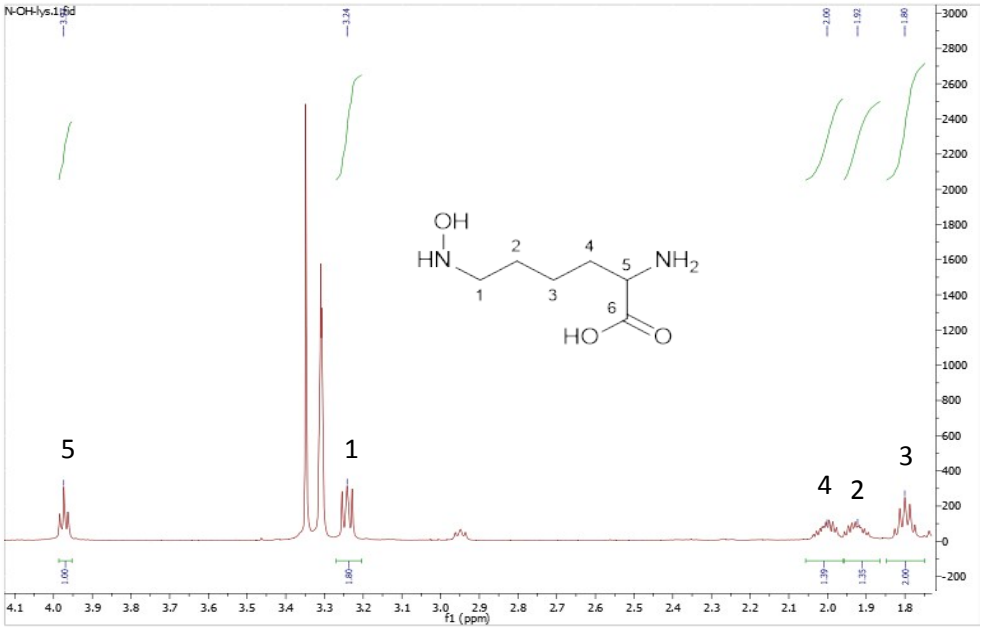


N1-Boc-N6-(3-phenyloxaziridinyl)-lysine **25**

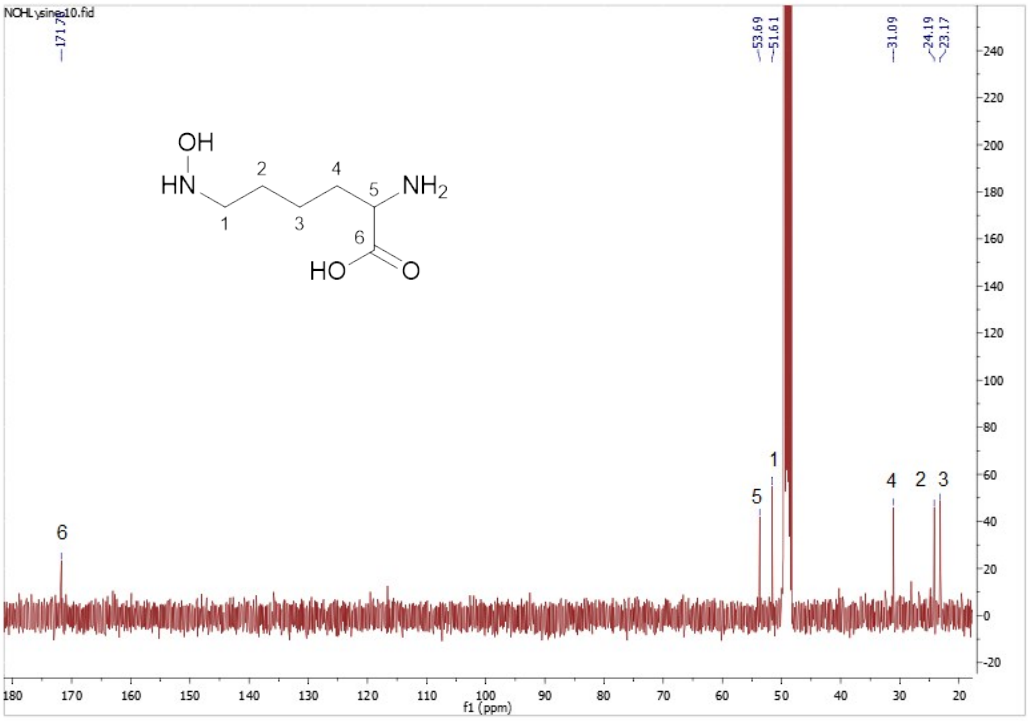
N-hydroxyl-lysine **10**

A solution of oxaziridine **25** (40 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) was treated with TFA/H_2O (0.58 mL, v/v, 4/1) at room temperature. The reaction mixture was stirred for 4h, H_2O (5mL) was added, and the layers were separated. The aqueous layer was washed with CH_2Cl_2 (2x3 mL) and concentrated to yield 6 mg (86%) of *N*-hydroxy-lysine **10**.

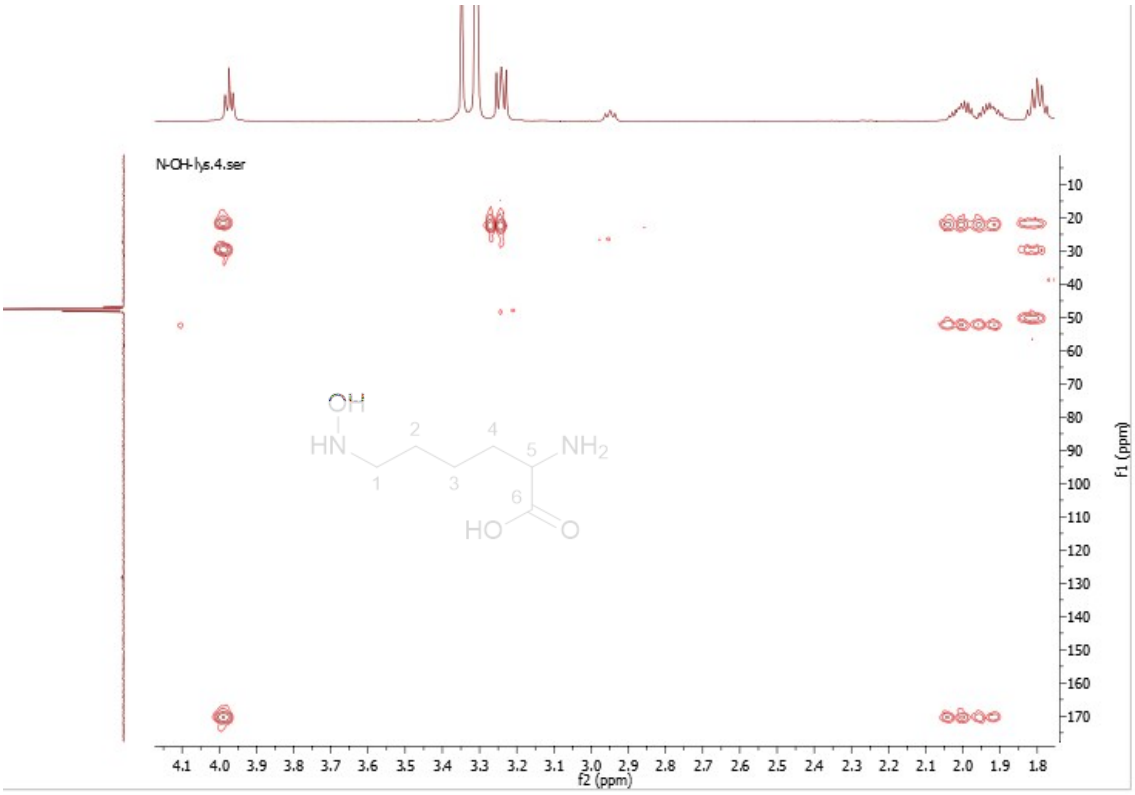
^{1}H NMR (400 MHz, CD_3OD) δ 3.97 (tr, 1H), 3.24 (tr, 2H), 2.00 (m, 2H), 1.92 (m, 2H), 1.80 (m, 2H); ^{13}C-NMR (400 MHz, CD_3OD) δ 171.7, 53.7, 51.6, 31.1, 24.2, 23.2; HRESIMS calculated for $C_6H_{15}N_2O_3^+$ $[M+H]^+$ 163.1077, found 163.1081; Δ=2.4826ppm.



^{1}H-NMR of *N*-hydroxy-lysine **10** (600MHz, CD_3OD)



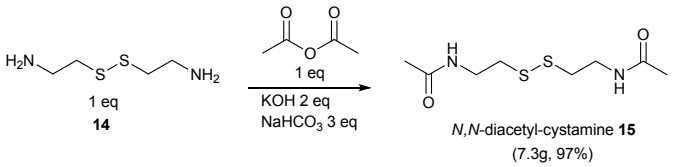
^{13}C-NMR of *N*-hydroxy-lysine **10** (600MHz, CD_3OD)



HMBC of *N*-hydroxy-lysine **10** (600MHz, CD_3OD)

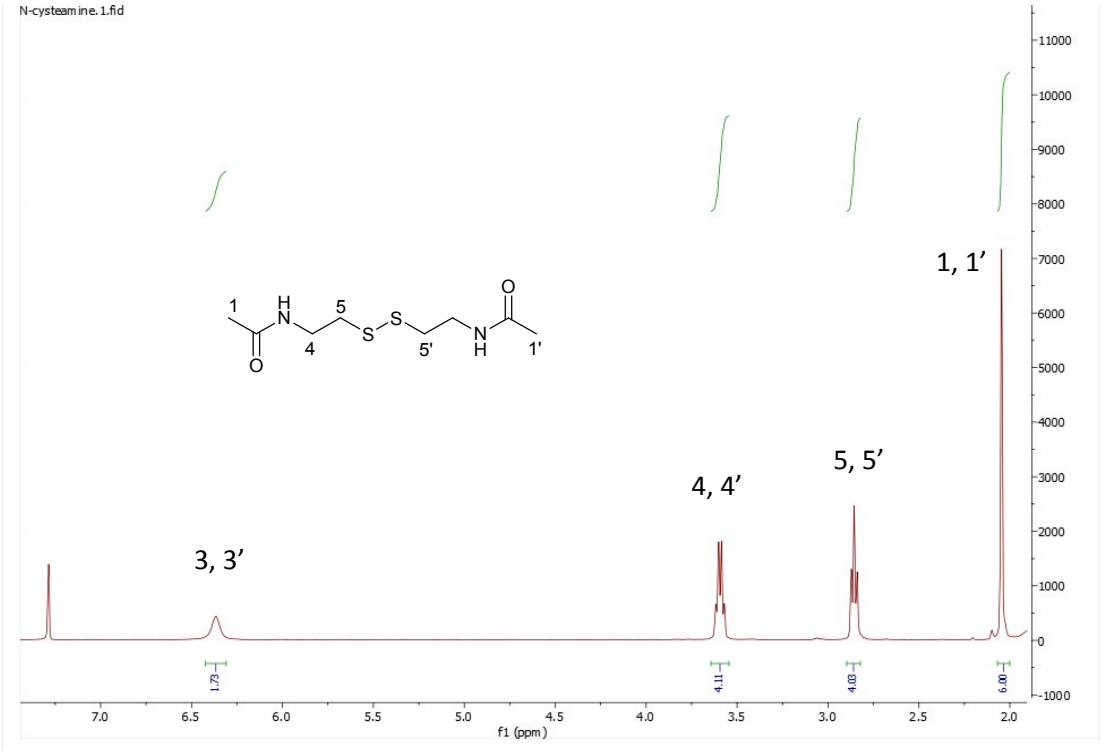
6. Synthesis of SNAC substrates

*6.1 Synthesis of N,N-diacetyl-cystamine **15***

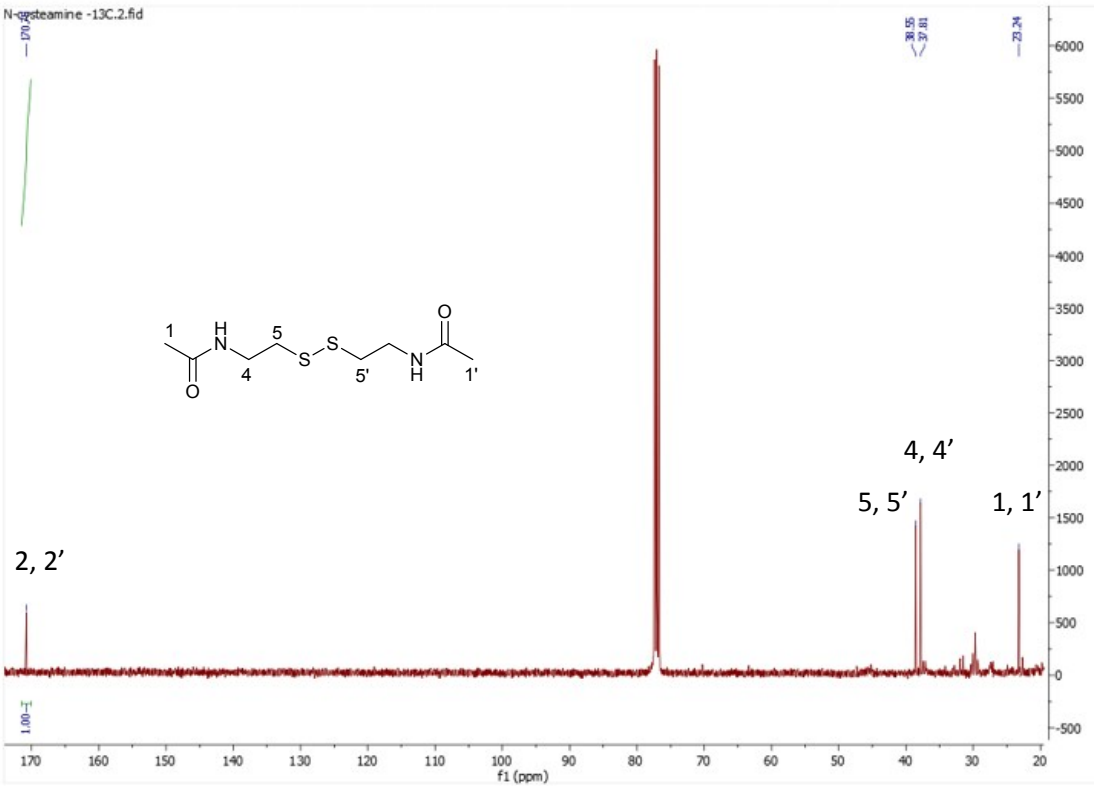


A total of cysteamine hydrochloride (50 mmol, 8.1g, 1.0 eq.), KOH (100 mmol, 5.6g, 2.0 eq.), and $NaHCO_3$ (150 mmol, 12.6g, 3.0 eq) were dissolved in a round-bottom flask containing 50 mL/ water. After the dropwise addition of acetic anhydride (50 mmol, 5.5g, 1.0 eq.), the solution was stirred at 22°C for 10 min. The pH was then adjusted to 7.3 using 12.0 N HCl. The resulting mixture was then added with saturated NaCl, followed by extraction with 150 mL ethyl acetate three times. The organic layer was dried using $MgSO_4$, and after filtration a quantitative yield of *N,N*-diacetylcystamine **15** (7.3g, 97%) was isolated in vacuo.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.37 (s, 2NH), 3.62 (q, 4H), 2.86 (t, Hz, 4H),2.05 (s, Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ170.7 (2C), 38.6 (2C), 37.8 (2C), 23.24 (2C); HRESIMS *m/z* 237.0725 $[M+H]^+$ (calculated 237.0726 for $C_8H_{17}N_2O_2S_2^+$; Δ =-1.964ppm)

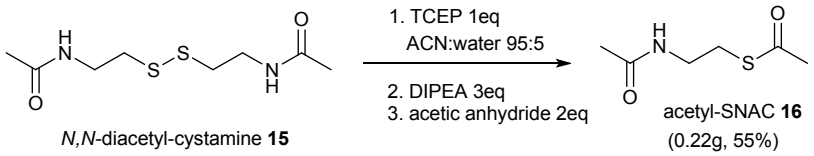


^{1}H-NMR of *N, N*-diacetyl-cystamine **15** (600MHz, CD_3OD)



^{13}C-NMR of *N, N*-diacetyl-cystamine **15** (600MHz, CD_3OD)

*6.2 Synthesis acetyl-SNAC **16***

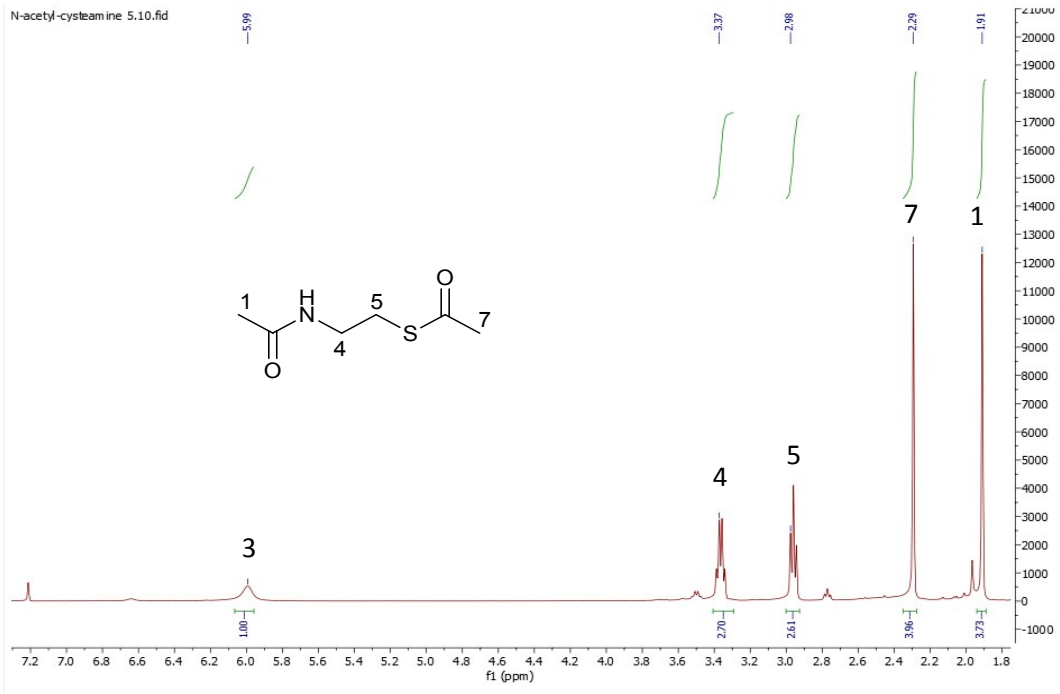


N,N-diacetyl-cystamine **15**

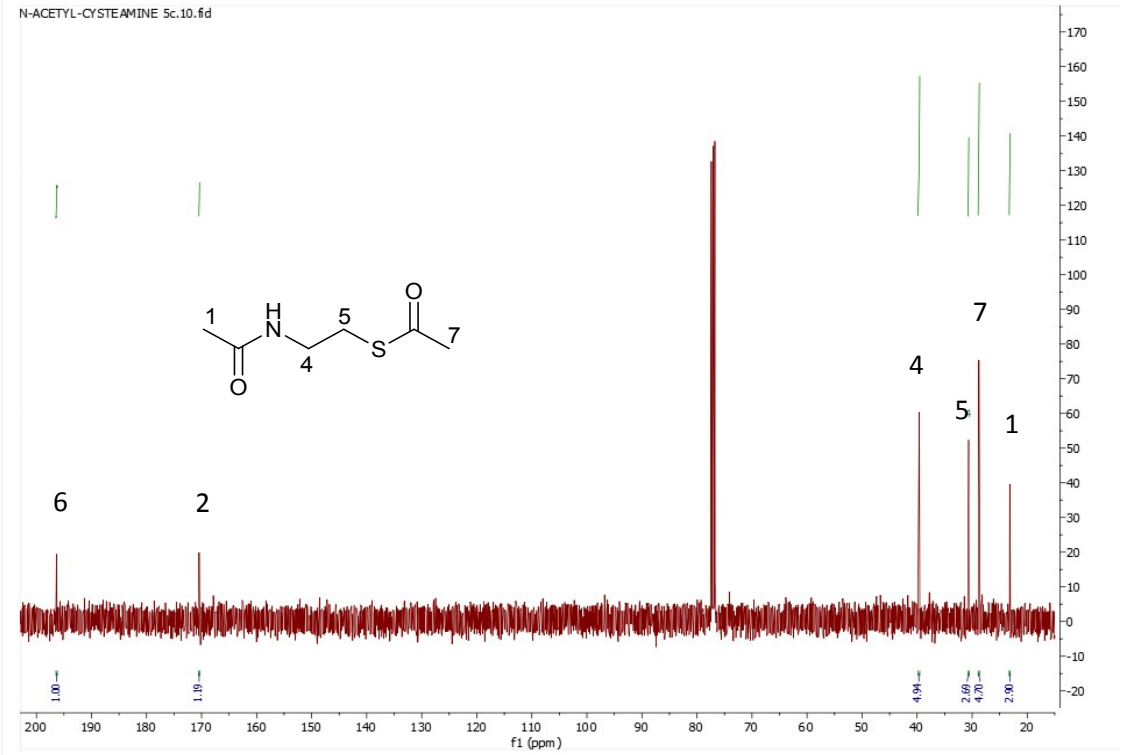
acetyl-SNAC **16**
(0.22g, 55%)

N, N-diacetyl-cystamine **15** (2.5 mmol, 0.59 g, 1 eq.) and TCEP (tris(2-carboxyethyl) phosphine) (2.5 mmol, 0.63g, 1 eq.) were dissolved in a round-bottom flask containing 10 mL acetonitrile: water (95:15). The solution was stirred at room temperature (RT) for 30 min under the argon gas protection. *N, N*-diisopropylethylamine (DIPEA) (7.5 mmol, 0.97g, 3 eq) base to activate thiol group of SNACs was added into the reaction mixture by syringe, followed by the addition of acetic anhydride (5 mmol, 0.51g, 2 eq.). The solution was well stirred at room temperature under argon gas. The progress of the reaction was monitored by TLC. After completion, acetonitrile was removed from the resulting mixture in vacuo and then saturated NaCl added. This was followed by extraction with ethyl acetate (200 mL x 3). The organic layer was dried using $MgSO_4$. The solvent was removed under reduced pressure and the crude product acetyl-SNAC **16** (0.22 g, 55%) was dried overnight.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.99 (s, NH), 3.37 (q, 2H), 2.96 (tr, 2H), 2.29 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 196.4, 170.5, 39.6, 30.6, 28.8, 23.2; HRESIMS observed *m/z* 162.0586 $[M+H]^+$ (calculated 162.0583 for $C_6H_{12}NO_2S^+$, Δ = -0.406ppm)

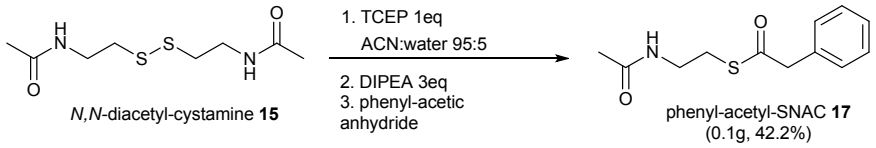


^{1}H-NMR of acetyl-SNAC **16** (600MHz, CD_3OD)



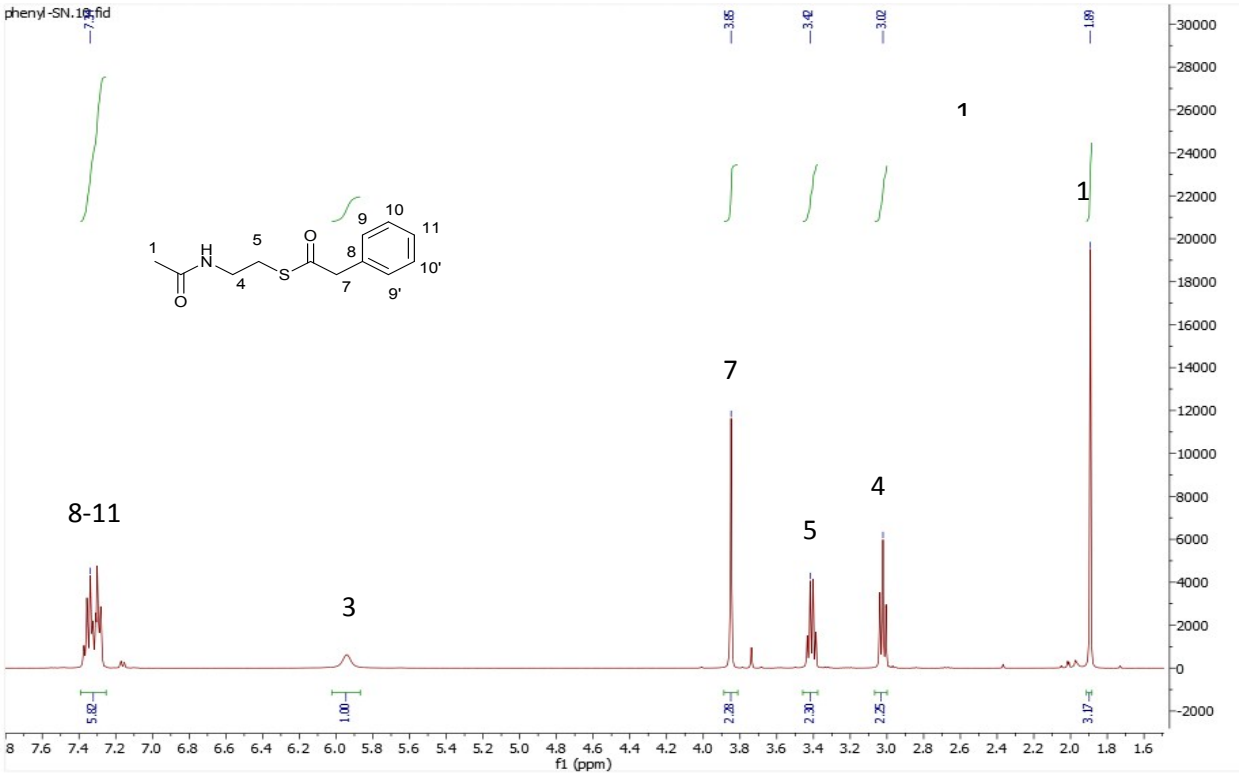
^{13}C-NMR of acetyl-SNAC **16** (600MHz, CD_3OD)

*6.3 Synthesis of phenyl-acetyl-SNAC **17***

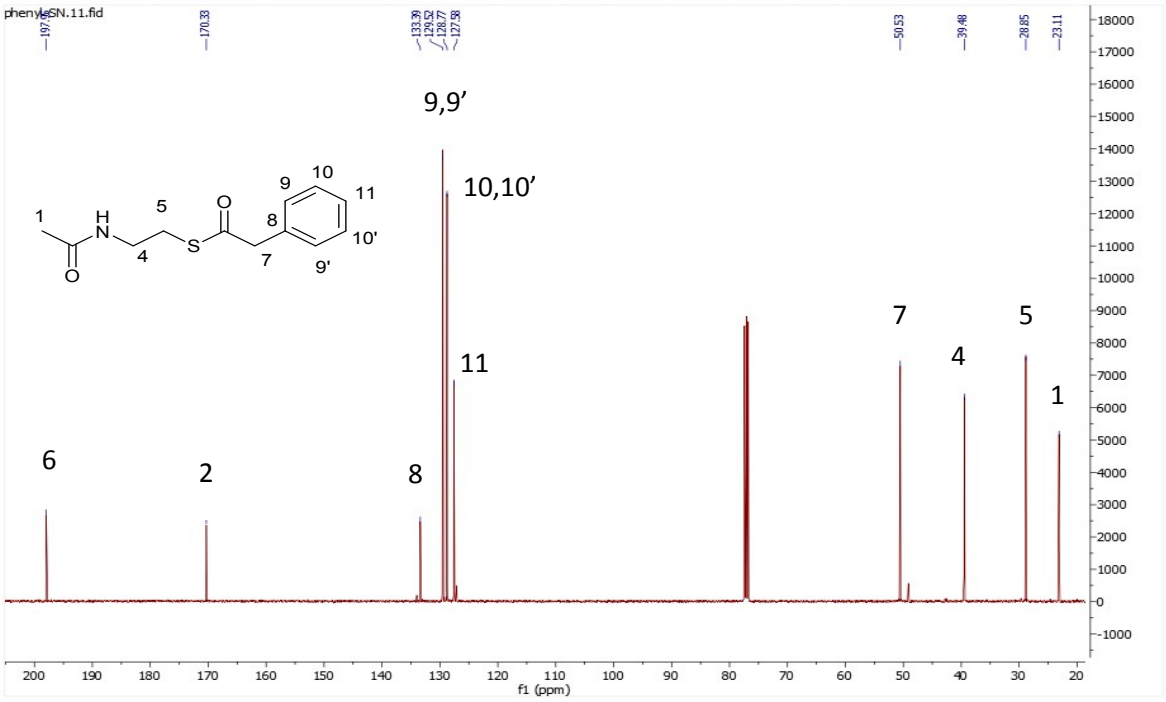


The same procedure described for the synthesis of **16** was applied, using N, N-diacetyl-cystamine **15** (2.5mmol, 0.59g, 1eq.) and phenyl acetic anhydride (5 mmol, 0.93g, 2 eq.) to obtain phenyl-acetyl-SNAC **17** (0.1g, 42.2%).

^{1}H NMR (400 MHz, $CDCl_3$) δ7.40 (m, 2H), 7.26 (m, 3H), 5.94 (s, NH), 3.85 (tr, 2H), 3.41 (s, 2H), 3.02 (tr, 2H), 1.89 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ198.0, 170.3, 133.4, 129.5, 128.7, 127.6, 39.5, 28.9, 23.1; HRESIMS *m/z* 238.0901 $[M+H]^+$ (calculated 238.0896 for $C_{12}H_{16}NO_2S^+$)

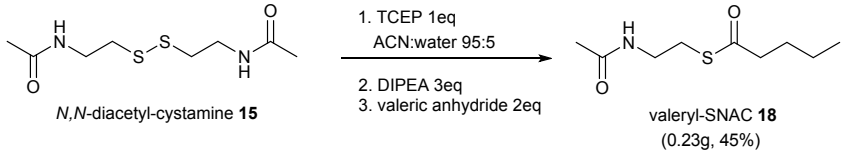


^{1}H-NMR of phenyl-acetyl-SNAC **17** (600MHz, CD_3OD)



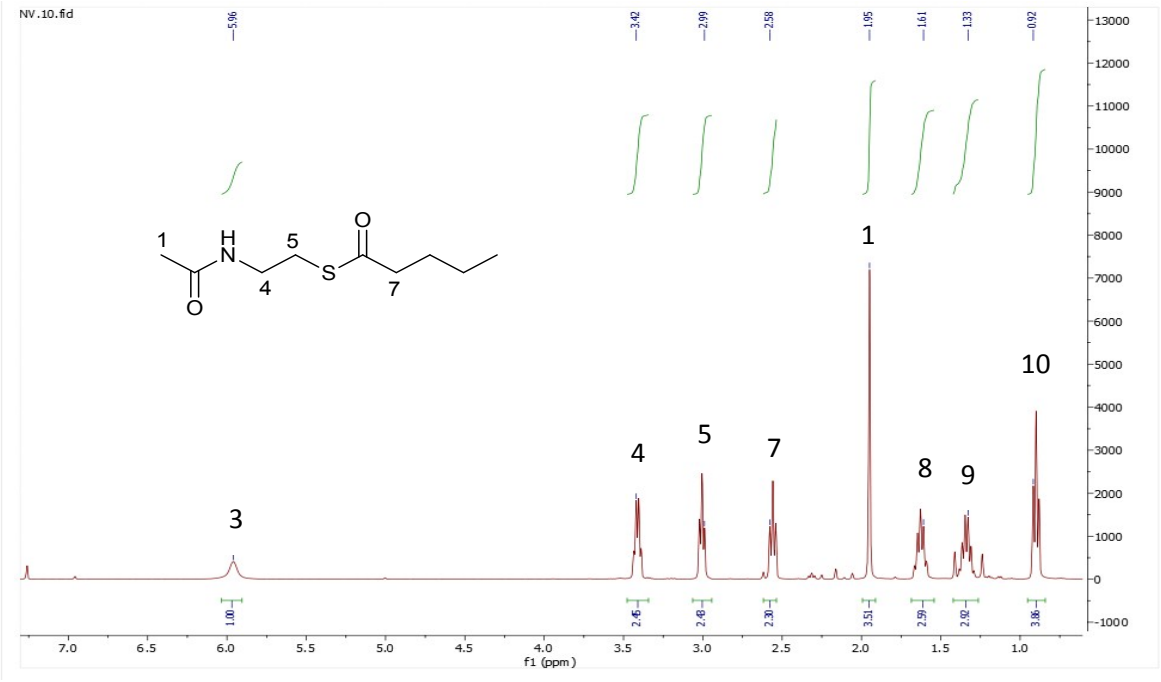
^{13}C-NMR of phenyl-acetyl-SNAC **17** (600MHz, CD_3OD)

*6.4 Synthesis of valeryl-SNAC **18***

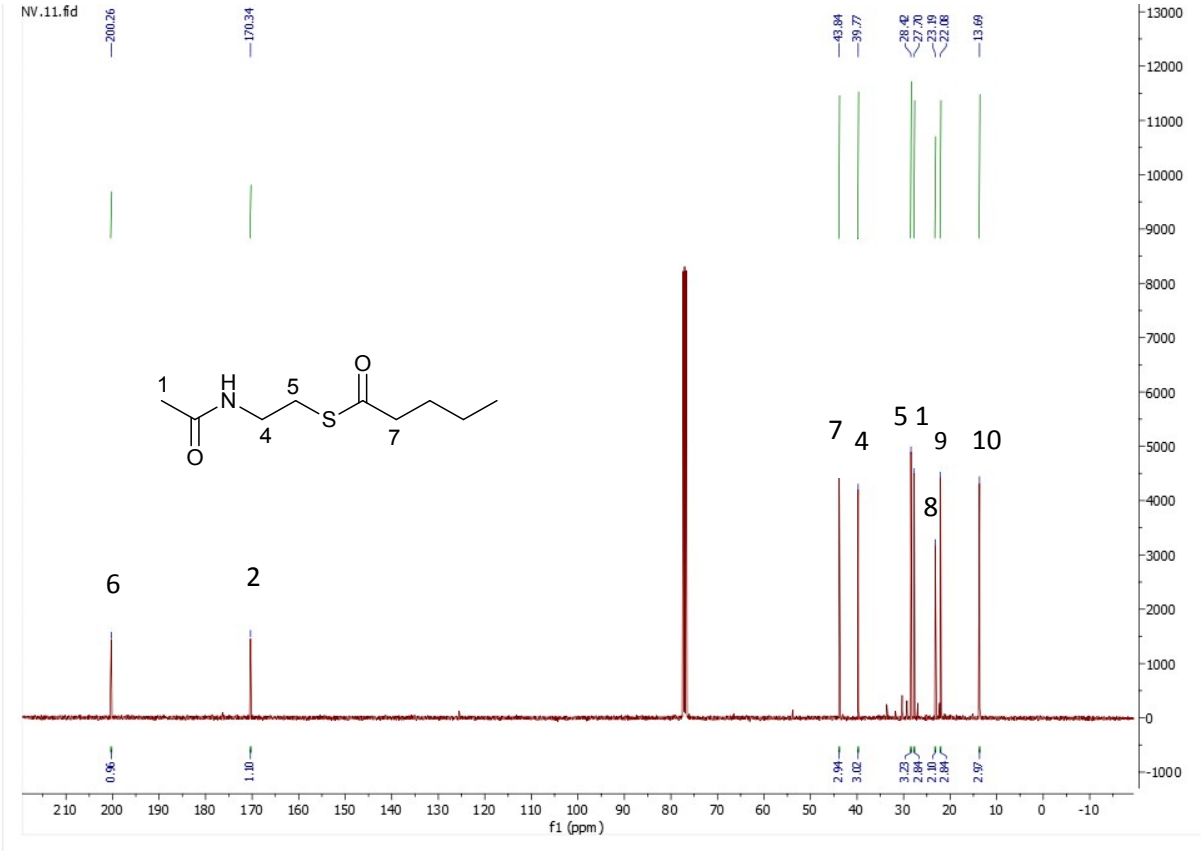


The same procedure described for the synthesis of **16** was applied, using *N, N*-diacetyl-cystamine **15** (2.5mmol, 0.59g, 1eq.) and valeric anhydride (5 mmol, 0.93g, 2 eq.) to obtain valeryl-SNAC **18** (0.23g, 45%).

^{1}H NMR (400 MHz, $CDCl_3$) δ5.96 (s, NH), 3.42 (q, 2H), 2.99 (tr, 2H), 2.58 (tr, 2H), 1.95 (m, 2H), 1.61 (m, 2H), 1.33 (m, 2H), 0.92 (tr, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 200.3, 170.3, 43.8, 39.7, 28.4, 27.7, 23.2, 22.1, 13.9; HRESIMS *m/z* $[M+H]^+$ 204.1064 (calculated 204.1053 for $C_9H_{18}NO_2S^+$; Δ = 0.6451ppm)

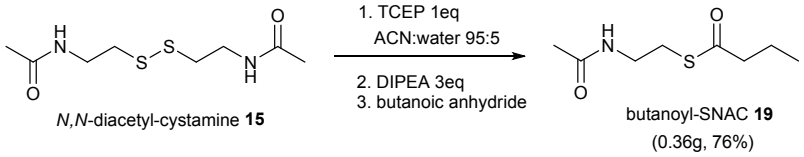


^{1}H-NMR of valeryl-SNAC **18** (600MHz, CD_3OD)



^{13}C-NMR of valeryl-SNAC **18** (600MHz, CD_3OD)

*6.5 Synthesis of butanoyl-SNAC **19***

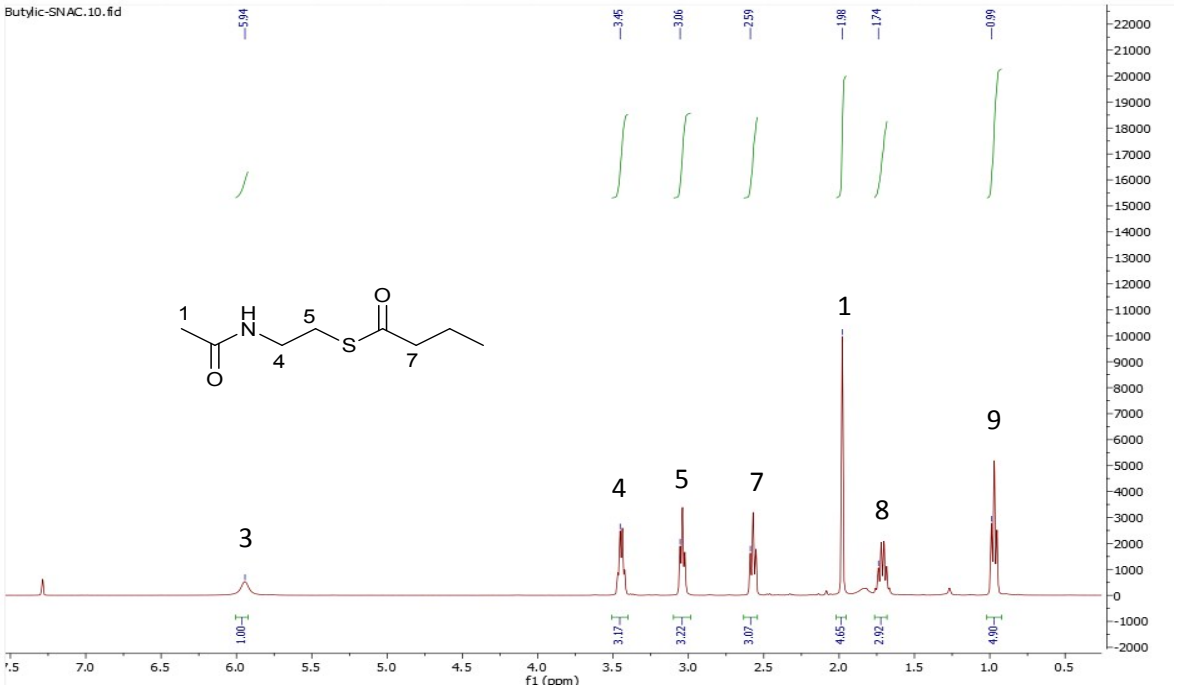


N,N-diacetyl-cystamine **15**

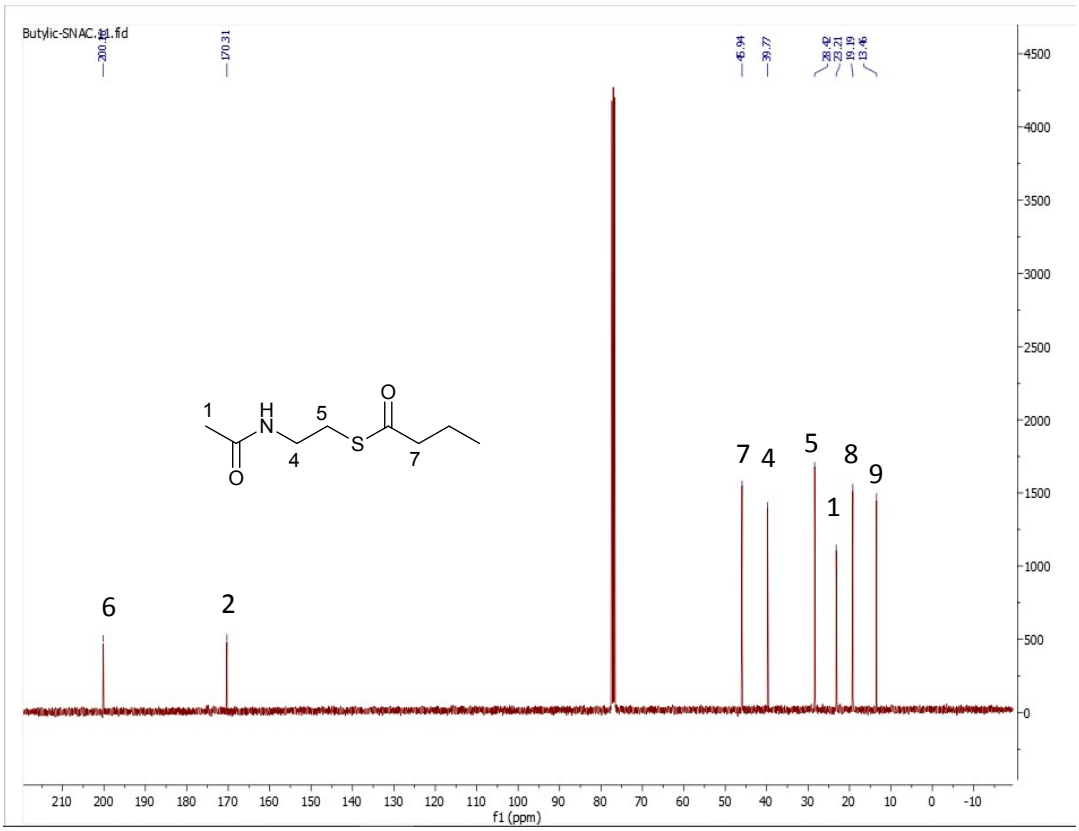
butanoyl-SNAC **19**
(0.36g, 76%)

The same procedure described for the synthesis of **16** was applied, using *N, N*-diacetyl-cystamine **15** (2.5mmol, 0.59g, 1eq.) and butanoic anhydride (5 mmol, 0.79g, 2eq.) to obtain butanoyl-SNAC **19** (0.36g, 76%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.94 (s, NH), 3.45 (q, 2H), 3.06 (tr, 2H), 2.59 (tr, 2H), 1.98 (s, 3H), 1.74 (m, 2H), 0.99 (tr, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 200.1, 170.3, 45.9, 39.8, 28.4, 23.2, 19.2, 13.5; HRESIMS observed *m/z* $[M+H]^+$ 190.0909 (calculated 190.0896 for $C_8H_{16}NO_2S^+$; Δ = 2.1089ppm)

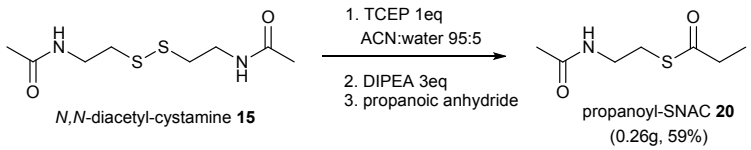


^{1}H-NMR of butanoyl-SNAC **19** (600MHz, CD_3OD)



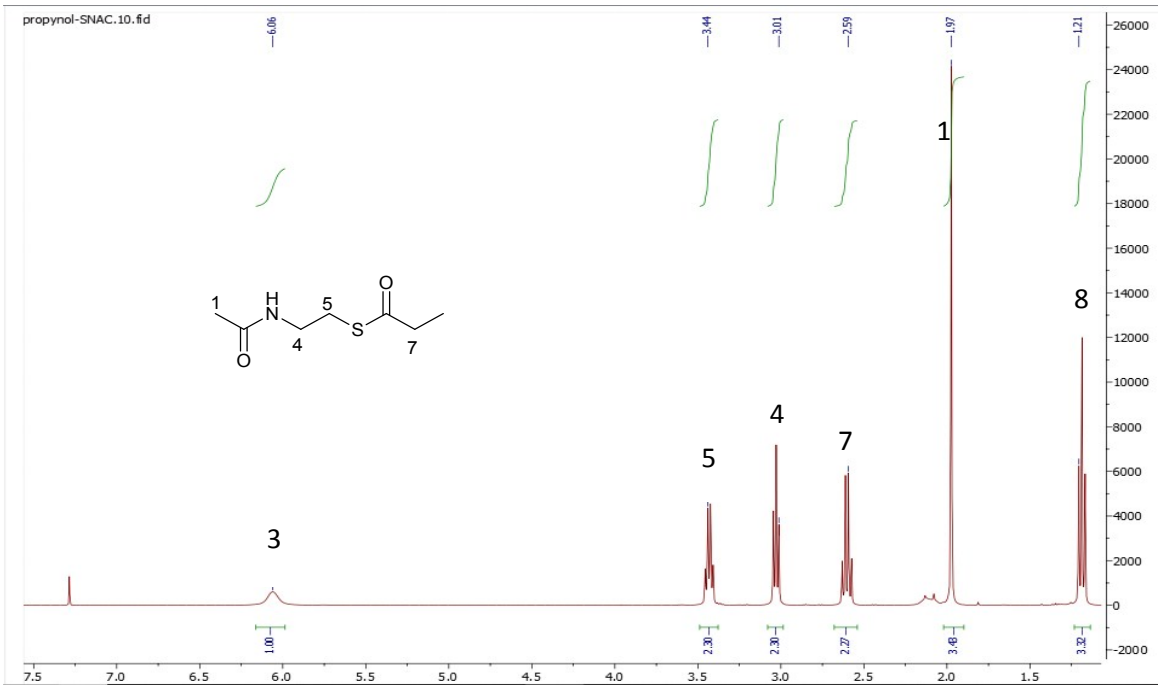
^{13}C-NMR of butanoyl-SNAC **19** (600MHz, CD_3OD)

*6.6 Synthesis of propanoyl-SNAC **20***

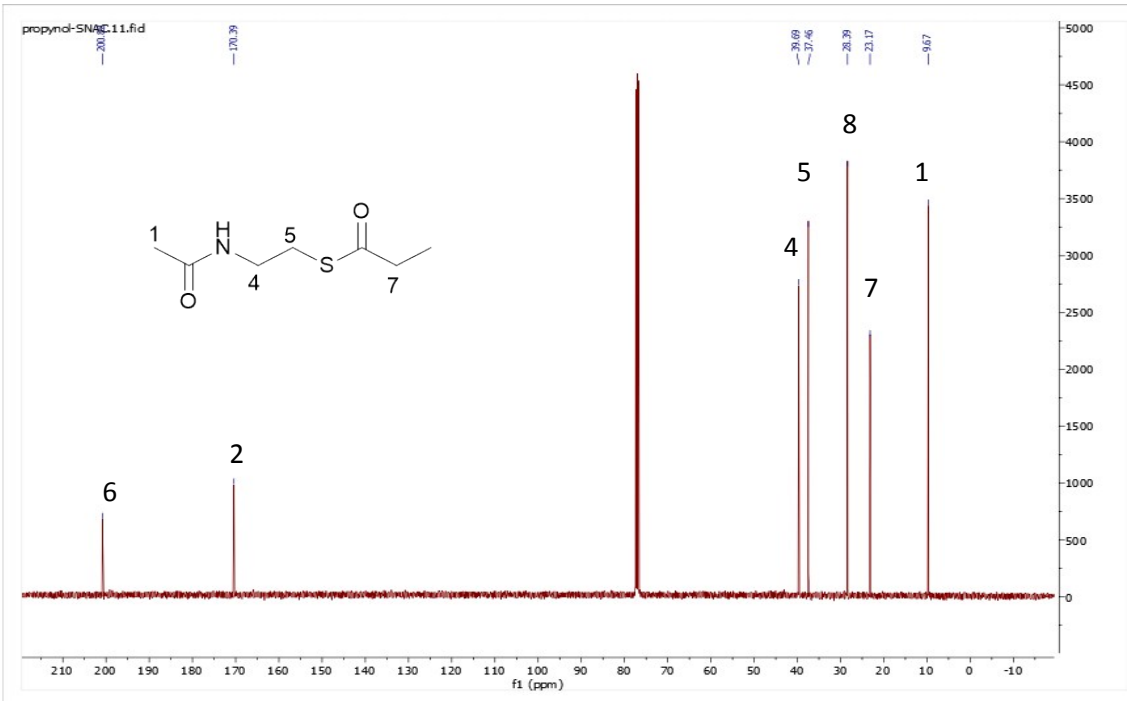


The same procedure described for the synthesis of **16** was applied, using *N, N*-diacetyl-cystamine **15** (2.5 mmol, 0.59 g, 1 eq.) and propanoic anhydride (5 mmol, 0.65g, 2 eq) to obtain propanoyl-SNAC **20** (0.26g, 59%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.06 (s, NH), 3.44 (q, 2H), 3.01 (tr, 2H), 2.59 (tr, 2H), 1.97 (s, 3H), 1.21 (tr, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ200.8, 170.4, 39.7, 37.5, 28.4, 23.2, 9.7; HRESIMS m/z 176.0742 $[M+H]^+$ (calculated 176.0740 for $C_7H_{14}NO_2S^+$; Δ = 1.2925ppm)

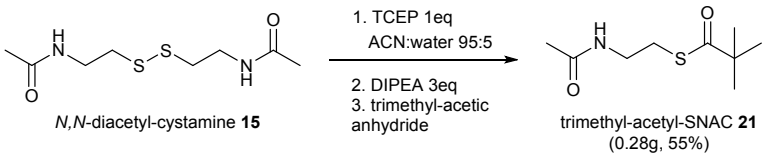


^{1}H-NMR of propanoyl-SNAC **20** (600MHz, CD_3OD)



^{13}C-NMR of propanoyl-SNAC **20** (600MHz, CD_3OD)

*6.7 Synthesis of trimethyl-acetyl-SNAC **21***

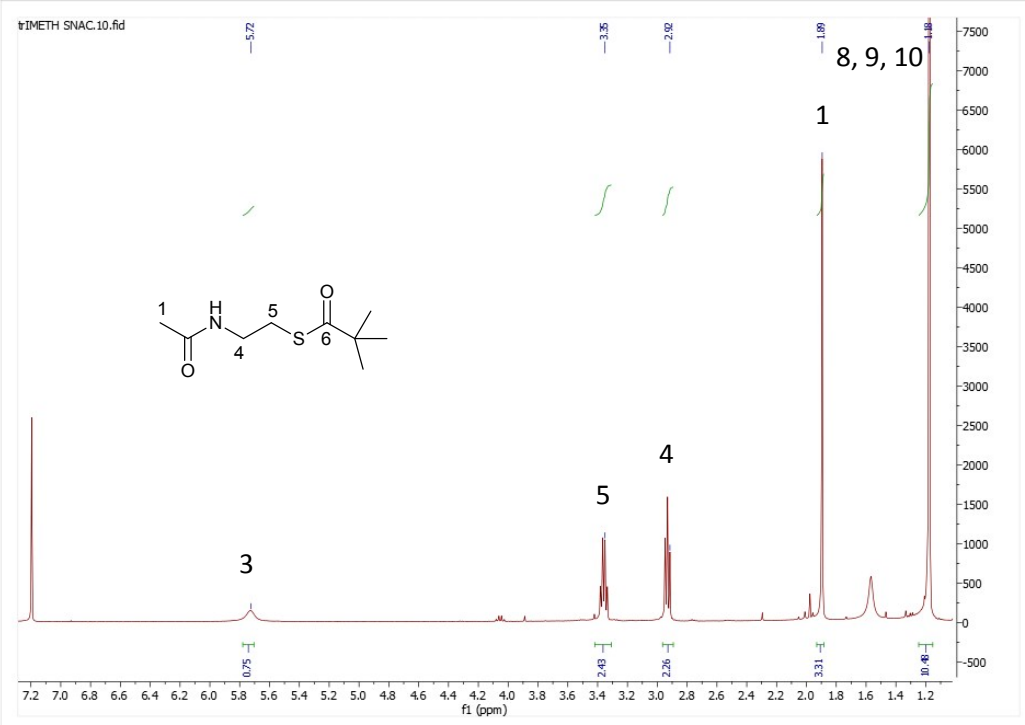


N,N-diacetyl-cystamine **15**

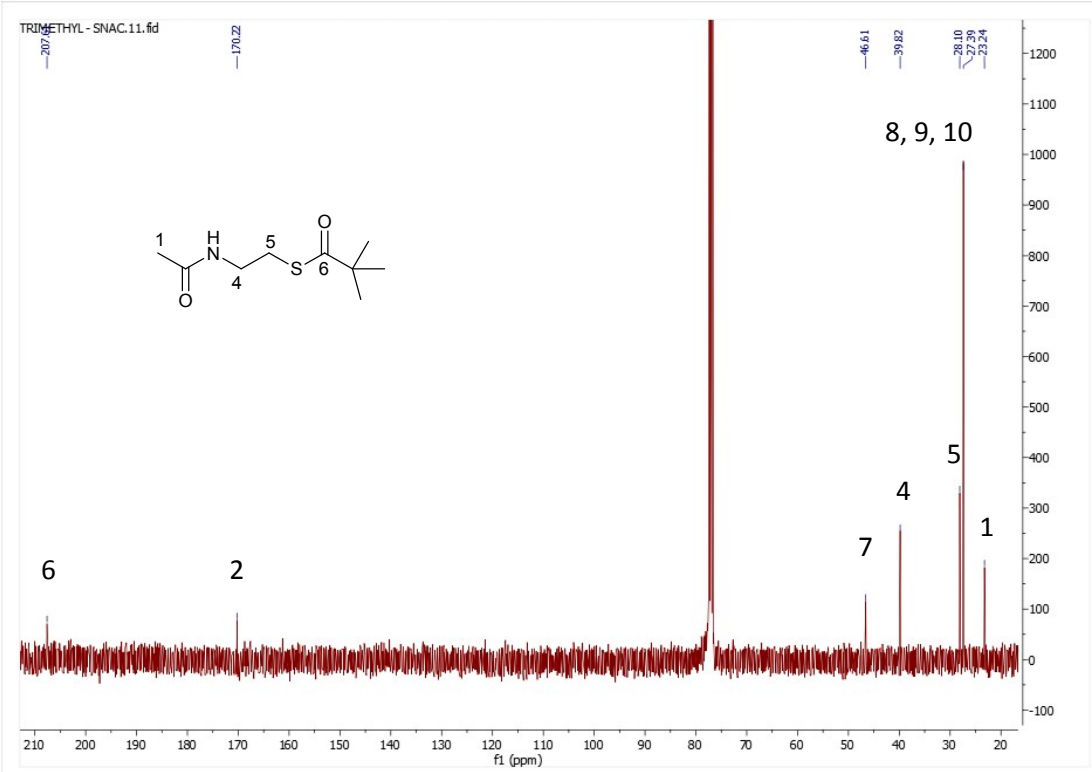
trimethyl-acetyl-SNAC **21** (0.28g, 55%)

The same procedure described for the synthesis of **16** was applied, using *N, N*-diacetyl-cystamine **15** (2.5mmol, 0.59g, 1eq) and methacrylic anhydride (5 mmol, 0.51g, 1 eq) to obtain the oily, trimethyl-acetyl-SNAC **21** (0.28g, 55%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (s, 1H), 3.36 (q, *J* = 6.1 Hz, 2H), 2.93 (t, *J* = 6.8, 5.9 Hz, 2H), 1.89 (s, 3H), 1.18 (s, 9H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 207.61, 170.22, 46.61, 39.82, 28.10, 27.39, 23.24. HRESIMS m/z 204.1056 $[M+H]^+$ (calculated $C_9H_{18}NO_2S^+$; Δ = 1.391ppm).



^{1}H-NMR of trimethyl-acetyl-SNAC **21** (600MHz, CD_3OD)



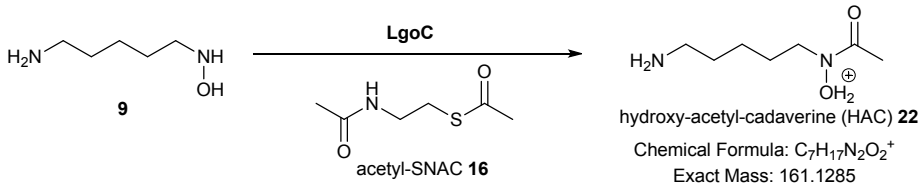
^{13}C-NMR of trimethyl-acetyl-SNAC **21** (600MHz, CD_3OD, Me_4Si)

6.8 LgoB activity determination

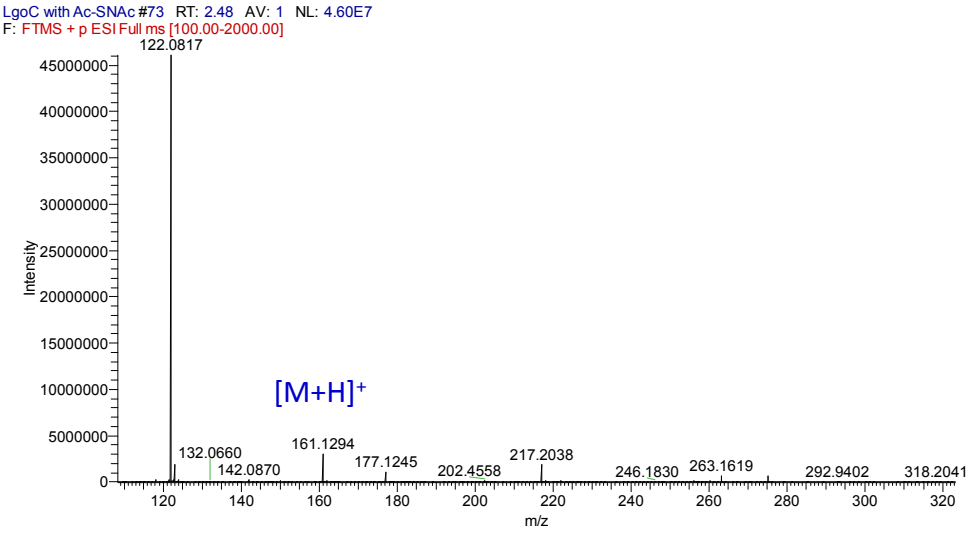
The activity of LgoB was carried out following the protocols described by Salomone-Stagni, *et al.*[2] The activity was estimated from the NAD(P)H oxidation followed by absorption at 340nm using an extinction coefficient of 6220 M^{-1} cm^{-1}. Absorption readings were taken every minute using the UV-vis spectrophotometer (Jenway 6300/05/20D). The reaction solution consisted of MOPS (pH 7.0, 100mM), FAD 50µM, substrate (2mM, cadaverine, L-lysine hydrochloride, L-ornithine hydrochloride, putrescine, 5-amino-1-pentanol, spermidine), LgoB (0.5 µM). Reactants were carefully mixed and incubated for 5min before addition of the NAD(P)H cofactor up to 500µM to initiate. Considering that NAD(P)H oxidation can be promoted by free FAD, the blank consisted of a solution with FAD, NAD(P)H, no substrate and no enzyme. The control consisted of the cofactors, substrate and no enzyme. The assays were carried out in volumes of 200µL. The specific activity (U mg^{-1} of LgoB) is expressed in µmol min^{-1} $NADP^+$ formed per mg of LgoB at 25^0C in 200µL of 50µM FAD, 100mM MOPS pH 7.0, and 500µM NADPH

6.9 Incubation of LgoC with acetyl-SNAC and phenyl-acetyl-SNAC substrates

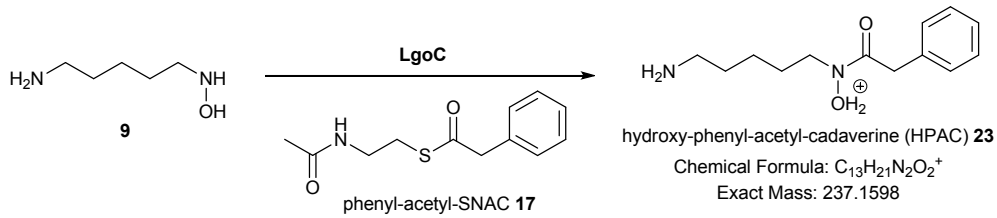
N-hydroxy cadaverine **9** (50µM) and acetyl-SNAC (100µM) were incubated with TRIS-HCl pH8.0 (0.5 mM) and LgoC (10µM) in a final volume of 200µL for 10 minutes at 37^0C. The reaction was initiated by addition of the enzyme and was stopped by addition of equal amount of 100% methanol. The controls were carried out without enzyme and boiled enzyme. Likewise, a similar reaction was carried out as described above using phenylacetyl-SNAC in lieu of acetyl-SNAC. The formation of the *N*-hydroxy-acetyl-cadaverine (HAC) **22** and *N*-hydroxy-phenyl-acetyl-cadaverine (HPAC) **23** were confirmed by HR ESIMS.



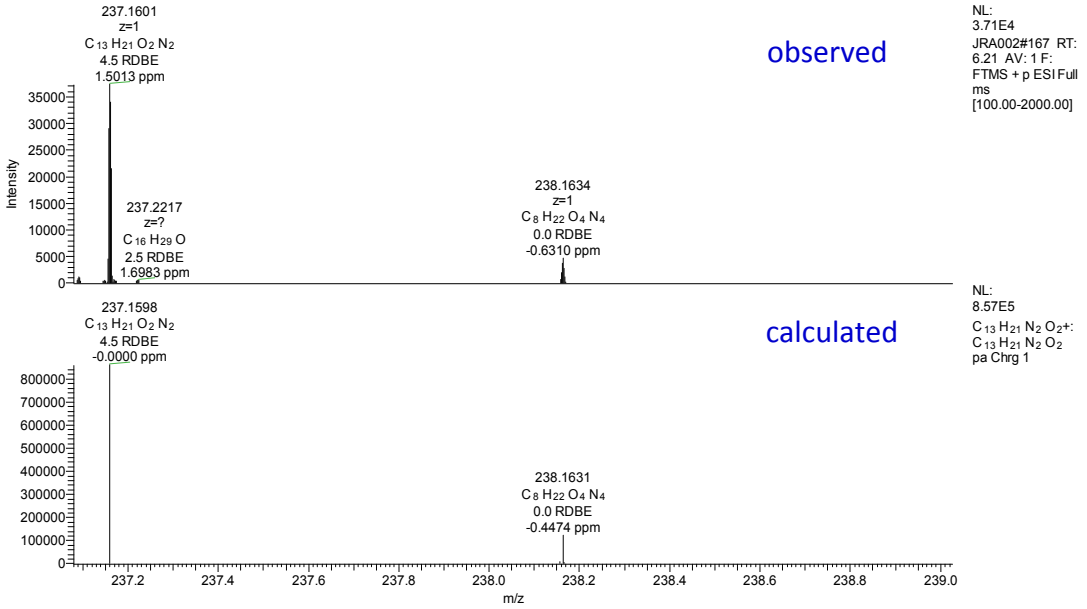
LgoC-catalysed reaction of *N*-hydroxy-cadaverine with acetyl-SNAC



HR ESIMS of LgoC catalysed enzymatic product, *N*-hydroxy-acetyl-cadaverine (HAC)



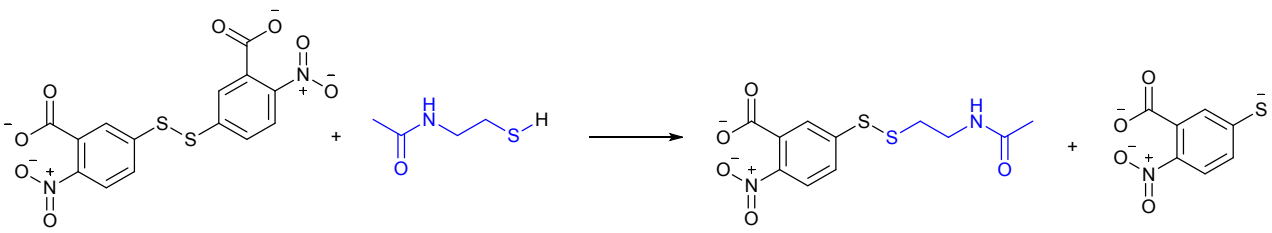
LgoC-catalysed reaction of *N*-hydroxy-cadaverine with phenyl-acetyl-SNAC



HR ESIMS of LgoC catalysed enzymatic product, *N*-hydroxy-phenyl-acetyl-cadaverine (HPAC)

6.10 DTNB Assay

For detection of the free sulfhydryl group release, 5,5'-dithio-bis-(2-nitrobenzoic acid) or DTNB assay was performed.[3,4] The DTNB (Ellman's reagent, Sigma) was freshly prepared by dissolving in 0.5mM TRIS-HCl pH buffer. The reactions were carried out at 37^0C in a disposable semi-micro cuvette (15mm BrandTech™ UV-Cuvette). The reaction mixture (200 µL) consisted of TRIS-HCl (pH 8.0, 0.50 mM), *N*-hydroxy cadaverine (50µM), LgoC (10 µM), and acetyl-SNAC in varying concentrations (25 µM, 50 µM, 75 µM, 100 µM, 300 µM). The controls were carried out without enzyme and without SNAC. The reaction was monitored by measuring the increase in absorbance due to the formation of 5-thio-2-nitrobenzoic acid (TNB^{2-}) at 412nm (extinction coefficient = 14,150 /M.cm) in UV-vis spectrophotometer (Jenway 6300/05/20D). The assay was performed in triplicates. Steady state parameters k_{cat} and k_m were determined by nonlinear fitting of Michaelis-Menten equation.



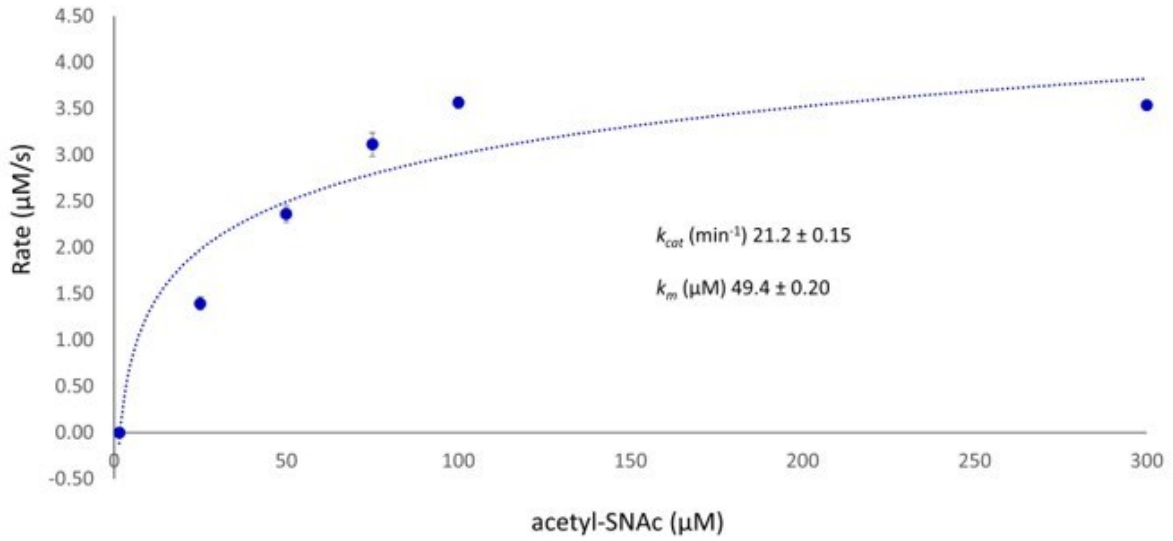
$DTNB^{2-}$

5,5-dithio-bis-(2-nitrobenzoic acid)

TNB^{2-}

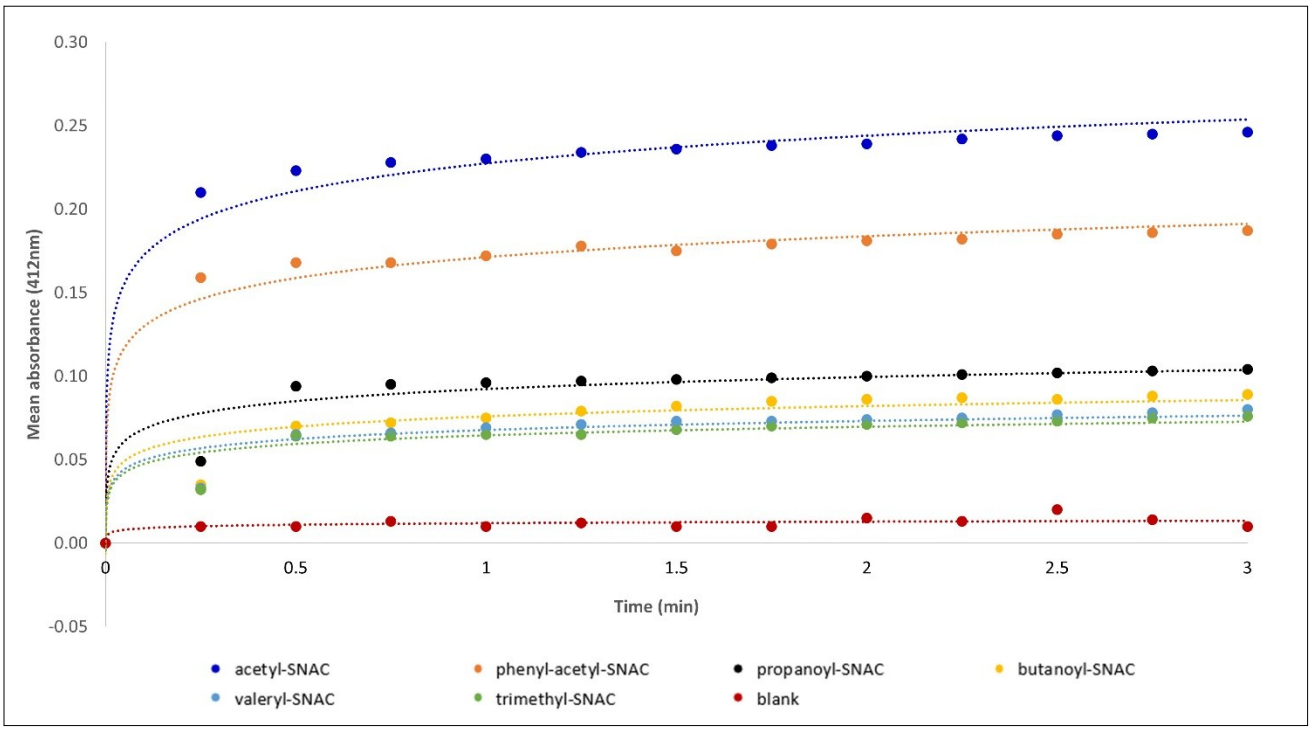
Extinction coefficient = 14,150 /M.cm
412nm

Reaction of DTNB reagent and thiol by-product



Determination of LgoC kinetic parameters by the DTNB assay. Error bars represent standard deviations from three independent experiments. Each assay was performed with a no-enzyme control

The DTNB assay was repeated as described above using various SNAC substrates (phenyl-acetyl-SNAC, propanoyl-SNAC, butanoyl-SNAC, valeryl-SNAC, trimethyl-SNAC) (100µM), and their rates of reaction were determined relative to acetyl-SNAC. Assays were performed in triplicates.



Absorbance spectra curves derived from the LgoC-catalysed incubation of *N*-hydroxy-cadaverine and DTNB with SNACs substrates monitored at 412nm

References

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2 M. Salomone-Stagni, J. D. Bartho, I. Polsinelli, D. Bellini, M. A. Walsh, N. Demitri and S. Benini, *J. Struct. Biol.*, 2018, **202**, 236–249.

3 D. Andrew Skaff and H. M. Miziorko, *Anal. Biochem.*, 2010, **396**, 96–102.

4 G. L. Ellman, *Arch. Biochem. Phys.*, 1959, **82**, 70–77.