**Communication**

**Oxygenated Analogues of Santacruzamate A**

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**Abstract:** A new approach for the synthesis of Santacruzamate A analogues is demonstrated. The method allows functionalization at position 3 of the gamma-aminobutyric fragment and carbon chain variation.

**Keywords:** Santacruzamate A; β-ketoamide; β-hydroxyamide

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1. **Introduction**

Santacruzamate A (Figure 1a) is a structurally simple natural product isolated from marine cyanobacterium *Symplaca* sp. [1]. This natural product bears some structural similarity to the clinically used histone deacetylase (HDAC) inhibitor vorinostat (SAHA) [2]. The initial publication about its discovery also reported picomolar-level selective inhibitory activity against HDAC2 (IC50 = 119 pM), with a relatively weak inhibition of HDAC4 and HDAC6 [1]. Although these data were not entirely corroborated by later publications from the same group [3] and others [4–6], the interest drawn by this natural product has led to the preparation of many analogues, some with promising bioactivity [4–8]. Considering the current interest in this topic and the ongoing investigations of the structure-activity relationships, we saw an opportunity to contribute to the availability of structurally diverse Santacruzamate A analogues with our method for β-keto amide synthesis [9].

In a previous publication we demonstrated that this enamine-based domino approach provides access to β-keto amides IV functionalized with protected amino group in the side chain (Scheme 1, R1 = H, Ph) [10]. If N-ethoxycarbonyl glycine is used as the amino acid component III in this methodology and R1 is set to phenethyl, then the products IV would be structurally similar to Santacruzamate A, with introduced carbonyl functionality in the gamma-aminobutyric part and possible variation of the chain length by the choice of an appropriate R2 substituent in the acetocetamide I (Figure 1b).

![Figure 1](https://example.com/fig1.png)

**Figure 1.** Santacruzamate A (a) and oxygenated analogues (b).
To synthesize the oxo-analogue of Santacruzamate A, we first prepared the enaminoamide 2a (Scheme 2, \( m = 2 \)) by condensation of \( N \)-phenethyl acetoacetamide with Boc-monoprotected ethylenediamine. This compound was then reacted with mixed carbonic anhydride of \( N \)-ethoxycarbonyl glycine to provide the intermediate 3a in 85% yield. Upon the subsequent Boc-deprotection and buffering with NaOAc solution, 3a gave the expected analogue 4a in 80% yield. By analogy, this procedure was applied for the preparation of \( N \)-methylated analogue 4b and chain-shifted analogue 4c (Scheme 2, Table 1). The entire synthetic sequence was carried out without any chromatographic purification of the intermediates. The final step gave practically pure keto amides 4 with only small proportion of the enol tautomer visible in the \(^{1}H\) NMR spectra (Supplementary materials).

### Table 1. Yields of keto amides 4, prepared according to Scheme 2.

| 4    | \( n \) | \( m \) | R    | Yield (%) \(^{1}\) |
|------|--------|--------|------|------------------|
| a    | 1      | 2      | H    | 68               |
| b    | 1      | 2      | \( \text{CH}_{3} \) | 76               |
| c    | 2      | 1      | H    | 71               |

\(^{1}\) Overall yield after three steps, based on the starting acetoacetamide 1.

The introduction of carbonyl group in the gamma-aminobutyric fragment of Santacruzamate A provides a useful handle for various further manipulations. For example, the reduction of 4a,b with NaBH\(_{4}\) was straightforward and gave the corresponding alcohols 5a,b in quantitative yields (Scheme 3).
Scheme 3. Reduction of oxo-analogues to hydroxy-analogues.

3. Materials and Methods

All reagents and solvents were purchased from commercial suppliers (Sigma-Aldrich or Merck, Darmstadt, Germany) and were used without further purification. Boc-monoprotected ethylenediamine [11], acetoacetamides 1 [12,13], and enaminoamides 2 [9] were prepared according to the published procedures. NMR spectra were run on Bruker Avance AV600 (600/150 MHz $^1$H/$^1^3$C) spectrometer (Bruker, Billerica, MA, USA) at BAS-IOCCP—Sofia and chemical shifts (δ, ppm) are downfield from TMS. High resolution mass spectral measurements were performed on a Thermo Scientific Q Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). TLC was done on aluminium-backed Silica gel 60 sheets (Merck) with KMnO$_4$ staining; melting points were measured on Boetius hot stage apparatus (Boetius, Germany) and are not corrected.

Synthetic Procedures

Oxo-analogues of Santacruzamate A (4a–c), general procedure: To a magnetically stirred solution of the corresponding N-ethoxycarbonyl amino acid (1 mmol) in CH$_2$Cl$_2$ (5 mL), N-methylmorpholine (1 mmol, 0.11 mL) was added. The solution was then put in an ice bath and ethyl chloroformate (1 mmol, 0.1 mL) was added. The mixture was left to stir for 5 min and after that a solution of enamino amide 2 (1 mmol) and DMAP (0.2 mmol) in CH$_2$Cl$_2$ (10 mL) was added in one go. The ice bath was then removed, and the reaction mixture was left to stir for one more hour at r.t. The reaction mixture was then transferred to a separatory funnel with additional 30 mL of CH$_2$Cl$_2$ and washed with aqueous (10:1) HCl. The aqueous layer was extracted with 30 more mL of CH$_2$Cl$_2$, the combined organic layers were dried with anhydrous sodium sulfate, the drying agent was removed by filtration, and the solvent was evaporated under reduced pressure. The intermediates 3 solidified upon trituration with small volume of diethyl ether. The ethereal washings were filtered off and the crude compounds 3 were dissolved in TFA (1 mL TFA per 100 mg of 3). The TFA solutions were stirred for 5 min at r.t. and then 3 mol/L aqueous solution of NaOAc (10 mL for each mL of TFA) was added, followed by CH$_2$Cl$_2$ (30–50 mL). The mixture was left to stir intensely for 2 h. The layers were then separated, and the aqueous layer was extracted two more times with CH$_2$Cl$_2$. The organic layers were combined, washed with saturated aqueous NaHCO$_3$ (20 mL), and then dried over Na$_2$SO$_4$. The solvent was removed on a rotary evaporator. Compounds 4a and 4c crystallized and were rinsed with small volumes of diethyl ether or ether-petroleum. Compound 4b was isolated as clear oil.

Chromatography of the ethereal washings through a short plug of silica gel can afford small additional amounts of 4a,c.

(2-Oxo-3-phenylcarbamoyl-propyl)-carbamic acid ethyl ester (4a): white solid, mp 137–139 °C; R$_f$ = 0.55 (Et$_2$O:CH$_2$OH 20:1); $^1$H-NMR (600 MHz, CDCl$_3$, δ ppm, J Hz): 7.34–7.20 (m, 5H), 6.71 (br s, 1H), 5.40 (br s, 1H), 4.15 (q, $J$ = 7.0, 2H), 3.55 (dt, $J$ = 6.4, $J$ = 7.0, 2H), 3.39 (s, 2H), 2.84 (t, $J$ = 7.0, 2H), 1.27 (t, $J$ = 7.0, 3H); $^{13}$C-NMR (150 MHz, CDCl$_3$, δ ppm): 201.6, 164.8, 156.5, 138.6, 128.8, 128.7, 126.6, 61.4, 51.1, 47.0, 40.9, 35.5, 14.6; ESI-MS (m/z): 315.1320 [M + Na$^+$] (calcd for C$_{15}$H$_{20}$N$_2$NaO$_4$ $^+$ 315.1315); 291.1353 [M – Na$^-$] (calcd for C$_{15}$H$_{19}$N$_2$O$_4$ $^-$ 291.1350).

Methyl-(2-oxo-3-phenylcarbamoyl-propyl)-carbamic acid ethyl ester (4b): clear oil; R$_f$ = 0.35 (Et$_2$O:CH$_2$OH 20:1); $^1$H-NMR (600 MHz, CDCl$_3$, δ ppm, J Hz), only signals for the major rotamer are listed: 7.34–7.21 (m, 5H), 6.87 (br s, 1H), 4.17–4.09 (m, 4H) 3.55 (m, 2H), 3.38
(s, 2H), 2.94 (s, 3H), 2.85 (t, J = 7.0, 2H), 1.30 (t, J = 7.0, 3H); 13C-NMR (150 MHz, CDCl3, δ ppm): 202.0, 165.1, 157.0, 138.7, 128.8, 128.6, 126.6, 62.0, 58.9, 46.7, 41.0, 35.6, 35.5, 14.6; ESI-MS (m/z): 329.1475 [M + Na]+ (calcd for C16H22N2NaO4+ 329.1472).

(4-Benzylcarbamoyl-3-oxo-buty)-carbamic acid ethyl ester (4c): white solid, mp 100–102 °C; Rf = 0.50 (Et2O:CH3OH 20:1); 1H-NMR (600 MHz, DMSO-d6, δ ppm, J Hz): 8.33 (br t, J = 5.9, 1H), 7.15–7.04 (m, 5H), 6.86 (br t, J = 5.9, 1H), 4.10 (d, J = 5.9, 2H) 3.77 (q, J = 7.0, 2H) 3.21 (s, 2H), 2.98(dt, J = 5.9, J = 7.0, 2H), 2.50 (t, J = 7.0, 2H), 0.95 (t, J = 7.0, 3H); 13C-NMR (150 MHz, DMSO-d6, δ ppm): 204.3, 166.5, 156.6, 139.6, 128.8, 127.7, 127.3, 60.0, 50.9, 42.9, 42.7, 35.7, 15.1; ESI-MS (m/z): 315.1317 [M + Na]+ (calcd for C15H20N2NaO4+ 315.1315).

Hydroxy-analogues of Santacruzamate A (5): To the corresponding keto amide 4 (100 mg) in methanol (10 mL) was added NaBH4 in small portions (5–7 mg every 10 min) until TLC indicated the absence of the starting material. The mixture was then diluted with water (50 mL) and extracted with CH2Cl2 (3 × 20 mL). The organic layers were combined, dried over Na2SO4 and the solvent was removed on a rotary evaporator to afford practically clean hydroxy amides 5.

(2-Hydroxy-3-phenethylcarbamoyl-propyl)-carbamic acid ethyl ester (5a): white solid, mp 104–105 °C; Rf = 0.50 (Et2O:CH3OH 20:1); 1H-NMR (600 MHz, CDCl3, δ ppm, J Hz): 7.25–7.11 (m, 5H), 6.20 (br s, 1H), 5.24 (br s, 1H), 4.02 (q, J = 7.0, 2H), 3.96 (m, 1H), 3.45 (dt, J = 5.9, J = 7.0, 2H), 3.22 (dt, J = 14.1, J = 4.7, 1H), 3.09 (dt, J = 14.1, J = 5.9, 1H), 2.75 (t, J = 7.0, 2H), 2.24 (m, 2H), 1.16 (t, J = 7.0, 3H); 13C-NMR (150 MHz, CDCl3, δ ppm): 172.04, 157.6, 138.6, 128.74, 128.70, 126.6, 68.2, 61.1, 45.9, 40.6, 39.6, 35.5, 14.6; ESI-MS (m/z): 317.1475 [M + Na]+ (calcd for C15H20N2NaO4+ 317.1472).

(2-Hydroxy-3-phenethylcarbamoyl-propyl)-methyl-carbamic acid ethyl ester (5b): white solid, mp 79–81 °C; Rf = 0.33 (Et2O:CH3OH 20:1); 1H-NMR (600 MHz, CDCl3, δ ppm, J Hz), only signals for the major rotamer are listed: 7.33–7.21 (m, 5H), 6.47 (br s, 1H), 4.15 (br s, 1H) overlapped with 4.13 (q, J = 7.0, 2H), 3.55 (m, 2H), 3.33 (m, 2H), 2.99 (s, 3H) 2.84 (t, J = 7.0, 2H), 2.33 (m, 2H), 1.28 (t, J = 7.0, 3H); 13C-NMR (150 MHz, CDCl3, δ ppm): 171.9, 158.0, 138.7, 128.8, 128.6, 126.6, 68.2, 61.8, 54.6, 40.6, 40.2, 36.2, 35.6, 14.7; ESI-MS (m/z): 331.1630 [M + Na]+ (calcd for C16H24N2NaO4+ 331.1628).

4. Conclusions

We successfully prepared new oxygenated analogues of Santacruzamate A. This extends the scope of the enamine-based domino approach to functionalized β-keto amides and demonstrates a viable route to many more analogues of the natural product.

Supplementary Materials: The following are available online, S1.PDF—processed 1H and 13C NMR spectra. S2.zip—Raw NMR data. S3.zip—mol files.

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Data Availability Statement: The data presented in this study are available in this article and supporting supplementary material.

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Conflicts of Interest: The authors declare no conflict of interest.
References

1. Pavlik, C.M.; Wong, C.Y.B.; Ononye, S.; Lopez, D.D.; Engene, N.; McPhail, K.L.; Gerwick, W.H.; Balunas, M.J. Santacruzamate A, a Potent and Selective Histone Deacetylase Inhibitor from the Panamanian Marine Cyanobacterium cf. Symploca sp. *J. Nat. Prod.* 2013, 76, 2026–2033. [CrossRef] [PubMed]

2. Marks, P.A. Discovery and development of SAHA as an anticancer agent. *Oncogene* 2007, 26, 1351–1356. [CrossRef]

3. Gromek, S.M.; deMayo, J.A.; Maxwell, A.T.; West, A.M.; Pavlik, C.M.; Zhao, Z.; Li, J.; Wiemer, A.J.; Zweifach, A.; Balunas, M.J. Synthesis and biological evaluation of santacruzamate A analogues for anti-proliferative and immunomodulatory activity. *Bioorg. Med. Chem.* 2016, 24, 5183–5196. [CrossRef] [PubMed]

4. Liu, Q.; Lu, W.; Ma, M.; Liao, J.; Ganesan, A.; Hu, Y.; Wen, S.; Huang, P. Synthesis and biological evaluation of santacruzamate A and analogs as potential anticancer agents. *RSC Adv.* 2015, 5, 1109–1112. [CrossRef] [PubMed]

5. Randino, R.; Gazzero, P.; Mazitschek, R.; Rodriguez, M. Synthesis and biological evaluation of Santacruzamate-A based analogues. *Bioorg. Med. Chem.* 2017, 25, 6486–6491. [CrossRef] [PubMed]

6. Krieger, V.; Hamacher, A.; Gertzen, C.G.W.; Senger, J.; Zwinderman, M.R.H.; Marek, M.; Romier, C.; Dekker, F.J.; Kurz, T.; Jung, M.; et al. Design, Multicomponent Synthesis, and Anticancer Activity of a Focused Histone Deacetylase (HDAC) Inhibitor Library with Peptoid-Based Cap Groups. *J. Med. Chem.* 2017, 60, 5493–5506. [CrossRef] [PubMed]

7. Andrade, S.N.; Evangelista, F.C.G.; Seckler, D.; Marques, D.R.; Freitas, T.R.; Nunes, R.R.; Oliveira, J.T.; Ribeiro, R.I.M.A.; Santos, H.B.; Thomé, R.G.; et al. Synthesis, cytotoxic activity, and mode of action of new Santacruzamate A analogs. *Med. Chem. Res.* 2018, 27, 2397–2413. [CrossRef]

8. Balunas, M.J.; Pavlik, C.M.; Gerwick, G.H. Santacruzamate A compositions and analogs and methods of use. Patent WO2014018913A2, 30 January 2014. Available online: https://patents.google.com/patent/WO2014018913A2/en. (accessed on 1 January 2021).

9. Angelov, P. Enamine-Based Domino Strategy for C-Acylation/Deacetylation of Acetoacetamides: A Practical Synthesis of β-Keto Amides. *Synlett* 2010, 1273–1275. [CrossRef]

10. Yanev, P.; Angelov, P. Synthesis of functionalised β-keto amides by aminocacylation/domino fragmentation of β-enamino amides. *Beilstein J. Org. Chem.* 2018, 14, 2602–2606. [CrossRef] [PubMed]

11. Kofod, T.; Hansen, H.F.; Orum, H.; Koch, T. PNA synthesis using a novel Boc/acyl protecting group strategy. *J. Pept. Sci.* 2001, 7, 402–412. [CrossRef] [PubMed]

12. Clemens, R.J.; Hyatt, J.A. Acetoacetylation with 2,2,6-trimethyl-4H-1,3-dioxin-4-one: A convenient alternative to diketene. *J. Org. Chem.* 1985, 50, 2431–2435. [CrossRef]

13. Witzeman, J.S.; Nottingham, W.D. Transacetoacetylation with tert-butyl acetoacetate: Synthetic applications. *J. Org. Chem.* 1991, 56, 1713–1718. [CrossRef]