An Expedient, Scalable Synthesis of the Natural Product L-Anserine

Charlotte Wiles, Paul Watts*

Department of Chemistry, The University of Hull, Cottingham Road, Hull, HU6 7RX, UK
Fax +44(1482)466416; E-mail: p.watts@hull.ac.uk

Received 15 May 2007

Abstract: To date, the synthesis of L-anserine has relied on the use of naturally occurring 1-methyl-L-histidine as a precursor, however its high cost prevents its use in the large-scale synthesis of L-anserine. With this in mind, we report herein a concise and efficient synthetic strategy, employing the 160-fold less expensive, L-histidine methyl ester dihydrochloride as a starting material, to afford the title compound in an overall yield of 88%, over four reaction steps.

Key words: natural products, peptides, amino acids, coupling

In 1929, Ackermann and co-workers[1] reported the isolation of L-anserine (**1**) from goose muscle tissue, proposing that the compound was a monomethylated carnosine derivative. Following extensive synthetic evaluation, Keil et al.[2] reported that the compound was, in fact, a dipeptide comprising of β-alanine and 1-methyl-L-histidine (β-alanyl-1-methyl-L-histidine; Figure 1).

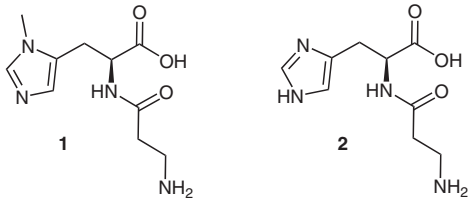


Figure 1 Target molecule β-alanyl-1-methyl-L-histidine, more commonly known as L-anserine (**1**) and the analogue L-carnosine (β-alanyl-L-histidine; **2**).

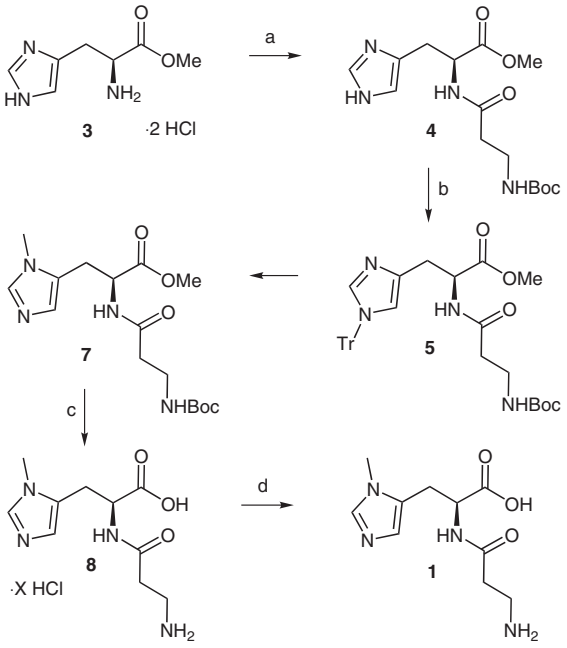
Since these preliminary accounts, L-anserine has been found to occur, in varying proportions, in the skeletal muscle and brain tissue of many species including dogs, birds, reptiles and fish.[3,4,5] Further biochemical investigations highlighted three pathways through which L-anserine **1** is synthesised in vivo: (i) from dietary histidine and bioavailable β-alanine (in the presence of carnosine-anserine synthetase),[6] (ii) direct *N*-methylation of carnosine **2** (using *S*-adenosylmethionine as the methyl donor) and (iii) β-alanyl transfer from carnosine **2** to 1-methyl histidine.[7] Although the precise metabolic function of L-anserine **1** and its analogue carnosine **2** is not known, it is widely believed that the histidine moiety (pKa 7.04) is an effective buffer, maintaining physiological pH during and after prolonged periods of exercise. Halliwell and co-workers[8] also reported a study that demonstrated the role of L-anserine **1** as a physiological antioxidant. Surprisingly however, the authors found that the compound did not possess a broad spectrum of antioxidant activity, scavenging only hydroxyl radicals (OH) in the presence of the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). Early trials in rats also demonstrated the compound's potential to protect against suppressed haematopoiesis in cancer radio- and chemotherapy.[9] More recently, it has been proposed that such histidine-containing compounds could be administered in the form of dietary supplements as a means of preventing and/or regulating muscular fatigue in athletes.[10] Owing to the lack of efficient synthetic protocols reported in the literature, at present, the compound is obtained from natural sources using a laborious extraction procedure.[11] Consequently, an efficient synthetic route would be of great industrial interest.

In 1937, Behrens and du Vingneaud[12] demonstrated the synthesis of L-anserine **1** via the condensation of 1-methyl-L-histidine methyl ester (obtained from the hydrolysis of naturally occurring L-anserine **1**) with *N*-carbobenzoxy-β-alanyl azide, followed by isolation of L-anserine **1** as the copper salt. Although this report unequivocally confirmed the structure of L-anserine **1** to be that illustrated in Figure 1, the synthesis relied on the natural product as a source of 1-methyl-L-histidine. Rinderknecht and co-workers[13] subsequently reported the total synthesis of L-anserine **1**, via the coupling of phthalyl-β-alanine and 1-methyl-L-histidine (prepared by the treatment of L-histidine with sodium and methyl iodide in liquid ammonia);[14] hydrazinolysis of the resulting *N*-phthalyl-β-alanyl-1-methyl-L-histidine afforded L-anserine, which was isolated as the respective nitrate salt. Treatment of the nitrate derivative with Dowex-3 resin afforded free base **1** in 36% yield (over 4 steps). With no truly scalable synthetic routes described, we were interested in developing an efficient, cost effective technique suitable for the large-scale synthesis of L-anserine **1**, the results of such an investigation are presented herein.

As Scheme 1 illustrates, the first step in our synthetic strategy comprised of a carbodiimide-mediated coupling reaction between L-histidine methyl ester dihydrochloride (**3**) and *tert*-butoxycarbonylamino-β-alanine to afford 2-(3-*tert*-butoxycarbonylaminopropionylamino)-3-(1*H*-imidazol-4-yl)propionic acid methyl ester (**4**) as a white crystalline solid (99%). Owing to the fact that the key reaction intermediates employed herein were previously uncharacterised, the optical rotation of each compound was measured, however, we were only able to determine at the

SYNTHESIS 2007, No. 17, pp 2608–2610
Advanced online publication: 30.07.2007
DOI: 10.1055/s-2007-983826; Art ID: P05507SS

final step that no racemisation had occurred throughout the synthesis. In this instance, *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDCI) was used as the coupling reagent, in order to simplify reaction work-up, however as a more economical alternative, this could readily be replaced with a cheaper coupling reagent such as dicyclohexylcarbodiimide (DCC).

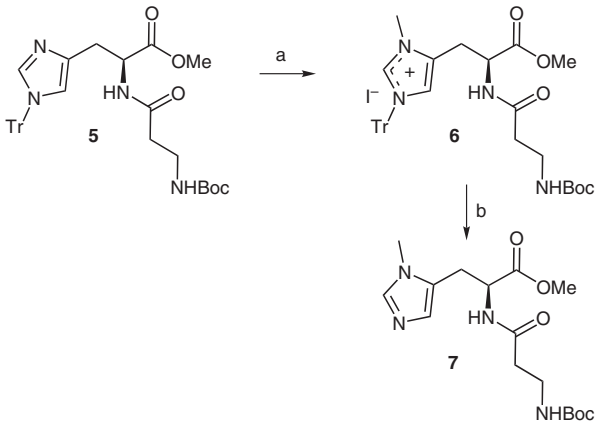


Scheme 1 *Reagents and conditions*: (a) Boc-β-alanine, EDCI, Et_3N, CH_2Cl_2, r.t., 24 h; (b) Ph_3CCl, Et_3N, benzene, reflux, 1 h; (c) aq NaOH, aq HCl; (d) aq $NaHCO_3$.

Deprotection at this stage, afforded a rapid, inexpensive route to L-carnosine **2** (Figure 1),[15] however, to obtain the desired L-anserine **1**, methylation of the histidine moiety must first be achieved.

Based on a procedure developed by Jones et al.[16] for the protection of histidine and its derivatives against racemisation, the regioselective N-methylation of the imidazole moiety in the 1-position (t-), was subsequently investigated. As Scheme 2 illustrates, the strategy involved N-tritylation in the 3-position (α-), via treatment of the dipeptide **4** with trityl chloride and triethylamine, to afford 2-(3-*tert*-butoxycarbonylaminopropionylamino)-3-(1-trityl-1*H*-imidazol-4-yl)propionic acid methyl ester (**5**; 98% yield). Addition of methyl iodide to a warmed solution of the trityl derivative **5** afforded the corresponding imidazolium iodide **6**, which was trityl-deprotected without isolation using silver acetate (AgOAc) in aqueous acetic acid. Removal of the reaction solvent under vacuum, followed by chromatographic separation of the crude material, afforded 2-(3-*tert*-butoxycarbonylaminopropionylamino)-3-(3-methyl-3*H*-imidazol-4-yl)propionic acid methyl ester (**7**) in 94% yield.

In a one-pot transformation, the methyl ester and Boc protecting groups were subsequently removed via treatment of the N-methylated derivative **7** with aqueous sodium hydroxide, followed by acidification (to pH 3.8) with aqueous hydrochloric acid. Treatment with a saturated solution of sodium bicarbonate afforded the free base **1** as a pale yellow solid. Recrystallisation of the crude solid from methanol and diethyl ether, afforded L-anserine **1** as a cream solid in 97% yield; representing an 88% yield over four key reaction steps. Spectroscopic data and physical properties of the final product were shown to be in excellent agreement with those reported for the naturally occurring compound.[3,17] Depending on the reaction conditions employed for the Boc deprotection, the respective TFA or HCl salt of **8** could be synthesised with ease. Furthermore, upon isolation of the free base **1**, the nitrate[18] and copper[3] salts are also readily available.



Scheme 2 *Reagents and conditions*: (a) MeI (10 equiv), THF, reflux, 1 h then AgOAc, AcOH–H_2O (80:20), 5 min.

In summary, we have completed a concise, four-step synthesis of L-anserine employing a commercially available precursor, whereby the target compound was obtained in an overall yield of 88%. Compared to previous syntheses, the route described herein is advantageous as it provides a protocol that can be conducted at scale and without the need for extensive chromatographic separation of key intermediates, making it suitable for application to the dietary supplement and pharmaceutical markets.

^1H and ^{13}C NMR spectra were recorded on a Jeol GX400 spectrometer as solutions in $CDCl_3$, using either TMS or CD_3OD as an internal standard; chemical shifts (δ) are given in ppm. Optical rotations were measured using the sodium D line on an Optical Activity AA-10 Automatic Polarimeter and specific rotations are recorded in 10^{-1} deg cm^2g^{-1}. Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. MS data were obtained using a Shimadzu QP5050A instrument and a EI ionisation source.

2-(3-*tert*-Butoxycarbonylaminopropionylamino)-3-(1*H*-imidazol-4-yl)propionic Acid Methyl Ester (4)

A solution of EDCI (19.16 g, 99.43 mmol) was added to a stirred solution of L-histidine methyl ester dihydrochloride (**3**; 21.90 g, 90.46 mmol), boc-β-alanine (**4**; 17.12 g, 90.46 mmol) and Et_3N (19.60 ml, 140.82 mmol) in CH_2Cl_2 (700 mL) at r.t. under N_2. After 24 h, the solvent was washed with sat. $NaHCO_3$ (700 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The com-

bined organic extracts were dried over $MgSO_4$ and concentrated under vacuo to afford ester **4**.

Yield: 30.32 g (99%); colourless crystalline solid; mp 76–78 °C; $[\alpha]_D^{22}$ +5.85 (*c* 0.29, MeOH).

^{1}H NMR ($CDCl_3$): δ = 1.42 (s, 9 H), 2.38 (t, *J* = 6.5 Hz, 2 H), 2.98 (dd, *J* = 8.4, 14.9 Hz, 1 H), 3.09 (dd, *J* = 8.4, 14.9 Hz, 1 H), 3.25 (br t, *J* = 6.5 Hz, 2 H), 3.69 (s, 3 H), 4.66 (m, 1 H), 6.58 (s, 1 H), 7.58 (s, 1 H). NH signals were not observed.

^{13}C NMR ($CDCl_3$): δ = 27.4, 28.8, 35.6, 36.6, 51.4, 52.8, 78.9, 123.2, 134.8, 135.1, 156.9, 172.1, 172.5.

MS: *m*/*z* = 341 [M$^+$].

2-(3-*tert*-Butoxycarbonylaminopropionylamino)-3-(1-trityl-1*H*-imidazol-4-yl)propionic Acid Methyl Ester (5)

Trityl chloride (19.88 g, 71.32 mmol) and Et_3N (9.93 mL, 71.32 mmol) were dissolved in anhydrous benzene (60 mL) and ester **4** (24.25 g, 71.32 mmol) was added. The resulting reaction mixture was refluxed for 1 h then the precipitated triethylammonium chloride was removed by filtration. The filtrate was concentrated under vacuo to afford ester **5**.

Yield: 40.50 g (98%); pale-yellow gum; $[\alpha]_D^{22}$ +5.3 (*c* 0.53, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 1.28 (s, 9 H), 2.34 (m, 2 H), 2.95 (m, 2 H), 3.32 (m, 2 H), 3.71 (s, 3 H), 4.71 (m, 1 H), 5.86 (br s, 1 H), 6.46 (s, 1 H), 7.13–7.29 (m, 15 H) and 7.26 (s, 1 H). NH signals were not observed.

^{13}C NMR ($CDCl_3$): δ = 28.5, 28.9, 36.5, 36.7, 52.4, 52.7, 69.3, 78.9, 123.4, 128.0, 128.4, 129.2, 129.8, 139.9, 143.2, 156.8, 171.2, 173.0.

MS: *m*/*z* = 583 [M$^+$].

2-(3-*tert*-Butoxycarbonylaminopropionylamino)-3-(3-methyl-3*H*-imidazol-4-yl)propionic Acid Methyl Ester (7)

MeI (17.43 mL, 279.9 mmol) was added to a solution of ester **5** (32.58 g, 55.98 mmol) in THF (50 mL) and the reaction mixture was refluxed for 1 h. Concentration under vacuo afforded the respective *N*-methyl imidazolium iodide **6** as a pale yellow gum. Trityl deprotection was achieved via dissolution of the *N*-methyl imidazolium iodide **6** in aq AcOH (AcOH–H_2O, 80:20), followed by the addition of AgOAc (9.34 g, 55.98 mmol). The reaction mixture was subsequently stirred for 5 min then the precipitated silver iodide was removed by filtration, washed with aq AcOH (2 × 50 mL) and the combined filtrates were concentrated under vacuo. The resulting pale yellow solid was purified by flash chromatography (EtOAc–hexane, 10% then MeOH–CH_2Cl_2, 50%) to give ester **7**.

Yield: (18.7 g, 94%); pale-yellow gum; $[\alpha]_D^{22}$ –12.4 (*c* 0.7, MeOH).

^{1}H NMR ($CDCl_3$): δ = 1.43 (s, 9 H), 2.37 (t, *J* = 5.8 Hz, 2 H), 3.04 (dd, *J* = 6.7, 15.4 Hz, 1 H), 3.10 (dd, *J* = 6.7, 15.4 Hz, 1 H), 3.31 (m, 2 H), 3.57 (s, 3 H), 3.71 (s, 3 H), 4.76 (m, 1 H), 6.77 (s, 1 H), 7.50 (s, 1 H).

^{13}C NMR ($CDCl_3$): δ = 26.5, 28.5, 31.7, 36.1, 36.6, 51.6, 52.8, 79.9, 126.9, 128.3, 138.1, 156.2, 171.2, 172.5.

MS: *m*/*z* = 355 [M$^+$].

l-Anserine (1)[12]

Aq NaOH (1.0 M, 35.6 mL) was added to a solution of ester **7** (12.62 g, 35.6 mmol) in MeOH (5 mL) and the reaction mixture was stirred at r.t. overnight. Evaporation gave a residual gum that was dissolved in distilled H_2O (10 mL) and the reaction mixture was acidified to pH 3.8 (HCl, 1.0 M). The acidified mixture was stirred for a further 1 h then neutralised with sat. $NaHCO_3$ and concentrated under vacuo. The organic residue was dissolved in warm EtOH and, after cooling to r.t., the solution was filtered in order to remove any residual NaCl and $NaHCO_3$. Evaporation of the filtrate afforded a pale-yellow foam. The crude material was subsequently precipitated from MeOH–Et_2O, which, due to the hygroscopic nature of the product, was subsequently washed with Et_2O (3 × 200 mL), the excess decanted off, and the residue concentrated under vacuo to afford L-anserine **1**.

Yield: 8.27 g (97%); colourless solid; mp 268 °C (dec.) [Lit.[13] 238–239 °C]; $[\alpha]_D^{22}$ +11.4 (*c* 0.5, H_2O) [Lit.[3] +11.3 (*c* 10, H_2O)].

^{1}H NMR (CD_3OH): δ = 2.33 (m, 2 H), 2.82 (t, *J* = 6.6 Hz, 2 H), 2.92 (dd, *J* = 8.8, 15.4 Hz, 1 H), 3.20 (dd, *J* = 8.8, 15.4 Hz, 1 H), 3.66 (s, 3 H), 4.51 (br dd, *J* = 4.5, 8.8 Hz, 1 H), 6.74 (s, 1 H), 7.47 (s, 1 H). Signals arising from NH, NH_2 and OH were not observed.

^{13}C NMR (CD_3OH): δ = 26.7, 30.5, 37.6, 38.3, 53.6, 126.5, 129.0, 137.7, 172.8, 177.0.

MS: *m*/*z* = 241 [M$^+$].

Acknowledgment

Financial support from The University of Hull is greatly acknowledged (C.W.).

References

(1) Ackermann, D.; Timpe, O.; Poller, K. *Z. Physiol. Chem.* **1929**, *183*, 1.
(2) Linneweh, W.; Keil, A. W.; Hoppe-Seyler, F. A. *Z. Physiol. Chem.* **1929**, *183*, 11.
(3) Wolff, W. A.; Wilson, D. W. *J. Biol. Chem.* **1932**, *95*, 495.
(4) Wolff, W. A.; Wilson, D. W. *J. Biol. Chem.* **1935**, *109*, 565.
(5) (a) Zapp, J. A.; Wilson, D. W. *J. Biol. Chem.* **1938**, *126*, 9. (b) Zapp, J. A.; Wilson, D. W. *J. Biol. Chem.* **1938**, *126*, 19.
(6) Stenesh, J. J.; Winnick, T. *Biochem. J.* **1960**, *77*, 575.
(7) McManus, I. R. *J. Biol. Chem.* **1962**, *237*, 1207.
(8) Aruma, O. I.; Laughton, M. J.; Halliwell, B. *Biochem. J.* **1989**, *264*, 863.
(9) Harada, M.; Yang, Z.; Higuchi, N.; Tanaka, T.; Iwata, S. *Proceedings of The 5th International Symposium on Clinical Nutrition*, Vol. 5(4); HEC Press: Australia, **1996**, 68.
(10) Matahira, Y.; Kikuchi, K. US 6855727, **2005**.
(11) L-Anserine (**1**) is obtained from natural sources using a hot water extraction procedure, affording typical recoveries of 45 g of L-anserine hydrochloride (**8**) from 10 kg of bonito extract (0.45 wt%).
(12) Behrens, O. K.; du Vigneaud, V. *J. Biol. Chem.* **1937**, *120*, 517.
(13) Rinderknecht, H.; Rebane, T.; Ma, V. *J. Org. Chem.* **1964**, *29*, 1968.
(14) Tallan, H. H.; Stein, W. H.; Moore, S. *J. Biol. Chem.* **1954**, *206*, 825.
(15) Vinick, F. J.; Jung, S. *J. Org. Chem.* **1983**, *48*, 392.
(16) Fletcher, A. R.; Jones, J. H.; Ramage, W. I.; Stachuiski, A. V. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2261.
(17) The free amine **1** was found to be stable for extended periods of time when stored under N_2, however, it rapidly sublimed when exposed to air; structural analysis confirmed that no decomposition had occurred.