Tetrahedron Vol 47, No 30, pp 5835-5840, 1991
Printed in Great Britain

0040-4020/91 $3 00+ 00

The Stereospecific Synthesis Of Alahopcin

Jack E Baldwin, Robert M Adlington, Christopher R A Godfrey,[a]
David W Gollins, and Christopher J Schofield

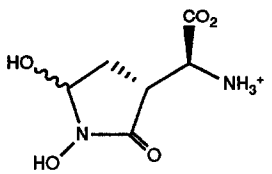
The Dyson Perrins Laboratory, University of Oxford,
South Parks Road, Oxford, OX1 3QY

[a] ICI Agrochemicals, Jealott's Hill Research Station,
Bracknell, Berkshire, RG12 6EY

(*Received in UK* 16 *April* 1991)

Abstract: *The first stereospecific synthesis of the naturally occuring dipeptide antibiotic, alahopcin, starting from (L)-aspartic acid is described*

Recently we reported the first total synthesis of the novel amino-acid dealanylalahopcin **1** [1] We now describe the first total synthesis of the parent dipeptide alahopcin **2**, *via* the common intermediate **3** [1]



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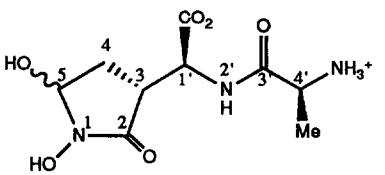

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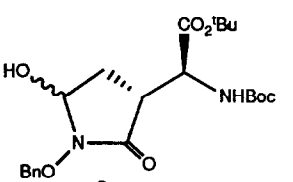
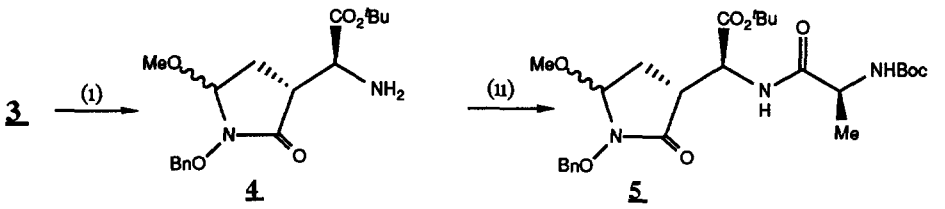


3

Alahopcin (B-52653) **2** was first isolated by Higashide *et al* from a culture of a sub-species of *Streptomyces albulus*[2] and was shown to be an active antibiotic against both Gram-positive and -negative bacteria, with especially strong activity against *Staphylococcus aureus* 4R, previously an antibiotic resistant mutant It was also found to inhibit prolyl collagen hydroxylase both *in vivo* and *in vitro* and to have a stimulatory effect on the production of bacterial α-amylase in mice [2] Also, alahopcin **2** has been shown to be identical to nourseimycin, an antimetabolite of (L)-proline [3,4]

Initially, intermediate **3** was reacted with *p*-toluenesulphonic acid (2 1eq) in methanol for 18 hours yielding the free α-amino 2-oxopyrrolidine **4** in 94% yield Coupling of the two C-5 epimers of the free amine **4** with the requisite protected (L)-alanine was carried out using the 1-hydroxybenzotriazole (HOBt)-catalysed dicyclohexylcarbodiimide (DCCI) coupling procedure [5] Thus, *N*-Boc-(L)-alanine, HOBt and DCCI were stirred in chloroform at 0°C After 2 hours, a solution of the α-amino 2-oxopyrrolidine **4** in chloroform was added and the resultant suspension stirred for a further 20 minutes before being filtered (Scheme 1) The filtrate was evaporated to dryness and the residue purified by flash chromatography to yield the two C-5 epimers of fully

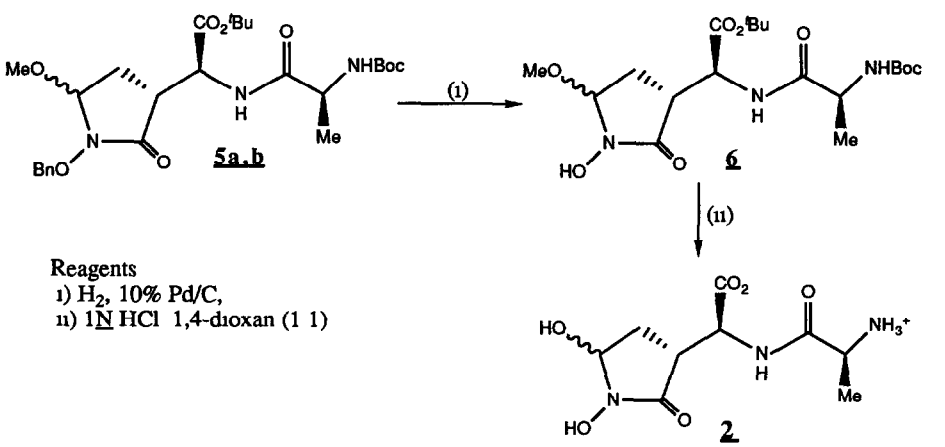
protected dipeptide **5a, 5b** (ratio of 1 1.1) in a 71% yield from the partially protected amino acid **3** The coupling was seen to proceed with no evidence of racemisation at either α-centre as judged by 500MHz ^{1}H NMR analysis of the crude coupled product.



Reagents
i) *p*TSA (2.1eq), MeOH, then extract into CH_2Cl_2 washing with aqueous NH_4OH,
ii) *N*-Boc (L)-alanine, HOBt, DCCI, chloroform

Scheme 1

A two step deprotection scheme was then employed to release alahopcin **2** (Scheme 2) Hydrogenolysis of the two epimers of the protected dipeptide **5a,b** (H_2, 10% Pd/C, $NaHCO_3$) for 3 hours gave the hydroxamic acid **6**, which was not purified, the remaining protecting groups were then efficiently removed using aqueous acid (1M HCl) in 1,4-dioxan, over 24 hours The crude product was purified by ion exchange chromatography on a Dowex-50W-X8(H) column with 1N ammonium hydroxide as the eluent The ninhydrin-active fractions were lyophilised, re-dissolved in water and then loaded onto a column of Amberlite IRA-68 which was eluted with a gradient from water to 0 2M aqueous acetic acid Ninhydrin active fractions were lyophilised to yield alahopcin **2** as an off-white powder (59% from dipeptide **5**) which displayed consistent spectral data [^{1}H NMR (figure 1), ^{13}C NMR, and *m/z*] and specific optical rotations $[\alpha]_D^{20}$ +50 3° (c=1 0, H_2O), +64 9° (c=1 0, 1N HCl) [lit ,[2] $[\alpha]_D^{20}$ +52 7° (c=1 0, H_2O), +62 0° (c=1 0, 1N HCl)] to those reported in the literature [2]



Reagents
i) H_2, 10% Pd/C,
ii) 1N HCl 1,4-dioxan (1 1)

Scheme 2

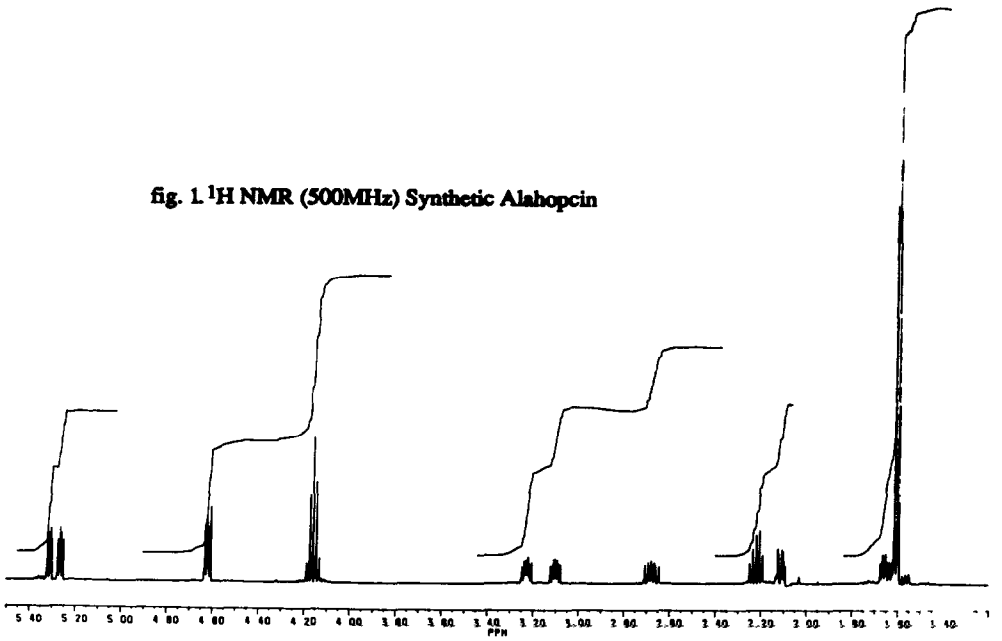


fig. 1. ^{1}H NMR (500MHz) Synthetic Alahopcin

Experimental Section

Infrared (IR) spectra were recorded on either a Perkin-Elmer 681 or Perkin-Elmer 1710 FT-IR spectrometers with only selected absorptions being reported Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-500 spectrometer Spectra were measured using $CDCl_3$ as solvent with chemical shifts quoted in parts per million (δ p p m) using the residual solvent peak as an internal reference, except when stated otherwise Coupling constants (*J*) are quoted to the nearest 0 5Hz ^{13}C spectra were run using DEPT editing, except when otherwise stated Mass spectra were recorded on V G Micromass ZAB 1F (DCI), V G 20-250 (DCI/CI/FAB$^+$) or V G BIO-Q(Electrospray) spectrometers, percentage intensities are recorded in parenthesis Optical rotation were measured using a Perkin-Elmer 241 polarimeter, at 20°C with a pathlength of 1dm with concentrations given in g/100ml Melting points were obtained using a Buchi 510 capillary melting point apparatus and are uncorrected Microanalyses were performed within the Dyson Perrins

Flash chromatography was accomplished on silica gel using Sorbsil™ C60 Thin layer chromatography was performed on aluminium sheets pre-coated with Merck silica gel 60 F_{254}, plates being visualised with UV (254nm) or 10%w/v ammonium molybate in 2M sulphuric acid, followed by heat Amino acids were located on t l c by 3%w/v ninhydrin in ethanol

Ion exchange resins were prewashed before use Dowex-50W-X8(H) (50-100 mesh) was washed with 1N HCl, water (to pH 7), 1N ammonium hydroxide, water (to pH 7), 1N HCl, and finally water (to pH 7), Amberlite IRA-68 was washed with 1N ammonium hydroxide, water (to pH 7), 0 2M acetic acid, water (to pH 7), 1N ammonium hydroxide, and finally water (to pH 7)

Methanol was dried before use by distillation from magnesium methoxide Anhydrous *p*-toluenesulphonic acid was prepared by refluxing *p*-toluenesulphonic acid monohydrate in toluene using a Dean and Stark apparatus

(3R)-3-[(1'S)-t-*Butyl 1'-(amino)acetate]-1-benzyloxy-5-methoxy-2-oxopyrrolidine* 4

(3R)-3-[(1'S)-*t*-Butyl 1'-(*t*-butyloxycarbonylamino)acetate]-1-benzyloxy-5-hydroxy-2-oxopyrrolidine **3** (841mg, 1.85mmol) was dissolved in methanol (20ml) and anhydrous *p*-toluenesulphonic acid (678mg, 3 94mmol) was added After stirring for 18 hours the solvent was removed The oily residue was taken into dichloromethane (50ml) and washed with 1M ammonium hydroxide (2x60ml), brine (60ml) and dried ($MgSO_4$) Removal of the dichloromethane yielded **4** (646mg, 94%) as a pale brown oil, R_f 0-0 1 (Et_2O), ν_{max} ($CHCl_3$) 3080-2800(m, N-H), 1730(C=O), 1370(s), and 1090(m); δ_H(500MHz, $CDCl_3$) 1 51 (9H, s, $C(CH_3)_3$), 1 64 (2H, s, NH_2, both epimers), 1 65-1 87 & 2 31-2 45 (2H, 2xm, 4-H, minor epimer), 1 93-2 22 (2H, m, 4-H, major epimer), 2.77-2 88 (1H, m, 3-H, minor epimer), 3 08-3 20 (1H, m, 3-H, major epimer), 3 43 (3H, s, OCH_3, major epimer), 3.52 (3H, s, OCH_3, minor epimer), 4 54 (1H, d, *J* 6Hz, α-H, major epimer), 4 67 (1H, dd, *J* 5, 10Hz, α-H, minor epimer), 5 04 (2H, s, CH_2Ph, major epimer), 5 00 & 5 18 (2H, *AB*q, *J* 10Hz, CH_2Ph, minor epimer), and 7 37-7 48 (5H, m, Ph), δ_C(125 8MHz, $CDCl_3$), 27 1 & 27 6 (4-C), 27 8 ($C(CH_3)_3$), 40 8 (3-C), 55 1 & 55 8 (α-C), 56 5 & 57 0 (OCH_3), 77 3 & 78 1 (CH_2Ph), 81 9 ($C(CH_3)_3$), 88 3 & 88 8 (5-C), 128 6-129 9 (Ph), 135 2 (Ph *ipso* C), and 163 4, 170 0 & 172 7 (C=O), *m/z* [Direct CI(NH_3)] 351 (MH^+, 90), 295 (100), 249 (33), 111 (53), and 91 (62%)

Coupling of (3R)-*3-[(1'S)-t-butyl 1'-(amino)acetate]-1-benzyloxy-5-methoxy-2-oxopyrrolidine* 4 with N-*Boc*-(L)-*alanine*

N-Boc-(L)-Alanine (384mg, 2 03mmol), 1-hydroxybenzotriazole (275mg, 2 03mmol) and dicyclohexylcarbodiimide (419mg, 2 03mmol) were stirred at 0°C in chloroform (15ml) for 1 hour The solution was then allowed to warm to room temperature and stirred for a further hour A solution of (3R)-3-[(1'S)-*t*-butyl 1'-(amino)acetate]-1-benzyloxy-5-methoxy-2-oxopyrrolidine **4** (646mg, 1 84mmol) in chloroform (10ml) was added and the resultant suspension was stirred for 20 minutes Removal of the the chloroform yielded a pale brown solid which was washed with diethyl ether The ether washings were combined and then evaporated to afford a pale brown foam which was then purified by flash chromatography [SiO_2 (40g), eluting with diethyl ether petrol 1 3] to yield (3R)-3-{(1'S)-*t*-butyl 1'-*N*-[(*t*-butoxycarbonyl)-(L)-alanylamino]acetate}-1-benzyloxy-5-methoxy-2-oxopyrrolidine [720mg (major epimer, **5a**, 372mg, minor epimer, **5b** 348mg, 71% from **3**] as white foams [Found (major epimer), C, 59 86, H, 7 66, N, 8 12 $C_{26}H_{39}O_8N_3$ requires C, 59 87, H, 7 67, N, 8.07%), Rf 0 5 (major epimer), 0 3 (minor epimer) (1 1 diethyl ether petrol), $[\alpha]_D^{20}$ (major epimer) -45 2° (c=0 84, $CHCl_3$), ν_{max} ($CHCl_3$) (both epimers) 3400(w), 1725(s, C=O), 1715(s, C=O), 1500(m), 1370(m), 1240(m), 1160(s), and 1080(m), δ_H(500MHz, $CDCl_3$) (major epimer) 1 38 (3H, d, *J* 7Hz, 5'-H), 1 44 & 1 49 (2x9H, 2xs, 2x$C(CH_3)_3$), 1 94-1 97 & 2 02-2 06 (2x1H, 2xm, 4-H), 3 35-3 40 [4H(3H+1H), m, OCH_3 + 3-H], 4 12-4 15 (1H, m, 4'-H), 4 52-4 55 (1H, m, 1'-H), 4 62-4 64 (1H, m, 5-H), 5 02 (2H, s, CH_2Ph), 5 06 (1H, d, *J* 7Hz, N-H), 6 49 (1H, d, *J* 8Hz, N-H), and 7 34-7 42 (5H, m, Ph), δ_H(500MHz, $CDCl_3$) (minor epimer) 1 38 (3H, d, *J* 7 Hz, 5'-H), 1 44 & 1 51 (2x9H, 2xs, 2x$C(CH_3)_3$), 1 81-1 86 & 2 31-2 37 (2x1H, 2xm, 4-H), 3 10-3 14 (1H, m, 3-H), 3 43 (3H, s, OCH_3), 4 22 (1H, m, 4'-H), 4 56 (1H, dd, *J* 3, 8Hz, 1'-H),

4 71 (1H, dd, *J* 4, 8Hz, 5-H), 5.01 & 5 11 (2H, ABq, *J* 10Hz, C<u>H</u>$_2$Ph), 5 11 (1H, d, *J* 9Hz, N-H), 6 65 (1H, d, *J* 8Hz, N-H), and 7.37-7 45 (5H, m, Ph), δ_C(125 8MHz, $CDCl_3$) (major epimer) 18 8 (5'C), 27 5 (4-C), 27 9 & 28 3 (2xC(<u>C</u>H$_3$)$_3$), 40 2 (3-C), 50 4 & 51 9 (both α-C), 56 6 (O<u>C</u>H$_3$), 77 9 (<u>C</u>H$_2$Ph), 83 0 (both <u>C</u>(CH$_3$)$_3$), 88 1 (5-C), 128 5-129 4 (Ph), 135 0 (Ph *ipso* C), and 155 1, 168 5, 169 1, & 173 8 (C=O), δ_C(125 8MHz, $CDCl_3$) (minor epimer) 18 9 (5'-C), 26 5 (4-C), 27 9 & 28 3 (2xC(<u>C</u>H$_3$)$_3$), 40 3 (3-C), 50.5 & 52 8 (both α-C), 56 9 (O<u>C</u>H$_3$), 78 0 (<u>C</u>H$_2$Ph), 83 0 (both <u>C</u>(CH$_3$)$_3$), 88 7 (5-C), 128 5-129 5 (Ph), 134.9 (Ph *ipso* C), and 155 1, 168 3, 173 2 (C=O), *m/z* [DCI(NH_3)] 522 (MH^+, 14), 466 (18), 410 (14), 151 (40), 108 (41), 91 (100), and 90 (80%)

(3<u>R</u>)-*3-[(1'S)-1'-((<u>L</u>)-Alanylamino)acetate]-1,5-dihydroxy-2-oxopyrrolidine* (Alahopcin) <u>2</u>

(3<u>R</u>)-3-{(1'<u>S</u>)-*t*-Butyl 1'-*N*-[(*t*-butoxycarbonyl)-(<u>L</u>)-alanylamino]acetate}-1-benzyloxy-5-methoxy-2-oxopyrrolidine **<u>5a,b</u>** (716mg, 1 37mmol), and sodium hydrogen carbonate (250mg) were added to a suspension of 10% Pd/C (100mg) in methanol (20ml) and placed under a balloon of hydrogen for three hours The resultant suspension was filtered through Celite® and the solvent removed to yield crude (3<u>R</u>)-3-{(1'<u>S</u>)-*t*-butyl 1'-[*N*-(*t*-butoxycarbonyl)-(<u>L</u>)-alanylamino)acetate]-1-hydroxy-5-methoxy-2-oxopyrrolidine (<u>6</u>) as a pale yellow oil (510mg) δ_H(500MHz, $CDCl_3$) 1 40 & 1 42 (2x3H, 2xd, *J* 7 Hz, 5'-H), 1 44-1 51 (4x9H+1H, 4xs+m, 4xC(C<u>H</u>$_3$)$_3$, 4-H), 1 58-2 00 & 2 51-2 58 (3x1H, 3xm, 4-H), 3 03-3 10 (2H, m, 2x3-H), 3 48 (6H, s, 2xOMe), 4 30-4 41 (2H, m, 2x4'-H), 4 66-4 71 & 4 73-4 79 (2x1H, 2xm, 1'-H), 4 83-4 92 (2x1H, 2xm, H-5), 5 30 (1H, d, *J* 5Hz, N-H), and 5 48 (1H, d, *J* 5Hz, N-H) This oil was immediately dissolved in 1,4-dioxan (15ml) 1<u>N</u> HCl (15ml) was added and the resultant solution was stirred for 24 hours The solution was then washed with ethyl acetate (2x20ml) and the remaining aqueous portion was lyophilised to yield crude alahopcin <u>2</u> (400 mg) This crude product was then dissolved in water and passed through a pre-washed Dowex-50W-X8(H) (20ml) column, washing with water then eluting with 1<u>N</u> ammonium hydroxide After lyophilisation of the ninhydrin-active fractions, the resultant pale brown solid (250mg) obtained was dissolved in water and passed down a pre-washed Amberlite IRA-68 (20ml) column, eluting with a gradient from water to 0 2<u>M</u> aqueous acetic acid The ninhydrin active fractions were collected and lyophilised to yield <u>2</u> (210mg, 59% from **<u>5a</u>**, **<u>b</u>**) as an off-white powder; $[\alpha]_D^{20}$ +50 3° (c=1 0, H_2O), +64 9° (c=1 0, 1<u>N</u> HCl) [lit ,[2] $[\alpha]_D^{20}$ +52 7° (c=1 0, H_2O), +62 0° (c=1 0, 1<u>N</u> HCl)], δ_H(500MHz, D_2O, ref <u>H</u>OD δ 3 63) 1 48 (6H(2x3H), d, *J* 7Hz, 5'-H, both epimers), 1 51-1 65 & 2 52-2 68 (2H, 2xm, 4-H, minor epimer), 1 95-2 00 & 2 07-2 11 (2H, 2xm, 4-H, major epimer), 2 94-2 97 (1H, m, 3-H, minor epimer), 3 07-3 10 (1H, m, 3-H, major epimer), 4 02 (2H(2x1H), q, *J* 7Hz, 4'-H, both epimers), 4 47-4 49 (2H(2x1H), m, 1'-H, both epimers), 5 13 (1H, dd, *J* 3, 4Hz, 5-H, minor epimer), and 5 19 (1H, m, 5-H, major epimer), δ_C(125 8MHz, D_2O, ref 1,4 dioxan δ 67 3) 17 1 (5'-C, Me), 28 8 (4-C, <u>C</u>H$_2$), 40 3 & 40 9 (3-C), 49 9 (4'-C), 55 8 & 56 1 (1'-C), 82 0 & 82 2 (5-C), and 171 0, 171 1 & 171 7 (C=O), *m/z* (Electrospray) 279 ($M+NH_4^+$, 10), and 262 (MH^+, 100%)

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