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An efficient stereoselective synthesis of *Z*-(2*S*)- and *Z*-(2*R*)-2-*tert*-butoxycarbonylamino-6-hydroxyhex-4-enoic acid, key intermediates in the synthesis of (2*S*,4*S*,5*R*)-(−)- and (2*R*,4*R*,5*S*)-(+)-bulgecinine

Karen E. Holt,* Jonathan P. Swift, Mark E. B. Smith, Stephen J. C. Taylor and Raymond McCague

Chirotech Technology Ltd., The Dow Chemical Company, Cambridge Science Park, Milton Road, Cambridge CB4 0WG, UK

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Abstract—A concise, scaleable route to both isomers of *Z*-2-*tert*-butoxycarbonylamino-6-hydroxyhex-4-enoic acid from 2-butyne-1,4-diol, utilizing L- and D-acylase enzymes is presented. These intermediates were readily converted to multigram quantities of *N*-Boc-(2*S*,4*S*,5*R*)- and *N*-Boc-(2*R*,4*R*,5*S*)-bulgecinine.

1. Introduction

Bulgecins are glycopeptide bacterial metabolites isolated from cultures of *Pseudomonas acidophila* and *Pseudomonas mesoacidophila*.[1] They have no antibacterial activity themselves but when used in conjunction with β-lactam antibiotics, they enhance the antibiotic effect. (2*S*,4*S*,5*R*)-(−)-Bulgecinine (**1**, Fig. 1) is a constituent amino acid of the bulgecins, making it an attractive synthetic target.[2] Previous workers have discovered an efficient halolactonisation of *cis*-allylic alcohol (*S*)-**2**, to give the lactone (2*S*,4*S*,5*S*)-**3** with good diastereoselectivity.[3] The conditions of Oppolzer[4] can then be used to convert lactone (2*S*,4*S*,5*S*)-**3** to (−)-bulgecinine **1**.

The key intermediate, *cis*-allylic alcohol (*S*)-**2** has previously been prepared from *N*-Boc-(*S*)-allylglycine methyl ester via a seven-step procedure[3] but this synthesis is not readily scaleable and the starting material is very expensive. Thus, we sought to devise a more scaleable synthesis of **2** from the cheap and readily available 2-butyne-1,4-diol (Scheme 1). Use of L-acylase[5] and D-acylase[6] enzymes at a strategic point in the synthesis gives access to both isomers of the alkynic alcohol **4**, which can be easily converted to the key *cis*-allylic alcohols, **2**. This allows synthesis of both the (2*S*,4*S*,5*R*)- and (2*R*,4*R*,5*S*)-isomers of bulgecinine. In addition, we considered that the amino acids (*S*)-**4**, (*R*)-**4**, (*S*)-**2** and (*R*)-**2** would be useful synthons for further diversification to other building blocks.

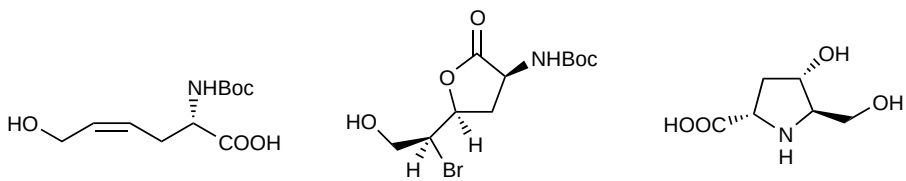

Figure 1.

* Corresponding author. E-mail: keholt-tiffin@dow.com

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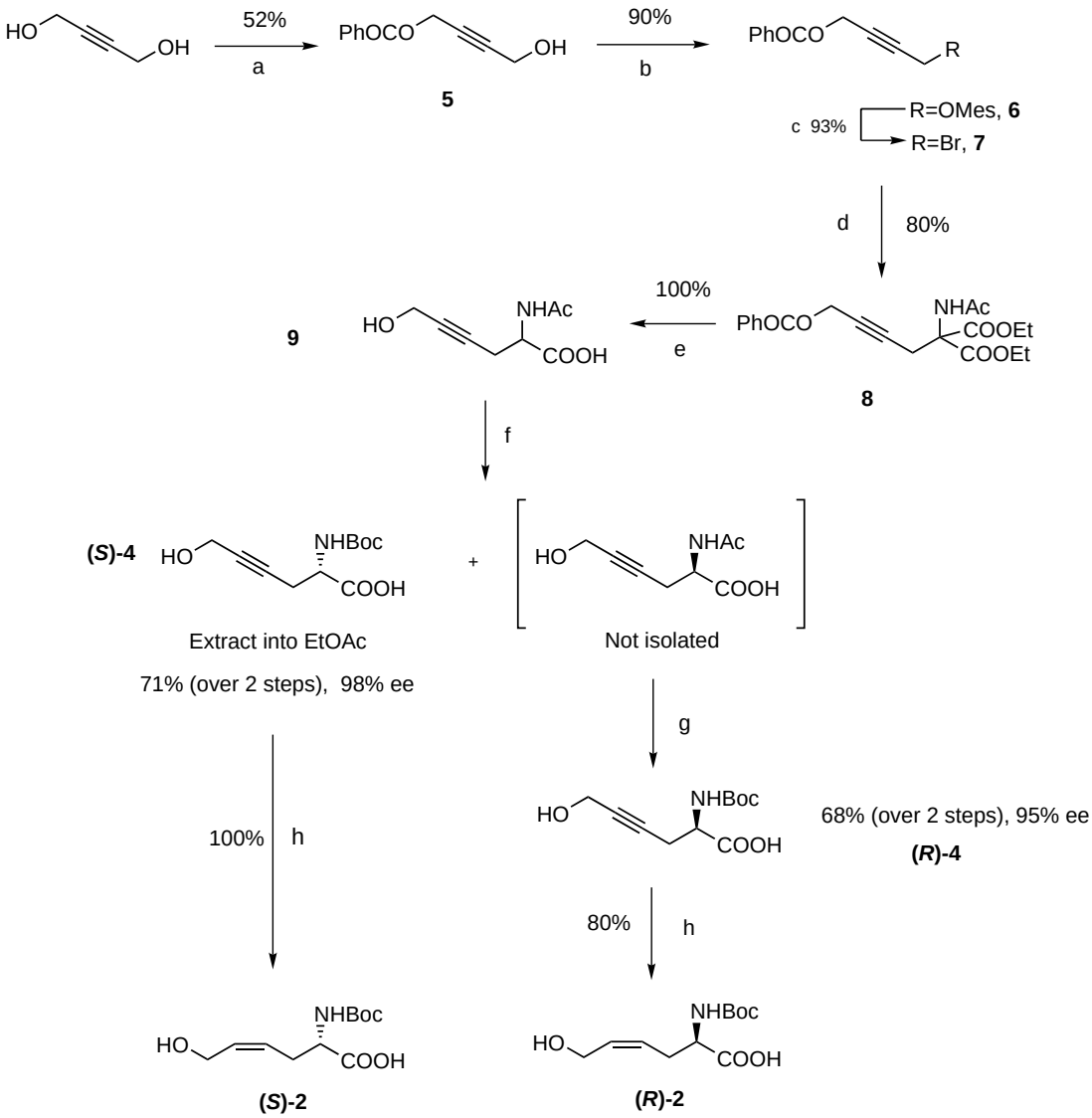
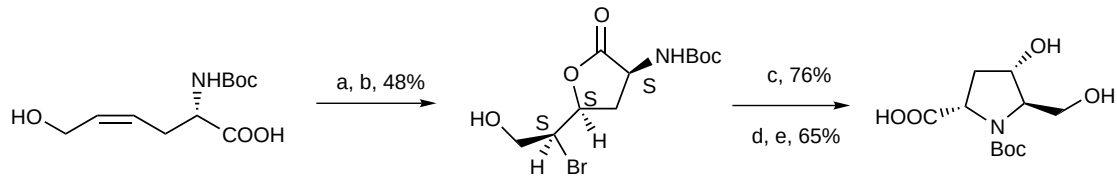


Scheme 1. *Reagents and conditions*: (a) PhCOCl (1 equiv.), Py (1 equiv.), DCM, 10°C to rt, o/n; (b) TEA (1.1 equiv.), DMAP (0.025 equiv.), MesCl (1.1 equiv.), DCM, 0–5°C, 2 h; (c) LiBr (2 equiv.), acetone, 10–15°C, 1.5 h; (d) diethyl acetamidomalonate (1.4 equiv.), KO^{t}Bu (1.4 equiv.), THF, reflux, 22 h; (e) NaOH (3 equiv.), MeOH, reflux, 3.5 h, then acidify to pH 3.5 with conc. HCl, reflux, 22 h; (f) L-acylase (200 U/g substrate), 30 mM KH_2PO_4, pH 7, 65°C, 2.5 h, then Boc_2O (0.5 equiv. cf. racemic *N*-Ac acid), THF, 5 M NaOH to maintain pH at 10, rt, 3.5 h; (g) D-acylase (40 U/g substrate), aqueous NaOH to adjust to pH 8, rt, 21 h, then Boc_2O (0.5 equiv. cf. racemic *N*-Ac acid), THF, 5 M NaOH to maintain pH at 10, rt, o/n; (h) 10% wt. Lindlar catalyst (5% wt. Pd on $CaCO_3$ poisoned with Pb), MeOH, 1 bar hydrogen, 20°C, 1.5 h.

2. Results and discussion

2.1. Preparation of *Z*-2-*tert*-butoxycarbonylamino-6-hydroxyhex-4-enoic acids

Treatment of 2-butyne-1,4-diol (£20/kg from Aldrich) with 1 equiv. of benzoyl chloride and pyridine gave a 2:1 mixture of the mono-:di-benzoate. This was easily purified by treatment of the mixture with ethanol, whereby the majority of the dibenzoate precipitated from solution. This was removed by filtration, thus allowing easy isolation of the monobenzoate **5** in 52% overall yield (92% pure). The monobenzoate **5** was derivatised to the mesylate **6** under standard conditions in 90% yield. The mesylate was then reacted with lithium bromide in acetone at 10–15°C to give the bromide **7** in 93% yield. Condensation of the bromide **7** with the diethyl acetamidomalonate anion at reflux gave the diester **8** in 80% isolated yield. This was hydrolysed with aqueous sodium hydroxide and then decarboxylated at pH 3 and reflux. Isolation of the resulting *N*-acetyl acid **9** was not straightforward since it did not extract into organic solvents from the aqueous reaction mixture. Thus, it was isolated by concentration of the aqueous phase (previously extracted with EtOAc to remove benzoic acid), precipitation of the inorganic salts with MeOH and concentration of the filtrate. This gave the racemic 2-acetylamino-6-hydroxyhex-4-ynoic acid **9** in quantitative crude yield from the diester (pure by ^{1}H NMR). In



(*S*)-2 **(2*S*,4*S*,5*S*)-3** ***N*-Boc-(2*S*,4*S*,5*R*)-1**

Scheme 2. *Reagents and conditions*: (a) NBS (1.1 equiv.), THF, −10 to 0°C, 20 min; (b) 40% EtOAc/60% heptane slurry, rt, 15 min–0.5 h; (c) *p*-TsOH·H_2O (2 equiv.), EtOAc, rt, 1 h; (d) H_2O, 1 M LiOH to pH 9, rt, 16 h; (e) Boc_2O (1 equiv.), THF, 1 M LiOH to maintain pH 9, rt, 4.5 h.

this way good quality racemic *N*-acetyl acid **9** can be easily prepared from butyne-1,4-diol without the need for any chromatography or excessively low temperature reactions.

Small-scale reactions revealed *N*-acetyl acid **9** to be a good substrate for both of our proprietary L-acylase[5] (extremophile) and D-acylase[6] enzymes.[7] This allowed us to develop an efficient 'one-pot' procedure[8] to (*S*)-**4** and (*R*)-**4** in 71% theoretical yield, 98% ee[9] and 68% theoretical yield, 95% ee,[9] respectively, from **9**. Thus, treatment of **9** with extremophilic L-acylase at 65°C and pH 7 gave a mixture of (*S*)-amino acid and unreacted (*R*)-*N*-acetyl acid. This mixture was treated with di-*tert*-butyl dicarbonate (Boc_2O) at pH 10 and the so formed (*S*)-*N*-Boc acid, (*S*)-**4**[10] extracted from the acidified solution with EtOAc. The remaining aqueous solution containing the (*R*)-*N*-acetyl acid was then treated with D-acylase at pH 8 and room temperature to give the (*R*)-amino acid. This was transformed to the extractable *N*-Boc derivative, (*R*)-**4**[10] by addition of Boc_2O to the reaction mixture at pH 10, as above.

Lindlar hydrogenation[11] of the alkyne **4** gave the desired *cis*-allylic alcohol **2** in 80% purified yield (column chromatography). It should be noted that the crude product is obtained in quantitative yield and can be used in the subsequent steps without further purification, if desired.

2.2. Preparation of *N*-Boc-(2*S*,4*S*,5*R*)-bulgecinine

Following the method of Ohfune,[3] treatment of (*S*)-**2** with *N*-bromosuccinimide (NBS) gave the desired bromo-γ-lactone (2*S*,4*S*,5*S*)-**3** and its (2*S*,4*R*,5*S*) diastereoisomer as a solid in an 8.3:1.7 ratio (by ^1H NMR), after flash column silica chromatography (Scheme 2). A slurry of this solid mixture in ethyl acetate/heptane at room temperature removed the (2*S*,4*R*,5*S*) diastereoisomer impurity to give enantiopure[12] (2*S*,4*S*,5*S*)-**3**[13] in 48% overall yield (with respect to *S*-**(2)**). In our hands, deprotection of the *N*-Boc lactone with trifluoroacetic acid according to Ref. 4 gave a mixture of compounds. However, pleasingly, treatment of the *N*-Boc lactone with *p*-toluenesulfonic acid in EtOAc at room temperature precipitated the pure amine tosylate salt in 76% isolated yield. Cyclisation to give the water-soluble (−)-bulgecinine was then accomplished with aqueous lithium hydroxide at pH 9. Laborious isolation of the bulgecinine using ion exchange chromatography[4] was avoided by forming the *N*-Boc derivative in situ, which was easily isolated via extraction into EtOAc to give multigram quantities of *N*-Boc-(2*S*,4*S*,5*R*)-bulgecinine (*N*-Boc-(2*S*,4*S*,5*R*)-**1**)[14], 65% over two steps with 97% ee[15] and >95% de.[16]

The *N*-Boc-(2*R*,4*R*,5*S*)-bulgecinine isomer [*N*-Boc-(2*R*,4*R*,5*S*)-**1**] was prepared from the *Z*-(2*R*)-*tert*-butoxycarbonylamino-6-hydroxyhex-4-enoic acid (*R*)-**2** in an identical manner.

3. Conclusion

We have demonstrated an efficient scaleable route to both isomers of *Z*-2-*tert*-butoxycarbonylamino-6-hydroxyhex-4-enoic acid 2 from butyne-1,4-diol, utilizing L- and D-acylase enzymes to supply (*S*)- and (*R*)-alkynyl amino acid **4** in a 'one-pot' reaction. These intermediates were readily converted to *N*-Boc-(2*S*,4*S*,5*R*)- and *N*-Boc-(2*R*,4*R*,5*S*)-bulgecinine using a key electrophilic bromolactonisation, allowing multigram quantities of these complex scaffolds to be prepared.

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8. *Experimental for biotransformations*: **9** (125 g, 0.67 mol) in 30 mM KH_2PO_4 (1.7 L) at pH 7 was treated with

L-acylase solution (200 U/g substrate) and the solution stirred at 65°C and pH 7 until ^{1}H NMR of an evaporated sample showed >40% conversion (44 h). Boc_2O (47 g, 0.27 mol) in THF (200 mL) was then added at rt, and the reaction maintained at pH 10 until the pH was static. The mixture was extracted with MTBE (500 mL), then the remaining aqueous acidified to pH 3 with $KHSO_4$ and extracted with EtOAc (3×1 L). The EtOAc portions were combined, dried ($MgSO_4$), filtered and evaporated in vacuo to give 44 g, 71% theoretical, *Z*-(2*S*)-*tert*-butoxycarbonylamino-6-hydroxyhex-4-ynoic acid (*S*)-**4**, 98% ee. The remaining aqueous solution from above was then adjusted to pH 8 with 48% NaOH, and D-acylase solution added (40 U/g substrate). The reaction mixture was stirred at rt and pH maintained at pH 8 until the reaction was complete by ^{1}H NMR (21 h). The pH was then adjusted to 10 and the mixture treated with Boc_2O (54 g, 0.31 mol) and worked up as above to give 48 g, 68% theoretical, *Z*-(2*R*)-*tert*-butoxycarbonyl-6-hydroxyhex-4-ynoic acid (*R*)-**4**, 95% ee.

9. Chiral HPLC conditions for separation of amino acid isomers: D-penicillamine (150×4.6 mm); 100% 2 mM $CuSO_4$; 0.15 ml/min; 254 nm; (*S*)-isomer 17.2 min, (*R*)-isomer 19.1 min.
10. ^{1}H NMR (DMSO-d_6) for compound **4** was consistent with the structure. However, this compound appears to be relatively unstable and was used without further purification.
11. Pelter, A.; Ward, R. S.; Little, G. M. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2775.
12. Chiral HPLC conditions: Chiralpak AD (250×4.6 mm); 15% IPA, 85% heptane; 1 ml/min; 220 nm; (*R*,*R*,*R*) and (*S*,*R*,*S*) isomers 10 min, (*R*,*S*,*R*) isomer 13.5 min, (*S*,*S*,*S*) isomer 15.0 min.
13. Optical rotation for (2*S*,4*S*,5*S*)-**3**: $[\alpha]_D = +41$ (*c* 0.8, MeOH) cf. Ref. 3: $[\alpha]_D = +44.7$ (*c* 0.78, MeOH) for the (2*S*,4*S*,5*S*)-isomer.
14. Selected data for *N*-Boc-(2*S*,4*S*,5*R*)-**1**: ^{1}H NMR (DMSO): δ 1.5 (s, 9H, $(CH_3)_3$), 1.7 (m, 1H, CH_aH_bCHOH), 1.95 (m, 1H, CH_aH_bCHOH), 3.75 (m, 2H, CH_2OH), 3.85 (m, 1H, $CHCOOH$), 4.05 (m, 1H, $CHCH_2OH$), 4.16 (m, 1H, $CHOH$), 5.2 (t, 1H, $J=3$ Hz, CH_2OH), 7.1 (d, 1H, $J=9$ Hz, $CHOH$); TOF-MS ES $(M-H^+)=260.1145$, $C_{11}H_{18}NO_6$ requires 260.1134; $[\alpha]_D^{25} = -13.2$ (*c* 1, MeOH); e.e. = 97%.
15. Chiral HPLC conditions: Chiralpak AD (250×4.6 mm); 20% IPA, 0.1% TFA, 79.9% heptane; 1 ml/min; 210 nm; (2*R*,4*R*,5*S*) isomer 5.8 min, (2*S*,4*S*,5*R*) isomer 7.8 min.
16. Determined by ^{1}H NMR (DMSO-d_6).