Pergamon

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Tetrahedron Letters 44 (2003) 973–976

TETRAHEDRON LETTERS

Convenient syntheses of (3*S*,5*S*)-carbapenam-3-carboxylates and their biosynthetic relevance

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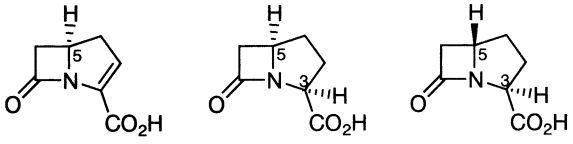
Received 8 November 2002; accepted 29 November 2002

Abstract—Starting from readily available glutamate derivatives, facile stereoselective syntheses of (3*S*,5*S*)-carbapenam-3-carboxylates have been developed. Their significance in confirming the absolute configuration of the natural material is discussed. © 2003 Published by Elsevier Science Ltd.

Some years ago we reported the isolation, from various bacterial species of *Erwinia* and *Serratia*, of the known antibiotic, (*R*)-carbapen-2-em-3-carboxylic acid **1** together with both the *cis*- and *trans*-isomers of carbapenam-3-carboxylic acid.[1] The *cis*-isomer **2** was assigned the (3*S*,5*R*) configuration by direct chemical correlation with **1**, the chirality of which had previously been established.[2] The major *trans*-isomer which was obviously epimeric with **2** at either C-3 or C-5 was provisionally assigned the (3*R*,5*R*) configuration, not least because all naturally occurring carbapenem antibiotics known at the time possessed the *R*-configuration at the ring junction.[3] However, synthesis of the *trans*-isomer starting from D-glutamic acid, to confirm the assigned *R*-stereochemistry at C-3, followed by direct comparison of the circular dichroism (CD) plots of the *p*-nitrobenzyl (PNB) esters of both the synthetic and natural material surprisingly revealed a mirror image relationship.[4] It followed therefore that the natural *trans*-isomer possessed the unique (3*S*,5*S*) configuration **3a**.



1 **2**

3a R = H
3b R = Bn
3c R = Me
3d R = PNB

This observation has been particularly relevant to our subsequent extensive biosynthetic studies. Initially we established that **1**, **2** and **3a** were all derived from an acetate unit, or an equivalent, and L-glutamate.[5] The genes encoding the elements required for their production have been mapped, sequenced and analysed.[6,7] Those designated *Car* A–E within the cassette were shown to be essential to the pathway and we[8] and others[9] have proposed that the products of these genes mediate the unusual sequence outlined in Scheme 1. Intermediate **3a**, the product of the CarA protein, upon derivatisation to its PNB ester was shown to be identical by ^{1}H NMR spectroscopy to that synthesised from L-glutamate.[9] However the chirality of the natural material was not definitively established but inferred on the basis of our original data for **3d**.

Recently the assignment of absolute configuration to **3a** has been questioned by Tanaka and co-workers.[10] This was based on their lengthy synthesis of the methyl ester **3c** and a comparison of its $[\alpha]_D$ value with that of our reported synthetic material but not with the *p*-nitrobenzyl ester derived from natural sources. While we were confident that our assignment based on the CD data described above was correct, the importance with respect to the biosynthetic proposals required that this discrepancy was resolved. In relation to our ongoing interest in the biosynthetic pathways for carbapenem antibiotics, we have already adapted our original synthesis to provide a more versatile and efficient procedure. We now report its application for the diastereoselective syntheses of **3a–d** from L-glutamate, which unequivocally confirms our original assignment and resolves the discrepancy.

Keywords: carbapenam-3-carboxylate; biosynthesis; absolute configuration; β-lactam; tris(1,3-dihydro-2-benzoxazolin-3-yl)phosphine oxide.

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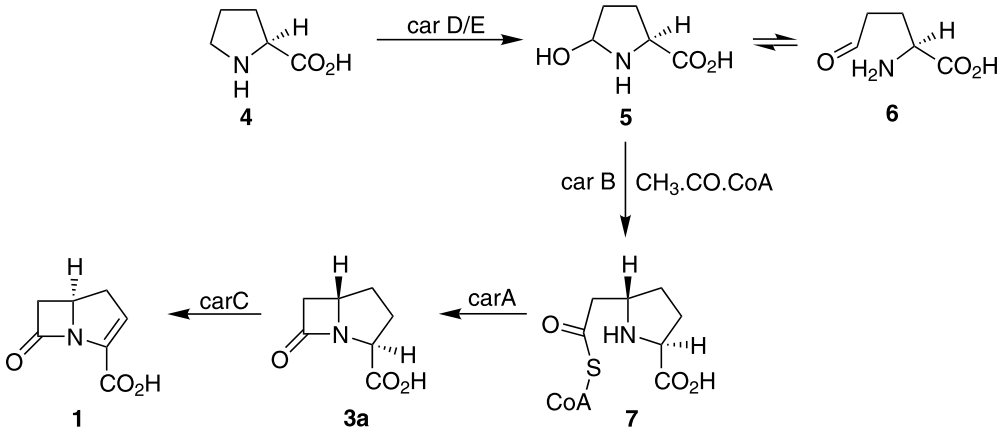
0040-4039/03/$ - see front matter © 2003 Published by Elsevier Science Ltd.
PII: S0040-4039(02)02731-4



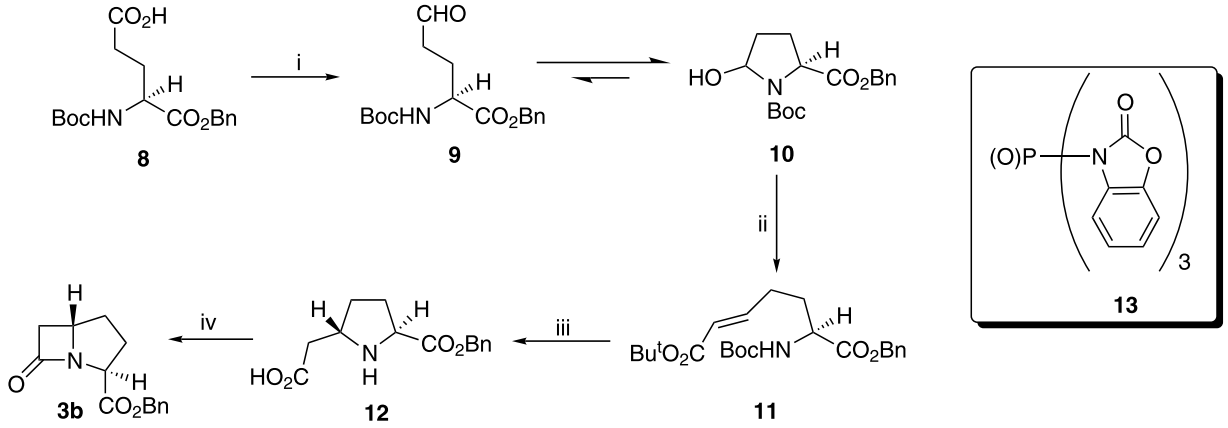
Scheme 1. Proposed biosynthetic pathway to (*R*)-carbapen-2-em-3-carboxylic acid **1**.

As shown in Scheme 2, aldehyde **9**, which exists predominantly as the protected cyclic hemi-aminal **10**, was readily prepared in good yield from the corresponding protected L-glutamate derivative **8** using the reductive conditions previously described.[3] Wittig olefination using *t*-butyl (triphenylphosphoranylidene)acetate afforded the *trans*-alkene **11**. Following removal of the *t*-butyl and Boc protecting groups and subsequent treatment with base, the product cyclised to give almost exclusively the *trans*-pyrrolidine derivative **12** in approximately 50% overall yield from **8**.

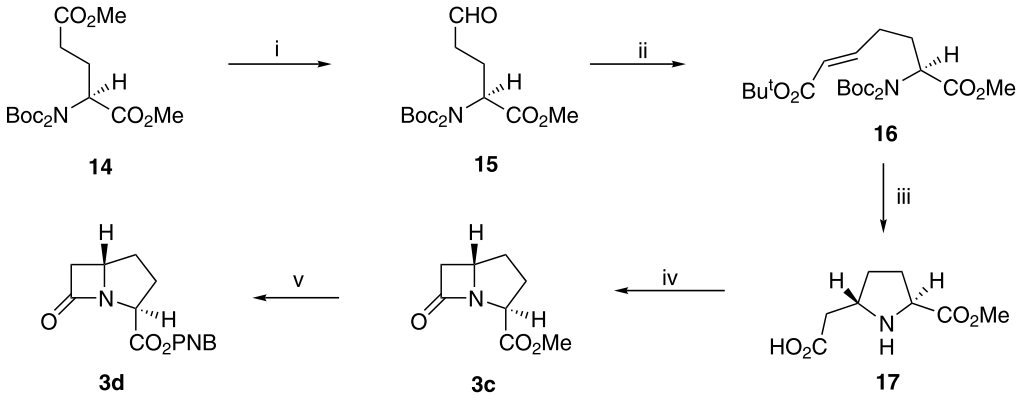
Earlier studies by others and ourselves used Mukaiyama's reagent to afford esters of **3a** from the corresponding β-amino acid, e.g. **12**, albeit in poor yield. We now report that after evaluating a range of other reagents, tris(1,3 - dihydro - 2 - oxobenzoxazolin - 3 - yl)phosphine oxide[11] **13** has proved to be the only method by which **3b**[12] can be obtained in acceptable yields (up to 70%). The reagent **13** had previously been shown to cyclise β-amino acids to the corresponding β-lactams and had been reported for the cyclisation of the racemic *cis*-isomer of **12**.[13]

While Scheme 2 provides ready access to esters such as **3b**, surprisingly it has not been possible via hydrogenation conditions to obtain the corresponding free acid **3a**. The apparent instability of **3a** to these mild conditions has also been noted by others for related carbapenam-3-carboxylic acids.[14] For our biosynthetic studies we have only been able to generate aqueous solutions of **3a** via enzymatic hydrolysis of the corresponding esters, preferably **3c**. This led to a modification of the initial step of the synthesis, which is shown in Scheme 3.

Dimethyl *N*,*N*-bisBoc-protected L-glutamate **14** was prepared from commercially available L-glutamic acid dimethyl ester hydrochloride as previously reported.[15] DIBAL-H reduction of **14** afforded aldehyde **15** in high yield, which was then transformed into the desired methyl (3*S*,5*S*)-1-carbapenam-3-carboxylate **3c** in good yield as described earlier.[16] Enzymatic hydrolysis of **3c** using pig liver esterase (PLE) on Eupergit® afforded **3a**, its presence in solution being confirmed by treatment with 4-nitrobenzyl bromide followed by the isolation and purification of *p*-nitrobenzyl (3*S*,5*S*)-1-carbapenam-3-carboxylate **3d**.[17] The CD curve obtained from **3d** was superimposable on that of the PNB ester of the material derived from natural sources ($\Delta\varepsilon$ −3.46 at 230 nm), thus confirming our original assignment of the absolute configuration of **3a** found in *Erwinia* and



Scheme 2. *Reagents and conditions*: (i) $(COCl)_2$, DMF, MeCN, THF, −30°C, 1 h., then 1 M $LiAlH(O^tBu)_3$, −78°C, 1 h, 82%; (ii) $Ph_3PCHCO_2{}^tBu$, toluene, 110°C, 12 h, 86%; (iii) HCl/Et_2O, 5 h, Et_3N, THF, 48 h, 69%, 92% de; (iv) **13**, Et_3N, MeCN, 24 h, 67%.



Scheme 3. *Reagents and conditions*: (i), DIBAL-H, Et_2O, −78°C, 30 min, 85%; (ii) $Ph_3PCHCO_2{}^tBu$, toluene, 110°C, 12 h., 85%; (iii) HCl/Et_2O, 5 h, then Et_3N, THF, 48 h, 60%, 92% de; (iv) tris(2,3-dihydro-2-oxobenzoxazol-3-yl) phosphine oxide, Et_3N, MeCN, 24 h, 45%; (v) PLE immobilised on Eupergit®, phosphate buffer pH 8.0, 3.5 h; then Aliquat® 336, DCM, 5 min; then 4-nitrobenzyl bromide, DCM, 20 h.

Serratia. The (3*S*,5*S*)-methyl ester **3c**, $[\alpha]_D^{25}$ −194.0 (*c* 0.30, $CHCl_3$), obtained from the above synthesis possessed a sign of optical rotation consistent with that reported by Tanaka and co-workers[10] for the enantiomer. We have carefully checked the $[\alpha]_D$ values of the intermediates in our original communication, and all of which are correct with exception of **3c**. It appears that we inadvertently misreported the sign of rotation for the synthetic (3*R*,5*R*)-methyl ester.[4] However, this error has no impact on the assignment of the absolute stereochemistry of the naturally occurring material.

In summary, we have developed efficient enantioselective syntheses of 1-carbapenam-3-carboxylate esters and a convenient means of generating the unstable free acid in solution. CD measurement on the PNB ester confirms our original assignment of the absolute configuration for the natural material as (3*S*,5*S*). Application of the new synthetic routes for the preparation of substituted carbapenams is underway and will be reported in due course.

Acknowledgements

We would like to thank the BBSRC for a studentship to PS and Drs Mark Searle and Stephen Simpson of the School of Chemistry, University of Nottingham for CD measurements.

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12. **Benzyl (3*S*,5*S*)-1-carbapenam-3-carboxylate 3b**: mp 32–33°C; *m*/*z* (+ES) 263.9 (MNa^+ requires 264.3); $[\alpha]_D^{20}$ −202.4 (*c* 0.25, $CHCl_3$); ν_{max} (KBr): 1746 (β-lactam and ester C=O) cm^{-1}; δ_H ($CDCl_3$): 1.48–1.63 (1H, m, 1β-H), 2.17–2.39 (2H, m, 1α- and 2α-H), 2.53–2.60 (1H, m, 2β-H), 2.65 (1H, dd, *J* 15.8 and 2.1 Hz, 6β-H), 3.30 (1H, dd, *J* 15.8 and 5.0 Hz, 6α-H), 3.85–3.93 (1H, m, 5β-H), 4.48 (1H, t, *J* 7.0 Hz, 3α-H), 5.20 (2H, s, $PhCH_2$), 7.40 (5H, s, Ph); δ_C ($CDCl_3$): 31.0 (1-C), 35.5 (2-C), 42.7 (6-C), 53.1 (5-C), 59.2 (3-C), 67.0 ($PhCH_2$), 128.2, 128.4, 128.6 (phenyl C), 135.4 (benzyl *i*-C), 171.3 (ester CO), 176.2 (β-lactam CO).
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16. **Methyl (3*S*,5*S*)-1-carbapenam-3-carboxylate 3c**: mp 32–33°C; $[\alpha]_D^{25}$ −194.0 (*c* 0.30, $CHCl_3$) {lit.[10] $[\alpha]_D^{25}$ +199.1 (*c* 0.20, $CHCl_3$) for (3*R*,5*R*) enantiomer}; *m*/*z* (+ES) 170.0 (MH^+ requires 170.1); ν_{max} (KBr): 1762 (β-lactam C=O), 1735 (ester C=O) cm^{-1}; δ_H ($CDCl_3$): 1.48–1.62 (1H, m,

1β-H), 2.18–2.36 (2H, m, 1α- and 2α-H), 2.52–2.61 (1H, m, 2β-H), 2.65 (1H, dd, *J* 15.8 and 2.0 Hz, 6β-H), 3.29 (1H, dd, *J* 15.8 and 4.9 Hz, 6α-H), 3.75 (3H, s, Me), 3.84–3.90 (1H, m, 5β-H), 4.41 (1H, t, *J* 7.7 Hz, 3α-H); δ_C ($CDCl_3$): 31.1 (1-C), 35.4 (2-C), 42.6 (6-C), 52.4 (Me), 53.1 (5-C), 59.1 (3-C), 171.9 (ester C=O), 176.3 (β-lactam C=O)

17. ***p*-Nitrobenzyl (3*S*,5*S*)-1-carbapenam-3-carboxylate 3d**: δ_H ($CDCl_3$): 1.48–1.62 (1H, m, 1β-H), 2.27–2.33 (2H, m, 1α- and 2α-H), 2.59–2.71 (2H, m, 2β-H and 6β-H), 3.31 (1H, dd, *J* 15.9 and 4.8 Hz, 6α-H), 3.85–3.89 (1H, m, 5β-H), 4.49 (1H, t, *J* 7.6 Hz, 3α-H), 5.26 (2H, s, CH_2Ar), 7.52 (2H, d, *J* 8.8 Hz, Ar 2′,6′-H_2), 8.24 (2H, d, *J* 8.8 Hz, Ar 3′,5′-H_2).