Stereocontrolled Enantiospecific Synthesis of Anticapsin: Revision of the Configuration

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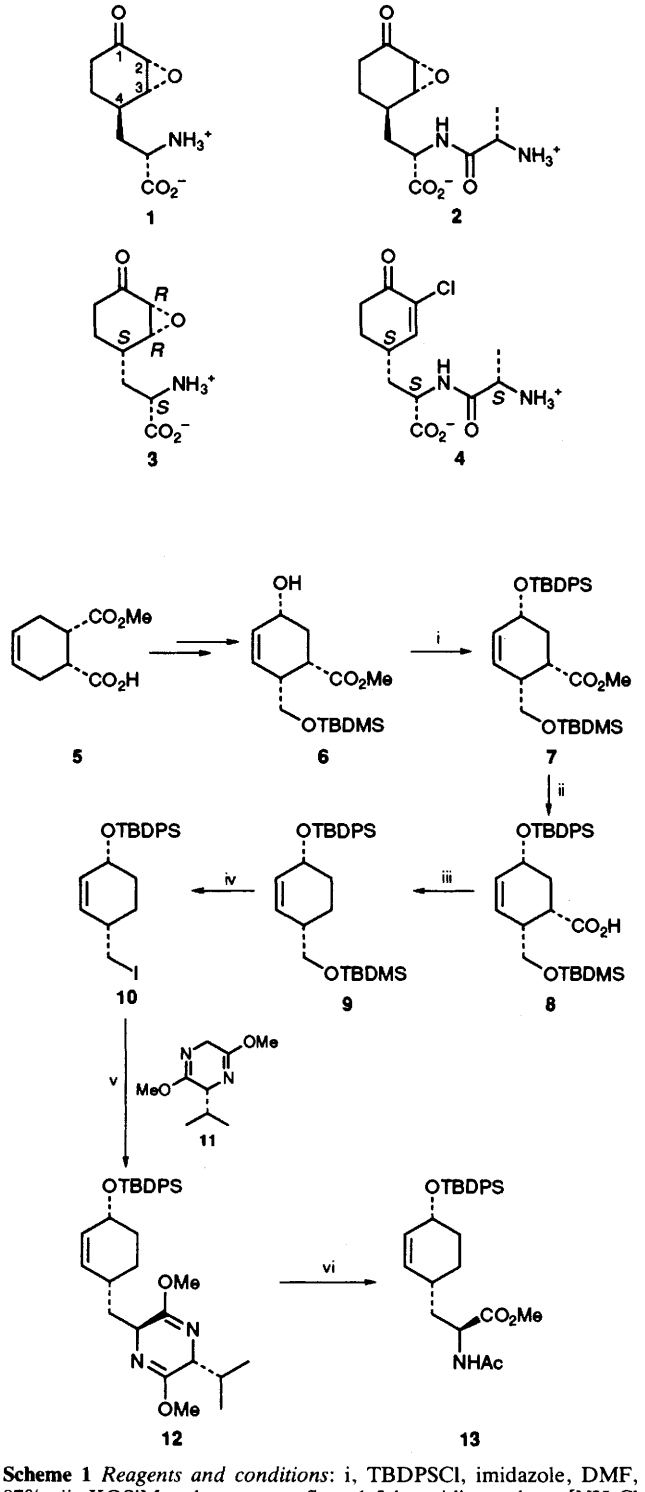
A stereocontrolled enantiospecific synthesis of anticapsin results in a revision of the C-4 configuration to that in structure **3**; the carbonyl group of anticapsin has also been observed to show a high propensity for hydration and enolisation.

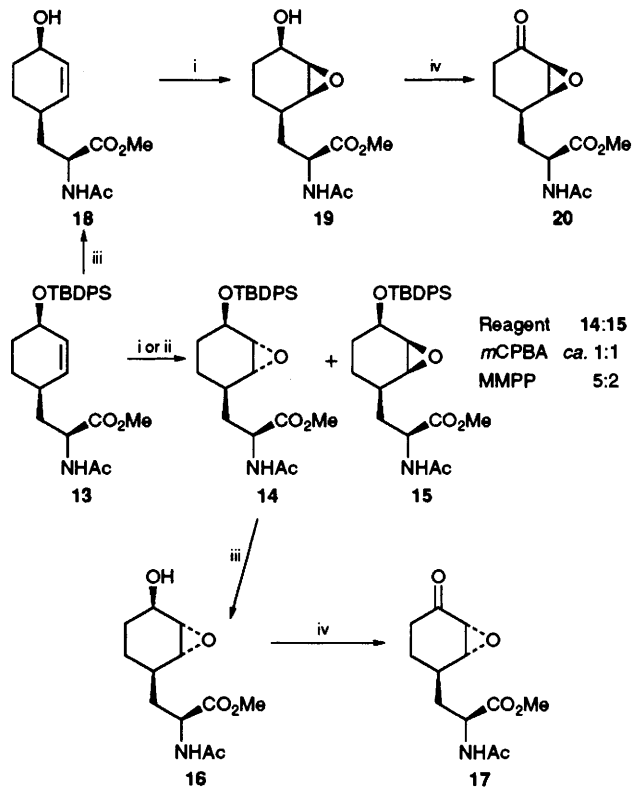
Anticapsin, a non-proteinogenic amino acid obtained from culture filtrates of *Bacillus subtilis*[1] and *Streptomyces griseoplanus*,[2] was assigned structure **1**, and is a component of the dipeptide bacilysin[1] **2**. The absolute configuration of the epoxide (C-2, C-3) was determined from ORD and CD measurements,[3] and the configuration of C-4 from coupling constants in the ^{1}H NMR spectrum.[3] Acidic hydrolysis of anticapsin afforded (*S*)-tyrosine, enabling the amino acid centre to be assigned an (*S*) configuration.[2,3] We have synthesised this structure **1** and found that it is not identical to the natural product. Further synthetic studies have shown that anticapsin is in fact structure **3**.

Previously claimed syntheses[4–6] of anticapsin suffered from a lack of stereochemical control affording mixtures of diastereoisomers, data for which were compared to literature data on the natural product, assumed to possess structure **1**. In



Scheme 1 *Reagents and conditions*: i, TBDPSCl, imidazole, DMF, 97%; ii, $KOSiMe_3$, benzene, reflux, 1.5 h, acidic work-up [NH_4Cl (sat. aq. soln.)], 89% (ref. 10); iii (*a*) oxalyl chloride, DMF (cat.), toluene, −5 to 10 °C, 30 min; (*b*) sodium 2-mercaptopyridine *N*-oxide, DMAP (cat.), benzene, 30 min at room temp. followed by the addition of *tert*-dodecanethiol (5 equiv.), *hν* (200 W tungsten lamp), 20–30 °C, 1 h, 75% (ref. 11); iv, (*a*) TsOH·H_2O (cat.), THF–H_2O, 81%; (*b*) MsCl, pyridine, 91%; (*c*) NaI, acetone, reflux, 18 h, 93%; v, **11** (1 equiv.), Bu^nLi (1 equiv.), THF, −78 °C; CuCN (1 equiv.), 2 min, 0 °C then −55 °C; **10** (1 equiv.) then DMPU (2 equiv.), −55 °C, 18 h, 30% yield plus 61% recovery of unreacted electrophile **10**, vi, (*a*) 0.25 mol dm^{-3} HCl (5 equiv.), MeCN, 2 h, room temp.; (*b*) acetic anhydride, pyridine, 3 h, room temp., 64% (2 steps).

TBDPS = Bu^tPh_2Si; DMF = dimethylformamide; DMAP = 4-*N*,*N*-dimethylaminopyridine; Ts = *p*-$MeC_6H_4SO_2$; THF = tetrahydrofuran; Ms = $MeSO_2$; DPMU = 1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-2(1*H*)-one (dimethylpropylene urea); TBDMS = Bu^tMe_2Si.



Scheme 2 *Reagents and conditions*: i, *m*CPBA, $CHCl_3$; ii, MMPP, Pr^iOH–H_2O; iii, NH_4F, MeOH, 50 °C, 18 h; iv, TPAP (cat.), *N*-methylmorpholine *N*-oxide, MeCN

previous 'syntheses' no comparable specific optical rotation data for synthetic and natural material was obtained.† Our new finding is consistent with the structure revision recently reported for the related compound chlorotetaine **4**, also shown[7] to possess (*S*) rather than (*R*) configuration at C-4.

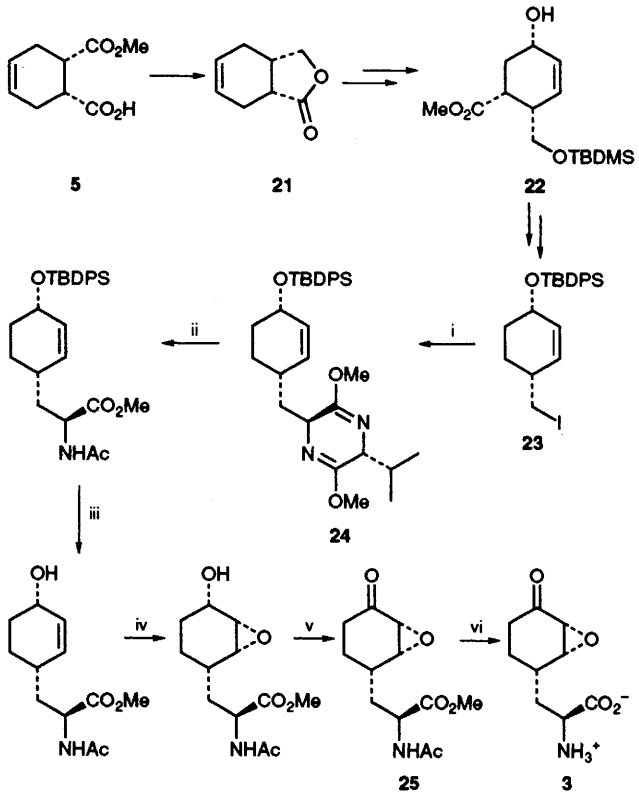
The starting point in our syntheses was chiral ester[8] **5**, which was converted to the alcohol **6** using chemistry described[9] by Ohno *et al.* (Scheme 1).

The amino acid residue was introduced by alkylation of the iodide **10** using the bislactim ether[12] **11**. The lithium azaenolate of **11** gave only 6% coupled material **12**, the main reaction being elimination (*ca.* 91%). Hence we prepared the less basic lithium cyanocuprate[13] of bislactim ether **11**. The iodide **10** was relatively unreactive towards this cuprate yielding only 30% of coupled material, but without elimination permitting recovery of 61% of unreacted **10**. The hydrolysis product **13** was epoxidised with *m*-chloroperbenzoic acid (*m*CPBA) to a 1 : 1 mixture of diastereoisomeric epoxides **14** and **15**.

We had expected steric approach control by the *tert*-butyldiphenylsilyl group (TBDPS) to give **14**;[14] however it seems that a directing effect of the amido group was operating.[15] Use of magnesium monoperoxy phthalate[16] (MMPP) in Pr^iOH–H_2O gave a 5 : 2 excess of the desired epoxide **14**, desilylated to the major product **16**, and purified by silica gel chromatography. Confirmation that **16** was the *trans*-epoxide was obtained when alcohol **18** was subjected to directed epoxidation[17] using *m*CPBA–chloroform affording exclusively epoxide **19** (Scheme 2).

Oxidation of **16** with the TPAP (tetrapropylammonium perruthenate) reagent[18] afforded *N*-acetyl methyl ester **17**,

† Natural material $[\alpha]_D^{25}$ +125 (*c* 1, H_2O)[2] *cf.* Ganem *et al.* $[\alpha]_D^{25}$ +4 (*c* 0.2, H_2O) for synthetic material and +21 (*c* 0.2, H_2O) for natural material;[6] Souchet *et al.* $[\alpha]_D^{20}$ +25 (*c* 0.2, H_2O) for synthetic material;[5] Rickards *et al.*: 'Comparison of CD spectra of synthetic and authentic anticapsin indicated a content of 87% of the natural enantiomer'.[4]



Scheme 3 *Reagents and conditions*: i, **11** (2 equiv.), Bu^{n}Li (2 equiv.), THF, −78 °C; CuCN (1 equiv.), 2 min at 0 °C then −21 °C; **22** (1 equiv.), −21 °C, 24 h, 71%; ii, (*a*) 0.25 mol dm^{-3} HCl (5 equiv.), MeCN, 2 h, room temp.; (*b*) acetic anhydride, pyridine, 3 h, room temp., 60% (2 steps); iii, NH_4F, MeOH, 50 °C, 18 h, 88%; iv, *m*CPBA, $CHCl_3$, 86%; v, TPAP (cat.), *N*-methylmorpholine *N*-oxide, MeCN, 89%; vi, (*a*) pronase E, phosphate buffer (≈2 : 3 ratio of 0.1 mol dm^{-3} and KD_2PO_4 and 0.1 mol dm^{-3} Na_2DPO_4 in D_2O), pH 7.5, 30 °C, 3 h; (*b*) acylase I from *Aspergillus sp.* immobilised on Eupergrit C, phosphate buffer (≈2 : 3 ratio of 0.1 mol dm^{-3} KD_2PO_4 and 0.1 mol dm^{-3} Na_2DPO_4 in D_2O), pH 7.5, 30 °C, 30 h, then cellulose chromatography (80% aqueous propan-2-ol as eluent), 80% (2 steps)

which did not have NMR characteristics‡ consistent with those reported for anticapsin *N*-acetyl methyl ester obtained from the natural material.[4] The diastereoisomeric structure **20** prepared similarly also did not have the expected NMR characteristics.‡ Combining these results with NMR data previously published[4] by Rickards for all the possible diastereoisomers we deduced that anticapsin must have structure **3**.

Access to **3** was achieved by way of the chiral ester **5**, converted *via* lactone[19] **21** to alcohol **22** (the enantiomer of **6**) and subsequently transformed to the iodide **23** by a sequence completely analogous to that in Scheme 1 (Scheme 3). Alkylation of **23** with the previously described lower order bislactim ether lithium cyanocuprate proceeded in very low yield; however the corresponding higher order lithium cyanocuprate afforded the coupled product **24** in excellent yield (71%) along with a small amount of eliminated material (12%). The bislactim ether **24** was converted to **25**, which had NMR data identical with that reported for anticapsin *N*-acetyl methyl ester.[4] A key stereochemical feature of this sequence

‡ Selected ^{1} NMR data: **17** δ_H (200 MHz, $CDCl_3$) 3.55 (dd, *J* 2, 4 Hz), 3.23 (d, *J* 4 Hz); **20** δ_H (500 MHz, $CDCl_3$) 3.71 (d, *J* 4 Hz), 3.25 (d, *J* 4 Hz).

Authentic anticapsin *N*-acetyl methyl ester:[4] δ_H ($CDCl_3$) 3.41 (d, *J* 4 Hz), 3.22 (d, *J* 4 Hz).

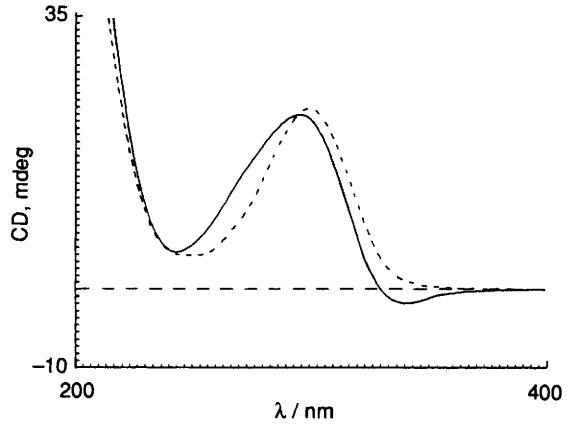


Fig. 1 CD spectra: —— natural anticapsin; - - - - synthetic anticapsin

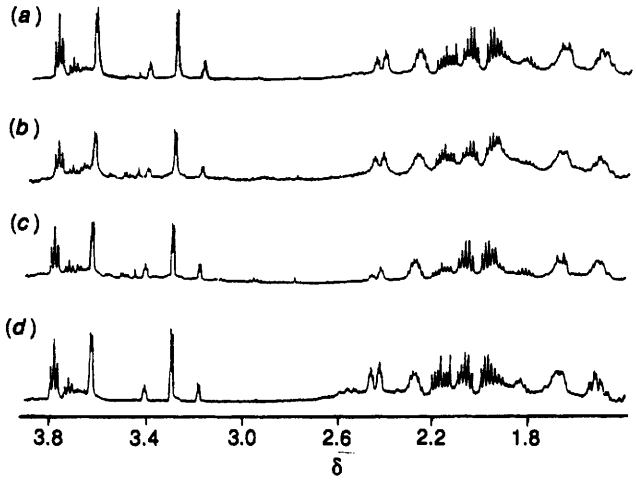


Fig. 2 500 MHz ^{1}H NMR spectra: (*a*) doped spectrum (natural and synthetic); (*b*) synthetic anticapsin (after freeze-drying from H_2O at pH 7.5); (*c*) synthetic anticapsin (after step vi, Scheme 3); (*d*) natural anticapsin

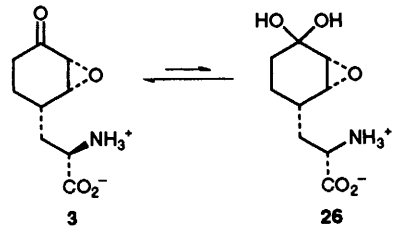


Fig. 3 Anticapsin hydrate **26**

was a *cis*-directed epoxidation (step iv, Scheme 3). Deprotection of **25** was achieved by the sequential application of the enzymes pronase E[20] and acylase I[21] from *Aspergillus sp.* The synthetic anticapsin **3** had spectroscopic data§ (^{1}H NMR, IR, MS, CD and $[\alpha]_D$) consistent with natural anticapsin obtained from Eli Lilly and Co. The positive Cotton effect observed in the CD spectrum (Fig. 1) is indicative of the epoxide configuration depicted in structure **3** on the basis of the reverse octant rule.[22]

§ Specific optical rotation data: natural anticapsin (in our hands) $[\alpha]_D^{20}$ +51 (*c* 0.1, H_2O); synthetic anticapsin $[\alpha]_D^{20}$ +45 (*c* 0.1, H_2O). The minor differences in synthetic and natural material CD and $[\alpha]_D$ values may be due to contaminants associated with the natural product consistent with additional peaks in the ^{1}H 500 MHz NMR spectrum of natural material at for example δ 2.50–2.62 and 3.97–4.18.

A comparison of synthetic and natural anticapsin by 500 MHz ^{1}H NMR spectroscopy is illustrated in Fig. 2. A feature which has not previously been reported is the appearance of minor signals at δ 3.72 (t, *J* 7.0 Hz), consistent with an α-proton, and δ 3.41 (*ca.* t, *J* 4.0 Hz) and 3.18 (d, *J* 4.0 Hz), consistent with epoxide protons. We believe these resonances are due to the hydrate **26** which would be in equilibrium with anticapsin **3** in aqueous solution ¶ (Fig. 3). That this ketone is also highly enolisable is shown by the deuteriation of the adjacent methylene group (δ 2.44, 2.16) in D_2O at pH 7.5 (Fig. 2, spectrum *C*).

The above results require revision of the previously reported structure **1** of anticapsin to **3**. A similar revision of the structure of bacilysin is implicit. Anticapsin inhibits glucosamine-6-phosphate synthetase and hence chitin biosynthesis. It has been suggested that anticapsin is a glutamine analogue[23] which binds covalently to the active site thiol of these amidotransferases.[24] Our new configurational assignment and observation on the hydration characteristics of the ketone group may be helpful in understanding the precise mechanism of this inhibition.

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¶ Further evidence in support of the hydrate is based upon an NMR study of anticapsin *N*-acetyl methyl ester **25**. Using $CDCl_3$ as solvent, the 500 MHz ^{1}H NMR consists of two doublets in the epoxide region and a single α-proton. On changing the solvent to D_2O high-field satellite peaks of the α and epoxide protons [δ_H 4.44 (dd, *J* 5.0, 10.0 Hz) and 3.36 (*ca.* t, *J* 4.0 Hz), 3.20 (d, *J* 4.0 Hz)] of analogous intensity to those seen for the free amino acid **3** were observed. The presence of the hydrate was confirmed by a signal at δ 92.4 in the 125 MHz ^{13}C NMR spectrum of **25** taken in D_2O.