Synthesis, X-ray crystal structures and biological evaluation of some mono- and bi-cyclic 1,3-diazetidin-2-ones: non-natural β-lactam analogues

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Mono- and bi-cyclic 1,3-diazetidin-2-ones (aza-β-lactams) are synthesised and evaluated as non-natural analogues of β-lactams. The aza-β-lactams are designed on the principle that their reaction with active site serine hydroxy will form a carbamoyl–enzyme intermediate that is sluggish to hydrolysis. The synthesis of racemic mono- and bi-cyclic aza-β-lactams is carried out starting from pyrimidinone 18 which is transformed to the densely functionalised substrate 20. The chemical reactivity of tricarbonyl 20 for selective functional group manipulation was first assessed and then it was transformed to amino alcohol 24. Cyclisation of 24 affords aza-carbapenams and its homologation followed by aldol cyclisation provides access to aza-carbacephams. The X-ray structures of aza-carbapenam 35 and aza-carbacepham 42 suggest that the structural requirements for biological activity in β-lactams are fulfilled. An unexpected ozonolysis product, phenol 52 resolves spontaneously during crystallisation and its crystal structure was also determined. The biological activity of the novel mono- and bi-cyclic aza-β-lactams was evaluated with potent gram-positive bacterial strain, *Bacillus subtilis* and compared with β-lactam antibiotics, ampicillin and penicillin G. Of the 19 aza-β-lactams tested, eight compounds show inhibition better than the standards while another eight are of comparable activity. This study shows that aza-β-lactams represent a novel and non-natural lead towards serine peptidase inhibitors.

1 Introduction

The β-lactam antibiotics have been in widespread use as chemotherapeutic agents to treat diverse bacterial infections and microbial diseases.[1] Despite the fact that there are about 150 β-lactam antibiotics in the market today, the threat that microbes are able to neutralise them is a major and growing concern to medicinal chemists and clinicians.[2] One of the reasons cited for the spread of antibiotic resistance is the lack of diversity in the chemical structures of β-lactam drugs. The β-lactam ring is the common moiety in penicillins, cephalosporins, thienamycins and related antimicrobial agents.[3] These antibiotics are close structural analogues of naturally occurring β-lactams and this could be one of the reasons that microbes in the air and soil have found ways to counter them.[4] The extensive use of classical β-lactam antibiotics in medicine has given rise to an increasing number of resistant bacterial strains through mutation and β-lactamase gene transfer. In recent years there has been a keen interest in the design, synthesis and biological testing of novel structural skeletons which will target the penicillin binding proteins (PBPs) and also overcome the defense mechanisms of bacteria. Non-traditional β-lactams are a possible solution to the problem of bacterial resistance. These compounds are non-natural analogues of penicillins and cephalosporins that contain a different four- or five-membered heterocycle in place of the usual 2-azetidin-1-one ring. Several research groups have investigated the synthesis and biological effects of such compounds to inhibit the β-lactamases, and these efforts have been summarised in an excellent review.[5]

The γ-lactam analogues **1–3** (Fig. 1), synthesised by Baldwin and co-workers,[6] exhibit weak antibacterial activity against both gram-positive and gram-negative organisms. Mono- and bi-cyclic 1,2-diazetidin-3-ones, such as **4** and **5** were found to be ineffective against representative strains of bacteria (MIC > 128 μM) but the sulfonyl derivative **6** displayed some

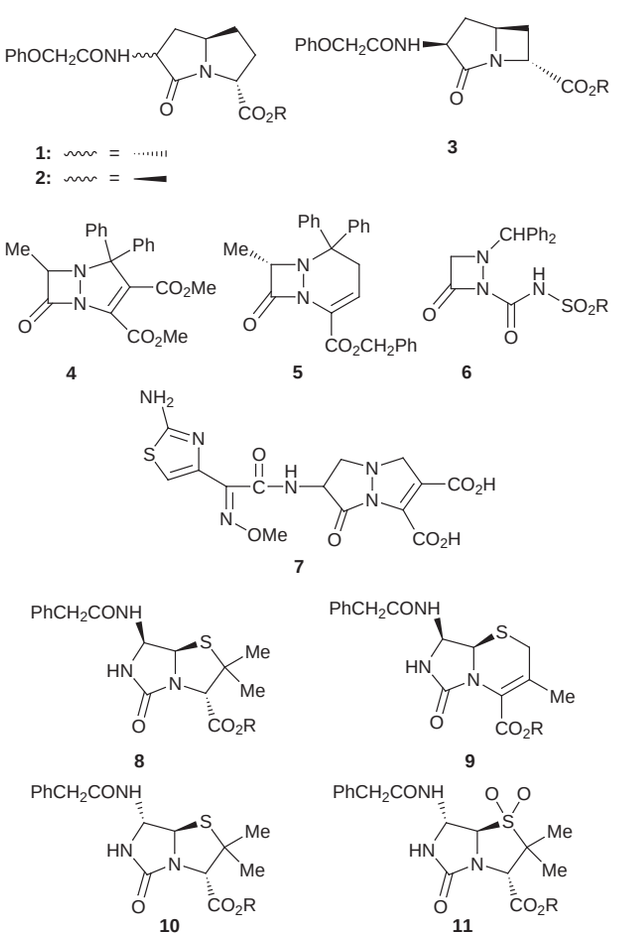


Fig. 1 Structures of some non-traditional β-lactam analogues

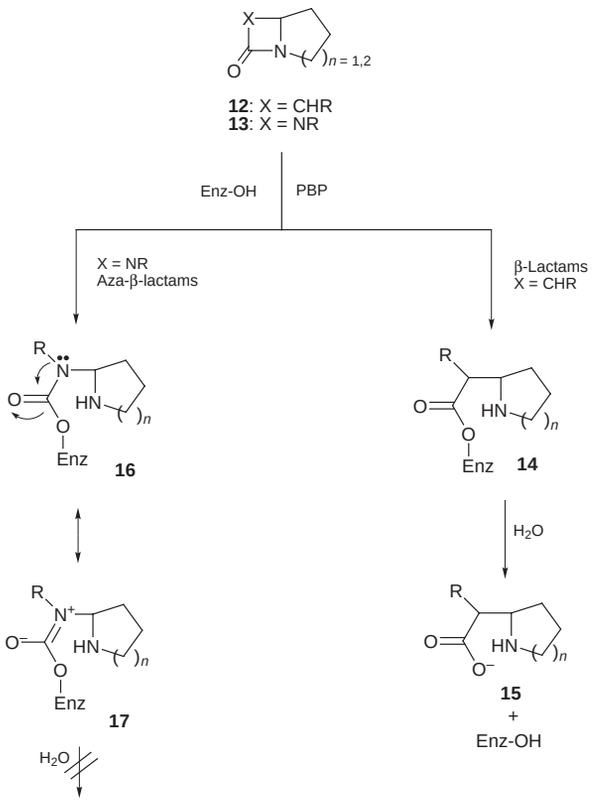


Fig. 2 Mechanism for the inactivation of PBP enzyme by aza-β-lactams

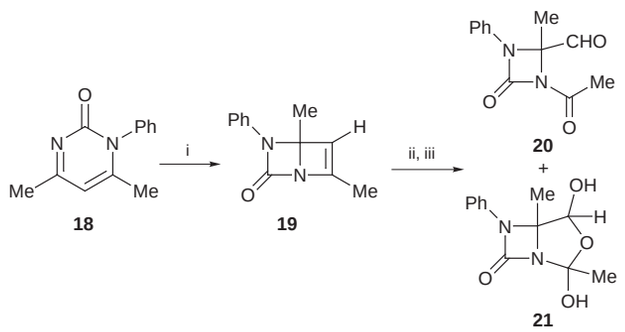
antifungal activity.[7] Among the pyrazolidinone containing compounds the carbapenem analogue **7**, prepared by the Eli Lilly group,[8] showed good binding to bacterial PBPs and exhibited *in vitro* and *in vivo* antibacterial properties at useful levels. Bicyclic imidazolidinones **8–10** were synthesised by a Lossen rearrangement on clinically active penicillins and cephalosporins and tested as antibacterial agents and β-lactamase inhibitors. Although imidazolidinones **8–10** were devoid of antimicrobial activity, the sulfone **11** showed 30–60% β-lactamase inhibition at 1 mM concentration.[9]

In this paper we report the synthesis of molecules that belong to the 1,3-diazetidin-2-one (aza-β-lactam) structural type as non-natural analogues of β-lactams.[10] The X-ray crystal structure and bioassays on selected compounds suggest that aza-β-lactams are potential serine peptidase inhibitors. The rationale for the design of aza-β-lactams as transpeptidase and β-lactamase inhibitors has been proposed earlier by us.[11] Thus, attack of active site Ser-OH on the aza-β-lactam nucleus **13** should lead to carbamoyl–enzyme intermediate **16** which is stabilised by partial amino-donation (Fig. 2). The resonance stabilisation in intermediate **17** will retard the normal deacylation pathway and provide hydrolytic stability to aza-β-lactams thereby making them less susceptible to the destructive action of β-lactamases. Such a stabilisation is not available on the acyl–enzyme intermediate **14** derived from the natural β-lactams **12**, and its hydrolysis to inactive **15** renders the antibiotic ineffective. Ghosez and co-workers evaluated the 1,3-imidazolidinones **8–11** for a similar reason[9] but found the molecules to be inactive, presumably because the topological changes from a four- to a five-membered heterocycle are too severe for effective drug–receptor recognition.[2c] The aza-β-lactam analogues **13** are closer structural mimics of β-lactams and also benefit from resonance stabilisation in the carbamoyl–PBP intermediate. Moreover, being non-natural they should be less vulnerable to the defence strategies of microorganisms.[4]

2 Results and discussion

2.1 Synthesis

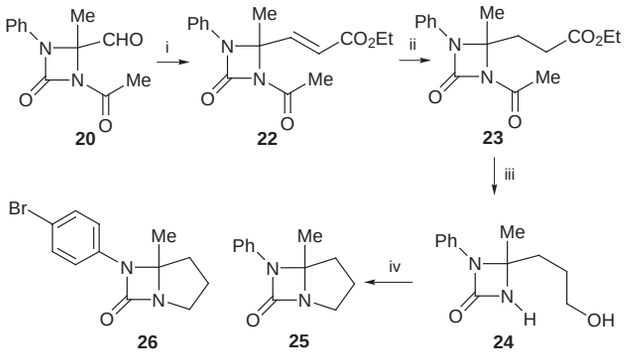
Photolysis of 1-phenyl-4,6-dimethylpyrimidin-2-one **18** afforded 3-phenyl-4,6-dimethyl-2-oxo-1,3-diazabicyclo[2.2.0]-hex-5-ene **19** in 42% yield after silica gel chromatography.[12] Ozonolysis and reductive work-up with Me_2S provided an easily separable >4:1 mixture of aldehyde **20** and acetal **21** (Scheme 1). The isolated yield of acetal **21** was significantly



Scheme 1 *Reagents and conditions*: i, *hν*, λ > 300 nm, PhH, 3 h, 46%; ii, O_3, CH_2Cl_2, −78 °C; iii, Me_2S (69%) or Et_3N

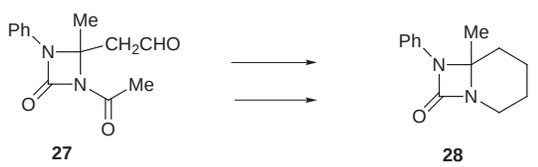
improved (1:1) when the ozonide was treated with Et_3N[13] instead of Me_2S. Of the four possible diastereomers of **21**, three were isolated in pure form and characterised by their acetal C–H singlets at δ 5.85, 5.43 and 4.98. The three carbonyl groups in **20** are chemically distinct and these were identified by their different IR stretching frequencies at 1807 cm^{-1} (urea), 1742 cm^{-1} (acetamide) and 1690 cm^{-1} (aldehyde). Heterocycle **20** is a densely functionalised aza-β-lactam and serves a useful synthon for the synthesis of racemic aza-β-lactams.[11c] Furthermore, differences in the chemical reactivity of aldehyde, amide and diazetidinone groups in **20** should permit selective transformations for elaboration to the target substrates.

The first target was the bicyclic aza-carbapenam **25** (Scheme 2). Treatment of tricarbonyl **20** with stabilised phosphorane or phosphonate reagent [$Ph_3P{=}CH{-}CO_2Et$, $(EtO)_2P(O)$-CH_2CO_2Et–NaH] afforded the *trans*-ester **22** after condensation with the aldehyde group. The reaction was not complicated by competition from the amide and urea carbonyl groups. The unsaturated ester was hydrogenated to **23** and reduced with $LiAlH_4$ at 0 °C. Under the standard ester group reduction conditions, the *N*-acetyl group was cleaved off to afford amino alcohol **24** directly. The concomitant deacetylation proved beneficial in the subsequent step. Exposure of amino alcohol **24** to Mitsunobu conditions[14] cleanly furnished the desired bicyclic aza-β-lactam **25**, an analogue of carbapenam antibiotics[15] but devoid of the crucial carboxy group at C3 (penicillin numbering). The structure of **25** was established by ^1H, ^{13}C, ^1H–^1H COSY NMR,[16] IR spectra and also by comparison with a related structure.[17] In **25**, the diazetidinone carbonyl appears at 1774 cm^{-1} and the N–CH_2 diastereotopic protons exhibit well separated multiplets at δ 3.62–3.51 and 2.82–2.65. The complex couplings between the CH_2 protons were revealed in the 2D COSY spectrum. The aza-carbapenam **25** is the first compound in the bicyclic aza-β-lactam series and an examination of its X-ray crystal structure seemed desirable. However, attempts to crystallise the viscous liquid were unsuccessful and attention shifted therefore to the 4-bromophenyl derivative **26**. The same protocol was followed for the synthesis of **26** except that the hydrogenation was carried out with Pt_2O instead of Pd/C[18] since the latter catalyst leads to hydrogenolysis of the bromophenyl group. Unfortunately, the 4-bromophenyl carbapenam **26** too did not yield single crystals suitable for X-ray analysis despite repeated attempts at recrystallisation from a number of solvents.

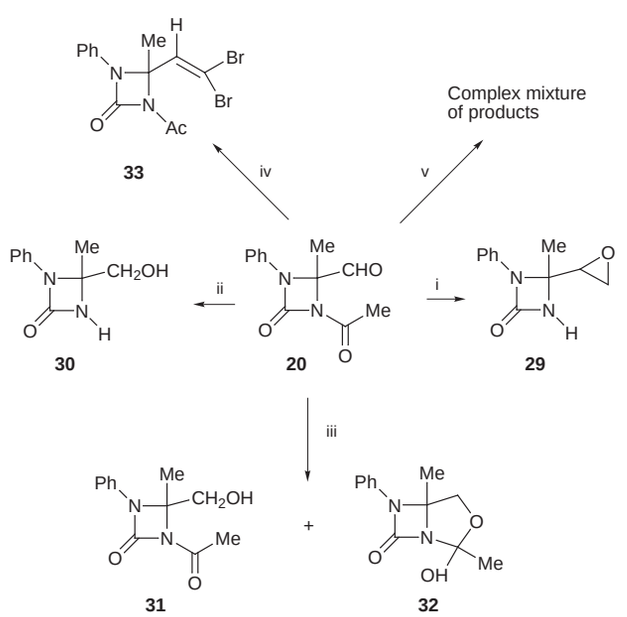


Scheme 2 *Reagents and conditions*: i, $Ph_3P{=}CHCO_2Et$, PhH, rt, 1 h, 80%; ii, H_2, 10% Pd/C, EtOAc, rt, 4 h, 99%; iii, $LiAlH_4$, Et_2O, 0 °C, 1 h, 68%; iv, Ph_3P, $EtO_2C{-}N{=}N{-}CO_2Et$, PhH, rt, 1 h, 84%

Based on the synthesis of aza-carbapenam **25** from heterocycle **20**, the synthesis of aza-carbacepham **28** from the homologated aldehyde **27** appeared to be a viable extension



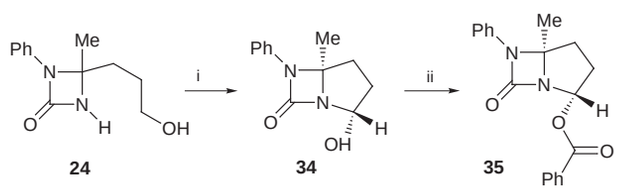
and so the synthesis of this aldehyde became our next target. Treatment of aldehyde **20** with unstabilised Wittig phosphoranes, $Ph_3P{=}CH_2$ or $Ph_3P{=}CH{-}OMe$[19] in THF afforded a complex mixture of products whose characterisation suggested that the diazetidinone ring was cleaved during the reaction. Related efforts at homologation of aldehyde **20** with Corey–Chaykovsky reagents, $Me_2S{=}CH_2$ and $Me_2S(O){=}CH_2$ gave epoxide **29** (Scheme 3) with concomitant loss of acetyl group.



Scheme 3 *Reagents and conditions*: i, $Me_3S^+I^-$, NaH, $Me_2S{=}O$, −10 to 0 °C, 40 min, 30%; ii, $LiAlH_4$, Et_2O, 0 °C, 1 h, 29%; iii, $NaBH_4$, MeOH, 0 °C, 1 h, 44%; iv, Ph_3P, CBr_4, CH_2Cl_2, 0 °C, 10 min, 35%; v, $Ph_3P{=}CH_2$ or $Ph_3P{=}CH{-}OMe$, THF, 0 °C

Attempted isomerisation of the epoxide to the aldehyde **27** was unsuccessful.[20] Though the above experiments did not advance the synthesis towards the target molecule **28**, they provided a better understanding of the chemical reactivity of the 1,3-diazetidin-2-one ring, a heterocyclic system that has been synthesised earlier[21] but its reactions not well-studied. The above studies suggest that: (i) strongly nucleophilic reagents ($Ph_3P{=}CH_2$, $Ph_3P{=}CH{-}OMe$) react indiscriminately and completely destroy the sensitive tricarbonyl molecule **20**; (ii) moderately nucleophilic reagents [$LiAlH_4$, $Me_2S{=}CH_2$, $Me_2S(O){=}CH_2$] react at the aldehyde group but also result in simultaneous deacetylation at the amide group, and; (iii) weakly nucleophilic reagents [$Ph_3P{=}CH{-}CO_2Et$, $(EtO)_2P(O){-}CH_2CO_2Et$–NaH] react at the aldehyde group selectively. The order of carbonyl reactivity in **20**, that is diazetidinone < amide < aldehyde, was further assessed by treatment of **20** with the weaker $NaBH_4$ reducing agent and milder dibromo-Wittig conditions.[22] A mixture of hydroxy amide **31** and isoclavam **32** in the former reaction and the dibromo olefin **33** in the latter case were isolated. The above observation, namely that the diazetidinone ring is the least reactive and the aldehyde group is the most reactive in heterocycle **20** guided further synthetic planning in the project.

The homologation of amino alcohol **24** was attempted next with a view to synthesise aza-carbacepham **28**. Oxidation of amino alcohol **24** with PCC afforded a product which was devoid of the expected downfield C–H resonance in the NMR spectrum and the aldehyde carbonyl stretch in the IR spectrum. The spontaneous cyclisation of the intermediate amino aldehyde afforded aminal **34** (Scheme 4) as a single diastereomer

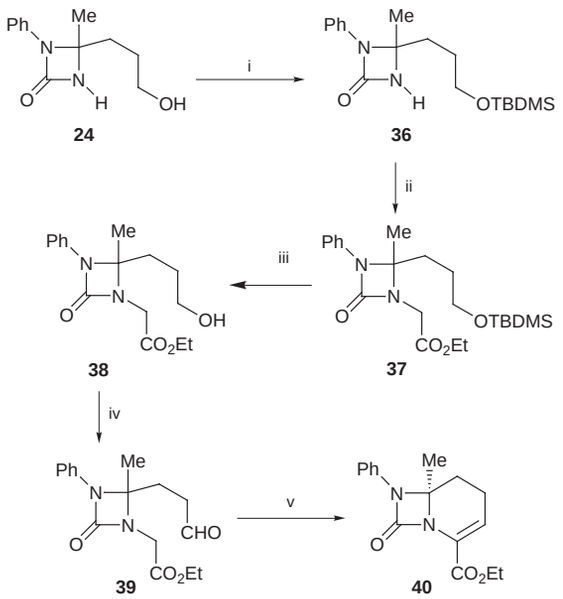


Scheme 4 *Reagents and conditions*: i, PCC, CH_2Cl_2, 0 °C to rt, 2 h, 65%; ii, PhCOCl, pyridine, CH_2Cl_2, 0 °C to rt, 2 h, 62%

after PCC oxidation.[23] The stereochemistry of **34** was assigned by its 2D NOESY spectrum in which the broad OH signal at δ 2.84 showed an NOE with the Me singlet at δ 1.85 and additionally, irradiation of the methine NCH resonance at δ 5.32 did not show enhancement of the methyl signal. The observed stereochemistry in the product results from cyclisation under equilibrium control in the acidic environment of PCC to afford the more stable *exo* oriented hydroxy group. Standard *O*-benzoylation (PhCOCl–pyridine) of the hydroxy amide **34** yielded the benzoyl derivative **35**. Fortunately, this benzoate afforded X-ray quality crystals and its crystal structure is discussed in the next section.

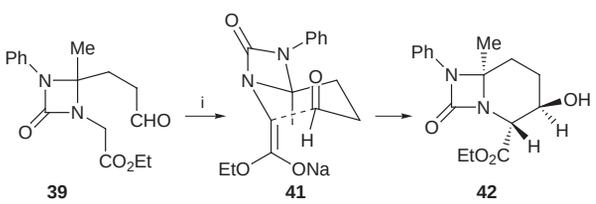
The bicyclic aza-carbapenams **25** and **34** lack the crucial carboxy group for recognition and binding between the β-lactam and the receptor protein.[2d,24] In order to fulfill the structural requirements for biological activity, the synthesis of bicyclic aza-β-lactams with a carboxy group was targeted. From the above results it is clear that the urea NH reacts intramolecularly with electrophilic groups and that it should be suitably protected. The derivatisation of the urea NH was planned in such a way that it protects the free NH and also provides a functionality for aldol cyclisation that later becomes a part of the product molecule.[25] Thus, amino alcohol **24** was transformed to aldehyde ester **39**, which was identified as an advanced precursor for the synthesis of aza-carbacephams with a carboxy group at C4 (cephalosporin numbering).[26]

In the event, selective *O*-protection of amino alcohol **24** with TBDMSCl–Et_3N produced the *O*-TBDMS ether **36**. The diazetidinone NH in **36** was deprotonated with NaH and the anion treated with $BrCH_2CO_2Et$ in THF to afford the *N*-alkylated ester **37** (Scheme 5). The homologation with acetic ester residue protects the reactive urea NH and also installs the functionalised carbon fragment required to prepare the target compound. Desilylation of **37** with TBAF produced alcohol ester **38**, which on oxidation with PCC in CH_2Cl_2 furnished aldehyde ester **39** in 55% overall yield. Intramolecular aldol



Scheme 5 *Reagents and conditions*: i, Bu′Me$_2$SiCl, Et$_3$N, DMAP (cat.), 0 °C, 1 h, 90%; ii, NaH, BrCH$_2$CO$_2$Et, THF, 0 °C to rt, 2 h, 98%; iii, Bu$^n_4$NF, THF, 0 °C, 1 h, 82%; iv, PCC, CH$_2$Cl$_2$, 0 °C to rt, 2 h, 76%; v, NaH, THF, 0 °C, 45 min, 35%

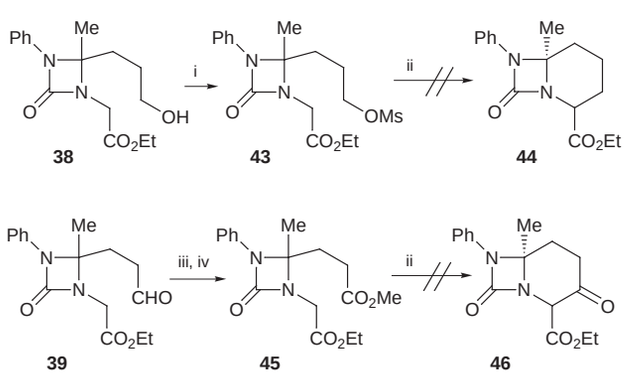
reaction of **39** with Bu′OK or NaH and dehydration of the intermediate hydroxy ester at 0 °C for 45 min furnished the desired aza-carbacephem **40**. The intermediate aldol product, β-hydroxy ester **42** was obtained as a single diastereomer when the reaction was conducted at 0 °C for a shorter duration of 20 min (Scheme 6). The stereospecificity in the aldol cyclisation



Scheme 6 *Reagents and conditions*: i, NaH, THF, 0 °C, 20 min, 60%

to afford the *anti* product exclusively is ascribed to the transition state **41** in which the aldehyde carbonyl group is axially oriented for attack by the ester enolate. The stereochemistry of the aldol product was assigned based on the 2D NOESY spectrum [16] and confirmed by the single crystal X-ray analysis of β-hydroxy ester **42**.

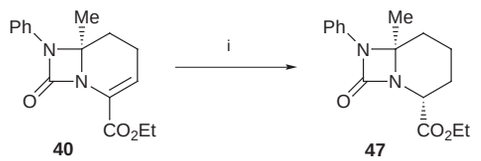
The synthesis of related aza-carbacephams **44** and **46** from mesylate ester **43** and diester **45** (Scheme 7), respectively, was attempted next. When mesylate ester **43** and diester **45** were subjected to cyclisation under basic conditions, the expected



Scheme 7 *Reagents and conditions*: i, MeSO$_2$Cl, Et$_3$N, CH$_2$Cl$_2$, 0 °C, 1 h, 95%; ii, NaH or Bu′OK, THF; iii, PDC, DMF, rt, 8 h, 94%; iv, CH$_2$N$_2$ (excess), 0 °C, 80%

products were not obtained. The failure of these cyclisations is attributed to the lower electrophilicity of the mesylate and ester groups when compared to the aldehyde for attack by the same ester enolate, shown in **41**. It is known that alkylation and acylation are less facile than aldol condensation [19a,b] and this could be the reason for the failure of related cyclisations. In view of these results, the synthesis of aza-carbacepham **44** was planned by the reduction of carbacephem **40** and that of **46** by the oxidation of hydroxy ester **42**.

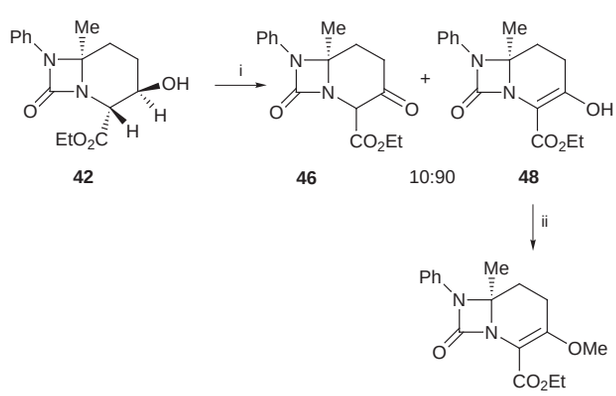
Hydrogenation of unsaturated ester **40** produced aza-carbacepham **47** (Scheme 8) as a single stereoisomer with an



Scheme 8 *Reagents and conditions*: i, H$_2$, 10% Pd/C, EtOAc, rt, 4 h, 86%

exo oriented ester group. The structure of **47** was confirmed by its 2D ^{1}H–^{1}H NOESY spectrum.[16] The presence of an NOE between the OCH$_2$ multiplet (δ 4.25) and the quaternary methyl singlet (δ 1.71) and furthermore, the absence of an NOE between the NCHCO$_2$Et resonance (δ 4.59) and the methyl group confirmed the *exo* orientation of the ester group. The observed stereochemistry in the product arises by the reduction of **40** from the *endo* face exclusively. This stereospecificity may be due to the fact that in the somewhat flat bicyclic aza-carbacepham molecule (AM1 $\Sigma N = 335$–$340°$),[11b] the *exo* oriented methyl group shields the convex face and directs hydrogenation [18] from the less hindered concave face. As a result, the carboxy group has the *exo* orientation of the natural β-lactams.

Carbacephems [27] with a C3-hydroxy group have been identified as useful precursors for the synthesis of biologically active carbacephems with various side-chains. For example, the 3-methoxy- and 3-chloro-cephalosporins with a phenylglycine side chain are orally active antimicrobial agents.[28] The hydroxy aza-carbacephem **48** appeared to be an accessible precursor for such targets in the aza-β-lactam family. Attempted oxidation of the secondary alcohol in β-hydroxy ester **42** under PCC, PDC and Swern conditions did not yield the ketone product **46**, presumably because of the proximal electron withdrawing ester and diazetidinone groups. However, oxidation with the more powerful Jones reagent at 0 °C for 1 h produced a 1:1 mixture of keto and enol isomers **46** and **48** (Scheme 9).



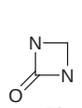
Scheme 9 *Reagents and conditions*: i, 8 M Jones reagent, 0 °C to rt, 2 h, then rt, 2 days, 88%; ii, CH$_2$N$_2$ (excess), 0 °C, 63%

When the 1:1 keto–enol mixture was left for 2 days at room temperature, the solution contained the enol form predominantly (90:10). This enriched mixture was immediately methylated with diazomethane to afford the methyl enol ether

49. The C3-oxygenated aza-carbacephems should serve as advanced precursors for the synthesis of biologically active aza-carbacephem analogues.

2.2 X-Ray crystallography

It has been shown by us that the Cambridge Structural Database (CSD)[29] can be profitably utilised to obtain structural parameters favourable for biological activity in the β-lactam class of drug molecules.[30] The motivation for the determination of X-ray crystal structures of aza-β-lactams was thus threefold: (i) to confirm the chemical structure and the stereochemistry of the molecules in this study; (ii) to compare the structural parameters in aza-β-lactams with those found in β-lactams, and; (iii) to examine the hydrogen bonding and crystal packing motifs in this class of molecules. A search of the CSD (Version 5.11, April 1996 update, 167 797 entries) for the aza-β-lactam fragment **50** furnished five hits, all of which belong to the 1,3-diazetidin-2,4-dione skeleton **51**. The absence of



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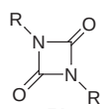

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1,3-diazetidin-2-ones in the CSD suggested that a study of their structural features should be interesting. The crystal structures of benzoate **35**, hydroxy ester **42** and phenolic alcohol **52** are discussed in this section.

Benzoate 35. The benzoate was recrystallised from a mixture of CH_2Cl_2 and hexane at room temperature to afford X-ray quality crystals. The compound crystallises in the monoclinic space group $P2_1/c$. The *exo* and *cis* stereochemistry of the methyl and benzoate groups deduced from the NOE data on the hydroxy precursor **34** are confirmed (Fig. 3). Based on the C–N distance (r), the Woodward parameter (h)[31] and the sum of angles at the N-atom (ΣN) values from the X-ray analysis, it is evident that N1 is in a pyramidal environment with inhibited

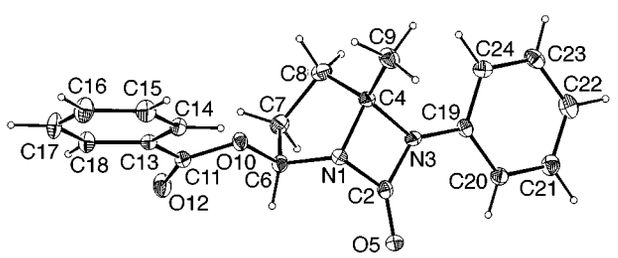


Fig. 3 Molecular structure of benzoate **35** showing ellipsoids at 50% probability level

conjugation and that N3 is in maximum resonance with the π-framework of the adjacent C=O group. This is reflected in a shorter C2–N3 bond [1.375(2) Å] compared to the C2–N1 bond distance [1.438(2) Å] and by their ΣN values ($\Sigma N3 = 359.99°$, $\Sigma N1 = 311.74°$). An h value of 0.601 Å and a C–N bond of intermediate strength with hindered amide resonance suggest that structural parameters favourable for antibiotic activity in β-lactams[30] are found in aza-β-lactam **35**. These observations from the crystallographic data support some of the conclusions derived from AM1 calculations,[11b] notably that N1 and N3 atoms are in chemically distinct environments and form C–N bonds of different strengths. This could have a bearing on the regioselectivity in the cleavage of the diazetidinone ring C–N bond upon attack by the active site Ser-OH group and other nucleophiles.

There are no strong hydrogen bonding groups (OH, NH_2) in **35** and the structure is stabilised by the weaker C–H···O, C–H···N and C–H···π hydrogen bonds.[32] The geometrical parameters of some of these interactions are listed in Table 1.

β-Hydroxy ester 42. The compound was recrystallised from a mixture of CH_2Cl_2 and hexane. The space group is $P2_1/c$ and there are two molecules, A and B, in the asymmetric unit that have similar bond distances, angles and conformations. The stereochemistry of the aldol product, β-hydroxy ester **42**, assigned from NOE experiments is confirmed from its X-ray structure (Fig. 4). The quaternary methyl and the ester groups are *exo* and *cis* to each other while the hydroxy group is on the *endo* face and *trans* to the ester and methyl groups. The crystal structure is stabilised by many strong (O–H···O) and weak (C–H···O) hydrogen bonds, some of which are listed in Table 2. It may be noted that the C–H···O hydrogen bonds in which the donor C–H is acidic, that is activated by an ester group or part of a phenyl C–H group, are shorter ($d < 2.40$ Å) than the other C–H···O bonds. This is in keeping with general trends for C–H···O hydrogen bonds.[32] The overall patterns of interactions that constitute the crystal structure are quite complex and the details are not discussed here. The h and c (Cohen distance)[33] values of the two molecules of hydroxy ester **42** (A: $h = 0.33$ Å, $c = 4.35$ Å; B: $h = 0.36$ Å, $c = 4.35$ Å) are in the range favourable for biological activity.[30]

Phenolic alcohol 52. The synthesis of alcohol **31** detailed

Table 1 Geometrical parameters for X-ray structure of benzoate **35**

Interaction	d/Å	D/Å	θ/°
C(16)–H···O(10)	2.760	3.659	140.20
C(20)–H···O(12)	2.535	3.370	133.21
C(22)–H···O(5)	2.690	3.632	145.04
C(23)–H···O(10)	2.529	3.422	139.19
C(23)–H···N(1)	2.771	3.703	144.16

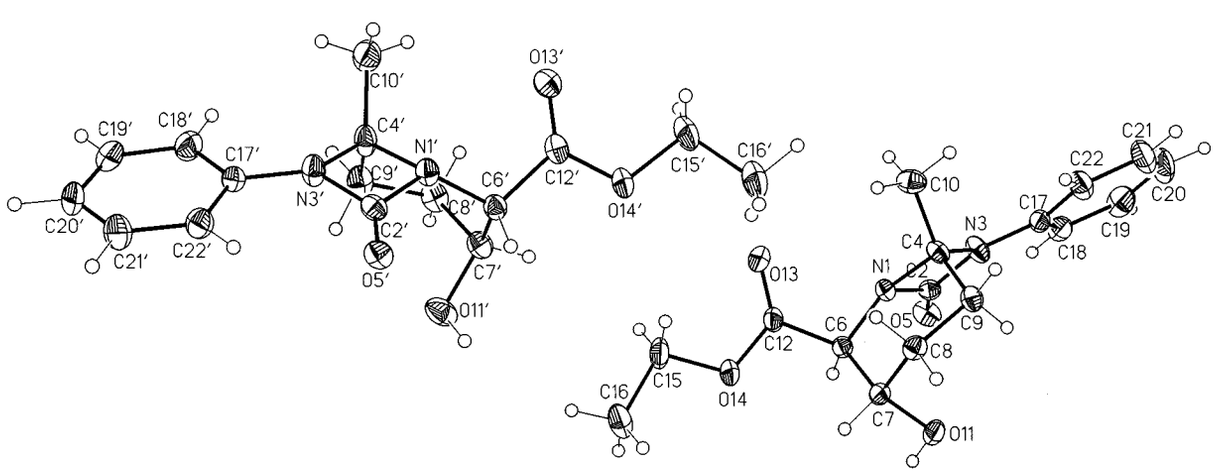
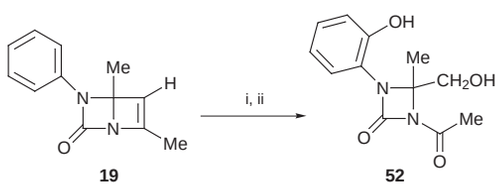


Fig. 4 Molecular structure of the two molecules of ester **42** showing ellipsoids at 50% probability level

Table 2 Geometrical parameters for X-ray structure of β-hydroxy ester **42**

Interaction	*d*/Å	*D*/Å	*θ*/°
O(11)–H···O(5)	1.839	2.768	156.46
O(11′)–H···O(5′)	1.851	2.813	165.34
C(6)–H···O(5)	2.289	3.141	134.10
C(6′)–H···O(5′)	2.377	3.293	141.34
C(8′)–H···O(11′)	2.557	3.589	158.92
C(8)–H···O(11)	2.549	3.607	165.26
C(9)–H···O(13′)	2.471	3.359	138.41
C(19)–H···O(13)	2.356	3.322	147.64
C(20′)–H···O(11)	2.350	3.420	169.38
C(10′)–H···O(5)	2.847	3.925	173.45
C(10)–H···O(5′)	2.542	3.445	140.22
C(16′)–H···N(3′)	2.977	3.924	146.26
C(16′)–H···O(13)	2.473	3.510	159.87

in Scheme 3 was carried out in three steps: ozonolysis of the alkene **19**, reductive work-up with Me_2S, and reduction of the aldehyde **20** with $NaBH_4$. We felt that an alternative and shorter synthesis of alcohol **31** could be the reduction of ozonide derived from alkene **19** with $NaBH_4$ to obtain the alcohol directly in a single pot (Scheme 10). Instead of the



19 52

Scheme 10 *Reagents and conditions*: i, O_3, CH_2Cl_2–MeOH, −78 °C; ii, $NaBH_4$, 0 °C, 1 h, 40%

expected alcohol, a different compound was isolated that contained an additional oxygen atom based on its mass spectral analysis.

The structure of this unexpected compound was unambiguously determined as **52** by X-ray analysis. Though the exact mechanism for the direct formation of **52** from **19** is not clear, it is likely that some sort of electrophilic hydroxylation of the phenyl group with ozone or the ozonide is involved.[34] Recrystallisation of racemic **52** from CH_2Cl_2 and hexane afforded single crystals in the non-centrosymmetric and enantiomorphous orthorhombic space group $P2_12_12_1$. The phenomenon of spontaneous resolution,[35] that is the crystallisation of a racemic molecule in separate enantiomorphous crystals, rather than as a racemic (and centrosymmetric) crystal is still poorly understood, despite its antiquity. It is known, however, that the presence of phenolic and alcoholic OH groups in a molecule tends to favour crystallisation in enantiomorphous space groups.[36] Spontaneous resolution is a related phenomenon and in this case, the presence of both these types of OH groups in the molecule could be the reason for its occurrence. The packing of molecules in the crystal structure of **52** is shown in Fig. 5. The phenolic OH is intramolecularly hydrogen bonded to the alcoholic OH which in turn is hydrogen bonded to the acetyl C=O of a 2_1 screw-related molecule. Additionally, the phenyl C–H is hydrogen bonded to the phenolic OH and the hydroxymethyl C–H is bonded to the diazetidinone C=O of distinct 2_1-related molecules. Molecules translated along [010] are connected by C–H···O hydrogen bonds between the phenol rings. The geometrical parameters of these interactions are listed in Table 3.

Table 3 Geometrical parameters for X-ray structure of phenolic alcohol **52**

Interaction	*d*/Å	*D*/Å	*θ*/°
O(18)–H···O(7)[a]	1.721	2.681	164.04
O(7)–H···O(17)[b]	1.723	2.694	168.58
C(8)–H···O(5)[c]	2.580	3.626	162.12
C(13)–H···O(18)[d]	2.475	3.364	138.52
C(11)–H···O(18)	2.754	3.691	144.65
C(16)–H···O(5)	2.951	3.960	155.09

[a–d] Represent interactions shown in Fig. 5.

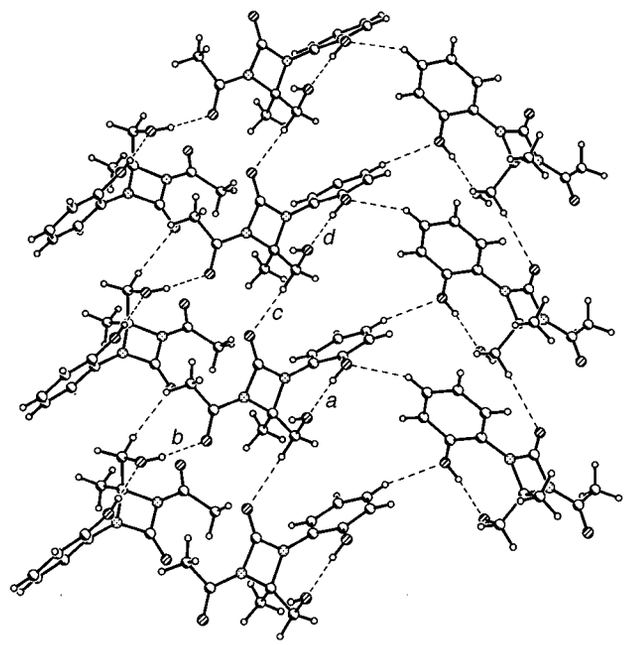
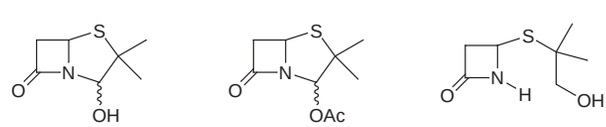


Fig. 5 Hydrogen bonding in the crystal structure of phenol **52**. The molecules have the *R* configuration in the crystal chosen for study.

2.3 Bioassay

The 1,3-diazetidinones described in the paper as well as other related compounds synthesised in this study[37] were tested for antibacterial activity against *Bacillus subtilis*, a gram-positive bacterium of a highly potent and evolved species. The inhibition of bacterial growth was studied in wells of microtitre plates, the growth being monitored by measuring the turbidity of the cultures with a microplate reader at 405 nm.[38] An enzyme-linked immunosorbent assay (ELISA) was used for measuring the turbidity as optical density (OD) in yeast extract peptone dextrose (YEPD) as the medium for bacterial growth. The inhibition was measured against two standard β-lactam drugs, ampicillin and penicillin G of known activity. The structures of the mono and bicyclic aza-β-lactams evaluated against *B. subtilis* are shown in Fig. 6 with the percentage inhibition at a concentration 330 μg ml^{-1} being displayed in Fig. 7.

Examination of the biological activity data suggests that of the 19 compounds tested, eight compounds (**22**, **26**, **35**, **47**, **49**, **53**, **54**, **57**) show inhibition better than the standards (>40%), eight compounds (**21**, **23**, **24**, **25**, **34**, **40**, **55**, **56**) are comparable to or slightly lower than the standards in their activity (20–40%), while three compounds (**19**, **42**, **58**) show poor inhibition (<20%). The *p*-bromophenylcarbapenam **26** exhibited excellent activity and is the most active of all compounds evaluated, showing >90% bacterial growth inhibition at 150 μg ml^{-1} and 100% inhibition at 300 μg ml^{-1} concentration (IC_{50} = 60 μg ml^{-1}). The activity of carbapenam benzoate **35**, whose X-ray structure has been determined is quite good (80% inhibition) and is better than the precursor hydroxy compounds **34** and **24**. While studying the effect of bicyclic skeleton and hydroxy group on biological activity, Sheehan *et al.*[23] found aminal **59** to be more potent than its acetate **60** and the open form **61**. In



59 60 61

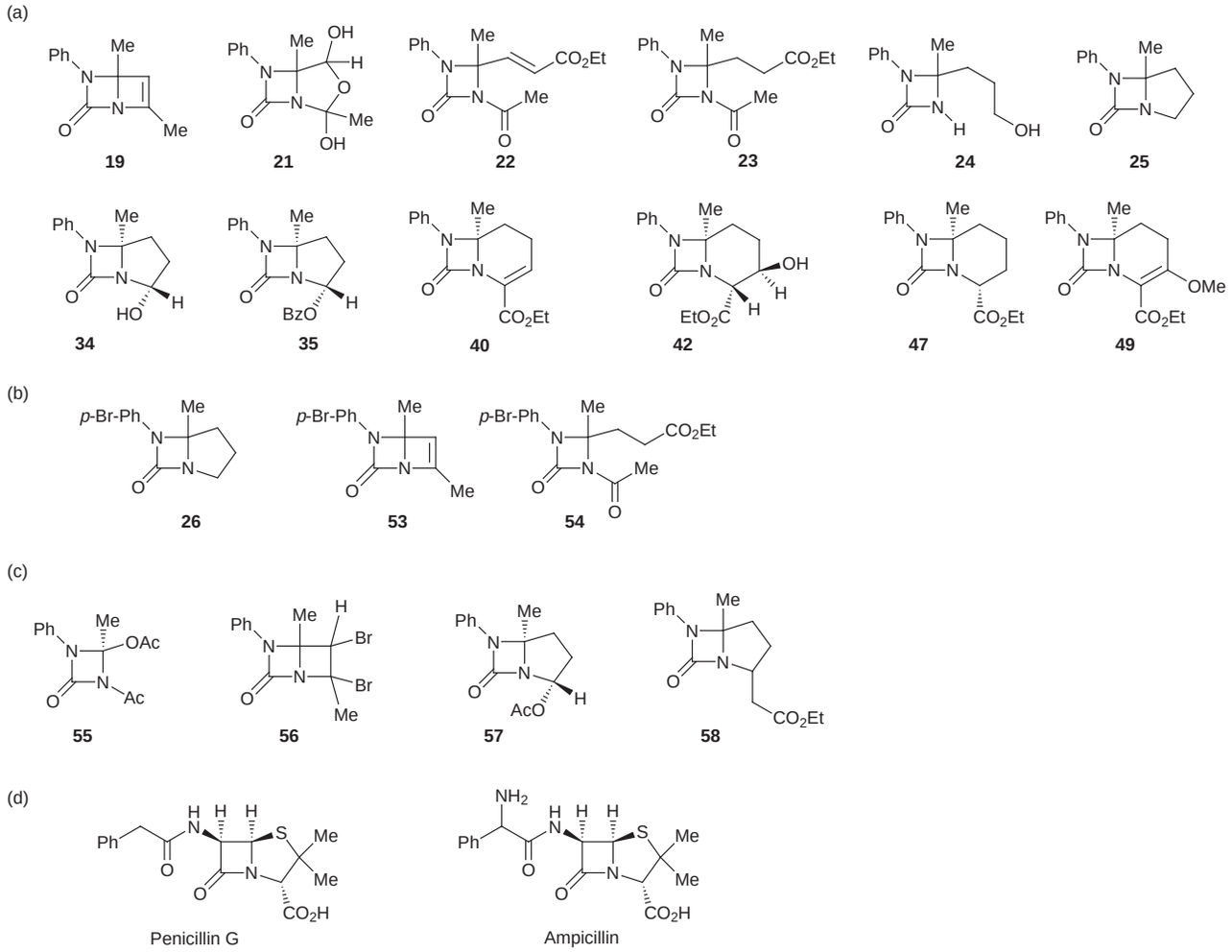


Fig. 6 Chemical structures of aza-β-lactams chosen for biological screening: (a) parent molecules; (b) bromophenyl derivatives; (c) additional compounds; (d) standard antibiotics

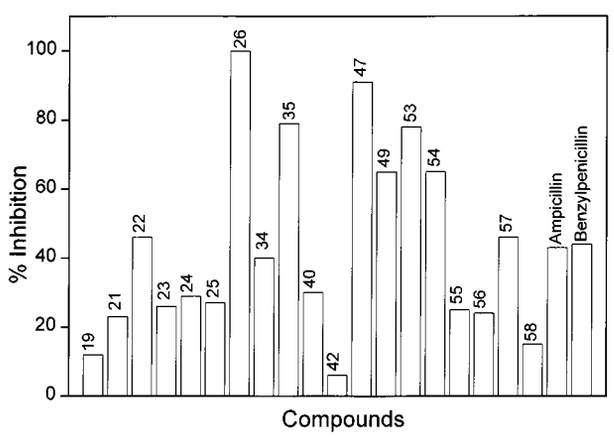


Fig. 7 Histogram for the percentage inhibition of *Bacillus subtilis* at a concentration of 330 μg ml^{-1} by the compounds shown in Fig. 6. Notice that out of the 19 compounds tested, eight show inhibition better than the standards.

this study, we find that the inhibition of *B. subtilis* is in the order: benzoate **35** > acetate **57** > aminal **34** > open form **24**. In addition to the bioassay results on aza-β-lactams, it may be noted that the diazetidinone carbonyl IR stretching frequency of these molecules (see Experimental section) is in the range favourable for active β-lactam antibiotics (1770–1800 cm^{-1}).[39]

While the above results show that mono- and bi-cyclic aza-β-lactams inhibit bacterial growth, the exact mechanism by which these molecules exert their antibacterial activity cannot be deduced from the limited structure–activity data. The comparison with ampicillin and penicillin G is to show that the novel skeleton is intrinsically active, even as it is understood that the mode of action of aza-β-lactams could be different from that of β-lactams.[40] Likewise, the similarity of the carbonyl stretching frequency between well-known β-lactams and the newly synthesised aza-β-lactams suggests that this structural parameter is in a favourable range. In summary, the bioassays though limited in scope are encouraging enough to initiate further work on the biological activity and mechanism of action of these molecules.

3 Conclusions

The aza-β-lactams discussed in this paper are non-natural structural mimics of β-lactams derived from the isosteric replacement of CH group by N-atom in the four-membered heterocycle. The target molecules are synthesised by the photochemical electrocyclisation of a pyrimidinone to afford the 1,3-diazetidin-2-one heterocycle. The chemical reactivity of diazetidinones has been studied with a variety of synthetic reagents for selective functional group manipulation. Mono- and bi-cyclic aza-β-lactams have been synthesised in a few steps with minimal use of protecting groups and in good overall yields. Carbapenam analogues without the carboxy group at C3 and carbacepham analogues with the carboxy group at C4 have been synthesised. The X-ray crystal structures of selected compounds have been determined to confirm their molecular structure and also to study the structural parameters that influence biological activity in β-lactam antibiotics.

The biological activity has been assayed by measuring the inhibition of bacterial growth in gram-positive strain, *B. subtilis*. A majority of the molecules tested show higher inhibition

Table 4 Crystallographic details for aza-β-lactams **35**, **42** and **52**

Compound	**35**	**42**	**52**
Molecular formula	$C_{19}H_{18}N_2O_3$	$C_{16}H_{20}N_2O_4$	$C_{12}H_{14}N_2O_4$
Crystal size/mm	0.10 × 0.20 × 0.25	0.25 × 0.25 × 0.25	0.25 × 0.25 × 0.15
Formula weight	322.35	304.34	250.25
Crystal system	Monoclinic	Monoclinic	Orthorhombic
Space group	$P2_1/c$ (No. 14)	$P2_1/c$ (No. 14)	$P2_12_12_1$ (No. 19)
Unit cell parameters/Å, °	a = 10.332(2)	a = 9.715(3)	a = 6.814(2)
	b = 9.140(2)	b = 20.211(6)	b = 6.905(3)
	c = 17.363(3)	c = 15.923(7)	c = 25.579(9)
	β = 98.170(10)	β = 90.92(3)	
No. mols. in unit cell (Z)	4	4	4
Volume/Å^3	1623.0(6)	3126(2)	1203.5(8)
Density (calc.)/mg m^{-3}	1.319	1.293	1.381
F(000)	680	1296	528
Diffractometer used	Enraf Nonius	Enraf Nonius	Enraf Nonius
Temperature/K	120	120	120
Absorption coeff./mm^{-1}	0.090	0.094	0.105
Collection range/°	2.52 to 27.48	2.39 to 28.96	3.06 to 28.95
Total reflections	13 851	22 515	11 943
Unique data measured	3708	7702	1798
Goodness-of-fit on F^2	1.045	0.754	0.885
Final R indices [$I > 2\sigma(I)$]	0.0456	0.0462	0.0375
R indices (all data)	0.0565	0.0903	0.0434

than the well-known standard antibiotics, ampicillin and penicillin G. Since this is an exploratory study on the antibacterial activity of a novel skeleton, synthetic ease dictated that the bioassays be carried out on racemic molecules. This raises the interesting possibility of testing the biological activity of chiral aza-β-lactams, which will naturally be higher than that found for the racemic molecules. The present study suggests directions for further work on a novel and non-natural class of serine peptidase inhibitors.

Experimental

Synthesis

IR spectra were recorded on Jasco 5300 FT-spectrometer. ^{1}H and ^{13}C spectra were recorded as a solution in $CDCl_3$ on Bruker ACF 200 instrument at 200 and 50 MHz, respectively and reported as δ_H and δ_C values. J Values are given in Hz. Elemental analysis was performed on Perkin-Elmer 240C, LRMS on JEOL JMS DX303 and HRMS on Micromass VG70/70H instruments. Photolysis and ozonolysis were carried out on the models available from Ace Glass and Welsbach.

Work up means drying of organic extracts with $MgSO_4$, solvent removal on rotary evaporator and concentration *in vacuo*. All reactions were carried out using standard syringe-septum techniques in an inert N_2 atmosphere with magnetic stirring. All reagents and solvents were dried and distilled prior to use.[41]

Crystallography

The intensity data were collected on an Enraf Nonius FAST area detector with no absorption correction. Mo-Kα (λ = 0.710 73 Å) radiation was used in the ω–2θ scan mode. The structures were solved by automatic direct methods (SHELXS 86)[42] and refined by full-matrix least-squares refinement (SHELXL 93).[43] H-atoms were refined isotropically. Normalised H-atom distances (C–H 1.083 Å; O–H 0.983 Å) are used in the hydrogen bond geometries detailed in Tables 1–3. The crystallographic data for aza-β-lactams **35**, **42** and **52** are summarised in Table 4.

Full crystallographic details, excluding structure factor tables, have been deposited at the Cambridge Crystallographic Data Centre (CCDC). For details of the deposition scheme, see 'Instructions for Authors', *J. Chem. Soc.*, *Perkin Trans. 1*, available *via* the RSC Web pages (http://www.rsc.org/authors). Any request to the CCDC for this material should quote the full literature citation and the reference number 207/230.

Version 5.11 (April 1996 update, 167 797 entries) of the CSD[29] was used to search for fragment **50**. No screens were applied.

Bioassays

The yeast extract peptone dextrose (YEPD) medium was prepared from the following: yeast extract 10 g, peptone 20 g, dextrose 5 g, double distilled water 1000 ml (pH 7). *B. subtilis* was cultured in YEPD medium. Each well of the microtitre plate was loaded with 25 µl of mid-log phase bacterial culture, YEPD medium and the aza-β-lactam compounds at different concentrations in DMSO to a final volume of 150 µl and incubated at 30 °C. Triplicate readings were recorded for each concentration and repeated twice. Every alternate well was kept empty to prevent interference and cross-contamination. The change in absorbance was monitored at 405 nm every 8 h up to 24 h. A total of 125 µl of YEPD medium and DMSO (1–10 µl) inoculated with 25 µl of mid log-phase culture of *B. subtilis* served as control for the same concentration of the compound in DMSO. The compounds were dissolved in DMSO such that 1 µl of the solution contained 10 µg of the compound. The optical density (OD) values were determined by taking the average of triplicate observations calculated by subtracting the values of the first measurement from those at a specific time. The percentage inhibition of bacterial growth was calculated from the difference in OD after 16 h (mid-log phase) using eqn. (1). In this way the IC_{50} (concentration

$$\%\text{inhibition} = \frac{OD_{control} - OD_{compound}}{OD_{control}} \times 100 \qquad (1)$$

for 50% inhibition) values for different aza-β-lactams were determined.

3-Phenyl-4,6-dimethyl-2-oxo-1,3-diazabicyclo[2.2.0]hex-5-ene 19

A solution of pyrimidinone **18** (1.20 g, 6.0 mmol) in 350 cm^3 of degassed benzene in a quartz vessel was irradiated with a high pressure mercury lamp using a Pyrex filter for 3 h. The solvent was removed *in vacuo* to provide a 1 : 1 photostationary mixture which upon purification by silica gel chromatography (hexane to 10% EtOAc–hexane) gave pure alkene **19** (560 mg, 46%). The recovered starting material (~50%) was recycled. Mp 62–64 °C; ν_{max}/cm^{-1} 3123, 3040, 2988, 1766, 1643, 1599, 1500, 1373, 1215, 1184 and 754; δ_H 7.38–7.05 (5 H, m, Ph), 6.10 (1 H, q, J 2, vinyl CH), 2.12 (3 H, d, J 2, CH_3) and 1.86 (3 H, s, CH_3).

1-Phenyl-3-acetyl-4-formyl-4-methyl-1,3-diazetidin-2-one 20
Through a solution of alkene **19** (100 mg, 0.5 mmol) in 20 cm^3 of CH_2Cl_2 was passed a stream of ozone at −78 °C until the light blue colour persisted. The reaction mixture was flushed with oxygen for 5 min. Me_2S (150 μl, 124 mg, 2 mmol) was added dropwise to the reaction mixture at 0 °C and then stirred at 0 °C to room temperature for 1 h. Removal of the solvent *in vacuo* gave acetamido aldehyde **20** (80 mg, 69%) after column purification (hexane to 30% EtOAc–hexane) (Found: C, 62.47; H, 5.16; N, 12.81. $C_{12}H_{12}N_2O_3$ requires C, 62.07; H, 5.17; N, 12.07%); Mp 173–174 °C; ν_{max}/cm^{-1} 3000, 1807, 1741, 1691, 1599, 1512, 1317, 1047 and 756; δ_H 9.44 (1 H, s, CHO), 7.35–7.14 (5 H, m, Ph), 2.46 (3 H, s, $COCH_3$) and 1.93 (3 H, s, CH_3).

Acetal 21
Through a solution of alkene **19** (50 mg, 0.25 mmol) in 10 cm^3 of dry CH_2Cl_2 was passed a stream of ozone at −78 °C until the blue colour persisted. The reaction was flushed with oxygen for 5 min. Et_3N (70 μl, 50 mg, 0.5 mmol) was added to the mixture which was then warmed to room temperature and stirred for 1 h. The reaction mixture was washed with 5% aq. HCl and brine. Work up gave a 1 : 1 mixture of aldehyde **20** and acetal **21**. Purification by column chromatography on silica (hexane to 20% EtOAc–hexane) afforded a mixture of three acetals as judged from its ^1H NMR spectrum (15 mg, 26%). Mp 173–175 °C; ν_{max}/cm^{-1} 3320, 1813, 1680, 1602, 1246, 1116, 1032, 966 and 760; δ_H 7.32–6.95 (5 H, s, Ph), 5.85, 5.43, 4.98 (1 H, s, acetal CHs), 2.37 (3 H, s, CH_5) and 1.83 (3 H, s, CH_3); *m/z* 250 (HRMS).

***trans*-Unsaturated ester 22**
To a solution of aldehyde **20** (58 mg, 0.25 mmol) in 2 cm^3 of dry benzene was added $Ph_3P{=}CHCO_2Et$ (174 mg, 0.5 mmol) and the mixture was stirred at room temperature for 1 h. Removal of solvent *in vacuo* and purification by column chromatography on silica (hexane to 20% EtOAc–hexane) provided unsaturated ester **22** (60 mg, 80%). ν_{max}/cm^{-1} 3072, 2995, 1803, 1718, 1601, 1512, 1037, 985 and 754; δ_H 7.34–7.05 (5 H, m, Ph), 7.05 (1H, d, *J* 16, vinyl CH), 6.34 (1 H, d, *J* 16, vinyl CH), 4.28–4.17 (2 H, q, *J* 8, OCH_2), 2.41 (3 H, s, CH_3), 2.06 (3 H, s, CH_3) and 1.31 (3 H, t, *J* 8, CH_2CH_3).

Saturated ester 23
To a solution of unsaturated ester **22** (60 mg, 0.2 mmol) in 5 cm^3 of EtOAc was added 10% Pd/C (15 mg), the mixture was flushed with H_2 and then stirred at room temperature under a H_2 atmosphere for 4 h. The reaction mixture was filtered through Celite. Work up afforded saturated ester **23** which was used in the next step without further purification (60 mg, 99%). ν_{max}/cm^{-1} 2900, 1780, 1720, 1680, 1330, 1240, 1160, 1100, 1060 and 750; δ_H 7.36–7.05 (5 H, m, Ph), 4.02 (2 H, q, *J* 8, OCH_2), 2.85–2.28 (4 H, m, 2 × CH_2), 2.41 (3 H, s, $COCH_3$), 1.93 (3 H, s, CH_3) and 1.22 (3 H, t, *J* 8, CH_2CH_3); δ_C 171.61, 165.95, 145.67, 135.41, 129.29, 123.65, 115.98, 81.58, 60.47, 29.70, 27.86, 22.64, 22.25 and 13.77.

Amino alcohol 24
To a suspension of $LiAlH_4$ (15 mg, 0.4 mmol) in 4 cm^3 of dry diethyl ether at 0 °C was added a solution of ester **23** (30 mg, 0.1 mmol) in 1 cm^3 of dry diethyl ether dropwise. The reaction mixture was stirred for 1 h at 0 °C and quenched with H_2O (15 μl), 15% aq. NaOH (15 μl) and then H_2O (45 μl). The mixture was diluted with diethyl ether, dried with $MgSO_4$ and filtered through Celite. Work up and purification by column chromatography on silica (hexane to 60% EtOAc–hexane) afforded amino alcohol **24** (30 mg, 68%) (Found: C, 65.50; H, 7.20; N, 12.68. $C_{12}H_{16}N_2O_2$ requires C, 65.45; H, 7.27; N, 12.73%); ν_{max}/cm^{-1} 3312, 2932, 2876, 1755, 1600, 1505, 1392, 1059, 754 and 694; δ_H 7.35–6.96 (5 H, m, Ph), 3.75–3.65 (2 H, m, OCH_2), 2.15 (2 H, t, *J* 9, CH_2), 1.85–1.54 (2 H, m, CH_2) and 1.76 (3 H, s, CH_3); δ_C 153.99, 137.44, 129.35, 122.63, 115.54, 76.44, 61.95, 33.88, 26.25 and 24.25.

Aza-carbapenam 25
A solution of $EtO_2C{-}N{=}N{-}CO_2Et$ (DEAD) (15 μl, 17 mg, 0.1 mmol) in 0.5 cm^3 of dry benzene was added to a solution of amino alcohol **24** (11 mg, 0.05 mmol) and Ph_3P (26 mg, 0.1 mmol) in 0.5 cm^3 of dry benzene. The reaction mixture was stirred at room temperature for 1 h. Removal of solvent *in vacuo* and purification by column chromatography on silica (hexane to 10% EtOAc–hexane) provided pure aza-carbapenam **25** (9 mg, 84%). ν_{max}/cm^{-1} 3053, 2970, 2878, 1774, 1601, 1504, 1386, 1261, 1165 and 754; δ_H 7.35–6.98 (5 H, m, Ph), 3.62–3.51 (1 H, m, CH_2), 2.82–2.65 (1 H, m, CH_2), 2.45–2.35 (1 H, m, CH_2), 2.15–1.92 (2 H, m, CH_2), 1.78 (3 H, s, CH_3) and 1.72–1.60 (1 H, m, CH_2); δ_C 158.12, 137.61, 129.40, 123.04, 115.76, 84.17, 46.41, 32.18, 26.94 and 22.86; *m/z* 202 (LRMS).

4-Bromophenyl-aza-carbapenam 26
Synthesised by a protocol similar to that adopted for **25**. ν_{max}/cm^{-1} 2969, 2930, 2856, 1777, 1593, 1495, 1385, 1265, 1165, 1072 and 824; δ_H 7.42 (2 H, d, *J* 9, aromatic CH), 7.12 (2 H, d, *J* 9, aromatic CH), 3.64–3.52 (1 H, m), 2.84–2.69 (1 H, m), 2.44–2.32 (1 H, m), 2.12–1.95 (2 H, m), 1.76 (3 H, s, CH_3) and 1.75–1.64 (1 H, m).

Epoxide 29
A 50% dispersion of NaH in mineral oil (19 mg, 0.4 mmol) under a N_2 atmosphere was washed with dry hexane (2 × 1 cm^3) to remove the oil and 0.8 cm^3 of dry DMSO was added. The mixture was heated to 70–75 °C for 30 min until the evolution of H_2 ceased and a somewhat cloudy pale yellow–grey solution of the sodium salt was formed. The solution was cooled to room temperature and diluted with an equal volume of dry THF (0.8 cm^3), the flask was cooled in an ice–salt bath (−10 °C) then a solution of $(CH_3)_3S^+I^-$ (102 mg, 0.5 mmol) in 0.4 cm^3 of DMSO was added and stirring was continued for 10 min at −10 °C. Aldehyde **20** (23 mg, 0.1 mmol) in 0.5 cm^3 of dry THF was added to the sulfur ylide and stirred at −10 to 0 °C for 40 min. The reaction mixture was extracted with diethyl ether. Work up and purification by column chromatography on silica (hexane to 30% EtOAc–hexane) gave pure epoxide **29** (6 mg, 30%). ν_{max}/cm^{-1} 3059, 2961, 2930, 1770, 1601, 1504, 1385, 1265, 1128, 1074, 739 and 694; δ_H 7.35–6.98 (5 H, m, Ph), 5.20 (1 H, s, NH), 3.31–3.26 (1 H, m, oxirane CH), 2.92–2.81 (2 H, m, oxirane CH_2) and 1.61 (3 H, s, C_3); δ_C 153.20, 136.73, 129.35, 123.06, 115.93, 74.65, 54.10, 44.28 and 17.62; *m/z* 204 (LRMS).

Alcohol 30
$LiAlH_4$ (15 mg, 0.4 mmol) and acetamido aldehyde **20** (23.2 mg, 0.1 mmol) in 5 cm^3 of dry diethyl ether were stirred at 0 °C for 1 h. Purification by column chromatography on silica (hexane to 50% EtOAc–hexane) gave alcohol **30** (6 mg, 29%). ν_{max}/cm^{-1} 3325, 3053, 2928, 1757, 1600, 1504, 1385, 1072, 754 and 694; δ_H 7.32–6.95 (5 H, m, Ph), 5.40 (1 H, s, NH), 3.92 (2 H, q, J_{AB} 12, OCH_2) and 1.68 (3 H, s, CH_3).

Alcohol 31 and γ-lactol 32
Acetamido aldehyde **20** (232 mg, 1 mmol) was taken up in 4 cm^3 of CH_3OH, cooled to 0 °C and $NaBH_4$ (152 mg, 4 mmol) added slowly in portions and stirred for 1 h. The reaction mixture was acidified with 5% aq. HCl to pH 4 and extracted with diethyl ether. Work up afforded a 4 : 1 mixture of γ-lactol **32** and hydroxy amide **31**. Purification by column chromatography on silica (hexane to 50% EtOAc–hexane) gave pure γ-lactol **32** (103 mg, 44%) followed by alcohol **31**. ν_{max}/cm^{-1} (**32**) 3342, 2935, 1797, 1691, 1340, 1072 and 752; δ_H (**32**) 7.38–6.85 (5 H, m, Ph), 4.28 (1H, d, *J* 12, OCH_2), 3.76 (1 H, d, *J* 12, OCH_2), 2.41 (3 H, s, CH_3) and 1.71 (3 H, s, CH_3); δ_H (**31**) 7.38–6.85 (5 H,

m, Ph), 4.22 (1 H, d, *J* 12, OCH_2), 3.98 (1 H, d, *J* 12, OCH_2), 2.42 (3 H, s, CH_3) and 1.87 (3 H, s, CH_3); δ_C (mixture of **31** and **32**) 167.57, 152.44, 148.58, 129.78, 120.73, 119.56, 118.21, 84.64, 61.57, 22.61 and 18.08; *m*/*z* 234 (LRMS).

Dibromo olefin 33

To a mixture of PPh_3 (105 mg, 0.4 mmol) and CBr_4 (66 mg, 0.2 mmol) in 1 cm^3 of dry CH_2Cl_2 was added aldehyde **20** (23 mg, 0.1 mmol) in 0.5 cm^3 of CH_2Cl_2 and stirred for 10 min at 0 °C. The reaction mixture was quenched with brine and extracted with diethyl ether (3 × 5 cm^3). Work up provided crude dibromo olefin **33** which was purified by column chromatography on silica (hexane to 20% EtOAc–hexane) (12 mg, 35% unoptimised). ν_{max}/cm^{-1} 2926, 2855, 1802, 1695, 1599, 1512, 1367, 1315, 1244, 1163 and 748; δ_H 7.41–7.08 (5 H, m, Ph), 6.91 (1 H, s, vinyl CH), 2.41 (3 H, s, CH_3) and 1.99 (3 H, s, CH_3).

Hydroxy amide 34

To a stirred solution of amino alcohol **24** (33 mg, 0.15 mmol) in 2 cm^3 of dry CH_2Cl_2 was added PCC (130 mg, 0.6 mmol) at 0 °C. The resulting brown solution was stirred for 2 h at 0 °C to room temperature. The reaction mixture was diluted with 5 cm^3 dry diethyl ether and filtered through Celite. Work up and purification by column chromatography on silica (hexane to 30% EtOAc–hexane) afforded pure hydroxy amide **34** (21 mg, 65%). Mp 130–131 °C (Found: C, 66.35; H, 6.64; N, 13.87. $C_{12}H_{14}N_2O_2$ requires C, 66.02; H, 6.47; N, 12.84%); ν_{max}/cm^{-1} 3383, 3055, 2924, 2852, 1771, 1601, 1504, 1388, 1263, 1170, 1078 and 739; δ_H 7.35–6.99 (5 H, m, Ph), 5.32 (1 H, d, *J* 3, NCH), 2.84 (1 H, br s, OH), 2.45–2.05 (4 H, m, 2 × CH_2) and 1.85 (3 H, s, CH_3); δ_C 154.86, 136.36, 129.39, 123.41, 115.98, 85.46, 83.46, 33.96, 29.46 and 23.55; *m*/*z* 218 (LRMS).

Benzoate 35

To a solution of aminal **34** (13 mg, 0.06 mmol) in 2 cm^3 of dry CH_2Cl_2 was added pyridine (48 μl, 47 mg, 0.6 mmol) and DMAP (cat.) at 0 °C. The reaction mixture was stirred for 10 min and to this was added benzoyl chloride (34 μl, 42 mg, 0.3 mmol) and stirring continued for 2 h at 0 °C to room temperature. The reaction mixture was diluted with sat. aq. NH_4Cl and work up gave 40 mg of crude benzoate. Purification by column chromatography on silica (hexane to 20% EtOAc–hexane) afforded pure benzoate **35** (12 mg, 62%). Mp 100–102 °C; δ_H 8.06 (2 H, d, *J* 6, COPh), 7.65–7.05 (8 H, m, Ph), 6.42 (1 H, d, *J* 3, NCHO), 2.62–1.99 (4 H, m, 2 × CH_2) and 1.91 (3 H, s, CH_3).

TBDMS ether 36

Amino alcohol **24** (44 mg, 0.2 mmol) was taken up in 2 cm^3 of dry CH_2Cl_2, cooled to 0 °C and then Et_3N (420 μl, 330 mg, 3 mmol) and catalytic DMAP were added and the mixture was stirred for 10 min. TBDMSCl (151 mg, 1 mmol) was added and stirring continued for 1 h. The reaction mixture was diluted with H_2O and extracted with diethyl ether. Work up afforded TBDMS ether **36** which was used as such without further purification (60 mg, 90%). ν_{max}/cm^{-1} 3288, 2955, 2930, 2856, 1763, 1601, 1504, 1390, 1255, 1101 and 837; δ_H 7.36–6.95 (5 H, m, Ph), 5.42 (1 H, s, NH), 3.62 (2 H, t, *J* 6, OCH_2), 2.1 (2 H, t, *J* 9, CH_2), 1.75 (3 H, s, CH_3), 1.85–1.46 (2 H, m, CH_2), 0.88 [9 H, s, $SiC(CH_3)_3$], 0.03 (3 H, s, $SiCH_3$) and 0.02 (3 H, s, $SiCH_3$); δ_C 153.76, 137.60, 129.26, 122.49, 115.61, 76.42, 62.48, 34.12, 26.48, 25.94, 24.34, 18.28 and −5.36.

Ester 37

A 50% dispersion of NaH in mineral oil (17 mg, 0.36 mmol) under a N_2 atmosphere was washed with dry hexane (2 × 2 cm^3) to remove the oil, 1 cm^3 of dry THF was added and the mixture was cooled to 0 °C. A solution of TBDMS ether **36** (30 mg, 0.09 mmol) in 1 cm^3 of dry THF was added and stirred for 10 min, then $BrCH_2CO_2Et$ (50 μl, 75 mg, 0.45 mmol) was added and stirring continued for 2 h at 0 °C to room temperature. The reaction mixture was quenched with H_2O and extracted with diethyl ether. Work up afforded TBDMS ester **37** which was used as such for the next reaction without further purification (37 mg, 98%). ν_{max}/cm^{-1} 2955, 2930, 2856, 1776, 1743, 1601, 1508, 1363, 1199, 1099, 837 and 750; δ_H 7.35–6.94 (5 H, m, Ph), 4.22 (2 H, q, *J* 9, OCH_2CH_3), 4.02 (2 H, s, NCH_2), 3.58 (2 H, t, *J* 6, $SiOCH_2$), 2.19–2.02 (2 H, m, CH_2), 1.73 (3 H, s, CH_3), 1.69–1.38 (2 H, m, CH_2), 1.31 (3 H, t, *J* 9, OCH_2CH_3), 0.88 [9 H, s, $Si(CH_3)_3$], 0.01 (3 H, s, $SiCH_3$) and 0.00 (3 H, s, $SiCH_3$); δ_C 169.02, 152.99, 137.82, 129.25, 122.17, 115.52, 81.53, 62.26, 61.33, 41.89, 32.25, 26.17, 25.89, 22.59, 18.24, 14.13 and −5.42.

Alcohol ester 38

A solution of TBDMS ester **37** (42 mg, 0.1 mmol) in 2 cm^3 of dry THF was cooled to 0 °C, then tetrabutylammonium fluoride (TBAF) (63 mg, 0.2 mmol) was added and the mixture was stirred at 0 °C for 1 h. The reaction mixture was diluted with brine and extracted with diethyl ether. Work up and purification by column chromatography on silica (hexane to 60% EtOAc–hexane) afforded pure alcohol ester **38** (25 mg, 82%). ν_{max}/cm^{-1} 3454, 2935, 1776, 1743, 1601, 1510, 1371, 1205, 1062 and 1024; δ_H 7.35–6.95 (5 H, m, Ph), 4.21 (2 H, q, *J* 9, OCH_2CH_3), 4.22 (1 H, d, *J* 18, NCH_2), 3.77 (1 H, d, *J* 18, NCH_2), 3.75–3.50 (2 H, m, OCH_2), 2.21–1.98 (2 H, m, CH_2), 1.85–1.35 (2 H, m, CH_2), 1.69 (3 H, s, CH_3) and 1.28 (3 H, t, *J* 9, OCH_2CH_3); δ_C 169.32, 152.99, 137.75, 129.33, 122.28, 115.37, 81.46, 61.91, 61.53, 41.78, 32.07, 25.91, 22.29 and 14.09.

Aldehyde ester 39

Alcohol ester **38** (40 mg, 0.13 mmol) was taken up in 2 cm^3 of dry CH_2Cl_2 and cooled to 0 °C, PCC (56 mg, 0.26 mmol) was added and the mixture was stirred at 0 °C to room temperature for 2 h. The reaction mixture was diluted with distilled diethyl ether and filtered through Celite. Removal of solvent *in vacuo* and purification by column chromatography on silica (hexane to 40% EtOAc–hexane) afforded aldehyde ester **39** (30 mg, 76%). ν_{max}/cm^{-1} 2976, 2932, 1774, 1745, 1601, 1508, 1369, 1203 and 1093; δ_H 9.74 (1 H, s, CO), 7.36–6.95 (5 H, m, Ph), 4.20 (2 H, q, *J* 9, OCH_2), 4.12 (1 H, d, *J* 18, NCH), 3.79 (1 H, d, *J* 18, NCH), 2.98–2.76 (1 H, m, CH_2), 2.42–2.04 (3 H, m, CH_2), 1.75 (3 H, s, CH_3) and 1.28 (3 H, t, *J* 9, OCH_2CH_3); δ_C 200.66, 168.91, 152.77, 137.88, 129.45, 122.58, 115.34, 80.48, 61.51, 41.67, 31.39, 27.71, 21.99 and 14.09.

Aza-carbacephem 40

A 50% dispersion of NaH in mineral oil (5 mg, 0.12 mmol) in 0.5 cm^3 of dry THF was taken in a dry two neck flask and cooled to 0 °C. Aldehyde ester **39** (9 mg, 0.03 mmol) in 0.5 cm^3 of dry THF was added slowly and stirred for 45 min at 0 °C. The reaction mixture was diluted with diethyl ether and filtered through Celite. Removal of solvent *in vacuo* gave 5 mg of crude aza-carbacephem **40** which was purified by column chromatography on silica (hexane to 20% EtOAc–hexane) (3 mg, 35%). ν_{max}/cm^{-1} 3021, 2928, 1784, 1732, 1601, 1508, 1373 and 1217; δ_H 7.36–6.98 (5 H, m, Ph), 6.34 (1 H, dd, *J* 6, 3, vinyl CH), 4.42–4.36 (2 H, m, OCH_2), 2.55–1.76 (4 H, m, 2 × CH_2), 1.66 (3 H, s, CH_3) and 1.38 (3 H, t, *J* 9, CH_3); δ_C 162.48, 149.39, 136.65, 129.90, 129.37, 122.85, 118.82, 115.96, 74.82, 61.46, 31.79, 21.13, 20.89 and 14.18; *m*/*z* 286 (LRMS).

β-Hydroxy ester 42

A 50% dispersion of NaH in mineral oil (7 mg, 0.16 mmol) was taken up in 1 cm^3 of dry THF and cooled to 0 °C. A solution of aldehyde ester **39** (12 mg, 0.04 mmol) in 0.5 cm^3 of dry THF was added and stirred at 0 °C for 20 min. The reaction mixture was diluted with distilled diethyl ether and filtered through

Celite. Removal of solvent *in vacuo* and purification by column chromatography on silica (hexane to 20% EtOAc–hexane) afforded pure β-hydroxy ester **42** (7 mg, 60%). ν_{max}/cm^{-1} 3441, 3059, 2934, 1774, 1743, 1601, 1508, 1361, 1199, 1059 and 752; δ_H 7.35–6.95 (5 H, m, Ph), 4.54 (1 H, d, *J* 2, NCH), 4.35–4.12 (3 H, m, OCH_2CH_3 and C*H*OH), 3.10 (1 H, br s, OH), 2.64–2.45 (2 H, m, CH_2), 2.12–1.62 (2 H, m, CH_2), 1.78 (3 H, s, CH_3) and 1.30 (3 H, t, *J* 9, OCH_2CH_3); δ_C 170.16, 154.21, 137.01, 129.28, 122.37, 115.82, 76.06, 66.38, 61.54, 60.28, 28.19, 24.35, 23.56 and 14.12; *m*/*z* 304 (LRMS).

Aza-carbacepham 47

To a solution of unsaturated ester **40** (7 mg, 0.025 mmol) in 2 cm^3 of EtOAc was added 10% Pd/C (5 mg), the mixture was flushed with H_2 and then stirred at room temperature under a H_2 atmosphere for 4 h. The reaction mixture was filtered through Celite. Work up afforded 6 mg of crude aza-carbacepham **47** which was purified by column chromatography on silica (hexane to 20% EtOAc–hexane) (6 mg, 86%). ν_{max}/cm^{-1} 2936, 2863, 1771, 1732, 1601, 1508, 1354, 1194 and 752; δ_H 7.35–6.95 (5 H, m, Ph), 4.59 (1 H, dd, *J* 8, 4, NCH), 4.28–4.12 (2 H, m, OCH_2), 2.28–1.55 (4 H, m, 2 × CH_2), 1.71 (3 H, s, CH_3) and 1.30 (3 H, t, *J* 9, CH_3); δ_C 171.91, 153.29, 137.19, 122.29, 115.80, 61.25, 51.87, 35.16, 26.87, 23.33, 17.13 and 14.17; *m*/*z* 288 (LRMS).

3-Hydroxy-aza-carbacephem 48

β-Hydroxy ester **42** (12 mg, 0.04 mmol) in 1 cm^3 of acetone was cooled to 0 °C, Jones reagent (100 μl, 0.8 mmol, 8 M) was added and stirring continued for 2 h at 0 °C to room temperature. The reaction mixture was diluted with brine and extracted with diethyl ether. Work up provided a 1 : 1 mixture of keto–enol isomers which when left at room temperature for 2 days afforded enol **48** which was purified by chromatography on silica (hexane to 40% EtOAc–hexane) (11 mg, 88%). ν_{max}/cm^{-1} 3418, 2924, 2855, 1807, 1749, 1694, 1601, 1506, 1352, 1256 and 1157; δ_H 11.64 (1 H, s, enol H), 7.42–7.06 (5 H, m, Ph), 4.44 (2 H, q, *J* 9, OCH_2), 2.94–2.05 (4 H, m, 2 × CH_2), 2.00 (3 H, s, CH_3), 1.66 (3 H, s, CH_3) and 1.42 (3 H, t, *J* 9, OCH_2CH_3); *m*/*z* 208 (LRMS).

Methyl enol ether 49

Diazomethane was generated by adding *N*-nitroso-*N*-methylurea (200 mg, 2 mmol) to a solution of 50% aq. KOH (20 cm^3) in 10 cm^3 of diethyl ether at 0 °C, the solution was left for a few minutes till the yellow colour developed. The diethyl ether layer was distilled and CH_2N_2 was bubbled through a solution of enol **48** (12 mg, 0.04 mmol) in 10 cm^3 of distilled diethyl ether at 0 °C. When the solution turned yellow it was slowly warmed to room temperature and left to dry. The crude methyl enol ether **49** was purified by column chromatography on silica (hexane to 20% EtOAc–hexane) (8 mg, 63%). ν_{max}/cm^{-1} 2938, 1807, 1742, 1601, 1510, 1445, 1352, 1257, 1091, 754 and 692; δ_H 7.42–7.12 (5 H, m, Ph), 4.45 (2 H, q, *J* 9, OCH_2), 3.60 (3 H, s, OCH_3), 2.90–2.36 (4 H, m, 2 × CH_2), 2.00 (3 H, s, CH_3) and 1.42 (3 H, t, *J* 9, CH_3); δ_C 172.06, 159.54, 135.07, 129.69, 129.35, 124.56, 116.54, 115.96, 82.54, 63.17, 51.87, 30.21, 27.75, 22.45, 13.85; *m*/*z* 317 (LRMS).

Phenolic alcohol 52

Through a solution of alkene (100 mg, 0.5 mmol) in 20 cm^3 of CH_2Cl_2 was passed a stream of ozone at −78 °C until the light blue colour persisted. The reaction mixture was flushed with oxygen for 5 min, $NaBH_4$ (76 mg, 2 mmol) added at 0 °C and stirring continued for 1 h at 0 °C. The reaction mixture was acidified with 5% aq. HCl to pH 4 and extracted with diethyl ether. Work-up and purification by column chromatography on silica (hexane to 50% EtOAc–hexane) afforded pure phenolic alcohol **52** (50 mg, 40%). δ_H 7.42–6.88 (5 H, m, Ph), 4.42 (1 H, d, *J* 12, OCH_2), 3.88 (1 H, d, *J* 12, OCH_2), 2.48 (3 H, s, CH_3), 1.85 (3 H, s, CH_3); *m*/*z* 250 (LRMS).

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