Clavulanic Acid Biosynthesis: the Stereochemical Course of β-Lactam Formation from Chiral Glycerol

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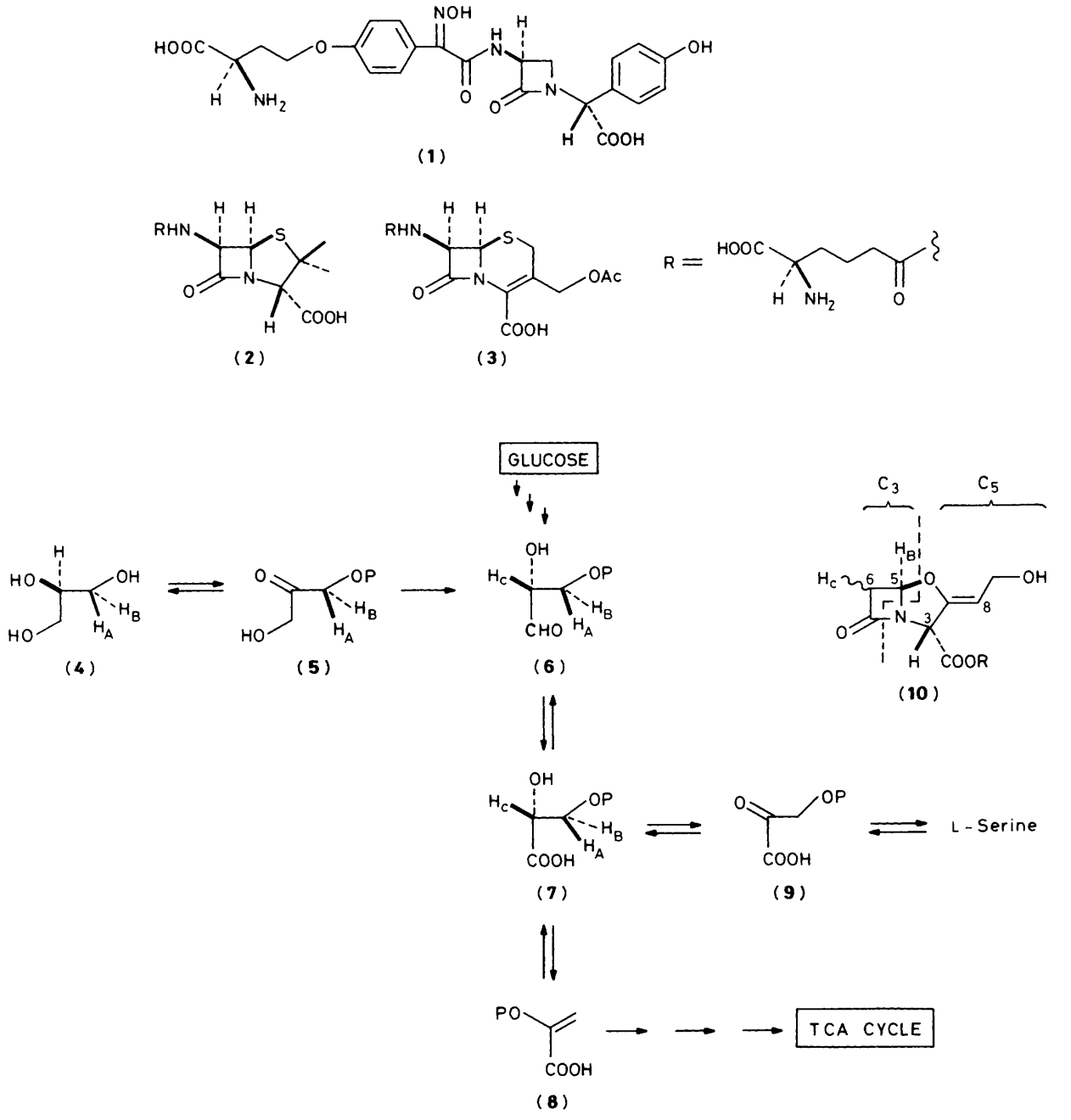
The overall stereochemical course of β-lactam formation in clavulanic acid was determined to be retention from (1*R*,2*R*)- and (1*S*,2*R*)-[1-^{3}H],[1,3-^{14}C]glycerol.

The monocyclic β-lactam ring of nocardicin A (**1**) is assembled from L-serine[1,2] in a reaction path occuring with inversion of configuration[3] and no change in oxidation state at the seryl β-carbon.[3] In contrast, the corresponding four-membered rings of penicillin N (**2**) and cephalosporin C (**3**) are derived from L-cysteine in a sequence involving oxidative cyclization of a tripeptide precursor[4] and overall retention of configuration[5] at the cysteinyl β-carbon. We record in this communication a notable parallel to the latter process where the *pro*-(*R*)-hydroxymethylene of glycerol (**4**,–CH_AH_BOH), diastereotopically labelled with tritium, is shown to give rise to clavulanic acid (**10**, R=H) with overall retention of configuration.

The incorporation of [1,3-^{3}H],[1,3-^{14}C]glycerol (**4**) into the β-lactam carbons (C_3-unit ≡ C-5—7) of clavulanic acid (**10**, R = H) is known to take place with retention of one quarter of

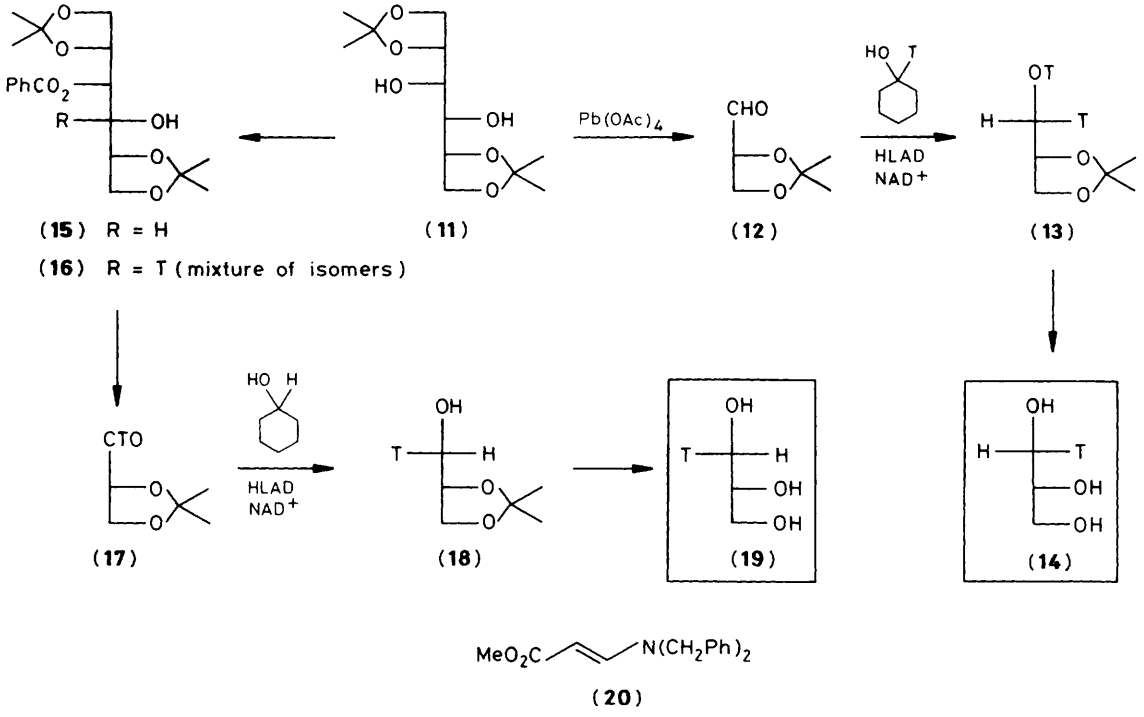


Scheme 1

its tritium activity;[6,7] that is, of the four methylene hydrogens of glycerol, one was shown by unambiguous degradation to label H-5. In contrast, tritium label from [2-^{3}H],[1,3-^{14}C]glycerol was completely lost on incorporation into (**10**).[6] A broad screening of potential C_3-intermediates identified[6] glyceric acid (**7**)† as the likely source from primary metabolism of the β-lactam carbons. While specific incorporations of radiolabel were low from (**7**), owing presumably to rapid flux through the glycolytic pathway, specificity of labelling in the C_3-unit of (**10**) was successfully demonstrated. In particular D-[2-^{3}H],[1-^{14}C]glyceric acid (**7**, H_C) was shown by careful degradation to selectively label H-6 (**10**, H_C).[6] Therefore, the present purpose of determining the stereochemical course of β-lactam formation in clavulanic acid was made experimentally accessible from glycerol by the conservation of the *pro*-(*R*)-hydroxymethylene stereocentre (**4**,–CH_AH_BOH) through the glycolytic intermediates (**6**) and (**7**) (see Scheme 1).

D-Mannitol was converted into its bis-acetonide (**11**) (Scheme 2).[7] Oxidative cleavage[7] to the acetonide of D-glyceraldehyde (**12**) and reduction with horse liver alcohol dehydrogenase (HLAD)[8] in the presence[9] of [1-^{3}H]cyclohexanol

† In strict terms the double-label incorporation experiments described in ref. 6 indicating the intermediacy of D-glycerate (**7**) could also be satisfied in principle by D-glyceraldehyde (**6**) as they do not address the issue of oxidation state at C-1 in these equilibrating glycolytic intermediates.



Scheme 2

Table 1. Incorporation of diastereotopically-labelled glycerols (**4**) into *p*-bromobenzyl clavulanate (**10**, R = PBB).

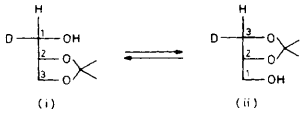
	$^3H/^{14}C$ (% ^3H retained)		
Substrate	(**14**) : (**19**)[a]	(**10**, R = PBB)	(**20**)
(1*R*,2*R*)-[1-^{3}H],[1,3-^{14}C]Glycerol (**14**)	12.28	6.71 (55%)	10.73 (87%)
(1*S*,2*R*)-[1-^{3}H],[1,3-^{14}C]Glycerol (**19**)	10.85	0.78 (7%)	0.98 (9%)

[a] Doubly-labelled glycerols were accurately counted as their tris(*p*-nitrobenzoyl) esters.

and NAD$^+$ gave the (1*R*,2*R*)-alcohol (**13**).‡ Mild acid hydrolysis afforded (1*R*,2*R*)-[1-^{3}H]glycerol (**14**). The diastereoisomeric (1*S*,2*R*)-[1-^{3}H]glycerol (**19**) was more difficult to obtain.[10] Attempted preparation of tritiated aldehyde (**17**) by reduction of (**12**) with sodium borotritide and reoxidation under a variety of conditions was unsatisfactory. Finally, the D-mannitol derivative (**11**) was converted into its monobenzoate (**14**).[11] Oxidation[11] of the remaining secondary hydroxy group followed by sodium borotritide reduction gave (**16**) (3:1 mixture of diastereoisomers favouring the isomer shown). After saponification of the benzoate (68% overall yield from introduction of the radioisotope), (**16**) was cleaved as above to give the desired tritiated aldehyde (**17**). Enzymic reduction and acetal hydrolysis (Scheme 2) afforded (1*S*,2*R*)-[1-^{3}H]glycerol (**19**).

The diastereomerically labelled glycerols (**14**) and (**19**) were separately combined with [1,3-^{14}C]glycerol and administered to cultures of *Streptomyces clavuligerus* (ATCC 27064) grown in a triglyceride medium.[12,13] The results obtained are shown in Table 1. Glycerol labels not only the C_3-unit of clavulanic acid, but also the C_5-unit by circulation of radioisotope around the TCA cycle and incorporation as a C_5-amino acid derived from α-ketoglutarate (Scheme 1).[13] Significant losses of tritium that attend these latter metabolic events account for the low $^3H:^{14}C$-ratios observed for (**10**, R = PBB). However, methanolysis of (**10**, R = PBB) in the presence of dibenzylamine isolates the β-lactam carbons as the crystalline derivative (**20**) (Table 1).[13,14] The data in the last column for this derivative show excellent complementarity, the glycerol *pro*-(1*S*) label (**4**, H_A) being largely lost while the *pro*-(1*R*) tritium (**4**, H_B) is largely retained in clavulanate.

‡ While expected, based on ample precedent, the structure assigned to (**13**) was supported by independent ^{1}H n.m.r. experiments with the corresponding deuteriated materials. At 400 MHz the methylene hydrogens of glycerol acetonide give four well separated doublets of doublets (C-1: δ 3.59 and 3.74, J_{gem} 11.8 Hz; C-3: δ 3.79 and 4.04, J_{gem} 8.3 Hz). The deuteriated material (i) corresponding to (**13**) showed



disappearance of the resonance at δ 3.74 and significant broadening of that at the δ 3.58. Equilibration of the acetonide in acetone (cat. dry *p*-$MeC_6H_4SO_3H$, 3 Å molecular sieves, room temp., 5 days) gave a mixture of (i) and (ii) where the site of deuteriation in the latter could be securely assigned to the (3*S*)-locus [T. D. Inch and N. Williams, *J. Chem. Soc.* (*C*), 1970, 263].

In conclusion, the normal metabolic course of glycerol to the glycolytic intermediates (**6**) and (**7**) is borne out in the biosynthesis of clavulanic acid in that the *pro*-(*R*) hydroxymethylene of (**4**, $-CH_AH_BOH$) becomes C-5 in (**10**). The absolute sense of chirality at this centre is maintained through the biosynthetic pathway to label clavulanate with overall retention of stereochemistry at C-5. The diastereotopic tritium labels in (**4**, H_A and H_B) do not become homotopic, thereby ruling out the intermediacy of, *e.g.*, free pyruvate. The observation of stereochemical retention in the β-lactam ring closure of clavulanic acid is an important parallel to the oxidative cyclizations that characterize penicillin and cephalosporin biosynthesis.[4,5] The extent of the similarities between these two pathways will be further defined in due course.

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