Synthetic studies towards peptidyl nucleoside antibiotics: first synthesis of a polyoxamic acid derivative enabling direct coupling with α-amino acid esters

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The reaction of the Mukaiyama's aldehyde-derived *N*-benzyl imine with an hydroxyketene equivalent followed by exposure of the resulting α-hydroxy β-lactam to NaOCl and 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO) provides a novel α-amino acid *N*-carboxy anhydride formally derived from polyoxamic acid.

The polyoxin **1** family of nucleoside antibiotics, Fig. 1, has as a common structural feature a dipeptide comprised of an unusual hydroxylated α-amino acid, commonly named polyoxamic acid **2**, linked to one of the related nucleoside α-amino acids.[1] As a consequence of the potent antifungal activity associated with this type of peptidyl nucleosides[1,2] many methods for the preparation of **2** have been proposed over the last few years.[3] However, most of them involve a large number of steps and, therefore, a practical short route to this amino acid still remains of interest. On the other hand, and most important, all of these methods deal with the synthesis of the amino acid in its free form rather than with the generation of a simultaneously amino-protected and carboxy-activated species ready for a subsequent acylation reaction. Consequently, it would be more desirable and also a conceptually new approach to peptidyl nucleoside antibiotics if the synthesis of the polyoxamic moiety could be combined with a peptide coupling step.[4] Here we disclose our initial results on the first synthesis of polyoxamic acid that fulfils all of these requirements.

As Scheme 1 illustrates, the approach takes advantage of the facile construction of an α-hydroxy β-lactam *via* standard [2 + 2] cycloaddition of hydroxyketene equivalents with imines,[5] followed by its quantitative conversion into an α-amino acid *N*-carboxy anhydride (NCA) according to our recently reported method.[6] For the first step of the synthesis we elected to use the imine **3** derived from Mukaiyama's aldehyde, already incorporating the trihydroxylated subunit of the desired amino acid and, at the same time, providing chirality to the corresponding NCA precursor.† Thus, the cycloaddition reaction of acetoxyketene, generated from acetoxyacetyl chloride and triethylamine, with the imine **3** and subsequent saponification of the acetoxy group of the resulting crude product furnished the crystalline α-hydroxy β-lactam **4** [mp 98–102 °C, $[\alpha]_D^{25}$ −12.9 (CH_2Cl_2, *c* 1.0)] in 90% yield over the two steps. Likewise, the reaction of benzyloxyketene, generated from benzyloxyacetyl chloride and triethylamine, with the imine **3** gave **5** [mp 74–76 °C, $[\alpha]_D^{25}$ +1.6 (CH_2Cl_2, *c* 1.0)] in 85% yield. In each case a single diastereoisomer was produced as judged by ^{1}H NMR analysis of the crude reaction products. The relative *cis*-disposition of the substituents at the C_3 and the C_4 positions of the β-lactam ring was determined by the value of the proton NMR coupling constant ($J_{3,4}$ = 5 Hz) and the absolute configuration of both β-lactams **4** and **5** was primarily established by the assumption of an uniform stereochemical outcome with regard to that observed in closely related reactions.[7,8] Further, chemical correlation of both **4** and **5** with the known 4-formyl derivative **6**[8] confirmed the assignment.

To complete the synthesis of the polyoxamic acid skeleton the 3-hydroxy β-lactam **4** was converted into the NCA **7** by using a solution of commercial bleach and a catalytic amount of 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO). The transformation occurred almost instantaneously (1–2 min)‡ *via* an intermediate α-keto β-lactam which underwent chemoselective Baeyer–Villiger rearrangement to produce **7** in almost quantitative yield in a single pot operation. That is, from the readily available imine **3** a simultaneously amino-protected and carboxy-activated form of polyoxamic acid is obtained in 90% overall yield. From this approach two key elements are especially noteworthy. First, the creation of the α-amino stereogenic centre of the polyoxamic acid with virtually complete diastereoselectivity as compared with the reported procedures employing Mukaiyama's aldehyde as the starting

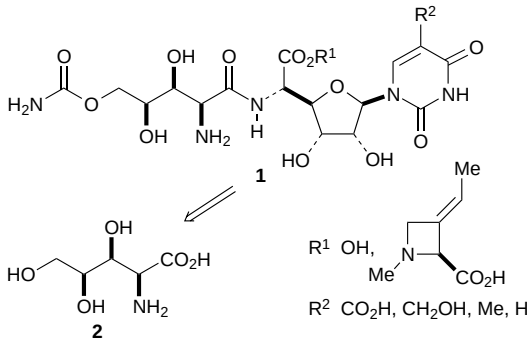
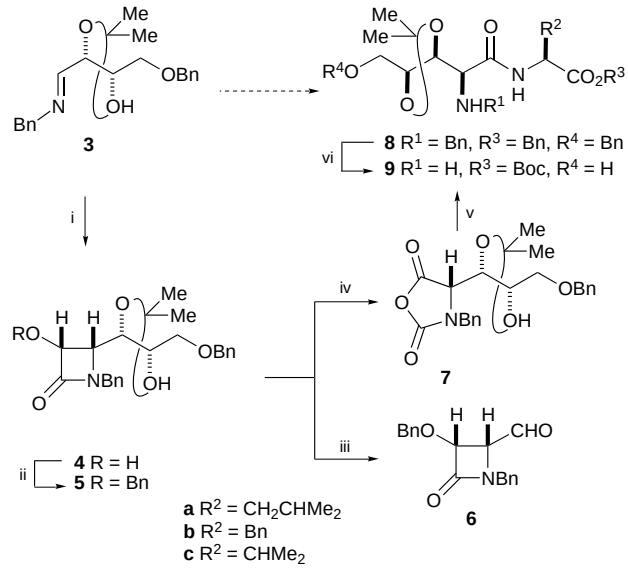


Fig. 1



Scheme 1 *Reagents and conditions*: i, $AcOCH_2COCl$, Et_3N, CH_2Cl_2, 0 °C → room temp., 16 h then LiOH (6 equiv.), H_2O_2 (18 equiv.), THF–H_2O, 0 °C, 3 h, 90% or $BnOCH_2COCl$, Et_3N, CH_2Cl_2, 0 °C → room temp., 16 h, 85%; ii, BnBr, NaH, DMF, −20 °C; iii, **5** with HO_4Cl, wet THF, room temp., 2–4 h, then $NaIO_4$, H_2O–Me_2CO, 0 °C, 2 h; iv, **4** with NaOCl, CH_2Cl_2, TEMPO (cat.), $NaHCO_3$, KBr, KH_2PO_4–K_2HPO_4 (pH 7.0), 0 °C, 1 min; v, (*S*)-$H_2NCH(R^2)CO_2Bn$, CH_2Cl_2, room temp., 24 h; vi, H_2, Pd–C (10% w/w), $(Boc)_2O$, EtOH, room temp., 24 h

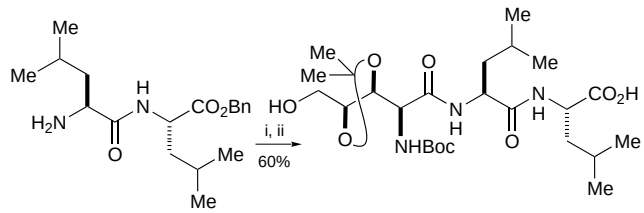
material[3c–g] and, second, the generation, for the first time, of the polyoxamic acid **2** as an active species allowing further coupling reactions. On pursuing this latter aspect, however, the coupling of **7** with an α-amino acid ester was essentially a question of whether epimerization at the α-centre of the NCA **7** could take place; if not, the approach would open up a new and short route to peptidyl nucleoside antibiotics. We were gratified to observe that the reaction of freshly prepared **7** with (*S*)-leucine benzyl ester in dichloromethane as solvent at room temperature for 24 h proceeded with no appreciable epimerization§ to give the desired peptide **8** in 85% yield. The reaction of **7** with both (*S*)-phenylalanine and (*S*)-valine benzyl ester also led to the formation of the corresponding coupling peptides in 86 and 90% yield, respectively. The peptide products were then transformed into their Boc-derivatives **9** for characterization purposes.

The reaction time for the coupling process could be shortened by using a more polar solvent such as DMF, but under these conditions isomerization at the C_α of the NCA occurred¶ giving rise to an equimolar mixture of epimers. This observation prompted us to examine further the influence of the nature of the solvent upon the degree of isomerization. To this end we carried out parallel experiments using an array of common solvents with different polarities. As the results in the Table 1 show, an increase of μ, the dipolar moment of the solvent, leads to increased isomerization, the largest isomerization being when the coupling was performed in HMPA. It thus appears that dipeptide products with the unnatural configuration at the C_α position of the polyoxamic acid might also be obtained from this approach, albeit some further improvement would be required. Finally, as Scheme 2 illustrates, the method can also be applied to the synthesis of tripeptides containing the polyoxamic framework as part of the backbone. Therefore, it is expected that more complex amino acid derived peptides could be made accessible from this approach without the necessity to previously prepare each individual non-proteinogenic α-amino acid.

Table 1 Solvent effect on the coupling of **7** with (*S*)-LeuOBn[a]

Entry	Solvent	μ[b]	**8** : *epi*-**8**[c]
1	Et_2O	4.34	100 : 0[d]
2	CH_2Cl_2	5.17	100 : 0[d]
3	MeCN	11.48	85 : 15
4	$MeNO_2$	11.88	92 : 8
5	DMF	12.88	50 : 50
6	Me_2SO	13.00	51 : 49
7	HMPA	18.48	28 : 72

[a] Reactions conducted on a 0.25 mmol scale at room temp. for 24 h in the corresponding solvent (2.5 ml); **7** : (*S*)-LeuOBn, 1 : 2. [b] From C. Reichardt in *Solvent Effects in Organic Chemistry*, ed. H. F. Ebel, Verlag Chemie Gmbh, Weinheim, 1979, p. 270. [c] Determined by ^{1}H NMR analysis of the crude products. [d] Corroborated by HPLC analysis.



Scheme 2. *Reagents and conditions*: i, **7**, CH_2Cl_2, room temp., 24 h, 80%; ii, H_2, Pd–C (10% w/w), Boc_2O, EtOH, room temp., 24 h, 75%

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Footnotes

† For a conceptually different β-lactam route to the unnatural enantiomer of polyoxamic acid, see ref. 3(*k*).

‡ Prolonged exposure of **4** to the reaction conditions causes a reduction in chemical yield.

§ Under these reaction conditions the degree of isomerization was determined to be less than 0.5% by HPLC analysis of the crude reaction nature and comparison with the chromatogram of a sample containing the mixture of epimers, the latter prepared by carrying out the coupling reaction in DMF as solvent, see text.

¶ The possibility of the isomerization of the other labile Cα stereocentre of the amino ester residue under these reaction conditions was ruled out by comparison of the HPLC retention times and NMR spectra of the crude product obtained by coupling of the NCA **7** with both optically pure and racemic amino esters.

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