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TRI- AND TETRA-PEPTIDES INCORPORATING AN α-AMINO ACID AT THE ANOMERIC POSITION OF MANNOFURANOSE

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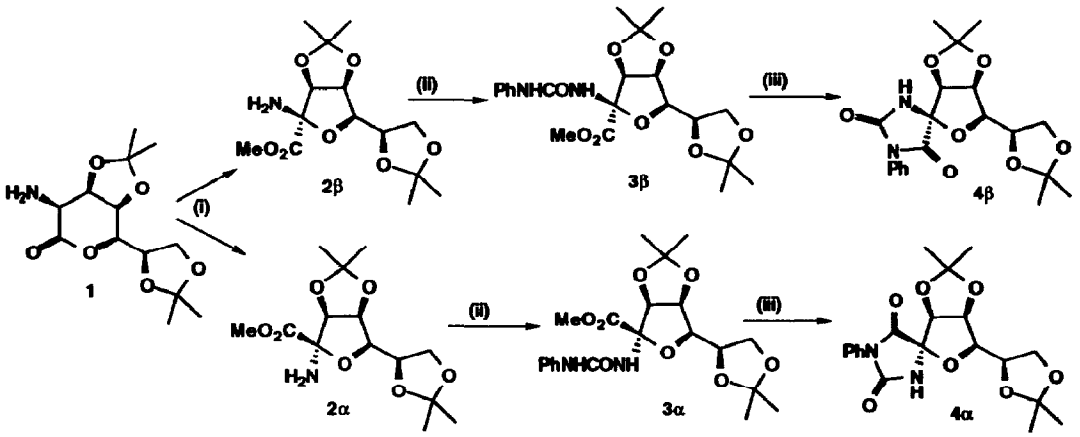
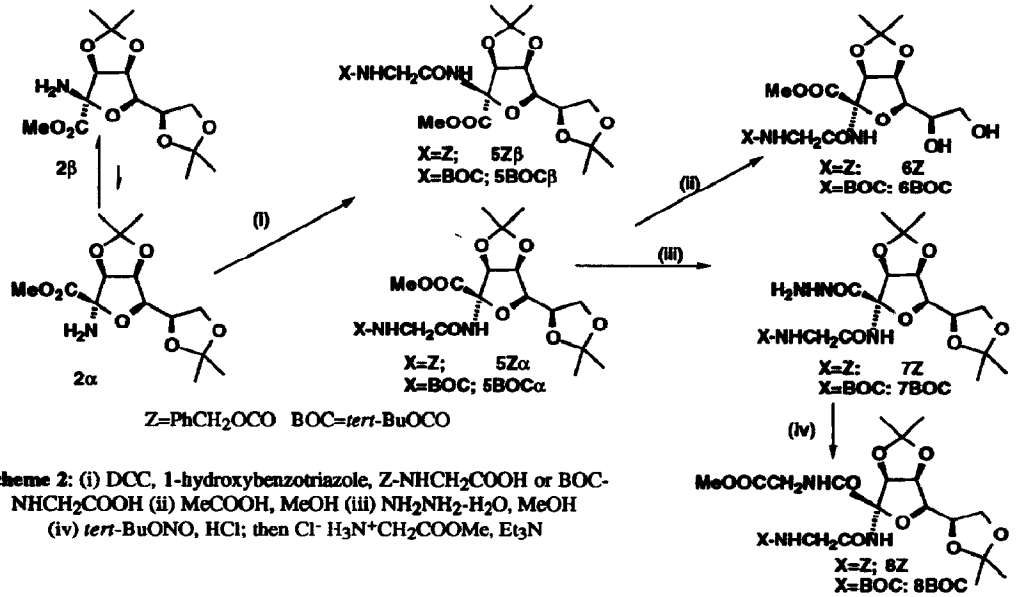
Abstract: The first examples of peptides containing an α-amino acid residue in which the α-carbon is also the anomeric position of a carbohydrate are described.

The bromine-induced oxidative ring contraction of the aminolactone **1** to give the epimeric amino esters **2β** and **2α** in 60% and 17% yields, respectively, was the key step in the synthesis of some mannofuranose spirohydantoins[1] as analogues of the naturally occurring plant growth regulator, hydantocidin.[2] Although **2α** and **2β** slowly equilibrate in solution, reaction of the β anomer with phenyl isocyanate [Scheme 1] gave the urea **3β** which cyclised in methanol to give the protected hydantoin **4β**; the minor α epimer on similar treatment gave **3α** which cyclised to the epimeric hydantoin **4α**, again without any apparent epimerisation. The reaction of the amines with phenyl isocyanate was faster than equilibration of **2α** and **2β**; also, it was clear that equilibration of the epimeric ureas **3** and hydantoins **4** was negligible other than under highly acidic or basic conditions.



Scheme 1: (i) Br_2, NaOAc, MeOH; then Et_3N (ii) PhNCO, THF (iii) MeOH

The chemical stability of **3** and **4**, together with the stereointegrity of the different anomers, indicated that it might be possible to incorporate amino acids at the anomeric position of sugars, such as **2**, as components of peptides. Such amino acids would be of interest, *inter alia*, as N-linked glycoprotein analogues, as α,α-disubstituted amino acids with the ability to control the secondary structure of short peptides,[3] and for the introduction of carbohydrate recognition sites in synthetic peptides. This paper describes the synthesis and chemical stability of tri- and tetra-peptides which have as one of their components an amino acid at the anomeric position of protected and unprotected mannofuranose derivatives.



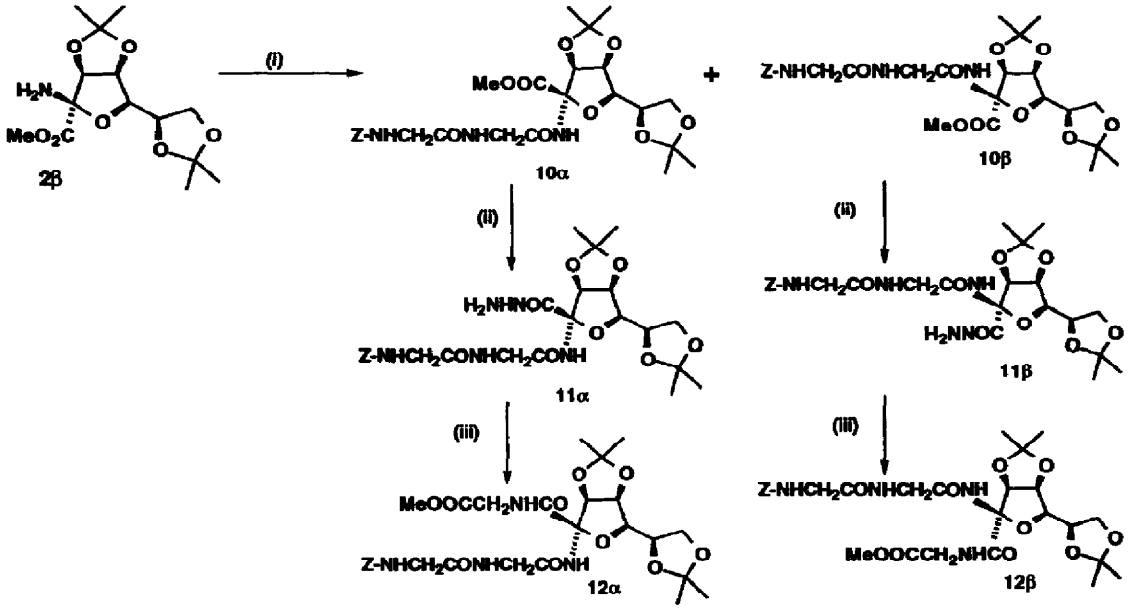
Scheme 2: (i) DCC, 1-hydroxybenzotriazole, Z-NHCH$_2$COOH or BOC-NHCH$_2$COOH (ii) MeCOOH, MeOH (iii) NH$_2$NH$_2$-H$_2$O, MeOH (iv) *tert*-BuONO, HCl; then Cl$^-$ H$_3$N$^+$CH$_2$COOMe, Et$_3$N

The individual epimeric amino esters **2α** and **2β** in dimethylformamide were separately coupled with benzyloxycarbonylglycine [ZGlyOH] activated by treatment with dicyclohexylcarbodiimide [DCC] and 1-hydroxybenzotriazole to give an epimeric mixture of the protected dipeptides **5Zα** and **5Zβ** in 75% to 80% overall yield and a ratio of approximately 10:1 [Scheme 2].The anomeric configuration of **5Zα** and **5Zβ** was determined by measurements of inter-proton distances from quantitative analysis of 500 MHz NMR NOE data.[4] In particular, the mannose NH..H2 distance is diagnostic having possible values of 2.3 to 3.7 Å for *cis* H2/NH and 3.6 to 4.3 Å for *trans* H2/NH; from NMR data, **5Zα** has a NH..H2 distance of 2.4 Å and **5Zβ** a distance of 3.8 Å. In addition **5Zα** gives NH-H3 and NH-H4 NOEs, whereas **5Zβ** gives an NOE between NH and one of the ketal methyl groups. This is consistent only with **5Zα** having a cis H2/NH configuration and **5Zβ** having a *trans* H2/NH configuration..

Since the ratio of **5Z** was the same regardless of which epimer of **2** was used, the rate of equilibration of the amino esters **2** was much faster than their rate of coupling; this is in marked contrast to the reactions in Scheme 1 where the reaction of the amines **2** with phenyl isocyanate was much faster than their equilibration. The thermodynamically less stable - and less hindered amine - **2α** reacts significantly faster with the activated glycine ester. There was no equilibration of the dipeptides **5Z** under the reaction conditions; the anomeric centre of N-acylated derivatives of **2** appears to be configurationally secure under a wide variety of acid and base conditions. The side chain acetonide in **5Zα** was removed by acetic acid in methanol to give the diol **6Z**,[5] m.p. 228-229°C, $[\alpha]_D^{20}$ +92.5 (*c* 0.8, MeOH), in 80% yield; the diol **6Z** could be converted back into **5Zα** in quantitative yield by reaction with 2,2-dimethoxypropane in acetone in the presence of tosic acid.

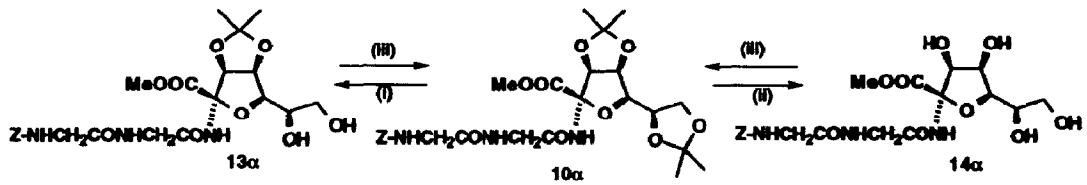
The amines **2** were also coupled with BOC-gly-OH under the same conditions to again give the α anomer **5BOCα** as the major product (75% yield) with a small amount of **5BOCβ** (7% yield). Mild acid hydrolysis of **5BOCα** gave the diol **6BOC**, m.p. 224-225°C, $[\alpha]_D^{20}$ +98.5 (*c* 1.0, MeOH); further acid hydrolysis removed the *tert*-butyl ester rather the second ketal protecting group. No equilibration of the anomeric position of the N-acylated mannofuranose derivatives occurred during any of these transformations.

Further coupling through the C-terminus of the dipeptide **5Zα** was undertaken to demonstrate that amino acids such as **2** could be incorporated into the middle of a peptide sequence. Reaction of **5Zα** with hydrazine hydrate in methanol gave the hydrazide **7Z** as an unstable white solid, $[\alpha]_D^{20}$ +49.4 (*c* 0.5, $CHCl_3$), in 87% yield. Treatment of a solution of **7Z** in dimethylformamide with hydrogen chloride in dioxan, followed by *tert*-butyl nitrite,[6] gave the acyl azide which was reacted *in situ* with glycine methyl ester hydrochloride and triethylamine to afford the protected tripeptide **8Z**, m.p. 227-228°C, $[\alpha]_D^{20}$ +62.0 (*c* 1.0, MeOH), in 80% yield. Similar coupling of **5BOCα** , via the unstable hydrazide **7BOC**, $[\alpha]_D^{20}$ +62.0 (*c* 1.0, MeOH), gave the BOC-protected tripeptide **8BOC**, m.p. 80-90(dec)°C, $[\alpha]_D^{20}$ +52.4 (*c* 0.5, $CHCl_3$), in 70% overall yield.



Scheme 3 (i) DCC, 1-hydroxybenzotriazole, $Z\text{-}NHCH_2CONHCH_2COOH$ (ii) $NH_2NH_2\text{-}H_2O$, MeOH (iii) *tert*-BuONO, HCl; then $Cl^- H_3N^+CH_2COOMe$, Et_3N

The aminoester **2β** was also coupled with ZGlyGlyOH activated by DCC and 1-hydroxybenzotriazole to afford as the major product **10α**, m.p. 99-100°C, $[\alpha]_D^{20}$ +55.0 (*c* 0.66, MeCN), in 74% yield, together with **10β**, an oil, $[\alpha]_D^{20}$ -10.8 (*c* 0.98, $CHCl_3$) in 17% yield [Scheme 3]. The anomeric amine **2α** gave **10α** in 79% yield, together with **10β** in 7% yield. Extension of the tripeptide **10α** from the C-terminal end was effected by initial treatment with hydrazine hydrate to give the unstable hydrazide **11α**, oil, $[\alpha]_D^{20}$ +46.8 (*c*, 1.55 CH_3CN) (68% yield); subsequent conversion to the acyl azide followed by reaction with glycine methyl ester gave the tetrapeptide **12α**, $[\alpha]_D^{20}$ +57.6 (*c* 0.87, MeCN), in 80% yield. Similar transformations on **10β** gave the epimeric tetrapeptide **12β** ,$[\alpha]_D^{20}$ -31.3 (*c* 1.08, $CHCl_3$), in 51% overall yield. No evidence of any epimerisation at the anomeric centre was found during any transformation after the initial acylation of the aminoesters **2**.



Scheme 4: (i) MeCOOH, MeOH (ii) Amberlyst 15 (H^+), aqueous dioxan (iii) $Me_2C(OMe)_2$, Me_2CO, CSA

The stability of the furanose ring under hydrolytic conditions was studied to determine whether completely unprotected sugar analogues could be readily incorporated into peptide chains. The side chain acetonide in **10α** was selectively removed by treatment with acetic acid: methanol (1:1) to give the diol **13α**, colourless oil, $[\alpha]_D^{20}$ +55.6° (*c*, 1.04 $CHCl_3$) in 94% yield [Scheme 4]. Reaction of **10α** with Amberlyst 15 (H^+) ion exchange resin in dioxan:water (1:1) at 60°C removed both ketals to afford the tripeptide **14α**,[7] m.p. >300°C, $[\alpha]_D^{20}$ +53.0 (*c* 0.82, H_2O) in 78% yield. Both **13α** and **14α** could be converted back to **10α** on treatment with dimethoxypropane in acetone in the presence of camphor sulphonic acid (CSA) in 96% and 66% yields respectively. The lack of formation of any **10β** under these conditions indicated there was neither epimerisation at the anomeric centre nor interconversion of the furanose to a pyranose form during any of the hydrolysis or reprotection transformations.

In summary this paper reports for the first time the incorporation of an amino acid functionality at the anomeric position of a sugar into an oligopeptide; such materials appear to be configurationally stable at the anomeric centre to acid hydrolysis conditions, and there is no evidence of equilibration of furanose and pyranose forms. The formation of a spiro-diketopiperazine derived from **5Zα** is described in the accompanying paper.[8, 9]

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[5]All new compounds in this paper have spectral data consistent with the structures proposed. Satisfactory microanalytical data has been obtained for **6Z**, **6BOC**, **8Z**, **8BOC**, **10α**, **10β**, **11α**, **12α**, **13α**; it has not been possible to remove the last traces of DCCurea from **5α** and **5β**.

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[7]Selected data for **14α**: δ_H (D_2O, 200 MHz) 7.22 (5H, bs, H-Ph), 4.94 (2H, s, CH_2-Z), 4.30-4.21 (2H, m), 3.87-3.61 (7H, m), 3.55 (3H, s, CH_3-O), 3.51-3.40 (1H, m); δ_C (D_2O): 128.8, 128.5, 127.7 (3xd, CH-Ph), 91.1 (s,C-2), 79.3, 77.4, 70.1, 68.8 (4xd, C-3,4,5,6), 67.2 (t, C-7), 62.7 (t,CH_2-Z), 53.2 (q, CH_3-O), 43.8, 41.8(2xt, CH_2-Gly); *m/z* (FAB+) 524 ($M+K^+$, 15%), 508 ($M+Na^+$, 22%), 486 ($M+H^+$, 7%), 193 (100%)

[8]Estevez, J. C., Ardron, H., Wormald, M. R., Brown, D., Fleet, G. W. J., *Tetrahedron Lett.*, 1994, **35**, following paper.

[9]This work has been supported by the Spanish Education Secretary (MEC-FPU) and the Xunta de Galicia (for a fellowship to JCE), and a CASE (to HA) graduate award. We are very grateful for helpful discussions with Professor L N Johnson and Dr J H Jones.

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