



Preliminary communication

# A route to new galactosyl peptides: application to the synthesis of a galactosyl pentapeptide analogue of leu-enkephalin

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## Abstract

The syntheses of a new galactosyl  $\alpha$ -amino acid building block and modified peptides including this moiety are described. The versatility of the method is illustrated by the preparation of a galactosyl pentapeptide analogue of Leu-enkephalin. Conformational studies of dipeptide models in the galactose series show an intramolecular hydrogen bond interaction between the backbone and the galactosyl side chain of the models. *To cite this article: F. Coutrot et al., C. R. Chimie 7 (2004).*

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## Résumé

Nous décrivons la synthèse d'un nouveau synthon  $\alpha$ -amino acide galactosylé et de galactopeptides. La synthèse d'un galactopentapeptide analogue de la Leu-enképhaline est réalisée comme exemple d'application biologique. La conformation de dipeptides modèles incluant un motif D-galactose est étudiée et indique une interaction par liaison hydrogène entre la chaîne principale et la chaîne latérale glucidique des modèles. *Pour citer cet article : F. Coutrot et al., C. R. Chimie 7 (2004).*

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**Keywords:** C-glycopeptide; glycosyl amino  $\alpha$ -acid;  $\alpha$ -ketoester; conformation; stereoselective reduction

**Mots clés :** C-glycopeptide ; glycosyl  $\alpha$ -amino acide ;  $\alpha$ -cétoester ; conformation ; réduction stéréosélective

## 1. Introduction

Glycoproteins play essential roles in many biological processes, especially in the domain of recognition sites at the surface of cells.[1] It follows that much

research concerns the synthesis of glycopeptides, which can mimic natural fragments of glycoproteins. In this case, the anomeric carbon of the glucidic moieties is directly linked to the peptide backbone by an *O*, *N*, or *S*-glycosidic linkage via the side chain of a serine or a threonine, the nitrogen of the side carboxamide of an asparagine, or the side sulfur of a cysteine respectively [2]. However, such anomeric links between pep-

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tide and glucide can be sensitive to basic or acidic conditions, as well as towards glycosidases [2,3]. Consequently, many chemists are interested in the synthesis of *C*-glycopeptides which contain a more stable carbon-carbon anomeric link between the glucide and the peptide and are the direct analogues of the fragments of natural glycoproteins [4]. Here we describe the diastereoselective synthesis of a new  $\alpha$ -galactosyl  $\alpha$ -amino acid, in which the alpha carbon of the amino acid moiety is directly linked to the carbon 6 of the pyranose. The preparation of this building block was extended to furanic and linear glucides to constitute a library of molecules, which are homologues of polyoxine scaffolds. Indeed, polyoxines contain an  $\alpha$ -amino acid motif from which the alpha carbon is linked to the carbon 4' of the ribose moiety [5].

Introduction of the galactosyl  $\alpha$ -amino acid building block in peptides was carried out, and as a biological example, we describe the preparation of a galactoenkephalin where the first tyrosine residue is replaced by a galactosyl amino acid chiron. The conformation of a galactose dipeptide model was studied by  $^1\text{H}$  NMR, infrared spectroscopy and molecular modelling, with the aim of investigating the possible

interactions between the glucidic motif and the peptide backbone.

The retrosynthetic route for the glycopeptides is described in Fig. 1. Glycopeptides were prepared from a galactosyl amino acid, from which protections were chosen to be chemoselectively and separately removed. Thus, diisopropylidene ketal was used as a suitable protecting group for glucidic hydroxyls, whereas a benzyl moiety was used to protect the amine function and the acid was protected as an ester.

The galactosyl  $\alpha$ -amino ester **6** was diastereoselectively synthesised from the galactosyl  $\alpha$ -ketoester **4** or  $\alpha$ -hydroxyester **7**. These latter compounds were derived from glycidic ester **2**, which was readily prepared from the protected dialdogalactose **1** using a modified Darzens reaction.

## 2. Results and discussion

### 2.1. Synthesis of galactosyl $\alpha$ -ketoester **4**

As part of our studies, we previously described the direct transformation in very good yields of protected dialdoses into the glucidic pyruvic ester via a glycidic

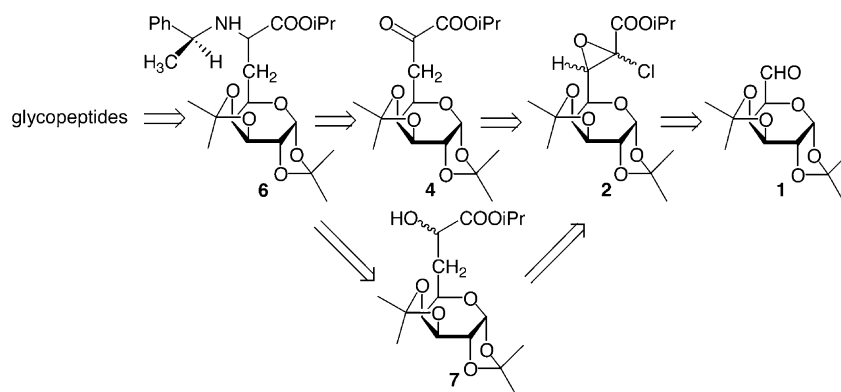


Fig. 1. Retrosynthetic route.

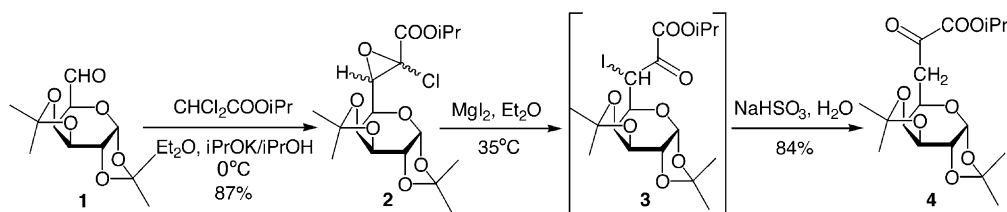


Fig. 2. Preparation of the  $\alpha$ -ketoesters **4**.

ester (Fig. 2) [6]. A modified Darzens reaction using the potassium anion of isopropyl dichloroacetate in isopropanol and diethylether led to the isolated glycidic ester, which was further transformed into the  $\alpha$ -ketoester using magnesium iodide followed by treatment with an aqueous solution of sodium hydrogensulfite. It is interesting to note that the enolic form of **4** was obtained first in the crude, and the tautomeric equilibrium was completely displaced towards the desired ketoester **4** after silicagel column chromatography. We assumed that the mechanism for the formation of the enol from **3** would involve an attack of sodium hydrogensulfite on the ketyl group to yield a bisulfite combination that would lead to an enol sulfate by rearrangement and after hydrolysis to the enol (analogously with a Perkov reaction in phosphorus chemistry between an  $\alpha$ -iodoketone and triethyl phosphite that leads to an enol phosphate).

## 2.2. Reductive amination of glycosyl $\alpha$ -ketoesters

A reductive amination of the  $\alpha$ -ketoester **4** to yield the  $\alpha$ -amino ester **6** was carried out in a first approach [7]. Whereas the use of benzylamine yielded a side product of transamidification, the more hindered  $\alpha$ -methylbenzylamine chemoselectively attacked the ketyl group located on the  $\alpha$  position of the ester, resulting in the formation of imines **5a/5b**, which were not isolated, but directly reduced (Fig. 3). Hydrogena-

tion of the imines in the presence of Pd as a catalyst led to the amino esters **6a/6b**. These aminoesters were synthesised in good to very good yields, depending on the conditions employed in the two steps. However, the diastereoselectivity of the reaction was found to be very low, in contrast to the results observed by Harada for a series of aliphatic  $\alpha$ -ketoesters. Neither the stereochemistry (*R*) or (*S*) of the added  $\alpha$ -methylbenzylamine nor the variation in polarity of the solvent increased the stereoselectivity or the yield. The use of two equivalents of (*S*)- $\alpha$ -methylbenzylamine at reflux in benzene, followed by hydrogenation under pressure using Palladium as a catalyst were found to be the best conditions for this reaction (Table 1).

Interestingly, and in comparison to the pentose series [8], no hydrogenolysis of the benzyl moiety was observed in the galactose series.

The analysis of the crude after imine formation showed a mixture of diastereoisomers due to the presence of *Z/E* isomers of the intermediate imine, which could not be separated due to their sensitivity towards hydrolysis. A non-chelated model described by Harada and applied to the present case could explain the lack of stereoselectivity observed, and indicate that the asymmetric sugar moiety is actually located too far away from the reactive site to participate in the differentiation of the two diastereotopic faces of the imine (Fig. 4).

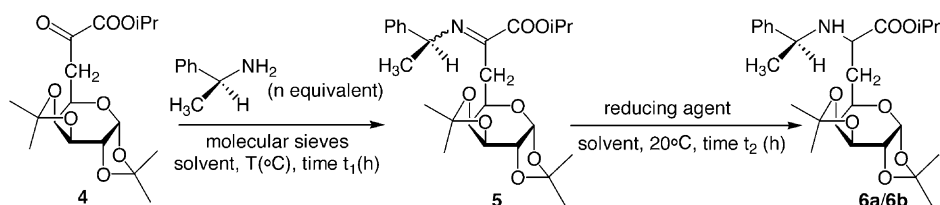


Fig. 3. Reductive amination of glycosyl  $\alpha$ -ketoesters.

Table 1  
Reductive amination of glycosyl  $\alpha$ -ketoesters

Entry	Product	$n^c$	Solvent	$T$ (°C)	$t_1$ (h)	Reducing agent	$t_2$ (h)	Overall yield % <sup>a</sup>	Diastereomeric ratio <sup>b</sup>
1	<b>6a/6b</b>	1	iPrOH	20	24	H <sub>2</sub> , 1 bar, Pd/C	24	30	43/57
2	<b>6a/6b</b>	1	AcOEt	20	24	H <sub>2</sub> , 1 bar, Pd/C	24	36	50/50
3	<b>6a/6b</b>	1	C <sub>6</sub> H <sub>6</sub>	20	24	H <sub>2</sub> , 1 bar, Pd/C	24	0	—
4	<b>6a/6b</b>	2	iPrOH	20	24	H <sub>2</sub> , 1 bar, Pd/C	24	70	49/51
5	<b>6a/6b</b>	2	iPrOH	20	24	H <sub>2</sub> , 10 bar, Pd/C	8	86	49/51
6	<b>6a/6b</b>	2	C <sub>6</sub> H <sub>6</sub>	reflux	13	H <sub>2</sub> , 10 bar, Pd/C	8	92	45/55

<sup>a</sup> Yield of purified products; <sup>b</sup> the diastereomeric ratio was calculated by <sup>1</sup>H NMR (250 MHz); <sup>c</sup> equivalents of (*S*)- $\alpha$ -methylbenzylamine.

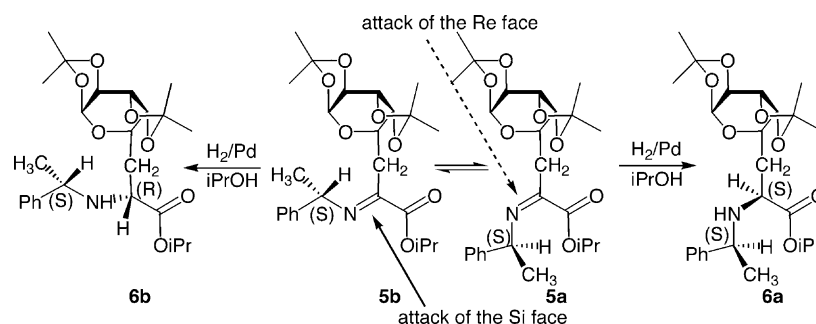


Fig. 4. Stereoselectivity of the reduction of imines **5a** and **5b**.

Nevertheless, it is noteworthy that the two stereoisomers **6a** and **6b** could easily be separated by selective precipitation in diethyl ether. Crystals of **6a** were grown by slow evaporation of a solution of **6** in chloroform and the  $\alpha$  carbon  $C_7$  of the amino ester was found to be (*S*) in the corresponding X-ray crystal structure (Fig. 5) [9].

No intramolecular hydrogen bonding was observed. The structure of the pyranose is not flexible due to the presence of the two isopropylidene ketal groups, and is locked in a twist-boat conformation.

To prevent the side reaction of debenzoylation, which occurred in the less hindered pentose series, a reductive amination using sodium cyanoborohydride was

achieved successfully [8]. Interestingly, a typical one pot reductive amination [10], using  $NaBH_3CN$  and the ketoester **4** was attempted, but proved unsuccessful, since the hydride surprisingly attacked the  $\alpha$ -ketyl group before the formation of the imine, thus leading to the exclusive formation of  $\alpha$ -hydroxyesters. Although the reduction of ketone using  $NaBH_3CN$  was known to require acidic pH as activator [11], it is noteworthy that in this case, the reaction could be carried out in high yield without the use of any activating additive, since the  $\alpha$ -keto moiety was highly reactive due to the presence of the ester at the  $\alpha$  site. This interesting result allowed us to investigate the diastereoselective reduction of  $\alpha$ -ketoester **4**.

### 2.3. Synthesis of galactosyl $\alpha$ -aminoesters via the asymmetric synthesis of galactosyl $\alpha$ -hydroxyesters

In order to improve the stereoselectivity of the synthesis of the glucidic amino ester **6**, a different route was studied, consisting first of a diastereoselective reduction of the  $\alpha$ -keto ester **4**, followed by activation of the hydroxyl group and  $S_N2$  nucleophilic substitution of the created leaving group using sodium azide or (*S*)- $\alpha$ -methylbenzyl amine (Fig. 6) [12]. Since the presence of the glucide moiety could induce a stereodifferentiation of the two ketyl faces, achiral reducing agents such as hydrogen using palladium as a catalyst or metal hydrides were used in a first instance (Table 2).

The highest yield and diastereomeric excess of the reduction using an achiral reducing agent was observed by hydrogenation under one atmosphere using palladium over charcoal as a catalyst (entry 1). The

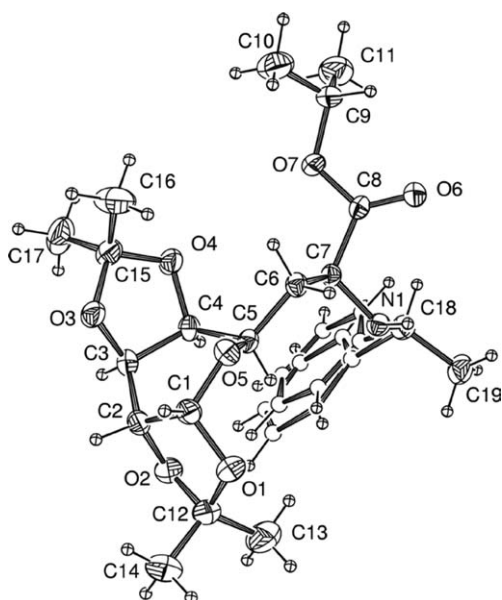
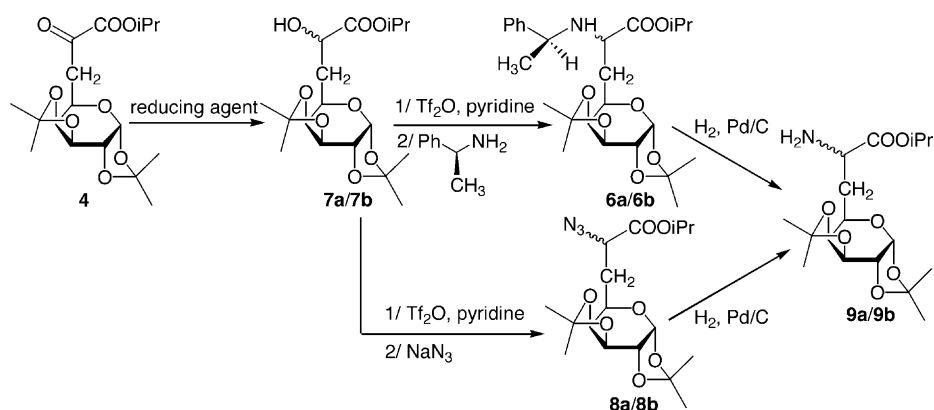


Fig. 5. X-ray crystal structure of **6a**.

Fig. 6. Diastereoselective synthesis of galactosyl  $\alpha$ -aminoester.Table 2  
Diastereoselective reduction of galactosyl  $\alpha$ -ketoester **4**

Entry	Starting substrate	Product	Reducing agent and catalyst	conditions	Yield <sup>a</sup> (%)	Diastereomeric ratio <sup>b</sup> <b>7a/7b</b>	de <sup>b</sup> (%)	C <sub>7</sub> <sup>c</sup>
1	<b>4</b>	<b>7a/7b</b>	H <sub>2</sub> , Pd/C 10%	P = 1 bar 48 h, iPrOH	73	80/20	60	S
2	<b>4</b>	<b>7a/7b</b>	H <sub>2</sub> , Pd/C 10%	P = 50 bar 24 h, iPrOH	85	60/40	20	S
3	<b>4</b>	<b>7a/7b</b>	NaBH <sub>4</sub>	iPrOH, 1 h	47	67/33	34	S
4	<b>4</b>	<b>7a/7b</b>	catecholborane-(R)oxazaborolidine	CH <sub>2</sub> Cl <sub>2</sub> , 18 h	81	95/05	90	S
5	<b>4</b>	<b>7a/7b</b>	catecholborane-(S)oxazaborolidine	CH <sub>2</sub> Cl <sub>2</sub> , 18 h	94	11/89	78	R
6	<b>4</b>	<b>7a/7b</b>	Bakers' yeast	H <sub>2</sub> O, 17 h	44	0/100 <sup>d</sup>	>98	R
7	<b>4</b>	<b>7a/7b</b>	Bakers' yeast	H <sub>2</sub> O, 69 h	61	0/100 <sup>d</sup>	>98	R

<sup>a</sup> Yield of isolated products after column chromatography using a silica gel column; <sup>b</sup> determined on the crude product by <sup>1</sup>H NMR analysis; <sup>c</sup> configuration of the C<sub>7</sub> of the major diastereomer **7**. The absolute configurations of C<sub>7</sub> were determined by the chemical correlation leading to **6a/6b**; <sup>d</sup> only one diastereomer could be observed by <sup>1</sup>H NMR spectroscopy.

$\alpha$ -hydroxyester **7** could then be obtained with a diastereomeric excess of 60%. Although increasing the pressure (entry 2) led to a shorter reaction time, it resulted in the decrease in diastereomeric excess. An attempt using sodium borohydride as the reducing reagent (entry 3) neither improved the diastereomeric excess nor the yield for **7**. The reason for this poor yield was due to a side reaction that occurred between sodium borohydride and isopropyl ester **4**, leading to the corresponding diol. The C<sub>7</sub> configuration of the major diastereomer product **7a** was again (*S*), as a result of an attack on the *Re* side of the  $\alpha$ -keto moiety. This configuration was relatively deduced from the <sup>1</sup>H NMR analysis of **6a/6b** obtained from **7a/7b** with a stereospecific reaction sequence (Fig. 6), the absolute configuration of **6a** being known (Fig. 5).

Preliminary results showed that the carbohydrate moiety induced a relatively moderate asymmetric ef-

fect on the  $\alpha$ -keto moiety of **4**, in contrast to the well-known powerful asymmetric induction of a chiral ester moiety on the reduction of aliphatic alkyl pyruvates in the corresponding  $\alpha$ -hydroxyesters (it has to be noted that the preparation of starting glycidic ester **2** was limited to methyl, isopropyl or *t*-butyl ester (the best results being obtained with isopropyl ester) and did not allowed us to prepare glucidic  $\alpha$ -ketoester with a chiral ester moiety). As a consequence, we studied the asymmetric reduction of  $\alpha$ -ketoester **4** with a chiral borohydride reagent, catecholborane assisted by (*R*) or (*S*)-5,5-diphenyl-2-methyl-3,4-propano-1,3,2-oxazaborolidine as a chiral catalyst auxiliary [13]. By this method, only reported in the ketone series, compound **7a** was obtained with high diastereomeric excess. Changing the catalyst configuration induced an inversion of the diastereoselectivity in favour of the other diastereomer **7b** in approximately the same

ratio, which characterised a stereospecific catalysis (entries 4–5). The diastereomeric excess increased by using chiral reducing agents and resulted in the attack at the less hindered *Re* face of the ketyl group when catecholborane-(*R*) oxazaborolidine was used ( $C_7$  (*S*)) (entry 4), whereas *Si* face appeared more favoured with catecholborane-(*S*) oxazaborolidine ( $C_7$  (*R*)) (entry 5).

These results provided additional evidence for the transition state assembly postulated by Itsuno et al. [14], and verified on the basis of NMR [14a] and X-ray crystallography studies [15]. They illustrated a novel, interesting case of enzyme-like behaviour of such oxazaborolidine that brought together the reductant and the carbonyl substrate [16]. The reduction of  $\alpha$ -ketoester **4** using a large amount of Baker's yeast (*Saccharomyces cerevisiae*) was also studied (entries 6–7). The highest diastereomeric excess (> 98%) was observed, but the reaction was not complete, even after 69 h.

In summary, diastereoselective reduction of  $\alpha$ -ketoester **4** into the corresponding  $\alpha$ -hydroxy ester **7** was achieved using two efficient reagents: the catecholborane-(*S*)- or (*R*)-5,5-diphenyl-2-methyl-3,4-propano-1,3,2-oxazaborolidine which gave galactosyl  $\alpha$ -hydroxyester diastereomers with good diastereomeric excess (78–90%) and in very good yields (81–94%), and the microorganism Baker's yeast which reduced the galactosyl  $\alpha$ -ketoester in a relatively modest yield (61%) but with complete diastereomeric excess (> 98%).

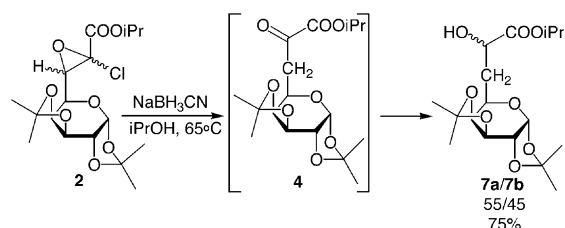


Fig. 7. Preparation of galactosyl  $\alpha$ -ketoesters **7** from galactosyl glycidic esters **2**.

Table 3  
Synthesis of *N*-deprotected building block **9**

Entry	<b>7a/7b</b>	$C_7^c$	<b>6a/6b</b> or <b>8a/8b</b>	$C_7^c$	de <sup>b</sup> (%)	Yield (%) <sup>a</sup>	<b>9a/9b</b>	Yield (%)
1	<b>7a/7b</b> (95/05)	S	<b>8a/8b</b> (05/95)	R	90	60	<b>9a/9b</b> (05/95)	93
2	<b>7b</b> <sup>d</sup>	R	<b>8a</b> <sup>d</sup>	S	> 98	42	<b>9a</b> <sup>d</sup>	98
3	<b>7a/7b</b> (35/65)	R	<b>6a/6b</b> (61/39)	S	22	70	<b>9a/9b</b> (61/39)	97

<sup>a</sup> Yield of isolated products after chromatography using a silica gel column; <sup>b</sup> determined on the crude product by <sup>1</sup>H NMR analysis; <sup>c</sup> configuration of the  $C_7$  of the major diastereomer; <sup>d</sup> only one diastereomer could be seen by <sup>1</sup>H NMR spectroscopy

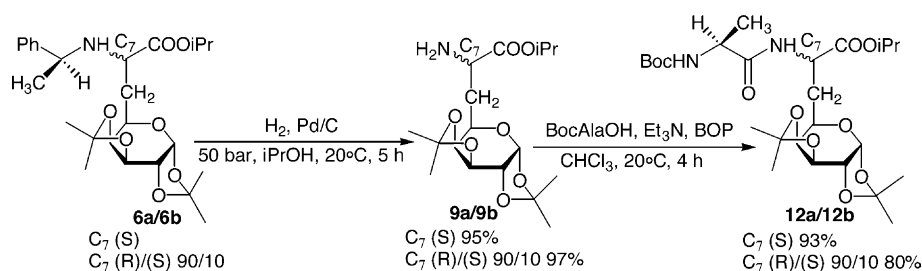
The direct synthesis of  $\alpha$ -hydroxyester from glycidic ester was also studied successfully (Fig. 7) [17]. Published results in aliphatic series indicated that lithium aluminium hydride or Grignard derivatives were not chemoselective for the epoxide and attack occurred first on the ester moiety [18]. In the present case, the use of two equivalents of sodium cyanoborohydride allowed for the chemoselective attack of the hydride at the  $\beta$  position of the ester, leading to the formation of the  $\alpha$ -ketoester **4**, which could not be isolated since it was immediately trapped by the reducing agent. By this route,  $\alpha$ -hydroxyesters were obtained in very high yields in both the glucidic series and the aliphatic series. The use of chiral hydrides to both open the epoxide and reduce **4** is in progress to improve the diastereomeric excess of the reaction.

The hydroxyl of the  $\alpha$ -hydroxyester **7** was then activated by triflation, and submitted to a pure  $S_N2$  reaction using either sodium azide or  $\alpha$ -methylbenzylamine (Fig. 6, Table 3). The azide **8** and the aminoester **6** were hydrogenolysed quantitatively to give **9** (Fig. 6, Table 3).

#### 2.4. *N*- and *C*-deprotections and couplings

To illustrate the synthetic utility of this building block for the preparation of new glycopeptides, *N*- and *C*-terminal deprotections and couplings were investigated. Hydrogenolysis under a pressure of hydrogen of 50 bar during 5 h readily afforded the primary amine **9** in very good yields. The *N*-terminal coupling was then achieved by stirring a mixture of Boc-Ala-OH, Et<sub>3</sub>N, BOP and compound **9** in chloroform for 4 h at 20 °C (Fig. 8).

The *C*-terminal deprotection proved to be difficult. Whereas it was not possible to reach the carboxylate by classical saponification of compound **6** using potassium hydroxide, galactosyl carboxylates could nevertheless be obtained in very good yields in two steps by first carrying out a transesterification of the isopropyl-

Fig. 8. N-deprotection and N-elongation: preparation of dipeptide **12**.

lester into the less hindered methyl ester **10** using MeOLi at reflux in THF/MeOH 1:3, followed by classical saponification using potassium hydroxide at reflux in MeOH/THF 1:3. The carboxylate was not isolated and directly submitted to a peptide coupling with H-Gly-OMe in chloroform at room temperature in the presence of Et<sub>3</sub>N and BOP as the activating agent. (Fig. 9)

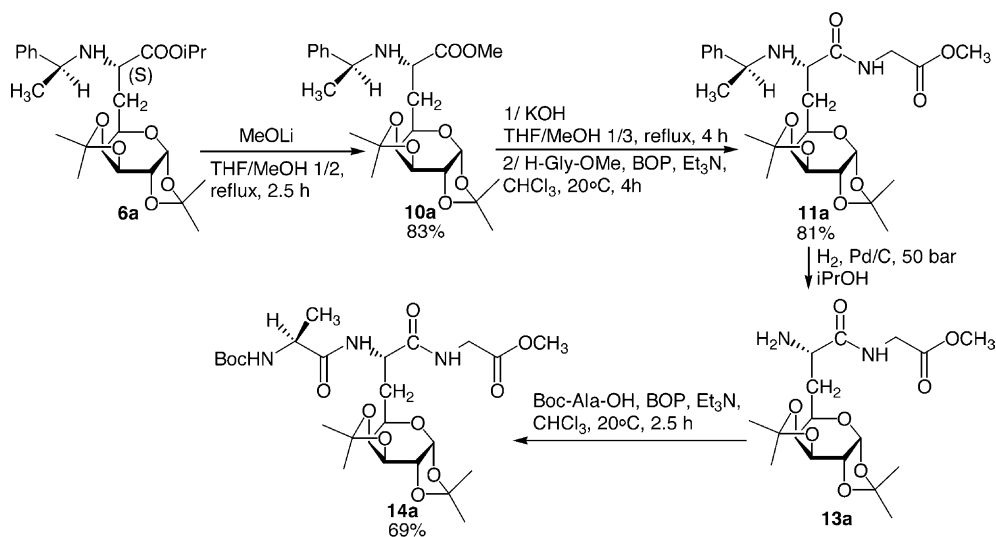
No epimerisation of any of the asymmetric carbons occurred during the reported deprotection and coupling reactions (only one stereomer was observed in <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra on the crude products). To illustrate the versatility of this synthesis of glycopeptides, we proposed the preparation of a tripeptide where the galactosyl moiety is located between two natural amino acids (Fig. 9). Thus, and in order to avoid any racemisation due to the formation of an oxazolone, the elongation of the backbone was achieved at the N-terminal side. Dipeptide **11** was

easily hydrogenolysed to give quantitatively **13**, which was then elongated using Boc-Ala-OH using the same procedure reported above. The preparation of dipeptides consisting of two identical or different glucidic amino acid building blocks was achieved [8] (Fig. 10).

Preparation of the dipeptide derivatives was achieved using the same conditions as above. Despite the bulkiness of the glucidic side chain of the amino acids **6** and **9**, dipeptides **15** and **16** were obtained in very good yields, thus allowing the preparation of a wide range of new glycopeptides.

### 2.5. Glycopeptide synthesis: preparation of a galactoenkephalin

With the aim of obtaining an analogue of a biologically active peptide incorporating a glucidic side chain which would induce conformational restraints on the peptide backbone and serve to protect the peptide

Fig. 9. C-deprotection, C-coupling, and N-elongation: preparation of tripeptide **14**.

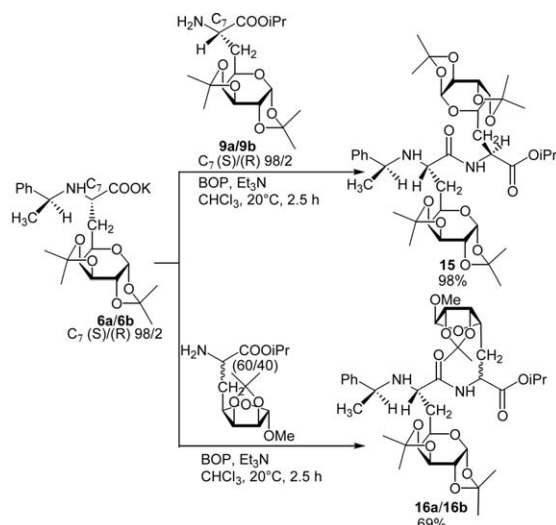
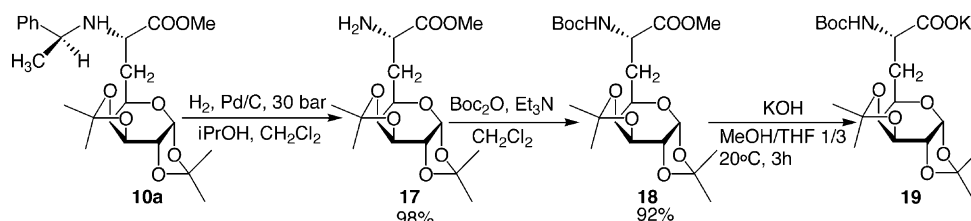
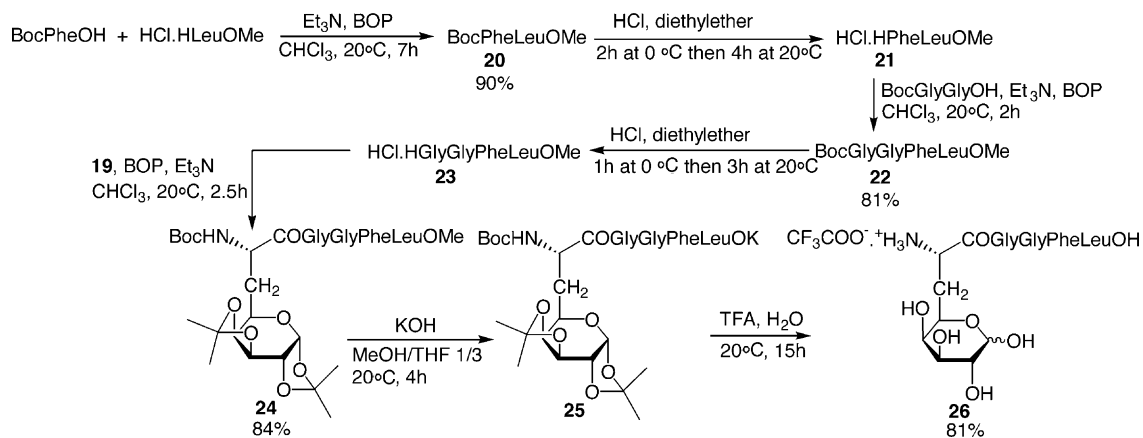


Fig. 10. Synthesis of galactodipeptides.

against enzymatic biodegradation, the preparation of a galactosyl pentapeptide, where the galactosyl moiety replaced the first tyrosine residue of the Leu-enkephalin, was synthesised successfully. Although the *N*-benzyl deprotection was carried out efficiently by hydrogenolysis of compounds **6** and **11** (Fig. 8 and 9), it was not possible to remove the benzyl moiety on the final galactoenkephalin, indicating a possible folding of the peptide backbone which could hinder the *N*-terminal side. As a result, *N*-terminal Boc protection was chosen, which was eventually removed at the same time as the ketal groups. Thus, compound **10a** was hydrogenolysed and the primary amine was then protected using a Boc group (Fig. 11).

The *N*-free amino tetrapeptide **23** was synthesised efficiently *via* classical peptide couplings without any epimerisation. Galactosyl amino acid **19** was elongated at the C-terminal side with **23**, then saponified and hydrolysed to afford the fully deprotected galactoenkephalin **26** (Fig. 12).

Fig. 11. Preparation of the *N*-Boc-protected galactosyl  $\alpha$ -amino carboxylate **19**.Fig. 12. Preparation of the galactopentapeptide **26** analogue of the Leu-enkephalin.



### 3. Synthesis and conformational study of a galactosyl dipeptide model

The conformational restraint as a result of the introduction of a glucide moiety in a peptide was investigated using models of galactosyl dipeptides **27**, **28** and **29** by infrared, NMR, X-ray, and molecular modelling and compared to the corresponding analogues in the leucine series (Fig. 13) [19].

These compounds are commonly denoted model dipeptides since they contain two peptide bonds connected to the  $\alpha$  carbon and are the simplest peptide molecules to study the possible interactions between consecutive peptide bonds.

Compounds **27**, **28** and **29** were readily obtained from building block **10a** in a four-step route: methyl ester **10a** was saponified and submitted to a C-terminal coupling using either isopropylamine or methylamine. The intermediate amide was then hydrogenolysed and amidified at the N-terminal side with PivCl or *i*PrCOCl (Fig. 14).

Crystals of **29** were grown by slow evaporation of a solution of **29** in diisopropylether/dichloromethane (Fig. 15).

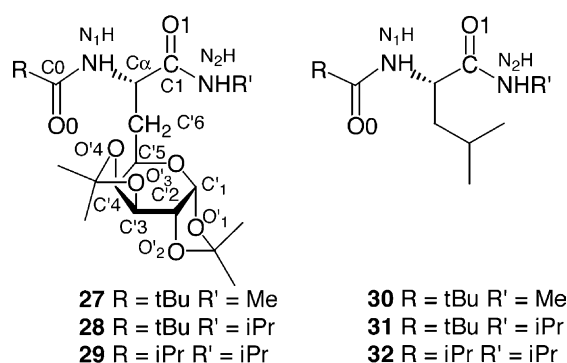


Fig. 13. Model dipeptides

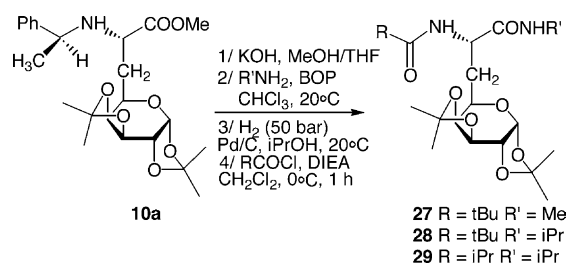


Fig. 14. Synthesis of the galactosyl model dipeptides **27–29**.

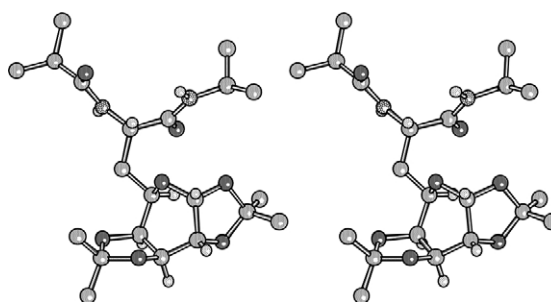


Fig. 15. X-ray crystal structure of **29**.

The peptide part assumes a classical, nearly extended conformation where the NH are intermolecularly H-bonded to the carbonyls of parallel neighbouring molecules. The side chain bearing the carbohydrate is oriented so that the pyranic ring is anti to the peptide nitrogen N<sub>1</sub>. Due to the quasi planarity of the dioxolane rings (Cremer–Pople parameters) [20] which imposes a nearly eclipsed disposition of the C'<sub>1</sub>–C'<sub>2</sub> and C'<sub>3</sub>–C'<sub>4</sub> bonds, the pyranose ring assumes a twist-boat conformation similar to that found for 1,2:3,4-di-*O*-isopropylidene-*L*- $\alpha$ -D-galactopyranose [21]. The four extracyclic oxygens are axially oriented while the bulky peptide part is equatorial. In CDCl<sub>3</sub> solution, the proton vicinal constants for the pyranose ring supports the retention of the twist-boat conformation on the basis of Karplus–Conroy correlation.

The NH stretching frequencies were explored in CCl<sub>4</sub> and CH<sub>2</sub>Cl<sub>2</sub>, two solvents allowing the formation of intramolecular H-bonding in the model dipeptides to occur, and also in the more polar, NH-solvating MeCN and DMSO solvents.

In CCl<sub>4</sub>, the leucine model peptides **30–32** are known to adopt two rapidly interconverting conformations: the major folded C<sub>7</sub> conformation stabilised by an *i* + 3  $\rightarrow$  *i* H-bond to give a 7-membered ring, and the minor extended C<sub>5</sub> conformer containing a weak *i*  $\rightarrow$  *i* H-bond giving a 5-membered ring. The C<sub>7</sub> conformer is characterised by a broad N<sub>2</sub>H absorption at 3330–3360 cm<sup>-1</sup>, depending on the Me/*i*Pr C-terminus. Due to the bulkiness of the *i*Bu Leu-side chain, the minor extended C<sub>5</sub> conformation is denoted by a weak, much less shifted N<sub>1</sub>H contribution (3420–3430 cm<sup>-1</sup>) depending on the *i*Pr/*t*Bu N-terminus. For both conformers, the free, sharp N<sub>1</sub>H and N<sub>2</sub>H absorptions are observed at higher frequencies (3430–3470 cm<sup>-1</sup>), which depend on the branching of the N- and C-terminal

groups and may overlap the weakly H-bonded  $N_1H$  contribution as for **31**. In  $CH_2Cl_2$ , the absorption frequencies are lowered by about  $10\text{--}20\text{ cm}^{-1}$  and the weak contribution for the  $N_1H\cdots O_1$  H-bond ( $C_5$  conformer) completely disappears whereas that for the  $N_2H\cdots O_0$  H-bond ( $C_7$  conformer) is much weaker. Due to NH-solvation by MeCN and DMSO, all the NH absorptions are shifted to low frequencies, indicating that the  $C_5$  and  $C_7$  conformers are not retained in strong solvating media.

The NH absorption profiles for glycopeptides **27–29** essentially differ from the preceding ones by an additional absorption in the  $3400\text{--}3420\text{ cm}^{-1}$  domain, whose frequency depends on the N-terminal group. The  $N_1H$  frequency typical of the intrapeptidic  $i\rightarrow i$  interaction is expected at a higher frequency and does not account for a shift of about  $40\text{ cm}^{-1}$  with reference to the free  $N_1H$  absorption. Previous work on the methyleneoxy peptide Piv-Pro $\psi$ [ $CH_2\text{--}O$ ]Gly-NHiPr indicates that an interaction between the amide NH and the ether oxygen induces a shift of approximately  $40\text{ cm}^{-1}$  for the NH stretching. We therefore conclude that  $N_1H$  in **27–29** is engaged in a rather strong interaction with one of the galactose oxygens, which cannot be easily identified by experimental techniques. We note that the afore-mentioned absorption is still present in  $CH_2Cl_2$  but disappears in MeCN and DMSO.

With the aim of determining the implicated galactose oxygen atom in the  $N_1H$  interaction, molecular modelling has been applied to compound **27**. Among the 500 conformers generated by the SA procedure, those presenting an H-bond between  $N_1H$  and a carbohydrate oxygen were retained and next clustered in two families with regards to the root mean square (rms) deviation for the backbone atoms. The most populated and stable family contains the classical  $i + 3 \rightarrow i$  interaction, observed experimentally by IR spectroscopy and typical of the  $C_7$  conformation. The side carbohydrate is equatorially oriented with reference to the  $\gamma$ -folded peptide chain so that  $N_1H$  is H-bonded to the ketal  $O'_4$  oxygen ( $d_{(H\cdots O)} = 1.94\text{ \AA}$ ), and closes a second seven-membered ring. In a second less populated and stable family, the peptide assumes an  $\alpha$ -helical like conformation, and  $N_1H$  is H-bonded to the  $O'_5$  pyranic oxygen ( $d_{(H\cdots O)} = 2.3\text{ \AA}$ ). The two average conformers extracted from each family (Fig. 16) essentially differ in the  $\psi$  angle and the disposition of the carbohydrate with reference to the peptide chain, denoted by the  $\chi^1$  and  $\chi^2$  torsion angles.

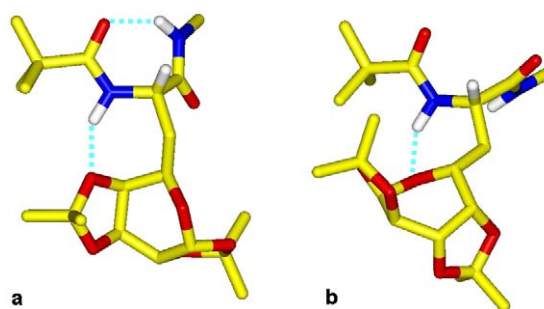


Fig. 16. Averaged modelled molecular structures of **27** showing the peptide to galactose H bonds in family A (a) and B (b)

#### 4. Conclusions

We have described the diastereoselective routes to new glucidic  $\alpha$ -amino acids from dialdoles, and their use in peptide chemistry. These routes have been exemplified in this paper with a galactosyl  $\alpha$ -amino acid. This strategy offers many possibilities to introduce glucidic  $\alpha$ -amino acids in any position along a peptide, and to combine two or more of these compounds together. It allows for the synthesis of a wide library of new glycopeptides which can have potent biological activities.

The influence of the glucide on the peptide backbone has been studied: the glucide plays a role in the conformation of the backbone, which could be useful in the design of new glycopeptides drugs.

The synthesis has been extended to four other series of sugars, differing by their cyclic (pyranic or furanic) or linear structures [8,12]. The versatility and the convenience of the method appear very useful for the preparation of this type of new glycopeptides.

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