

Designing disaccharide derivatives of Leu-Enkephalin for nasal drug delivery

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Introduction

The aim of this study is to chemically modify the opioid peptide leu-enkephalin in order to increase its bioavailability after nasal administration. Opioid peptides are endogenous compounds with potent pain modulating activity but they suffer from poor metabolic stability and membrane permeability.

The chemical modification involved the addition of the lipid and/or disaccharide moiety to the N-terminus of the peptide. The lipid used was a lipoamino acid which is an 8 carbons synthetic amino acid [1]. Lactose and maltose have been chosen as disaccharides. It is believed that their use could increase the interactions between the drug and the glycoproteins of the nasal mucus. In order to make their use in solid phase peptide synthesis possible, they were chemically modified into lactose succinate (4-oxo-4-[(2,3,6-tri-O-acetyl-4-O- β -D-galactopyranosyl)- β -D-glucopyranosyl]amino]butanoic acid) **1** and maltose succinate (4-oxo-4-[(2,3,6-tri-O-acetyl-4-O- α -D-glucopyranosyl)- α -D-glucopyranosyl]amino]butanoic acid) **2** (Fig. 1).

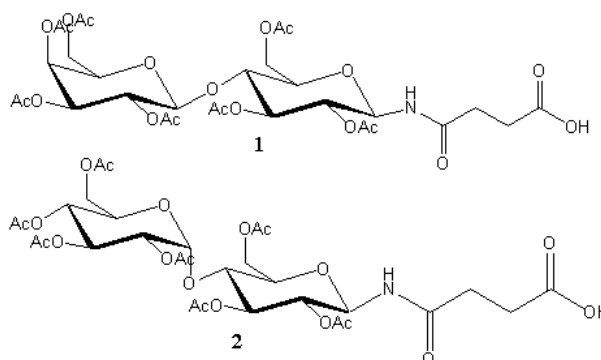


Fig. 1. Structure of Lactose succinate (**1**) and maltose succinate (**2**).

Results and Discussion

Lactose and Maltose succinate were successfully synthesised following the procedure described in Fig.2.

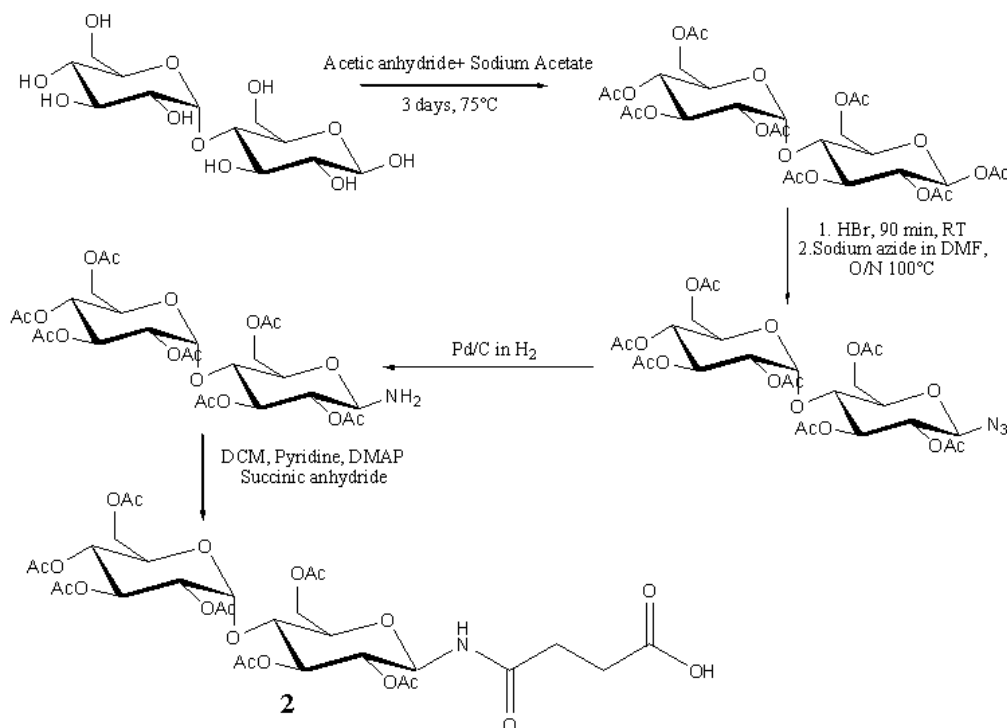


Fig. 2. Synthesis of maltose succinate (**2**). *Synthesis of lactose Succinate (**1**) follows the same procedure.

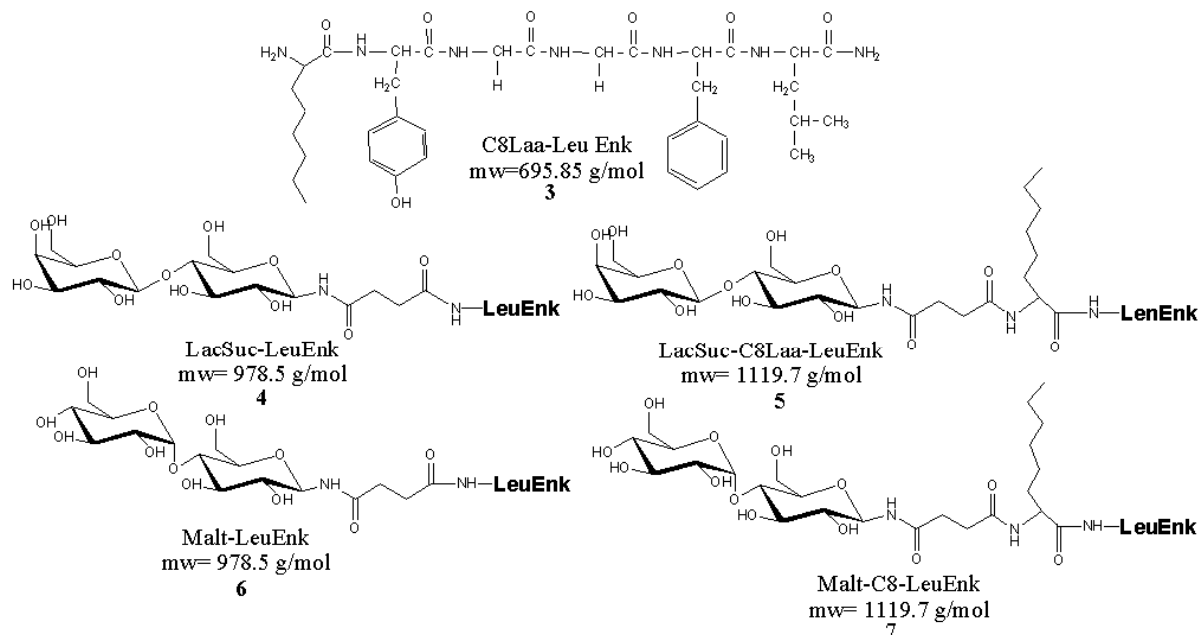


Fig. 3. Leu-enkephalin peptide derivatives

Each of the modified disaccharide was synthesised with a yield of 50% for lactose succinate and 58% for maltose succinate after purification by column chromatography

The purified disaccharides were then coupled to the N-terminus of the leu-enkephalin peptide on solid phase peptide synthesis. Classic Fmoc chemistry was used to synthesise the 5 amino acid peptide onto an MBHA rink amide resin. Once all the amino acids were coupled, a C8 lipoamino acid and/or a disaccharide was coupled using standard coupling reagents (HBTU, DIEA). This resulted in 5 leu-enkephalin derivatives (Fig. 3).

After completion of the synthesis, the peptides were purified by preparative RP-HPLC using a gradient of solvent A (H₂O, 0.01% TFA) and solvent B (90% Acetonitrile, 10% H₂O, 0.01% TFA).

Analytical RP-HPLC retention times of peptides 3 to 7 are reported in Table 1. The maltose derivatives exhibited a higher retention time compared to the lactose derivatives. This increased lipophilicity may be due to the closer proximity of the OH groups on C₃ and C₂' and the glycosidic O atom in the maltose derivative. This proximity allows for increased intramolecular hydrogen bonding compared to that of the lactose derivative, causing the maltose compounds to appear more lipophilic.

Table 1. Retention time of the leu-enkephalins derivatives

Peptide Derivatives	Retention time* (min)
3	15.5/15.9**
4	20.4
5	23.9
6	21.4
7	25.1

* gradient: 10-100% B over 30 minutes

**gradient: 10-100% B over 20 minutes, lipoamino acid synthesised as a mixture of diastereoisomers.

The aim of these modifications is to improve the bioavailability and stability of the peptide leu-enkephalin.

Preliminary results on the caco-2 cell permeability experiment shows an improved permeability for derivative 3 and 4 compared to the native Leu-enkephalin (Fig 4.).

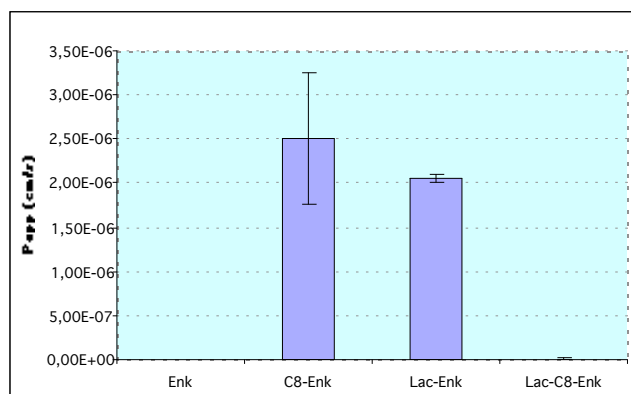


Fig 4. Apparent permeability in caco-2 cell monolayer of Leu-enkephalin [2], C8-Enk (3), Lac-Enk (4), and Lac-C8-Enk (5).

However, compound 5 did not show any significant permeability through the caco-2 monolayer. The detection limit of the LC/MS/MS method was reached after the first sampling time (30min after the start of the experiment).

Preliminary results on an *in vitro* plasma stability assay [3] showed an improved stability for the lipidic derivative 3 as well as for the lactose and maltose derivatives 4 and 6. However, the derivatives combining disaccharide and lipid (5 and 7) showed a low stability, compared to that observed for the native peptide (Table 2.)

Table 2. In vitro plasma stability of leu-enkephalin derivatives

Peptide Derivatives	Plasma Half life (Min)
Leu-Enk [4]	1
3	9
4	40
5	2
6	10
7	2

It is possible that the addition of a bulky group, such as a lipid or a disaccharide, to the N-terminal of the peptide could enhanced the stability of compounds **3**, **4** and **6**. Due to their bulk, those groups would obstruct the action of the enzymes, aminopeptidases, enkephalinases A and B [5] usually responsible for the degradation of Leu-enkephalin. The combination of a lipid and a disaccharide in compound **5** and **7** might stop the action of those enzymes. However, their decreased stability might suggest that this combination also triggers the action of a different enzyme that would cleave the peptide bond between the sugar and the lipid. Efforts are now targeted at identifying the products of the degradation of the liposaccharide derivatives **5** and **7**.

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