New synthetic approach to 1,4-asymmetrically functionalized β-cyclodextrins as drug carriers and receptor mimics via amino acids dipeptides analogues

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Abstract. Cyclodextrins make up a family of well-known, synthetic α-1,4 linked cyclic oligosaccharides that have found widespread use in clinical, industrial and environmental applications. Modified cyclodextrins may also be used as enzyme models for laboratory research. Our study was focused on the selective functionalization of two primary hydroxyl groups in positions 1 and 4 of a β-cyclodextrin, with an Asparagine and a Lysine amino acid residue, respectively.

The project was to control the insertion on the desired positions of the β-cyclodextrin by using a conveniently sized intermediate, obtained by condensation between two suitable derivatives of the two amino acids. This idea ensures at the same time both the functionalization of the right position of the β-cyclodextrin ring and the functionalization of the two positions with the two different amino acids.

Selective protection and functionalization of the two amino acids, in order to have the right substrates to synthetize the intermediate and to perform the insertion in the cyclodextrin, has been the primary goal of our work in the laboratory.

Key words: asymmetric functionalization, β-cyclodextrin, dipeptides, drug carriers, receptor mimics

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides notoriously biosynthesized starting from the starch through an enzymatic process of intramolecular trans-glycosylation by the action of the enzyme cyclodextrin glycosyltransferase (CGTase).[1]

The correct structure of cyclodextrins was discovered by Freudeberg in 1936;[2,3] they are constituted by several units of glucopyranose linked by α-1,4-glycosidic bonds that impart to the molecule the typical shape of a truncated hollow cone.

The three most common structures, isolated for the first time by Villiers(1891) and subsequently classified in 1903 by Scardinger, are the α-cyclodextrin (α-CD), the β-cyclodextrin (β-CD) and the γ-cyclodextrin (γ-CD), respectively constituted by 6,7 or 8 α-glucopyranoside residues.
Certainly, the most important characteristic of cyclodextrins is their ability to form supramolecular inclusion complexes with a wide range of organic substrates (guests). This is due to the fact that the guest migration from the solvent to the hydrophobic cavity of the cyclodextrin (host) is generally a thermodynamically favoured process.

Thanks to this capability, cyclodextrins are widely used as drug carriers, due to their ability to alter physical, chemical and biological properties of guest drug molecules. In particular, this offers a great tool to enhance the solubility of poorly water-soluble drugs. However, this is not the only advantage CDs bring to the pharmaceutical industry and they offer many other features: enhancement of bioavailability; improvement of chemical, physical and thermal stability; reduction of irritation; prevention of incompatibility between different drugs; odour and taste masking; and material handling benefits so that complexation with CDs may convert liquid substances into microcrystalline or amorphous powders.

Cyclodextrins exhibit also catalysis in many organic reactions. This can be divided into two categories: 1) catalysis by the hydroxyl groups, in which the hydroxyl groups of the CD function as intracomplex catalysts toward the substrates included in the cavity of the cyclodextrin; 2) effect of the reaction field, in which the cavity of the cyclodextrin serves as an apolar and sterically restricted reaction field.

However, CDs as enzyme models suffer from a shortcoming, namely their only catalytic group is the hydroxyl group, which restricts the scope of their applicability. Thus, many attempts have been made to introduce other catalytic functional groups into cyclodextrins. In these modified cyclodextrins, the introduced groups function as catalytic sites and the cavities of cyclodextrins serve as the binding sites for the substrates.

**Development of the strategy**

**Preliminary studies**

Some computational studies have shown that the distance between the primary hydroxyl groups in positions 1,4 of a β-cyclodextrin, if suitably functionalized with amino acid residues, allow to build enzyme models particularly useful for the development of new drugs or for the study of interactions between these receptors with some biologically active molecules.

The aim of this project was, first, to develop a synthetic strategy to obtain the β-cyclodextrin 1 difunctionalized in positions 6^A^ and 6^D^ with one unit of L-Asparagine and one of L-lysine, respectively.

A particularly efficient method to obtain disubstituted CDs in the required position is the one that provides a capping of the position
1 and 4 of the cyclodextrin using arenesulfonyl chlorides. In particular, the use of a biphenyl-4,4′-disulfonyl chloride would lead to the synthesis of derivative 2 without the protection of other free hydroxyl groups (Scheme 1).\cite{4}
The idea of a capped cyclodextrin has suggested a possible strategy to introduce a derivative, analogous to the biphenyl-4,4’-disulfonyl chloride, constituted by the two amino acids required for the functionalization of the β-cyclodextrin target, which, thanks to subsequent transformations, allow us to get molecule 1. By computational approximate calculations we found that the distance between two sulfur atoms in molecule 3 is equal to 10.49 Å. Therefore, through the formation of an imine deriving from the two amino acids, it is possible to think about getting a β-CD capped in both positions 1 and 4 as shown in structure 4. Next, with the hydrolysis of the imine, it is possible to get the difunctional cyclodextrin 5. With subsequent oxidation of the aldehyde functionality and amidation, it is possible to obtain molecule 1 (Scheme 2). This strategy allows not only to avoid protection / deprotection steps of the entire cyclodextrin but also to have in the same step at the same time both the two different amino acids bound to the CD.
The dimer could be synthesized by reaction of the aldehyde 6, resulting from the Aspartate, with suitably protected Lysine 7. By performing calculations similar to those conducted for the disulfonyl chloride 3, we can derive the dimensions of this imine being 10.71 Å, a value very close to that of the disulfonyl derivative. For this reason this seemed a potential synthetic strategy (Scheme 3).

Results and discussion

The first goal was to functionalize both amino acids in order to have the right substrates to synthesize the dimer. The first step was the protection of the α-NH₂ of the Lysine. Since this amino acid possesses two NH₂ groups, in order to selectively protect the desired one we have to “lock” the terminal amine by reacting the aminoacid with benzaldehyde forming imine 9. This reaction is almost quantitative since the terminal amino group reacts readily with benzaldehyde both for its position (less steric hindrance) and for its enhanced nucleophilicity with respect to the other NH₂ which is next to an electron withdrawing group. Once imine 9 was obtained, in a single step, the benzyl chloroformate (CbzCl) protection of the free amino group was carried out and, by adding concentrated HCl and heating the reaction mixture, the imine could
be hydrolysed, unlocking the terminal NH$_2$ (Scheme 4). Since different reactions were carried out under acid or basic pH, Cbz as protecting group seemed the best choice because it is resistant to these conditions and is simply removed by Pd/C catalyzed hydrogenolysis.

Scheme 4.

Conversion of the carboxylic acid to its methyl ester derivative was performed afterwards, in order to lower the possibility of side reactions during all the steps of the synthesis (Scheme 5).

Scheme 5.

Functionalization of the L-Asparagine starts with the protection of the amino group with Cbz and conversion to the methyl ester derivative 12 (Scheme 6).

This substrate was treated with t-BuONO to obtain the carboxylic acid derivative N-(Benzyloxy carbonyl)-aspartic acid α-methyl ester (13) from the amide.

The next two steps involve reduction of the carboxylic acid to alcohol and oxidation of the hydroxyl group to the corresponding aldehyde (6) by using Dess-Martin Periodinane (DMP).

Scheme 6.

Experimental Section

Reactions were monitored through thin layer chromatography on Merck silica gel plates Kieselgel 60 F254. The separation and purification of compounds were performed through flash chromatography on silica gel Merck (0,040-0,063 mm). Characterization of products was performed through infrared spectroscopy, $^1$H and $^{13}$C nuclear magnetic resonance. NMR spectra were acquired with a spectrometer Varian Mercury Plus 400, operating at 400 MHz, using CDCl$_3$, D$_2$O and DMSO-d$_6$ as solvents. Chemical shifts are expressed in $\delta$ (ppm) referred to the undeuterated solvent. The following abbreviation are used: s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, tt = triple triplet, m = multiplet, br = broad. Substrates, reagents and solvents were acquired from common commercial sources and used as received. If dry solvents were used, these were dried according to standard procedures.

$N^\varepsilon$-benzylidene-L-lysine (9). To a solution of L-lysine (1.46 g, 10 mmol) in 2N lithium hydroxide (4 ml) at 0°C was added benzaldehyde (0.84 ml, 12 mmol, 1.2 eq.).
reaction flask was stirred at the same temperature until the benzaldehyde had dissolved and a white solid precipitated. After standing in the refrigerator for several hours, the solid was filtered, washed with cold ethanol and dried over CaCl₂ under vacuo for one day to give N²-benzylidene-L-lysine as a white solid in 80% yield. It was used in the next step without further purification. The spectroscopic data of the product obtained are in accordance to the literature.⁶ M.p. = 187-189 °C. ¹H NMR: (400 MHz, D₂O) δH = 1.12-1.29 (m, 2H), 1.43-1.60 (m, 4H), 2.83 (t, 2H), 3.25 (m, 1H), 3.47 (m, 1H), 7.30-7.62 (m, 5H).

N²-carbobenzyloxy-L-lysine (10). N²-benzylidene-L-lysine (1.868 g, 8 mmol) was dissolved in a mixture of 1N sodium hydroxide solution (7.8 ml) and ethanol (7.8 ml) below -5 °C. A cooled mixture of 1N sodium hydroxide solution and ethanol (1:1, 31 ml) and benzyl chloroformate (1.52 ml, 10,4 mmol, 1.3 eq.) were added in two portions over 5 minutes at -10°C with vigorous stirring. The mixture was stirred at the same temperature for 10 min and then at room temperature for further 30 min.

Concentrated hydrochloric acid (2.34 ml) was added and the resulting mixture was heated at 50 °C for 30 min. The mixture was then extracted with diethyl ether (3x20 ml) and the aqueous layer was adjusted to pH 6.2 with a NaOH/KH₂PO₄ buffer. The resulting mixture was concentrated in vacuo to a volume approximately half of the starting volume. After standing in the refrigerator for one day, some white crystals precipitated. The remaining solvent was removed in vacuo and the solid was dried over CaCl₂ in vacuo for one day to give N²-carbobenzyloxy-L-lysine in 78% as a white solid, which was used in next step without purification. ¹H NMR: (400 MHz, D₂O) δH = 1.22-1.41 (m, 2H), 1.49-1.79 (m, 4H), 2.9 (t, 2H), 3.61 (m, 1H), 5.0 (m, 2H), 7.25-7.34 (m, 5H). ¹³C NMR: (100MHz, D₂O) δC = 21.8 (γ-CH₂), 26.0 (δ-CH₂), 30.9 (β-CH₂), 39.0 (α-CH₂), 54.1 (α-CH₂), 66.4 (CH₂-Ar), 127.0 (Ar), 127.8 (Ar), 128.4 (Ar), 135.8 (Ar), 157.6 (NCO-Cbz), 178 (CO).

N²-carbobenzyloxy-L-lysine methyl ester (7). SOCl₂ (0.583 ml, 8 mmol, 4eq.) was added dropwise to MeOH (6 ml) at 0 °C and stirred 5 min. N²-Cbz-L-lysine (0.562 g, 2 mmol) was added and the reaction stirred 15 min at 0 °C and at reflux overnight. MeOH was removed under reduced pressure, and the residue dissolved in sat. NaHCO₃ (50 ml) and extracted with CH₂Cl₂ (3x40 ml). The combined organic layers were dried (Na₂SO₄) and the solvent was removed in vacuo to afford a yellowish oil, which was used in next step without purification. The spectroscopic data of the product obtained are in accordance to the literature.⁷ ¹H NMR: (400 MHz, CDCl₃) δH = 1.45-1.86 (m, 6H), 2.87 (t, 2H), 3.68 (s, 3H), 4.31 (m, 1H), 5.1 (s, 2H), 7.38-7.47 (m, 5H). ¹³C NMR: (100MHz, CDCl₃) δC = 22.3 (γ-CH₂), 26.7 (δ-CH₂), 30.8 (β-CH₂), 40.4 (α-CH₂), 51.9 (OCH₃) 54.7 (α-CH₂), (CH₂-Ar), 127.1 (Ar), 128.1 (Ar) 128.5 (Ar), 157.4 (NCO-Cbz), 174.5 (CO).

N-carbobenzyloxy-L-asparagine (11). To a mixture of L-asparagine (1.32 g, 10 mmol) and MgO (0.86 g, 21 mmol, 2.1 eq.) in 10 ml of H₂O was added in 4 portions at 5 °C benzyl chloroformate (1.713 ml, 12 mmol, 1.2 eq.). After stirring 15 min at the same temperature, the thick reaction mixture was stirred at r.t. for 3 hours. It was then acidified with 2N HCl to pH 1-2, filtered and the solid was washed with H₂O and dried over CaCl₂ giving 2.1 g of product. The entire material was recrystallized from 40 ml of MeOH to yield 1.3 g of N-carbobenzyloxy-L-asparagine. Recrystallization in the same manner of the residue obtained from the concentration of
mother liquors to dryness gave other 0.65 g of the product for a total yield of 73%. M.p. = 164-165 °C. $^1$H NMR: (400 MHz, DMSO) $\delta_H = 2.41-2.58$ (m, 2H), 4.41-4.49 (m, 1H), 5.14 (s, 2H), 7.11-7.41 (m, 6H), 12.23 (br, 1H).

**N-carbobenzyloxy-L-asparagine methyl ester (12).** To a solution of N-carbobenzyloxy-L-asparagine (1.95 g, 7.3 mmol) in dry DMF (18 ml) was added NaHCO$_3$ (1.23 g, 14.6 mmol, 2 eq.) and iodomethane (1.23 g, 14.6 mmol) in dry THF (1 ml) and cooled to 5°C with an ice-salt bath. Borane in THF (1 M, 1.422 ml, 2 eq.) was added dropwise over a period of 30 minutes. The solution was allowed to warm up to room temperature and stirring was continued overnight. After checking the reaction TLC, excess borane was quenched with citric acid (10% w/v, 40 ml) and the mixture was extracted with Et$_2$O (4x50 ml). The combined organic layers were washed with brine, dried (Na$_2$SO$_4$) and the solvent was removed in vacuo to afford a yellow oily crude product, which was subject to column chromatography eluting with Exane:EtOAc 60:40 to obtain pure 14 as a yellow oil in 48% yield. $^1$H NMR: (400 MHz, DMSO) $\delta_H = 1.65-1.7$ (m, 1H), 1.79-1.85 (m, 1H), 3.36-3.46 (m, 2H), 3.61 (s, 3H), 4.18-4.21 (m, 1H), 4.54 (t, 1H), 5.02 (s, 2H), 7.28-7.39 (m, 5H), 7.64 (d, 1H). $^{13}$C NMR: (100 MHz, DMSO) $\delta_C = 33.9$ (CH$_2$OH), 51.1 (NHCHCO), 51.9 (OCH$_3$), 57.1 (CH$_2$OH), 65.6 (PhCH$_2$O), 127.85 (Ar), 127.93 (Ar), 128.48, (Ar), 137.01 (Ar), 156.2 (OCONH), 173.35 (COOCH$_3$).

Conclusions and Future Work

A new strategy for the total synthesis of asymmetrically selectively difunctionalized β-
cyclodextrins was designed entirely by our research group. Next short-term goals will cover surely the completion of the functionalization of amino acids with the last oxidation step and subsequently the formation of the dimer. Afterwards, the attack on the cyclodextrin will be tried with conditions used for the Steglich esterification and, if it’s going to work, hydrolysis of the imine present in the dimer will be performed and the desired amino acids functionalization will be restored. Also, one can concretely think to improve some steps of the synthesis to optimize the reaction conditions and obtain more satisfactory yields.

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References