Tetrahedron Letters, Vol.33, No.2, pp. 209-212, 1992
Printed in Great Britain

0040-4039/92 $3.00 + .00
Pergamon Press plc

Vectorised Transport of Drugs :Synthesis of a New Glycosyl Derivative of β-Cyclodextrin.

Hélène Parrot-Lopez,*[1] Hervé Galons,[1] Anthony W. Coleman,[2] Jacqueline Mahuteau,[2] and Marcel Miocque[2]

[1] Laboratoire de Chimie Organique 2, Faculté de Pharmacie, Université René Descartes, 4 Avenue de l'Observatoire, 75270 Paris Cedex 06 (France)

[2] Laboratoire de Chimie Organique, Centre Pharmaceutique, Université de Paris-Sud, Chatenay-Malabry, 92296 Cedex (France)

Key Words: *β-cyclodextrin; synthesis; spacer; β-glycosamide;*

Abstract: *Monosubstitution at the O-6 position of β-cyclodextrin by a β-N-glucosyl residue has been achieved with a spacer C_9 diamide as the interglycosidic linkage. The new glycosyl derivative has been characterised by ^1H N.M.R. (COSY-RCT), is much more soluble (200g.l^{-1}) in water and has been shown to retain the capacity to include and to enhance the solubility of pharmacologically active molecules.*

The solubilisation and transport of hydrophobic drugs can be readily performed using inclusion in β-cyclodextrin [1] (β-CD). Such systems however, do not possess the capability for molecular recognition within the organism and in consequence the drug delivery is not targeted at a specific site. As glycoconjugates [2] play a central role in cellular recognition, as well as bacterial and viral adhesion, coupling of saccharide antennae to the β-cyclodextrin is a key step in the use of inclusion compounds as vectored carriers of pharmacologically active molecules. Recent work has focused on the direct coupling of carbohydrate groups onto the primary hydroxyl group of the cyclodextrin.[3] The incorporation of such receptor-bound systems into the cell membrane may prove sterically difficult and to avoid the problems of such rigid pivots we have used the flexible C_9 spacer of Lemieux[4] to link the saccharide antenna to the cyclodextrin. In this communication, we describe the synthesis of a novel glycoconjugate* derivative of β-cyclodextrin, its NMR characterization and the retention, by this derivative, of the ability to include and solubilise active molecules.

The β-N-glycosidic linkage [5,6] appears to be particularly important in large number of natural glycoconjugates: membrane glycoproteins, globulins and blood plasma proteins.[7] The model β-cyclodextrin-based system incorporating such a linkage was synthesized as shown in Figure 1.

The reaction of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate [8] 1 with a slight excess of monomethyl nonanedicarboxylate 2 in the presence of 0.1 molar equivalent of triethylamine in dry toluene at

* Glycoconjugates are defined as saccharides bound to a carrier, generally lipids or proteins, in the case we consider the cyclodextrin to be an aglycone molecular transport system.

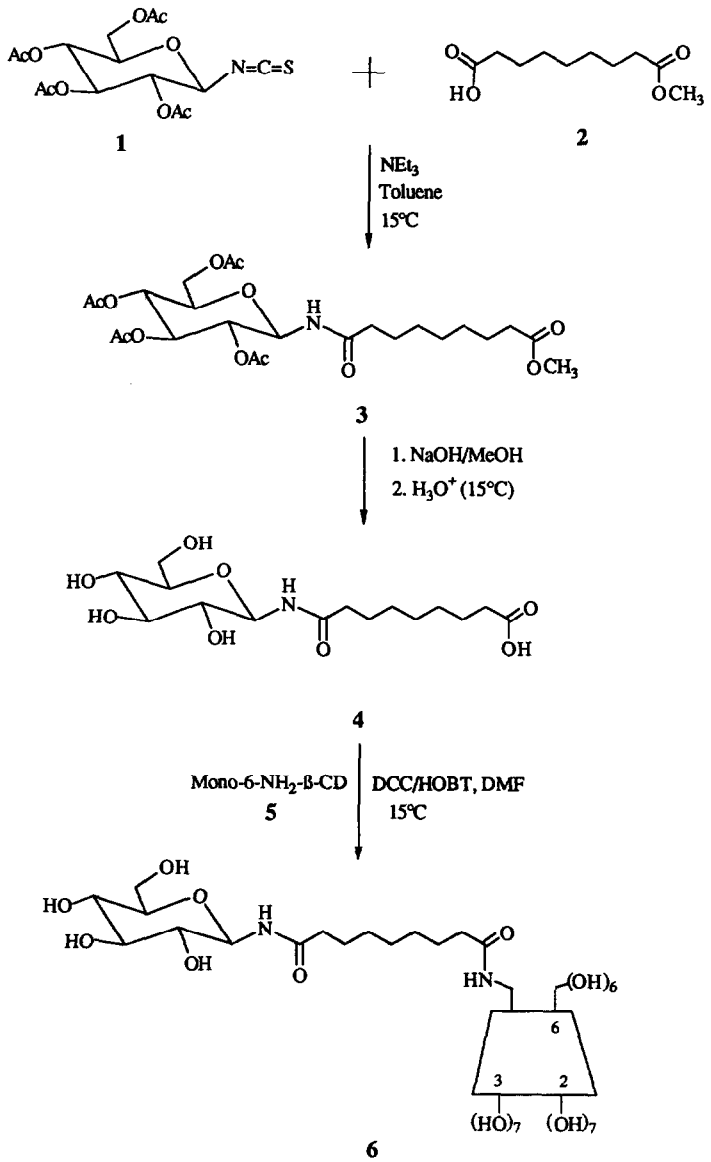
OAc
AcO
AcO
OAc
N=C=S
1
HO
OCH3
2
NEt3
Toluene
15°C
3
1. NaOH/MeOH
2. H3O+ (15°C)
4
Mono-6-NH2-ß-CD
5
DCC/HOBT, DMF
15°C
(OH)6
6
3
2
(HO)7
(OH)7
6

Figure 1

room temperature during 3 days produces the β-glucosylamide 3 as a glass in 46 % yield, after chromatographic separation (eluent toluene / acetone, 9:1).

Deacetylation and de-esterification were carried out by stirring in methanolic sodium hydroxide (1M) for 4 hours to yield the sodium salt of the acid 4. The free acid 4 was released by a simple neutralisation.The β-cyclodextrin model system 6 was synthesized by condensation of 4 (1.2 mmol) with mono 6-amino β-cyclodextrin 5 in N,N-dimethylformamide (DMF) at 15°C using dicyclohexylcarbodiimide/1-hydroxybenzotriazole hydrate (DCC/HOBT) as coupling reagents. Column chromatography using as eluent n-butanol/ ethanol/ water, (5:4:3) allows isolation of the pure β-cyclodextrin derivative 6 in 40% yield.

The NMR analysis of 3 (COSY,RCT) shows the retention of the β-anomeric form with H-1 as a triplet (J H1-H2= 9.6Hz; J H1-NH= 9.6Hz), and a clear distinction in the nonanedioic chain between protons α to the amide and ester functions. A detailed analysis of the ^1H NMR spectra of 6, carried out in DMSO-d_6 and D_2O [11] has allowed assignment of the glucosamide antenna signals, the anomeric proton H-1 appearing in DMSO-d_6 as a triplet at 4.7 ppm, H-2 at 3.05 ppm and H-3 at 3.15 ppm. For systems in which strong interactions occur between the β-cyclodextrin ring and a group linked to the primary face, considerable pertubation of the axial symmetry occurs and there exists strong pertubation of the anomeric H-1 proton signals, leading in extreme cases, such as the auto-included mono-6-phenylalanylamino β-cyclodextrin derivatives, to the observation of 7 doublets for these protons.[9] In contrast for 6 the anomeric protons H-1 are observed as a single non-perturbated doublet; this, coupled with the lack of spectral change between DMSO-d_6 and D_2O, allows us to propose a structure in which there is no interaction between the antenna and the β-cyclodextrin host.

The enhancement of the solubility of β-cyclodextrin (19g.l^{-1}) by substitution with O- and S-glucosyl residues has been reported,[3] with the β-anomers having generally much lower solubilities (S-β, 30g.l^{-1}) than the α-anomers (S-α, 430g.l^{-1}; O-α, 970g.l^{-1}). These differences are probably explained by different orientations of the glycosidic group with regard to the solvent. We have observed for the β-NHCO-hydrocarbon spacer linked 6, an enhanced solubility of 200g.l^{-1}, intermediate between the two cases, as the lipid spacer chain-water interactions will disfavour solubilisation. This solubilisation supports a conformation of the carbohydrate antenna in which it may interact freely with the external environment.

In view of the above, 6 should retain the inclusion properties of β-cyclodextrin coupled with increased transport capacities. To test this, we investigated the interaction with nicardipine, a anti-hypertensive drug, inclusion of which has already been observed with 2,6-dimethyl-β-cyclodextrin. Comparison of the ^1H NMR spectrum of 7 in D_2O and that obtained in the presence of 6 (1:2), shows upfield shifts (0.2 ppm) of the signals arising from the nitro-phenyl groups of 7, an effect typical for inclusion in the cavity. There is also a considerable gain in solubility with respect to the 2,6- dimethyl-β-cyclodextrin complex.[10]

In view of the above results 6 may represent a model for a new series of β-cyclodextrin derivatives possibly capable of vectoring active molecules, which would have enhanced transport properties coupled with possible molecular recognition functions and a flexible pivot to allow cellular incorporation.We are currently developping other β-cyclodextrin derivatives possessing biologically active saccharide antennae.

References

1) J. Szejtli, J.L. Atwood, J.E.D. Davies and D.D.Mc Nicol, *Inclusion Compounds*, Vol. 2, Academic Press, London, **1984**.

2) J. Montreuil, *Adv. Carbohydr. Chem. Biochem.*, **1980**, *37*, 157-223.

3) J. Defaye, A. Gadelle, A. Guiller, R. Darcy and T. O'Sullivan, *Carbohydr. Res.*, **1989**, *192*, 251-258; S. Cottaz, H. Driguez, *Synthesis*, **1989**, 755-758; P.Fugedi, P. Nanasi, J. Szejtli, *Carbohydr. Res.*, **1988**, *175*, 178-181.

4) J. Lehmann and S. Petry, *Carbohydr. Res.*, **1990**, *204*, 141-144.

5) J. Montreuil, *Angew. Chem. Int. Ed. Engl.*, **1990**, *29*, 823.

6) H. Kunz, *Angew. Chem. Int. Ed. Engl.*, **1987**, *26*, 294-308.

7) R. U. Lemieux, D. R. Bundle and D. A. Baker, *J. Am. Chem. Soc.*, **1975**, *97*, 4076.

8) A. Y. Khorlin, S.E. Zurabyan and R.G. Macharadze, *Carbohydr. Res.*, **1980**, *85*, 201-208.

9) H. Parrot-Lopez, H. Galons, A.W. Coleman, F. Djedaïni, N. Keller and B. Perly, *Tetrahedron Assym.*, **1990**, *1*, 367-370.

10) D.Tsoucaris-Kupfer and M. Legrand, *Proceedings of the 5th Int. Symposium on Cyclodextrins*, Paris **1990**, 464-469.

11) **Chemical and Physical data:** NMR data (200 MHz for ^1H):

2,3,4,6-tetra-O-acetyl-1-N-(8-methoxycarbonyloctanoyl)-β-D-glucopyranosylamide 3, ($CDCl_3$): ^1H: 6.25 (1H, d, J =9.6Hz, NH), 5.28 (1H, t, H-3, $J_{H\text{-}3\text{-}H\text{-}2} = J_{H\text{-}3\text{-}H\text{-}4} = 9.6Hz$), 5.21 (1H,t, H-1, $J_{H\text{-}1\text{-}H\text{-}2} = 9.6Hz$), 3.64 (3H, s, OCH_3), 2.27 (2H, t, H α to ester), 2.15 (2H, m, H α to amide). Rf: 0.59 (toluene-acetone 9:3). **1-N-(8-octanoyl)-β-D-glucopyranosylamide** 4, (DMSO-d_6):^1H: 8.6 (1H, d, J =9.6Hz, NH), 5.1 (4H, brd, OH), 4.6 (1H, t, H-1), 2.05 (4H, t, 2 CH_2 α το C=O). Hygroscopic.

Mono 6-(1-octanoyl-β-D-glucopyranosylamido) amino-6-deoxy-β-cyclodextrin 6, RCT2*(DMSO-d_6): ^1H: 8.2 (1H, d, NH glc.), 8.05 (1H, brd, NH), 5.8-5.6 (14H, m, OH-2 and OH-3 β-CD), 4.9 (1H, d, OH-3 glc.), 4.8 (7H, d, H-1 β-CD), 4.7 (1H, t, H-1 glc.), 4.45 (6H, m, OH-6 β-CD), 3.7-3.2 (H-5, H-4, H-3, H-6,6' β-CD), 3.15 (1H, H-3 glc.), 3.05 (1H, m, H-2 glc.), 2.1 (4H, t, CH_2 α to C=O), 1.3 (4H, brd, CH_2 β το C=O), 1.0 (6H, brd, $3CH_2$). Rf: 0.3 (n-butanol-ethanol-water 5:4:3). m.p.$_{dec.}$= 250°C. (α)$_D^{20}$ = + 134°.

*RCT2 is the double relay correlation transfer experiment.

Acknowledgements: The DRED is thanked for partial support in the context of the " Réseau de Recherche- Innovation Thérapeutique" of the Centre Pharmaceutique of the University Paris -Sud, and CNRS for financial help.

(Received in France 16 September 1991)