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Stereospecific Synthesis of Retinoic Acid glucoconjugates, as Pseudo-Substrates of Epidermal β-Glucocerebrosidase.

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Abstract

The synthesis of glucocerebrosides (precursors of skin lipids) analogues bearing the bioactive compound retinoic acid is described ; the two diastereoisomeric gluco-conjugates glucose-glycerol-retinoic acid are pseudo-subtrates for the title enzyme.

The use of retinoic acid in the treatment of dermatosis such as acne vulgaris [1] suffers from limitations due to local accumulation responsible for undesirable side effects such as skin irritation, erythema, scaling and puritis [2]. To overcome these drawbacks, we developed a delivery strategy based on the enzymatic activity present in the first epidermis layers [3,4], with a specific interest in β-Glucocerebrosidase owing to its prevalence in the formation of skin lipids [5]. The glycosidic bond clivage catalysed by this enzyme in the glucoconjugate of an active compound allows its slow release [4]. Moreover, gluco-conjugation may improve the diffusion and stability of the compound, as shown for retinoic acid by other authors [6].

Working along these lines, the synthesis and the enzymatic assay of a retinoïc acid glucoconjugate close in structure to a glucocerebroside, actual substrate of β-Glucocerebrosidase is described in the present work. This includes a glucose moiety linked at position 1 to a glycerol unit, the two other hydroxyl groups of which bear retinoic acid through ester bonds. The scheme gives the synthetic route and the structure of the two isomeric target compounds. The delivery of free retinoic acid requires the action of a second enzyme, an esterase also present in the epidermis [7]. Owing to the high sensitivity of retinoic acid conjugated double bonds [8], particularly to Lewis acid catalysis used to form the glycosidic linkage and also acid or base used to protect and deprotect the hydroxyl groups on the glucose part, the following strategy was adopted :

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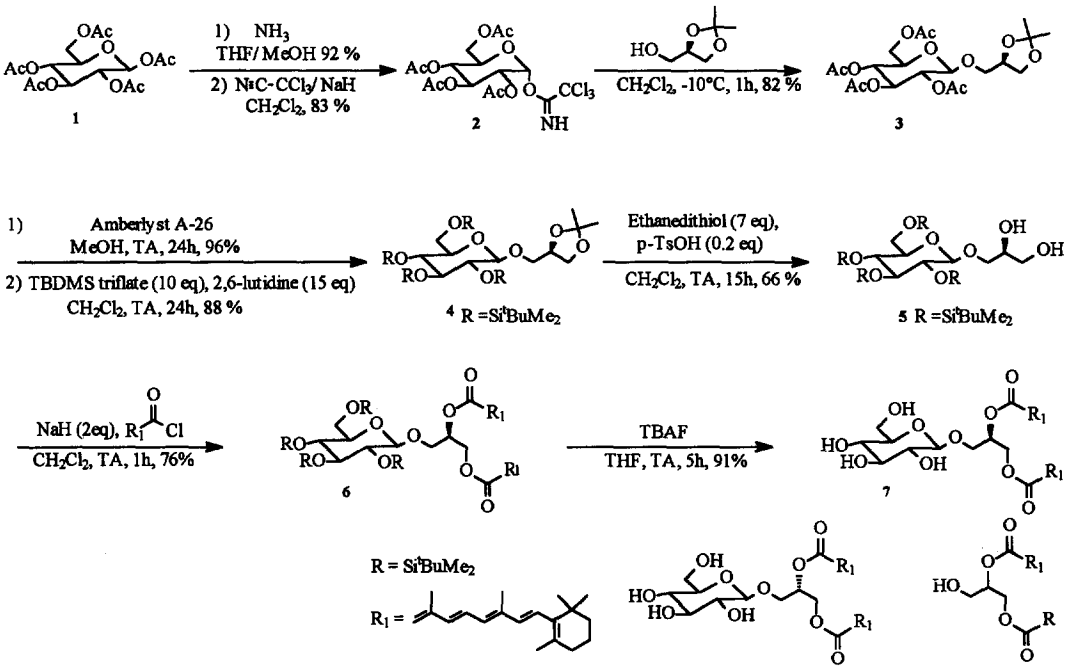
i binding the glycerol unit at C_1 center in glucopyranose protected in the acetylated form ;

ii changing this protection from acetyl to silyl groups bearing bulky substituents more stable in the acid conditions [9] required for acetal deprotection; indeed deprotection of acetyl or benzyl groups in the last step led to low yields and side products in all investigated conditions (basic or acidic for acetyl deprotection, hydrogenolysis on Pd/C or in Birch conditions with lithium or calcium in liquid ammonia for benzyl deprotection) ;

iii solving the key point of acetal deprotection without interference with the protecting groups on the glucopyranose ring ; this was performed via an ethanedithiol reaction ;

iv suitable activation of retinoic acid functions ; this was first done in the form of corresponding acid fluoride since described as a stable and easily handled solid [10] ; however the more reactive acid chloride was preferred since it was stable enough in the short reaction time that allowed the glycerol hydroxyl groups' activation by sodium hydride ;

v deprotection of the silyl groups with treatment by tetrabutylammonium fluoride, without any damage for the remaining part of the molecule



Scheme 1

Specific deprotection at carbon 1 by ammonia in a THF-methanol (7/3) mixture over 20mn in **1** allowed **2** [11], the reaction of which with trichloroacetonitrile in the presence of sodium hydride in dichloromethane leading to the corresponding imidate (C=N : 1676 cm^{-1}) in a 83% yield [12] ; only the α isomer was obtained as indicated by the unique signal in ^{13} C NMR (92.9 ppm) and doublet in ^{1}H NMR at 6.6ppm (J=3.5Hz). Purification was made on a 2 cm silica column with fast elution by a 1/1 hexane-ethylacetate mixture ; no change in structure was observed.

Imidate **2** was then reacted under Lewis acid catalysis with the suitably protected glycerol leading to **3** in the β-configuration ($\delta^{13}C$ = 105 ppm ; $\delta^{1}H$ = 5 ppm , J=8Hz). Acetyl groups in **3** were deprotected using an ion exchange resin (Amberlist A-26) at room temperature over 24 hours [13] ; no change on the glycerol part was observed, signals at 1.39 and 1.33 ppm of the isopropylidene group. The deprotected compound was obtained in a 96% yield after solvent evaporation and vacuum drying. Subsequently, complete silylation with the bulky TEST triflate of the four hydroxyl groups required a large excess of reagent and base, and a 30h reaction time. In the conditions previously described [14], as in many others attempted, only 3 hydroxyl groups were protected. Complete silylation was confirmed by full disappearance of the OH group and the mass spectrum (FAB ; MNa^+ at m/z = 774) on compound **4** (Rf = 0.15 purified by flash chromatography Hex/AcOEt 30/1 as eluant). Several typical reactions for acetal deprotection including hydrogen fluoride in pyridine were then attempted, all leading to partial deprotection of the silyl groups ; a route was found using conditions derived from those described by Williams et al. [15], but by using lower temperature (1.33 mmole of compound **4**, and 9.33 mmoles of ethane-dithiol in the presence of 25mg of PTSA, in 20ml dichloromethane) ; as shown by these authors, the reaction proceeds with configuration retention. Product **5** was purified as for **4**, (Rf = 0.2 ; Hex/AcOEt : 8/1) and characterized by NMR (complete disappearance of the isopropylidene bridge) and mass spectrum (FAB ; m/z MNa^+ = 733). The coupling step leading to **6** resulted from reaction of retinoic acid chloride (formed from the acid by $SOCl_2$, pyridine in dichloromethane, 2h at room temperature νC=O = 1752 cm^{-1}) and compound **5** treated by 2 equivalents of sodium hydride at room temperature 1h in dichloromethane. **6** was purified by flash chromatography (Rf = 0.2 ; eluant Hex/AcOEt 25/1) and characterized by ^{13}C, ^{1}H NMR spectra and mass (FAB ; m/z MNa^+=841). Deprotection by TBAF (3.7 mmoles for 0.26 mmole of compound **6** in anhydrous THF ; 5 h room temperature) led to **7**, purified by flash chromatography (Rf = 0.3 ; eluant CH_2Cl_2 MeOH 95/5) obtained as red crystals. Compound **7** was fully characterized by spectroscopic techniques [16]. The same strategy was applied for the synthesis of the S isomer ; measurements of the optical rotations characterized the two diastereoisomeric structures α_D = 12° (c 0.3 CH_2Cl_2) for the R form **7** ; α_D = -8° (c 0.5 CH_2Cl_2) for compound **8**.

Synthesis of 9

To follow the glycosidic bond enzymatic cleavage in **7** and **8**, the corresponding product **9** (as racemic) was synthetised. This was done by using reactions developed for the synthesis of **7** and **8**, namely by coupling retinoic acid chloride to glycerol protected at position 1 by TBDMS (obtained from isopropolidene glycerol). **9** was purified by flash chromatography (Rf = 0.65 ; eluant Hex/AcOEt 3/2) and fully characterized by spectroscopic analyses [17].

Enzymatic assays

These were performed using an over-expressed β-Glucocerebrosidase [18]. The reaction was followed by HPLC on samples extracted from the reaction medium by ethyl acetate. Results are as follows,

	K_M (μM)	V_M (nmoles.h^{-1}.mg protein^{-1})
7	8.6 ± 2.5	74 ± 7
8	5 ± 0.4	17 ± 0.4

The two compounds have an affinity for the enzyme close to that of a glucocerebroside (K_M in the range of: 10μM), compound 7 (R at C2 in the glycerol part) being hydrolyzed four times faster than **8**. Both are able to deliver retinoic acid although with different rates and the racemic form can be eventually considered for further industrial development.

This strategy is being extended to glucoconjugates of other active compounds with possible association of effects, if the linker (here glycerol) is by itself a biologically active compound.

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(16) **RMN ^{1}H** ($CDCl_3$, 200 MHz) δ ppm: 6.97 (dd, 2H, H-11',11"-C=CH, JJ= 14Hz), 6.3-6.08 (m, 8H, H-7', 7", 8', 8"-HC=CH, H-10',10", 12', 12"-C=CH), 5.74 (s, 2H, H-14', 14"-CH=CH,), 4.37-4.32 (m,2H, H-1,8), 3.96-3.23 (m, 14H, H-2, 3, 4, 5, 2H6, 2H7, 2H9), 2.3 (s, 6H, H-20', 20" -CH_3), 2.03-1.97 (1s et m, 10H, H-19',19" -CH_3, H-4', 4" -CH_2), 1.68-1.36 (1m, 1s, 14H, H-2', 3', 2", 3" -$(CH_2)_2$, H-18', 18" -CH_3), 0.98 et 1 (2s, 12H, H-16', 16", 17', 17" -CMe_2).
RMN ^{13}C ($CDCl_3$, 50 MHz) δ ppm : 167, 166.5 (C-15', 15"), 154.3 (C-13', 13"), 140 (C-9', 9"), 137.7 (C-6,' 6"), 137.3 (C-8, 8"), 135.1 (C-12', 12"), 131.6 (C-11', 11"), 130.4 (C-5', 5"), 129.6 et 128.8 (C-10', 10", 7', 7"), 117.9 (C-14', 14"),103.7 (C-1), 76.1 (C-8), 73.7 (C-3,5), 70 (C-2,4), 68.3 (C-7), 62.5 (C-9), 62 (C-6), 39.6 (C-2', 2"), 34.3 (C-1', 1"), 33.2 (C-4', 4"), 29 (C-16', 16", 17', 17"), 21.8 (C-18', 18"), 19.3 (C-3', 3"), 13.8 et 13 (C-20', 20", 19', 19")

(17) **RMN ^{1}H** ($CDCl_3$, 200 MHz) δ ppm: 7 (dd, 2H, H-11',11"-C=CH, J = 14Hz), 6.36-6.07 (m, 8H, H-7', 7", 8', 8"-HC=CH, H-10',10", 12', 12"-C=CH), 5.8 (s, 2H, H-14', 14"-CH=CH,), 4.36-4.05 (m, 5H, H-2, 2H3, 2H1), 2.33 (s, 6H, H-20', 20" -CH_3), 2.01-1.96 (1s et m, 10H, H-19',19" -CH_3, H-4', 4" -CH_2), 1.7 (s, 6H, H-18', 18" -CH_3), 1.64-1.41 (1m, 8H, H-2', 3', 2", 3" -$(CH_2)_2$,), 1.02 (s, 12H, H-16', 16", 17', 17" -CMe_2).
RMN ^{13}C ($CDCl_3$, 50 MHz) δ ppm : 167.2 (C-15', 15"), 154.3 (C-13', 13"), 140 ;1 (C-9', 9"), 137.7 (C-6,' 6"), 137.3 (C-8, 8"), 134.5 (C-12', 12"), 131.6 (C-11', 11"), 130.2 (C-5', 5"), 129.5 et 129 (C-10', 10", 7', 7"), 117.5 (C-14', 14"), 68.7 (C-2), 64.8 (C-3), 62.2 (C-1), 39.6 (C-2', 2"), 34.3 (C-1', 1"), 33.2 (C-4', 4"), 29 (C-16', 16", 17', 17"), 21.8 (C-18', 18"), 19.3 (C-3', 3"), 14 et 13 (C-20', 20", 19', 19")

(18) We thank Pr D. Fournier for providing the enzyme.