Stereoselective synthesis of D-galactal-derived *N*-ethoxycarbonyl aziridine, as a new, improved synthetic protocol to glycal-derived *N*-activated vinyl aziridines

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Abstract: A new protocol for the synthesis of D-galactal-derived *N*-ethoxycarbonyl vinyl aziridine **1β-CO$_2$Et**, starting from tri-*O*-acetyl-D-glucal, is described. The new protocol constitutes a simple and fast access, with a satisfactory overall yield (5 steps, 36%), to a D-galactal-derived vinyl aziridine with a clear improvement compared with the previously described procedure leading to the structurally related *N*-nosyl aziridine **1β-Ns** which, starting from the same precursor, proceeded through 13 steps with a low, decidedly unsatisfactory, overall yield (3%).

Keywords: *Vinyl aziridines, Glycals, Glycosylation, 4-N-(protected)-O-glycosides, Stereoselectivity*

1. Introduction

Aminosugars, consisting in carbohydrates bearing a free or *N*-protected amino functionality in different ring positions of a glycoside scaffold, are structures widely present in nature,[1] either alone or also as constituents of crucial glycoproteins, glycosaminoglycans and GPI anchors.[2] In particular, 4-aminosugars are found as glycoconjugates, in naturally occurring antibiotics, and their presence has been closely related to the activity of this type of antibiotics.[3] Moreover, following the glycodiversification approach, the synthesis of naturally occurring antibiotic analogs with the original sugars replaced by synthetic ones, currently represents an interesting challenge.[4]

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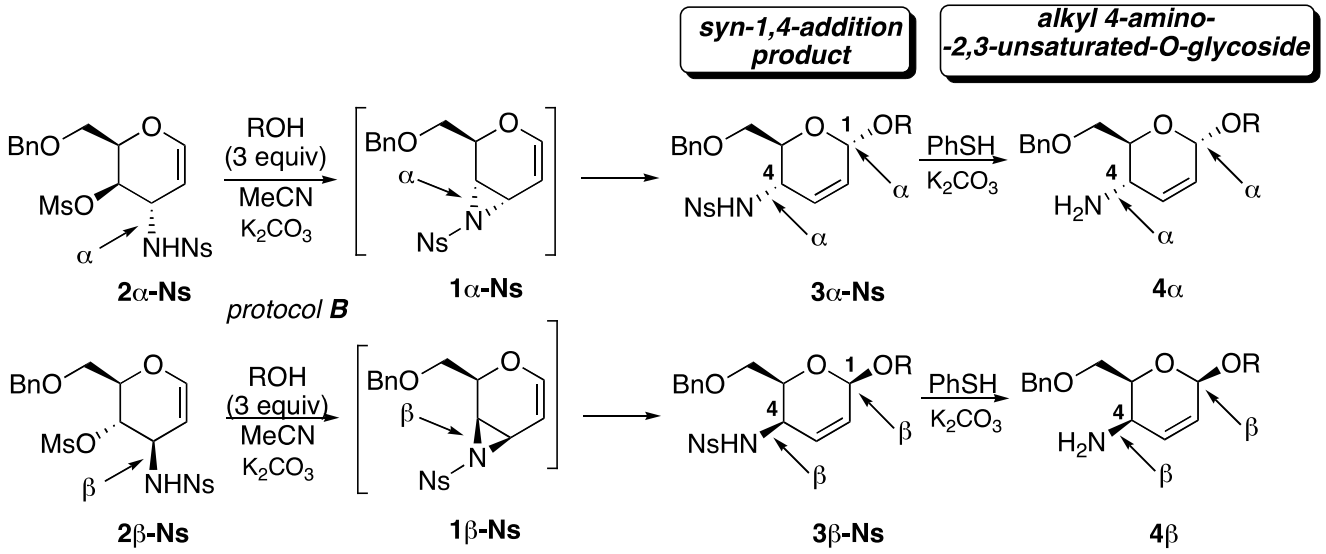
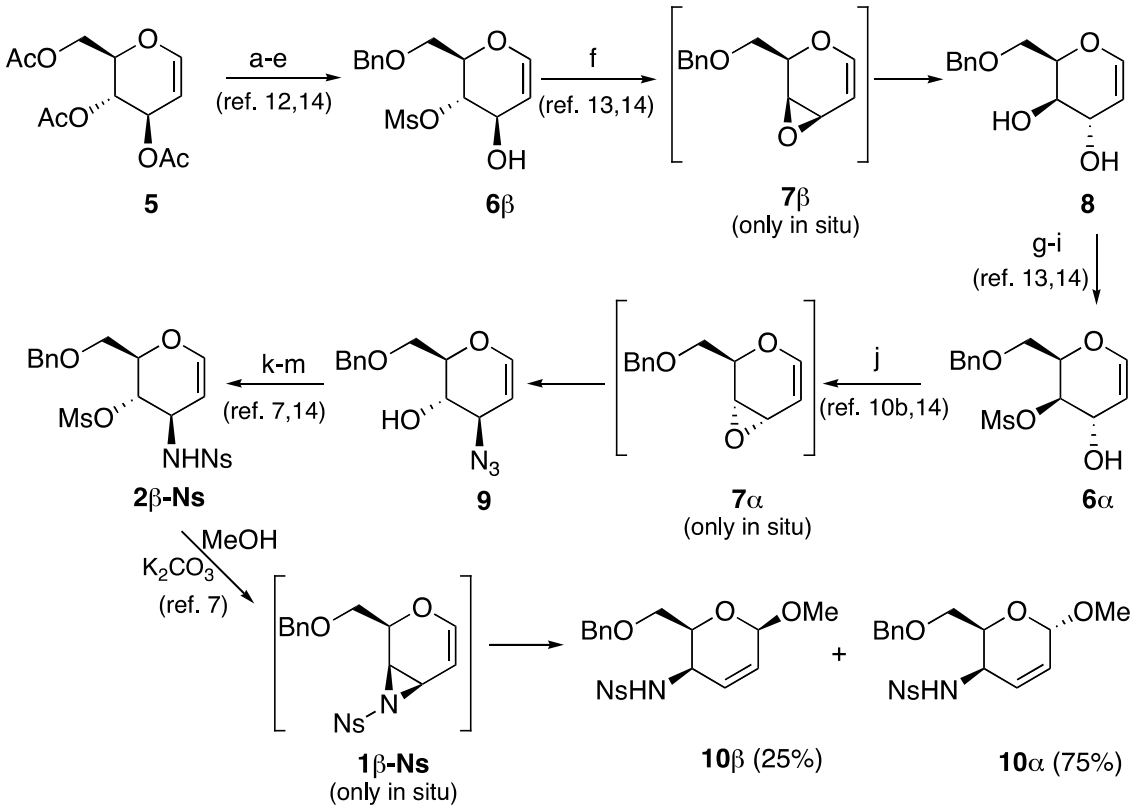
Due to the importance of unusual 4-aminosugars, extensive synthetic studies have been carried out towards the production of these compounds and a divergent synthesis of aminosugar libraries has also been proposed.[5, 6]

In view of the obtainment of a new synthetic protocol to introduce an *N*-protected amino functionality at C(4)-carbon of a pyranoside system with the simultaneous construction of the glicosydic bond, the use of an *N*-protected vinyl aziridine inserted in a glycal system appeared particularly attractive. Actually, we have recently found that D-allal- and D-galactal-derived *N*-nosyl aziridines **1α-Ns** and **1β-Ns**,[7] prepared *in situ* by cyclization of the corresponding stable precursors *trans N*-nosyl-*O*-mesyl derivatives **2α-Ns** and **2β-Ns**, respectively, are very effective glycosyl donors, able to glycosylate (under *protocol B* reaction conditions)[8] *O*-nucleophiles (alcohols, phenol and partially *O*-protected monosaccharides) in a completely 1,4-regio- and syn-stereoselective fashion.[7] In this way, alkyl 4-*N*-(nosylamino)-2,3-unsaturated-*O*-glycosides **3α-Ns** and **3β-Ns**, having the same configuration as the starting azidridine (*syn-1,4-addition products*, Scheme 1), are obtained in an uncatalyzed, directly *substrate-dependent*, stereospecific glycosylation process with simultaneous, completely regioselective introduction at C(4)-carbon of an α- or β-directed *N*-nosylamino group (–NHNs) from aziridine **1α-Ns** or **1β-Ns**, respectively, in a completely stereoselective fashion (Scheme 1).[7,9,10] Considering that alkyl *O*-glycosides **3α-Ns** and **3β-Ns** can be easily deprotected to corresponding 4-amino-2,3-unsaturated-*O*-glycosides **4α** and **4β** with complete retention of configuration, *N*-nosyl aziridines **1α-Ns** and **1β-Ns** can be considered as effective synthetic tools for the completely regio- and stereoselective introduction of a free amino group at C(4) of a pseudoglycal system (Scheme 1).[7] Moreover, the presence of the unsaturation makes alkyl *O*-glycosides **3α,β-Ns** and **4α,β** susceptible to further elaborations toward more complex structures bearing a free or *N*-protected-amino group with defined regiochemistry and configuration.[11]



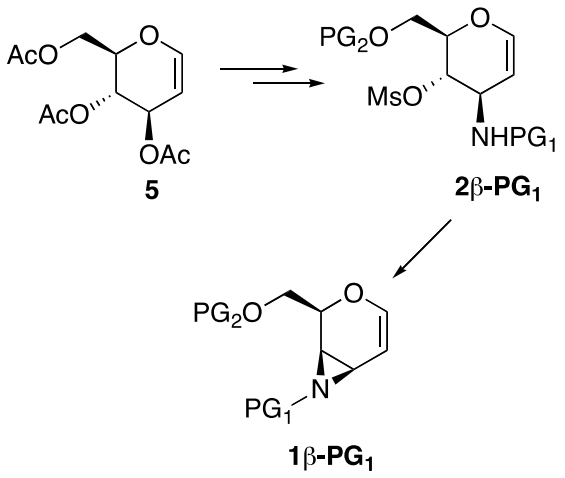
Scheme 1. 1,4-Regio- and syn-stereoselective glycosylation of alcohols by *N*-nosyl aziridines **1α-Ns** and **1β-Ns** (*protocol B* reaction conditions) and stereoselective synthesis of alkyl 4-amino-2,3-unsaturated-*O*-glycosides **4α** and **4β** (see ref. 7).

Although *N*-nosyl aziridines **1α-Ns** and **1β-Ns** are interesting and synthetically useful, there are problems related to their preparation, particularly as aziridine **1β-Ns** is concerned (Scheme 2). By using tri-*O*-acetyl-D-glucal (**5)**, as a useful and not expensive starting material and chiral source, the synthesis of aziridine **1β-Ns** proceeds through the formation of *trans* hydroxy mesylate **6β**, the stable precursor of D-galactal-derived epoxide **7β**[12] and then, through D-gulal-derived *trans* diol **8,** of diastereoisomeric *trans* hydroxy mesylate **6α**, a precursor of D-allal-derived epoxide **7α**.[13] Azidolysis of epoxide **7α** to *trans* azido alcohol **9**,[10b] followed by the usual protocol (reduction of azido group to amino group, *N*-nosylation and *O*-mesylation) leads to *trans N*-nosyl-*O*-mesylate **2β-Ns,** the ultimate stable precursor of the desired aziridine **1β-Ns**.[7,14,15] Aziridine **1β-Ns** is not stable and can be prepared only *in situ* by cyclization of **2β-Ns** under basic conditions (*t*-BuOK or K_2CO_3) and left to react immediately with a nucleophile, as shown in Scheme 2, where MeOH is taken as an example of solvent/nucleophile (*protocol A* reaction conditions).[8] Under these conditions, the addition reaction is still completely 1,4-regioselective, but not stereoselective and a 75:25 mixture of methyl α-*O*- **10α** and β-*O*-glycoside **10β** is obtained.[7,9] As a whole, the preparation of *N*-nosyl aziridine **1β-Ns** from tri-*O*-acetyl-D-glucal (**5**) is decidedly long with a low overall yield[16] (13 steps, 3%)[7,10b,12-15] and, as a consequence, requires improvement (Scheme 2).



Scheme 2. Synthesis of *trans N*-nosyl-*O*-mesylate **2β-Ns** from tri-*O*-acetyl-D-glucal (**5**) (13 steps, 3% overall yield) and base-catalyzed cyclization (K_2CO_3) to aziridine **1β-Ns** in MeOH (*protocol A* reaction conditions): a) MeONa/MeOH (97%, ref.12); b) 1M LHMDS in THF/BnBr/DMF (39%, ref. 12 and 14); c) TBDMS-Cl/imidazole/DMF (84%, ref. 7); d) MsCl/Py (79%, ref. 12 and 14); e) TBAF/THF (74%, ref. 12); f) *t*-BuOK/$Bu_4N^+Me_3SiO^-$/THF (85%, ref. 13 and 14); g) TBDMS-Cl/imidazole/DMF (82%, ref. 13 and 14); h) MsCl/Py (82%, ref. 13); i) TBAF/THF (79%, ref. 13 and 14); j) *t*-BuOK/TMGA (74%, ref. 10b and 14); k) Resin supported-PPh_3/THF-H_2O (23:1) **(**82%, ref. 7, 14 and 15); l) NsCl/Et_3N/CH_2Cl_2 (80%, ref. 7); m) MsCl/Et_3N/CH_2Cl_2 (78%, ref. 7 and 14).

For these reasons, a study was started to find a new, more convenient protocol which, still starting from tri-*O*-acetyl-D-glucal (**5**), could lead to a stable D-glucal-derived mesylate **2β-PG$_1$**, precursor of a new D-galactal-derived aziridine **1β-PG$_1$** structurally related to *trans*-*N*-nosyl-*O*-mesylate **2β-Ns** and *N*-nosyl aziridine **1β-Ns**, respectively. The generic new aziridine **1β-PG$_1$** and corresponding precursor **2β-PG$_1$** should have easily removable 6-*O*-protecting (PG_2) and *N*-protecting/activating group (PG_1) (Scheme 3).

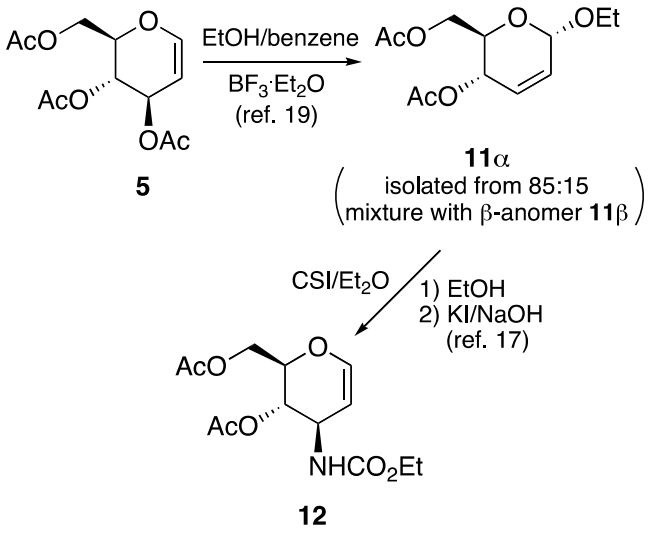


Scheme 3. Generic structures of the new D-galactal-derived aziridine **1β-PG1** and its stable D-glucal-derived precursor **2β-PG1**, bearing easily removable 6-*O*-protecting (PG_2) and *N*-protecting/activating group (PG_1).

2. Results and Discussion

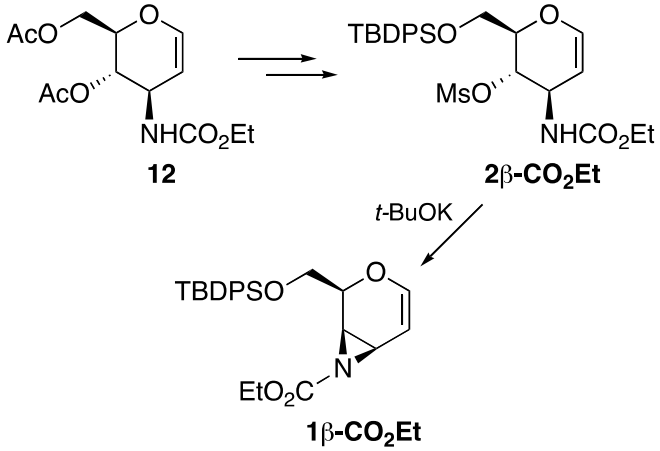
2.1. Synthesis of 4-*O*-mesyl-3-deoxy-3-*N*-(ethoxycarbonylamino)-D-glucal-derivative 2β-CO_2Et, the stable precursor of D-galactal-derived *N*-ethoxycarbonyl vinyl aziridine 1β-CO_2Et

In the course of literature research into D-glucal-derivatives useful to our study, D-glucal-derived urethane **12**, structurally related to *trans N*-nosyl-*O*-mesylate **2β-Ns**, was found to be easily prepared by reaction with chlorosulfonyl isocyanate (CSI)[17,18] of ethyl α-*O*-glycoside **11α**, itself prepared in an 85:15 mixture with diastereoisomeric ethyl β-*O*-glycoside **11β**, by Ferrier rearrangement reaction of tri-*O*-acetyl-D-glucal (**5**) with EtOH/benzene in the presence of $BF_3{\cdot}Et_2O$ (Scheme 4).[19]



Scheme 4. Previously described synthesis of D-glucal-derived urethane **12** by reaction of ethyl α-*O*-glycoside **11α** with CSI.

Urethane **12** appeared decidedly interesting to our purpose, provided that some modifications were necessarily introduced. Actually, the acyl protection of the primary alcohol of **12** had to be replaced with a different, still easily removable protective group, as *t*-butyl-diphenylsilyl group (-OTBDPS), more stable to basic conditions and to nucleophiles, whereas the secondary acetoxy group had to be substituted with a mesyloxy group. In this way, the obtained *trans O*-mesyl-*N*-(ethoxycarbonylamino)-derivative **2β-CO_2Et** could hopefully act as the ultimate precursor of the new D-galactal-derived *N*-CO_2Et vinyl aziridine **1β-CO_2Et** by base-catalyzed intramolecular cyclization process, as shown in Scheme 5 (*vide infra*).

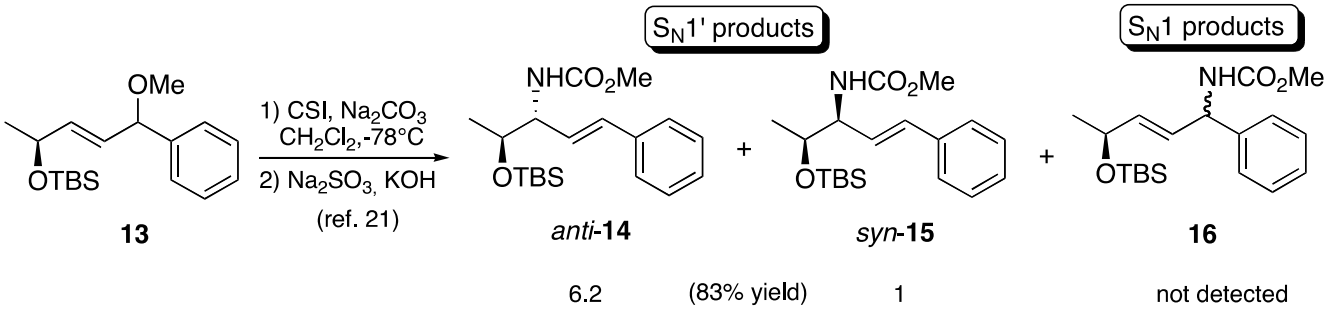


Scheme 5. Necessary modifications of *O*-Ac protections in order to make urethane **12** useful for the preparation of D-galactal-derived *N*-CO_2Et vinyl aziridine **1β-CO_2Et**.

In this framework, the new protocol to D-galactal-derived vinyl aziridine **1β-CO_2Et** (see Schemes 4 and 5) was started by repeating the previously described Ferrier reaction consisting in the treatment of a 9:1 benzene/EtOH solution of tri-*O*-acetyl-D-glucal (**5**) with $BF_3{\cdot}Et_2O$.[19] Ethyl α-*O*-glycoside **11α** was not separated and the obtained 85:15 mixture of ethyl α- and β-*O*-glycosides **11α** and **11β** (90% yield) was directly subjected to the subsequent reaction with CSI following the previously described protocol (CSI/Et_2O in the presence of EtOH, followed by reduction with KI/NaOH), originally applied only to ethyl α-*O*-glycoside **11α**. (Scheme 4).[17a,20]

Contrary to expectations and from the result described by the original authors, only a complex reaction mixture was obtained and a corresponding unsatisfactory result was achieved also when the same reaction was repeated in MeCN, as the solvent.[17b]

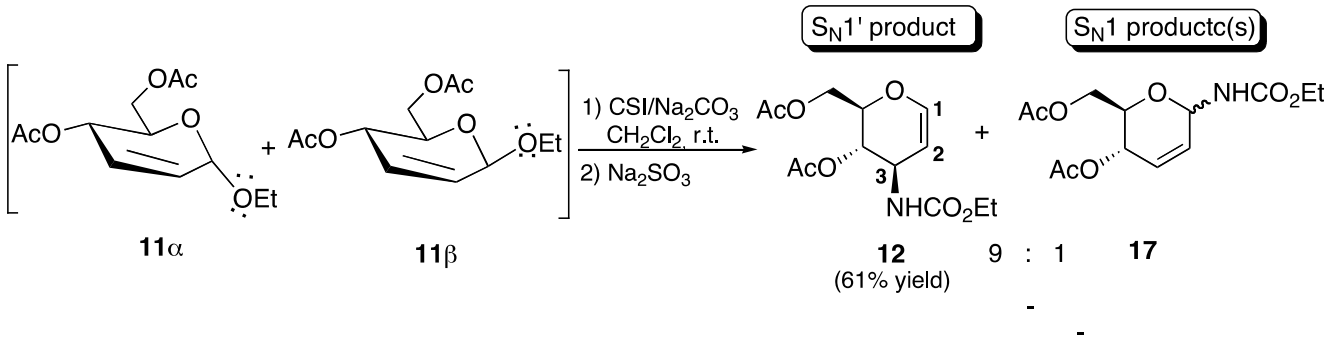
Owing to these negative results, the presence in the literature of some related use of CSI, with possible effective application to our system, constituted by ethyl α- and β-*O*-glycosides **11α** and **11β**, was checked. In this way, a CSI application in the allyl amination of allyl methyl ether **13**,[21] a compound functionally very similar to both ethyl *O*-glycosides **11α** and **11β**, which essentially are mixed dialkyl acetals around an allyl carbon, appeared particularly interesting. The new protocol consisted in the treatment of a solution of methyl allyl ether **13** in CH_2Cl_2 with CSI/Na_2CO_3 at –78 °C followed by reduction with Na_2SO_3/KOH. Under these conditions, a 6.2:1 mixture of diastereoisomeric allyl methoxycarbonylamino-substituted derivatives *anti*-**14** and *syn*-**15** (S_N1' products) had been obtained, in a completely regioselective fashion (83% yield, Scheme 6).[21]



Scheme 6. Allyl amination of -OTBS-protected methyl allyl ether **13** by CSI (see ref. 21).

Considering that we were looking for a result corresponding to the one reported in Scheme 6, the reaction of the mixture of ethyl α- and β-*O*-glycosides **11α** and **11β** with CSI was repeated under the above described new conditions (CSI/Na_2CO_3, CH_2Cl_2, –78 °C).[20,21] However, also in this case a positive result was not initially obtained and we thought that the described low temperature conditions (–78 °C) could be the reason of the unsuccess and that a warmer temperature was necessary for the reaction to occur in our system.

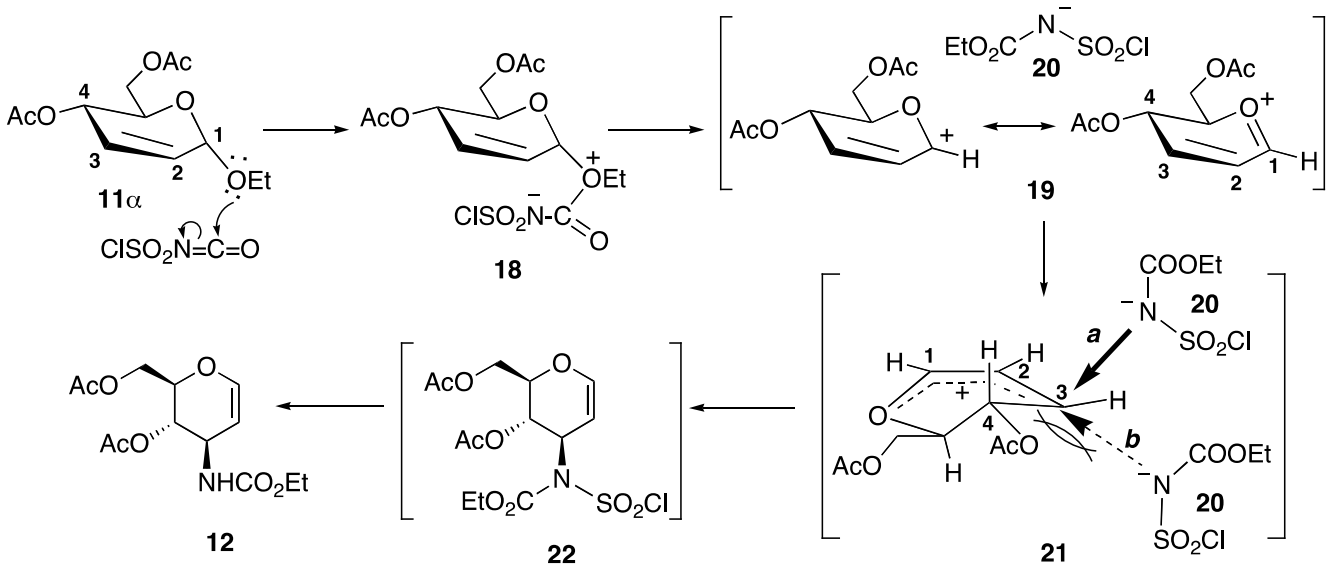
By carrying out the reaction of the 85:15 mixture of ethyl *O*-glycosides **11α** and **11β** with CSI/Na_2CO_3 in CH_2Cl_2 at room temperature and by affecting the subsequent reduction/hydrolysis only with Na_2SO_3, the desired *anti*-S_N1' product, D-glucal-derived allyl *N*-ethoxycarbonylamino derivative **12**, was obtained as the major reaction product in a highly regio- and complete stereoselective fashion [β-direction of *N*-CO_2Et group at C(3) carbon] accompanied by a small amount of corresponding regioisomeric S_N1 product(s), pseudoglycal(s) **17 (12** : **17** ratio = 9 : 1, Scheme 7).[22,23] Subsequent flash column chromatography afforded pure D-glucal-derived allyl *N*-ethoxycarbonylamino derivative **12** in good yield (61%).



Scheme 7. Reaction of ethyl *O*-glycosides **11α** and **11β** with CSI/Na_2CO_3-Na_2SO_3 protocol at room temperature.

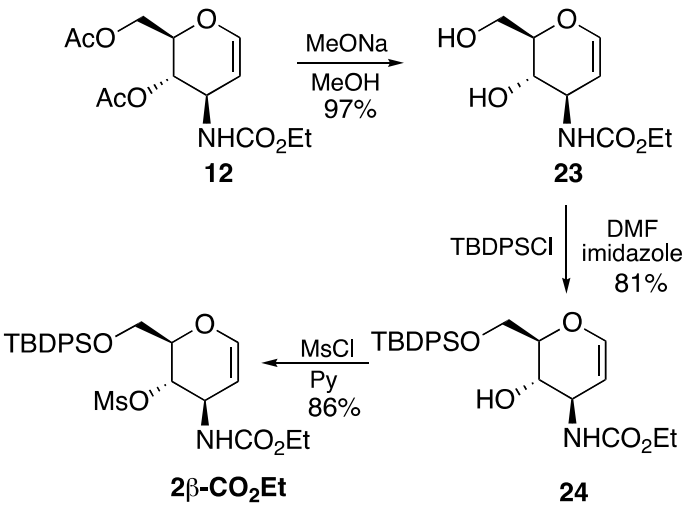
As previously found in similar cases,[17,21] the regio- and stereoselectivity observed in this reaction can be rationalized by the initial coordination of CSI, through its electrophilic carbon, with the acetal oxygen of the pseudoaxial –OEt group of α-*O*-glycoside **11α** (structure **18**, Scheme 8) which determines the lengthening and subsequent breaking of the anomeric C-OEt bond with the formation of allyl carbocation **19** and nitrogen-based anion **20**, as good leaving group. Subsequent S_N1'-type attack by **20**, acting as a nucleophile, at C(3) carbon of the delocalized carbocation **19** exclusively occurs from the less hindered β-face as shown in **21** (*route **a***, Scheme 8), to give, through the intermediate *N*-

chlorosulfonyl derivative **22**, D-glucal-derived urethane **12**, as the main reaction product. Actually, a corresponding alternative/competitive nucleophilic attack by **20** from the α-face (*route **b*** in **21**, Scheme 8) would suffer of torsional tension with the vicinal equatorial C(4)-OAc bond and, as a consequence, is not observed.



Scheme 8. Rationalization of the formation of diacetoxy urethane **12** by reaction of ethyl α-*O*-glycoside **11α** with CSI.

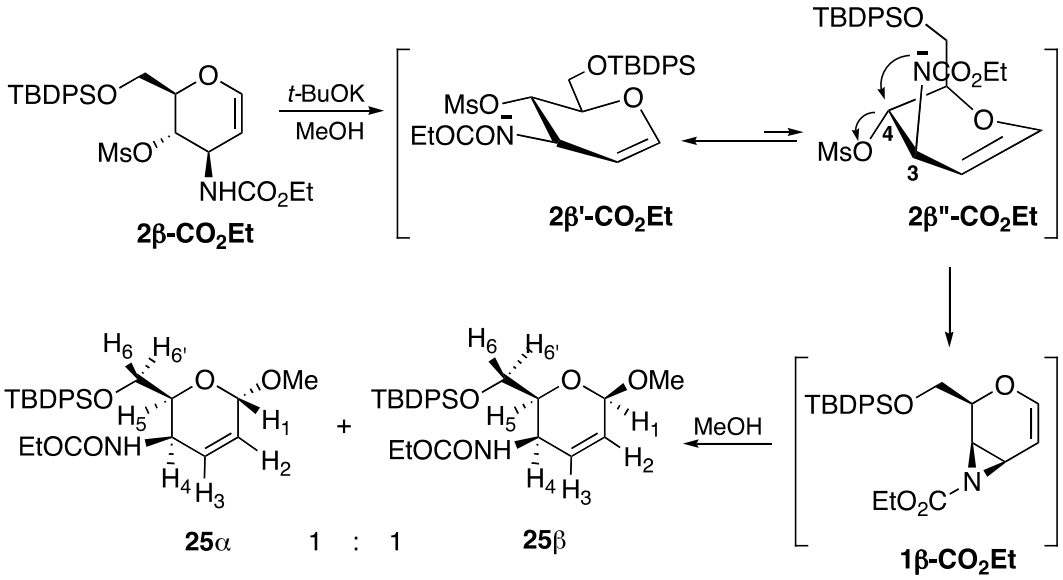
The MeONa/MeOH-catalyzed transesterification of diacetoxy urethane **12** afforded the corresponding dihydroxy urethane **23** (97% yield) which was protected at the primary hydroxy group by treatment with *t*-butyl-diphenylsilyl chloride (TBDPSCl)/imidazole protocol in anhydrous DMF to give corresponding 6-*O*-[*t*-(butyl)-diphenysilyl]- (6-OTBDPS) derivative **24** (78% yield, Scheme 9). The residual secondary –OH group of mono *O*-TBDPS derivative **24** was mesylated (MsCl/Py) to give *trans* 3-*N*-(ethoxycarbonylamino)-4-*O*-mesyl derivative **2β-CO$_2$Et** (86% yield) the supposed ultimate stable precursor of the corresponding *N*-ethoxycarbonyl vinyl aziridine **1β-CO$_2$Et** (Schemes 5 and 9).



Scheme 9. Transformation of diacetoxy urethane **12** into aziridine precursor **2β-CO$_2$Et**.

2.2. Formation *in situ* of vinyl aziridine 1β-CO$_2$Et and regio- and stereoselectivity of its reaction with MeOH under *protocol A* and *protocol B* reaction conditions

Considering that the expected *N*-CO$_2$Et vinyl aziridine **1β-CO$_2$Et** (Scheme 5) could reasonably be unstable and, as a consequence, only be obtained *in situ*, as was the case of the previously studied *N*-nosyl aziridine **1β-Ns**,[7,10] the only way to check its formation *in situ* was to make aziridine **1β-CO$_2$Et** react with a nucleophile, such as MeOH, immediately after its formation. As a consequence, a solution of *trans*-*N*-(ethoxycarbonyl)-*O*-mesyl derivative **2β-CO$_2$Et** in MeOH (*protocol A* reaction conditions) was treated with *t*-BuOK (1 equiv) in order to determine the deprotonation of the residual acid N-H bond. Subsequent intramolecular S_N2 substitution on the vicinal C(4) carbon bearing the good leaving group (-OMs) in the required trans-diaxial relationship, necessary for the cyclization to occur and possible in conformer **2β"-CO$_2$Et**, could have led to the desired aziridine **1β-CO$_2$Et** (Scheme 10).



Scheme 10. Cyclization of ultimate stable precursor **2β-CO₂Et** to aziridine **1β-CO₂Et** and glycosylation of MeOH (*protocol A* reaction conditions) with the formation of methyl α- and β-*O*-glycosides **25α** and **25β**.

A few minutes after the addition of the base, TLC analysis of the reaction mixture showed the gradual disappearance of the starting material [*trans*-*N*-(ethoxycarbonyl)-*O*-mesylate **2β-CO₂Et**] and the contemporary formation of a product with a higher R_f. After 16 h stirring at room temperature, NMR analysis of the crude reaction product, by combined use of scalar and dipolar 1D and 2D techniques, indicated the presence of an almost 1:1 mixture of *anti*- and *syn-1,4-addition products*, methyl α- **25α** and β-*O*-glycoside **25β**, respectively (94%) which turned out to be not separable by any chromatographic conditions tried (Scheme 10).[24] The formation of methyl *O*-glycosides **25α** and **25β** clearly indicated that, under the reaction conditions tried, the desired D-galactal-derived *N*-CO_2Et aziridine **1β-CO₂Et** was formed, with *trans N*-methoxycarbonyl-*O*-mesyl- derivative **2β-CO₂Et** as the ultimate stable precursor.

The complete 1,4-regioselectivity observed in the reaction of aziridine **1β-CO₂Et** with MeOH under *protocol A* reaction conditions is in accordance with what observed with the previously examined *N*-nosyl aziridine **1β-Ns** in the corresponding reaction with MeOH under the same conditions.[7,10] Typically, under *protocol A* reaction conditions, nucleophilic attack by MeOH, solvent/nucleophile of the reaction, exclusively occurs at C(1) vinyl carbon of aziridine **1β-CO₂Et** which, in the presently adopted non-catalyzed conditions and in the presence of a weak nucleophile (MeOH), is the more

reactive site in the molecule. In this way, only corresponding *anti*- **25α**, by *route* ***a***, and *syn-1,4-addition product* **25β**, reasonably by *route* ***b*** and aziridine nitrogen-nucleophile coordinate *route* ***c***, are obtained in a completely regioselective way, with aziridine reacting through conformer **1β'-CO_2Me** (Scheme 11).[25] The observed lack of stereoselectivity (a 1:1 mixture of α- and β-anomer is obtained) is the consequence of the large excess of MeOH, solvent of the reaction, which can attack C(1) carbon indifferently from the α and β side of the molecule.[26] Significantly, corresponding *anti-1,2-addition product* (*route* ***d***, Scheme 11) or any other reaction products turned out to be completely absent.

The demonstrated formation *in situ* of aziridine **1β-CO_2Et** in the methanolysis carried out under *protocol A* reaction conditions, made a preliminary examination possible of the regio- and stereoselective behavior under the synthetically more interesting *protocol B* reaction conditions, that is under conditions which had shown for *N*-nosyl aziridine **1β-Ns** a completely 1,4-syn-stereoselective result (Scheme 1).[7] As a consequence, following the common protocol,[7] a solution of *trans N*-CO_2Et-*O*-mesylate **2β-CO_2Et** in anhydrous MeCN, containing MeOH (3 equiv), was treated with *t*-BuOK. ^{1}H NMR examination of the crude reaction product showed the presence of methyl β-*O*-glycoside **25β,** having the same configuration as the starting aziridine (*syn-1,4-addition product*), as the only reaction product (Scheme 11, *route* ***c***). This result, superposable to that previously obtained with *N*-nosyl aziridine **1β-**Ns under the same conditions (Scheme 1),[7] permits to consider aziridine **1β-CO_2Et** an effective, easy to prepare, glycosyl donor potentially able to glycosylate alcohols in a completely stereoselective, directly *substrate-dependent* fashion and, thus, a valid synthetic tool to be preferred to the corresponding aziridine **1β-Ns**.

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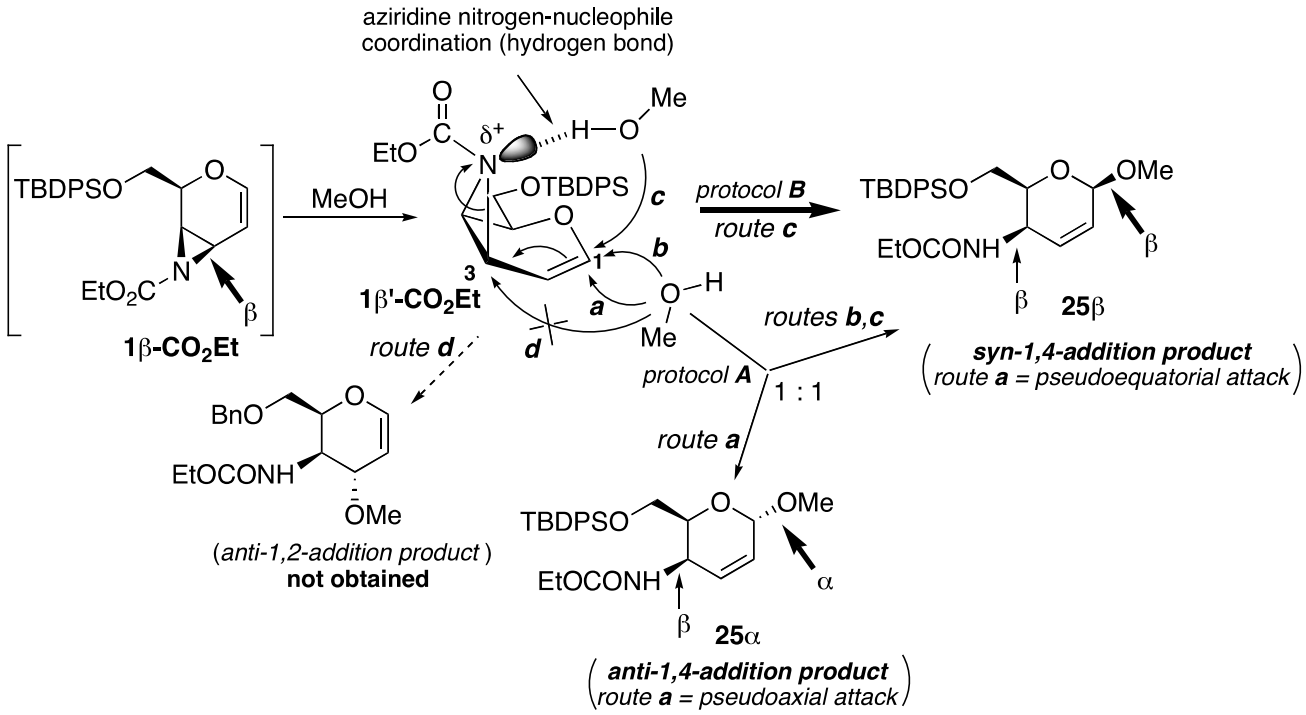

Scheme 11. Regio- and stereoselectivity of the glycosylation of MeOH by vinyl aziridine **1β-CO_2Et** under *protocol A* and *B* reaction conditions.

As previously admitted for aziridine **1β-Ns**,[7] the completely 1,4-regio- and syn-stereoselectivity observed in the reaction of aziridine **1β-CO_2Et** with MeOH under *protocol B* can be rationalized by the occurrence of a coordination between MeOH and the aziridine nitrogen in the form of an hydrogen bond, as shown in Scheme 11. In this way, the nucleophile is appropriately disposed for an entropically-favored attack on C(1) carbon from the same side of the heterocyclic functionality through a pathway (*route* ***c***) which, due to the small amount of MeOH present, is the only active addition process with consequent exclusive formation of methyl β-*O*-glycoside **25β** in a completely regio- and stereoselective fashion, as experimentally found.[27,28]

2.3. Conclusion

The results obtained in the present study are of considerable synthetic importance because a new, simple and fast synthetic protocol to access D-galactal-derived *N*-activated vinyl aziridines, as **1β-CO_2Et,** has been found.

By using tri-*O*-acetyl-D-glucal (**5**) as the starting material and the chiral source, and by noting, as previously done in the case of **1β-Ns**,[7] that the base-catalyzed cyclization of the stable precursor to the corresponding aziridine is quantitative, the new protocol to *N*-ethoxycarbonyl-*O*-mesylate **2β-CO$_2$Et**, (the ultimate precursor of aziridine **1β-CO$_2$Et**) requires only 5 steps (Schemes 4, 7 and 9) with a overall yield of 36%, this particularly pleasing if compared with the yield previously obtained in the case of the structurally related *N*-nosyl aziridine **1β-Ns** and corresponding stable precursor **2β-Ns** (13 steps, 3% overal yield, Scheme 2).[7,10b,12-14] The preliminary results obtained with the new aziridine **1β-CO$_2$Et** in the glycosylation of MeOH, as a model *O*-nucleophile, are identical to those previously obtained with the corresponding *N*-nosyl azidine **1β-Ns**. In particular, the exclusive formation of the corresponding *syn-1,4-addition product* (methyl β-*O*-glycoside **25β**) observed under *protocol B* reaction conditions, indicated that aziridine **1β-CO$_2$Et** is an effective, completely 1,4-regio- and syn-stereoselective glycosyl donor.

3. Experimental

3.1.General.

All reactions were performed in a flame-dried modified Schlenk (Kjeldahl shape) flask fitted with a glass stopper or rubber septa under a positive pressure of argon. Flash column chromatography was performed employing 230-400 mesh silica gel (Macherey-Nagel). Analytical TLC were performed on Alugram SIL G/UV_{254} silica gel sheets (Macherey-Nagel) with detection by 0.5% phosphomolybdic acid solution in 95% EtOH. Benzene and Et_2O were distilled from sodium/benzophenone. HPLC grade MeOH, EtOH and CH_2Cl_2 were used without any further purification.

3.2. Synthesis of mesylate 2β-CO_2Et, stable precursor of *N*-CO_2Et vinyl aziridine 1β-CO_2Et

*3.2.1 Ethyl α- and β-O-glycosides **11α** and **11β**.* Following a previously described procedure,[19] a solution of tri-*O*-acetyl-D-glucal **(5)** (3.0 g, 11.03 mmol) in a 9:1 anhydrous benzene: EtOH mixture (13.30 mL) was treated at 0 °C with $BF_3{\cdot}Et_2O$ (0.60 mL, 4.74 mmol). After 2 h stirring at the same temperature, the reaction mixture was diluted with Et_2O. Evaporation of the washed (saturated aqueous $NaHCO_3$) organic solution afforded a crude product consisting of an 85:15 mixture of ethyl α- and β-*O*-glycosides **11α** and **11β** (2.56 g, 90% yield) (^{1}H NMR)[19] which was directly used in the next step, without any further purification.

*3.2.2. 4,6-Di-O-acetyl-3-deoxy-3-N-(ethoxycarbonylamino)-D-glucal **(12)**.* Following a previously described procedure,[21] a suspension of Na_2CO_3 (0.183 g, 1.73 mmol) in anhydrous CH_2Cl_2 (2.0 mL) containing chlorosulfonylisocyanate (0.24 g, 0.15 mL, 1.70 mmol) was treated at room temperature with a solution of the mixture of ethyl α- and β- *O*-glycosides **11α** and **11β** (0.20 g, 0.77 mmol) in anhydrous CH_2Cl_2 (2.0 mL) and the reaction mixture was stirred at the same temperature for 90 min. Water (3 mL) was added and the reaction mixture was extracted with AcOEt (3 mL x 2). The combined organic extracts were added to an aqueous solution of Na_2SO_3 (25%) and KOH (10%) (2.5 mL) and the reaction mixture was stirred at room temperature for 18 h. Evaporation of the separated organic phase afforded a crude mixture (0.204 g) consisting of a 90:10 mixture of ethyl carbamate **12** and *1,4-addition product(s)* **17** (^{1}H NMR) which was subjected to flash chromatography. Elution with a 6:4 hexane/AcOEt mixture afforded pure diacetoxy ethyl carbamate **12** (0.142 g, 61%),[17a] as a solid, m.p. 113-115 °C (lit.[17a], m.p. 113-115 °C): FTIR(Nujol) ν_{max} 3430, 1740, 1720, 1650 cm^{-1}. ^{1}H NMR (250

MHz, $CDCl_3$) δ 6.41 (dd, 1H, *J* = 5.9, 2.1 Hz), 5.08 (dd, 1H, *J* = 10.2, 8.4 Hz), 4.73-4.78 (m, 1H), 4.74 (dd, 1H, *J* = 5.9, 2.3 Hz), 4.47-4.58 (m, 1H), 4.43 (dd, 1H, *J* = 11.7, 4.7 Hz), 4.17-4.24 (m, 2H), 4.12 (q, 2H, *J* = 7.1 Hz), 2.11 (s, 6H), 1.26 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 171.0, 169.1, 155.8, 146.6, 100.2, 77.2, 68.5, 62.8, 61.6, 49.5, 29.9, 20.9, 14.7. Anal. Calcd for $C_{13}H_{19}NO_7$: C, 51.82; H, 6.36; N, 4.65. Found: C, 51.99; H, 6.57; N, 4.41.

*3.2.3. 6-O-(t-Butyldiphenylsilyl)-3-deoxy-3-N-(ethoxycarbonylamino)-D-glucal (**24**).* A solution of diacetoxy ethyl carbamate **12** (0.136 g, 0.45 mmol) in MeOH (2.0 mL) was treated with MeONa (0.016 g, 0.30 mmol) and the reaction mixture was stirred for 18 h at room temperature. Evaporation of the filtered organic solvent afforded a crude product consisting of practically pure *trans* diol **23**, as a pale yellow oil (0.095 g, 0.437 mmol, 97%): FTIR(liquid film) ν_{max} 3398, 1695, 1531, 1232, 1126, 1050 cm^{-1}; ^{1}H NMR (250 MHz, MeOD) δ 6.37 (dd, 1H, *J* = 6.0, 2.0 Hz), 4.95 (broad s, 1H), 4.57 (dd, 1H, *J* = 6.0, 2.0 Hz), 4.09 (q, 2H, *J* = 7.1 Hz), 3.90-3.71 (m, 5H), 1.23 (t, 3H, *J* = 7.1 Hz).

Crude diol **23** was dissolved in anhydrous DMF (2.5 mL) and treated at 0 °C with TBDPSCl (0.126 g, 0.12 mL, 0.46 mmol) in the presence of imidazole (0.063 g, 0.92 mmol). After 18 h stirring at room temperature, the reaction mixture was diluted with AcOEt. Evaporation of the washed (saturated aqueous $NaHCO_3$ and brine) organic solution afforded a crude product (0.20 g) consisting of *O*-TBDPS derivative **24** which was subjected to flash chromatography. Elution with an 8:2 hexane/AcOEt mixture afforded mono *O*-TBDPS derivative **24** (0.155 g, 78%), pure as a colourless oil, R_f = 0.13 (8:2 hexane/AcOEt): FTIR(liquid film) ν_{max} 3387, 1695, 1529, 1233, 1048, 701 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.62-7.75 (m, 4H), 7.30-7.48 (m, 6H), 6.42 (dd, 1H, *J* = 5.9, 2.2 Hz), 4.74-4.83 (m, 1H), 4.48 (dd, 1H, *J* = 5.9, 2.2 Hz), 4.23-4.31 (m, 1H), 4.14 (q, 2H, *J* = 7.1 Hz), 4.12 (d, 1H, *J* = 4.5 Hz), 3.97-4.01(m, 1H), 3.78-3.88 (m, 2H), 1.24 (t, 3H, *J* = 7.1 Hz), 1.06 (s, 9H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 155.9, 145.1, 135.9, 129.9, 129.8, 127.7, 99.4, 79.1, 70.7, 63.1, 61.7, 52.5, 26.9, 19.5, 14.6. Anal. Calcd for $C_{25}H_{33}NO_5Si$: C, 65.90; H, 7.30; N, 3.07. Found: C, 65.79; H, 7.18; N, 2.89.

*3.2.4. 6-O-(t-Butyldiphenylsilyl)-3-deoxy-3-N-(ethoxycarbonylamino)-4-O-mesyl-D-glucal (**2β-CO$_2$Et**).* A solution of mono *O*-TBDPS derivative **24** (0.091 g, 0.20 mmol) in a 1:1 CH_2Cl_2/pyridine mixture (2.0 mL) was treated with MsCl (0.060 mL, 0.30 mmol) at 0°C and the resulting reaction mixture was stirred at the same temperature for 18 h. After dilution with CH_2Cl_2, evaporation of the washed (saturated aqueous $NaHCO_3$ and brine) and filtered organic solution afforded a liquid residue which was co-evaporated at the rotary evaporator (40 °C) with toluene (3 mL x 2) to give a crude product

consisting of practically pure O-mesyl derivative **2β-CO_2Et** (0.092 g, 86%), as a pale yellow oil: R_f = 0.12 (8:2 hexane/AcOEt): FTIR(liquid film) ν_{max} 3413, 1653, 1360, 1234, 1176, 1100 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.67-7.78 (m, 4H), 7.31-7.51 (m, 6H), 6.40 (dd, 1H, *J* = 6.1, 1.8 Hz), 5.08 (t, 1H, *J* = 6.5 Hz), 4.93-5.05 (m, 1H), 4.69 (dd, 1H, *J* = 6.1, 3.1 Hz), 4.41-4.51 (m, 1H), 4.05-4.20 (m, 3H), 3.70-3.93 (m, 2H), 3.08 (s, 3H), 1.23 (t, 3H, *J* = 7.1 Hz), 1.07 (s, 9H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.1, 145.6, 135.8, 130.0, 129.8, 127.9, 99.2, 76.4, 75.5, 61.6, 61.4, 48.2, 38.8, 26.8, 19.3, 14.7. Anal. Calcd for $C_{26}H_{35}NO_7SSi$: C, 58.51; H, 6.61; N, 2.62. Found: C, 58.72; H, 6.85; N, 2.85.

*3.2.5. Treatment of mesylate **2β-CO_2Et** with t-BuOK in MeOH (protocol A).* A solution of *trans N*-CO_2Et-*O*-mesyl derivative **2β-CO_2Et** (0.080 g, 0.15 mmol) in MeOH (2.5 mL) was treated with *t*-BuOK (0.018 g, 0.16 mmol) and the reaction mixture was stirred for 18 h at room temperature. Dilution with Et_2O and evaporation of the filtered organic solution afforded a crude product (0.066 g, 94%) consisting of an 1:1 mixture of methyl α- and β-*O*-glycosides **25α** and **25β** (^{1}H NMR**)** which turned out to be not separable under any TLC conditions tried. ^{1}H NMR (600 MHz, CD_3CN, 25 °C) data for the mixture of methyl α- and β-*O*-glycosides **25α** and **25β**: δ 5.98 (dd, 1H, *J* = 9.9 Hz, *J* = 5.8 Hz, H(3), **25α**], 5.96 [dd, 1H, *J* = 10.1 Hz, *J* = 5.7 Hz, H(3), **25β**), 5.85 (dd, 1H, *J* = 9.9 Hz, *J* = 2.3 Hz, H(2), **25α**), 5.77 (d, 1H, *J* = 10.1 Hz, H(2), **25β**), 5.48 (d, 1H, *J* = 8.7 Hz, NH, **25β**), 5.41 (d, 1H, *J* = 9.5 Hz, NH, **25α**), 4.99 (s, 1H, H(1), **25β**), 4.87 (d, 1H, *J* = 2.3 Hz, H(1), **25α**), 4.18-3.90 (m, 6H, CH_2(Et), H(4) and H(5), **25α** and **25β**), 3.82 and 3.80 (m, 2H, H(6), **25α** and **25β**), 3.73 (dd, 1H, *J* = 10.9 Hz, *J* = 6.7 Hz, H(6'), **25β**), 3.69 (dd, 1H, *J* = 10.9 Hz, *J* = 7.4 Hz, H(6'), **25α**), 3.40 (s, 3H, OMe, **25β**), 3.39 (s, 3H, OMe, **25α**), 1.17 (t, 3H, *J* = 7.0 Hz, Me, **25α** and **25β**).[24]

*3.2.6. Reaction of aziridine **1β-CO_2Et** with MeOH in anhydrous MeCN (protocol B).* A solution of *trans N*-CO_2Et-*O*-mesyl derivative **2β-CO_2Et** (0.040 g, 0.075 mmol) in anhydrous MeCN (0.90 mL) containing MeOH (9.1 μL, 0.225 mmol, 3 equiv) was treated with *t*-BuOK (0.009 g, 0.080 mmol) and the reaction mixture was stirred for 18 h at room temperature. Dilution with Et_2O and evaporation of the filtered organic solution afforded a crude product consisting of practically pure methyl β-*O*-glycoside **25β** (^{1}H NMR**)** (0.032 g, 95% yield) which was subjected to flash chromatography. Elution with an 1:1 hexane/AcOEt mixture afforded pure methyl *6-O-(t-butyldiphenylsilyl)-2,3,4-trideoxy-4-N-(ethoxycarbonylamino)-β-D-threo-hex-2-enopyranoside* (**25β**) (0.022 g, 62%), as a pale yellow liquid: R_f = 0.29 (1:1 hexane/AcOEt); FTIR(liquid film) ν_{max} 3359, 1660, 1540, 1417, 1120, 1058 cm^{-1}. ^{1}H

NMR (600 MHz, CD_3CN) δ 7.65-7.82 (m, 3H), 7.38-7.58 (m, 7H), 5.96 (dd, 1H, *J* = 10.1, 5.7 Hz), 5.77 (d, 1H, *J* = 10.1, 5.7 Hz), 5.48 (d, 1H, *J* = 8.7 Hz, NH), 4.99 (broad s, 1H), 4.20 (q, 2H, *J* = 7.1 Hz), 4.12-4.18 (m, 1H), 3.91-3.97 (m, 1H), 3.75-3.82 (m, 1H), 3.73 (dd, 1H, *J* = 10.9, 6.7 Hz), 3.40 (s, 3H), 1.27 (t, 3H, *J* = 7.1), 1.03 (s, 9H); ^{13}C NMR δ (150 MHz CD_3CN) 150.8, 145.8, 135.4, 130.0, 129.9, 128.1, 127.7, 98.4, 74.8, 63.7, 63.6, 54.7, 45.0, 26.3, 19.2, 13.4. Anal. Calcd for $C_{26}H_{35}NO_5Si$: C, 66.49; H, 7.51; N, 2.98. Found: C, 66.35; H, 7.29; N, 2.73.

3.3. NMR Measurements

NMR measurements were performed on a spectrometer operating at 600 MHz, for ^1H nuclei. The temperature was controlled to ± 0.1 °C. The 2D NMR spectra were obtained by using standard sequences with the minimum spectral width required. Proton 2D gCOSY (gradient COrrelated SpectroscopY) spectra were recorded with 128-200 increments of 4-16 scans and 2K data points. The relaxation delay was 1 s. 2D TOCSY (TOtal Correlation SpectroscopY) spectra were recorded by employing a mixing time of 80-120 ms. The pulse delay was 1 s; 256-512 increments of 16-32 scans and 2K data points each were collected. 1D TOCSY spectra were acquired with 1024-4096 scans in 32K data points with a 1 s relaxation delay and a 100 ms mixing time. The 2D NOESY (Nuclear Overhauser Enhancement SpectroscopY) experiments were performed by employing a 1 s mixing time. The pulse delay was 1 s; 200 increments of 16scans and 2K data points each were collected. 1D NOESY spectra were recorded using the selective pulse SEDUCE generated by means of the Agilent Pandora Software. The selective 1D NOESY spectra were acquired with 1024 scans in 32K data points with a 1-5 s relaxation delay and a 1 s mixing time. gHSQC (gradient Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Correlation) spectra were recorded by employing a pulse delay of 1.2 s and 128 increments of 64 scans. HMBC experiments were optimized for a long-range ^1H-^{13}C coupling constant of 8 Hz.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at…...

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8. *Protocol A* reaction conditions: the aziridine stable precursor, *trans N*-nosyl-*O*-mesylate **2α-Ns** or **2β-Ns** as an example, is dissolved in the glycosyl acceptor (low boiling alcohol as MeOH, EtOH, *i*-PrOH or *t*-BuOH) and treated with a base (*t*-BuOK or K_2CO_3, 1 equiv). Under these conditions, the glycosylation occurs in the presence of a large excess of nucleophile, solvent of the reaction. *Protocol B* reaction conditions: the aziridine stable precursor, *trans N*-nosyl-*O*-mesylate **2α-Ns** or **2β-Ns** as an example, is dissolved in a non-nucleophilic solvent (MeCN, THF, or toluene) containing the glycosyl acceptor (alcohols, also complex, phenol or partially *O*-protected monosaccharide, 3 equiv) and the reaction mixture is treated with a base (*t*-BuOK or K_2CO_3, 1 equiv). Under these conditions, the glycosylation occurs in the presence of only a small excess of *O*-nucleophile.

9. Glycosylation of alcohols by aziridines **1α-Ns**, **1β-Ns**, **1α-Ms** and **1β-Ms** under *protocol A* reaction conditions are always completely 1,4-regioselective (exclusive formation of *syn*- and *anti-1,4-addition products*) but not stereoselective and mixtures of corresponding α- and β-anomers are commonly obtained. Corresponding *1,2-addition products* are never observed under these conditions.

10. Similar results had been previously obtained with the corresponding *N*-mesyl aziridines. See: a) Di Bussolo, V.; Romano, M. R; Pineschi, M.; Crotti, P. *Org. Lett.* **2005**, *7*, 1299-1302. b) Di Bussolo, V.; Romano, M. R.; Favero, L.; Pineschi, M.; Crotti, P. *J. Org. Chem.* **2006**, *71*, 1696-1699.

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14. Previously described particularly low yielding steps [b (32%, ref. 12), d (72%, ref. 12), f (68%, ref. 13), g (70%, ref. 13), i (68%, ref. 13), j (65%, ref. 10b), m (65%, ref. 7)] were re-examined and the corresponding more satisfactory yields obtained are indicated in Scheme 2. Moreover, the previously reported protocol ($SnCl_2$/PhSH/CH_2Cl_2, 70% yield, k step, ref. 10b) for the reduction of *trans* azido alcohol **9** to the corresponding *trans* amino alcohol has been substituted by the simpler, higher yielding resin supported-PPh_3 /THF/H_2O protocol, as reported in Scheme 2 (see ref.15).

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16. The overall yield (3%) has been calculated on the obtainment of the ultimate stable precursor, *trans N*-nosyl-*O*-mesylate **2β-Ns**, on the assumption that its cyclyzation to aziridine **1β-Ns**, not isolable, is quantitative.

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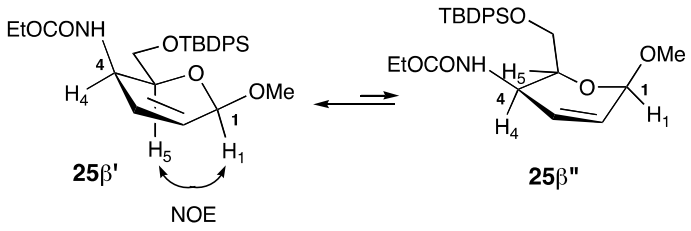
20. The use of the 85:15 mixture of ethyl α- and β-*O*-glycosides **11α** and **11β** was justified by the good quality of the crude reaction product [only **11α** and **11β** were present (^{1}H NMR)] and by considering that a separation/purification of **11α** from the low amount of diastereoisomeric **11β** would have lowered the yield. Moreover, the possibility that **11β** reacts with CSI as **11α**, making the separation not necessary, could not be excluded. In the contrary case, unreacted **11β** or an undesired side product deriving from its reaction with CSI could be separated subsequently or, in the most favorable case, could be lost in the work-up.

21. Kim, J. D.; Zee, O. P.; Jung, Y. H. *J. Org. Chem*. **2003**, *68*, 3721-3724. The formation of a small amount of undesired corresponding regioisomeric S_N1 product(s) **16** (9%, Scheme 6), when the same reaction was carried out in $CHCl_3$, is also reported.

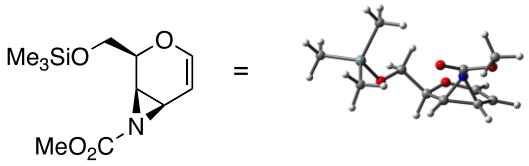
22. S_N1 product(s) **17** was not separated pure from the reaction mixture. However, its formation has been tentatively admitted in accordance with a previously observed behavior of CSI in similar systems (see Scheme 6, ref. 21) and by the presence in the ^1H NMR spectrum of the crude reaction mixture of a multiplet at δ 5.75-5.95 corresponding to the vinyl protons of a pseudoglycal system as **17** actually is.

23. The strict correspondence between the starting 85:15 mixture of ethyl α- and β-*O*-glycosides **11α** and **11β** and the final 9:1 mixture of allyl *N*-ethoxycarbonylamino derivative **12** (S_N1' product) and the supposed S_N1 product(s) **17** could indicate the completely regioselective behavior of α- and β-*O*-glycosides **11α** and **11β**: **11α** (pseudoaxial-oriented –OEt group) leads to S_N1' product (*N*-ethoxycarbonylamino derivative **12**), whereas **11β** (pseudoequatorial-oriented –OEt group) leads to S_N1 product(s) **17** (Schemes 7 and 8).

24. Accurate NMR analysis of the crude reaction product made the structural identification of the two compounds present in a 1:1 mixture possible. In particular the absence in both compounds of *O*-mesyl group and the presence of: *a*) an anomeric H(1) proton, *b*) a methoxy group at C(1) anomeric carbon, *c*) a *N*-(ethoxycarbonyl)-amino group (-$NHCO_2Et$) at C(4) carbon, and *d*) two vinyl proton signals at δ 5.90-6.30 ppm clearly indicated their nature of *1,4-addition products*. Subsequent appropriate 1D-NOESY experiments made the exact regio- and stereochemical assignement to both compounds possible indicating a structure corresponding to anomer **25α** (*anti-1,4-addition product*) and **25β** (*syni-1,4-addition product)* (Schemes 10 and 11). In particular, spatial proximity between H(1) and H(5) protons was confirmed for β-*O*-glycoside **25β** by detecting their mutual dipolar interaction, as expected for protons in a syn 1,3-diaxial arrangement, possible in the corresponding more stable conformer **25β'** with –CH_2OTBDPS side chain equatorial, as here shown:



25. Theoretical conformational studies in a vacuum, carried out, for simplicity, on the simplified 6-$OSiMe_3$- and *N*-CO_2Me substituted aziridine **1β-CO_2Me-OTMS**, structurally related to aziridine **1β-CO_2Et**, indicated that aziridine model **1β-CO_2Me-OTMS** exists only as the corresponding conformer **1β'-CO_2Me-OTMS** with the –CH_2OTMS side chain equatorial and N-CO_2Me group in the exo position (see Supplementary data).



1β-CO_2Me-OTMS **1β'-CO_2Me-OTMS**

The results obtained with aziridine **1β-CO_2Me-OTMS,** reasonably extended to aziridine **1β-CO_2Et**, indicate that also the presently studied aziridine exists only as the corresponding conformer **1β'-CO_2Et** with the –CH_2OTBDPS side chain equatorial and the exo-directed N-CO_2Et group as shown in Scheme 11 (see Supplementary data).

26. In addition to the presence of a large excess of MeOH, the absence of stereoselectivity found in the glycosylation of MeOH by aziridine **1β-CO_2Et,** under *protocol A* reaction conditions, could be the consequence of a complete balance between two opposite directing effects. Actually, if on one hand, the formation of **25α** through a stereoelectronically favored pseudoaxial attack by MeOH (*route **a***) should be preferred with respect to **25β** which is formed by a less favored pseudoequatorial attack by MeOH (*routes **b*** and ***c***), on the other hand, the formation of **25β**, at least through *route **c***, appears to be entropically favored by the occurrence of an aziridine nitrogen-nucleophile (MeOH) coordination (hydrogen bond), as shown in Scheme 11.

27. Because arising from a coordination process, methyl β-*O*-glycoside **25β** can be called also *coordination product* (see ref. 7)

28. It is interesting to note that the presence of the bulky –OTBDPS group in **1β-CO_2Et** has no influence on the completely 1,4-regio- and syn-stereoselective result observed in the methanolysis under *protocol B* reaction conditions. However, appropriate studies will be carried out in order to have a complete examination of the regio- and stereoselective behavior of aziridine **1β-CO_2Et** with different types of *O*-nucleophiles under different reaction conditions.