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[**Impact of Aromatic Stacking on Glycoside Reactivity: Balancing CH/π and Cation/π Interactions for the Stabilization of Glycosyl-Oxocarbenium Ions.**](https://www.ncbi.nlm.nih.gov/pubmed/31390207)

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# ABSTRACT

Carbohydrate/aromatic stacking represents a recurring key motif for the molecular recognition of glycosides, either by protein binding domains, enzymes or synthetic receptors. Interestingly, it has been proposed that aromatic residues might also assist in the formation/cleavage of glycosidic bonds by stabilizing positively charged oxocarbenium-like intermediates/transition states through cation/ interactions. While the significance of aromatic stacking on glycoside recognition is well stablished, its impact on the reactivity of glycosyl donors is yet to be explored. Herein, we report the first experimental study on this relevant topic. Our strategy is based on the design, synthesis and reactivity evaluation of a large number of model systems, comprising a wide range of glycosidic donor/aromatic complexes. Different stacking geometries and dynamic features, anomeric leaving groups, sugar configurations, and reaction conditions have been explicitly considered. The obtained results underline the opposing influence exerted by van der Waals and coulombic forces on the reactivity of the carbohydrate/aromatic complex: depending on the outcome of this balance, aromatic platforms can indeed exert a variety of effects, stretching from reaction inhibition all the way to rate enhancements. Unfortunately, reaction stereoselectivity seems to follow an opposite trend suggesting that aromatic/glycosyl cation contacts must be loose and/or highly dynamic. The conclusions of our study indicate that, although tricky, aromatic catalysis in glycosidation should be attainable, which presents far reaching implications in enzyme engineering and organocatalysis.

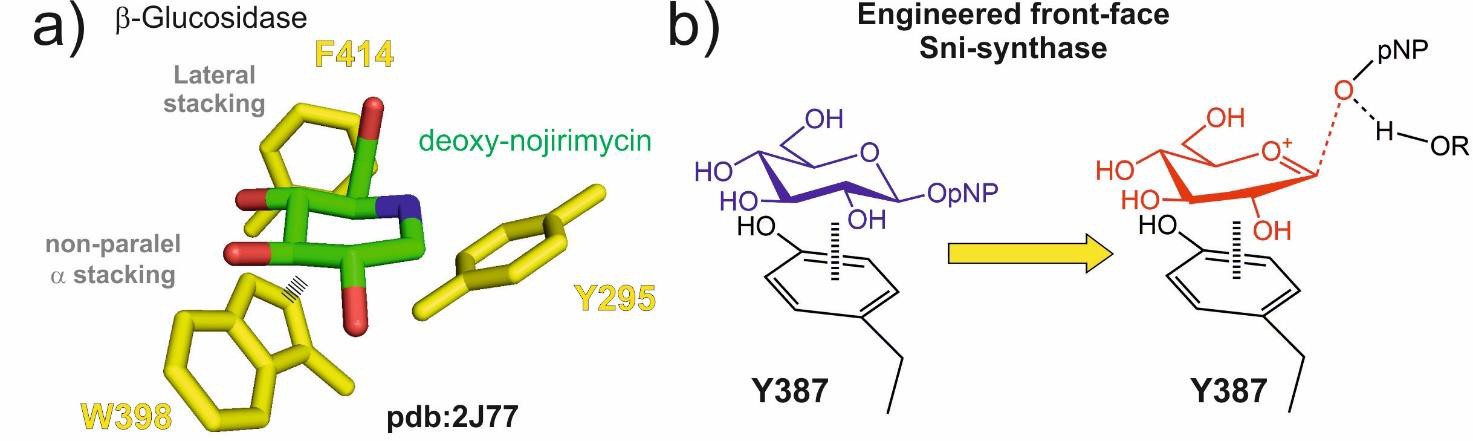
# INTRODUCTION

Aromatic stacking plays a prominent role in the molecular recognition of saccharidic ligands either by protein, nucleic acids or synthetic receptors.1-3 These contacts result from a combination of dispersive, hydrophobic and electrostatic forces, involving polarized pyranose CH groups and the aromatic electronic quadrupole,4,5 and can exhibit a variety of geometries. Their relevance is highlighted by data-mining studies which show the extraordinary high frequency of aromatic amino acids, especially tryptophan, in the carbohydrate binding sites of proteins. Of note, tryptophan, tyrosine and phenylalanine residues are also common within the catalytic sites of glycosidases and glycosyiltransferases, prompting the question of whether they might also assist the cleavage or formation of glycosidic bonds (Figure 1).

Regarding the potential influence of aromatic stacking on the reactivity of pyranoses, different scenarios could be envisaged. Thus, for concerted SN2 processes, shielding of the anomeric centre by the aromatic platform would be expected to exert a protective influence, leading to a sharp decrease in reactivity. On the contrary, more dissociative transition states might benefit from favourable electrostatic forces arising between the aromatic quadrupole and the pyranose developing positive charge (a so-called cation/interaction), 6,7 especially in low dielectric environments (Figure 1). In addition, aromatic/glycosyl cation interactions might direct the stereochemical course of the reaction by preventing nucleophilic attack through either the or pyranose face. Fittingly, oxocarbenium- like intermediates or transition states have been commonly invoked to explain the outcome of glycosylation and hydrolytic reactions both in a chemical8-12 or enzymatic context.13-15 As a further consideration, formation of glycosyl cations is usually accompanied by substantial conformational changes of the reactive pyranose ring.8 Conceivably, this conformational adjustment could be either impeded or facilitated by pre-organized CH/bonds depending on the interaction geometry and dynamic behaviour of the complex. Additional factors such as the access of solvent molecules to the catalytic region, its local dielectric constant or the presence of counter ions should also be taken into account, drawing a more complex scenario. In fact, while the relevance of aromatic platforms in carbohydrate recognition is undisputable, their possible role in catalysis remains uncertain.

Despite the relative lack of experimental information available on this topic, some examples have been reported in the literature in which tyrosine or phenylalanine residues seem to assist the cleavage/formation of glycosidic bonds by presenting favourable interactions with carbohydrate transition states. Thus, a *hydrophobic platform* comprising a phenylalanine residue, highly conserved in the active centre of *all* glycoside hydrolases, has been proposed as a *mechanistically relevant transition state stabilising factor*.16 More recently, a seminal contribution by Prof. Davis and col. puts forward a novel mechanism for a ‘SNi synthase’ engineered from a retaining ‘double-SN2’ hydrolase.17 According to kinetic, structural and theoretical considerations, this mutant protein makes use of a tyrosine residue to stabilize the oxocarbenium-like transition state generated during the front-

face attack of the glycosidic acceptor to the acetalic centre (Figure 1b). Interestingly, this tyrosine residue is originally involved in a parallel stacking with the -face of the glycosidic donor and therefore seems to play a dual role, both participating in substrate recognition and catalysis. These examples illustrate that under appropriate conditions aromatic platforms can be employed to stabilize glycoside transition states. However, the precise structural and chemical requirements for this task are presently unknown.



**Figure 1.-***a) Aromatic interactions in a -glucosidase/DNJ complex as revealed by X-ray. b) Aromatic assistance to catalysis in the context of an engineered front-face SNi-synthase.17*

The objective of this work is to explore the influence of carbohydrate/aromatic stacking on the pyranose reactivity. With this purpose in mind, we have employed a bio-organic approach based on the design, synthesis and evaluation of appropriate molecular models, in conjunction with the extensive use of NMR as a monitorization and detection tool, facilitated in particular cases by 13C- labelled samples. Despite its significant relevance, from both an applied and a fundamental perspective, this represents, to the best of our knowledge, the first systematic experimental study reported on this topic.

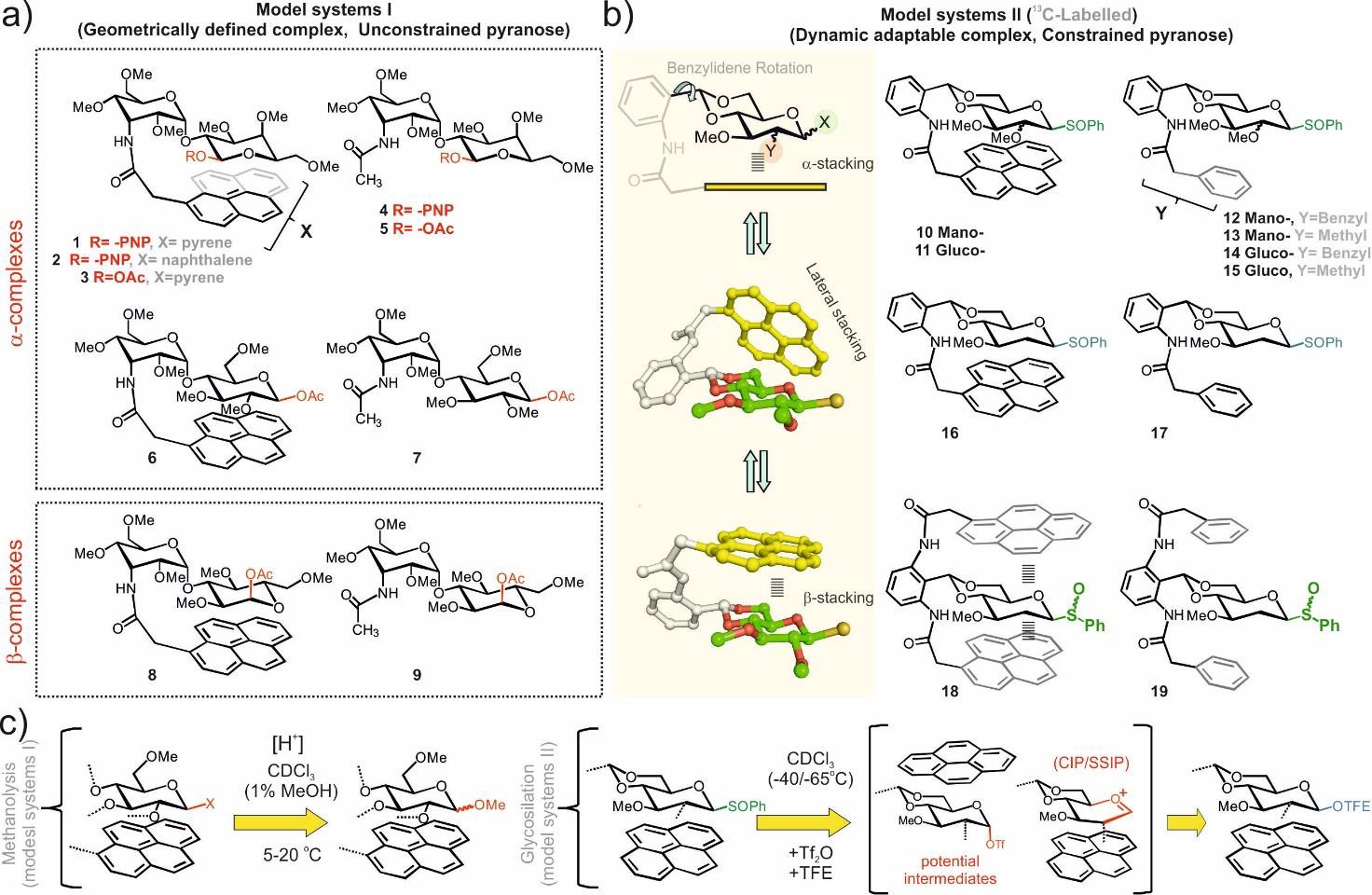
# RESULTS

* 1. ***Model compounds comprising glycosidic-donor/aromatic complexes: Design principles. –*** In order to enhance any potential electrostatic interaction between the aromatic quadrupole and cationic intermediates/transition states and to avoid competition by solvent molecules,18 we decided to carry out our studies in a low dielectric environment such as chloroform (=4.8). It is worth mentioning that comparable dielectric constants have been previously theorized to model the inner protein environment in binding sites or catalytic regions.19 Accordingly, designed scaffolds encompassed pyranose units equipped with several non-participating MeO- groups, providing higher solubility in organic solvents.

Two alternative families of model systems were conceived (Figure 2a). Compounds in family I comprise a disaccharide scaffold. Reactive pyranoses, bearing alternative leaving groups at the anomeric centre, participate in contacts with a pyrene platform attached to a vicinal 3-amino-allose

unit through an amide bond (compounds **1**-**3**, **6** and **8**). Compounds in family II (Figure 2b) include

a glycosyl donor involved in a stacking complex with a pyrene unit tethered to a 4,6-*O*-benzylidene protecting group. Considering the key influence exerted by pyranose position 2 on the reactivity of the acetalic centre, manno- (**10**) gluco- (**11**) and 2-deoxy (**16**) derivatives were explicitly considered. Moreover, to maximize the influence wielded by the aromatic ring on the pyranose behaviour, derivatives comprising a 2-deoxy-gluco donor sandwiched between two pyrene platforms were also prepared (**18**). For comparison purposes, both families included reference compounds equipped with smaller or non-aromatic units (derivatives **4**, **5**, **7** and **9** for models type I and **12**-**15**, **17** and **19** for models type II). Finally, selected type II models were also prepared incorporating 13C atoms, either at all pyranose positions (**11**, **14**, **15**, **16** and **17**) or just at the anomeric centre (**10** and **13**).By employing these latter derivatives, relatively fast reactions could be conveniently monitored with 2D HSQC NMR experiments, also allowing sensitive detection of transient species even in highly overlapped spectra (see the experimental section).



**Figure 2.-** *Chemical structures of model systems I (a) and II (b). Conformational variability for the established carbohydrate/aromatic complexes II are illustrated. c) Chemical reactions selected to evaluate the impact of aromatic stacking on glycoside reactivity.*

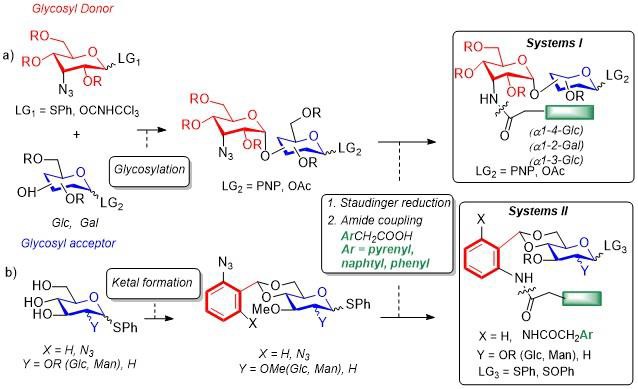
Two important differences between models type I and II, relevant from a reactivity perspective, deserve special attention. First, family I scaffolds form stable carbohydrate/aromatic complexes with a defined geometry, involving either the - (**1**-**3**, **6**) or the - (**8**) face of the pyranose (Figure 2a). On the contrary, for type II rotation of the benzylidene moiety with respect to the pyranose unit is allowed, which determines a highly dynamical and therefore, geometrically adaptable

carbohydrate/aromatic stacking. Molecular dynamic simulations confirm that indeed, several interaction geometries are possible. This includes parallel and parallel-shifted complexes mediated by the - or face of the glycoside, together with lateral, edge-to-face aromatic stacking modes involving the hydroxymethyl moiety (Figure 2b and S1). As a second consideration, family I derivatives encompass an unconstrained pyranose donor, while for family II pyranose units are fixed by the 4,6-*O*-benzilidene moiety, and consequently only a reduced subset of the piranose conformational space is available for the oxocarbenium-like transition states/intermediates. The resulting combinations (fixed stacking/flexible pyranose, in models type I and adaptable stacking/constrained pyranose, in models type II) allow dissecting the influence of aromatic stacking on the glycoside reactivity in complementary scenarios.

Anomeric leaving groups for both scaffolds were selected based on chemical considerations (Figure 2c). Family I derivatives, structurally more complex and more challenging to obtain with 13C- labels, were tested in straightforward acid catalysed methanolysis reactions, for which acid labile *p*- nitro-phenyl or acetyl anomeric substituents were incorporated at the reactive site. These processes are currently assumed to proceed through cationic transition states.9 On the other hand, family II scaffold, affording less overlapped NMR spectra and 13C-labelled for particular models, offered the opportunity to carry out more demanding, low temperature, assays involving a relevant glycosidation reaction widely employed by the carbohydrate community. Consequently, these models were equipped with phenyl sulfoxide fragments as leaving group. It should be mentioned that glycosidations may proceed through a continuum of mechanisms spanning the gap between pure SN2 and SN1 processes as shown by extensive studies by Prof. Crich and col.8 These reactions require the participation of several key intermediates (Figure 2c) including glycosyl triflates20 and/or glycosyl oxocarbenium ions (either solvent equilibrated or forming close ion pairs with the corresponding counterions).8 Fortunately, more dissociative processes, involving cationic transition states, can be favoured by an appropriate choice of the acceptor alcohol as recently shown by Codée and col.21 Taking this into account, we selected for our analyses a weakly nucleophilic acceptor such as trifluoroethanol (TFE), unless explicitly stated.

*b)* ***General synthetic strategy. -*** The synthetic route for the preparation of all model systems used in this study is represented in Scheme 1. Glycosylation reaction between a glycosyl donor containing a phenyl 3-azido-3-deoxy -D-*allo*-thiopyranoside unit22 and the appropriately protected glycosyl acceptor (2-OH-*galacto*-, 3-OH-*gluco-*, and 4-OH-*gluco*-pyranosides including a leaving group at the anomeric position from which the oxocarbenium ion could be generated)23 gave the desired (1→2)-, (1→3)- and (1→4)-linked disaccharides. Subsequent steps related to Staudinger reduction of the azide moiety and amide-coupling with the corresponding (aryl)acetic acid granted access to the model sytems type I, **1**-**9**. In a similar manner, 4,6-benzylidenation of thioglycosides (gluco-, manno- and 2-deoxy-) with the appropriate arylaldehyde dimethyl acetal, followed by the Staudinger

reduction/amide-coupling sequence provided constrained pyranose systems type II, **10-19**. A detailed description of the synthetic protocols together with the characterization of products and intermediates is included in the supplementary material.



***Scheme 1****. General synthetic route for model systems I and II.*

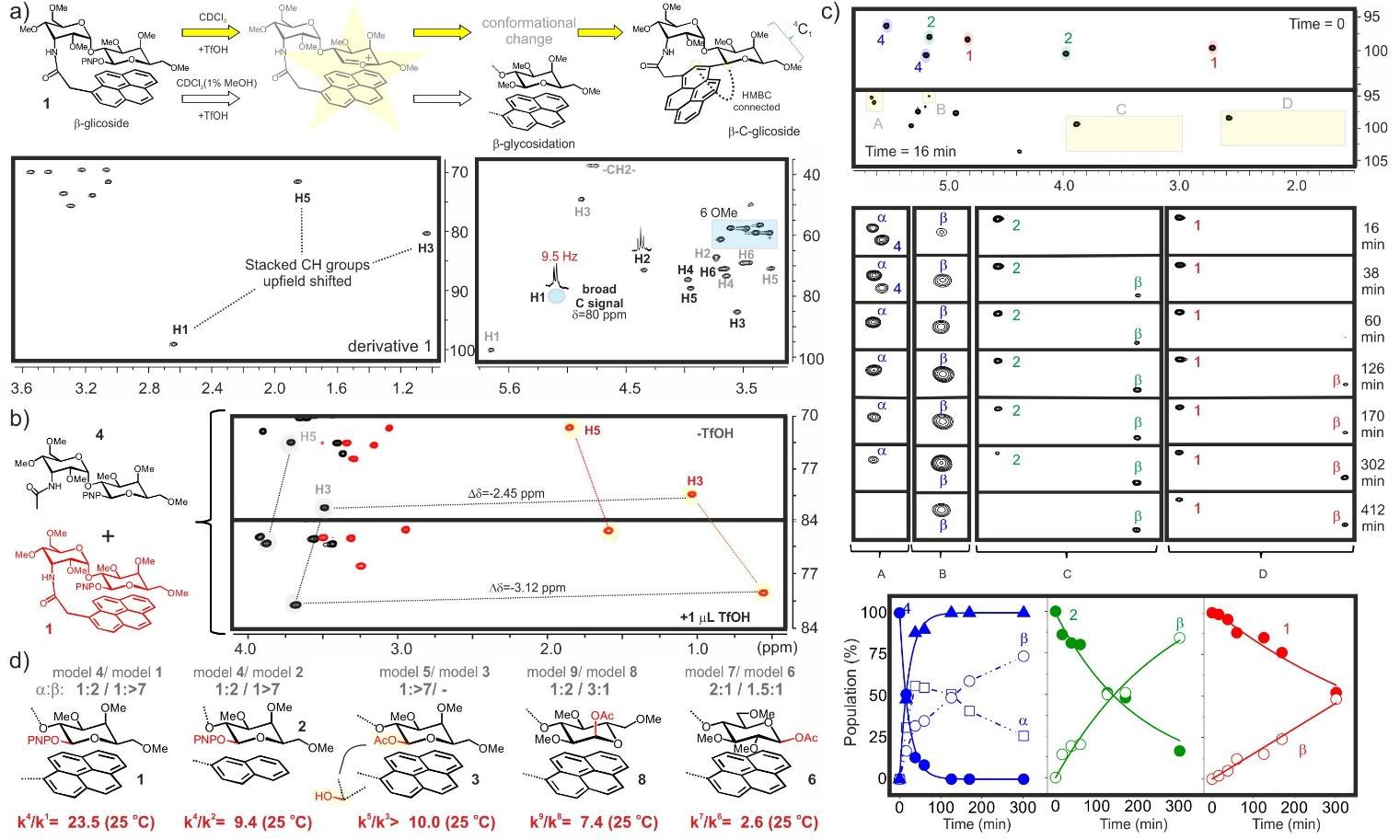
c) ***Methanolysis assays with family I models.-*** Reactivity of compounds **1-9** was assayed in chloroform at temperatures in the 5-20 oC range (see below) and monitored by 2D-HSQC experiments. Obtained results with derivative **1** (and references **2** and **4**) are summarized in Figure 3. As expected from modelling studies (Figure S1), spectroscopy data were consistent with the formation of a well-defined complex involving the pyrene platform and the -face of the reactive unit. Thus, HSQC signals for galactose protons H1, H3 and H5 appear extremely up-field shifted in the proton dimension (Figure 3a, Left), reflecting their involvement in strong CH/bonds with the aromatic unit.

Attempts to perform the methanolysis in two steps were unsuccessful. Instead, the addition of 1

l of triflic acid to the disaccharide solution led to an extreme broadening of the NMR proton signals, consistent with the presence of several species in intermediate exchange on the NMR time-scale. Quenching of the sample with 5 L of MeOD-d4 yielded a major product, with no carbohydrate/aromatic stacking, as judged from chemical shift data. This derivative proved to be extremely unstable, rendering all isolation efforts fruitless. Fortunately, both NMR and mass spectroscopy analyses of the reaction mixture provided key structural information, strongly pointing to the highly strained macrocyclic *C*-glycoside shown in Figure 3a (see also Figure S2). Thus, galactose anomeric carbon (with a broad *C*-glycosidic peak at 80 ppm) presented clear HMBC and NOE connections with the pyrene unit. Moreover, *3JHH* coupling constants were fully consistent with a 4C1 conformation for both pyranose units. More intriguingly, the final *C*-glycoside exhibits a -

configuration as shown by its 9.5 Hz coupling constant. This was totally unanticipated from the

structure of the original carbohydrate/pyrene complex (with the aromatic unit stacked on the -face of the galactose), suggesting that formation of the glycosyl cation must be followed by a significant conformational rearrangement.



**Figure 3*.-****a) Top.- Chemical evolution of derivative* ***1*** *upon treatment with triflic acid, both in the absence and presence of 1% MeOD-d4. Bottom.- Key region of HSQC spectra for* ***1****, and the proposed C-glycosidic structure. b) HSQC spectra of a* ***1****(red)/****4****(black) mixture before and after triflic acid addition. values (ppm) for representative signals are indicated. c) Reactivity assays with a* ***1****(black)/****2****(green)/****4****(red) equimolecular mixture in CDCl3 (1% CD3OD) at 25 oC. Top.- Initial data sets (anomeric region). Middle.- Regions A-D (highlighted with yellow boxes) at different times. Bottom.- Reaction profiles derived from the integration of anomeric cross-peaks. d) Kinetic constant ratios measured for* ***1****-****3****,* ***6*** *and* ***8*** *with respect to the corresponding reference derivatives. The resulting anomeric :ratios are also shown.*

In contrast, addition of 4 L of triflic acid in the presence of 1% MeOD-d4 triggers the desired methanolysis process, which can be conveniently monitored by NMR. Interestingly, this lead to partial protonation of the disaccharides, with distinct behaviours for pyrene-bearing compounds and reference derivatives. As an example, Figure 3b displays HSQC spectra acquired for an equimolecular mixture of derivatives **1** (red) and **4** (black) in the absence and presence of TfOH (1

L). It can be observed that the acidic conditions induce slight down-field shifts in model **4** signals. On the contrary, for derivative **1** protons already involved in CH/interactions with the aromatic platform are shielded even further. As a consequence, chemical shift perturbations promoted by aromatic stacking on the reactive pyranose unit (with respect to reference **4**. See Figure 3b) are significantly enhanced, reaching unusually large values higher than > -3 ppm. In conclusion, partial

protonation of the reactive pyranose turns the original CH/interactions into a much stronger

cation/interactions, leading to a significant stabilization of the carbohydrate/aromatic complex. A similar behaviour was observed for all the stacking complexes tested regardless of the pyranose interacting face (or . See Figure 3b and S3-S6)

The chemical evolution of an equimolecular mixture containing compounds **1**, **2** and **4**, upon triflic acid addition is represented in Figure 3c. Integration of relevant reagent and product cross- peaks with time allowed to build reaction time-courses for the three derivatives assayed. According to this data, reference compound **4**, in which no aromatic unit is present, rapidly evolves to yield a mixture of methyl - and *O*-glycosides with a half reaction time (t1/2) ca. 15 min. A slower methanolysis process is apparent for derivatives **1** and **2**, containing a pyrenyl and naphthyl residue (t1/2 values of 353 and 140 minutes, respectively). Interestingly, in these latter cases, a single O-Me product was detected. Reaction time-courses were translated into kinetic constants assuming pseudo- first order conditions, and the influence of aromatic stacking on reactivity was expressed in terms of kinetic constant ratios (kreference/k+aromatic). Employing this general protocol, we tested all family I model systems. Obtained results are summarized in Figure 3d and reveal a consistent inhibition of the glycoside reactivity by aromatic stacking. Indeed, reactivity falls are in the 2- to 20-fold range depending on the strength and geometry of the original carbohydrate/aromatic interaction. Significantly, this effect is, in some cases, accompanied with an increase in the stereoselectivity of the process (which is particularly clear for the strongest/less reactive complexes). For example, methanolysis of reference **4** produces an initial 2:1 :mixture that under the acidic reaction conditions equilibrates to the final 1:2 stereomeric ratio. On the contrary, donor **1**, involved in a strong stacking complex with a pyrene unit, yields a unique retention -product. This observation is in accordance with the behaviour recently described for an engineered SNi-synthase (Figure 1b)17 and might also point to an SNi mechanism for the substitution process.

In summary, according to the obtained data the aromatic systems present in family I models exert a preferential stabilization of the ground state and partially charged pyranoses over the oxocarbenium- like transition state, leading to a net increase in the global activation energy of the process. This conclusion seems consistent with the apparent strength of the observed CH/contacts, specially under acidic conditions (as judged by values) and suggests that geometrical and dynamical properties of the initial complex are of critical importance for reactivity (see below).

***Familiy II model systems in glycosydation reactions. -*** With these results in hand, we decided to explore the influence of the pyrene unit in a rather different context (Figure S7). As previously mentioned, compounds type II combine weaker/more dynamic carbohydrate/aromatic interactions with a conformationally constrained reactive pyranose. The transient/dynamic character of the stablished CH/bonds is reflected in the reduced chemical shift perturbations promoted by the

aromatic systems at the sugar unit (lower than -2 ppm for derivatives **10**, **11** and **16** even at temperatures below -50 oC. See Figures 4, 5 and S8-S24).

Reactivity assays were individually performed for derivatives **10**-**19** at temperatures in the -65/- 50 oC range and monitored by 1D and/or 2D-HSQC experiments NMR, employing unlabelled or 13C- labelled molecules.24 As internal standard for integrations, equimolecular amounts of 4,4,5,5,- tetramethyl-2-(1-naphthalenyl)-1,3-dioxolane was included in the reaction mixtures.25 Considering the increased complexity of these muti-component reactions, a minimum of three runs, under identical reaction conditions, were completed with each derivative. Competition experiments in pairs were also carried out for qualitative comparison purposes. In all cases, glycosyl donors were activated with triflic anhydride in the presence of 5-8 equivalents of TFE depending on the model compound (for particular models, we performed additional pre-activation experiments as a control. See the experimental section).

*The mannose case.-* The major conformation of derivative **10** is represented in Figure 4a and comprises a preferred stacking geometry which involves the -face of the mannose unit (as shown by chemical shift data. See also Figure S8). Glycosylation reactions carried out in both, NMR tubes and preparative scale in the lab, furnished a mixture of **20**and ****products with an overall yield in the 70-80% range (Figure S9). This is slightly lower than that obtained from the reference compounds **12** and **13** (75%-85%). Strikingly, in the presence of the pyrene system the stereoselectivity of the process completely vanishes (from 1:4 to 1:1).

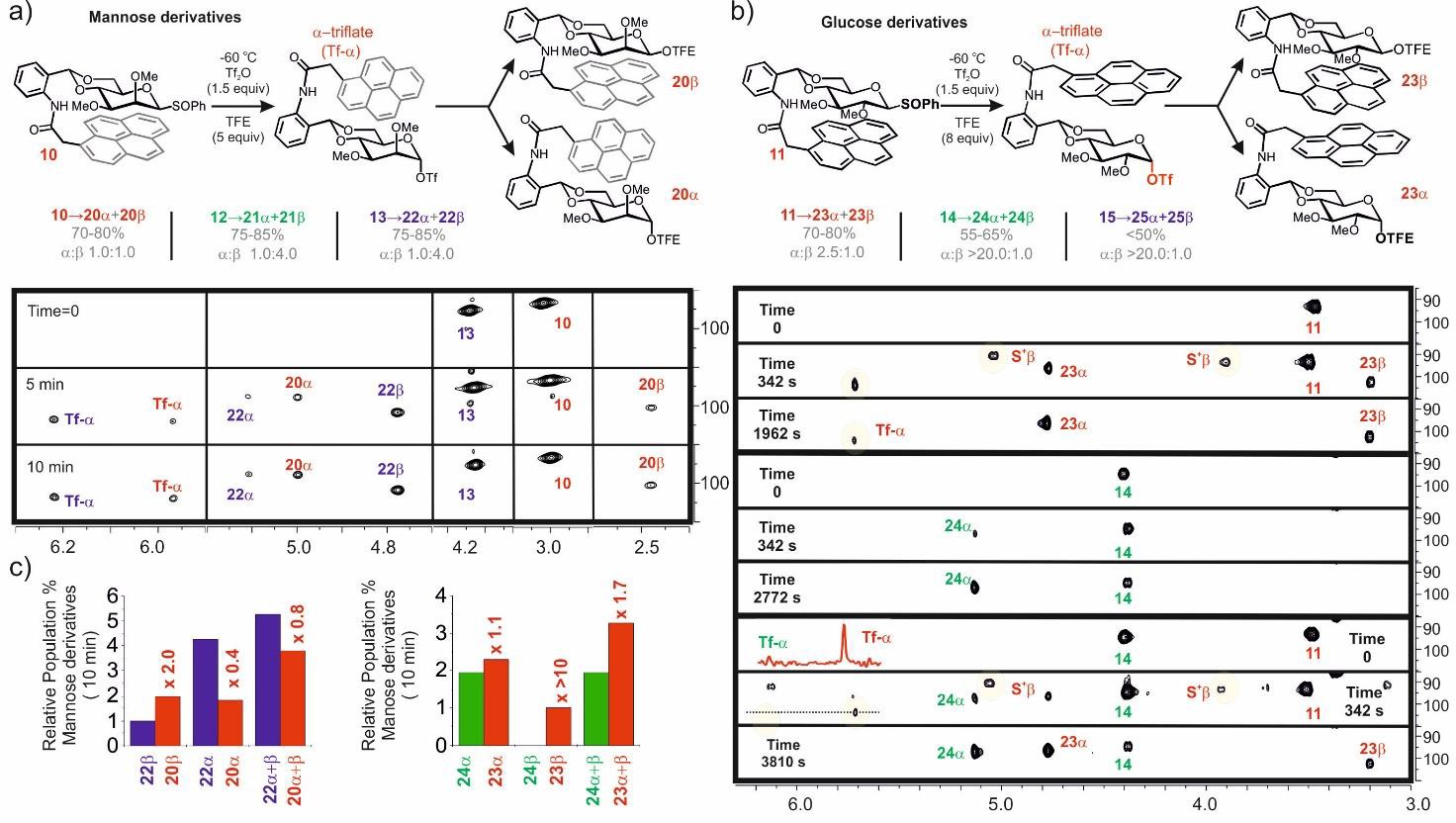
NMR assays performed both with individual donors and equimolecular **10**/**13** 13C-labelled mixtures allowed us to estimate relative concentrations for products **20** (/and **22** (/at short reaction times (5-10 min). Obtained values, proportional to the relative formation rates, are represented as a column chart in Figure 4a (see also Figure S10). According to these data, aromatic stacking has an opposite effect on the individual or product formation rates: two-fold increase for the -anomer and 2-fold decrease for the -derivative, consistent with the observed degradation of the stereoselectivity. Interestingly, even in the presence of five equivalents of TFE, glycosyl - triflates formed from both **10** and **13** were detected throughout the reaction, displaying comparable steady concentrations. This behaviour reflects the higher nucleophilic character of the triflate anion with respect to TFE or the aromatic unit itself.8,26 It should be noted that formation the of an -triflate from **10** requires a rearrangement of the initial carbohydrate/aromatic complex, which is greatly facilitated by the adaptable nature of the designed scaffold. Indeed, the pyrene unit is displaced from the mannose -face, to a lateral disposition, stablishing CH/bonds with the hydroxymethyl moiety, now shielded up to = -1.7 ppm (these intermediates were also formed and characterized by NMR employing a pre-activation protocol. See Figure S8). A similar outcome was obtained employing

**10**/**12** mixtures (Figure S11) These unexpected results prompted us to further interrogate alternative aromatic/donor complexes also belonging to family II model systems.

*The Glucose case.-* Derivative **11** exhibits a highly dynamic conformational behaviour, due to the co-existence of - and - stacking complexes in equilibrium (as judged from the significant induced

values involving hydrogens from both pyranose faces, as well as supporting molecular dynamics simulations. Figure S1 and S12-S14). Glycosidations with donors **11**, **14** and **15** (Figure S15-S18) revealed an even more dramatic effect of the pyrene unit on the stereoselectivity, which drops from

>20:1 to 2:1, with an overall improvement of the reaction yield. 13C-labelled donors allowed to pinpoint further reactivity differences between **11** and the reference derivatives **14** and **15**.



**Figure 4*.-****a) Glycosylation of* ***10*** *with TFE. Yields and stereoselectivities obtained from* ***10****,* ***12*** *and* ***13*** *are shown below. Representative HSQC regions of an NMR competition carried out with an equimolecular 13C-labelled* ***10****/****13*** *mixture at -60 oC, at three different reaction times are displayed.*

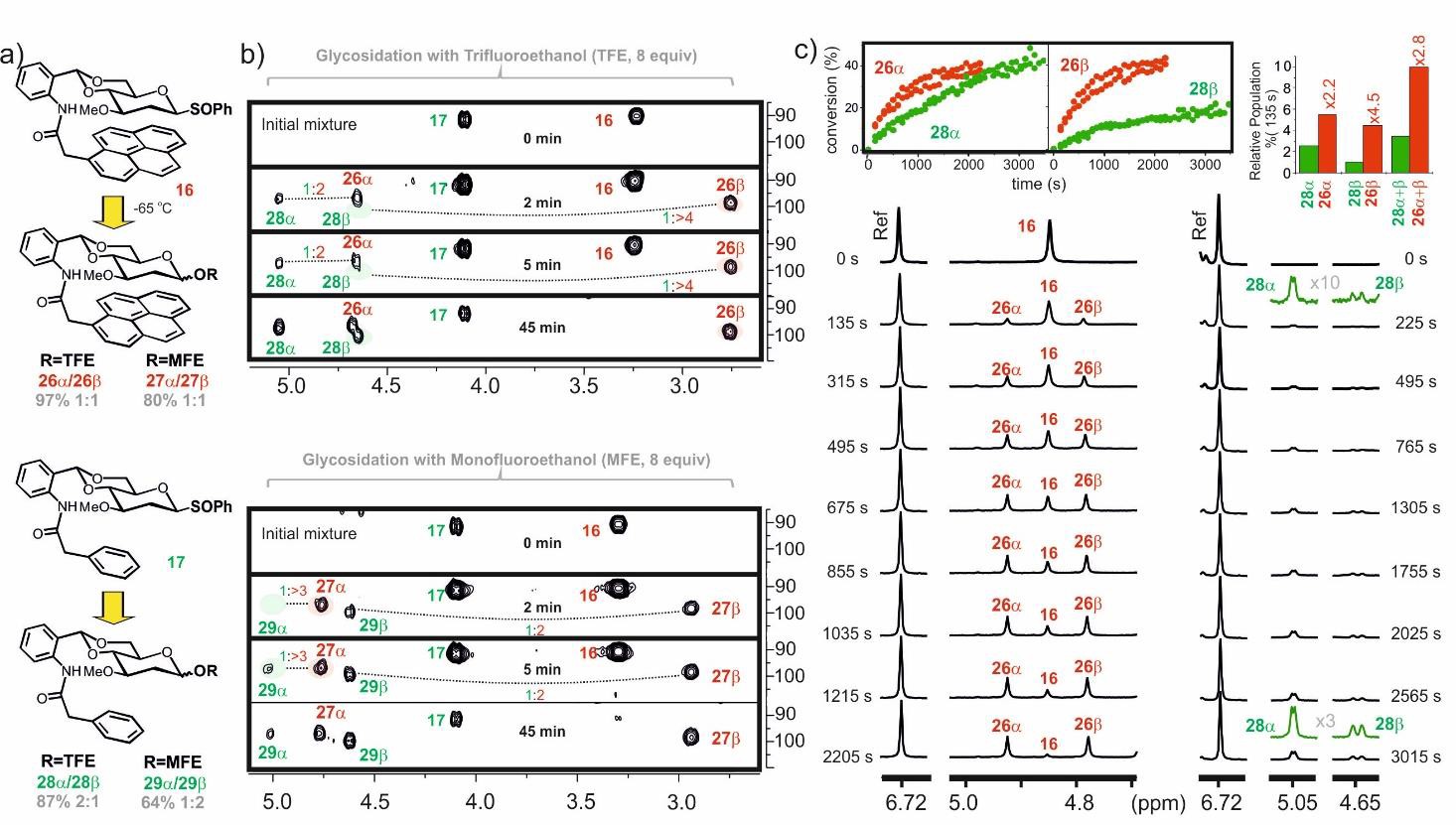
*b) Glycosylation of* ***11*** *with TFE. Yields and stereoselectivities obtained from* ***11****,* ***12*** *and* ***13*** *are shown below. Representative HSQC regions of glycosylations performed with13C-labelled* ***11*** *(top),* ***14*** *(middle) and mixture* ***11****/****14*** *(bottom) at -60oC, at three different reaction times are displayed. c) Bar charts represent relative populations for the corresponding products at short reaction times.*

Thus, for the stacked donor **11** the process seems to proceed with a substantial accumulation of three transient species: an -glycosyl triflate and two unidentified -derivatives (labelled as S+. Figure 4b. Right), whose carbon chemical shifts and heteronuclear *1JHC* constants (168 Hz) strongly point to sulfonium species. On the contrary, analogous intermediates were barely detectable in the absence of a stacked aromatic unit (from **14** and **15**). Of note, the triflate species formed from **11** (also prepared with a pre-activation protocol and characterized. See Figure S14), presents clear CH/ interactions with the pyrene platform, which in this case are mediated by the pyranose -face CH groups. Regarding the reaction kinetics, both individual assays and competition experiments (with

equimolecular 13C-labelled donor mixtures) revealed that while the formation rate of the major - stereomer is marginally increased by the pyrene unit (Figure 4b, **23**vs **24**), that of the minor - anomer is largely enhanced, which accounts for the observed stereoselectivity degradation.

As a final point, the larger accumulation of intermediate species formed from **11** indicates that their formation and/or consumption rates must be significantly altered by carbohydrate/aromatic stacking. Fittingly, pre-activation experiments (see Figure 4b and S19-S20) confirmed that triflate formation is significantly accelerated in the presence of the pyrene unit, pointing to a transition state with a significant cationic character for this step.

*Reactions with 2-deoxy-donors.-* Among common glycoside donors, 2-deoxy-derivatives, lacking an electron-withdrawing substituent at the neighbouring position to the acetalic center, present the highest tendency to evolve through dissociative, cationic transition states. This would ensure an SN1- like behaviour even when more nucleophilic alcohols are considered. Accordingly, for the reactivity analysis of these models, we incorporated monofluoroethanol (MFE) as an additional acceptor.



**Figure 5*.-****a) Reactions with* ***16*** *(up) and* ***17*** *(down) at -65 oC employing TFE or MFE. Yields and stereoselectivities are shown. b) Competition experiments with equimolecular 13C-labelled* ***16****/****17*** *mixtures with TFE and MFE monitored by HSQCs. Data sets measured at different reaction times are displayed. Intensity ratios for reaction products are indicated. c) Reactions performed either with* ***16*** *(left) or* ***17*** *(right) employing TFE, monitored through sequential 1D-NMR experiments at -65 oC. Kinetics of formation for the corresponding and products, derived from two independent experiments, are superimposed above. Bar chart represents relative populations for the corresponding products at short reaction times.*

NMR reactivity experiments performed with C-labelled models revealed, in all cases, a clean evolution from reagents to products, with no detectable intermediates, which is consistent with the higher reactivity expected for any potential transient species involved. Glycosylation yields, either

with TFE or with MFE, were somewhat improved in the presence of the pyrene platform (see Figure 5a and S21-S22). On the contrary, the /stereoselectivity of the processes, already marginal for reference compounds (2:1 and 1:2 with TFE and MFE, respectively) was completely abolished in these cases. Interestingly, competition experiments monitored by HSQC spectra showed that this effect relies on a substantial acceleration in the production of the minor anomer, regardless of their stereochemistry or the alcohol employed in each case. That is, while pyrene significantly accelerates

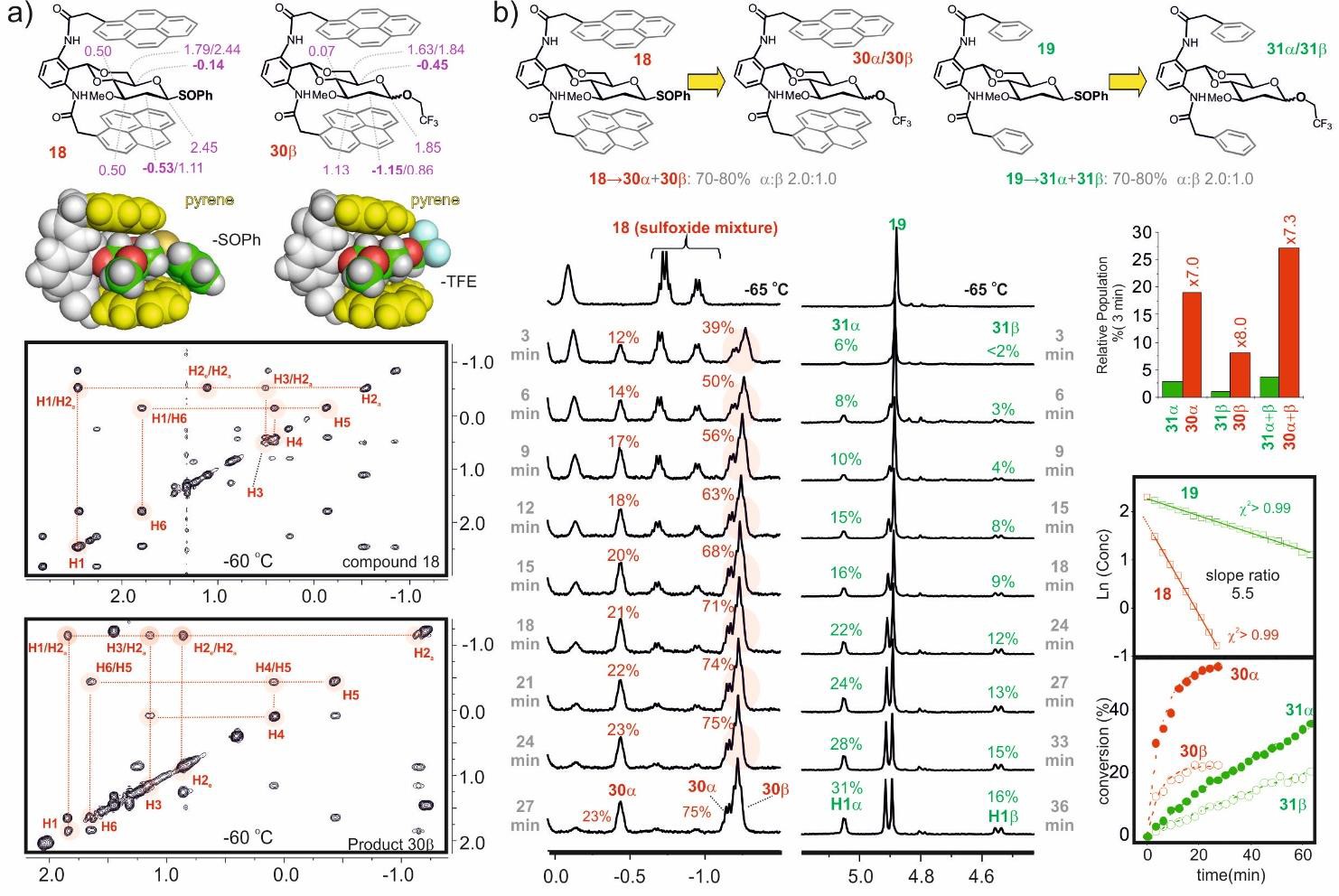
-product formation with TFE (**26**vs **28**), it promotes the same effect but for the -anomer with MFE (**27**vs **29**Figure 5a).

Regarding glycosidations with TFE, the presence of well resolved-proton NMR signals for both reagents and products prompted us to carry out additional experiments and monitor the individual reaction kinetics through sequential low-temperature 1D-NMR. Representative time courses obtained independently for **16** and **17** are shown in Figure 5b, together with relative initial reaction rates, estimated from the concentration of - and - reaction products at short reaction times (less than 20% evolution). These data sets showed a modest 2-fold increase for the -anomer **26**formation rate, and a concomitant 4-fold increase for the -anomer **26**, thus equating both formation rates and explaining the observed 1:1 stereoselectivity. Overall, the glycosidation reaction is accelerated around 3-fold (+ products) in the presence of the pyrene platform, as shown in Figure 5b (bar chart).

Encouraged by these promising results we decided to push these latter models one step further into the SN1 realm by the incorporation of a second interacting pyrene moiety. To our delight, unprecedented shieldings (with proton signals appearing with negative chemical shifts up to = -1.3 ppm) were induced by this optimized covalent scaffold for **18** and the corresponding glycosidation products **30 /**(Figure 6a). Of note, MD simulations show that this effect results from the additive influence of both aromatic platforms but does not imply a significantly tighter, more rigid geometry of the CH/complex (see below).

Reactions of both **18** and the reference derivative **19** with TFE yielded the final glycosylation products, **30 /**and **31 /**with identical yields (in the 70-80% range) and stereoselectivities (1:2). Satisfactorily, careful monitorization of the glycosylation progress through sequential low- temperature 1D-NMR experiments was greatly facilitated by the presence of several proton signals with negative values. *Analysis of the resulting time courses assuming a first order kinetics model showed a increase in glycosylation rates for - and -anomer promoted by the two pyrene platforms in the 5-6-fold range (products* ***30 /****. Figure 6b). Similarly, product concentrations at the shortest reaction time tested (already showing a 40% evolution for* ***18****) were also consistent with a 7-fold enhancement.*

In summary, the combination of a 2-deoxy-glucose donor (prone to participate in dissociative SN1-like substitutions) with appropriately positioned aromatic platforms have permitted, *for the first time, the observation of glycosidation rate enhancements associated to carbohydrate/aromatic interactions*. Intriguingly, the presence of one or two bulky aromatic substituents proximate to the acetalic reactive centre has a null (compound **18**) or negative influence (derivatives **10**, **11** and **17**) on the stereoselectivity of the reaction. Indeed, degradation of stereoselectivity seems to be a common observation across family II model systems herein analysed.



**Figure 6*.-****a) Pyranose chemical shifts for* ***18*** *and* ***30****at -65 oC. CPK models for relevant conformations are shown below together with assigned COSY experiments. b) Reactions with* ***18*** *(Left, in red) and* ***19*** *(Right, in green) with TFE, monitored by 1D-NMR at -65 oC. Yields and stereoselectivities are displayed above. Kinetics of reaction for* ***19*** *and* ***18*** *(expressed as Lg C vs time) and formation for the corresponding and products, are shown on the right. Bar chart represents relative populations for the glicosilation products at short reaction times.*

# DISCUSSION

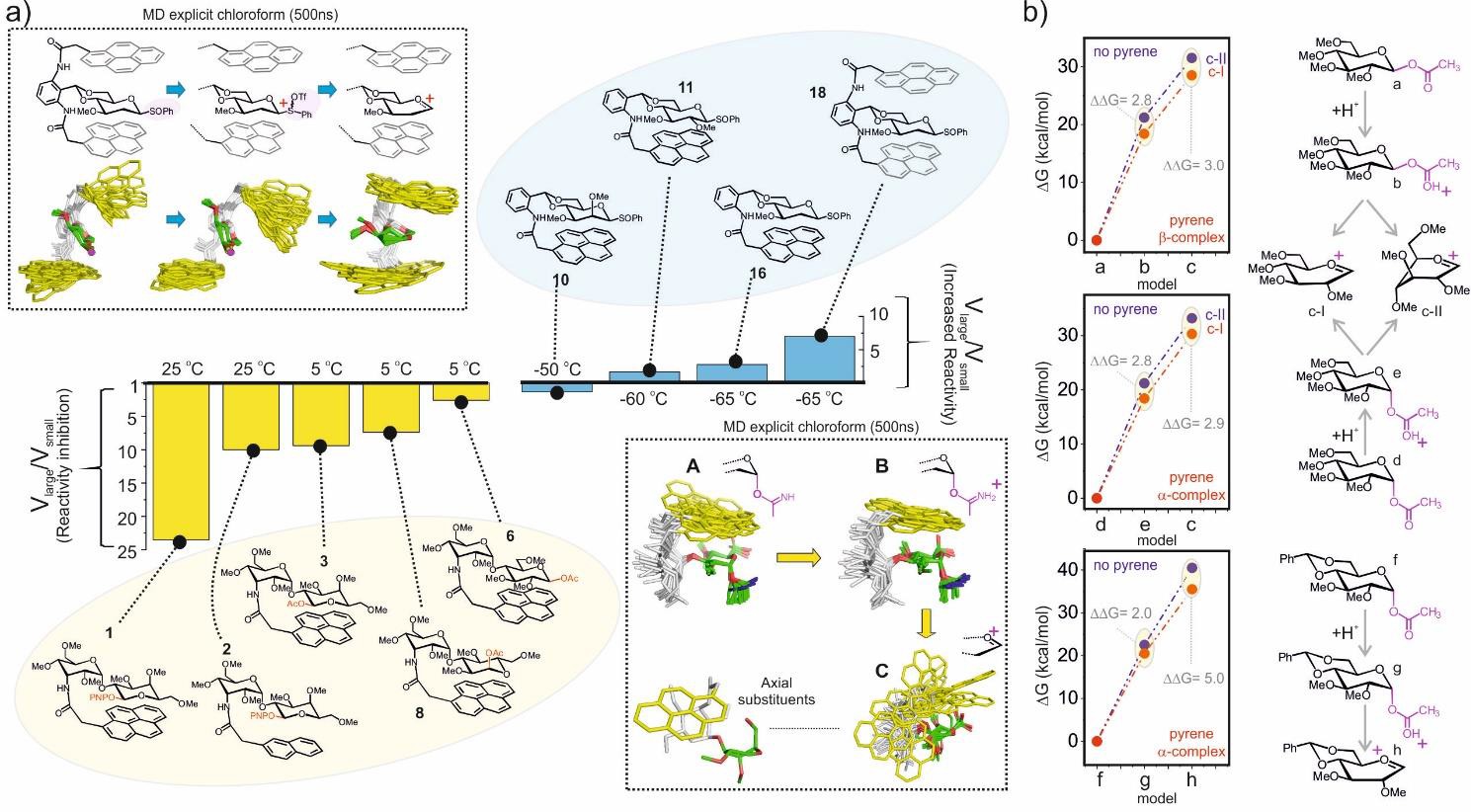
Figure 7 summarizes the results obtained for both families I (Left) and II (Right) model systems and illustrates that aromatic platforms can exert a variety of effects on the pyranose reactivity, ranging from significant reactivity inhibition to moderate rate enhancement. These data can be globally understood by considering the main driving forces that stabilize aromatic/carbohydrate complexes and how they evolve throughout the chemical reactions. On the one hand, development of positive charge at the anomeric position reinforces attractive electrostatic interactions with the aromatic electronic quadrupole. This effect would be expected to be most significant under low

dielectric environments and for highly dissociative SN1-like processes. On the other hand, as the anomeric leaving group departs, the reactive pyranose distorts. In the simplest scenario, anomeric sp3 to sp2 rehybridation would imply that shape complementarity between the pyranose and the aromatic platform, optimal for a 4C1 chair, is decreased, determining the disruption of at least one CH/bond (the one involving the anomeric CH). Furthermore, for unconstrained pyranoses, more dramatic conformational changes could take place, thus leading to an overall deterioration of the van der Waals interactions with the aromatic platform. *According to this view, the net influence of aromatic stacking on the pyranose reactivity depends on a delicate balance between two conflicting energy contributions: electrostatic and van der Waals, favouring and opposing chemical evolution, respectively.*

Regarding family I models, they encompass an *unconstrained reactive* 4C1 *pyranose* participating in strong CH/interactions with the aromatic unit, in both the ground and activated (protonated) states. The decrease in reactivity experimentally observed for the carbohydrate/aromatic complexes indicates that the weakening of the van der Waals interactions at the transition state must not be compensated by any potential electrostatic improvement. Indeed, both CH/and electrostatic contributions seem to be optimal for the activated species rather than for the presumably, highly distorted transition state, totalling in a net increase in the activation energy. This view is supported by solvated molecular dynamic simulations carried out with a modified version of model **8** (with an anomeric imidate instead of acetate. Figure 7a) employing explicit chloroform (and triflate counter ions when required. See the experimental section). According to this theoretical data, protonation of the anomeric substituent determines a reinforcement of the existing CH/bonds, leading to a somewhat more defined interaction mode (in agreement with the chemical shift perturbations described in Figure 2b). Significantly, on departure of the leaving group the internal mobility of the complex is largely increased with frequent disruptions of the carbohydrate/aromatic contacts. This effect appears to reflect the lack of an appropriate shape complementarity between the aromatic platform and the newly formed glycosyl cation. Indeed, this latter species seems to exhibit a significant number of conformational states characterized by the presence of axially oriented substituents and, therefore, incompatible with the stacking requirements.

On the contrary, for family II model systems the presence of weaker, more dynamic CH/ bonds in the ground state *involving a constrained pyranose* determines a distinct behaviour. Three common trends are apparent from the obtained data: First, the larger the SN1 character of the substitution process, the more favourable the influence exerted by the aromatic platform is. Accordingly, clear enhancements in the glycosylation rates are observed only for 2-deoxy-donors. Second, conformational restriction of the donor pyranose allows for a more optimized glycosyl cation/aromatic complex thereby explaining the observed behaviour. This point is supported by MD simulations performed in explicit chloroform and shown in Figure 7a. Three, despite the improved

shape complementarity between the pyrene unit and the nascent glycosyl oxocarbenium intermediate/transition state, stablished interactions must still be highly mobile.



**Figure 7*.-*** a) *Summary of the results obtained for family I (Left, in yellow) and II (Right, in cyan). Aromatic influences are expressed as initial reaction rate ratios for model and reference derivatives, with the larger value divided by the smaller (Vlarge/Vsmall). Conformational ensembles derived from solvated MD simulations (explicit CHCl3) with a modified version of model* ***6*** *(Bottom-right corner. See the main text) and* ***18*** (Upper-Left corner)*, in their ground, activated and glycosyl cation states are represented. b) G cost for the protonation of models* ***a****,* ***d*** *,****f*** *(from top to bottom) and the subsequent glycosyl cation formation, in the presence (red) and absence (blue) of a complexed pyrene platform, calculated by quantum mechanics. Complex geometry (either or ) is indicated. The influences exerted by the pyrene unit are expressed as G (kcal/mol). Most stable conformation of the glycosyl cations were consider for both the free and complexed states.*

Indeed, the aromatic system seems to provide a nurturing, more polar local environment for the developing pyranose charge, rather than fixing in a preferred complex. Accordingly, pyrene units have null to negative impacts on the stereoselectivity of the process. In particular, for compound **18**, with the donor unit sandwiched between two pyrenes, no improvement in the stereoselectivity of the reaction was detected. Further, for donors **10**, **11** and **16** a preferential increase in the formation rate of the minor anomer is observed, which determines a sharp decrease in the reaction stereoselectivity. This observation could be interpreted in terms of a *shift in the reaction mechanism toward a more dissociative SN1-like process*. The apparent dynamic character of the glycosyl cation/aromatic contacts is intriguing and might partially reflect the detrimental influence of limited pyranose distortions. Alternatively, this dynamic behaviour could result from competing electrostatic cation/triflate interactions, not correctly accounted for by the simulations. Overall, this represents a consistent and general conclusion for family II model compounds.

In summary, while aromatic quadrupole platforms seem to facilitate formation of glycosylic cations by electrostatic forces, pyranose distortion and its detrimental influence on CH/ contacts oppose this process. Significantly, quantum mechanics calculations (method) performed with simplified 1-*O*-acetyl model glycosides (Figure 7b and S22), either constrained (**f**) or unconstrained (**a** and **d**), predict an enhanced stabilization of the glycosyl cation by the pyrene unit in the former case (G of 5 kcal/mol vs 2.9-3.0 kcal). While this trend is in agreement with our experimental observations, theoretical stabilizations seem, in all cases, largely overestimated, which might reflect limitations inherent to these theoretical methods (use of implicit solvation, employment of simplified intermolecular model complexes, and absence of triflate counterions).

Finally, it is worth noting that among all interactions participating in enzyme/glycoside complexes at the -1 site, carbohydrate/aromatic contacts seem to exhibit distinct geometrical features with respect to those typically observed in carbohydrate binding modules (CBMs). Thus, parallel stacking geometries (fully involving either the or - face of the reacting pyranose) are uncommon, in comparison with lateral, edge-to-face or parallel-shifted interactions (see Figure S24), which usually involve the formation of only one or two CH/bonds per aromatic. This phenotypic/recurring feature might reflect the need to maintain the sugar ring relatively unconstrained by the aromatic platform, allowing penalty-free pyranose distortions required for the reaction to proceed. In view of our results, aromatic moieties could only contribute to the catalysis of highly dissociative chemical transformations (involving positive charge development), if they allow pyranose distortions without associated energy costs.

# CONCLUSIONS

While the participation of aromatic platforms in the molecular recognition of carbohydrates is undeniable, its potential participation in catalysis remains an open question. Employing a bioorganic approach, based on the design, synthesis and evaluation of around twenty model systems spanning a range of carbohydrate/aromatic stacking geometries and dynamic features, anomeric leaving groups, sugar configurations and reaction conditions, we have been able to dissect the fundamental requirements of aromatic catalysis to glycosidic bond cleavage or formation. To the best of our knowledge, this represents the first experimental study on this relevant topic, with far reaching implications in catalysis and enzyme engineering.

# METHODS

A detailed description of the synthetic protocols together with the characterization of products and intermediates is included in the supplementary material.

*-****NMR tube methanolysis experiments.-*** A stock solution containing CD3OD (1% v/v) in CDCl3 was transferred to a 5 mm NMR tube fitted with a septum and purged with argon. Solutions

of the adequate donor (for individual assays) or donors (for competitions) in CDCl3 (50 L, 100 mM) were then added to the tube and shaken to mix. Both 1D and 2D-HSQC data sets were recorded for the starting mixture at the appropriate working temperature (5 or 25 oC). After addition of triflic acid (3-5 L) in an ice bath, the sample was transferred to the magnet.

Time evolution of the reaction mixtures was followed by sequential 1D-1H and 2D-HSQC data. These NMR experiments were acquired on a Bruker Avance 600 MHz spectrometer equipped with a cryo-probe. 1D-1H data sets were collected with 8-16 scans and a relaxation delay of 2 s. 2D- HSQC spectra were typically acquired with 64-128 increments and 8-16 scans per increment, which determines a total experimental time of 6-16 min per spectrum. The resulting time-resolution was found to be sufficient to monitor slow methanolysis processes.

Relative populations of reagents and products throughout the reactions were determined by integrating selected NMR signals in 1D and 2D-HSQC data sets at different reaction times. Indeed, these latter experiments proved especially useful for the analysis of competition assays, given the complexity of the resulting reaction mixtures, formed by up to six highly similar disaccharides.

The spectrum array obtained from each reactivity assay (either 1D or 2D) was processed using MestReNova. (v. 10.0, Mestrelab Research S.L.). Each spectrum was individually phased in the region of interest and baseline corrected. Integration ranges were selected to avoid overlap and to account for peak drifting over the course of the reaction. Absolute peak volumes (for 2D data sets) were normalized and converted to relative populations (0-100 %) with the assumption (supported by the NMR data) that no significant side reactions occurred. These were then represented with respect to the reaction time employing Origin.package. Reaction time courses were fitted assuming pseudo- first order kinetics, employing equations **1** and **2** for donor disappearance and product formation, respectively:

(1) D(t)=100e-kt

(2) P(t)= 100(1-e-kt)

where D(t) and P(t) stand for donor and product population (0-100%) at any particular time. The influence exerted by the aromatic platforms on the reaction rates was expressed in terms of kinetic constant ratios (kreference/k+aromatic).

***-NMR tube Glycosidation Experiments.-*** Considering the increased complexity of these muti- component reactions a minimum of three runs, under identical reaction conditions, were completed with each derivative. Additionally, competition experiments were performed for selected equimolecular donor mixtures. Thus, the internal standard 4,4,5,5-tetramethyl-2-(naphthalen-1-yl)- 1,3-dioxolanex (25.6 mg, 0.1 mmol), DTBMP (61.5 mg, 0.3 mM), the appropriate acceptor alcohol (5-8 equiv depending on the employed donor) and activated molecular sieves 4 Å (500 mg) were added to a septum-capped oven-dried vial. The vial was twice evacuated and purged with argon before the addition of CDCl3 (10 mL). The resulting stock solution (550 L) was transferred to a 5

mm NMR tube fitted with a septum and purged with argon. A solution of the adequate donor in CDCl3 (50 L, 100 mM) was then added to the tube and shaken to mix. Afterwards, the NMR tubes were cooled down in a liquid nitrogen/acetone bath to the selected reaction temperature before the treatment with triflic anhydride (1.5 equiv, 1.4 l) and immediately transferred to the NMR spectrometer, previously equilibrated at the proper working temperature. Before each run, locking, tuning, and shimming were carried out on a tube prepared in an identical fashion containing all reaction components.

Glycosidation reactions were followed by low-temperature (-65/-50 oC) NMR experiments, employing a Bruker Avance 500 MHz spectrometer. 1D-1H spectra were acquired with 300 excitation pulses and a relaxation delay of 1 s. The extra sensitivity provided by 13C-labelled samples allowed us to acquire 2D-HSQC data sets with just 2 scans per increment and 64 increments, limiting the total experimental time to 2-3 minutes. These conditions were found to be adequate to monitor relatively fast glycosidation reactions. Transmitter offsets were set to 5 and 90 ppm, in the proton and carbon dimensions, respectively, which allowed an optimum excitation of the anomeric CH groups. In addition, a delay corresponding to a *J* value of 165 Hz (between those expected values for anomeric

- and –CH fragments) was employed in all cases. Data processing was carried out with MestReNova, as previously described for methanolysis assays.

For mannose and glucose models **10**-**15**, reactions were carried out with one or two competing donors at a time. The absence of well resolved peaks for all glycosylation products in the 1D data sets (particularly for **10** and **11**), led us to mainly rely on 2D-HSQC experiments to derive relative populations at different reaction times. To assess the validity of this approach, control HSQC spectra were measured with increasing relaxation delays (in the 1-10 s range), showing that this parameter had a relatively minor influence (<10%) on the estimated volume ratios. When possible, values derived from 2D data sets were also confirmed with 1D experiments for selected non-overlapped signals. Relative concentrations at short reaction times (<10 min, when <20% evolution has occurred) were taken as indicative of the relative formation rates for the different products.

For derivatives **16**-**19**, showing the largest influence of the aromatic stacking on the donor reactivity, a more quantitative approach was attempted. This was greatly facilitated by the presence of well-resolved NMR signals for all the intervening species (extremely up-field shifted by the pyrene platforms in the case of **19**). Thus, peak areas corresponding to the different reaction products (**27**, **28**, **30**, and **31**) in 1D data sets were transformed into a percentage of conversion using the expression: C%= (Pt/St)\*100/(D0/S0)

where, D0 and S0 are the integration values of a donor peak and the internal standard in the

initial reaction mixture (time=0) and Pt and St are those of a given product peak and internal standard at a particular reaction time (time=t). Conversions were represented with respect to the reaction times employing Origin package (OriginLab Corporation).

The presence of well resolved peaks allowed us to also monitorize the decreasing concentrations C of **18** and **19** throughout the reaction course. Linear representations of Ln(C) vs Time were built form these data and fitted to the equation:

Ln(C)=Ln(Co) - kt

where Co represent the initial concentration of the model and the slope, k. a first order kinetic constant. Slope ratios for **18** and **19** was employed to estimate the influence of the aromatic platform on the reaction rate. Finally, relative formation rates for the different products were also estimated, without mechanistic assumptions, from their relative conversions at short reaction times.

***- Glycosidations in the laboratory.-*** XXX

# SUPPLEMENTARY MATERIALS

A detailed description of the synthetic protocols together with the characterization of products and intermediates. Figures S1-Sx showing details of the NMR and reactivity experiments.

# ACKNOWLEDGMENTS

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23.- Synthesis of each targeted substrate was not as straightforward as expected and a number of challenges were encountered in the experimental work. These specific aspects of the study are addressed in full detail in the Supporting Information

24.-Reactivity assays, were carried out with enantiomerically pure sulfoxides unless explicitly stated. This structural parameter was shown to have no influence on the obtained conclusions.

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# TOC

