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Systematic studies toward the synthesis of D-galactosamine-containing coumarin glycosides†

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An O-glycosylation method for accessing coumarin glycosides is presented. We report the reaction of 6,8-difluoro-7-hydroxy-4-methylcoumarin and 4-methylumbelliferone with a variety of glycosyl imidate donors using $\text{BF}_3 \cdot \text{Et}_2\text{O}$ as activator to access a series of coumarin glycosides in 64%–76% isolated yields. Several reaction parameters are evaluated including promoters, temperature and reagent equivalents. Following initial methodology development using simple D-glucose donors, D-galactosamino mono- and disaccharides are explored as substrates, showcasing applicability towards late-stage transformation of biologically relevant chondroitin sulfate glycosides. Glycosylation diastereoselectivity trends were also considered, proposing that the identity of the D-galactosamino N-protecting group and the coumarin acceptor contribute to observed anomeric product ratios. This methodology provides a convenient access to D-galactosamino-coumarin glycoconjugates and provides a benchmark for the development of related systems for biological evaluation.

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Introduction

Coumarins are naturally occurring heterocycles that have received attention due to their use as therapeutic agents. For example, warfarin and acenocoumarol are approved anti-coagulants, derived from coumarin.¹ Relatedly, coumarin glycosides, where coumarin is conjugated to a sugar, have been evaluated as anti-cancer,² anti-diabetic,³ anti-inflammatory,⁴ anti-microbial,⁵ and anti-viral agents.⁶ Additionally, coumarin glycosides contain a fluorogenic reporter group, and have thus been deployed as probes/inactivators for enzymes.^{7,8} Notable examples include esculin, an anti-inflammatory therapeutic, which undergoes hydrolysis by esculin hydrolase as a rapid test to detect Gram-positive or negative bacteria.⁹ Furthermore, 4-methylumbelliferone- α -D-galactopyranoside has been used to monitor α -galactosidase-A activity relating to Fabry disease.¹⁰ Access to coumarin-functionalised glycosides is thus of particular interest to advocate their use as biological tools in glycoscience. To achieve this, robust methods for their synthesis are required, particularly those that avoid harsh reaction conditions and are applicable beyond simple monosaccharides.

Methods for coumarin attachment to sugars have been reviewed,^{11,12} and often involve heating glycosyl acetates (the Helferich method), exemplified for D-Gal in Fig. 1a, using an

excess of Lewis acid to afford the glycoside. However, these reactions are often only moderately yielding and not applicable to more complex/precious saccharides.¹³ Methods for accessing coumarin conjugates of the aminosugar D-galactosamine (D-GalN) are surprisingly few. Glycosyl halide **3** has been employed as a D-GalN monosaccharide donor,^{14–16} undergoing appropriate phase-transfer activation to deliver coumarin glycoside **5** in 33% yield [Fig. 1b, method (a)].¹⁷ However, this method is incompatible with glycosyl halide substrates prone to elimination under basic conditions.¹⁸ Alternatively, coumarin glycoside **5** has been accessed by heating glycosyl acetate **4** with $\text{Cu}(\text{OTf})_2$ under microwave conditions,¹⁹ improving the yield to 53%. Finally, a D-GalN-containing α -linked 4-methylumbelliferone (4-MU) disaccharide (D-Gal- β (1,3)-D-GalN) **7** was prepared by Kiso and co-workers, who optimised a Mitsunobu reaction using glycosyl donor **6** containing an α -directing 4,6-O-di-*tert*-butylsilylene group. α -Selectivity was achieved, but as an inseparable mixture (9:1 α : β) in 80% yield, and required high temperature using an excess of 4-MU (Fig. 1c).²⁰

As part of a wider programme concerning the chemical synthesis of glycosaminoglycan mimetics,^{21–27} we required a general synthetic approach to access structurally defined chondroitin sulfate fragments containing a D-GalN-coumarin reducing end conjugate, particularly toward materials with orthogonal protecting group patterns. Considering the relative sparsity of methods available, our approach sought glycosyl imidate donors, which are reactive, accessible under mild conditions and have been employed previously for saccharide-coumarin attachment. For example, Ferro and co-workers syn-

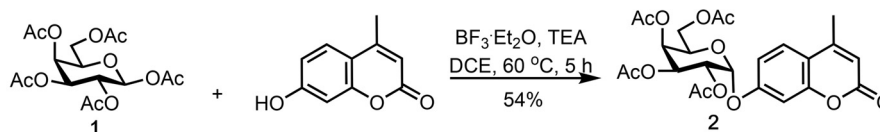
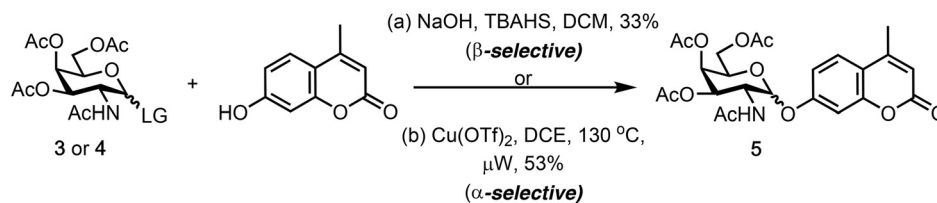
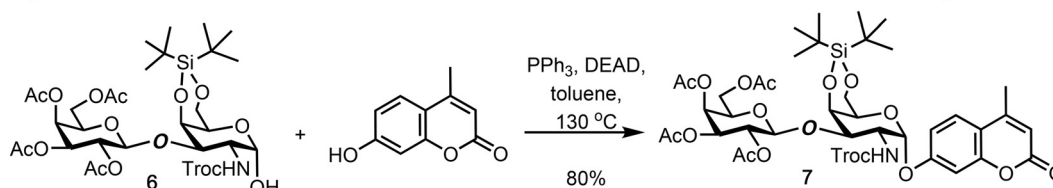
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† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d5ob00746a>



Previous Work

a) *D*-Gal Coumarin Monosaccharides

Molecules 2015

b) *D*-GalN Coumarin Monosaccharides(a) *Org Lett* 2007(b) *ACS Synth Biol* 2019c) *D*-GalN Coumarin Disaccharides*Org Lett* 2005

This work

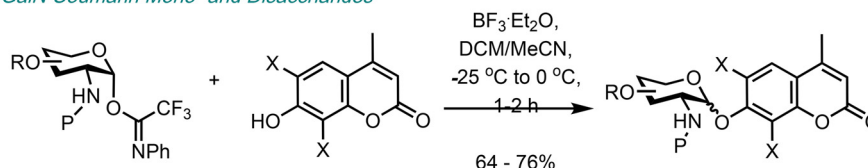
d) *D*-GalN Coumarin Mono- and Disaccharides

Fig. 1 Methods of *O*-glycosylation toward accessing *D*-galactosamine coumarin glycosides and the reaction conditions developed here for a range of mono- and disaccharides. LG = Cl (**3**) or OAc (**4**); X = H = 4-MU; X = F = DiFMU; P = Troc or Phth.

thesised a fluorogenic heparan sulfate disaccharide (*D*-GlcN- α (1,4)-*D*-GlcA) using a glycosyl *D*-glucuronate imidate in 76% yield. Notably this required prior TMS protection of 4-MU, successive addition of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ and acetylation of the product to facilitate purification,²⁸ highlighting further the challenges associated with this type of transformation. Reported herein is our development of a methodology to generate coumarin-functionalised chondroitin sulfate disaccharides (*D*-GlcA- β (1,3)-*D*-GlcN).

Results and discussion

D-Glucopyranosyl donors

To initiate a general method for coumarin attachment, a series of simple *D*-glucopyranosyl monosaccharide donors were synthesised (see ESI† for details) before exploring *D*-GalN derivatives. Briefly, commercially available peracetylated β -*D*-glucose **8** was diversified at the anomeric position to generate six different donors, which were then subjected to glycosylation

using 6,8-difluoro-7-hydroxy-4-methylcoumarin (DiFMU) as the acceptor (Table 1).

Reactions using acetate **8**, thioglycoside **9** and phosphate **10** donors were unsuccessful due to no reaction, decomposition or hemiacetal formation, respectively. Glycosyl bromide **11** and Ag_2O activation was more promising, generating the required coumarin derivative **14** in 30% yield (Table 1, entry 1), and this could be improved to 60% using a shorter reaction time (2 h *versus* 48 h) and the addition of TMSOTf (Table 1, entry 2), as adopted from work by Demchenko and co-workers.²⁹ Optimal results were achieved using *D*-glucopyranosyl imidate donors **12** and **13**. Whilst initially no reaction or donor hydrolysis occurred when using *N*-trichloroacetimidate (TCAI) donor **12** with catalytic TMSOTf or tris(pentafluorophenylborane) (BCF), the adoption of catalytic $\text{BF}_3 \cdot \text{Et}_2\text{O}$ gave the desired β -coumarin **14** in 69% yield (Table 1, entry 3). Increasing the amount of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ from 0.2 to 1.0 equivalent had little effect on reaction yield using TCAI donor **12** (Table 1, entry 4); these conditions could be similarly applied using donor **13** (Table 1, entry 5). Overall, *D*-glucopyranosyl donors **11–13** generated the derived



Table 1 Development of DiFMu attachment for D-glucopyranosyl donors

Coumarin Glucopyranosides

14

8 = X = OAc
 9 = X = SEt
 10 = X = OP(O)(OBu)₂
 11 = X = Br
 12 = X = OTCAI
 13 = X = OPTFAI

Entry	Substrate	Conditions	Promotor	Equiv.	α : β	Yield (%)
1	11	<i>b</i>	Ag ₂ O	20	0 : 1	30
2	11	<i>c</i>	Ag ₂ O/TMSOTf	3.0	0 : 1	60
3	12	<i>a</i>	BF ₃ ·Et ₂ O	0.2	0 : 1	69
4	12	<i>a</i>	BF ₃ ·Et ₂ O	1.0	0 : 1	64
5	13	<i>a</i>	BF ₃ ·Et ₂ O	1.0	0 : 1	63

Conditions: ^a 1–2 h, –20 °C–0 °C. ^b 48 h, RT. ^c 2 h, RT. General conditions: glycosyl donor (1.0 equiv.); DiFMu (1.2 equiv.); DCM.

difluorinated coumarin glucoside **14** in 60–69% yields with expected β-stereoselectivity and minimal by-product formation.

Towards D-galactosamine-coumarin conjugates

With a glycosylation method established, a panel of monosaccharide D-GalN imidate donors (**15–18**) were synthesised, including *N*-Phth, *N*-Ac and *N*-Troc amine protecting groups (see ESI† for details).

Glycosylation using *N*-Phth donor **15** and *N*-Ac donor **16** were unsuccessful, either generating inseparable mixtures or no product. Using *N*-Troc imidate **17**, glycosylation was suc-

cessful, generating disaccharide **19** in yields of up to 71% (Table 2, entries 1–3). β-Diastereoselectivity was improved using a DCM/MeCN solvent system, from a 1 : 2 α : β mixture (Table 2, entry 1) to 1 : 4 (Table 2, entry 2). Such mixtures were separable *via* column chromatography and displayed clear differences in derived ³J_{H1–H2} ¹H NMR coupling constants [*J* = 3.2 Hz (α) *versus* 8.3 Hz (β)] and coupled HSQC [¹J_{C1–H} = 183.5 Hz (α) *versus* 168.8 Hz (β)]. Similar patterns were observed for PTFAI donor **18** (Table 2, entries 4–7), albeit in reduced yield and a stoichiometric amount of BF₃·Et₂O proved optimal to deliver coumarin glucoside **19** in 65% yield (Table 2, entry 7). Finally, scalability was demonstrated (1.3 mmol, Table 2, entry

Table 2 Exploring coumarin attachment using D-galactosamine donors

Coumarin Galactopyranosides

19 = P = NHTroc

15 = P = NPhth, X = OTCAI
 16 = P = NHAc, X = OTCAI
 17 = P = NHTroc, X = OTCAI
 18 = P = NHTroc, X = OPTFAI

Entry	Substrate	Solvent	Promotor	Equiv.	α : β	Yield
1	17	<i>a</i>	BF ₃ ·Et ₂ O	0.2	1 : 2	60%
2	17	<i>b</i>	BF ₃ ·Et ₂ O	0.2	1 : 4	71%
3	17	<i>b</i>	BF ₃ ·Et ₂ O	1.0	1 : 4	66%
4	18	<i>a</i>	BF ₃ ·Et ₂ O	0.2	1 : 2	50%
5	18	<i>b</i>	BF ₃ ·Et ₂ O	0.2	1 : 5	54%
6	18	<i>c</i>	BF ₃ ·Et ₂ O	0.2	1 : 3	52%
7	18	<i>b</i>	BF ₃ ·Et ₂ O	1.0	1 : 4	65%
8 ^d	18	<i>b</i>	BF ₃ ·Et ₂ O	1.0	1 : 4	66%

Solvent conditions: ^a DCM. ^b DCM/MeCN (10/1). ^c DCM/MeCN (5/1). General conditions: glycosyl donor (1.0 equiv.); DiFMu (1.2 equiv.); 1–2 h, –20–0 °C. ^d 1.3 mmol scale.



8). Despite a less-than-optimal diastereoselectivity, these results using TCAI or PTFAI *D*-GalN donors encouraged us to explore disaccharide substrates.

Coumarin glycosylation with disaccharide *D*-GalN donors

The compatibility of late-stage disaccharide glycosylation with both DiFMu and 4-MU was explored next, utilising a selection of available chondroitin sulfate disaccharide precursors containing a variety of protecting groups (Table 3, see ESI† for details of donor preparation).²¹ Glycosyl donor **20** containing a *N*-Phth group generated β -**24** in 70% yield (Table 3, entries 1 & 2) with stoichiometric activator (using 0.2 equivalents of Lewis acid reduced the yield to 50%). Full characterisation of β -**24** was obtained, with notable coupling constants deduced from the relevant 1D and 2D NMR spectra. The β -configuration at the reducing end was determined by a $^3J_{\text{H1-H2}}$ coupling of 8.4 Hz and a large $^1J_{\text{C-H}}$ coupling of 169.4 Hz. Furthermore, characteristic ^{19}F coupling was observed, with $^1J_{\text{C-F}}$, $^3J_{\text{H-F}}$ and $^4J_{\text{F-F}}$ values of 251.5, 10.1 and 3.6 Hz respectively (Fig. 2).

Reaction with *N*-Troc TCAI donor **21** generated glycoside **25** (1 : 2 α : β) firstly in 20% yield (Table 3, entry 3) then 28% yield (Table 3, entry 4) using stoichiometric activator. The major product isolated from this reaction (in 50% yield) was the *N*-glycoside, formed through return nucleophilic addition of the acetamide leaving group at C1. Switching to *N*-Troc PTFAI donor **22** and DiFMu generated disaccharide **25** in a moderately improved 39% yield (Table 3, entry 5). Reducing the temperature to -50°C had a minimal impact on yield or diastereoselectivity (Table 3, entry 6). Stoichiometric activator afforded **25** in an improved 73% yield but was poorly diastereoselective, affording a separable 2 : 1 α : β mixture (Table 3, entry 7). Furthermore, when donor **22** was reacted with 4-MU under identical conditions, the

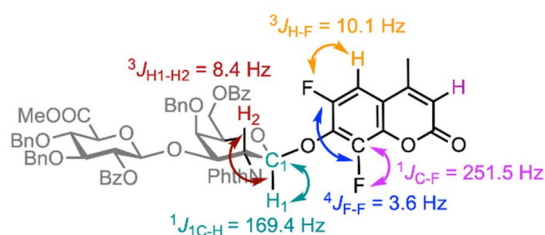


Fig. 2 Chemical structure of coumarin glycoside **24**, highlighting the key coupling constants observed in NMR spectroscopic data.

reaction again proceeded smoothly to generate **26** in 76% yield, but now as a 1 : 2 α : β mixture (Table 3, entry 8). Finally, 4,6-*O*-acetylated imidate **23** was reacted with DiFMu (Table 3, entry 9) which afforded disaccharide **27** in 64% yield in a 1.5 : 1 α : β ratio.

Several factors can influence the rate and stereochemical outcome of a glycosylation reaction, including the choice of anomeric leaving group, promotor system and acceptor nucleophilicity. For aminosugars, donor reactivity and glycosylation stereoselectivity can also be influenced by participation of a C2-amine protecting group. Indeed, this has been noted previously for *D*-GlcN derivatives, but less so for *D*-GalN.³⁰ Herein we observed that the outcome (20%–76% yields; 2 : 1–0 : 1 α : β ratio) of glycosylation reactions to install a coumarin aglycon was dependent upon both the identity of the galactosamino *N*-protecting group and the phenol nucleophilicity. An *N*-Phth protecting group effected complete β -stereoselectivity and an *N*-Troc group also promoted β -stereoselectivity using a monosaccharide donor. However, at disaccharide level a decrease in β -selectivity was observed; reaction of *N*-Troc disaccharide donor **22** with DiFMU generated compound **25** as a 2 : 1 α : β

Table 3 Optimisation of coumarin attachment to generate disaccharides **24**–**27**

Entry	Substrate	Conditions	Promotor	Equiv.	Product	α : β	Yield
1	20	<i>c</i>	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	0.2	24	0 : 1	50%
2	20	<i>c</i>	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	1.0	24	0 : 1	70%
3	21	<i>c</i>	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	0.2	25	1 : 2	20%
4	21	<i>c</i>	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	1.0	25	1 : 2	28%
5	22	<i>c</i>	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	0.2	25	1 : 1	39%
6	22	<i>d</i>	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	0.2	25	1 : 1	42%
7	22	<i>c</i>	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	1.0	25	2 : 1	73%
8	22	<i>c</i>	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	1.0	26	1 : 2	76%
9	23	<i>c</i>	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	1.0	27	1 : 1	64%

Reaction conditions: *a* DCM, RT. *b* MeCN, RT. *c* DCM/MeCN (10/1, v/v), -20°C to 0°C . *d* DCM/MeCN (10/1, v/v), -50°C to 0°C . General conditions: glycosyl donor (1.0 equiv.); coumarin (1.2 equiv.); 1–2 h.



Table 4 Comparison of this work and prior methods for D-GalN-coumarin glycosylation

Method	Donor (equiv.)	Coumarin (equiv.)	Promotor (equiv.)	Time (h)	Temp. (°C)	Scale (mmol)	Yield	α : β ratio
This work	1.0	1.2	1.0	2.0	-25	1.3	76%	2 : 1-0 : 1
Ref. 13 ^a	2.0	1.0	15	5.0	60	2.0	54%	α major
Ref. 17 ^b	1.0	2.0	2.0	3.0	RT	0.6	33%	0 : 1
Ref. 19 ^c	1.0	4.0	0.15	0.5	130	1.0	53%	α major
Ref. 20 ^d	1.0	8.0	1.3	10.0	130	0.05	80%	9 : 1

N.B. promotors for the described methods are as follows: this work. ^a BF₃·Et₂O. ^b NaOH. ^c Cu(OTf)₂. ^d PPh₃.

mixture. Notably reaction with 4-MU saw an improvement in glycosylation diastereoselectivity (to 1 : 2, α : β for **26**). Related studies have noted the effects of acceptor reactivity within glycosylation reactions, observing weaker nucleophiles to generate more α -product.³¹⁻³⁴ Using a DiFMU acceptor, containing electron withdrawing fluorine appears to follow this trend, promoting α -selectivity. Shifting to 4-MU acceptor improved β -selectivity (2 : 1, α : β in **25** to 1 : 2, α : β in **26**). Additionally, it has been proposed that an axial C4-*O*-acetate in D-Gal substrates promotes α -selectivity, due to participation.³⁵ Our results partially support this, evidenced by β -selectivity being reduced when D-GalN monosaccharide donors are used *versus* D-GlcN (Tables 1 and 2). However, at disaccharide level, reaction of DiFMU with 4,6-*O*-acetyl donor **23** showed an increase in β -selectivity compared to C4-benzylated donor **22** (2 : 1, α : β for **22** to 1 : 1, α : β for **23**). Whilst this methodology did not always afford diastereomerically pure glycosylation outcomes, disaccharide anomers were separable using conventional chromatography (see ESI† for details), providing access to stereopure materials for wider deprotection and biological evaluations.

Compared to previously reported methods (Table 4), this methodology requires low equivalents of both coumarin (1.2 equiv.) and glycosyl donor (1.0 equiv.) which is helpful for purification. Also, a low reaction temperature is employed compared to methods (a), (b) or (d) in Table 4 (-25 °C *versus* 60 °C or 130 °C), the reaction is scalable (1.3 mmol) and has a short duration. Furthermore, this methodology is applicable to a range of substrates utilising different *O*- and *N*-protecting groups which is useful for late-stage functionalisation. Notably here, β -selectivity is achieved in preference, whereby methods (a), (c) and (d) in Table 4 generate the α -anomers as the major product. Method (b) is β -selective but is lower yielding (33%) and incompatible with glycosyl substrates prone to elimination under basic conditions.

Conclusion

We have developed an *O*-glycosylation protocol for installing the commonly used aglycon coumarin onto D-GalN monosaccharides and disaccharides (chondroitin sulfate precursors). First optimising a methodology towards simple D-Glc donors, we selected imidates as the leaving group and BF₃·Et₂O as Lewis acid activator. Moving to D-GalN, the protecting group

used on nitrogen proved optimal as *N*-Troc at monosaccharide level and either *N*-Phth or *N*-Troc for disaccharides containing a D-GlcA-(1,3)- β -D-GalN motif. Here the diastereoselectivity outcome was also dependant on the coumarin acceptor used, with higher β -selectivity observed for 4-MU *versus* a difluorinated analogue. Overall, the methodology is accessible and applicable, harnessing mild reaction conditions, short reaction times and delivering good yields (64%–76%) across a panel of fourteen glycosyl donors screened. Furthermore, access to orthogonally protected disaccharides used in late-stage oligosaccharide synthesis is shown and serves to demonstrate a broader application potential for glycosidation of fluorogenic motifs to study carbohydrate active enzymes.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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