Preprint Title	Chemical synthesis of C6-tetrazole D-mannose building blocks and access to a bioisostere of mannuronic acid 1-phosphate
Authors	Eleni Dimitriou and Gavin J. Miller
Publication Date	22 März 2021
Article Type	Letter
Supporting Information File 1	Supporting Information File 1.pdf; 3.7 MB
ORCID® iDs	Gavin J. Miller - https://orcid.org/0000-0001-6533-3306

Chemical synthesis of C6-tetrazole D-mannose building blocks and access to a bioisostere of mannuronic acid 1-phosphate

Eleni Dimitriou and Gavin J. Miller*

Lennard-Jones Laboratory, School of Chemical and Physical Sciences, Keele University, Keele, Staffordshire, ST5 5BG, U. K.

Email: g.j.miller@keele.ac.uk

* Corresponding author

Abstract

Alginate, an anionic polysaccharide, is an important industrial biomaterial naturally harvested from seaweed. Many of its important physicochemical properties derive from the presence of charged carboxylate groups, presented as uronic acids, within the polysaccharide backbone. An ability to design and synthesise isosteres of these carboxylates would ultimately enable access to new alginate systems possessing different physicochemical properties. We present herein an approach to the chemical synthesis of alginate building blocks, modified at the carboxylate C6 position with bioisosteric tetrazole. The development of this synthesis enables utilisation of C6-tetrazole donors to deliver anomeric phosphate and 3-aminopropyl free sugars containing this motif. Access to these building blocks will further enable glycosylation methodologies to be explored that incorporate tetrazole as a bioisostere within uronic acid-containing carbohydrates.

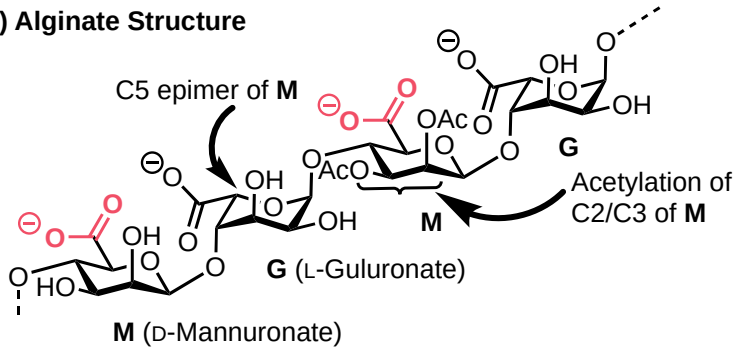
Keywords

alginate, tetrazole, non-native monosaccharide, glycosyl 1-phosphate, uronate

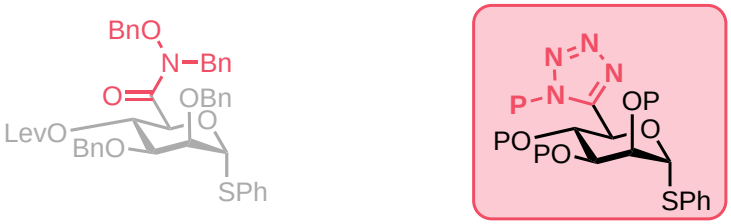
Introduction

Alginate, a heterogenous polysaccharide composed of β-1,4-linked D-mannuronic acid (M) and its C5 epimer α-L-guluronic acid (G) (Figure 1a), is an important industrial biomaterial. It is currently sourced from marine algae and applied as a stabiliser, viscosifier and gelling agent across the food, beverage, paper and pharmaceutical industries.[1–5] Within alginate sub-structure the relative proportions of M and G units, their homo- or heteropolymeric block-groupings and the possibility for acetylation at the C2 and/or C3 positions of M residues produces a structurally diverse biopolymer. This structural microheterogeneity varies depending on the alginate source and consequently affects the viscosity and gel-forming capacity of the final polysaccharide material. Such broad physicochemical properties present an opportunity to explore modifying the chemical structure of alginate, with a view to understanding and imparting changes upon its structure-to-function relationships.

a) Alginate Structure



b) Prospects for bioisosteric replacement of C5-mannuronic acid



Previously reported hydroxamate building blocks *This work: C6-tetrazole D-manno thioglycoside donors*

Figure 1: a) Chemical structure of alginate showing constituent M and G residues and C2/C3 acetylation for one M residue b) Introduction of bioisosteric carboxylate groups at C6 within a D-manno thioglycoside donor, P = appropriate protecting group.

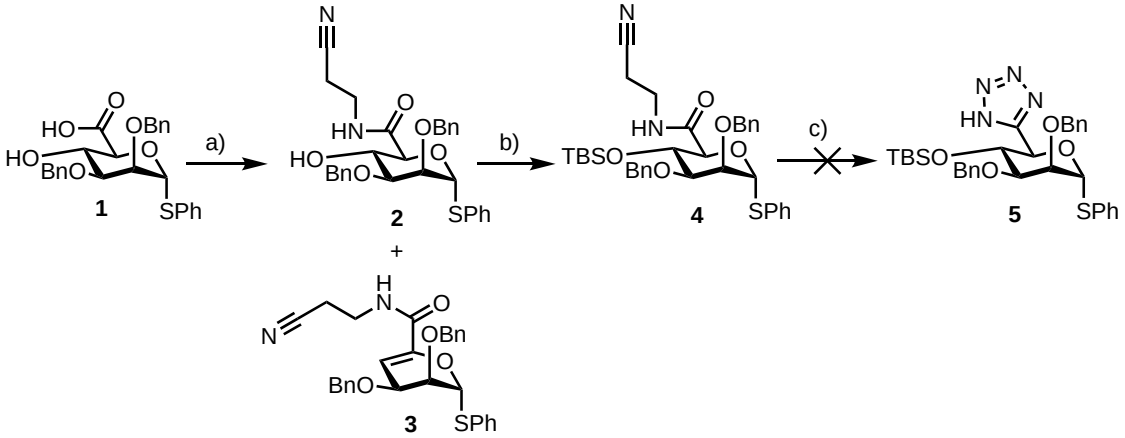
To this end, synthetic chemistry strategies (both solution and solid phase) have demonstrated exciting capabilities for access to native alginate oligosaccharides in recent years.[6–10] As part of a program to access non-native alginate oligosaccharide sequences, we targeted a synthetic approach to provide structurally defined building blocks containing bioisosteres of D-mannuronic acid. Building on our recently reported synthesis and glycosylation capability of hydroxamate modified D-mannuronate building blocks,[11] we now demonstrate the synthesis of a second carboxylate C6-bioisostere, tetrazole (Figure 1b).

As an established bioisostere for a carboxylic acid, tetrazole has found significant application within medicinal chemistry.[12] The aromatic tetrazole ring is considered a nonclassical bioisostere, differing in size and number of atoms from the carboxylic acid. The functional group has a similar pK_a to a carboxylate yet provides enhanced hydrogen bonding capability and alternative prospects for permeability due to a larger hydrophobic region enabling improved lipophilic contacts. These alternative properties and a prospect for their inclusion within new alginate fragments led us to explore the synthesis of a D-manno C6-tetrazole thioglycoside donor and examine its glycosidation capability to install C1 phosphate and anomeric linker groups.

Results and Discussion

An initial route towards a protected C6-tetrazole building block started from known mannuronic acid thioglycoside **1** (Scheme 1),[13] from which we recently

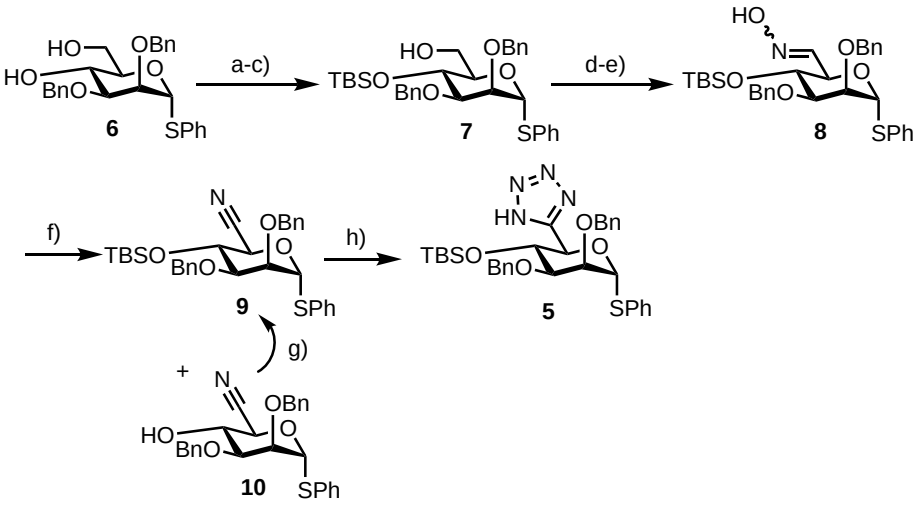
effected coupling to yield a protected C6 hydroxamate.[11] Accordingly, **1** was stirred with PyBOP and DIPEA in CH_2Cl_2 for 5 minutes, before 3-aminopropionitrile was added. After stirring for 1.5 hours at room temperature an undesired C4-C5 elimination material **3** was isolated as the major product in 35% yield, with only a trace amount of the desired **2** formed (7% yield). The ability of 3-aminopropionitrile to act as a base and trigger this elimination was comparable to results we observed using *N*,*O*-dibenzyl hydroxylamine as the coupling partner.[11] In the latter instance we were able to modify the nucleophile component to *O*-benzyl hydroxylamine and supress unwanted elimination. Unable to do this here, we instead reduced the reaction temperature to 0 °C, maintaining this for 40 minutes Pleasingly, the yield and product distribution were improved, affording separable amounts of **2** and **3** in 47% and 44% yields respectively.



Scheme 1: a) $H_2N(CH_2)_2CN$, PyBOP, DIPEA, CH_2Cl_2, 0 °C, 40 min., 47% (+44% **3**) b) TBSOTf, imidazole, DMAP, DMF, 40 °C, 24 h, 80% c) PPh_3, DIAD, $TMSN_3$, MeCN, 80 °C, 48 h.

Subsequent silyl protection of the C4-hydroxyl in **2** was completed using TBSOTf, furnishing **4** in 80% yield. Attempts to next convert **4** to **5** (*via* an *N*-cyanoethyl-protected tetrazole) using PPh_3, DIAD and $TMSN_3$ were unsuccessful, despite repeated attempts.[14] TLC and NMR analysis consistently indicated no conversion of **4**, even after stirring at 80 °C in MeCN for 48 hours. We therefore

proposed an alternative route to **5**, directly from reaction of a C6 nitrile with NaN_3, obviating the need for intermediate cyanoacetamide formation (Scheme 2).

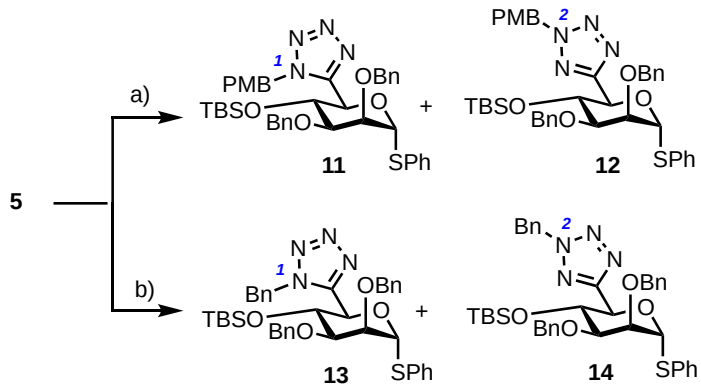


Scheme 2: a) BzCl, DMAP, pyridine, CH_2Cl_2, RT, 24 h, 90% b) TBSOTf, imidazole, DMAP, DMF, 40 °C, 24 h, 78% c) $Na_{(s)}$, MeOH, 16 h, 90% d) DMSO, SO_3•pyridine, Et_3N, RT, 1 h, 98% e) H_2NOH•HCl, THF, Na_2CO_3, 80% f) $POCl_3$, MeCN, RT, 40% g) TBSOTf, imidazole, DMAP, DMF, RT, 24 h, 87% h) $TMSN_3$, Bu_2SnO, toleune, 100 °C, 51%.

This second route commenced with a three-step protecting group manipulation of primary alcohol **6**, delivering **7** in 63% yield over three steps (Scheme 2). Alcohol **7** was then subjected to Parikh-Doering oxidation to deliver a crude aldehyde in 98% yield, from which oxime **8** was subsequently formed in 80% yield as a 6.7/1 mixture of *C=N*-isomers. Dehydration of **8** using $POCl_3$ gave **9** in a disappointing 40% yield, alongside **10** (26% yield). Formation of **10** was attributed to the acidic reaction conditions concomitantly effecting TBS removal. However, further amounts of **9** could be accessed through reprotection at C4 with TBSOTf in excellent yield. C6-nitrile **9** was then successfully converted into C6-tetrazole **5** in 51% yield using $TMSN_3$ and a catalytic amount of Bu_2SnO.[15] This method was recently utilised successfully by Bräse and colleagues for D-gluco configured C6-tetrazoles in their synthesis of modified hyaluronic acid fragments.[16] ^{13}C NMR of **5** confirmed the presence of a new

quaternary carbon (tetrazole C_q, δ_C = 155.8 ppm) alongside disappearance of the C6-nitrile (δ_C = 117.0 ppm). Furthermore, ^{1}H NMR analysis indicated the common H5 doublet was further downfield (δ_H = 5.64 ppm), compared to data observed previously for mannuronate ester (δ_H = 4.54 ppm) and hydroxamate (δ_H = 4.56 ppm) motifs.[11] Finally, the coupling constant calculated for H5 ($^3J_{H5\text{-}H4}$ = 8.9 Hz), indicated a $^4C_1$ pyranose conformation for newly formed **5**.

To explore improving the efficiency of the latter synthetic steps towards **5**, an alternative, one-pot three component procedure (H_2N-OH, NaN_3 and catalytic $[(NH_4)_4Ce(SO_4)_4]$) was attempted from the crude C6-aldehyde.[17] TLC analysis indicated C6-nitrile formation was evident after 36 h, however, the desired C6-tetrazole **5** was not observed. Repeated attempts were unable to indicate progress beyond mixtures of **8** and **9** in 35% and 14% yields and the procedure was abandoned, instead reverting to the successful route developed in Scheme 2.



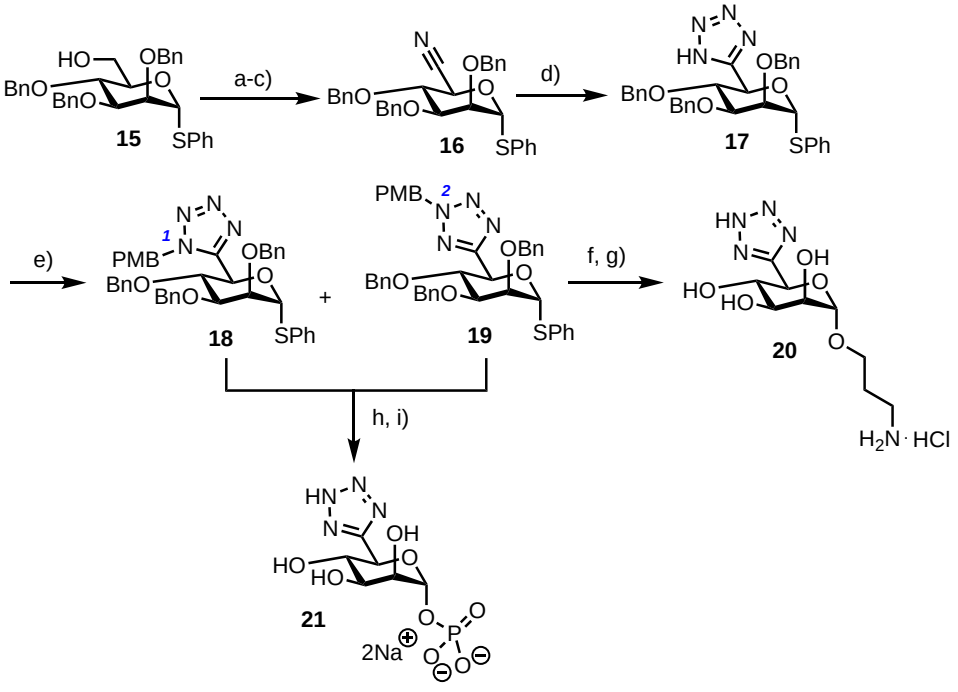
Scheme 3: a) PMBCl, KI, K_2CO_3, DMF, RT, 53% b) BnBr, DMF, Et_3N, DCM, RT, 31%.

The final step towards the synthesis of a fully protected C6-tetrazole glycosyl donor required nitrogen protection (Scheme 3). A first attempt to protect the tetrazole involved a reaction of **11** with PMBCl in DMF, using K_2CO_3 alongside KI. Two separable regioisomers **11** and **12** were isolated in an acceptable 53% overall yield and in a ratio of N_1-PMB/N_2-PMB = 1.1/1 (Scheme 3). HMBC NMR of **11** and **12**

clarified the position of the PMB group on the tetrazole ring for each compound. For **11**, a correlation of tetrazole C_q (δ_C = 150.4 ppm) with the benzylic protons of the PMB group (δ_H = 5.66 ppm, see SI, Figure S3), was observable. Similar analysis for **12** indicated no such correlation. In order to try and improve the N_1-PMB/N_2-PMB ratio, converting **5** to a triethylammonium salt form in 94% yield was adopted.[18] Subsequent reaction with PMBCl gave **11** and **12**, but in a largely unchanged ratio (N_1-PMB/N_2-PMB = 1/1.1). A comparative attempt to install a benzyl protecting group using this method afforded N_1-Bn and N_2-Bn tetrazoles **13** and **14** in low yield (31%) and again with little regiodiscrimination (N_1-Bn/N_2-Bn = 1/1.2).

The synthesis of appropriately protected C6-tetrazole donors **11**-**14** was accomplished to allow for regioselective deprotection and unveil C4-acceptor capability within an oligosaccharide synthesis strategy. With such capability effectively demonstrated, we next explored the provision of D-manno C6-tetrazoles without an orthogonal C4-protecting group.

Accordingly, a synthesis initiating from alcohol **15**[19] enabled access to C6-nitrile **16** in three steps and an improved yield of 50% (compared to 31% in accessing **9** from **7**). Nitrile **16** then underwent dipolar cycloaddition with NaN_3, converting it to C6-tetrazole thioglycoside **17** in 55% yield. This material was then protected at tetrazole nitrogen using PMBCl to give **18** and **19** (N_1-PMB/N_2-PMB = 1/1.2) as separable regioisomers and their structures were confirmed by HMBC, as previously demonstrated. Removal of the need to orthogonally protect C4 expectedly reduced the complexity of the synthetic route and nine steps for the synthesis of donors of type **11**/**12** was reduced to five steps for **18**/**19**. Moreover, the overall yield for the route increased from 9% to 21%.



Scheme 4: a) DMSO, SO_3•pyridine, Et_3N, RT, 1 h, 96% b) H_2NOH•HCl, THF, Na_2CO_3, 89% c) $POCl_3$, MeCN, RT, 59% d) $TMSN_3$, Bu_2SnO, toleune, 100 °C, 55% e) PMBCl, KI, K_2CO_3, DMF, 76% f) 3-(benzyloxycarbonylamino) propan-1-ol, NIS, AgOTf, CH_2Cl_2, -30 to -10 °C, 3 h, 34% g) $H_{2(g)}$, Pd/C, $Pd(OH)_2$/C, HCl, EtOH, THF, RT, 56 h, 96% h) Dibenzylphosphate, NIS, AgOTf, CH_2Cl_2, -30 to -10 °C, 3.5 h, 72% i) $H_{2(g)}$, Pd/C, $Pd(OH)_2$/C, 5% $NaHCO_3$, EtOH, THF, RT, 24 h, 72%.

To demonstrate capability for anomeric linker attachment and conversion to a biologically relevant analogue of mannuronic acid 1-phosphate, C1-aminopropyl **20** and glycosyl 1-phosphate **21** were synthesised (Scheme 4). Mixture **18**/**19** was first used for glycosylation of 3-(benzyloxycarbonylamino)-1-propanol and furnished a regioisomeric and anomeric mixture in low yield (34%, with 20% recovery of starting material and 18% hydrolysed donor). Isomeric separation of this mixture was not completed at this stage and the material was deprotected using hydrogenolysis to remove the benzyl protecting groups. This furnished **20** in excellent yield (96%) as a

3/1 mixture of α/β anomers at C1, indicating a preference for α-selective glycosylation using C6-tetrazole thioglycoside donors **18**/**19**.

Additionally, glycosylation of dibenzylphosphate using **18**/**19** was successful and furnished the expected mixture of tetrazole *N*-regioisomers in 72% yield. These materials were not separated and instead exposed to hydrogenolysis conditions to deliver free D-manno C6-tetrazole 1-phosphate **21**. Deprotection utilised 0.6 equiv. of Pd/C (0.1 equiv. per benzyl group) and 0.6 equiv. of $Pd(OH)_2$/C to afford **21** in 72% yield after 24 h. ^{13}C NMR of **21** confirmed the presence of a C6-tetrazole (δ_C = 160.8 ppm) alongside the anomeric phosphate (δ_P = -2.15 ppm). This material compliments a recently reported C6-hydroxamic acid derivative,[20] as a bioisostere for mannuronic acid 1-phosphate and will be enabling for evaluating non-native glycosyl 1-phosphates in appropriate chemoenzymatic syntheses.[21–23]

Conclusion

We have established synthetic access to a series of C6-tetrazole thioglycoside monosaccharide building blocks with capability for orthogonal C4- and tetrazole protecting groups. We demonstrate anomeric manipulation of these donors to new, biologically relevant 1-phosphate and conjugable, aminopropyl tethered materials as mimics of mannuronic acid. Evaluation of these C6-tetrazole thioglycosides as donors for non-native alginate fragment synthesis is currently underway and will be reported in due course.

Supporting Information

File Name: Supporting Information File

File Format: .pdf

Title: Detailed experimental protocols and characterisation data; spectral NMR data (^{1}H, ^{13}C, ^{31}P and HSQC NMR for compounds 2-5, 7-14, 16-18 and 20-21)

Acknowledgements

We thank the EPSRC UK National Mass Spectrometry Facility (NMSF) at Swansea University.

Funding

Keele University are thanked for a studentship to E. D.

References

1. Lee, K. Y.; Mooney, D. J. *Prog. Polymer. Sci.* **2012**, *37*, 106–126.

2. Ertesvåg, H. *Front. Microbiol.* **2015**, *6*, 523.

3. Sabra, W.; Zeng, A.-P.; Deckwer, W.-D. *Appl. Microbiol. Biotech.* **2001**, *56*, 315–325.

4. Jia, J.; Richards, D. J.; Pollard, S.; Tan, Y.; Rodriguez, J.; Visconti, R. P.; Trusk, T. C.; Yost, M. J.; Yao, H.; Markwald, R. R.; Mei, Y. *Acta Biomaterialia* **2014**, *10*, 4323–4331.

5. Sun, J.; Tan, H. *Materials* **2013**, *6*, 1285–1309.

6. Zhang, Q.; Rijssel, E. R. van; Walvoort, M. T. C.; Overkleeft, H. S.; Marel, G. A. van der; Codée, J. D. C. *Angew. Chem.* **2015**, *54*, 7670–7673.

7. Dinkelaar, J.; Bos, L. J. van den; Hogendorf, W. F. J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; Marel, G. A. van der. *Chem. Eur. J.* **2008**, *14*, 9400–9411.

8. Bos, L. J. van den; Dinkelaar, J.; Overkleeft, H. S.; Marel, G. A. van der. *J. Am. Chem. Soc.* **2006**, *128*, 13066–13067.

9. Pan, D.; Hua, Q.; Yang, Y. *Org. Biomol. Chem.* **2019**, *17*, 6174–6177.

10. Codée, J. D. C.; Bos, L. J. van den; Jong, A.-R. de; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; Marel, G. A. van der. *J. Org. Chem.* **2009**, *74*, 38–47.

11. Dimitriou, E.; Miller, G. J. *Org. Biomol. Chem.* **2019**, *17*, 9321–9335.

12. Ostrovskii, V. A.; Trifonov, R. E.; Popova, E. A. *Russ. Chem. Bull.* **2012**, *61*, 768–780.

13. Walvoort, M. T. C.; Elst, H. van den; Plante, O. J.; Kröck, L.; Seeberger, P. H.; Overkleeft, H. S.; Marel, G. A. van der; Codée, J. D. C. *Angew. Chem.* **2012**, *51*, 4393–4396.

14. Lassalas, P.; Gay, B.; Lasfargeas, C.; James, M. J.; Tran, V.; Vijayendran, K. G.; Brunden, K. R.; Kozlowski, M. C.; Thomas, C. J.; Smith, A. B.; Huryn, D. M.; Ballatore, C. *J. Med. Chem.* **2016**, *59*, 3183–3203.

15. Wittenberger, S. J.; Donner, B. G. *J. Org. Chem.* **1993**, *58*, 4139–4141.

16. Mende, M.; Nieger, M.; Bräse, S. *Chem. Eur. J.* **2017**, *23*, 12283–12296.

17. Mitra, B.; Mukherjee, S.; Pariyar, G. C.; Ghosh, P. *Tetrahedron Lett.* **2018**, *59*, 1385–

18. Ostrovskii, V. A.; Koren, A. O. *Heterocycles* **2000**, *53*, 1421–1448

19. Ahmadipour, S.; Pergolizzi, G.; Rejzek, M.; Field, R. A.; Miller, G. J. *Org. Lett.* **2019**, *21*, 4415–4419.

20. Beswick, L.; Dimitriou, E.; Ahmadipour, S.; Zafar, A.; Rejzek, M.; Reynisson, J.; Field, R. A.; Miller, G. J. *ACS Chem. Biol.* **2020**, *15*, 3086–3092.

21. Beswick, L.; Ahmadipour, S.; Dolan, J. P.; Rejzek, M.; Field, R. A.; Miller, G. J. *Carbohydr. Res.* **2019**, *485*, 107819.

22. Ahmadipour, S.; Beswick, L.; Miller, G. J. *Carbohydr. Res.* **2018**, *469*, 38–47.

23. Ahmadipour, S.; Miller, G. J. *Carbohydr. Res.* **2017**, *451*, 95–109.