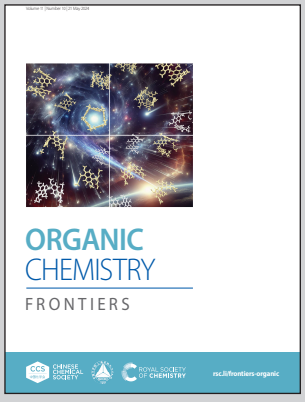
Showcasing research from Andrew Stachulski's laboratory, School of Chemistry, University of Liverpool, Merseyside, UK.

Convenient syntheses of *trans*-resveratrol 3-*O* and 4′-*O*-β-D-glucuronides and a study of their aqueous stability

Resveratrol, a naturally occurring stilbene triol, is found in a number of dietary substances including various fruits, nuts and wines. We describe convenient syntheses of its isomeric monoglucuronides and the properties of their aqueous solutions.

As featured in:



See Andrew V. Stachulski *et al.*, *Org. Chem. Front.*, 2024, **11**, 2720.

CCS 中国化学会 CHINESE CHEMICAL SOCIETY
SIOC 1950
ROYAL SOCIETY OF CHEMISTRY
rsc.li/frontiers-organic

ORGANIC CHEMISTRY FRONTIERS

RESEARCH ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Org. Chem. Front.*, 2024, **11**, 2720

Convenient syntheses of *trans*-resveratrol 3-*O* and 4′-*O*-β-D-glucuronides and a study of their aqueous stability†

Megan K. Fraser,‡[a] Aleksandra Gorecka,§[a] Edwin A. Yates,[b] Jonathan A. Iggo,[a] Krzysztof Baj [a] and Andrew V. Stachulski *[a]

The two isomeric monoglucuronides of the stilbene triol derivative, resveratrol, are thought to have potentially valuable biological activities, including possible antiproliferative properties, in common with resveratrol itself. In connection with a wider interest in naturally occurring phenolic glucuronides, we sought access to both of these conjugates from readily available resveratrol triesters. Selective monodeacylation using either chemical or enzymatic hydrolyses afforded the required diesters: subsequent glucuronidation using the trichloroacetimidate procedure, then mild hydrolysis, afforded the desired products. We also discovered a very mild, effective method for the anomeric deacetylation of the readily available methyl glucuronate β-tetraacetate using *N*-Me piperazine *en route* to the imidate. This reagent appears to have wider value in the deprotection of relatively activated acetates. We further studied the stability of the glucuronides in aqueous solution, and found remarkable differences in their properties, especially in the ready *E*/*Z* isomerisation of the 3-glucuronide.

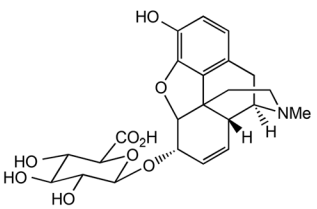
Received 23rd October 2023,
Accepted 22nd November 2023

DOI: 10.1039/d3qo01736b

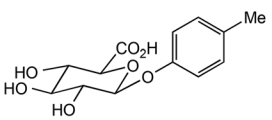
rsc.li/frontiers-organic

Introduction

While it is still commonly believed that glucuronides of xenobiotics and endogenous materials are mere biological waste byproducts, an increasing body of evidence has found that many glucuronides have biological activity *per se*.[1] A well-known example is morphine-6-glucuronide (M6G) **1**, which has some advantages over morphine itself as an analgesic: better tolerance, especially reduced respiratory depression, and longer duration of action.[2–4] We have recently shown that *p*-cresyl glucuronide **2**, an end product of Tyr metabolism in the gut, is significantly less toxic in human kidney cells than the parent phenol and may indeed possess cytoprotective properties.[5,6]



1



2

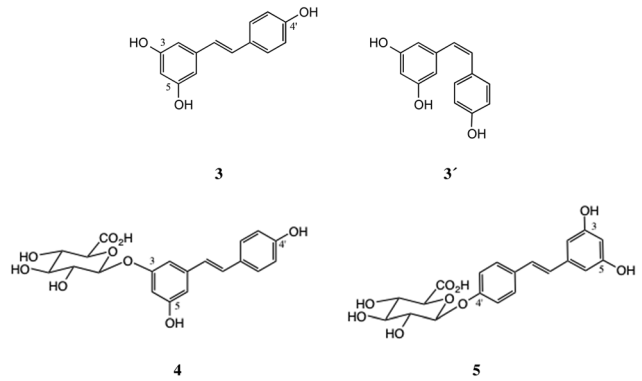
As part of our continuing programme, we sought to study the behaviour of other glucuronides of endogenous and dietary substances: the isomeric 3-*O* and 4′-*O*-β-D-glucuronides of the stilbenetriol, resveratrol **3** were of particular interest. Resveratrol, present in various plants, nuts and red wine skins, has been associated with diverse biological activities and health benefits,[7–9] generally thought to be associated with its radical scavenging properties. It occurs naturally mainly as the *trans* (*E*) isomer shown, but the *cis* (*Z*) isomer **3′** is always present in lesser amounts.[10] Interest in **3** was greatly spurred by the discovery of its cancer chemopreventive effect:[8b] more recently, the relative effects of **3**

[a]*Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, UK. E-mail: stachuls@liv.ac.uk; Tel: +44 (0)151-794-3482*
[b]*Department of Biochemistry, Cell and Systems Biology, ISMIB, Crown St., University of Liverpool, Liverpool L69 7ZB, UK*

†Electronic supplementary information (ESI) available: Details of preparation and characterisation of all compounds described, including photocopy ^{1}H and ^{13}C NMR spectra; detailed assignment and ^{1}H/^{13}C correlation of the *E*/*Z* isomers of **4**. See DOI: https://doi.org/10.1039/d3qo01736b

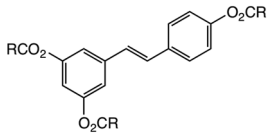
‡Current address: GSK Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY, UK.
§Current address: School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff, CF10 3AT, UK.

and its glucuronide metabolites in lowering hepatic cholesterol have been studied.[11]



3 3′ 4 5

Clearly, efficient syntheses of the two glucuronides **4** and **5** will depend on selective preparations of suitable di-protected intermediates. Other preparations of **4** and **5** have proceeded *via ab initio* synthesis of **3** from benzenoid precursors,[12] or the use of regiospecifically silylated derivatives.[13,14] Also Koenigs–Knorr glucuronidation of **3** itself, followed by deprotection and preparative HPLC of the resulting mixture of isomers has been used.[14] The enzymology and uridine diphosphate glucuronosyltransferase (UGT) specificity in the human glucuronidation of both **3** and **3′**, including gender differences, have been studied.[15,16]



6 R = Me
15 R = Pr^{i}

We considered that resveratrol triacetate **6**, commercially available or readily prepared from **3** in essentially quantitative yield,[17] should be a convenient precursor. Indeed, others have described selective monodeacylation of **6** using chemical and enzymatic methods,[18,19] with conversion of the 3,4′-diacetate into **4**,[18] and we aimed to build on these results.

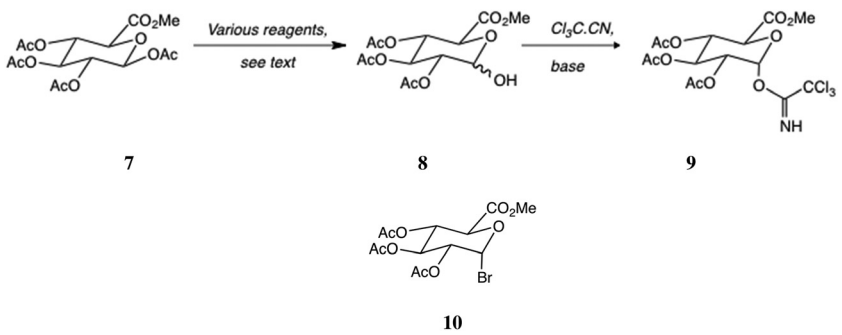
Regarding the conjugation step, our recent synthesis[5] of *p*-cresyl glucuronide **2** was a good example of successful glucuronidation of a phenol of low acidity with the anomeric tetraacetate **7**. However, the success of this straightforward method appears critically dependent on phenolic pK_a: any substrate more acidic than phenol itself (pK_a = 10.0) cannot be readily glucuronidated in this way, and we verified that this was true with resveratrol diacetates once available. A recent thorough study[20] gave a value of pK_{a1} = 9.2 for **3**, with the 4′-OH being the most acidic. Resorcinol (1,3-dihydroxybenzene) also has an experimental pK_{a1} of 9.2 [21] (the authors determined a theoretical value of 9.07) and the values for monoacylated derivatives are expected to be lower. We therefore selected the trichloroacetimidate method[22] and found a valuable new method for efficient anomeric deacylation of **7** under mild conditions, giving **8** which after conversion to **9** (Scheme 1) was conjugated with the appropriate diesters of **3** followed by deprotection of the products to afford the desired monoglucuronides.

Results and discussion

Carbohydrate intermediates

Several reagents have been used for the conversion of tetraester **7** into hemiacetal **8** (Scheme 1), including ammonium acetate,[23] ammonium carbonate,[24] hydrazine[25,26] (with or without buffering), morpholine,[27] benzylamine[28] and a tin-based reagent.[29] In addition the longer route *via* heavy metal-catalysed hydrolysis of the bromosugar **10** derived from **7** is still used.[1] We felt that there was still room for improvement in view of the toxicity of some of the reagents used and the inhomogeneity of the ammonium salts. As a result, we screened a number of amine reagents, aiming for homogeneous conditions and the avoidance of metal reagents as a minimum.

We were pleased to find that *N*-methylpiperazine, pK_{a1} = 9.14,[30] (and a very weakly basic pK_{a2} of 4.73) performed extremely well in this reaction, Scheme 1. The best conditions called for 3 eq. of the amine buffered with equimolar acetic acid in acetonitrile: after 24 h at 20 °C, reaction was complete, and after neutralization the hemiacetal **8** was extracted in 88% yield, pure enough for immediate progression. It was converted to the trichloroacetimidate **9** as described previously.[31]



7 8 9 10

Scheme 1 Carbohydrate intermediates in the glucuronic acid series.

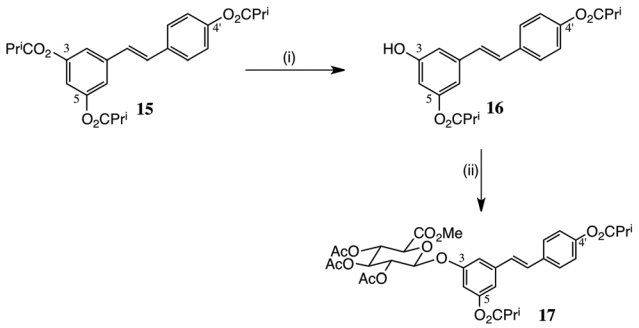
Resveratrol diesters: chemical hydrolysis

We studied both selective chemical and enzymic hydrolyses to convert triacetate **6** into the 3,4′-and 3,5-diesters (Scheme 2). Reaction of **6** with NH_4OAc has been used for synthesis of the 3,4′-diester **11** in 41% yield,[18] but the elaborate procedure called for two crystallizations in addition to chromatography. The authors reported other possible conditions: catalytic K_2CO_3/EtOH, catalytic HCl/EtOH, catalytic DBU/EtOH/CH_2Cl_2 and catalytic Ph_3P/EtOH at reflux. Using their optimum procedure, *viz*. NH_4OAc in MeOH/THF, we could only obtain 21% of **11**, together with the 3,5-diester and mixed monoesters. Here too, however, *N*-Me piperazine buffered with AcOH was a superior alternative and allowed **11** to be isolated in 36% yield by direct chromatography, in sufficient amount to obtain useful quantities of the 3-glucuronide.

We also studied resveratrol tri-isobutyrate **15**, Scheme 3, hoping that the additional bulk of the esters might allow a more controlled, selective hydrolysis. Compound **15** was obtained by acylation of **3** with isobutyryl chloride in essentially quantitative yield. However, there was only a slight improvement in the mono-deacylation step. The resulting 3,4′-diisobutyrate **16**, however, gave a better yield of **17** in the glucuronidation step.

Resveratrol diesters: enzymic hydrolysis

Nicolosi *et al.* described the preparation of a number of resveratrol esters using enzymatic transesterification:[19] in particular, the 3,5-diester **13**, ideal for the synthesis of the 4′-glucuronide, was obtained in high yield by treatment of **6** with *Burkholderia cepacia* and *n*-butanol. We did not have access to this particular enzyme, but we studied immobilized *Candida* enzymes, CAL A and CAL B, which are known to be valuable in transesterification.[32] While CAL A gave a virtually equal mixture of the 3,5- and 3,4′-diesters in moderate yield in a slow reaction (*ca.* 50% conversion after 72 h), CAL B showed high selectivity for removal of the 4′-acetate in just 1 h at 45 °C. In this way, following removal of the enzyme and chromatography, or direct crystallisation, the 3,5-diester **13** was obtained in 75% yield.



Scheme 3 Isobutyryl intermediates. Reagents: as Scheme 2.

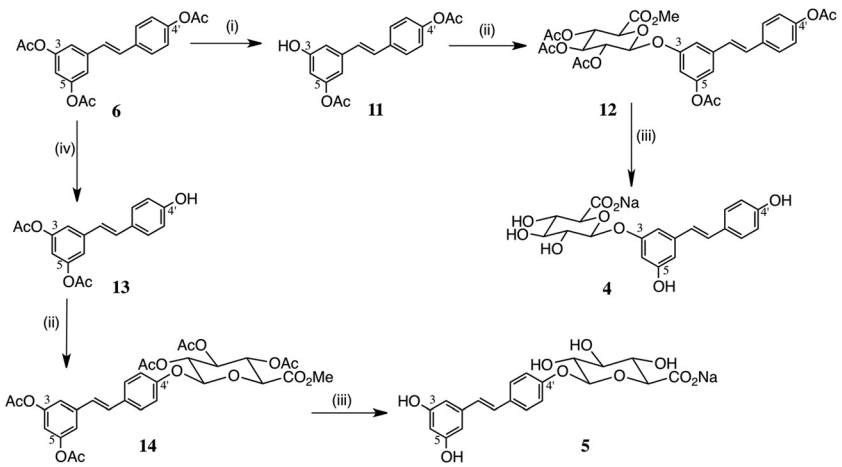
Glucuronidation

Both diesters **11** and **13** were successfully glucuronidated using trichloroacetimidate **9**, affording **12** and **14** in 53% and 56% yields respectively, employing $BF_3{\cdot}OEt_2$ as the Lewis acid catalyst. Similarly, the 3, 4′-diisobutyrate **16** gave the related conjugate **17** in 66% yield. Neither diester gave any useful product on Lewis acid-catalysed reaction with the β-tetraacetate **7**, using TMSOTf or $BF_3{\cdot}OEt_2$.

Hydrolysis was performed using the mild conditions of Na_2CO_3 in aq. methanol, and the products **4** and **5** were finally isolated as their Na salts.[31] Alternatively, in this series, somewhat purer products were obtained by first isolating the free glucuronic acid, following acidification to pH 3 using IR-120 (H^+) resin. The sodium salts were then obtained by adding an equimolar amount of aq. $NaHCO_3$. The NMR spectra (see ESI†) were consistent with published data; Na salts of glucuronic acids show an upfield shift of the H(5) proton, typically by about 0.1 ppm, compared to the free acid forms.

Aqueous stability of the glucuronides

When the ^1H NMR of the 3-glucuronide **4** was recorded in D_2O, we found that small extra signals (sc. <5%) were visible in the aryl region after leaving the solution at 20 °C for several



Scheme 2 Synthesis of resveratrol monoglucuronides. Reagents: (i) *N*-Me piperazine, AcOH, MeCN, 20 °C; (ii) **9**, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −20 °C; (iii) Na_2CO_3, aq. MeOH, 0 °C to 20 °C, then IR-120 (H^+) to pH 6.2, or see text; (iv) Cal-B, *n*-BuOH, TBME, 45 °C.

hours on the bench. The NMR spectrum showed two AB quartets which can be assigned to pairs of olefinic Hs. The multiplets have been simulated (see ESI†) to obtain the chemical shifts and coupling constants (δ 7.11, 6.93 and 6.61, 6.46) with J = 16.5 Hz and 12.1 Hz respectively An nOe is observed between the resonances of the AB quartet around 6.5 ppm but is not observed between the resonances of the AB quartet around 7 ppm consistent with the assignment of these resonances to the olefinic protons of the *Z* and *E* isomers of **4** respectively.

Transformation of the **4**(*E*) to the **4**(*Z*) isomer occurs on exposure of the solution in the NMR tube to light but is reversed on storage in the dark. Thus, after one week in dull ambient light, the spectrum showed the presence of two compounds in a roughly 2 : 1 ratio. On returning the NMR tube to the refrigerator, after *ca.* 7 days the ^{1}H NMR spectrum revealed the sample was >90% *E*, (Fig. 1a) and after a further 7 days in the dark of the NMR magnet the sample was 96% *E*, (Fig. 1b) *i.e.* the *Z* to *E* isomerization is slow and happens in the dark. On exposing the sample to bright sunlight for *ca.* 8 h the NMR spectrum revealed the sample had converted to *ca.* 90% *Z* together with a small amount of decomposition (Fig. 1c). Finally, after seven days in the NMR magnet conversion back to the *E* isomer, which was now present as *ca.* 45% of the sample, had occurred (Fig. 1d).

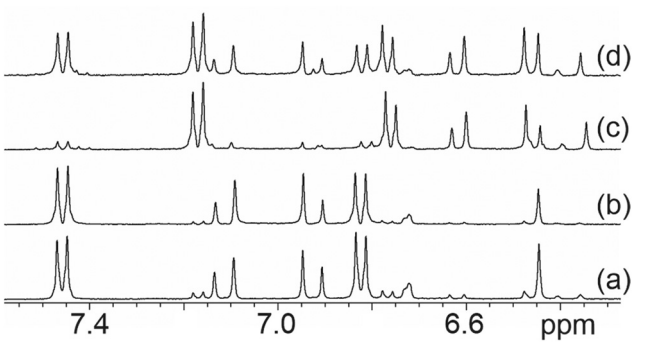
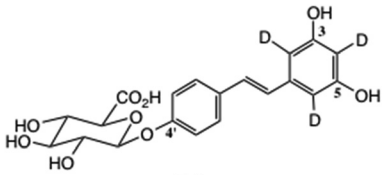


Fig. 1 Time course of the *E* to *Z* isomerization of **4**: ^{1}H NMR of the aryl region: (a) after *ca.* 1 week in the dark *E* 90%, *Z* 10%; (b) after 2 weeks *E* 96%, *Z* 4%; (c) after *ca.* 8 h bright sunlight *E* 11%, *Z* 89%; (d) after further 5 days in dark *E* 45%, *Z* 55%.

While such isomerization is well known for **3** and **3′** themselves,[10b] employing a 450 W medium pressure mercury lamp, it has not been reported for the glucuronides, and the conditions are remarkably mild. In the ESI† we present a full assignment of the ^{1}H and ^{13}C spectra of both isomers, with HSQC, HMBC and nOe difference analyses.



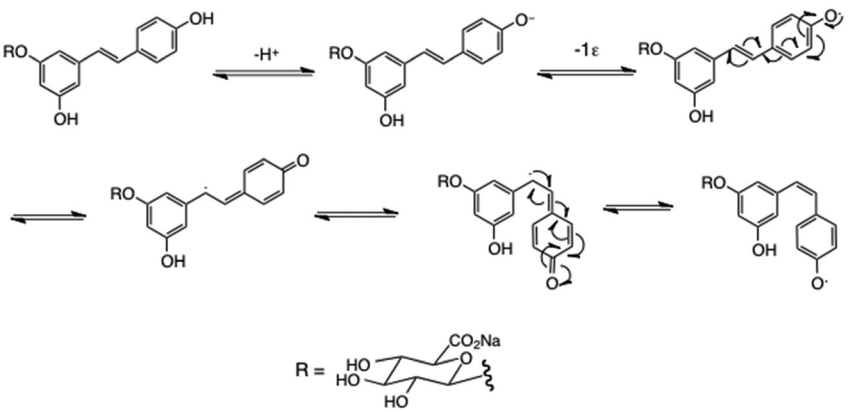
5-d$_3$

In contrast, under identical conditions the isomeric 4′-glucuronide **5** showed essentially no *E*/*Z* isomerisation after 120 h. Instead, complete H/D exchange of the aryl Hs of the resorcinol ring was observed (see the spectrum in ESI†) after just 24 h, giving the tri-deuterio species **5-d$_3$**.[33] While this is in a sense not surprising, as H/D exchange of aryl protons is quite facile in phenols, it is clear that exchange under these very mild conditions requires the free resorcinol group. The 3-glucuronide **4**, where only free *monophenols* are present, showed no observable H/D exchange under these conditions.

These results may be rationalized by postulating that the free 4-phenol is necessary for facile *E*/*Z* isomerisation: see Scheme 4. Ionisation of this phenol, followed by one-electron oxidation (or direct air/light-catalysed H abstraction from starting material) generates a phenoxy radical that is directly conjugated to the double bond, allowing an isomeric C radical to form, which is disposed to revert to the isomeric phenoxy radical. This mechanism is clearly inaccessible to the 4′-glucuronide **5**.

E/*Z* isomerisation of resveratrol diesters

We studied the pair of diesters **13** and **16** to look for similar behaviour, in particular to investigate whether the position of



Scheme 4 Proposed mechanism for the *E*/*Z* isomerisation of glucuronide **4**.

the free phenol was important for the rate of *E*/*Z* isomerisation as observed with **4** and **5**. These compounds are water-insoluble, but d_3-acetonitrile was a satisfactory solvent for both. When these solutions were stored at 20 °C in the dark, no significant *E*/*Z* isomerisation was observed for either isomer after 12 h, but in diffused sunlight over a further 12 h both underwent isomerisation, with the 3,5-diester **13** reacting at least 5× faster; see the spectra in the ESI.† While these further experiments have introduced new variables (substituent groups, solvent), the faster isomerisation of **13**, which contains a 4′-OH group, is consistent with the behaviour of **4** and **5**. We shall report our findings on *E*/*Z* isomerisation of a series of resveratrol derivatives elsewhere.

Conclusions

Convenient syntheses of both isomeric resveratrol monoglucuronides have been achieved from the readily available triacetate, using selective chemical and enzymatic deprotections to access the necessary diesters. The glucuronidation step was performed in good yields for both isomers using the trichloroacetimidate method, and mild final deprotection was carried out using aqueous Na_2CO_3 solution with methanol. In the course of these studies, we discovered that *N*-methylpiperazine was a highly suitable reagent for both selective deacetylation of the anomeric glucuronate perester and cleavage of a single ester from resveratrol triacetate.

The final glucuronides, as their Na salts in D_2O, showed very different properties, namely ready *E*/*Z* alkene isomerisation in the case of **4** and H/D exchange in the case of **5**, mutually exclusive. We shall report the results of biological investigations of the products in future publications.

Author contributions

MF: investigation, formal analysis, experimental write-up. AG: investigation, formal analysis, experimental write-up. EY: project administration, supervision, editing of MS. KB: investigation, formal analysis. JI: NMR experimental design and NMR methodology, formal analysis, editing of MS. AS: conceptualization, supervision, formal analysis, experimental verification, MS write-up.

Conflicts of interest

We have no conflicts of interest to declare.

Acknowledgements

We are grateful to the EPSRC for funding (summer internship to A. G.). M. K. F. was a final year M. Chem. project student at the University of Liverpool, 2021–2022.

References

1 A. V. Stachulski and X. L. Meng, Glucuronides from metabolites to medicines: a survey of the in vivo generation, chemical synthesis and properties of glucuronides, *Nat. Prod. Rep.*, 2013, **30**, 806–848.
2 (*a*) R. J. Osborne, S. P. Joel, N. S. Nixon and M. L. Slevin, The Analgesic Activity of Morphine-6-glucuronide, *Lancet*, 1988, **331**, 828; (*b*) R. J. Osborne, P. I. Thompson, S. P. Joel, D. Trew, N. Patel and M. Slevin, The Analgesic Activity of Morphine-6-glucuronide, *Br. J. Clin. Pharmacol.*, 1992, **34**, 130–138.
3 R. T. Brown, N. E. Carter, F. Scheinmann and N. J. Turner, Synthesis of morphine-6-glucuronide *via* a highly selective enzyme catalysed hydrolysis reaction, *Tetrahedron Lett.*, 1995, **36**, 1117–1120.
4 A. V. Stachulski, F. Scheinmann, J. R. Ferguson, J. L. Law, K. W. Lumbard, P. Hopkins, N. Patel, S. Clarke, A. Gloyne and S. P. Joel, Structure-Activity Relationships of Some Opiate Glycosides, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 1207–1214.
5 J. A. London, E. C. S. Wang, I. L. Barsukov, E. A. Yates and A. V. Stachulski, Synthesis and toxicity profile in 293 human embryonic kidney cells of the β-D-glucuronide derivatives of ortho-, meta- and para-cresol, *Carbohydr. Res.*, 2021, **499**, 108225.
6 A. V. Stachulski, T. B.-A. Knausenberger, S. N. Shah, L. Hoyles and S. McArthur, The host/gut microbial co-metabolite of aromatic amino acids, p-cresol glucuronide, promotes blood–brain barrier integrity in, vivo, *Tissue Barriers*, 2023, **11**, e2073175.
7 (*a*) C. Wilson, *XPharm: The Comprehensive Pharmacology Reference*, 2010, pp. 1–9; (*b*) T. Rezanka, I. Kolouchova, L. Gharwalova and K. Sigler, Metabolic Screening of Wine (Grapevine) resveratrol, *Stud. Nat. Prod. Chem.*, 2018, **59**, 1–30.
8 (*a*) J. A. Baur and D. A. Sinclair, Therapeutic Potential of Resveratrol: The in Vivo Evidence, *Nat. Rev. Drug Discovery*, 2006, **5**, 493–506; (*b*) M. S. Jang, E. N. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C. W. W. Beecher, H. H. S. Fong, N. R. Farnsworth, A. D. Kinghorn, R. G. Mehta, R. C. Moon and J. M. Pezzuto, Cancer chemopreventive activity of resveratrol, a natural product derived from grapes, *Science*, 1997, **275**, 218–220.
9 C. Iuga, J. R. Alvarez-Idaboy and N. Russo, Antioxidant activity of trans-resveratrol towards hydroxyl and hydroperoxyl radicals: a quantum chemical and computational kinetics study, *J. Org. Chem.*, 2012, **77**, 3868–3877.
10 (*a*) N. Y. Anisimova, M. V. Kiselevsky, A. V. Sosnov, S. V. Sadovnikov, I. N. Stankov and A. A. Gakh, Trans, cis, and dihydro-resveratrol: a comparative study, *Chem. Cent. J.*, 2011, **5**, 88. These authors point out that dihydro-3 is also present *in vivo*; (*b*) R. A. Rodriguez, I. R. Lahoz, O. N. Faza, M. M. Cid and C. S. Lopez, Theoretical and experimental exploration of the photochemistry of resveratrol: beyond the simple double bond isomerization, *Org. Biomol. Chem.*, 2012, **10**, 9175–9182.

11 D. Shao, Y. Wang, Q. Huang, J. Shi, H. Yang, Z. Pan, M. Jin, H. Zhao and X. Xu, Cholesterol-Lowering Effects and Mechanisms in view of Bile Acid Pathway of Resveratrol and Resveratrol Glucuronides, *J. Food Sci.*, 2016, **81**, H2841–H2848.

12 D. Learmonth, A Concise Synthesis of the 3-O-β-D- and 4′-O-β-D-Glucuronide Conjugates of *trans*-Resveratrol, *Bioconjugate Chem.*, 2003, **14**, 262–267.

13 R. Lucas, D. Alcantara and J. C. Morales, A concise synthesis of glucuronide metabolites of urolithin-B, resveratrol and hydroxytyrosol, *Carbohydr. Res.*, 2009, **344**, 1340–1346.

14 L.-X. Wang, A. Hereida, H. Song, Z. Zhang, B. Yu, C. Davis and R. Redfield, Resveratrol glucuronides as the metabolites of resveratrol in humans: Characterisation, synthesis and anti-HIV activity, *J. Pharm. Sci.*, 2004, **93**, 2448–2457.

15 V. Aumont, S. Krisa, E. Battaglia, P. Netter, T. Richard, J.-M. Merillon, J. Magdalou and N. Sabolovic, Regioselective and stereospecific glucuronidation of trans- and cis-resveratrol, *Arch. Biochem. Biophys.*, 2001, **393**, 281–289.

16 R. W. Dellinger, A. M. G. Garcia and F. L. Meyskens Jr., Differences in the glucuronidation of resveratrol and pterostilbene: altered enzyme stereospecificity and potential gender differences, *Drug Metab. Pharmacokinet.*, 2014, **29**, 112–119.

17 (*a*) G. S. Jayatilake, B. J. Baker and J. B. McClintock, Isolation and Identification of a Stilbene Derivative from the Antarctic Sponge Kirkpatrickia variolosa, *J. Nat. Prod.*, 1995, **58**, 1958–1960; (*b*) B. Ruan, X. Huang, H. Ding, C. Xu, H. Ge, H. Zhu and R. Tan, Synthesis and cytotoxic evaluation of a series of resveratrol derivatives, *Chem. Biodiversity*, 2006, **3**, 975–981.

18 C. S. Jungong and A. V. Novikov, Practical Preparation of Resveratrol 3-O-β-D-glucuronide, *Synth. Commun.*, 2012, **42**, 3589–3597.

19 G. Nicolosi, C. Spatafora and C. Tringali, Chemo-enzymatic preparation of resveratrol derivatives, *J. Mol. Catal. B: Enzym.*, 2002, **16**, 223–229.

20 (*a*) L. Zimanyi, S. Thekkan, B. Eckert, A. R. Condren, O. Dmitrenko, L. R. Kuhn, I. V. Alabugin and J. Saltiel, Determination of the pK_a Values of trans-Resveratrol, a Triphenolic Stilbene, by Singular Value Decomposition. Comparison with Theory, *J. Phys. Chem.*, 2020, **124**, 6294–6302; (*b*) A valuable broad study of the pK_as of many phenols, comparing theoretical and experimental values, was given by M D. Liptak, K. C. Gross, P. G. Seybold, S. Feldgus and G. C. Shields, Absolute pK_a determinations for Substituted Phenols, *J. Am. Chem. Soc.*, 2002, **124**, 6421–6427.

21 S. E. Blanco, M. C. Almandoz and F. H. Ferretti, Determination of the overlapping pK_a values of resorcinol using UV-visible spectroscopy, *Spectrochim. Acta, Part A*, 2005, **61**, 93–102.

22 (*a*) R. R. Schmidt, New Methods for the Synthesis of Glycosides and Oligosaccharides-Are There Alternatives to the Konigs-Knorr Method? [New Synthetic Methods (56)], *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 212–235; (*b*) A. Nudelman, J. Herzig, H. E. Gottlieb, E. Keinan and J. Sterling, Selective deacetylation of anomeric sugar acetates with tin alkoxides, *Carbohydr. Res.*, 1987, **162**, 145–152, as applied to the glucuronic acid series.

23 (*a*) Y. J. Lu, Y. Liu, M. Prashad and W.-C. Shieh, Synthesis of vildagliptin-β-O-glucuronide, *Adv. Chem. Eng. Sci.*, 2012, **2**, 379–383; (*b*) S. Chittaboina, B. Hodges and Q. Wang, A Facile Route for the Regioselective Deacetylation of Peracetylated Carbohydrates at Anomeric Position, *Lett. Org. Chem.*, 2006, **3**, 35–38.

24 (*a*) B. Wang, S. van Herck, Y. Chen, X. Bai, Z. Zhong, K. Keswarte, B. N. Lambrecht, N. N. Sanders, S. Lienenklaus, H. W. Scheeren, S. A. David, F. Kiessling, T. Lammers, B. G. de Geest and Y. Shi, Potent and Prolonged Innate Immune Activation by Enzyme-Responsive Imidazoquinoline TLR7/8 Agonist Prodrug Vesicles, *J. Am. Chem. Soc.*, 2020, **142**, 12133–12139; (*b*) Compound 8 was originally described by: G. N. Bollenback, J. W. Long, D. G. Benjamin and J. A. Lindquist, The Synthesis of Aryl-D-glucopyranosiduronic Acids, *J. Am. Chem. Soc.*, 1955, **78**, 3310–3315.

25 L. Kerins, S. Byrne, A. Gabba and P. V. Murphy, Anomeric Preferences for Glucuronic and Galacturonic Acid and Derivatives and Influence of Electron-Withdrawing Substituents, *J. Org. Chem.*, 2018, **83**, 7714–7729.

26 D. S.-E. K. Teki, A. Bil, V. Moreau, V. Chagnault, B. Fante, A. Adjou and J. Kovensky, Synthesis of multivalent *S*-glycoside analogues of a heparan sulfate sequence, *Org. Chem. Front.*, 2019, **6**, 2718–2725.

27 M. Eloranta, L. Hirvelae, L. Kangas, P. Koskimies, R. Lammintausta and M. Unkila, WO 2014207309, 2014.

28 Y. Chen, Y. Li, H. Yu, G. Sugiarto, V. Thon, J. Hwang, L. Ding, L. Hie and X. Chen, Tailored Design and Synthesis of Heparan Sulfate Oligosaccharide Analogues Using Sequential One-Pot Multienzyme Systems, *Angew. Chem., Int. Ed.*, 2013, **52**, 11852–11856.

29 (*a*) R. Nakajima, M. Ono, S. Aiso and H. Akita, Synthesis of Methyl 1-O-(4-hydroxymethamphetaminyl)-α-D-glucopyranouronate, *Chem. Pharm. Bull.*, 2005, **53**, 684–687; (*b*) J. A. Hayes, K. S. Eccles, S. E. Lawrence and H. E. Moynihan, Preparation and characterisation of solid state forms of paracetamol-O-glucuronide, *Carbohydr. Res.*, 2012, **349**, 108–112; (*c*) M. M. Cavalluzzi, G. Lentini, A. Lovece, C. Bruno, A. Catalano, A. Carocci and C. Franchini, First synthesis and full characterisation of mexiletene N-carbonyloxy β-D-glucuronide, *Tetrahedron Lett.*, 2010, **51**, 5265–5268.

30 N-Me, piperazine pK_as: 9.14, 4.73. F. Khalili, A. Henni and A. L. L. East, pK_a Values of Some Piperazines at (298, 303, 313, and 323) K, *J. Chem. Eng. Data*, 1990, **35**, 276–277.

31 (*a*) R. T. Brown, F. Scheinmann and A. V. Stachulski, Intermediates for Glucuronide Synthesis: 7-Hydroxycoumarin Glucuronide, *J. Chem. Res., Synop.*, 1997, 370–371; (*b*) B. Fischer, A. Nudelman, M. Ruse, J. Herzig, H. E. Gottlieb and E. Keinan, A Novel Method for

Stereoselective Glucuronidation, *J. Org. Chem.*, 1984, **49**, 4988–4993.

32 O. Kirk and M. W. Christensen, Lipases from Candida antarctica: Unique biocatalysts from a unique origin, *Org. Process Res. Dev.*, 2002, **6**, 446–451.

33 The D_2O solution of **5** was evaporated, then co-evaporated quickly with two small portions of H_2O to exchange any *O*-deuterio species, for mass spectrometric analysis. This product showed $[M + 3]^+$ as required.