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Synthesis of isotopically labelled SGLT inhibitors and their metabolites

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ABSTRACT

Isotopically labelled analogues of two structurally very similar SGLT inhibitors AVE2268 (**1a**) and AVE8887 (**1b**) have been synthesized by various routes. The radioactive labelled [^{14}C]-AVE2268 was prepared in five steps including a Friedel–Crafts acylation as the key step for the ^{14}C-label introduction. For [^{14}C]-AVE8887 the same synthetic approach was not successful and therefore an alternative thiophene metallation/Weinreb amide sequence was developed. This pathway was also applied to obtain stable isotopically labelled analogues of both AVE2268 and AVE8887. Finally, the synthesis of two metabolites, sulfate **12** and glucuronide **13** were achieved by applying interesting protecting group and oxidation strategies.

1. Introduction

Sodium glucose co-transporters (SGLT-2)[1] are membrane proteins. They play an important role in maintaining glucose equilibrium in the human body by *re*-absorption of glucose in the kidney. It is therefore expected that the inhibition of SGLT-2 transporters could decrease glucose blood levels by preventing *re*-absorption from the urine. The control of these transporters could be an effective tool in the normalisation of high blood glucose levels that are associated with diseases such as Type 1 and Type 2 diabetes. In combination with a negative energy balance, such a potential profile could be highly attractive for the treatment of diabetes mellitus and obesity. Other potential therapeutic applications like treatment of lipid disorders, atherosclerosis, cardiovascular diseases, high blood pressure and metabolic syndrome could also be relevant.[2] Consequently, the development of small-molecule inhibitors of SGLT has received widespread attention across the pharmaceutical industry (Fig. 1).[3]

Two new candidates are being developed by Sanofi–Aventis as novel SGLT-2 inhibitors for the treatment of type II diabetes mellitus. They are AVE2268 (**1a**) and AVE8887 (**1b**).[4] In animal models, both compounds have reduced the intestinal absorption of glucose and at the same time increased renal excretion of glucose. In the course of drug development the candidate's pharmacokinetic (PK) properties and the absorption, distribution, metabolism and elimination (ADME)[5] characteristics have to be evaluated in vitro, then in animals and finally in humans.[6] In order to keep track of the drug molecules throughout the body and in excreta, even after their transformation into different metabolites, the administration of radiolabelled drugs is considered essential.[7] Typically, ^{14}C is the label of choice because it can be introduced into a metabolically and chemically stable position in the backbone of the compound without changing the pharmacological profile of the drug candidate.[8]

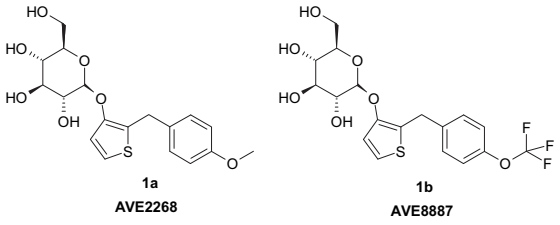

Figure 1.

In recent years, liquid chromatography coupled with tandem mass spectroscopy detection (LC–MS/MS) has become the most powerful bioanalytical tool for the investigation of samples from these animal and human toxico-, metabolism and pharmacokinetic studies.[9] For a quantitative LC–MS/MS analysis of the new drug candidates or relevant metabolites in complex matrices (like blood, urine, bile etc.,), stable isotopically labelled internal standards are considered essential.[10] That means, in conjunction with the planned development program for AVE2268 **1a** and AVE8887 **1b**, both ^{14}C- and stable isotopically labelled versions of these new drug candidates were required. In addition, two major metabolites were requested to be synthesised as the toxicological and pharmacological profile of relevant metabolites also needs to be assessed during drug development.[11]

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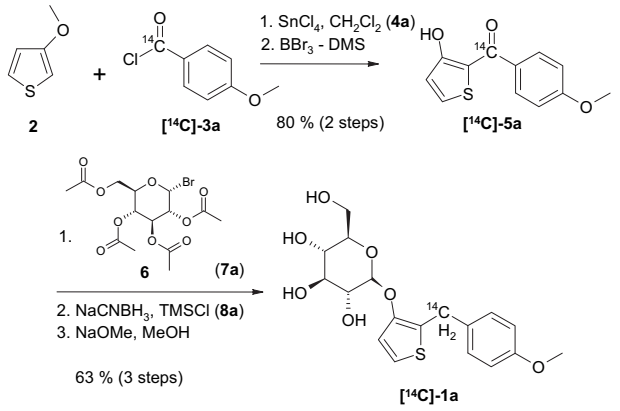
2. Results and discussion

2.1. Synthesis of ^{14}C-labelled AVE2268 1a and AVE8887 1b

In choosing the labelling position not only the chemical and metabolic stability have to be considered but also the availability of suitable ^{14}C-labelled precursors. For all these reasons placing the label into the benzylic position was preferred. Using thiophene ring labelling could have had the advantage of a common ^{14}C-building block for both candidates however it is less accessible for labelling and therefore much more expensive. Hence, ^{14}C-synthesis of AVE2268 **1a** was achieved following the simple five step synthesis path depicted in Scheme 1.[12] Starting from commercially available 3-methoxythiophene **2** and [^{14}CO]anisoyl chloride [**^{14}C**]-**3a**, purchased from Amersham Biosciences, a completely regioselective Friedel–Crafts acylation followed by a selective methyl ether cleavage of the 3-methoxythiophene derivative **4a** gave the hydroxyl-thiophene derivative [**^{14}C**]-**5a**. Subsequent alkylation with acetobromo-alpha-glucose under phase transfer conditions, carbonyl reduction with $NaCNBH_3$/TMSCl[13] and final basic acyl deprotection afforded the desired compound [**^{14}C**]-**1a** in a very good overall yield of 50%. The structure of **1a** was confirmed by an X-ray analysis of **8a** (Fig. 2).



Scheme 1. Synthesis of [^{14}C]-AVE2268 **1a**.

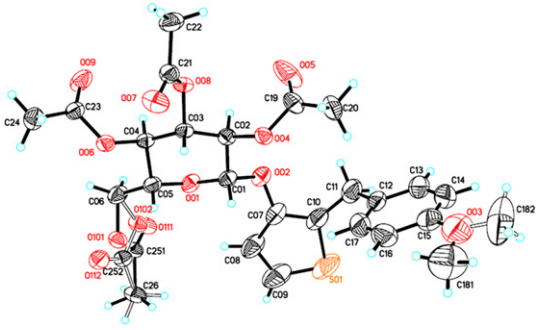
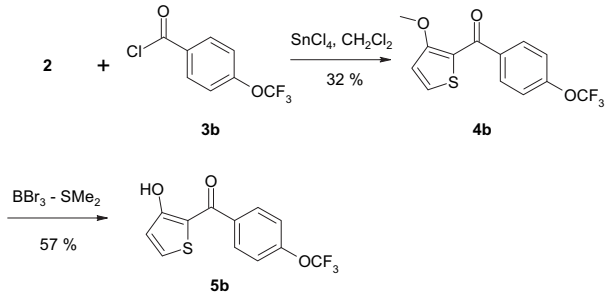


Figure 2. X-ray structure of **8a**.

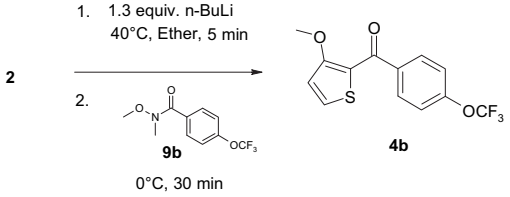
For ^{14}C-labelling of AVE8887 **1b** a very similar pathway was envisioned employing 4-trifluoromethoxy-[^{14}**CO**]benzoyl chloride [**^{14}C**]-**3b** instead of [**^{14}C**]-**3a**. Unfortunately we found in our cold elaboration experiments that more electron deficient benzoyl chlorides, such as **3b**, reacted much slower with thiophene **2** under Friedel–Crafts acylation conditions. Consequently the acid labile thiophene **2** started to decompose resulting in low yields for this reaction step. Even under optimised conditions and a stepwise addition (2–3 equiv) of **2**, the maximum yield achieved was only 32%, see also Scheme 2. In addition, work up and purification were difficult due to the numerous side products formed. Therefore, we started to look for alternative strategies for the synthesis of **5b** in which greater emphasise was taken in minimising decomposition by increased reactivity of the thiophene **2**.



Scheme 2. Synthesis of AVE8887 **1b** building block **5b**.

Stimulated by results reported by Miller et al. and Gronowitz et al.[14] we examined the regioselective lithiation of 3-methoxythiophene **2** in refluxing ether over 5 min. However, when quenching the lithiated thiophene **2** either with anisoyl chloride **3a** or 4-trifluoromethoxybenzoyl chloride **3b** only traces of the corresponding products **4a** and **4b**, respectively, could be observed. Besides acid chlorides the Weinreb amide is well known to be reactive in organometallic reactions and would be readily accessible also in labelled form.[15] Initial tests of **9b** in the reaction with the lithiated thiophene **2** resulted in the formation of **4b** in a promising 40% yield (Table 1, entry 1). Consequently, Weinreb amide **9b** was used during further optimisation of the reaction conditions in the lithiation step (Table 1). Under the described reaction conditions no regioisomer of **4b** was determined (LC–MS).

Table 1
Optimisation of lithiation conditions



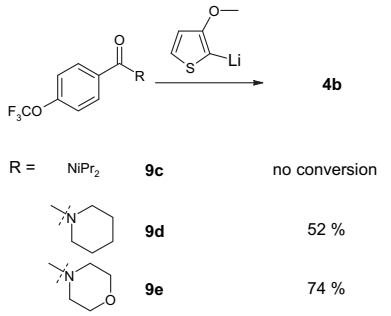
Entry	**2** (equiv)	Solvent	Yield **4b**[a] [%]
1	1.0	Et_2O	40
2	1.3	Et_2O	66
3	1.5	Et_2O	67
4	2.0	Et_2O	74
5	1.3	Toluene	9
6	1.3	THF	53
7	1.3	MTBE	62

[a] Yields are based on Weinreb amide **9b**.

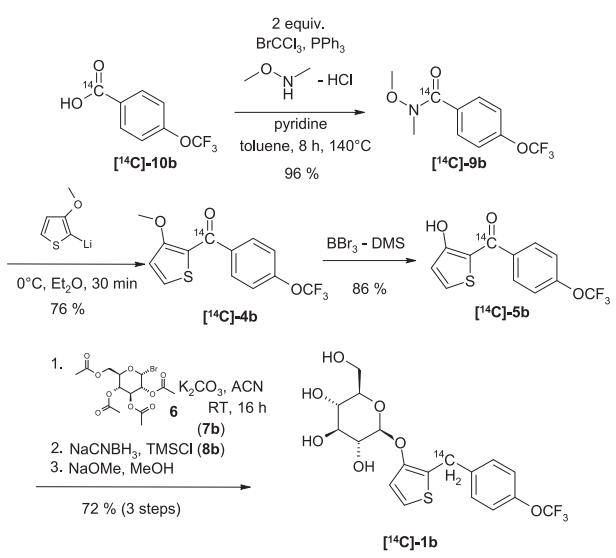
Initially the focus of this optimisation work was to achieve improved reaction conditions for the synthesis of only ^{14}C-labelled AVE8887, in which handling of refluxing ether is manageable due to the small scale. To this end the best results were achieved employing 2 equiv of *n*-BuLi (entry 4). However, for a multi-gram synthesis of AVE8887 both a safer solvent and a more convenient electrophile were preferred. Diethylether could be substituted by less flammable MTBE (entry 7) and the Weinreb amide **9b** by the less expensive piperidine **9d** or morpholine amide **9e**. Interestingly, reaction of the lithiated thiophene with the bulky diisopropylamide **9c** gave no conversion (Scheme 3).

The ^{14}C-synthesis of [**^{14}C**]-**1b** was finally accomplished following the pathway shown in Scheme 4. Starting from [^{14}CO]trifluoromethoxy benzoic acid [**^{14}C**]-**10b** the corresponding Weinreb amide [**^{14}C**]-**9b** was formed under Appel conditions.[16] Addition of

the lithiated thiophene to an ice–cold solution of the Weinreb amide [^{14}C]-**9b** yielded [^{14}C]-**4b** in 76%. Luckily the rest of the synthesis showed no significant deviation from the synthesis of [^{14}C]-**1a** reported above. The colourless compound was isolated after six reaction steps with an overall yield of 45%.



Scheme 3. Alternative amides for lithiation coupling step.

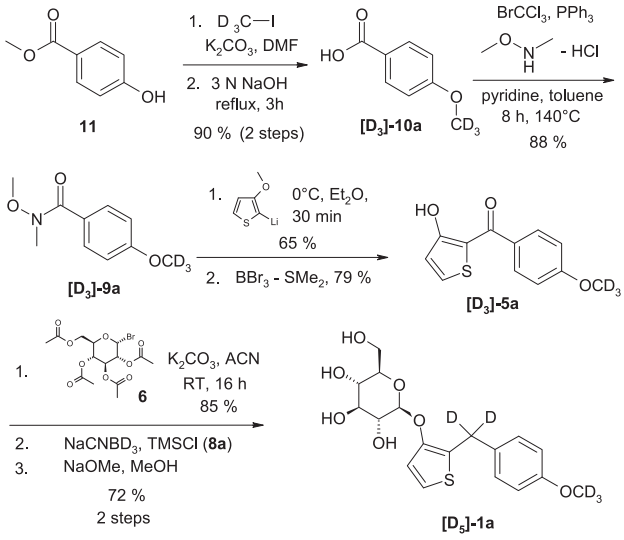


Scheme 4. Synthesis of [^{14}C]-AVE8887 **1b**.

2.2. Synthesis of stable isotopically labelled AVE2268 1a and AVE8887 1b

In order to avoid matrix effects in mass spectrum experiments,[17] such as ion suppression, stable isotopic labelled internal standards are of particular advantage due to the similarity of the physical and chemical properties to the investigated substance.[10] Both can be extracted from biological samples to the same extent, have identical retention time and ionisation behaviour in the LC–MS but differ on account of their mass difference, see also Figure 3. If this mass difference is selected to be large enough to avoid cross signal overlapping of the natural isotope pattern, quantitative determination is possible. Typically, for small molecules without chlorine, bromine or sulfur-containing functionalities, an incorporation of 3–4 mass units is sufficient.[18] In this case due to the thiophene ring moiety present in **1a** and **1b** at least five deuterium atoms were required.

Stable isotopically labelled **1a** was synthesised according to the path shown in Scheme 5 in eight chemical steps with an overall yield of 25%. Starting from 4-hydroxy benzoyl methyl ester **11** the hydroxy group was alkylated with deuterated methyliodide followed by basic ester hydrolysis to give the corresponding acid [**D$_3$**]-**10a** in 90% yield over two steps. The subsequent steps were described in the ^{14}C-synthesis above, however sodiumcyanoborodeuteride was employed in the carbonyl reduction to introduce two further deuterium atoms. A co-injection of AVE2268 and [D$_5$]-AVE2268 revealed the expected mass difference but identical retention time in the LC–MS (Fig. 3).



Scheme 5. Synthesis of [D$_5$]-AVE2268 **1a**.

The synthesis of stable isotopically labelled AVE8887 **1b** was achieved applying exactly the same pathway starting from ^{13}C-labelled-trifluoromethoxy benzoic acid **10b**.

2.3. Synthesis of sulfate and glucoronite metabolites 12 and 13

During preclinical development the synthesis of two metabolites, sulfate **12** and glucuronide **13**, became necessary (Fig. 4).

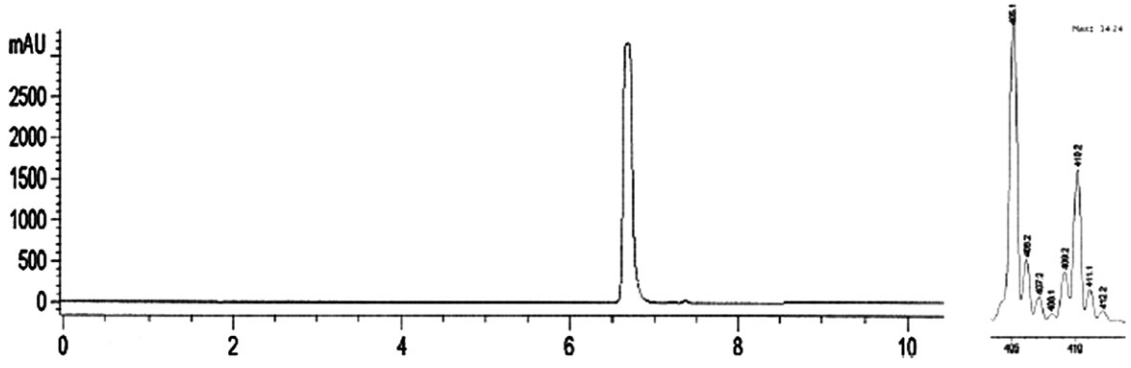


Figure 3. LC–MS-spectra of a 1:1 mixture of AVE2268 and [D$_5$]-AVE2268.

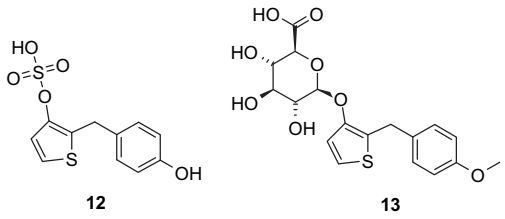
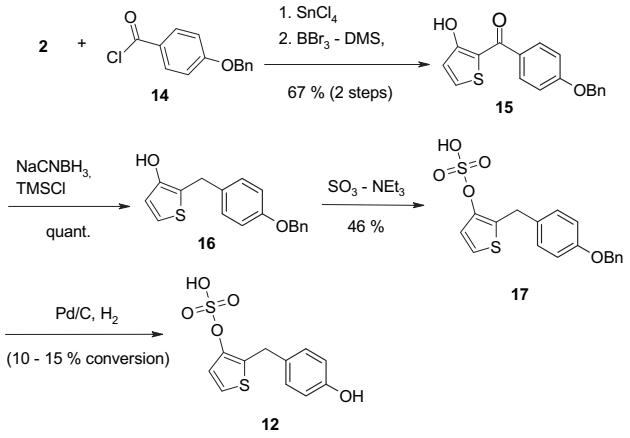


Figure 4. Structure of AVE2268 metabolites **12** and **13**.

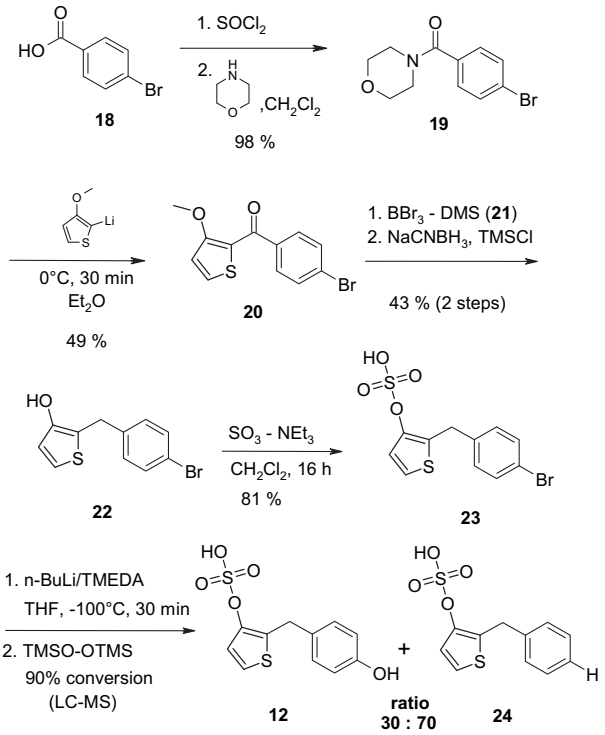
Based on our knowledge from the described syntheses above we choose a benzyl protection of the phenol to avoid the strong acidic conditions that would be required for demethylation. Starting from 4-benzyloxybenzoyl chloride **14** Friedel–Crafts acylation with **2** and subsequent methyl ether cleavage with BBr_3–DMS complex the 3-hydroxy thiophene derivative **15** was obtained as stable crystalline intermediate without benzyl-group removal (Scheme 6).



Scheme 6. Synthesis of sulfate metabolite **12**, route 1.

Compared to the previous labelling syntheses a higher excess (10 equiv) of $NaBH_3CN$ was necessary for complete reduction of the carbonyl function. The corresponding product **16** proved to be highly unstable and completely decomposed after three days at room temperature. It was therefore immediately used after synthesis in the next step. Sulfate **17** was formed quantitatively using excess of sulfurtrioxide–triethylamine-complex. However, in the last reaction step (debenzylation) we only observed 10–15% conversion under all conditions tested: (a) 0.1 equiv Pd/C 10%, H_2 (40 bar), THF, 6 h, rt and 50 °C; (b) 1 equiv Pd/C 10%, H_2 (40 bar), THF; (c) 1 equiv Pd/C 10%, H_2 (40 bar), THF, 1 equiv NEt_3; (d) 1 equiv Pd/C 10%, H_2 (40 bar), THF, 1 equiv HOAc. Unfortunately, we were neither successful in further optimisation of the reaction conditions (temperature, pressure, solvent, etc.,) nor in a semi-preparative HPLC separation of the small amounts of metabolite **12** formed. In order to provide the requested amount (around 200 mg) of **12** in time we started working on two alternative approaches in parallel. Firstly, we planned to use a bromo-atom as an anchor for a late hydroxyl-group introduction and secondly, we choose allyl-protection due to a higher liability than the benzyl-group under reductive conditions.

Route 2 (Scheme 7) started from commercially available 4-bromo benzoic acid **18**, which was transformed into the morpholine amide **19** and subsequently reacted with lithiated thiophene **2** to give ketone **20** in moderate yields. After methyl ether cleavage and reduction of the carbonyl function, the unstable thiophenol **22** was immediately reacted with sulfurtrioxide–triethylamine-complex to give the sulfate **23** as a colourless salt. In the last reaction step a halogen–lithium exchange was performed at −100 °C and then bistrimethylsilyl peroxide was added as electrophile. Unfortunately, we obtained a mixture of phenol **12** and the reduced starting material **24** in the ratio 30:70. We couldn't prevent the reductive formation of the by-product even by performing the halogen exchange directly in the presence of the electrophile at −100 °C. No further optimisation of this reaction was performed as separating even small amounts of **24** from compound **12** under reversed phase HPLC conditions proved to be difficult and time consuming.

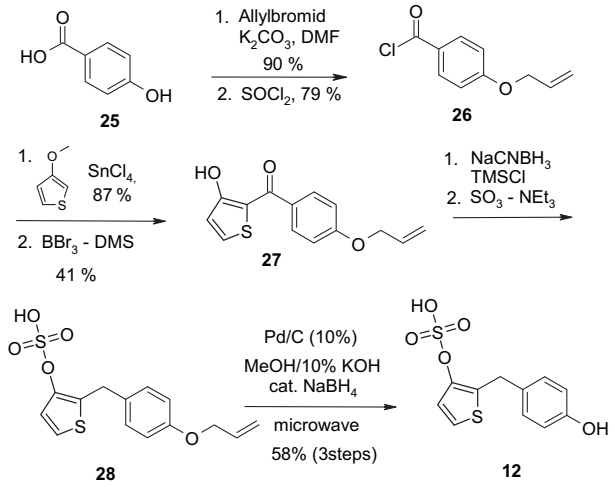


Scheme 7. Synthesis of sulfate metabolite **12**, route 2.

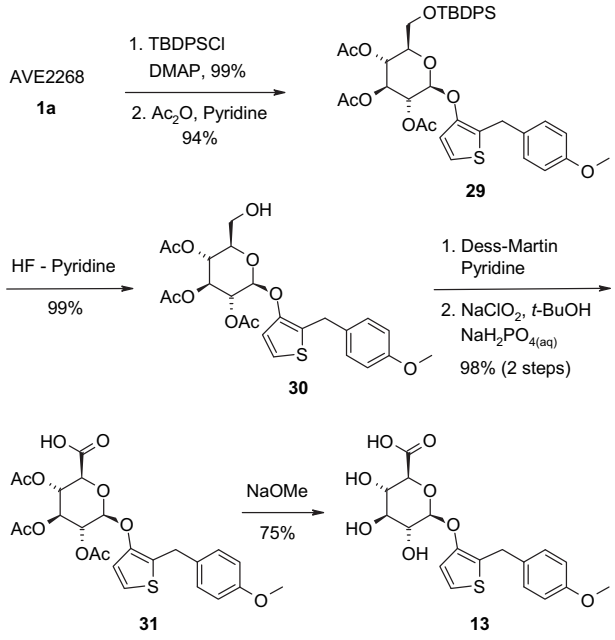
In parallel to route 2 we followed route 3 (Scheme 8) starting from 4-hydroxybenzoic acid **25**, which was alkylated with allylbromide and converted to the corresponding acylchloride **26**. Friedel–Crafts acylation with thiophene **2** and selective methyl ether cleavage resulted in ketone **27**. The reduction of the carbonyl group was followed by the successful formation of the allyl-protected sulfate **28**. After initial unsuccessful attempts to remove the allyl-group we surprisingly succeeded by using combined conditions of Hara et al.[19a] and Zhu et al.[19b] and addition of catalytic amounts of $NaBH_4$. Accordingly **28** was heated in the presence of $NaBH_4$-activated Pd/C catalyst in MeOH/KOH under microwave conditions in a closed vial for 80 min. We found that only the combination of high temperature, strong basic conditions and $NaBH_4$-activated Pd/C catalyst gave reasonable yields for the deprotection of **28**.

For the synthesis of metabolite **13** we initially envisioned a one step synthesis approach starting from parent drug AVE2268 **1a**. Firstly, we tried to directly oxidise the glucoside moiety to afford the corresponding glucuronide. However, any attempts using reported methods like $KMnO_4$, SeO_2, PtO_2/O_2 TEMPO/NaOCl/Oxone, peroxidases, etc., failed. Secondly, any trials to perform a reacetalisation of AVE2268 **1a**–**13** by adding glucuronic acid in the presence of a weak acid (*p*-toluenesulfonic acid) were unsuccessful. Applying an orthogonal protecting group strategy finally allowed the selective oxidation of the C6-position. However, several problems had to be considered; (a) any traces of acid had to be avoided to prevent acetyl migration, (b) in the first oxidation step utilising the Dess–Martin periodinane, a very weak base had to be used in order to

prevent double bond formation by elimination of acetic acid, and (c) the generated aldehyde was unstable and had to be directly oxidised to the corresponding acid (Scheme 9).



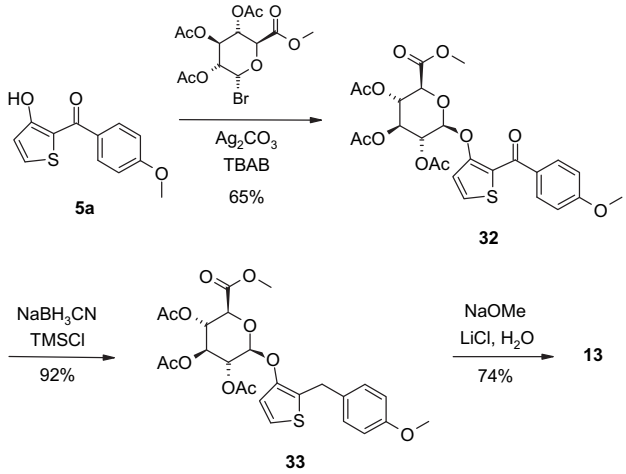
Scheme 8. Synthesis of sulfate metabolite **12**, route 3.



Scheme 9. Synthesis of glucuronide metabolite **13**, route 1.

Starting from AVE2268 **1a** the primary alcohol functionality could be selectively silylated using TBDPSCl (*tert*-butyldiphenylchlorosilane). Afterwards the secondary alcohol functionalities were acetylated to give **29**, which was then reacted with HF-pyridine complex to deprotect the primary alcohol again. Compound **30** could be oxidised to the carboxylic acid **31** in two steps applying Dess–Martin periodinane and sodium chlorite. Final cleavage of the acetyl groups with sodium methoxide led to **13** after six steps in 68% overall yield.

In parallel a second route was developed (Scheme 10). While the alkylation of the corresponding des-keto aglycon precursor did not proceed the keto aglycon **5a** allowed the introduction of the protected glucuronic acid. The reaction succeeded under phase transfer conditions using an excess of silver carbonate (Koenigs–Knorr conditions). Subsequent reduction of the benzylic carbonyl function in **32** with $NaBH_3CN$ and TMSCl gave **33**. After removing the acetyl groups with sodium methoxide, water and lithium chloride had to be added to cleave the methyl ester. Performing this route enabled the metabolite **13** to be synthesised in only three steps with 44% overall yield.



Scheme 10. Synthesis of glucuronide metabolite **13**, route 2.

3. Conclusions

We have described in detail the challenges of the synthesis of isotopically labelled compounds of SGLT inhibitors **1a** and **1b**. Although very similar in their chemical structure, different synthetic pathways had to be optimised. Furthermore we have shown in the synthesis of metabolites **12** and **13** unexpected challenges and possible new strategies to overcome them.

4. Experimental section

4.1. General

*4.1.1. [**^{14}C**]-**4a** (4-Methoxy-phenyl)-(3-methoxy-thiophen-2-yl)-methane-[^{14}CO]one.* In a flask tin-(IV)-tetrachloride (786 μL, 6.72 mmol,) was dissolved in dichloromethane (54 mL) and at 5–10 °C [^{14}CO]anisoyl chloride [**^{14}C**]-**3a** (915 mg, 5.37 mmol, 11,100 MBq) was added. Then 3-methoxythiophene (768 mg, 6.72 mmol) at 5–10 °C was slowly added and the reaction mixture was stirred at rt for 4 h. After complete conversion (LC–MS) ice-cooled water (30 mL) and 30%ige HCl (2.5 ml) was added. The different layers were separated and the organic layer was washed with water (20 mL), sodium bicarbonate solution (8 wt % in water, 20 mL) and water (20 mL). The organic phase was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by chromatography using heptane/ethyl acetate 3:1 as mobile phase. The product [**^{14}C**]-**4a** (1.06 g, 4.29 mmol, 8880 MBq, 80%, purity 97% (HPLC, 254 nm)) was isolated as a yellow solid: mp 98–99 °C; ^{1}H NMR ($CDCl_3$): δ=8.37 (d, J=6.3 Hz, 1H), 7.96 (d, J=6.9 Hz, 2H), 6.96 (d, J=6.9 Hz, 2H), 6.37 (d, J=6.3 Hz, 1H), 3.91, 3.88 (s, 6H) ppm.

*4.1.2. [**^{14}C**]-**5a** (3-Hydroxy-thiophen-2-yl)-(4-methoxy-phenyl)-methane- [^{14}CO]one.* In a 250 mL flask were weighed BBr_3–DMS (1.20 g, 3.83 mmol) and the flask was filled with argon over 10 min. The solid was dissolved in dichloromethane (62 mL) and at rt [**^{14}C**]-**4a** (887 mg, 3.57 mmol, 7389 MBq) in dichloromethane (10 mL) was added dropwise. The dark solution was stirred for 7 h at rt (TLC-control) and then satd sodium bicarbonate solution (8 mL) was added. The layers were separated and the organic was washed with

water (10 mL), dried over Na_2SO_4 and the solvent evaporated in vacuo. The crude, slightly wet product was directly used in the next reaction step.

*4.1.3. [**^{14}C**]-**7a** 3-(2-(4-Methoxy-[^{14}CO]benzoyl)-thiophenyl)-ß-D-4,5,6-acetyl-glucopyranoside.* Into a 250 mL flask was filled [**^{14}C**]-**5a** (836 mg, 3.57 mmol, 7389 MBq) and dissolved in dichloromethane (42 mL). At rt tetrabutylammoniumbromide (571 mg, 1.77 mmol), potassium carbonate (4.49 g, 32.5 mmol) and water (2.1 mL) were added. Then at rt acetobrom-alpha-glucose **6** (3.07 g, 7.48 mmol) was added in portions during 1 h. The brown reaction mixture was stirred at rt over 16 h (TLC-control). The reaction mixture was filtered and the organic layer washed with water (10 mL) three times. Finally the organic layer was dried over Na_2SO_4 and the solvent removed in vacuo. The crude product was purified by chromatography (eluent: heptane/ethyl acetate 1:1) to give [**^{14}C**]-**7a** (1.70 g, 3.00 mmol, 6600 MBq, 84% in two steps, purity 98% (HPLC, 254 nm)) as a colourless solid; mp: 149–151 °C; ^{1}H NMR (DMSO-d_6): δ=8.02 (d, J=5.6 Hz, 1H), 7.71 (d, J=6.7 Hz, 2H), 7:1 (d, J=6.7 Hz, 2H), 7.04 (d, J=5.6 Hz, 1H), 5.62 (d, 1H), 5.34 (dd, 1H), 4.93 (m, 1H), 4.72 (dd, 1H), 4.24 (m, 2H), 4.12 (m, 1H), 3.81 (s, 3H, O–CH_3), 2.05, 2.00, 1.90, 1.85 (s, 12H, acetyl-CH_3) ppm.

*4.1.4. [**^{14}C**]-**8a** 3-(2-(4-Methoxy-[$^{14}CH_2$]benzyl)-thiophenyl)-ß-D-1,3,4,5-acetyl-glucopyranoside.* In a 100 mL two-necked flask [**^{14}C**]-**7a** (1.70 g, 3.00 mmol, 6600 MBq) was weighed and the flask was filled with argon. Then the compound was dissolved in dry acetonitrile (29 mL) and the reaction mixture was cooled to 0–5 °C. $NaCNBH_3$ (1.49 g, 23.7 mmol) was added in portions at max. 5 °C and the mixture was stirred for 30 min. Then trimethylsilylchloride (2.90 mL, 23.7 mmol) was added dropwise (internal temperature should not exceed 5 °C) and the mixture was stirred for 4 h at 5 °C (LC–MS control). Finally satd sodium bicarbonate solution (44 mL) was added and was stirred vigorously for 5 min. After addition of dichloromethane (400 mL) the phases were separated and the aqueous phase was extracted three times by dichloromethane (20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude material was purified by chromatography (SiO_2, heptane/ethyl acetate 1:1) to give [**^{14}C**]-**8a** (1.68 g, 3.00 mmol, 6600 MBq, 100%, purity 93% (HPLC, 254 nm)) as a colourless solid. Mp: 116–118 °C; ^{1}H NMR (DMSO-d_6): δ=7.29 (d, J=5.5 Hz, 1H), 7.09 (d, J=6.7 Hz, 2H), 6.87 (d, J=5.5 Hz, 1H), 6.84 (d, J=6.7 Hz, 2H), 5.41–5.33 (m, 2H), 5.07–4.97 (m, 2H), 4.21–4.17 (m, 2H), 4.09 (d, J=9.7 Hz, 1H), 3.91–3.79 (m, 2H), 3.71 (s, 3H), 2.00, 1.99, 1.96, 1.95 (s, 12H, acetyl–CH_3) ppm.

*4.1.5. [**^{14}C**]-**1a** 3-(2-(4-Methoxy-[$^{14}CH_2$]benzyl)-thiophenyl)-ß-D-glucopyranoside.* In a 100 mL flask acetyl-protected glucose derivative [**^{14}C**]-**8a** (1.68 g, 3.00 mmol, 6600 MBq) was dissolved in methanol (25 mL). To this solution a sodium methoxide solution (30% in MeOH, 2.90 mL, 15.9 mmol) was added at 0 °C and the reaction mixture was stirred for 2 h. Then the pH was adjusted to pH 6.7–6.9 by the addition of 2 N HCl in ethanol and subsequently the solvent evaporated in vacuo. The residue was suspended in water, two times centrifugated and dried in vacuo. This material was purified by chromatography using dichloromethane/methanol 10:1 as eluent, followed by semi-preparative HPLC (column: Luna RP18 column, eluents: acetonitrile/water 20:80 for 4 min, then up to 80:20 in 6 min, then 80:20 for 4 min and finally in 1 min down again to 20:80 for 1 min, 14 mL flow) to give [**^{14}C**]-**1a** (860 mg, 2.25 mmol, 4950 MBq, 75%, purity 99.4% (HPLC, 254 nm)) as a colourless solid. Mp: 154–155 °C; ^{1}H NMR (DMSO-d_6): δ=7.16–7.14 (m, 3H), 6.91 (d, J=5.5 Hz, 1H), 6.80 (d, J=8.6 Hz, 2H), 5.35 (s, 1H), 5.05 (s, 1H), 4.99 (s, 1H), 4.63–4.53 (m, 2H), 4.01–3.97 (m, 2H), 3.71 (s, 3H), 3.66 (s, 1H), 3.49–3.44 (m, 1H), 3.32–3.05 (m, 4H); ^{13}C NMR (DMSO-d_6): δ=157.6, 150.7, 132.7, 129.5, 123.8, 121.3, 120.2, 113.7, 103.4, 77.1, 76.5, 73.4, 69.8, 60.8, 54.9, 30.0 ppm; $[\alpha]_D^{20}$ −34.3 (*c* 5, methanol); MS (ESI, pos.) m/z (%)=383.2 [M+H$^+$] (100); HRMS (ESI-LTQ, neg.) $C_{18}H_{22}O_7S$ calcd 381.10135; found, 381.10110.

*4.1.6. [**^{14}C**]-**9b** 4-Hydroxy-N-methoxy-N-methyl-benzamide-[^{14}CO].* Into a 50 mL flask [^{14}CO]-4-trifluoromethoxy benzoic acid (721 mg, 3.50 mmol, 7400 MBq), *N,O*-dimethyl-hydroxylamine–HCl (683 mg, 7.00 mmol) and triphenylphosphine (1.83 g, 7.00 mmol) were added and the flask was filled with argon. The compounds were dissolved in dry THF (5 mL) and bromotrichloromethane (0.69 mL, 7.00 mmol) and pyridine (0.567 mL, 7.00 mmol) were added. The reaction mixture was heated to reflux (bath temperature 80–90 °C) for 5 h (TLC-control). After cooling to rt water (5 mL) was added, the phases were separated and the aqueous phase extracted three times with ethyl acetate (20 mL). The combined organic phases were dried over Na_2SO_4 and the solvent evaporated in vacuo. The crude product was purified by chromatography on SiO_2 using heptane/ethyl acetate 1:1 as eluent to give [**^{14}C**]-**9b** (830 mg, 3.33 mmol, 7030 MBq, 95%) as colourless solid. ^{1}H NMR (DMSO-d_6): δ=7.75 (d, J=8.8 Hz, 2H), 7.45 (d, J=8.8 Hz, 2H), 3.57 (s, 3H), 3.28 (s, 3H) ppm.

*4.1.7. [**^{14}C**]-**4b** [2-(4-Trifluoromethoxy-benzoyl)-(3-methoxy-thiophen-2-yl)-methane-[^{14}CO]one.* In a 50 mL flask 3-methoxythiophene **2** (0.7 mL, 7.0 mmol) was dissolved in diethylether (15 mL). Then the flask was filled with argon and at rt *n*-BuLi (0.80 mL, 1.6 M in hexane) was added via a syringe. The reaction mixture was stirred at 40 °C bath temperature for 30 min. Then the reaction mixture was added slowly to an ice-cooled solution of *N*-methoxy-*N*-methyl-4-trifluoromethoxy-[^{14}CO]benzamide [**^{14}C**]-**9b** (830 mg, 3.33 mmol, 7030 MBq) in diethylether (10 mL) via syringe. After one hour stirring at rt complete conversion was monitored by LC–MS and water (5 mL) was added. Then the layers were separated, the aqueous phase was extracted three times with dichloromethane (10 mL) and the combined organic layers were dried over Na_2SO_4 and the solvent evaporated in vacuo. The crude product was purified by chromatography heptane/ethyl acetate 3:1 to give [**^{14}C**]-**4b** (756 mg, 2.50 mmol, 5342 MBq, 76%) as a yellow oil. ^{1}H NMR (DMSO-d_6): δ=8.04 (d, J=5.5 Hz, 1H), 7.82 (d, J=8.6 Hz, 2H), 7.45 (d, J=8.6 Hz, 2H), 7.19 (d, J=5.5 Hz, 1H), 3.79 (s, 3H) ppm.

*4.1.8. [**^{14}C**]-**5b** (3-Hydroxy-thiophen-2-yl)-(4-trifluoromethoxy-phenyl)-methane-[^{14}CO]one.* Into a 250 mL flask was weighed BBr_3–DMS (821 mg, 2.63 mmol) and the flask was filled with argon over 10 min. The solid was dissolved in dichloromethane (50 mL) and at rt (3-methoxy-thiophen-2-yl)-(4-trifluoromethoxy-phenyl)-methanone [**^{14}C**]-**4b** (756 mg, 0.43 mmol, 5342 Mbq) in dichloromethane (10 mL) was added dropwise. The dark solution was stirred for 7 h at rt (TLC-control) and then satd sodium bicarbonate solution (8 mL) was added. The layers were separated and the organic phase was washed with water (10 mL), dried over Na_2SO_4 and the solvent was evaporated in vacuo. Chromatography (eluent heptane/ethyl acetate 3:1) of the crude product yielded [**^{14}C**]-**5b** (621 mg, 2.15 mmol, 4595 MBq, 86%) as a yellow solid. Mp: 67–70 °C; ^{1}H NMR (DMSO-d_6): δ=11.45 (br s, 1H, OH), 7.97 (d, J=5.4 Hz, 1H), 7.93 (d, J=8.7 Hz, 2H), 7.51 (d, J=8.7 Hz, 2H), 6.87 (d, J=5.4 Hz, 1H) ppm.

*4.1.9. [**^{14}C**]-**7b** 3-(2-(4-Trifluoromethoxy-[^{14}CO]benzoyl)-thiophenyl)-ß-D-1,3,4,5-acetyl-glucopyranoside.* Into a 50 mL flask was filled [**^{14}C**]-**5b** (625 mg, 2.15 mmol, 4595 MBq) and was dissolved in dichloromethane (20 mL). At rt tetrabutylammoniumbromide (346 mg, 1.07 mmol), potassium carbonate (2.30 g, 9.67 mmol) and water (0.2 mL) were added. Then at rt acetobromo-alpha-glucose **6** (1.50 g, 3.65 mmol) was added in portions during 1 h. The brown reaction mixture was stirred at rt over 16 h (TLC-control) then filtered and the organic layer washed three times with water (10 mL). Finally the organic layer was dried over Na_2SO_4 and the solvent

removed in vacuo. The crude product was purified by chromatography (eluent: heptane/ethyl acetate 1:1) to give [^{14}C]-**7b** (1.20 g, 1.94 mmol, 4135 MBq, 90%) as a colourless solid. Mp: 90–93 °C; ^{1}H NMR (DMSO-d_6): δ=8.09 (d, J=5.5 Hz, 1H), 7.78 (d, J=6.7 Hz, 2H), 7.43 (d, J=6.7 Hz, 2H), 7.13 (d, J=5.5 Hz, 1H), 5.60 (d, J=7.9 Hz, 1H), 5.27 (dd, J=9.5/9.5 Hz, 1H), 4.94–4.90 (m, 1H), 4.63 (dd, J=9.6/9.5 Hz, 1H), 4.21–4.17 (m, 2H), 4.06–4.04 (m, 1H), 2.02, 1.99, 1.90, 1.84 (s, 12H, acetyl-CH_3) ppm.

*4.1.10. [^{14}C]-**8b** 3-(2-(4-Trifluorormethoxy-[$^{14}CH_2$]benzyl)-thiophenyl)-ß-D-1,3,4,5-acetyl-glucopyranoside.* In a 250 mL flask ketone [^{14}C]-**7b** (1.20 g, 1.94 mmol, 4135 MBq) was weighed and the flask was filled with argon. Then the compound was dissolved in dry acetonitrile (50 mL) and the reaction mixture was cooled to 0–5 °C. $NaCNBH_3$ (2.51 g, 40.0 mmol) was added in portions at max. 5 °C and the mixture was stirred for 30 min. Then trimethylsilylchloride (5.11 mL, 40.0 mmol) was added dropwise (internal temperature should not exceed 5 °C) and the mixture was stirred for 4 h at 5 °C (LC–MS control). Finally satd sodium bicarbonate solution (10 mL) was added and the reaction mixture was stirred vigorously for 5 min. After addition of dichloromethane (400 mL) the phases were separated and the aqueous phase was extracted three times by dichloromethane (20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude material was purified by chromatography (SiO_2, heptane/ethyl acetate 1:1) to give [^{14}C]-**8b** (1.11 g, 1.84 mmol, 3928 MBq, 95%) as a colourless solid. Mp: 113–114 °C; ^{1}H NMR (DMSO-d_6): δ=7.47 (d, J=8.1 Hz, 2H), 7.40 (d, J=5.5 Hz, 1H), 7.30 (d, J=8.1 Hz, 2H), 6.86 (d, J=5.5 Hz, 1H), 5.89 (d, J=3.6 Hz, 1H), 5.45 (dd, J=9.8/9.3 Hz, 1H), 5.38 (d, J=8.0 Hz, 1H), 5.11 (dd, J=8.0/9.8 Hz, 1H), 5.04 (dd, J=9.3/9.3 Hz, 1H), 4.21–4.17 (m, 2H), 4.10 (dd, J=5.0/9.8 Hz, 1H), 3.33 (s, 2H), 2.09, 2.01, 2.00, 1.99 (s, 12H, acetyl-CH_3); ^{13}C NMR (DMSO-d_6): δ=170.0, 169.6, 169.3, 169.3, 148.9, 147.2, 144.1, 129.5, 127.4, 123.8, 120.7, 118.9, 99.6, 71.8, 70.9, 70.8, 68.1, 66.1, 61.7, 20.4, 20.4, 20.3, 20.3 ppm; HRMS (ESI-LTQ, pos.) $C_{26}H_{27}O_{11}F_3NaS$ calcd 627.11293; found, 627.11285.

*4.1.11. [^{14}C]-**1b** 3-(2-(4-Trifluoromethoxy-[$^{14}CH_2$]benzyl)-thiophenyl)-ß-D-glucopyranoside.* In a 250 mL flask acetyl-protected glucose derivative [^{14}C]-**8b** (1.11 g, 1.84 mmol, 3928 MBq) was dissolved in methanol (30 mL). To this solution sodium methoxide solution (1.14 mL, 6.00 mmol, 30% in MeOH) was added at 0 °C and the reaction mixture stirred for 3 h. Then the pH was adjusted to a value of 6.7–6.9 using 2 N HCl in ethanol and the solvent was evaporated in vacuo to a volume of 2–3 mL. The crude material was purified by chromatography using dichloromethane/methanol 8:1 as eluent, followed by semi-preparative HPLC (column: Luna RP18 column, eluents: acetonitrile/water 20:80 for 4 min, then up to 80:20 in 6 min, then 80:20 for 4 min and finally in 1 min down again to 20:80 for 1 min, 14 mL flow) to give [^{14}C]-**1b** (576 mg, 1.32 mmol, 2828 MBq, 72%, purity 99.2% (HPLC, 254 nm)) as a colourless solid. Mp: 144–145 °C; ^{1}H NMR (DMSO-d_6): δ=7.41 (d, J=8.5 Hz, 2H), 7.27 (d, J=8.5 Hz, 2H), 7.24 (d, J=5.5 Hz, 1H), 6.97 (d, J=5.5 Hz, 1H), 5.37 (d, J=4.9 Hz, 1H), 5.05 (d, J=4.5 Hz, 1H), 4.98 (d, J=5.3 Hz, 1H), 4.64 (d, J=7.3 Hz, 1H), 4.56 (dd, J=5.7/5.7 Hz, 1H), 4.12–4.04 (m, 2H), 3.72–3.68 (m, 1H), 3.51–3.47 (m, 1H), 3.32–3.12 (m, 4H); ^{19}F NMR (DMSO-d_6): δ=56.8 ppm; HRMS (ESI-LTQ, pos.) $C_{18}H_{21}O_7F_3S$ calcd 437.08763; found, 437.08752.

*4.1.12. [**D$_3$**]-**10a** [CD_3]anisic acid.* (a) Into a 250 mL flask were weighed methyl-4-hydroxy benzoate **11a** (3.80 g, 25.0 mmol) and potassium carbonate (6.90 g, 50.0 mmol) and the flask was filled with argon. Then the compounds were suspended in dry DMF (50 mL) and [D_3]methyliodide (5.0 g, 35 mmol) was added dropwise via syringe. The flask was closed and heated to 80 °C over 10 h. Then ethyl acetate (50 mL) was added and the organic phase was washed three times with 2 N HCl (10 mL). The combined organic phases were dried over Na_2SO_4 and subsequently the solvent evaporated to dryness and immediately used in the next reaction step.

(b) To the ester was dissolved in 3 N NaOH (40 mL) and ethanol (10 mL) to obtain a clear solution. This mixture was heated to reflux for 2 h. Under ice cooling a pH of 1 was obtained by the addition of 2 N HCl. The precipitated colourless solid was filtered, washed with water (3 mL) and dried in vacuo to give [**D$_3$**]-**10a** (3.49 g, 22.5 mmol, 90% in two steps). ^{1}H NMR (DMSO-d_6): δ=12.55 (br s, 1H), 7.88 (d, J=8.6 Hz, 2H), 7.03 (d, J=8.6 Hz, 2H); ^{2}H NMR (DMSO): δ=3.78 (s, 3D) ppm; MS (ESI, neg.) m/z (%)=154 $[M-H]^+$ (100).

*4.1.13. [**D$_3$**]-**9a** 4-[CD_3]Methoxy-N-methoxy-N-methyl-benzamide.* Into a 250 mL flask benzoic acid [**D$_3$**]-**11a** (3.20 g, 20.6 mmol), *N,O*-dimethyl-hydroxylamine–HCl (4.02 g, 41.2 mmol) and triphenylphosphine (10.8 g, 41.2 mmol) were added and the flask filled with argon. The compounds were dissolved in dry THF (30 mL) and bromotrichloromethane (3.97 mL, 41.2 mmol) and pyridine (3.33 mL, 41.2 mmol) were added. The reaction mixture was heated to reflux (bath temperature 80–90 °C) for 5 h (TLC-control). After cooling to rt water (30 mL) was added, the phases were separated and the aqueous phase extracted three times with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude product was purified by chromatography on SiO_2 using heptane/ethyl acetate 1:1 as eluent to give [**D$_3$**]-**9a** (3.60 g, 18.2 mmol, 88%, purity 97 % (HPLC, 254 nm)) as a colourless solid. ^{1}H NMR (DMSO-d_6): δ=7.64 (d, J=8.9 Hz, 2H), 6.99 (d, J=8.9 Hz, 2H), 3.55 (s, 3H), 3.24 (s, 3H); ^{2}H NMR (DMSO): δ=3.78 (s, 3D) ppm; MS (ESI, pos.) m/z (%)=199 $[M+H]^+$ (100).

*4.1.14. [**D$_3$**]-**4a** (4-[CD_3]Methoxy-phenyl)-(3-methoxy-thiophen-2-yl)-methan-one.* (a) In a 250 mL flask 3-methoxythiophene **2** (3.10 mL, 31.0 mmol) was dissolved in diethylether (50 mL). Then the flask was filled with argon and at rt *n*-BuLi (20.0 mL, 31.0 mmol, 1.6 M in hexane) was added. The reaction mixture was stirred at 35 °C bath temperature for 30 min. Then the reaction mixture was added to an ice-cooled solution of amide [**D$_3$**]-**9a** (3.00 g, 15.1 mmol) in diethylether (30 mL) via syringe. After one hour stirring at rt complete conversion was monitored by LC–MS and water (30 mL) was added. Then the layers were separated, the aqueous phase was extracted three times with dichloromethane (30 mL) and the combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude product was purified by chromatography heptane/ethyl acetate 1:1 to give [**D$_3$**]-**4a** (2.50 g, 9.90 mmol, 65%), which was used for the next step without further analysis.

*4.1.15. [**D$_3$**]-**5a** (3-Hydroxy-thiophen-2-yl)-(4-[CD_3]methoxy-phenyl)-methanone.* Into a 250 mL flask were weighed BBr_3–DMS (3.25 g, 10.4 mmol) and the flask filled with argon over 10 min. The solid was dissolved in dichloromethane (40 mL) and as solution of thiophene [**D$_3$**]-**4a** (2.50 g 9.97 mmol) in dichloromethane (10 mL) was added dropwise at rt. The dark solution was stirred for 3 h at rt (LC–MS control) and then satd sodium bicarbonate solution (20 mL) was added. The layers were separated and the organic layer was washed with water (10 mL), dried over Na_2SO_4 and the solvent was evaporated in vacuo. Chromatography (eluent heptane/ethyl acetate 4:1) of the crude product yielded [**D$_3$**]-**5a** (1.79 g, 7.54 mmol, 79%, purity 98% (HPLC, 254 nm)) as a colourless solid. ^{1}H NMR (DMSO-d_6): δ=11.85 (br s, 1H, OH), 7.96 (d, J=5.4 Hz, 1H), 7.89 (d, J=8.9 Hz, 2H), 7.10 (d, J=8.9 Hz, 2H), 6.93 (d, J=5.4 Hz, 1H); ^{2}H NMR (DMSO): δ=3.82 (s, 3D) ppm; MS (ESI, pos.) m/z (%)=238 $[M+H]^+$ (100).

*4.1.16. [**D$_5$**]-**1a** 3-(2-(4-[CD_3]Methoxy-[CD_2]benzyl)-thiophenyl)-ß-D-glucopyranoside.* (a) In a 250 mL flask [**D$_3$**]-**5a** (1.73 g, 7.30 mmol) was dissolved in dichloromethane (90 mL). At rt

tetrabutylammoniumbromide (1.18 g, 3.65 mmol), potassium carbonate (9.06 g, 65.7 mmol) and water (5 mL) were added. Then at rt acetobrom-alpha-glucose **6** (6.00 g, 14.6 mmol) was added in portions. The brown reaction mixture was stirred at rt over 16 h (TLC-control) and then water (20 mL) was added. The phases were separated, the aqueous phase was extracted three times with dichloromethane (20 mL), the combined organic phases were dried over Na_2SO_4 and the solvent removed in vacuo. The crude product was purified by chromatography (eluent: heptane/ethyl acetate 1:1) to give [**D_3**]-**7a** (3.53 g, 6.22 mmol, 85%) as a colourless solid.

(b) In a 250 mL flask ketone [**D_3**]-**7a** (2.00 g, 3.50 mmol) was weighed and the flask filled with argon. Then the compound was dissolved in dry acetonitrile (60 mL) and the reaction mixture was cooled to 0–5 °C. $NaCNBD_3$ (1.84 g, 28.0 mmol) was added in portions at max. 5 °C and the mixture was stirred for 30 min. Then trimethylsilylchloride (3.55 mL, 28.0 mmol) was added dropwise (internal temperature should not exceed 5 °C) and the mixture was stirred for 4 h at 5 °C (LC–MS control). Finally satd sodium bicarbonate solution (30 mL, in D_2O) was added and the solution was stirred vigorously over 5 min. After addition of dichloromethane (60 mL) the phases were separated and the aqueous phase was extracted three times with dichloromethane (20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. This crude material (2 g, >100%) was directly used in the next reaction step without further purification.

(c) In a 100 mL flask acetyl-protected glucose derivative [**D_5**]-**8a** (1.00 g, 1.80 mmol) was dissolved in methanol (15 mL). To this solution sodium methoxide solution (0.343 mL, 1.80 mmol, 30% in MeOH) was added at 0 °C and the reaction mixture was stirred for 2 h. Then with 2 N HCl in ethanol a pH of 6.7–6.9 was adjusted and water (100 mL) was added. 1/3 of the solvent was evaporated in vacuo and the resulting solid was isolated by centrifugation. The solid was washed twice with water and dried in vacuo. This material was further purified by chromatography using dichloromethane/methanol 6:1 as eluent, followed by semi-preparative HPLC (column: Luna RP18 column, eluents: acetonitrile/water 20:80 for 4 min, then up to 80:20 in 6 min, then 80:20 for 4 min and finally in 1 min down again to 20:80 for 1 min, 14 mL flow) to give [**D_5**]-**1a** (503 mg, 1.29 mmol, 72% in two steps, purity 99.6% (HPLC, 254 nm)) as a colourless solid. Mp: 151–152 °C; ^{1}H NMR (DMSO-d_6): δ=7.16–7.14 (m, 3H), 6.91 (d, J=5.5 Hz, 1H), 6.80 (d, J=8.6 Hz, 2H), 5.35 (s, 1H), 5.05 (s, 1H), 4.99 (s, 1H), 4.63–4.53 (m, 2H), 3.66 (s, 1H), 3.49–3.44 (m, 1H), 3.32–3.05 (m, 4H) ppm; MS (ESI, pos.) m/z (%)=408 [M+Na+3D] (3), 409 [M+Na+4D] (22), 410, [M+Na+5D] (53), 411 [M+Na+6D] (17), 412, [M+Na+7D] (5).

4.1.17. Compound ***15*** *(3-hydroxy-thiophen-2-yl)-(4-benzyloxy-phenyl)-methanone.* (a) In a 500 mL flask 3-methoxythiophene **2** (2.75 mL, 27.5 mmol) and 4-benzyloxy benzoic acid chloride (5.40 g, 21.9 mmol) were dissolved in dichloromethane (200 mL) and at 0–5 °C tin-(IV)-tetrachloride (5.52 mL, 46.8 mmol) was added. The reaction mixture was stirred at 5 °C for 2 h and a further 2 h at rt. After complete conversion (LC–MS) ice-cooled water (20 mL) was added. The different layers were separated and the organic layer was washed with water (10 mL), sodium bicarbonate solution (10 mL, 8 wt % in water) and brine (10 mL). The organic phase was dried over Na_2SO_4 and was evaporated in vacuo. The crude product was purified by chromatography using heptane/ethyl acetate 3:1 as mobile phase. The product (6.20 g, 19.1 mmol, 87%, purity 95% (HPLC, 254 nm)) was isolated as a yellow oil. ^{1}H NMR ($CDCl_3$): δ=7.87 (d, J=8.9 Hz, 2H), 7.58 (d, J=5.5 Hz, 1H), 7.48–7.35 (m, 5H), 7.14 (d, J=8.9 Hz, 2H), 6.93 (d, J=5.5 Hz, 1H), 5.17 (s, 2H), 3.86 (s, 3H) ppm; LC–MS (ESI, pos.) m/z (%)=325 $[M+H]^+$ (100), 347 $[M+H]^+$ (35).
b) Into a 250 mL flask was weighed BBr_3–DMS (1.99 g, 6.29 mmol) and the flask filled with argon over 10 min. The solid was dissolved in dichloromethane (130 mL) and (3-methoxy-thiophen-2-yl)-(4-benzyloxy-phenyl)-methanone (1.90 g, 5.86 mmol) in dichloromethane (10 mL) was added dropwise at rt. The dark solution was stirred for 3 h at rt (TLC-control) and then satd sodium bicarbonate solution (30 mL) was added. The layers were separated and the organic phase was washed with water (30 mL), dried over Na_2SO_4 and the solvent was evaporated in vacuo. Chromatography (eluent heptane/ethyl acetate 2:1) of the crude product yielded **15** (1.40 g, 4.51 mmol, 77%, purity 95% (HPLC, 254 nm)) as yellow oil, LC–MS (ESI, pos.) m/z (%)=311 $[M+H]^+$ (100).

4.1.18. Compound ***16*** *2-(4-benzyloxy-benzyl)-thiophen-3-ol.* In a 250 mL flask ketone **15** (1.90 g, 6.12 mmol) was weighed and the flask filled with argon. Then the compound was dissolved in dry acetonitrile (53 mL) and the reaction mixture was cooled to 0–5 °C. $NaCNBH_3$ (2.50 g, 37.8 mmol) was added in portions at max. 5 °C and the mixture was stirred for 30 min. Then trimethylsilylchloride (6.00 mL, 46.5 mmol) was added dropwise (internal temperature should not exceed 5 °C) and the mixture was stirred for 4 h at 5 °C (LC–MS control). Finally satd sodium bicarbonate solution (45 mL) was added and the mixture was stirred vigorously over 5 min. After addition of dichloromethane (400 mL) the phases were separated and the aqueous phase was extracted three times by dichloromethane (20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. This material was directly used in the next reaction step without further purification.

4.1.19. Compound ***17*** *sulfuric acid mono-[2-(4-benzyloxy-benzyl)-thiophen-3-yl] ester.* Thiophene **16** (1.81 g, 6.11 mmol) was dissolved in dichloromethane (20 mL) and triethylamine (TEA, 4.29 mL, 30.6 mmol). Then sulfurtrioxide–triethylamine-complex (2.21 g, 12.2 mmol) was added in one portion and the reaction mixture was stirred for 16 h at rt. Water (10 mL) was added and with 1 N HCl a pH of 1 was obtained. The phases were separated and the aqueous phase was extracted three times by dichloromethane (20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo to give sulfate **17** (1.50 g, 3.14 mmol, 46%). This material was used in the next reaction step without further purification. ^{1}H NMR (DMSO-d_6): δ=7.54–7.33 (m, 5H), 7.22 (d, J=8.9 Hz, 2H), 7.15 (d, J=5.5 Hz, 1H), 6.96 (d, J=5.5 Hz, 1H), 6.92 (d, J=8.9 Hz, 2H), 5.05 (s, 2H), 3.98 (s, 2H) 3.09 (q, 6H, TEA), 1.19 (t, 9H, TEA) ppm.

4.1.20. Compound ***20*** *[2-(4-bromo-phenyl)-(3-methoxy-thiophen-2-yl)-methanone.* In a 250 mL flask 3-methoxythiophene **2** (1.30 mL, 13.1 mmol) was dissolved in diethylether (30 mL). Then the flask was filled with argon and *n*-BuLi (7.00 mL, 17.5 mmol, 2.5 M in hexane) added at rt. The reaction mixture was stirred at 35 °C bath temperature for 10 min. Then the reaction mixture was slowly dropped into an ice-cooled solution of amide **19** (3.50 g, 13.0 mmol) in diethylether (50 mL) via syringe. After two hours stirring at rt complete conversion was monitored by LC–MS and water (25 mL) was added. Then the layers were separated, the aqueous phase was extracted three times with dichloromethane (30 mL) and the combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude product was purified by chromatography heptane/ethyl acetate 4:1 to give **20** (1.90 g, 6.39 mmol, 49%) as a yellow solid. ^{1}H NMR (DMSO-d_6): δ=8.06 (d, J=5.3 Hz, 1H), 7.71 (d, J=9.0 Hz, 2H), 7.67 (d, J=9.0 Hz, 2H), 7.19 (d, J=5.3 Hz, 1H), 3.80 (s, 3H) ppm; LC–MS (ESI, pos.) m/z (%)=297 $[M+H]^+$ (95), 299 $[M+H]^+$ (100).

4.1.21. Compound ***21*** *[2-(4-bromo-phenyl)-(3-hydroxy-thiophen-2-yl)-methanone.* Into a 250 mL flask were weighed BBr_3–DMS (2.79 g, 8.92 mmol) and the flask filled with argon over 10 min. The solid was dissolved in dichloromethane (100 mL) and a solution of ketone **20** (2.50 g, 8.41 mmol) in dichloromethane (10 mL) was

added dropwise at rt. The dark solution was stirred for 3 h at rt (LC–MS control) and then satd sodium bicarbonate solution (30 mL) was added. The layers were separated and the organic phase was washed with water (10 mL), dried over Na_2SO_4 and the solvent was evaporated in vacuo. Chromatography (eluent heptane/ethyl acetate 2:1) of the crude product yielded **21** (1.40 g, 4.96 mmol, 59%) as a yellow solid. ^{1}H NMR (DMSO-d_6): δ=11.45 (s, 1H, OH), 7.98 (d, J=5.3 Hz, 1H), 7.79–7.70 (m, 4H), 7.87 (d, J=5.3 Hz, 1H); LC–MS (ESI, pos.) m/z (%)=283 $[M+H]^+$ (95), 285 $[M+H]^+$ (100).

*4.1.22. Compound **22** 2-(4-bromo-benzyl)-thiophen-3-ol.* In a 250 mL flask ketone **21** (1.00 g, 3.53 mmol) was weighed and the flask filled with argon. Then the compound was dissolved in dry acetonitrile (15 mL) and the reaction mixture was cooled to 0–5 °C. $NaCNBH_3$ (1.71 g, 25.9 mmol) was added in portions at max. 5 °C and the mixture stirred for 30 min. Then trimethylsilylchloride (4.11 mL, 31.9 mmol) was added dropwise (internal temperature should not exceed 5 °C) and the mixture was stirred for 4 h at 5 °C (LC–MS control). Finally satd sodium bicarbonate solution (30 mL) was added and the reaction mixture was stirred vigorously for 5 min. After addition of dichloromethane (40 mL) the phases were separated and the aqueous phase was extracted three times with dichloromethane (20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. This crude material (684 mg, 2.54 mmol, 72%, purity 94% (HPLC, 254 nm)) was directly used in the next reaction step without further purification.

*4.1.23. Compound **23** sulfuric acid mono-[2-(4-bromo-benzyl)-thiophen-3-yl] ester.* Thiophene **22** (950 mg, 3.53 mmol) was dissolved in dichloromethane (10 mL) and triethylamine (2.49 mL, 5.01 mmol). Then sulfurtrioxide–triethylamine-complex (1.28 g, 7.06 mmol) was added in one portion and the reaction mixture was stirred for 16 h at rt. Water (10 mL) was added and with 1 N HCl a pH of 1 was obtained. The phases were separated and the aqueous phase was extracted three times by dichloromethane (20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude product was purified by chromatography using dichloromethane/EtOH/TEA 90:5:5 as solvent to give sulfate **23** (1.10 g, 2.44 mmol, 69%, purity 97% (HPLC, 254 nm)) as a slightly brown solid. ^{1}H NMR (DMSO-d_6): δ=8.91 (br s, 1H, SO_3H), 7.48 (d, J=9.0 Hz, 2H), 7.23 (d, J=9.0 Hz, 2H), 7.15 (d, J=5.4 Hz, 1H), 6.96 (d, J=5.4 Hz, 1H), 4.00 (s, 2H), 3.15–3.09 (m, 6H, TEA), 1.23–1.11 (m, 9H, TEA) ppm.

*4.1.24. Compound **24** sulfuric acid mono-[2-benzyl-thiophen-3-yl] ester.* Bromide **23** (400 mg, 0.89 mmol) was weighed into a 100 mL flask and the flask was filled with argon. Then **23** was dissolved in THF (20 mL) and TMEDA (0.3 mL, 2.0 mmol) was added. The solution was cooled to −100 °C in a dichloromethane/dry ice-cooling bath and *n*-BuLi (1.0 mL, 2.5 mmol, 2.5 M in hexane) was added dropwise via syringe. The mixture was stirred for 15 min at −100 °C and then bis(trimethylsilyl)peroxide (474 mg, 2.66 mmol) in THF (5 mL) was added via syringe. The resulting mixture was allowed to warm to rt during 3 h. (LC–MS control showed 90% conversion of starting material). Satd NH_4Cl solution (10 mL) was added and the layers were separated. The aqueous phase was extracted five times with dichloromethane/1-butanol 4:1 (20 mL). The two main products **24** and **12** were analysed by ^{1}H NMR and MS (for detailed analytical information of **12** see below). Attempts to separate both compounds by HPLC and RP columns on a preparative scale failed. Compounds **12** and **24** furthermore decomposed under acidic conditions. Compound **24**: ^{1}H NMR (DMSO-d_6): δ=9.6 (br s, 1H, SO_3H), 7.29–7.23 (m, 4H), 7.19–7.16 (m, 1H), 7.13 (d, J=5.4 Hz, 1H), 6.98 (d, J=5.4 Hz, 1H), 4.02 (s, 2H), 3.10–3.04 (m, 6H, TEA), 1.20 (t, J=7.3 Hz, 9H, TEA); ^{13}C NMR dept135 δ=128.4, 128.2, 125.8, 122.7, 120.1 (arom. *C*H), 45.3 (TEA, CH_2), 30.9 (CH_2), 8.3 (TEA, CH_3); MS (ESI, neg.) m/z (%)=269 $[M+H]^+$ (100).

*4.1.25. Compound **26** 4-allyloxy-benzoyl chloride.* (a) 4-hydroxybenzoic acid **25** (8.10 g, 58.6 mmol), allylbromide (10.7 mL, 123 mmol) and potassium carbonate (20.0 g, 145 mmol) were suspended in DMF (60 mL) and stirred for 48 h. Then water (20 mL) and dichloromethane (50 mL) were added and the layers were separated. The aqueous layer was extracted three times by dichloromethane (30 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. To the residue 2 N NaOH (100 mL) and ethanol (20 mL) were given and the solution was heated to reflux for 5 h (LC–MS control). A pH of 1 was obtained by conc HCl and the precipitated solid was filtered, washed with ice-cooled water-methanol 5:1 and dried in vacuo over $CaCl_2$ to give the allyl ether (9.40 g, 52.7 mmol, 90%).

(b) 4-allyloxy benzoic acid (6.40 g, 35.9 mmol) was suspended in thionyl chloride (25 mL) and heated to reflux for 8 h. Thionyl chloride was removed in vacuo. Toluene (20 mL) was added and again the solvent was removed in vacuo. This procedure was repeated twice. The residue **26** (5.60 g, 28.5 mmol, 79%) was directly used in the next reaction step.

*4.1.26. Compound **27** (4-allyloxy-phenyl)-(3-hydroxy-thiophen-2-yl)-methanone.* (a) In a 500 mL flask 3-methoxy-thiophene **2** (3.61 mL, 36.0 mmol) and 4-allyloxy benzoic chloride **26** (5.60 g, 28.5 mmol) were dissolved in dichloromethane (200 mL) and tin-(IV)-tetrachloride (5.52 mL, 46.8 mmol) added at 0–5 °C. The reaction mixture was stirred for 2 h at 5 °C and further 2 h at rt. After complete conversion (LC–MS) ice-cooled water (20 mL) was added. The different layers were separated and the organic layer was washed with water (10 mL), sodium bicarbonate solution (10 mL, 8 wt % in water) and brine (10 mL). The organic phase was dried over Na_2SO_4 and was evaporated in vacuo. The crude product was purified by chromatography using heptane/ethyl acetate 3:1 as mobile phase. The product (6.80 g, 24.8 mmol, 87%) was isolated as a yellow solid.

(b) Into a 250 mL flask were weighed BBr_3–DMS (22.5 g, 72.0 mmol) and the flask filled with argon over 10 min. The solid was dissolved in dichloromethane (200 mL) and a solution of (3-methoxy-thiophen-2-yl)-(4-allyloxy-phenyl)-methanone (9.88 g, 36.0 mmol) in dichloromethane (10 mL) added dropwise at rt. The dark solution was stirred for 3 h at rt (TLC-control) and then satd sodium bicarbonate solution (30 mL) was added. The layers were separated and the organic layer was washed with water (30 mL), dried over Na_2SO_4 and the solvent was evaporated in vacuo. Chromatography (eluent heptane/ethyl acetate 3:1) of the crude product yielded **27** (3.80 g, 14.6 mmol, 41%; purity 93% (HPLC, 254 nm)) as yellow oil. ^{1}H NMR (DMSO-d_6): δ=11.85 (s, 1H, OH), 7.95 (d, J=5.4 Hz, 1H), 7.88 (d, J=9.0 Hz, 2H), 7.12 (d, J=9.0 Hz, 2H), 6.93 (d, J=5.4 Hz, 1H), 6.11–6.03 (m, 1H, allyl-H), 5.45 (dd, J=1.6 Hz, J=17.2 Hz, 1H, allyl-H), 5.32 (dd, J=1.6 Hz, J=17.2 Hz, 1H, allyl-H), 4.68–4.66 (m, 2H) ppm; LC–MS (ESI, pos.) m/z (%)=261 $[M+H]^+$ (100).

*4.1.27. Compound **28** sulfuric acid mono-[2-(4-allyloxy-benzyl)-thiophen-3-yl] ester.* (a) In a 250 mL flask ketone **27** (3.80 g, 14.6 mmol) was weighed and the flask filled with argon. Then the compound was dissolved in dry acetonitrile (176 mL) and the reaction mixture was cooled to 0–5 °C. $NaCNBH_3$ (5.52 g, 87.8 mmol) was added in portions at max. 5 °C and the mixture was stirred for 30 min. Then trimethylsilylchloride (14.1 mL, 111 mmol) was added dropwise (internal temperature should not exceed 5 °C) and the mixture stirred for 4 h at 5 °C (LC–MS control). Finally satd sodium bicarbonate solution (9 mL) was added and the reaction mixture stirred vigorously over 5 min. After addition of dichloromethane

(400 mL) the phases were separated and the aqueous phase was extracted three times by dichloromethane (20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. This material was directly used in the next reaction step without further purification.

(b) Thiophene from step (a) (3.60 g, 14.6 mmol) was dissolved in dichloromethane (20 mL) and triethylamine (8.12 mL, 58.4 mmol). Then sulfurtrioxide–triethylamine-complex (7.95 g, 43.9 mmol) was added in one portion and the reaction mixture was stirred for 16 h at rt. Water (10 mL) was added and with 1 N HCl a pH of 1 was adjusted. The phases were separated and the aqueous phase was extracted three times by dichloromethane (20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude product was purified by chromatography using dichloromethane/MeOH/TEA 90:5:5 as solvent to give crude sulfate **28** (7.8 g, >100%) as brown oil (with excess TEA). This material was used in the next reaction step without further purification; MS (ESI, neg.) *m*/*z* (%)=325 $[M-H]^+$ (100).

*4.1.28. Compound **12** sulfuric acid mono-[2-(4-hydroxy-benzyl)-thiophen-3-yl] ester.* In a 20 mL microwave vial sulfate **28** (700 mg, 1.64 mmol) and 200 mg Pd/C (10% Pd, water free, Fa. Heraeus) were suspended in KOH (6 mL, 10% in MeOH). The vial was filled with argon and $NaBH_4$ (20 mg) was added in one portion. The reaction mixture was stirred for 3 min, and then the vial was closed and heated at 110 °C for 80 min (biotage Initiator microwave; absorption level — normal; pressure 10 atm). *Caution*: *Overpressure might be possible after reaction and must be removed carefully*. The reaction mixture was filtered, the filter cake washed twice with methanol (5 mL) and the filtrate concentrated in vacuo to give an orange oil. The crude product was purified by chromatography using dichloromethane/MeOH/TEA 90:5:5 as solvent, followed by semi-preparative HPLC (YMC-Basic RP8 column, 10 mL flow; acetonitrile/water/TEA gradient program) to give sulfate **12** — TEA (370 mg, 0.95 mmol, 58%, purity 99.6% (HPLC)) as colourless needles. ^{1}H NMR (DMSO-d_6): δ=9.18 (s, 1H, OH), 8.85 (br s, 1H, SO_3H), 7.09 (d, *J*=5.5 Hz, 1H), 7.04 (d, *J*=8.4 Hz, 2H), 6.96 (d, *J*=5.5 Hz, 1H), 6.66 04 (d, *J*=8.4 Hz, 2H), 3.88 (s, 2H) 3.11–3.04 (m, 6H, TEA), 1.20–1.17 (m, 9H, TEA); ^{13}C NMR (DMSO-d_6): δ=155.6, 146.0, 130.9, 129.4, 127.8, 122.9, 120.1, 115.0, 45.7, 30.4, 8.6 ppm; (MS (*m*/*z*) neg. ESI=285 (100); HPLC (UV, 230 nm)=99.5%); HRMS (ESI-LTQ, neg.) $C_{11}H_9O_5S_2$ calcd 284.98969; found, 284.98990.

*4.1.29. Compound **29** 3-(2-(4-methoxy-benzyl)-thiophenyl)-ß-D-1-tertbutyldiphenylsilyl,3,4,5-acetyl-glucopyranoside.* Dry dichloromethane (140 mL), TEA (3.72 mL, 26.4 mmol) and 4-dimethylaminopyridine (DMAP, 296 mg, 2.40 mmol) were added to a solution of AVE2268 **1a** (4.59 g, 12.0 mmol) in dry DMF (16 mL). Then *tert*-butyldiphenylchlorosilane (TBDPSCl, 6.67 mL, 25.2 mmol) in dichloromethane (10 mL) was added dropwise at 0 °C during 10 min and the reaction mixture was stirred overnight without further cooling. Afterwards water (120 mL) was added, the organic layer was separated and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with brine and dried over Na_2SO_4. The crude product was purified by column chromatography (heptane/ethyl acetate 1:2→1:4) to give TBDPS-protected AVE2268 (7.40 g, 11.9 mmol, 99%) as a white foam. LC–MS (ESI, pos.) *m*/*z* (%)=643 $[M+Na]^+$ (100).

TBDPS-protected AVE2268 (1.55 g, 2.50 mmol) was dissolved in dry pyridine (130 mL) and reacted with acetic acid anhydride (35.8 mL, 375 mmol). The mixture was stirred for 4 h before methanol (60 mL) was added. After one hour water (130 mL) was added and the mixture was stirred overnight. Afterwards the aqueous layer was extracted twice with ethyl acetate (50 mL) and the combined organic layers were dried over Na_2SO_4. The residue was purified by column chromatography (heptane/ethyl acetate 3:1) to give **29** (1.76 g, 2.35 mmol, 94%) as a white foam. ^{1}H NMR (DMSO-d_6): δ=7.64–7.60 (m, 4H), 7.45–7.39 (m, 4H), 7.34 (t, *J*=7.5 Hz, 2H), 7.23 (d, *J*=5,5 Hz, 1H), 7.09 (d, *J*=8.6 Hz, 2H), 6.95 (d, *J*=5.5 Hz, 1H), 6.82 (d, *J*=8.6 Hz, 2H), 5.41–5.38 (m, 2H), 5.17–5.14 (m, 1H), 5.08–5.03 (m, 1H), 4.09–4.04 (m, 1H), 3.93–3.83 (m, 2H), 3.73 (m, 2H), 3.70(s, 3H), 1.97 (s, 6H), 1.93 (s, 3H), 0.99 ppm (s, 9H); LC–MS (ESI, pos.) *m*/*z* (%)=769 $M+Na]^+$ (100).

*4.1.30. Compound **30** 3-(2-(4-methoxy-benzyl)-thiophenyl)-ß-D-3,4,5-acetyl-glucopyranoside.* To a solution of **29** (1.42 g, 1.90 mmol) in dry THF (20 mL) and dry pyridine (20 mL) hydrofluoric acid–pyridine-complex (3.42 mL, 24.7 mmol) was added slowly at 0 °C. After stirring for 30 min at 0 °C, the reaction mixture was allowed to come to rt. After one hour further HF-complex (1.31 mL, 9.50 mmol) was added to the mixture at 0 °C and stirring was continued for another hour at rt. Then ethyl acetate (60 mL) and water (60 mL) were added. The layers were separated and the aqueous layer was extracted twice with ethyl acetate (50 mL). The combined organic layers were dried over Na_2CO_3. The crude product was purified by column chromatography (heptane/ethyl acetate 3:2) to give **30** (956 mg, 1.88 mmol, 99%) as a white foam. ^{1}H NMR (DMSO-d_6): δ=7.27 (d, *J*=5.5 Hz, 1H), 7.10 (d, *J*=8.6 Hz, 2H), 6.95 (d, *J*=5.5 Hz, 1H), 6.84 (d, *J*=8.6 Hz, 2H), 5.37–5.30 (m, 2H), 5.04–4.95 (m, 2H), 4.89 (s, br s, OH), 3.91–3.86 (m, 1H), 3.84 (d, *J*=7.8 Hz, 2H), 3.71 (s, 3H), 3.57–3.53 (m, 1H), 3.46–3.42 (m, 1H), 2.00 (s, 3H), 1.95 (s, 3H), 1.93 ppm (s, 3H); LC–MS (ESI, pos.) *m*/*z* (%)=531 $[M+Na]^+$ (100).

*4.1.31. Compound **31** 3-(2-(4-methoxy-benzyl)-thiophenyl)-ß-D-3,4,5-acetyl-glucuronide.* Dry pyridine (37.6 µL, 0.46 mmol) and Dess–Martin periodinane (779 µL, 0.30 mmol, 12% in dichloromethane) were added to a solution of **30** (102 mg, 0.20 mmol) in dry dichloromethane (2 mL) under an argon atmosphere. After stirring for 2 h at rt the starting material had disappeared (LC–MS control). Due to the low stability of the formed aldehyde (unstable in LC–MS) the solvent was just evaporated and the residue directly redissolved in *tert*-butanol (2 mL) and satd NaH_2PO_4-solution (1.4 mL). Then 2-methyl-2-butene (224 µL, 2.00 mmol) and sodium chlorite ($NaClO_2$, 27.1 mg, 0.24 mmol) were added and the reaction mixture was stirred overnight. For work up water (3 mL) was added, the aqueous phase was extracted three times with ethyl acetate (20 mL) and the combined organic layers were dried over Na_2SO_4. The residue was purified by column chromatography (dichloromethane/methanol 9:1→4:1) to give **31** (103 mg, 0.20 mmol, 98%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6): δ=13.4 (s, br s, 1H), 7.28 (d, *J*=5.5 Hz, 1H), 7.10 (d, *J*=8.6 Hz, 2H), 6.87 (d, *J*=5.5 Hz, 1H), 6.83 (d, *J*=8.6 Hz, 2H), 5.43–5.39 (m, 2H), 5.13–5.07 (m, 2H), 4.50 (d, *J*=10.0 Hz, 1H), 3.84 (s, 2H), 3.71 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.97 ppm (s, 3H); LC–MS (ESI, pos.) *m*/*z* (%)=545 $[M+Na]^+$ (100).

*4.1.32. Compound **13** 3-(2-(4-methoxy-benzyl)-thiophenyl)-ß-D-glucuronide.* To a solution of **31** (310 mg, 0.59 mmol) in methanol (40 mL) sodium methylate (452 µL, 2.37 mmol, 30% in methanol) was added dropwise. After stirring for 1 h at rt the starting material had disappeared (LC–MS control) and the reaction mixture was adjusted to a pH value of 6.5 with hydrochloric acid (1.25 M in ethanol). The solvent was evaporated and the residue was purified first by column chromatography (dichloromethane/methanol 3:1→2:1→1:1) and afterwards by semi-preparative HPLC (column: YMC Pack Pro C18, eluents: acetonitrile/water 10:90 for 4 min, then up to 80:20 in 12 min, then 80:20 for 4 min and finally in 1 min down again to 10:90 for 1 min, flow: 15 mL/min, retention time: 10.5 min). Final freeze-drying of the product containing fractions yielded **13** (176 mg, 0.44 mmol, 75%, purity 99.6% (HPLC,

230 nm)) as a white solid. Mp: 185 °C (decomp.); ^{1}H NMR (DMSO-d_6): δ=7.21–7.18 (m, 3H), 6.92 (d, J=5.5 Hz, 1H), 6.83 (d, J=8.6 Hz, 2H), 5.38 (s, br s, 1H), 5.08 (s, br s, 1H), 4.67 (d, J=7.1 Hz, 1H), 3.98 (s, 2H), 3.71 (s, 3H), 3.49–3.47 (m, 2H), 3.26–3.23 ppm (m, 3H); LC–MS (ESI, pos.) m/z (%)=419, $[M+Na]^+$ (100); HRMS (ESI-LTQ, neg.) $C_{18}H_{19}O_8S$ calcd 395.08061; found, 395.08072.

4.1.33. Compound ***32*** *3-(2-(4-methoxy-benzoyl)-thiophenyl)-ß-D-3,4,5-acetyl-glucuronic methyl ester.* Potassium carbonate (3.70 g, 26.5 mmol) and tetrabutylammoniumbromide (TBAB, 863 mg, 2.65 mmol) dissolved in water (20 mL) were added to keto aglycon **5a** (1.24 g, 5.30 mmol) and triaceto-bromo-α-D-glucuronic acid methyl ester (4.62 g, 11.1 mmol) dissolved in dichloromethane (80 mL) and the reaction mixture was stirred for 24 h at rt. Then silver carbonate (1.11 g, 3.98 mmol) and TBAB (863 mg, 2.65 mmol) in water (15 mL) were added. After 3.5 h the addition of silver carbonate (1.11 g, 3.98 mmol) and TBAB (863 mg, 2.65 mmol) in water (15 mL) was repeated. After stirring over two nights water was added and the reaction mixture was extracted three times with ethyl acetate (30 mL). The combined organic layers were washed with brine and then dried over Na_2SO_4. The crude product was purified by column chromatography (heptane/ethyl acetate 3:2) to give **32** (1.89 g, 3.44 mmol, 65%) as a pale yellow foam. ^{1}H NMR (DMSO-d_6): δ=7.96 (d, J=5.5 Hz, 1H), 7.68 (d, J=6.9 Hz, 2H), 7.10 (d, J=5.5 Hz, 1H), 6.98 (d, J=6.9 Hz; 2H), 5.66 (d, J=7.7 Hz, 1H), 5.31 (t, J=9.6 Hz, 1H), 4.98 (t, J=9.6 Hz, 1H), 4.73–4.62 (m, 2H), 3.84 (s, 3H), 3.61 (s, 3H), 1.98 (s, 3H), 1.91 (s, 3H), 1.86 ppm (s, 3H); LC–MS (ESI, pos.) m/z (%)=573 $[M+Na]^+$ (100).

4.1.34. Compound ***33*** *3-(2-(4-methoxy-benzyl)-thiophenyl)-ß-D-glucuronic methyl ester.* To a solution of **32** (1.85 g, 3.36 mmol) in dry acetonitrile (90 mL) $Na(BH_3)CN$ (1.78 g, 26.9 mmol) was added at 0 °C and the reaction stirred for 30 min under cooling. Afterwards chlorotrimethylsilane (3.50 mL, 26.9 mmol) was dropped slowly into the reaction mixture (*caution*: *development of gases*). After stirring for further 3 h at 0–4 °C satd $NaHCO_3$-solution was added and the reaction mixture was extracted with dichloromethane. The combined organic layers were washed once with water and dried over Na_2SO_4. The crude product was purified by column chromatography (heptane/ethyl acetate 3:2) to give **33** (1.65 g, 3.09 mmol, 92%) as a white solid. ^{1}H NMR (DMSO-d_6): δ=7.27 (d, J=5.5 Hz, 1H), 7.08 (d, J=8.6 Hz, 2H), 6.85–6.81 (m, 3H), 5.48–5.42 (m, 2H), 5.12–5.02 (m, 2H), 4.63 (d, J=9.9 Hz, 1H), 3.83 (s, 2H), 3.70 (s, 3H), 3.63 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.96 ppm (s, 3H); LC–MS (ESI, pos.) m/z (%)=559 $[M+Na]^+$ (100).

4.1.35. Compound ***13*** *3-(2-(4-methoxy-benzyl)-thiophenyl)-ß-D-glucuronide.* Sodium methylate (2.54 mL, 13.4 mmol, 30% in methanol) was dropped into a suspension of **33** (1.60 g, 2.98 mmol) in methanol (200 mL). After 2 h stirring at rt lithium chloride (644 mg, 14.9 mmol) and water (70 mL) were added to the yellow solution and the reaction mixture was stirred overnight. Then the reaction mixture was adjusted to a pH value of 5.5 with hydrochloric acid (1.25 M in methanol). The solvent was evaporated in vacuo and the residue was purified firstly by column chromatography (dichloromethane/methanol 3:1→2:1→1:1) and secondly by semi-preparative HPLC (information: see above). Final freeze-drying of the product containing fractions yielded **13** (872 mg, 2.20 mmol, 74%, purity 98.3% (HPLC, 230 nm)) as a white solid. (Analytical data: see above).

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Supplementary data

^{1}H and/or ^{13}C NMR spectra of **1a**, **1b**, **4a**, **4b**, **5a**, **5b**, **7a**, **7b**, **8a**, **8b**, **9b**, **9e**, **12**, **13**, **15**, **17**, **20**, **23**, **24**, **27**, **28**, **29**, **30**, **31**, **32** and **33**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.12.003.

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