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Tetrahedron
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Tetrahedron 62 (2006) 3969–3976

Synthesis of stereochemical probes for new fluorogenic assays for yeast transketolase variants

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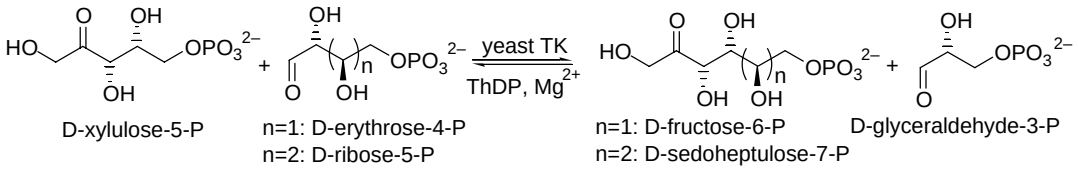
Received 16 November 2005; revised 12 January 2006; accepted 13 February 2006

Available online 9 March 2006

Abstract—For the screening of yeast transketolase (TK) variants with improved or new properties acquired by random mutagenesis, we report on the stereoselective synthesis of fluorogenic substrates as probes for measuring TK activity. Compound **1** (7-(2′,3′,5′-trihydroxy-4′-oxo-pentyl)oxycoumarine), prepared as previously described, [*Tetrahedron Lett.* **2003**, *44*, 827–830] enabled us to evaluate wild type TK velocity in a simple, specific and reproducible way. To select TK mutants able to produce D-*threo* aldoses, we prepared compound **2** (dihydroxy-4-O-(2′-oxo-benzopyran-7′-yl-D-threose) from dimethyl tartrate. Starting from D-ribose, we successfully obtained compound **3** (7′-(2,3,5-trihydroxy-4-oxo-pentyl)oxycoumarine) as a probe for TK mutants able to produce L-*erythro* ketoses.

1. Introduction

The discovery of new biocatalysts that mediate selective transformations is a growing field of research in organic chemistry. Diversity is either generated artificially by random mutagenesis from the gene that encodes an existing enzyme or collected directly from a natural reservoir.[1] Such libraries must be screened to identify suitable enzymes with desired properties. Recently, new sensitive detection methods for the evaluation of large numbers of catalysts have been reported. Efficient high throughput screening assays have been achieved using solid phase bound tests related to immunoassays[2] and a variety of spectroscopic methods, such as IR thermography[3] or mass spectrometry.[4] One of the most popular methods consists in using chromogenic or fluorogenic substrates as product formation sensors.[5] In this area, Reymond developed a simple stereospecific assay consisting of a fluorescence release based on the secondary release of umbelliferone by β-elimination catalysed by bovine serum albumin (BSA) from a primary or secondary carbonyl reaction product. The prototypical example of this technique was an enantioselective assay for alcohol dehydrogenase.[6] This approach was extended to the use of acylases, lipases, epoxide hydrolases, phophatases,[7] aldolase catalytic antibodies[8] and more recently for the transaldolase[9] enzyme catalysing C–C bond formation. In the latter case, the assay was based on microscopic reversibility, assuming that if transaldolase was able to cleave a C–C bond by retroaldolisation, it would be able to catalyse its formation by aldolisation. Our interest in this assay stemmed from our ongoing investigation of TK enzyme catalysing a stereocontrolled C–C bond formation, according to a similar natural reversible reaction (Scheme 1). Here we report on the stereoselective synthesis



Scheme 1. Natural reaction catalysed by transketolase.

Keywords: Enzyme evolution; Transketolase; Fluorogenic assays; C–C bond formation; High throughput screening.
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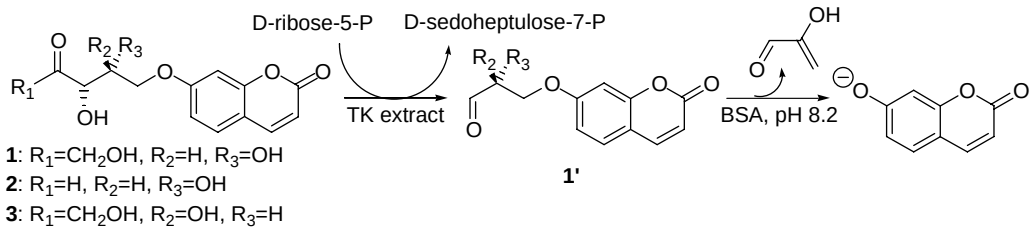
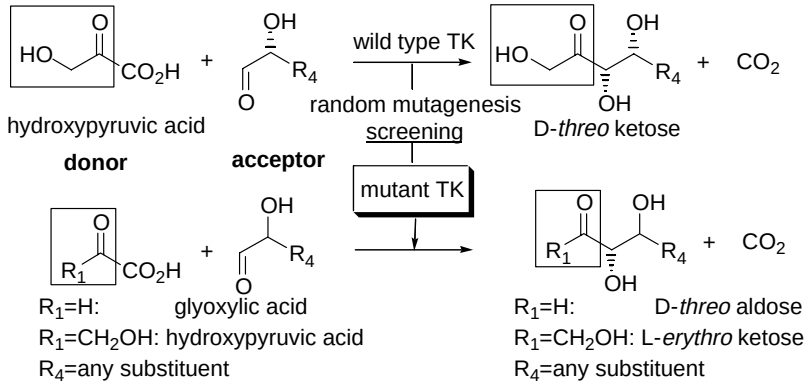
doi:10.1016/j.tet.2006.02.033



Scheme 2. Fluorescence assay based on umbelliferone release.



Scheme 3. Access to new D-*threo*-aldoses or L-*erythro*-ketoses by TK engineering.

of fluorogenic substrates as probes for measuring wild type or altered TK activity from variants with improved or new properties acquired by random mutagenesis.

2. Results and discussion

TK is a useful catalyst for ketoses syntheses. For this purpose, β-hydroxypyruvic acid was used as a donor substrate because it rendered the reaction irreversible. In this case, TK catalysed the irreversible transfer of a ketol unit from this donor substrate to various phosphorylated or non-phosphorylated aldehydes to generate D-*threo* (3*S*,4*R*) ketoses. TK isolated from spinach leaves,[10] baker's yeast,[11] *Escherichia coli*,[12] were investigated. Morerecently, we used *Saccharomyces cerevisiae* recombinant TK.[13]

2.1. Substrate design

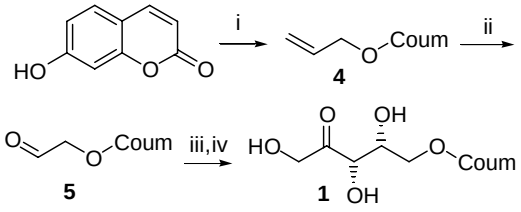
We have shown the possibility of measuring wild type TK activity from *S. cerevisiae* using the suitable stereoselective fluorogenic substrate **1**.[14] This compound (Scheme 2) was used as a donor substrate of the enzyme in the presence of D-ribose-5-phosphate as an acceptor substrate. TK cleaved the C_2–C_3 bond of **1** and generated **1′**. A fluorescent signal appeared because **1′** underwent a rapid β-elimination catalysed by bovine serum albumin (BSA) to release umbelliferone, a highly fluorescent compound. Our goal is to use this assay in the evaluation of TK mutants generated by random mutagenesis with fluorogenic substrates containing a sugar moiety depending on the enzymatic property desired.

We would like to modify the substrate specificity of TK to extend its synthetic potential to the L-*erythro* ketoses and D-*threo* aldoses series (Scheme 3). In the latter case, variants of TK would be able to accept glyoxylic acid as the donor substrate yielding D-*threo* aldoses while in the former case, variants would be able to accept (*S*)-hydroxyaldehydes as acceptor substrates yielding L-*erythro* ketoses.

Here we report the stereoselective syntheses of the fluorogenic compounds **1**, **2** and **3** as stereochemical probes for de novo TK activities. Compound **2** should be suitable for evaluating altered selectivity for the donor substrate, while compound **3** should be suitable for evaluating altered stereoselectivity for the acceptor substrate.

2.2. Synthesis

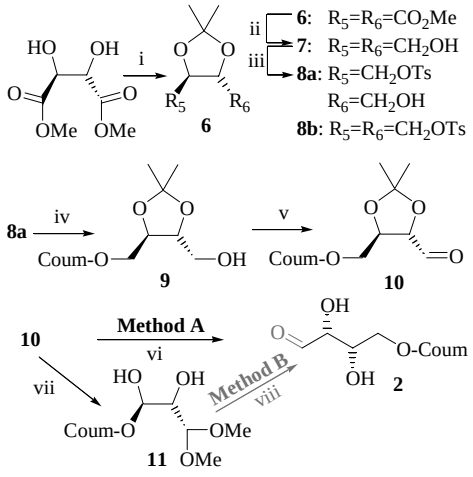
As previously described,[14] the fluorogenic compound **1** was prepared by a chemoenzymatic route from the known umbelliferone (Scheme 4). Olefin **4** was obtained in 96% yield after allylation in refluxing acetone.[15] As reported in the literature,[16] aldehyde **5** was obtained by a two-step procedure using OsO_4/NMMO to give the diol followed by overoxidation by $NaIO_4$. To carry out the reaction in a single step, we performed an ozonolysis of crude **4** at −30 °C in methylene chloride with 10% DMF. Reduction of the ozonide intermediate with dimethyl sulfide gave the aldehyde **5** in 62% yield with high selectivity for the exocyclic double bond versus the conjugated lactone double bond.



Scheme 4. Synthesis of **1**: (i) CH_2=CH–CH_2Br, K_2CO_3, (**4**, 96%); (ii) O_3, Me_2S, (**5**, 62%); (iii) RAMA, DHAP, mCD; (iv) acid phosphatase, (**1**, 35% overall for the last two steps).

Our strategy was to introduce both chiral centers (3*S*,4*R*) of the sugar moiety of the fluorogenic substrate **1** at once, by using fructose-1,6-bisphosphate aldolase from rabbit muscle (RAMA; E.C.4.1.2.13). The utility of this enzyme is well-documented[17] for the synthesis of D-*threo* ketoses by C–C bond formation in a highly stereoselective manner. Aldehyde **5** thus underwent aldol addition using RAMA with dihydroxyacetone phosphate (DHAP) as the donor substrate (DHAP was prepared and assayed according to Charmantray et al.[18]). Because of the high hydrophobicity of the coumarin part of the molecule, we added a modified cyclodextrin (mCD) to make the aldehyde water-soluble. In these conditions, the reaction proceeded smoothly giving higher yields than using co-solvents such as DMSO or MeOH. After dephosphorylation catalysed by acid phophatase (E.C.3.1.3.2) at pH 4.8, compound **1** was obtained in 35% overall yield for the two enzymatic steps.

To obtain compound **2**, we investigated the use of commercially available D-dimethyl tartrate (2*S*,3*S*) as a building block to set the chirality on C_2 and C_3 at an early stage (Scheme 5).



Scheme 5. Synthesis of **2**: (i) $(CH_3)_2C(OCH_3)_2$ (**6**, 85%); (ii) $NaBH_4$ (**7**, 73%); (iii) TsCl, NaOH (**8**, 83%); (iv) Coum-OH, NaH (**9**, 71%); (v) Dess–Martin periodinane (**10**, 49%); (vi) Dowex H^+ resin (**2**, 25%); (vii) Dowex H^+ resin (**11**, 43%); (viii) Dowex H^+ resin (**2**, 28%).

Compound **8a** was easily obtained from D-dimethyl tartrate (2*S*,3*S*) in a three-step sequence. Intermediate **6** was synthesised by transacetalisation in dimethoxypropane in 85% yield as described in the literature.[19a] Subsequent reduction of the diester was achieved using 1.5 equiv of $NaBH_4$ in ethanol instead of 2.0 equiv in methanol.[19b] In this way, the yield of compound **7** was increased from 30 to 73% without significant transesterification.

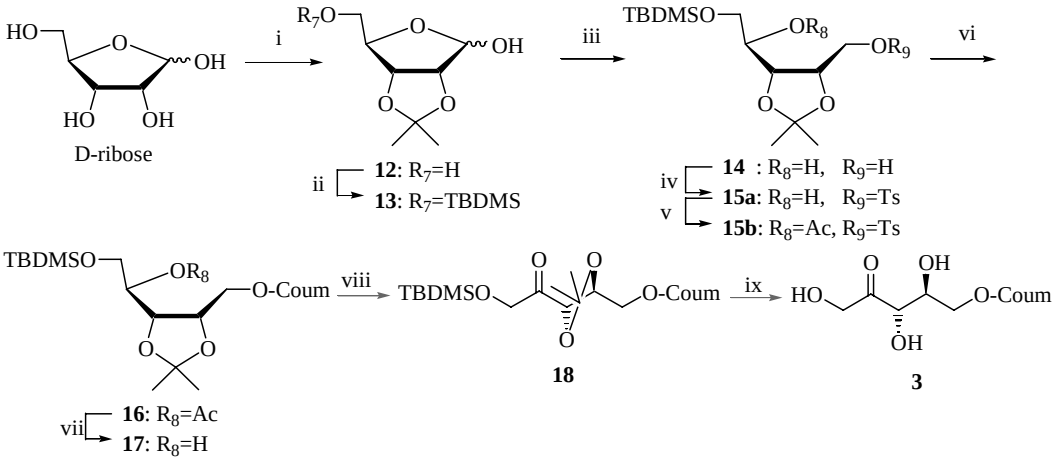
Monotosylation of compound **7** was described earlier in 89% yield.[20] Following this procedure, we failed to obtain **7** in more than 57% yield. Both increasing reaction time at room temperature to 5 h from 45 min and introducing strictly 1 equiv of tosyl chloride as the tosylating agent, we succesfully obtained compound **8a** in 83% yield along with 10% of the corresponding bistosylated compound **8b**, which were separable by column chromatography.

At this stage, it was necessary to convert the primary alcohol function from **8a** into a coumarinyl ether. We chose the sodium salt of umbelliferone as the nucleophile to substitute the tosylate **8a**, as described by Gonzalez-Garcia et al.[9] for the synthesis of 6-*O*-coumarinyl D-fructose from protected D-fructose furanoside. The new compound **9** was recovered in 71% yield after purification. None of our attempts to increase the yield by either modifying the reaction temperature (60, 70 and 90 °C) or changing the counter-ion (from sodium to *tert*-butyl ammonium)[21] was successful.

Oxidation of alcohol **9** was carried out using various oxidising reagents. Neither CrO_3/Pyr nor PCC/NaOAc gave any desired product, whereas Dess–Martin periodinane or Pyr-SO_3/DMSO/NEt_3 gave compound **10** with similar yields (50%). In these latter conditions, reaction monitoring by TLC showed that the starting material was fully converted to the aldehyde **10** but the moderate yield may be explained by the instability of compound **10** on silica gel during purification by chromatography. Finally, this step was achieved using the easily handled periodinane reagent.

The final step dealt with the deprotection of the diol **10**. We tried several usual acidic conditions such as AcOH, TFA, HCl, and Dowex H^+ resin to hydrolyse the acetonide. We noted that when using AcOH, TFA, and HCl in water, compound **10** was fully dissolved, whereas when Dowex H^+ resin was used in water, it was necessary to add a minimum amount of co-solvent to obtain a clear solution. The co-solvents used were DMSO, THF, MeOH and acetone. Only the combination of Dowex H^+ resin in acetone/H_2O at room temperature gave compound **2** in 25% yield (method A). It is noteworthy that when the reaction was carried out in the same conditions but in MeOH/H_2O as the solvent mixture, we characterised compound **11** as a single product resulting from the deprotection of the diol with concomitant acetalisation of the aldehyde **2**. We tried to increase the yield of compound **11** by using larger amounts of MeOH to favour the acetal formation. The highest yield of **11** (43%) was obtained with Dowex H^+ resin in MeOH/H_2O 99:1 as the solvent mixture. We thus investigated the deprotection of **11** as an alternative route to compound **2** (method B). For that purpose, we followed the same conditions as described for the reaction of **10** to **2**. Starting from **11**, we obtained compound **2** in 28% yield. Due to its propensity to oligomerise because of the presence of a hydroxy group alpha to a carbonyl, compound **2** was recovered in moderate yields whatever the route followed. It was characterised by NMR and HRMS. The presence of the aldehyde function was confirmed by derivatisation to the corresponding diphenylhydrazone **2′** according to the protocol described by Friestad et al.[22]

The synthesis of substrate **3** started from inexpensive D-ribose, useful for setting both C_3 and C_4 chirality in the final product[9] (Scheme 6). The first steps required prior protection of the two secondary alcohols in acetonide **12** followed by protection of the primary one in a silylated derivative according to the literature.[23–26] Compound **13** thus obtained was converted into **14** after ring opening by reduction over sodium borohydride in ethanol.[27]



Scheme 6. Synthesis of **3**: (i) acetone, PTSA, (**12**, 63%); (ii) TBDMSCl, imidazole, (**13**, 71%); (iii) $NaBH_4$, (**14**, 80%); (iv) TsCl, NEt_3, DMAP, (**15a**, not isolated) then (v) Ac_2O, NEt_3, (**15b**, 53% overall); (vi) $n$$Bu_4NBr$, umbelliferone, NaOH, (**16**, 60%); (vii) K_2CO_3, (**17**, 90%); (viii) Dess–Martin reagent, (**18**, 74%); (ix) I_2, MeOH, (**3**, 56%).

At this stage, it was necessary to activate selectively the primary alcohol function of **14** into a tosyl group. In our case, neither the use of NaOH[28] nor NEt_3/DMAP[29] as bases gave satisfactory yields. The tosylated compound **15a** was obtained in the reaction mixture but was sensitive to intramolecular cyclisation by nucleophilic attack of the free secondary alcohol on the tosyl group, leading to a furan type compound. As this side reaction occurred mainly during workup, this unwanted reaction was prevented by protecting the free secondary alcohol as an acetyl group directly in the reaction medium, just after formation of the tosyl derivative. Compound **15b** was then obtained in satisfactory yield (53% overall).

The tosyl displacement by umbelliferone was first attempted using the protocol described by Gonzalez-Garcia et al.,[10] that is, using NaH as base. Unfortunately, in our case, these conditions suffered from appearance of many byproducts resulting of a loss of silylated protecting group.

Finally, we chose to make the tetrabutylammonium salt of umbelliferone beforehand following the procedure described by Vasela et al.[21] to carry out the intermolecular nucleophilic substitution in neutral conditions at a lower reaction temperature. This gave the desired product **16** in 60% yield. A single alkaline hydrolysis of the acetyl group followed by an oxidation using the smooth Dess–Martin reagent[30] then gave **18** in 67% overall yield. Usual acidic hydrolysis of all the remaining protective groups using acidic resin, TFA or HCl in various co-solvents to solubilise the starting material (MeOH, acetone, DMF) at different reaction temperatures ranging from room temperature to 65 °C (the degradation threshold temperature) failed to give product **3**. The ether bond proved to be easily cleaved causing umbelliferone release. The highest yield (56%) was obtained using iodine in methanol,[31] an alternative method for the cleavage of acetals into carbohydrate derivatives, leading to the final L-*erythro* ketose **3**.

2.3. Yeast TK fluorogenic assays

Assay conditions were optimised with wild type TK[14] and fluorogenic compound **1**, bearing the glycosyl moiety of D-*threo* ketose configuration as the donor substrate. The reaction proceeded in the presence of D-ribose-5-phosphate as acceptor substrate, thiamine pyrophosphate and Mg^{2+} as cofactors and BSA as catalyst for umbelliferone β-elimination in Tris buffer (pH 8.2). We observed a fluorescent signal proportional to both TK quantity and compound **1** concentration.[14] Under the same conditions, fluorogenic compounds **2** and **3** bearing, respectively, D-*threo* aldose and L-*erythro* ketose moieties did not lead to a significant fluorescence signal in the presence of wild type TK. This experiment showed that wild type TK was able to discriminate between the natural (D-*threo*) configuration of compound **1** and the non-natural (L-*erythro*) configuration of compound **3**. Moreover, TK was able to discriminate between the natural hydroxyacetyl moiety of **1**, mimicking the natural substrate, and the non-natural formyl moiety of **2**.

3. Conclusion

In conclusion, three stereochemical fluorogenic probes for transketolase have been prepared. The fluorogenic compound **1** with natural D-*threo* ketose configuration enabled us to design a highly sensitive fluorogenic assay for wild type TK, in the presence of BSA as auxiliary protein. Our interest was to modify the substrate specificity of TK by random mutagenesis. In this field, the principle of this stereospecific fluorogenic assay could be used to screen TK variants able to recognize the D-*threo* aldose (compound **2**) and L-*erythro* ketose (compound **3**) moieties. Some experiments in this field are currently under investigation.

4. Experimental

4.1. General information

Chemicals and solvents were purchased from Aldrich and Acros and were reagent grade. Rabbit muscle aldolase (E.C.4.1.2.13), acid phosphatase (E.C.3.1.3.2) from wheat germ and BSA were purchased from Sigma. Transketolase from *S. cerevisiae* was produced and purified by us as

previously described.[32] Merck 60 F254 silica gel TLC plates and Merck 60/230–400 and 60/40–63 mesh silica gel for column chromatography were used. ^{1}H, and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$, D_2O, CD_3OD and CD_3COCD_3, δ values are given in parts per million and J values in Hertz. MS and HRMS were recorded on a Micromass Q-Tof spectrometer equipped with an electrospray ionisation source. Optical rotations were determined on a Jasco DIP-370 polarimeter using a 10 cm cell. Melting points are uncorrected and were measured on a Reichert apparatus.

4.1.1. 7-(2-Oxoethoxy) coumarine (5). Eight hundred and sixty milligrams of compound **4** (4.57 mmol) was dissolved in 2 mL of DMF and 20 mL of dichloromethane at room temperature, and then cooled to −40 °C and treated with a stream of ozone. When the reaction was complete as seen by TLC using cyclohexane/ethyl acetate 2:8, ozone was removed by flushing with an argon stream for 1 h. 1 mL of dimethylsulfide (13.4 mmol, 3 equiv) was added dropwise at −40 °C. The reaction mixture was then warmed to room temperature overnight and evaporated under vacuum. The crude material was dissolved in 50 mL of ethyl acetate and washed five times with 50 mL of water. After evaporation under vacuum, the product was chromatographed on silica gel using cyclohexane/ethyl acetate 1:1 as eluent. 560 mg of compound **5** was obtained as a white solid in 64% yield. The analytical data for characterisation are similar to those already reported in the literature.[16]

4.1.2. 7-(2′,3′,5′-Trihydroxy-4′oxo-pentyl)oxycoumarine (1). 0.878 g of (2-Hydroxypropyl)-β-cyclodextrin (0.63 mmol) and 100 mg (0.53 mmol, 1.2 equiv) of 7-(2-oxoethoxy)coumarin **5** were dissolved in 3.9 mL of methanol and stirred for 15 min. 2.6 mL of water were added and the methanol was evaporated. 1.3 mL of a DHAP solution (400 mM, 0.53 mmol, 1 equiv, pH 7.8) were then poured in (to give a 200 mM final substrate concentration) followed by 150 U of commercially available RAMA. The mixture was stirred for 48 h at room temperature. The reaction was followed by TLC with 1-propanol/ethylacetate/water/ethanol/pyridine/acetic acid 35:15:25:15:10:10 as eluent until complete disappearance of the starting aldehyde. The pH was adjusted to 4.8 and 150 U of acid phosphatase was added. The mixture was then stirred overnight. Three volumes of methanol were added to precipitate proteins, the mixture was centrifuged at 8000 rpm and the subsequent supernatant was evaporated to dryness under vacuum. Two flash chromatographies (methylene chloride/methanol 9:1) gave 56 mg of compound **1** as a white solid (35% yield).

^{1}H NMR (CD_3COCD_3, 400 MHz) δ (ppm): 4.15 (1H, dd, $J_{1'-2'}=6.2$ Hz, $J_{1'-1''}=9.8$ Hz, $H_{1'}$), 4.26 (1H, dd, $J_{1''-2}=6.2$ Hz, $J_{1''-1'}=9.8$ Hz, $H_{1''}$), 4.40 (1H, td, $J_{2'-3'}=2.4$ Hz, $J_{2'-1'}=6.2$ Hz, $J_{2'-1''}=6.2$ Hz, $H_{2'}$), 4.45 (1H, d, $J_{5'-5''}=19.6$ Hz, $H_{5'}$), 4.50 (1H, d, $J_{3'-2'}=2.4$ Hz, $H_{3'}$), 4.55 (1H, d, $J_{5''-5'}=19.6$ Hz, $H_{5''}$), 6.20 (1H, d, $J_{3-4}=9.2$ Hz, H_3), 6.8 (1H, s, H_8), 6.9 (1H, d, $J_{6-5}=8.5$ Hz, H_6), 7.54 (1H, d, $J_{5-6}=8.5$ Hz, H_5), 7.87 (1H, d, $J_{4-3}=9.2$ Hz, H_4). ^{13}C NMR (CD_3COCD_3, 100 MHz) δ (ppm): 66.8 ($C_{5'}$), 68.9 ($C_{1'}$), 70.1 ($C_{2'}$), 75.7 ($C_{3'}$), 101.3 (C_6), 112.5 (C_8), 112.7 (C_9), 112.8 (C_3), 129.3 (C_5), 143.7 (C_4), 155.8 (C_{10}), 160.1 (C_7), 161.9 (C_2), 211.9 ($C_{4'}$). HRMS (ESI+) calculated for $C_{14}H_{15}O_7$: $[M+H]^+$ 295.0818, found 295.0823.

4.1.3. 2,3-*O*-Isopropylidene-1-tosyl-D-threitol (8b). This compound was synthesised according to the method of Valverde et al.[20] Starting from 2.77 g (17.1 mmol) of compound **7**, we recovered compound **8a**, isolated in 83% yield as a colourless oil (4.48 g, 14.2 mmol) after column chromatography. Bistosylated compound **8b** was also isolated from the crude product in 10% yield.

4.1.4. 2,3-Isopropyliden-1-*O*-(2′-oxo-benzopyran-7′-yl)-D (9). To a solution of 1.25 g of umbelliferone (7.73 mmol, 1.5 equiv) in 30 mL of DMF under nitrogen was dissolved 0.31 g of sodium hydride (60% oil dispersion, 7.73 mmol, 1.5 equiv). The mixture was stirred at room temperature for 1 h. A mixture of compound **8b** (1.63 g, 5.15 mmol, 1 equiv) in DMF (12 mL) was then added. The reaction mixture was then heated under stirring at 80 °C and was followed by TLC (cyclohexane/AcOEt 4:6) until complete disappearance of the starting material (18 h). After extraction with ethyl acetate, the collected organic phases were washed with brine and evaporated under reduced pressure. The title compound **9** was purified from the crude product by column chromatography on silica gel, eluted in cyclohexane/AcOEt 6:4, 5:5, 4:6 gradient. Compound **9** was recovered as white needles (1.12 g, 3.65 mmol) in 71% yield.

TLC: R_f (cyclohexane/AcOEt 4:6)=0.30. $[\alpha]_D^{22}$ 24.3 (c 1.01, acetone). Mp 79–83 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45 (6H, 2s, $2H_6$), 2.31 (1H, s, 1OH), 3.75 (1H, dd, $J_{4-3}=4$ Hz, $J_{4-4'}=12$ Hz, H_4), 3.88 (1H, dd, $J_{4'-3}=4$ Hz, $J_{4'-4}=12$ Hz, $H_{4'}$), 4.10 (1H, ddd, $J_{3-4}=4$ Hz, $J_{3-4'}=4$ Hz, $J_{3-2}=8$ Hz, H_3), 4.14 (1H, dd, $J_{1-2}=5$ Hz, $J_{1-1'}=10$ Hz, H_1), 4.18 (1H, dd, $J_{1'-2}=5$ Hz, $J_{1'-1}=10$ Hz, $H_{1'}$), 4.31 (1H, ddd, $J_{2-1}=5$ Hz, $J_{2-1'}=5$ Hz, $J_{2-3}=8$ Hz, H_2), 6.24 (1H, d, $J_{3'-4'}=10$ Hz, $H_{3'}$), 6.81 (1H, s, $H_{8'}$), 6.85 (1H, d, $J_{6'-5'}=8$ Hz, $H_{6'}$), 7.35 (1H, d, $J_{5'-6'}=8$ Hz, $H_{5'}$), 7.62 (1H, d, $J_{4'-3'}=10$ Hz, $H_{4'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.0 ($2C_6$), 62.2 (C_4), 68.9 (C_1), 75.5 (C_3), 78.6 (C_2), 101.7 ($C_{6'}$), 110.7 (C_5), 112.9 ($C_{8'}$), 113.0 ($C_{9'}$), 113.5 ($C_{3'}$), 128.9 ($C_{5'}$), 143.5 ($C_{4'}$), 155.8 ($C_{10'}$), 161.2 ($C_{7'}$), 161.7 ($C_{2'}$). HRMS (ESI^+) m/z: $[M+H^+]$: calculated for $C_{16}H_{19}O_6$ 307.1182, found 307.1195.

4.1.5. 2,3-Isopropyliden-4-*O*-(2′-oxo-benzopyran-7′-yl)-D-threose (10). A cold (4 °C) 15% solution of Dess–Martin periodinane in CH_2Cl_2 (800 μL, 1.2 equiv) was added to a solution of compound **9** (97 mg, 0.32 mmol, 1 equiv) in dichloromethane (6.3 mL) at 5 °C. The reaction mixture was kept at 5 °C with stirring for 2 h, and then poured into an ice–0.1 N $NaHCO_3$ mixture (20 mL). After 3 extractions with ethyl acetate, the organic layer was successively washed with 0.1 N $NaHCO_3$ and brine. It was then dried on $MgSO_4$. After evaporation under reduced pressure the crude residue was purified by flash chromatography on silica gel (cyclohexane/AcOEt 1:1). Compound **10** (43 mg, 0.14 mmol) was isolated as white needles in 50% yield.

TLC: R_f (cyclohexane/AcOEt 4:6)=0.30. Mp 67–69 °C. $[\alpha]_D^{25}$ 4.95 (c 1.07, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.50 (6H, 2s, $2H_6$), 4.19 (1H, dd, $J_{4a-3}=5$ Hz,

$J_{4a-4b}=10$ Hz, H_{4a}), 4.26 (1H, dd, $J_{4b-3}=4$ Hz, $J_{4b-4}=10$ Hz, $H_{4'}$), 4.36 (1H, d, $J_{2-3}=7$ Hz, H_2), 4.47 (1H, m, H_3), 6.27 (1H, d, $J_{3'-4'}=10$ Hz, $H_{3'}$), 6.84 (1H, s, $H_{8'}$), 6.87 (1H, d, $J_{6'-5'}=9$ Hz, $H_{6'}$), 7.38 (1H, d, $J_{5'-6'}=9$ Hz, $H_{5'}$), 7.63 (1H, d, $J_{4'-3'}=10$ Hz, $H_{4'}$), 9.85 (1H, s, H_1). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 26.4, 26.7 ($2C_6$), 68.2 (C_4), 75.3 (C_3), 81.5 (C_2), 101.8 ($C_{6'}$), 112.2 (C_5), 112.8 ($C_{8'}$), 113.5 ($C_{9'}$), 113.6 ($C_{3'}$), 128.9 ($C_{5'}$), 143.3 ($C_{4'}$), 155.7 ($C_{10'}$), 161.0 ($C_{7'}$), 161.4 ($C_{2'}$), 200.8 (C_1). HRMS (ESI^+) *m/z*: [M+H^+]: calculated for $C_{16}H_{17}O_6$ 305.1025, found 305.1035.

4.1.6. 2,3-Dihydroxy-1,1-dimethylacetal-4-*O*-(2′-oxo-benzopyran-7′-yl)-D-threose (11). Fifty four milligrams (0.18 mmol, 1 equiv) of the starting compound was solubilised in 2 mL of methanol and 20 μL of water. 1.5 mL of Dowex H^+ resin (50WX8-400) was then added and the reaction mixture allowed to stir for 2 days. The reaction was monitored by TLC using CH_2Cl_2/MeOH 9:1 as eluent. After completion of the reaction, the resin was removed by filtration and the mixture evaporated under vacuum. The white residue thus obtained was purified by flash chromatography on silica gel using CH_2Cl_2/MeOH 95:5 as eluent. Compound **11** was isolated in 43% yield as white crystals.

TLC: R_f (DCM/MeOH 9:1)=0.75. Mp 112–114 °C. $[\alpha]_D^{25}$ −7.8 (*c* 0.92, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.75 (1H, s, OH), 2.92 (1H, s, OH), 3.5 (6H, 2s, $2H_5$), 3.72 (1H, dd, $J_{2-3}=2$ Hz, $J_{2-1}=6$ Hz, H_2), 4.11 (2H, d, $J_{4a-3}=6$ Hz, $H_{4a}+H_{4b}$), 4.23 (1H, td, $J_{3-2}=2$ Hz, $J_{3-4a}=6$ Hz, $J_{3-4b}=6$ Hz, H_3), 4.50 (1H, d, $J_{1-2}=6$ Hz, H_1), 6.23 (1H, d, $J=10$ Hz, $H_{3'}$), 6.82 (1H, s, $H_{8'}$), 6.85 (1H, d, $J_{6'-5'}=9$ Hz, $H_{6'}$), 7.34 (1H, d, $J_{5'-6'}=9$ Hz, $H_{5'}$), 7.61 (1H, d, $J_{4'-3'}=10$ Hz, $H_{4'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 53.3, 56.7 ($2C_5$), 68.5 (C_3), 69.6 (C_4), 70.2 (C_2), 101.9 ($C_{6'}$), 105.4 (C_1), 112.9 ($C_{8'}+C_{9'}$), 113.3 ($C_{3'}$), 128.9 ($C_{5'}$), 143.5 ($C_{4'}$), 155.8 ($C_{10'}$), 161.3 ($C_{7'}$), 161.9 ($C_{2'}$). HRMS (ESI^+) *m/z*: [M+H^+]: calculated for $C_{15}H_{19}O_7$ 311.1131, found 311.1139.

4.1.7. 2,3 Dihydroxy-4-*O*-(2′-oxo-benzopyran-7′-yl)-D-threose (2). General method for acidic hydrolysis of acetals.

Acetal was dissolved in binary system (water/acetone 3:1). Dowex H^+ resin (50WX8-400) was added and the reaction suspension stirred. The suspension was refluxed for 1 h with stirring under reflux until completion (reaction monitored by TLC). After cooling to room temperature, the reaction mixture was filtered and washed with 1 mL of acetone–water mixture (1/2). Acetone was removed under reduced pressure and the pH of the water layer was adjusted to 7 with an Amberlite resin (IRA-93). After lyophilisation, the crude material was flash chromatographed on silica gel using CH_2Cl_2–MeOH (97/3) as the eluent. The final compound **2** was obtained as a colourless oil.

When compound **10** was used as the starting acetal, title compound **2** was recovered in 25% yield whereas conversion of compound **11** afforded compound **2** in 28% yield.

TLC: R_f (CH_2Cl_2/MeOH 9:1)=0.33. $[\alpha]_D^{25}$ 1.84 (*c* 3.3, chloroform). ^{1}H NMR (400 MHz, CD_3OD) δ: 3.25–3.52 (1H, m, H_2), 3.54–4.13 (3H, m, H_3+2H_4), 4.71 (1H, d, $J_{1-2}=6$ Hz, H_1), 6.14 (1H, d, $J_{3'-4'}=9.6$ Hz, $H_{3'}$), 6.83 (1H, s, $H_{8'}$), 6.88 (1H, d, $J_{6'-5'}=8.4$ Hz, $H_{6'}$), 7.45 (1H, d, $J_{5'-6'}=8.4$ Hz, $H_{5'}$), 7.77 (1H, d, $J_{4'-3'}=9.6$ Hz, $H_{4'}$). ^{13}C NMR (100 MHz, CD_3OD) δ: 68.7 (C_2), 69.7 (C_4), 73.3 (C_3), 89.6 (C_1, hydrate form), 101.4 ($C_{6'}$), 112.7 ($C_{8'}$), 113.4 ($C_{9'}$), 113.8 ($C_{3'}$), 129.6 ($C_{5'}$), 145.9 ($C_{4'}$), 154.9 ($C_{10'}$), 163.9 ($C_{2'}+C_{7'}$). HRMS (ESI^+) *m/z*: [M+H^+]: calculated for $C_{13}H_{13}O_6$ 265.0712, found 265.0708.

4.1.8. Derivatisation of compound 2 into the corresponding *N,N*-diphenylhydrazone 2′. To a solution of compound **2** (56 mg, 0.21 mmol, 1 equiv) in 4 mL of toluene were successively added *N,N*-diphenylhydrazine hydrochloride (94 mg; 0.42 mmol; 2 equiv) and sodium sulphate Na_2SO_4 (1.26 g; 10.2 mmol) at room temperature. The reaction mixture was kept at room temperature under stirring for 24 h. After filtration, the crude mixture was concentrated under vacuum, and purified by flash chromatography on silica gel (cyclohexane/AcOEt 6:4). Hydrazone **2′** was recovered as a bright yellow oil in 36% yield.

TLC: R_f (cyclohexane/AcOEt 6:4)=0.13. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.63 (1H, d, $J=5$ Hz, OH), 3.35 (1H, d, $J=5$ Hz, OH), 4.04 (3H, m, H_4), 4.43 (1H, ddd, $J_{2-3}=10$ Hz, $J_{2-1}=4$ Hz, $J_{2-1'}=4$ Hz, H_2), 6.20 (1H, d, $J_{3'-4'}=9.6$ Hz, $H_{3'}$), 6.56 (1H, d, $J=3$ Hz, H_1), 6.74 (1H, s, $H_{8'}$), 6.77 (1H, d, $J_{6'-5'}=8.4$ Hz, $H_{6'}$), 7.11 (4H, m), 7.19 (2H, m), 7.33 (1H, d, $J_{5'-6'}=8.4$ Hz, $H_{5'}$), 7.35 (4H, m), 7.54 (1H, d, $J_{4'-3'}=9.6$ Hz, $H_{4'}$). ^{13}C NMR (100 MHz, CD_3COCD_3) δ: 70.6 (C_4), 72.4 (C_2), 72.9 (C_3), 102.3 ($C_{6'}$), 113.5 ($C_{8'}$), 113.7 ($C_{3'}$), 123.1 ($C_{2''}$), 125.2 ($C_{4''}$), 130.1 ($C_{5'}$), 130.6 ($C_{3''}$), 138.9 (C_1), 144.5 ($C_{4'}$), 144.7 ($C_{1''}$).

4.1.9. 4-*O*-Acetyl-2,3-*O*-isopropylidene-5-*O*-terbutyldimethylsilyl-1-*O*-tosyl-D-ribitol (15b). In 16 mL of anhydrous dichloromethane were dissolved 400 mg (1.3 mmol) of compound **14**, 273 mg (1.43 mmol, 1.1 equiv) of tosyl chloride, 192 μL (1.43 mmol, 1.1 equiv) of anhydrous triethylamine and 160 mg (1.3 mmol, 1 equiv) of DMAP. The reaction mixture was stirred for 30 min at room temperature and was monitored by TLC using cyclohexane/ethyl acetate 7:3 as eluent. 615 mg (6.5 mmol, 5 equiv) of acetic anhydride was then added followed by 364 μL (2.8 mmol, 2.2 equiv) of anhydrous triethylamine. The mixture was evaporated under vacuum. The crude product was dissolved in ethyl acetate and the organic phase was washed three times with water. After drying on $MgSO_4$, rotary evaporation and flash chromatography on silica gel (cyclohexane/ethyl acetate 8:2), 346 mg (53%) of product **15b** was obtained as a colourless oil.

TLC: R_f (cyclohexane/AcOEt 7:3)=0.65. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.02 (6H, s, $2H_8$), 0.87 (9H, s, $9H_{10}$), 1.30–1.31 (6H, 2s, $2H_7$), 2.04 (3H, s, H_{17}), 2.44 (3H, s, H_{15}), 3.74 (1H, dd, $J_{5-4}=4$ Hz, $J_{5-5'}=12$ Hz, H_5), 3.87 (1H, dd, $J_{5'-4}=2$ Hz, $J_{5'-5}=12$ Hz, $H_{5'}$), 3.92 (1H, dd, $J_{1-2}=7$ Hz, $J_{1-1'}=10$ Hz, H_1), 4.08 (1H, dd, $J_{1'-2}=6$ Hz, $J_{1'-1}=10$ Hz, $H_{1'}$), 4.38 (2H, m, H_2+H_3), 4.78 (1H, ddd, $J_{4-5'}=2$ Hz, $J_{4-5}=4$ Hz, $J_{4-3}=9$ Hz, H_4), 7.33 (2H, d, $J_{13-12}=8$ Hz, $2H_{13}$), 7.80 (2H, d, $J_{12-13}=8$ Hz, $2H_{12}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.4 ($2C_8$), 18.4 (C_9), 21.1,

21.8 ($2C_7$), 25.9 ($3C_{10}$), 27.0 (C_{15}), 27.9 (C_{17}), 62.0 (C_5), 68.2 (C_1), 71.6 (C_4), 73. 8 (C_3), 75.0 (C_2), 109.3 (C_6), 128.2, 130.0 ($C_{12}+C_{13}$), 132.8 (C_{14}), 145.0 (C_{11}), 170.0 (C_{16}). HRMS (ESI^+) *m/z*: $[M+H^+]$: calculated for $C_{23}H_{39}O_8SiS$ 503.2135, found 503.2141.

4.1.10. 4-*O*-Acetyl-2,3-*O*-isopropylidene-1-*O*-(2′-oxo-benzopyran-7′-yl)-5-*O*-terbutyldimethylsilyl-D (16). The tetrabutylammonium salt of umbelliferone was obtained by mixing 616 mg (3.8 mmol, 1 equiv) of umbelliferone with 1.22 g (3.8 mmol, 1 equiv) of tetrabutylammonium bromide in 10 mL of a NaOH solution (228 mg, 5.7 mmol, 1.5 equiv). After stirring for few minutes, the salt was extracted with chloroform and the organic phase was dried on $MgSO_4$ and evaporated under vacuum. Thus 1.5 g of crude salt was obtained and used without further purification. The salt previously obtained was transferred to a flask containing 200 mg of **15b** (0.38 mmol, 1 equiv) dissolved in 20 mL of anhydrous DMF. The mixture was then stirred for 96 h at 50 °C under argon. The reaction was monitored by TLC using cyclohexane/ethyl acetate 6:4 as eluent. After disappearance of the starting compound, 50 mL of water was added followed by 50 mL of ethyl acetate, and the aqueous phase was extracted twice with 2×50 mL of ethyl acetate. The organic phase was dried on $MgSO_4$ and evaporated under vacuum. The crude product was purified by column chromatography on silica gel using cyclohexane/ethyl acetate 6:4 as eluent. Compound **16** was isolated in 60% yield as a white solid.

TLC: R_f (cyclohexane/AcOEt 6:4)=0.42. Mp 95–96 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 0.03 (6H, s, $2H_8$), 0.87 (9H, s, $3H_{10}$), 1.39, 1.47 (6H, 2s, $2H_7$), 2.03 (3H, s, H_{12}), 3.81 (1H, dd, $J_{5a-4}=4$ Hz, $J_{5a-5b}=12$ Hz, H_{5a}), 3.92 (1H, dd, $J_{5b-4}=2$ Hz, $J_{5b-5a}=12$ Hz, H_{5b}), 4.09 (1H, dd, $J_{1a-2}=6$ Hz, $J_{1a-1b}=10$ Hz, H_{1a}), 4.15 (1H, dd, $J_{1b-2}=5$ Hz, $J_{1b-1a}=10$ Hz, H_{1b}), 4.48 (1H, dd, $J_{3-2}=6$ Hz, $J_{3-4}=8$ Hz, H_3), 4.56 (1H, m, H_2), 5.02 (1H, ddd, $J_{4-5b}=2$ Hz, $J_{4-5a}=4$ Hz, $J_{4-3}=8$ Hz, H_4), 6.24 (1H, d, $J_{3'-4'}=10$ Hz, $H_{3'}$), 6.78 (1H, s, $H_{8'}$), 6.82 (1H, d, $J_{6'-5'}=9$ Hz, $H_{6'}$), 7.36 (1H, d, $J_{5'-6'}=9$ Hz, $H_{5'}$), 7.62 (1H, d, $J_{4'-3'}=10$ Hz, $H_{4'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.4 ($2C_8$), 18.4 (C_9), 21.3 (C_{12}), 25.5 ($2C_7$), 25.9 ($3C_{10}$), 62.3 (C_5), 67.3 (C_1), 72.0 (C_2), 74.2 (C_4), 75.4 (C_3), 101.8 ($C_{6'}$), 109.2 (C_6), 112.8 ($C_{8'}$), 113.0 ($C_{9'}$), 113.5 ($C_{3'}$), 129.0 ($C_{5'}$), 143.4 ($C_{4'}$), 155.9 ($C_{10'}$), 161.2 ($C_{7'}$), 161.8 ($C_{2'}$), 170.0 (C_{11}). HRMS (ESI^+) *m/z*: $[M+H^+]$: calculated for $C_{25}H_{37}O_8Si$ 493.2258, found 493.2267.

4.1.11. 2,3-*O*-Isopropylidene-1-*O*-(2′-oxo-benzopyran-7′-yl)-5-*O*-terbutyl-dimethylsilyl-D (17). To a solution of 191 mg (0.39 mmol) of compound **16** in 9 mL of methanol were added 107 mg (0.77 mmol, 2 equiv) of potassium carbonate. The mixture was stirred at room temperature for 4 h. After disappearance of the starting compound (cyclohexane/ethyl acetate 4:6), a mixture of ethyl acetate and water (v/v 1:1) was added and the solution was extracted. The organic phase was dried under $MgSO_4$ and evaporated under vacuum to give 141 mg (90%) of compound **17** as a colourless oil, used without further purification.

TLC: R_f (cyclohexane/AcOEt 4:6)=0.65. $[\alpha]_D^{24}$ −22.75 (*c* 0.4, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.102 (6H, s, $2H_8$), 0.92 (9H, s, $3H_{10}$), 1.39, 1.47 (6H, 2s, $2H_7$), 3.70 (2H, m, H_4+H_{5a}), 3.85 (1H, dd, $J_{5b-4}=5$ Hz, $J_{5b-5a}=12$ Hz, H_{5b}), 4.12 (1H, dd, $J_{1a-2}=6$ Hz, $J_{1a-1b}=10$ Hz, H_{1a}), 4.19 (1H, dd, $J_{1b-2}=8$ Hz, $J_{1b-1a}=10$ Hz, $H_{1'}$), 4.48 (1H, dd, $J_{3-2}=3$ Hz, $J_{3-4}=10$ Hz, H_3), 4.60 (1H, ddd, $J_{2-3}=3$ Hz, $J_{2-1a}=6$ Hz, $J_{2-1b}=8$ Hz, H_2), 6.25 (1H, d, $J_{3'-4'}=9$ Hz, $H_{3'}$), 6.89 (1H, s, $H_{8'}$), 6.92 (1H, d, $J_{6'-5'}=8$ Hz, $H_{6'}$), 7.36 (1H, d, $J_{5'-6'}=8$ Hz, $H_{5'}$), 7.63 (1H, d, $J_{4'-3'}=9$ Hz, $H_{4'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.2 ($2C_8$), 18.5 (C_9), 25.7 ($2C_7$), 26.0 ($3C_{10}$), 64.4 (C_1), 68.1 (C_5), 69.5 (C_4), 76.1 (C_2+C_3), 101.9 ($C_{6'}$), 109.6 (C_6), 112.7 ($C_{8'}$), 112.8 ($C_{9'}$), 113.3 ($C_{3'}$), 128.8 ($C_{5'}$), 143.5 ($C_{4'}$), 155.8 ($C_{10'}$), 161.3 ($C_{7'}$), 162.2 ($C_{2'}$). MS *m/z* 473 ($M+Na^+$). HRMS *m/z*: $[M+Na^+]$: calculated for $C_{23}H_{34}NaO_7Si$ 473.1971, found 473.1965.

4.1.12. 2,3-*O*-Isopropylidene-4-oxo-5-*O*-terbutyl-dimethylsilyl-7′-(2,3,5-trihydroxy-4-oxo-pentyl)oxy-coumarine (18). Four hundred and ten milligrams (0.96 mmol, 1.4 equiv) of Dess–Martin reagent were dissolved in 6 mL of anhydrous dichloromethane. 310 mg (0.69 mmol, 1 equiv) of compound **17** dissolved in 4 mL of anhydrous dichloromethane were then added and the mixture was stirred for 5 h at room temperature. 30 mL of diethyl ether were poured in followed by 12 mL of a 1.3 M NaOH solution. The mixture was stirred for 10 min and the organic layer was washed with 20 mL of water, dried on $MgSO_4$ and evaporated under reduced pressure to yield 230 mg (74%) of yellow oil, used in the next step without further purification.

TLC: R_f (cyclohexane/AcOEt 4:6)=0.69. $[\alpha]_D^{24}$ −52.2 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.06 (9H, s, $2H_8$), 0.88 (9H, s, $3H_{10}$), 1.40, 1.58 (6H, 2s, $2H_7$), 4.00 (1H, dd, $J_{1-2}=4$ Hz, $J_{1-1'}=10$ Hz, H_1), 4.15 (1H, dd, $J_{1'-2}=4$ Hz, $J_{1'-1}=10$ Hz, $H_{1'}$), 4.52 (2H, 2s, $J_{5a-5b}=19$ Hz, $2H_5$), 4.77 (1H, ddd, $J_{2-1}=4$ Hz, $J_{2-1'}=4$ Hz, $J_{2-3}=8$ Hz, H_2), 4.87 (1H, d, $J_{3-2}=8$ Hz, H_3), 6.24 (1H, d, $J_{3'-4'}=9$ Hz, $H_{3'}$), 6.77 (1H, s, $H_{8'}$), 6.79 (1H, d, $J_{6'-5'}=8$ Hz, $H_{6'}$), 7.33 (1H, d, $J_{5'-6'}=8$ Hz, $H_{5'}$), 7.61 (1H, d, $J_{4'-3'}=9$ Hz, $H_{4'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.3 ($2C_8$), 18.4 (C_9), 24.9 ($2C_7$), 26.0 ($3C_{10}$), 66.5 (C_1), 68.5 (C_5), 76.0 (C_2), 76.5 (C_3), 102.2 ($C_{6'}$), 110.5 (C_6), 112.7 ($C_{8'}$), 113.2 ($C_{9'}$), 113.6 ($C_{3'}$), 128.9 ($C_{5'}$), 143.3 ($C_{4'}$), 154.8 ($C_{10'}$), 161.2 ($C_{2'}+C_{7'}$), 206.5 (C_4). HRMS *m/z* $[M+H^+]$: calculated for $C_{23}H_{33}O_7Si$ 449.1996, found 449.2002.

4.1.13. 7′-(2,3,5-Trihydroxy-4-oxo-pentyl)oxycoumarine (3). Eighty six milligrams (0.19 mmol) of compound **18** and 48 mg (0.19 mmol, 1 equiv) of iodine were dissolved in 8 mL of methanol. The mixture was stirred and refluxed for 2 h. After disappearance of the starting compound (dichloromethane/methanol 9:1), the reaction mixture was cooled in an ice bath and 30 mg (1.9 mmol, 10 equiv) of Na_2SO_3 was added under stirring. After evaporation of methanol under vacuum and flash chromatography on silica gel (dichloromethane/methanol 95:5), 32 mg of the final compound **3** was obtained (56%) as a colourless oil.

TLC: R_f (CH_2Cl_2/MeOH 9:1)=0.62. $[\alpha]_D^{24}$ −16.4 (*c* 1, MeOH). ^1H NMR (400 MHz, CD_3COCD_3) δ: 4.12 (1H, dd, $J_{1a-2}=6$ Hz, $J_{1a-1b}=10$ Hz, H_{1a}), 4.21 (2H, m, $H_{1b}+H_2$), 4.35 (1H, d, $J_{3-2}=5$ Hz, H_3), 4.52 (2H, 2 d, $J_{5a-5b}=19$ Hz, $2H_5$), 5.37 (1H, d, $J_{3'-4'}=10$ Hz, H3′), 6.05 (1H, s, $H_{8'}$),

6.07 (1H, d, $J_{6'-5'}=8$ Hz, $H_{6'}$), 6.66 (1H, d, $J_{5'-6'}=8$ Hz, $H_{5'}$), 7.00 (1H, d, $J_{4'-3'}=10$ Hz, $H_{4'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 67.9 (C_5), 69.7 (C_1), 72.3 (C_2), 77.2 (C_3), 102.5 ($C_{6'}$), 113.4 ($C_{8'}$), 113.6 ($C_{9'}$), 114.0 ($C_{3'}$), 130.5 ($C_{5'}$), 145.7 ($C_{4'}$), 156.8 ($C_{10'}$), 161.1 ($C_{7'}$), 162.8 ($C_{2'}$), 212.4 (C_4). HRMS *m/z*: $[M+Na^+]$: calculated for $C_{14}H_{14}NaO_7$ 317.0637, found 317.0627.

4.2. Yeast TK fluorogenic assays

TK enzyme (0–0.02 mg mL^{-1}) was incubated for 30 min in a reaction mixture containing 2 mM ThDP, 3 mM $MgCl_2$, 1 mM D-ribose-5-phosphate, 100 μM of fluorogenic substrate (**1**, **2** or **3**) and 2 mg mL^{-1} BSA in a 50 mM aq Tris buffer, pH 8.2. The reaction progress was followed by fluorescence detection at $\lambda_{em}=412$ nm ($\lambda_{ex}=390$ nm). Fluorescence was correlated with umbelliferone concentration by means of a calibration curve.

Acknowledgements

This work was supported by MENRT (PhD grant and postdoctoral fellowship).

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