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A Fluorogenic Screening for Enantio- and Diastereoselectivity of 2-Deoxy-D-ribose-5-phosphate Aldolases

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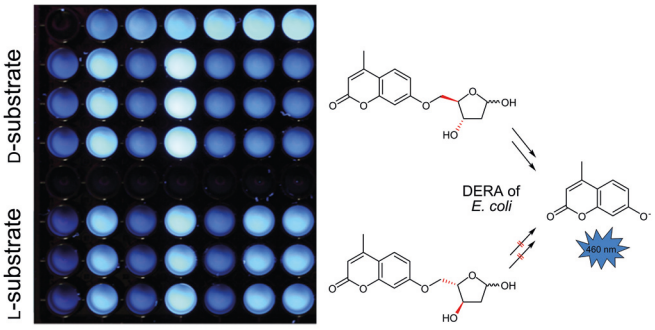
Dedicated to Prof. Steven V. Ley on the occasion of his 70th birthday



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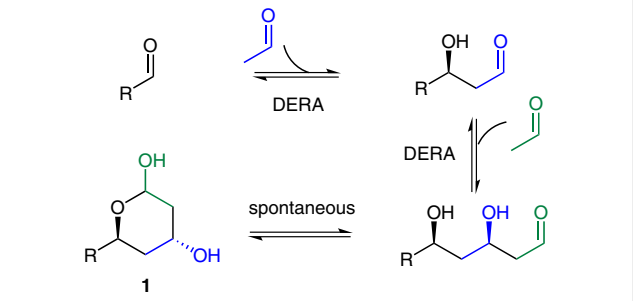
Abstract A highly sensitive, fluorescence-based selectivity screening system for 2-deoxy-D-ribose-5-phosphate aldolases was realized by installing short, straightforward syntheses to fluorophore-coupled carbohydrates as D-ribose, L-ribose, and D-xylose. The substrates allow the simultaneous determination of enantioselectivity and diastereoselectivity of DERA-catalyzed aldol reactions.

Key words 2-deoxy-D-ribose-5-phosphate aldolase, fluorogenic screening, enantioselectivity, diastereoselectivity, extremophilic aldolases



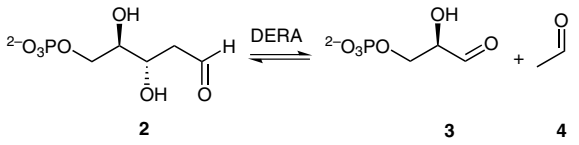
Scheme 1 DERA-catalyzed aldol reaction towards β-hydroxy-γ-lactols **1**

Enantiomerically pure aldol compounds represent a highly desirable class of building blocks in organic synthesis. However, the enantioselective synthesis by conventional methods often remains a complex challenge.[2] Besides different organic synthetic methods,[3] aldolases offer excellent stereo- and chemoselectivity under mild reaction conditions.[4] A particularly promising representative is the 2-deoxy-D-ribose-5-phophate aldolase (DERA), due to its unique ability to utilize an aldehyde (as a natural substrate) as the nucleophile, giving access to a double aldol reaction resulting in β-hydroxy-γ-lactols **1** (Scheme 1).[5] Applying this enzyme, various complex natural products have been synthesized with good yields and high enantioselectivity without harsh reaction conditions.[6] A prominent example is the key building block for the pharmaceutical blockbuster atorvastatin synthesized by Jennewein et al.[7]

For synthetic purposes, it is desirable to produce all possible stereoisomers of a given compound. Unfortunately, in the case of DERA, there is no natural stereocomplementary enzyme known. To complete the synthetic toolbox, enzymes with an inverted enantio- and diastereoselectivity are highly desired. Different methods, like metagenome approaches or protein engineering, are used to find new possible enzymatic catalysts.[8] Here, a fast and reliable high-throughput screening system is essential for the identification of positive hits.

Fluorogenic screenings are often favored over other methods, due to their high sensitivity, which allows low substrate and enzyme concentrations.[9] In 1998 Reymond and coworkers reported a fluorogenic screening for aldolases, based on a retroaldol reaction of umbelliferone-coupled aldols.[10] As activity assay for DERA, Greenberg used 2004 4-methylumbelliferone-coupled D-ribose,[11] whereas Fei et al. applied a green fluorescence coumarin derivative.[12]

Here we present a fluorescence screening system adapted from Reymond et al. and Greenberg et al., which we expanded to a enantio- and diastereoselectivity assay by affording efficient and straightforward syntheses to the fluorogenic substrates **5** and **6**. Based on the natural DERA reaction (Scheme 2) the enantio- and diastereoselectivity was assayed using 5-O-(4′-methylumbelliferyl)-2-deoxypentoses, such as 2-deoxy-D-ribose (D-**5**), 2-deoxy-L-ribose (L-**5**), and 2-deoxy-D-xylose (**6**). The aldose form **7** could be cleaved, depending on the configuration of the sugar moi-

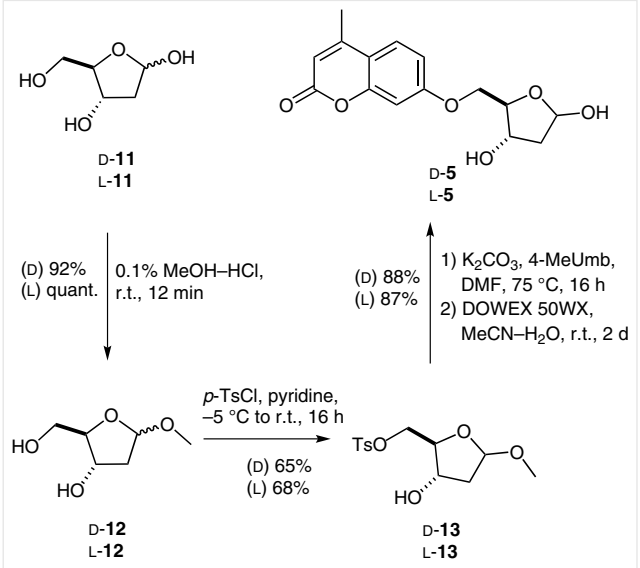


Scheme 2 DERA-catalyzed in vivo retro-aldol reaction of 2-deoxy-D-ribose-5-phosphate (**2**) to glyceraldehyde-3-phosphate (**3**) and acetaldehyde (**4**)

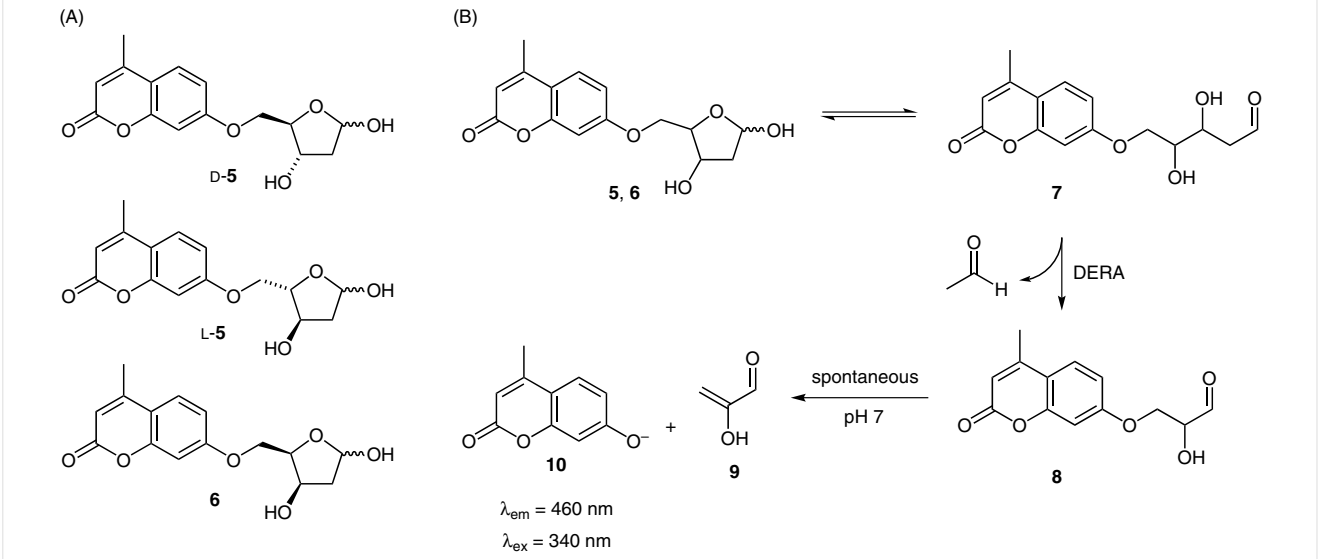
ety, to the corresponding glyceraldehyde derivative **8**. This compound **8** decomposes spontaneously by β-elimination to aldehyde **9** and the fluorophore **10**, which can be quantified even at low concentrations (Scheme 3).

The enantiocomplementary screening substrates D-**5** and L-**5** were synthesized in a short sequence from 2-deoxy-D-ribose (D-**11**) and 2-deoxy-L-ribose (L-**11**), respectively (Scheme 4). To stabilize the cyclic form of the furanoses, the anomeric hydroxyl group was methylated using methanolic hydrochloride acid, carefully monitoring the reaction to avoid undesired overmethylation of the primary and secondary hydroxy group.[13] The methylated D- and L-ribose **12** were isolated in good to excellent yields (92%–quant.).[14] To install a good leaving group at the 5-hydroxy position, a standard tosylation, using *p*-tosyl chloride in pyridine, was applied yielding the compounds D-**13** (65%) and L-**13** (68%).[15] Substitution with the fluorophore 4-methylumbelliferone (**10**) was performed in DMF using K_2CO_3 as base.[11] A following demethylation, applying an acidic cation exchange resin, provided the screening substrates in good yields (D-**5**, 88%; L-**5**, 87%) over two steps.[16]

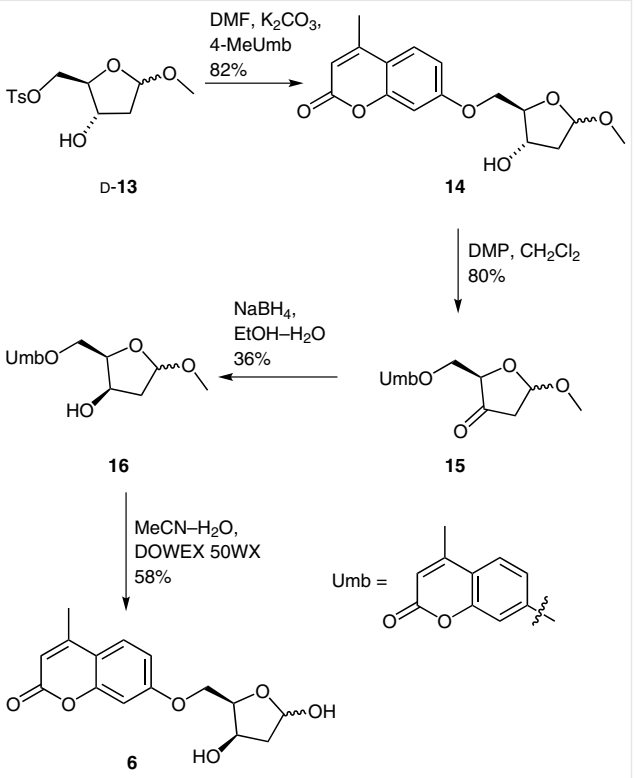
In order to analyze the diastereoselectivity of different DERA, it became necessary to synthesize 4-methylumbelliferone-coupled D-xylose substrate **6** (Scheme 5), since 2-deoxy-D-xylose is very expensive ($1250/gram, Carbosynth). Hence, it was synthesized based on 2-deoxy-D-ribose by inverting the configuration at the 3-position of the carbohydrate moiety. Analogously, the tosyl group was substituted with 4-methylumbelliferone, but without subsequent demethylation, to uphold the cyclic form of the furanose. The product **14** was isolated with a good yield of 80%.[17] A reaction with the Dess–Martin periodinane (DMP)[18] in dichloromethane led to oxidation of the unprotected 3-hydroxy group, yielding 82% of compound **15**.[19] After reduction with $NaBH_4$ 36% of the desired diastereomer was



Scheme 4 Three-step synthesis towards the enantiomeric fluorogenic substrates D-**5** and L-**5**



Scheme 3 (A) Synthesized screening substrates D-**5**, L-**5**, and **6**; (B) DERA-catalyzed screening reaction



Scheme 5 Synthesis of the 2-deoxy-D-xylose fluorogenic substrate **6** by inverting the configuration of the 2-deoxy-D-ribose moiety

isolated.[20] Final demethylation with DOWEX 50WX yielded the fluorogenic D-xylose product **6** in 58%.[21]

To afford a high throughput, the screening was realized in 96-well microtiter plates. The fluorescence was measured, applying an excitation wavelength of 340 nm and measuring an emission wavelength of 460 nm. The fluorogenic substrates D-**5**, L-**5**, and **6** were dissolved in acetonitrile–water to form stable stock solutions, which were used for the measurement in triethanolamine buffer (0.1 M, pH 7). To determine the volume activity of various DERA a calibration curve using 4-methylumbelliferone (**10**) was recorded.[22] The proteins were provided by their gene expression in *E. coli* BL21 (DE3) and inserted for the screening as cell-free crude extract.[23]

Applying this screening system, we tested DERA from *E. coli* and five additional aldolases from psychrophilic (*Colwellia psychrerythraea*, *Shewanella halifaxensis*), mesophilic (*Rhodococcus erythropolis*),[24] and hyperthermophilic (*Pyrobaculum aerophilum*, *Thermotoga maritima*)[25] organisms for their enantio- and diastereoselectivity (Figure 1). Extremophilic enzymes are interesting targets for various applications in biotechnology: While (hyper)thermophilic enzymes show high stability even under harsh reaction conditions,[26] their psychrophilic homologues have adapted to work very efficiently at low temperatures – resulting in an increased activity and a broader substrate spectrum (due to the high flexibility of the proteins).[27]

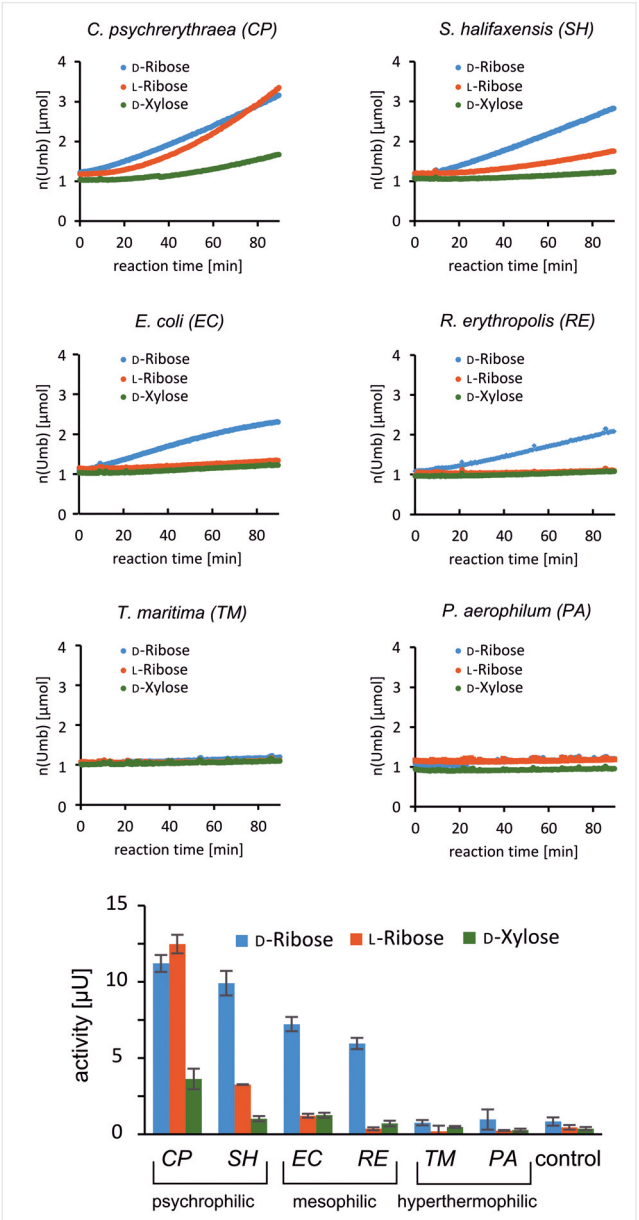


Figure 1 Activity of psychrophilic, mesophilic, and hyperthermophilic DERA enzymes for the 4-methylumbelliferone-coupled D-ribose D-**5**, L-ribose L-**5**,-and D-xylose **6** substrate. Error bars represent the standard deviation of triplet measurements. As control cells, transformed with an empty vector were used.

The screening results show a higher volume activity of the DERA from psychrophilic organisms compared to the usually used *E. coli* DERA at a similar expression level (see Supporting Information). It was found that the hyperthermophilic variants show nearly no conversion of the sub-

strates. As the expression level is low, but higher than in the negative control, these results indicate a low activity for the used substrates. Interestingly, under these screening conditions DERA from *C. psychrerythraea* showed an even higher activity for the L-ribose substrate L-**5** than for the D-ribose compound D-**5**. This corresponds to the theory that psychrophilic enzymes are more flexible than their *meso*- or (hyper)thermophilic homologues. Furthermore this enzyme might be a promising candidate to accept a broader substrate scope as the *E. coli* DERA.[28]

In summary, we showed short and straightforward syntheses to obtain useful fluorophore-coupled screening substrates, which can be applied in a reliable, fast, and highly sensitive microtiter-plate screening system. We expanded this fluorescence-based screening from an activity assay to a complex screening for enantio- as well as diastereoselectivity. This enables us to test a high number of DERA enzymes to find possible new catalysts for application in organic synthesis.

Acknowledgment

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1560723.

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(1) Authors contributed equally.
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(14) **(3*S*,4*R*)-4-(Hydroxymethyl)-1-methoxytetrahydrofuran-3-ol (D-12) and (3*R*,4*S*)-4-(Hydroxymethyl)-1-methoxytetrahydrofuran-3-ol (L-12)**
2-Deoxy-D-ribose (D-**11**, 0.5 g, 3.7 mmol) were dissolved in methanolic HCl (10 mL, 0.1%) and stirred at r.t. for 12 min. The reaction was controlled by rotary power and after full conversion neutralized with Ag_2CO_3. The mixture was filtrated, and the solvent was evaporated. The crude product was purified by Kugelrohr distillation; 506 mg (92%, dr A/B = 0.6:0.4) D-**12** were isolated as a colorless oil. Analogously 100 mg (0.75 mmol) 2-desoxy-L-ribose (L-**11**) were converted into 116 mg (0.78 mmol, quantitative yield, dr A/B = 0.6:0.4) L-**12**. R_f = 0.06 (PE–EtOAc, 40:60). IR (film): ν = 3383, 2918, 1444, 1206, 1050 cm^{-1}. GC–MS (EI, +, 70 eV): *m/z* (%) = 147 (1) $[M + H]^+$, 117 (100) $[C_5H_9O_3]^+$, 88 (52) $[C_5H_{10}O]^+$, 71 (26) $[C_4H_8O]^+$. ^1H NMR (600 MHz, $CDCl_3$): δ = 2.00 (m, 1 H, 2b-H_A), 2.08–2.14 (m, 3 H, 2b-H_B, 2a-H_A, 3-OH_A), 2.26 (ddd, *J* = 13.9, 6.9, 2.0 Hz, 1 H, 2a-H_B), 2.50 (d, *J* = 5.7 Hz, 1 H, 3-OH_B), 2.78 (m, 1 H, 5-OH_B), 2.90 (d, *J* = 10.23 Hz, 1 H, 5-OH_A), 3.38 (m_c, 6 H, OMe_A, OMe_B), 3.58–3.73 (m, 4 H, 5a-H_A, 5a-H_B, 5b-H_A, 5b-H_B), 4.05 (ddd, *J* = 3.5 Hz, 1 H, 4-H_B), 4.10–4.17 (m, 2 H, 4-H_A, 3-H_A), 4.51 (ddd, *J* = 8.4, 6.9, 3.5 Hz, 1 H, 3-H_B), 5.10 (dd, *J* = 4.5, 0.2 Hz, 1 H, 1-H_A), 5.11 (dd, *J* = 5.7, 2.0 Hz, 1 H, 1-H_B) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 41.5 (C-2_A), 42.5 (C-2_B), 54.9 (OMe_A), 55.4 (OMe_B), 63.1 (C-5_A), 63.5 (C-5_B), 72.1 (C-3_B), 72.8 (C-3_A), 87.4 (C-4_A), 87.5 (C-4_B), 105.5 (C-1_A), 105.6 (C-1_B)

ppm. HRMS (ESI, +): *m/z* (%) calcd for $C_6H_{12}O_4Na$ [M + Na]$^+$: 171.06333; found: 171.06275. D-**12**: $[\alpha]_D$ +39.4 (*c* 1.0, $CHCl_3$); lit.[13] $[\alpha]_D$ +38.4 (*c* 0.6, CH_3COOH). L-**12**: $[\alpha]_D$ –40 (*c* 0.93, $CHCl_3$).

(15) **[(3*S*,4*R*)-3-Hydroxy-1-methoxytetrahydrofuran-4-yl]methyl *p*-Tosylate (D-13) and [(3*R*,4*S*)-3-Hydroxy-1-methoxy-tetrahydrofuran-4-yl]methyl *p*-Tosylate (L-13)**

Under an argon atmosphere D-**12** (4.20 g, 28.4 mmol) was dissolved in pyridine (100 mL) and cooled to –5 °C. A solution of *p*-TsCl (5.22 g, 27.4 mmol, 1.00 equiv) in dry CH_2Cl_2 (40 mL) was added dropwise. The temperature of the reaction was maintained between –5 °C and 0 °C. The reaction was stirred for 1 h at 0 °C, warmed up to r.t. and stirred overnight. The conversion was controlled by TLC. Ice-cold H_2O (130 mL) and EtOAc were added, and the reaction was extracted with a sat. $CuSO_4$ solution. The aqueous phases were combined and extracted with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and filtered. The solvent was evaporated, and the crude product was purified by column chromatography (PE–EtOAc, 70:30); 5.60 g (18.5 mmol, 65%, dr A/B = 0.7:0.3) of D-**13** were isolated as a colorless oil. Analogously 1.00 g (8.75 mmol) of L-**12** were converted into 1.40 g (4.63 mmol, 68%, dr A/B = 0.7:0.3) of the colorless oil L-**13**. R_f = 1.9 (PE–EtOAc, 40:60). IR (film): ν = 3454, 2928, 1598, 1444, 1180, 1096, 665 cm^{-1}. GC–MS (EI, +, 70 eV): *m/z* (%) = 301 (1) [M – H]$^+$, 271 (1) [M – OCH_3]$^+$, 91 (71) $[C_7H_8]^+$. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.03–2.10 (m, 3 H, 2a-H_B, 2b-H_A, 2b-H_B), 2.21 (ddd, *J* = 13.4, 6.8, 1.7 Hz, 1 H, 2a-H_A), 2.45 (s, 6 H, Me_A, Me_B), 3.23 (s, 3 H, OMe_B), 3.34 (s, 3 H, OMe_A), 4.02–4.13 (m, 6 H, 5-H_A, 5-H_B, 4-H_A, 4-H_B), 4.20 (ddd, *J* = 4.06, 4.02, 1.7 Hz, 1 H, 3-H_A), 4.43 (ddd, *J* = 6.6, 6.5 Hz, 3.4 Hz, 1 H, 3-H_B), 5.01–5.04 (m, 2 H, 1-H_A, 1-H_B), 7.33–7.37 (m, 4 H, 3′-H_A, 3′-H_B), 7.76–7.83 (d, *J* = 8.3 Hz, 4 H, 2′-H_A, 2′-H_B) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 21.7 (Me), 41.0 (C-$2_{A/B}$), 41.4 (C-$2_{A/B}$), 55.0 ($OMe_{A/B}$), 55.1 ($OMe_{A/B}$), 69.4 (C-1′$_{A/B}$), 70.1 (C-1′$_{A/B}$), 72.7 (C-$3_{A/B}$), 72.8 (C-$3_{A/B}$), 83.0 (C-5/C-4), 84.6 (C-5/C-4), 106.4 (C-$1_{A/B}$), 106.7 (C-$1_{A/B}$), 128.0 (C-2′), 130.0 (C-3′), 145.1 (C-4′) ppm. HRMS (ESI, +): *m/z* (%) calcd for $C_{13}H_{18}O_6SNa$ [M + Na]$^+$: 325.07218; found: 325.07163. D-**13** $[\alpha]_D$ +30 (*c* 0.6, $CHCl_3$). L-**13** $[\alpha]_D$ –31.5 (*c* 0.9, $CHCl_3$).

(16) **7-{[(3*S*,4*R*)-1,3-Dihydroxytetrahydrofuran-4-yl]methoxy}-4-methyl-2*H*-chromen-2-one (D-5) and 7-{[(3*R*,4*S*)-1,3-Dihydroxytetrahydrofuran-4-yl]methoxy}-4-methyl-2*H*-chromen-2-one (L-5)**

Compound D-**13** (7.00 g, 23.2 mmol) was dissolved in DMF (70 mL). K_2CO_3 (7.00 g, 50.6 mmol) and 4-methylumbelliferone (5.30 g, 30.0 mmol, 1.3 equiv) were added. The reaction was stirred for 16 h at 75 °C, then quenched with H_2O (70 mL), extracted with EtOAc and washed with 0.1 M NaOH. The organic phase was dried over $MgSO_4$ and filtered. The solvent was evaporated. The crude product was dissolved in MeCN–H_2O (150 mL, 1:3), mixed with DOWEX 50WX 8-100 (4.5 g), stirred for 1.5 h, and stored for 2 d at r.t. Resulting MeOH was evaporated. The solution was filtered, and the solvent was evaporated. The crude product was purified by column chromatography (gradient PE–EtOAc, 70:30 → 40:60 → 10:90 → 0:100) yielding 6.40 g (21.9 mmol, 88%, dr A/B 0.8:0.2) D-**5**. Analogously 115 mg (0.38 mmol) of L-**13** was converted into 97 mg (0.33 mmol, 87%, dr A/B 0.8:0.2) L-**5**. R_f = 0.09 (*n*-pentane–EtOAc, 20:80). IR (film): ν = 3450, 2928, 1736, 1611, 1366, 1216 cm^{-1}. GC–MS (EI, +, 70 eV): *m/z* (%) = 293 (100) [M + H]$^+$, 246 (10) $[C_{14}H_{14}O_4]^+$. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.17–2.21 (m, 1 H, 2′a-H_B), 2.24 (ddd, *J* = 13.9, 5.9, 4.6 Hz, 1 H, 2′a-H_A), 2.32–2.37 (m, 2 H, 2′b-$H_{A,B}$), 2.39 (d, *J* = 1.5 Hz, 6 H, $Me_{A,B}$), 3.99 (dd, *J* = 10.0, 4.7 Hz, 1 H, 5′a-H_A), 4.06 (dd, *J* = 10.0, 4.8 Hz, 1 H, 5′b-H_A), 4.18 (dd, *J* = 5.3, 5.3 Hz, 1 H, 5′a-H_B), 4.26 (dd, *J* = 5.3, 4.9 Hz, 1 H, 5′b-H_B), 4.42 (ddd, *J* = 5.9, 1.5, 1.4 Hz, 1 H, 3′-H_A), 4.47 (ddd, *J* = 4.6, 4.5, 2.1 Hz, 1 H, 3′-H_B), 4.59 (ddd, *J* = 4.8, 4.7, 1.5 Hz, 1 H, 4′-H_A), 4.64 (ddd, *J* = 5.3, 4.9, 4.6 Hz, 1 H, 4′-H_B), 5.66 (dd, *J* = 4.6, 0.2 Hz, 1 H, 1′-H_A), 5.67 (dd, *J* = 5.6, 2.5 Hz, 1 H, 1′-H_B), 6.14 (d, *J* = 1.5 Hz, 2 H, 3-$H_{A,B}$), 6.79 (d, *J* = 2.6 Hz, 2 H, 8-$H_{A,B}$), 6.85 (dd, *J* = 8.8, 2.6 Hz, 1 H, 6-H_A), 6.89 (dd, *J* = 8.8, 2.6 Hz, 1 H, 6-H_B), 7.48 (d, *J* = 8.8 Hz, 2 H, 5-$H_{A,B}$) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 18.8 (Me), 41.6 (C-2′), 68.8 (C-5′), 73.5 (C-4′), 85.4 (C-3′), 99.8 (C-1′), 101.8 (C-8), 112.4 (C-3), 112.6 (C-6), 114.1 (C-4a), 125.8 (C-5), 152.7 (C-8a), 155.3 (C-7), 161.4 (C-2), 161.6 (C-4) ppm. HRMS (ESI, +): *m/z* (%) calcd for $C_{15}H_{17}O_6$ [M + H]$^+$: 293.10251; found: 293.10190. D-**5** $[\alpha]_D$ +30 (*c* 0.6, $CHCl_3$). L-**5** $[\alpha]_D$ –42.4 (*c* 0.23, $CHCl_3$).

(17) **7-[(3*S*,4*R*)-3-Hydroxy-1-methoxytetrahydrofuran-4-yl]methoxy-4-methyl-2*H*-chromen-2-one (14)**

Compound D-**13** (10 g, 33.1 mmol) was dissolved in DMF (25 mL). K_2CO_3 (2.5 g) and 4-methylumbelliferone (7.58 g, 43 mmol, 1.3 equiv) were added. The reaction was stirred for 16 h at 75 °C, then quenched with H_2O, extracted with EtOAc, and washed with 0.1 M NaOH. The organic phase was dried over $MgSO_4$, and the solvent was evaporated. The crude product was purified by column chromatography (*n*-pentane–EtOAc, 50:50), yielding 8.33 g (27.2 mmol, 82%, dr A/B = 0.4:0.6) of **14** as a colorless oil. R_f = 0.33 (*n*-pentane–EtOAc, 20:80). IR (film): ν = 3586, 3322, 3093, 2923, 2838, 1709, 1613, 1389, 1369, 1294, 1150, 1069, 1027, 971, 838 cm^{-1}. GC–MS (EI, +, 70 eV): *m/z* (%) = 133 (10) $[C_5H_9O_4]^+$, 147 (8) $[C_6H_{11}O_4]^+$. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.15 (ddd, *J* = 13.4, 6.4, 5.4 Hz, 1 H, 2′a-H_A), 2.22 (ddd, *J* = 13.8, 6.4, 4.6 Hz, 2 H, 2′a-H_B, 2′b-H_B), 2.32 (ddd, *J* = 13.4, 6.4, 1.8 Hz, 1 H, 2′b-H_A), 2.39 (d, *J* = 1.3 Hz, 3 H, $Me_{A,B}$), 3.33 (s, 3 H, OMe_A), 3.42 (s, 3 H, OMe_B), 4.03 (dd, *J* = 10.0, 4.5 Hz, 1 H, 5′a-H_B), 4.07–4.13 (m, 3 H, 5′a-H_A, 5′b-H_A, 5′b-H_B), 4.26 (ddd, *J* = 6.4, 6.4, 4.4 Hz, 1 H, 3′-H_A), 4.30 (ddd, *J* = 6.4, 1.6, 1.5 Hz, 1 H, 3′-H_B), 4.43 (ddd, *J* = 4.6, 4.5, 1.6 Hz, 1 H, 4′-H_B), 4.57 (ddd, *J* = 6.7, 6.6, 4.4 Hz, 1 H, 4′-H_A), 5.13 (dd, *J* = 5.4, 1.8 Hz, 1 H, 1′-H_A), 5.16 (dd, *J* = 4.6, 0.2 Hz, 1 H, 1′-H_B), 6.13 (d, *J* = 1.3 Hz, 2 H, 3-$H_{A,B}$), 6.81 (d, *J* = 2.5 Hz, 1 H, 8-H_B), 6.84 (d, *J* = 2.5 Hz, 1 H, 8-H_A), 6.86 (dd, *J* = 8.8, 2.5 Hz, 1 H, 6-H_B), 6.89 (dd, *J* = 8.8, 2.5 Hz, 1 H, 6-H_A), 7.48 (d, *J* = 8.8 Hz, 1 H, 5-H_B), 7.49 (d, *J* = 8.8 Hz, 1 H, 5-H_A) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 18.8 (Me), 41.2/41.7 (C-2′), 55.2/55.3 (OMe), 68.8/69.9 (C-5′), 73.0/73.3 (C-4′), 83.8/85.4 (C-3′), 101.7/101.8 (C-1′), 105.5/105.8 (C-8), 112.3/112.4 (C-3), 112.7 (C-6), 114.0/114.1 (C-4a), 125.7 (C-5), 152.6/152.7 (C-8a), 155.2/155.3 (C-7), 161.3/161.4 (C-2), 161.7/161.8 (C-4) ppm. HRMS (ESI, +): *m/z* (%) calcd for $C_{16}H_{20}O_6$ [M + H]$^+$: 307.11761; found: 307.11762. $[\alpha]_D$ +29.8 (*c* 1.0, $CDCl_3$).

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(19) **7-[(4*R*)-1-Methoxy-3-oxotetrahydrofuran-4-yl]methoxy-4-methyl-2*H*-chromen-2-one (15)**

To a solution of DMP (10.4 g, 24.5 mmol, 1.5 equiv) in CH_2Cl_2 (300 mL) compound **14** (5 g, 16.3 mmol) dissolved in CH_2Cl_2 (25 mL) were added. The reaction was stirred for 2 h at r.t. before it was quenched with 1 M $Na_2S_2O_3$ solution (100 mL). Sat. $NaHCO_3$ solution (100 mL) was added, and the reaction was stirred until both phases were clear. The mixture was diluted with CH_2Cl_2 and H_2O, the phases were separated, and the aqueous layer was extracted with CH_2Cl_2. The organic phases were combined, dried over $MgSO_4$, and the solvent was evaporated. The crude product was purified by column chromatography (PE–EtOAc, 60:40) yielding 3.97 g (13.1 mmol, 80%, dr A/B 0.7:0.3) of product **15**. R_f = 0.49 (PE–EtOAc, 50:50). IR (film): ν = 3079, 2939, 2359, 1752, 1707, 1610, 1391, 1268, 1087, 1063, 1016, 982, 953, 833, 806 cm^{-1}. GC–MS (EI, +, 70 eV): *m/z* (%) = 58 (100)

$[C_3H_6O]^+$. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.38 (d, *J* = 1.19 Hz, 3 H, Me_B), 2.39 (d, *J* = 1.28 Hz, 3 H, Me_B), 2.50 (dd, *J* = 18.21, 0.32 Hz, 1 H, 2′a-H_B), 2.52 (dd, *J* = 18.14, 1.12 Hz, 1 H, 2′a-H_A), 2.74 (dd, *J* = 18.21, 5.61 Hz, 1 H, 2′b-H_B), 2.83 (dd, *J* = 18.14, 5.58 Hz, 1 H, 2′b-H_A), 3.42 (s, 3 H, OMe_A), 3.48 (s, 3 H, OMe_B), 4.15 (dd, *J* = 10.3, 7.27 Hz, 1 H, 4′-H_A), 4.26–4.34 (m, 4 H, 5′a-H_A, 5″a-H_B, 5″b-H_B, 4′-H_B), 4.44 (dd, *J* = 7.27, 3.17 Hz, 1 H, 5′b-H_A), 5.40 (dd, *J* = 5.58, 1.12 Hz, 1 H, 1′-H_A), 5.43 (dd, *J* = 5.61, 0.32 Hz, 1 H, 1′-H_B), 6.14 (m_c, 2 H, 3-$H_{A,B}$), 6.83 (d, *J* = 2.88 Hz, 1 H, 8-H_B), 6.84 (d, *J* = 2.42 Hz, 1 H, 8-H_A), 6.84 (dd, *J* = 8.67, 2.88 Hz, 1 H, 6-H_B), 6.90 (dd, *J* = 8.84, *J* = 2.42, 1 H, 6-H_A), 7.48 (d, *J* = 8.67 Hz, 1 H, 5-H_B), 7.50 (d, *J* = 8.84 Hz, 1 H, 5-H_A) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 18.7 (Me), 43.6 (C-2′$_A$), 43.7 (C-2′$_B$), 55.2 (OMe_A), 55.3 (OMe_B), 67.2 (C-4′$_B$), 69.5 (C-4′$_A$), 76.1 (C-5′$_B$), 78.0 (C-5′$_A$), 101.6 (C-8$_B$), 101.9 (C-8$_A$), 101.9 (C-1′$_B$), 102.7 (C-1′$_A$), 112.3 (C-3$_A$), 112.4 (C-3$_A$), 112.5 (C-4a$_{A,B}$), 114.1 (C-6$_A$), 114.2 (C-6$_B$), 125.6 (C-5$_B$), 125.7 (C-5$_A$), 152.5 (C-4$_{A,B}$), 155.1 (C-8a$_B$), 155.2 (C-8a$_A$), 161.2 (C-7$_{A,B}$), 161.3 (C-2$_B$), 161.4 (C-2$_A$), 210.6 (C-3′$_A$), 210.8 (C-3′$_B$) ppm. HRMS (ESI, +): *m/z* (%) calcd for $C_{16}H_{18}O_6$ [M + H]$^+$: 305.10196; found: 305.10199. $[\alpha]_D$ +44.5 (*c* 1.1, MeCN).

(20) **7-[(3*R*,4*R*)-3-Hydroxy-1-methoxytetrahydrofuran-4-yl]methoxy-4-methyl-2*H*-chromen-2-one (16)**

Compound **15** (3.97 g 13.1 mmol) was dissolved in EtOH–H_2O (1:1) and cooled to 0 °C. $NaBH_4$ (1.23 g, 32.7 mmol, 2.5 equiv) was added, and the reaction was stirred for 1 h at r.t. The reaction was quenched with a sat. NH_4Cl solution and extracted with $CHCl_3$. The organic phases were washed with H_2O, dried over $MgSO_4$, and the solvent was evaporated. The diastereomers (dr 1:1) could be separated by column chromatography (PE–EtOAc, 50:50); 1.45 g (4.73 mmol, 36%) of the desired diastereomer **16** were isolated. R_f = 0.17 (PE–EtOAc, 50:50). IR (film): ν = 3395, 3060, 2946, 1716, 1609, 1370, 1208 cm^{-1}. GC–MS (EI, +, 70 eV): *m/z* (%) = 133 (19) [$(C_5H_9O_4)^+$], 147 (8) [$(C_6H_{11}O_4)^+$]. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.13–2.21 (m, 2 H, 2′-H), 2.37 (d, *J* = 1.2 Hz, 3 H, Me), 2.96 (d, *J* = 10.9 Hz, 1 H, 3′-OH), 3.38 (s, 3 H, OMe), 4.19 (dd, *J* = 9.7, 6.8 Hz, 1 H, 5′a-H), 4.34 (ddd, *J* = 6.8, 4.1 Hz, 1 H, 4′-H), 4.36–4.41 (m, 2 H, 5′b-H, 3′-H), 5.11 (dd, *J* = 4.0 Hz, 1 H, 1′-H), 6.10 (q, *J* = 1.2 Hz, 1 H, 3-H), 6.87 (d, *J* = 2.5 Hz, 1 H, 8-H), 6.90 (dd, *J* = 8.8, 2.5 Hz, 1 H, 6-H), 7.48 (d, *J* = 8.8 Hz, 1 H, 5-H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 18.8 (Me), 41.5 (C-2′), 55.3 (OMe), 69.0 (C-5′), 71.6 (C-3′), 82.5 (C-4′), 101.9 (C-8), 105.5 (C-1′), 112.2 (C-3), 112.6 (C-6), 113.9 (C-4a), 125.7 (C-5), 152.6 (C-4), 155.3 (C-8a), 161.4 (C-7), 161.9 (C-2) ppm. HRMS (ESI, +): *m/z* (%) calcd for $C_{16}H_{20}O_6$ [M + H]$^+$: 307.11761; found: 307.11769. $[\alpha]_D$ –67.4 (*c* 1.0, $CHCl_3$).

(21) **7-{[(3*R*,4*R*)-1,3-Dihydroxytetrahydrofuran-4-yl]methoxy}-4-methyl-2*H*-chromen-2-one (6)**

Compound **15** (980 mg, 3.2 mmol) was dissolved in MeCN–H_2O (3:1). DOWEX 50WX 8-100 (130 mg) was added, and the reaction was stirred for 1.5 h at r.t. Resulting MeOH was evaporated, and the mixture was stored at r.t. for 2 d. The solution was filtered, and the solvent was evaporated. The crude product was purified by column chromatography (PE–EtOAc, 70:30) yielding 544 mg (1.86 mmol, 58%, dr A/B 0.7:0.3) of product **6** as a white solid. R_f = 0.06 (PE–EtOAc, 50:50). IR (film): ν = 3356, 1705, 1609, 1364, 1207 cm^{-1}. GC–MS (EI, +, 70 eV): *m/z* (%) = 293 (100) [M + H]$^+$, 246 (12) $[C_{14}H_{14}O_4]^+$. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.19 (ddd, *J* = 13.8, 4.6, 4.6 Hz, 1 H, 2′a-H_A), 2.23–2.32 (m, 3 H, 2′b-H_A, 2′a-H_B, 2′b-H_B), 2.40 (d, *J* = 1.3 Hz, 6 H, $Me_{A,B}$), 4.28 (dd, *J* = 10.0 Hz, *J* = 6.2 Hz, 1 H, 5′a-H_B), 4.30–4.34 (m, 3 H, 5′a-H_B, 5′a-H_A, 5′b-H_A), 4.35 (dd, *J* = 6.9, 3.9 Hz, 1 H, 3′-H_B), 4.42 (d, *J* = 8.6, 4.6 Hz, 1 H, 3′-H_A),4.50–4.54 (m, 1 H, 4′-H_A), 4.69 (ddd, *J* = 6.2, 3.9, 3.0 Hz, 1 H, 4′-H_B), 5.61 (dd, *J* = 4.6, 0.3 Hz, 1 H, 1′-H_A), 5.78 (dd, *J* = 5.4, 3.3 Hz, 1 H, 1′-H_B), 6.14 (q, *J* = 1.3 Hz, 2 H, 3-$H_{A,B}$), 6.89 (d, *J* = 2.5 Hz, 2 H, 8-$H_{A,B}$), 6.91 (dd, *J* = 8.7, 2.5 Hz, 2 H, 6-$H_{A,B}$), 7.50 (d, *J* = 8.7 Hz, 2 H, 5-$H_{A,B}$) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 18.8 (Me), 42.1 (C-2′), 68.8 (C-5′), 71.9 (C-4′), 82.3 (C-3′), 99.4 (C-1′), 102.0 (C-8), 112.4 (C-3), 112.5 (C-6), 114.1 (C-4a), 125.8 (C-5), 152.7 (C-8a), 155.3 (C-7), 161.4 (C-2), 161.8 (C-4) ppm. HRMS (ESI, +): *m/z* (%) calcd for $C_{16}H_{20}O_6$ [M + H]$^+$: 293.10196; found: 293.10218. $[\alpha]_D$ +11.5 (*c* 0.2, MeCN).

(22) **Protocol of the Fluorogenic Assay**

Triethanolamine buffer (120 µL, 0.1 M, pH 7.0), the specific substrate (10 µL of a 10 mM solution), and BSA (10 µL of a solution 40 mg/mL in H_2O) were pipetted in a 96-well microtiterplate. Cell-free crude lysate (60 µL) was added and then directly measured with a GENios Microtiterplate Reader (Tecan, Switzerland) for 1–2 h, applying an excitation wavelength of 340 nm and an emission wavelength of 460 nm with a band-width of 5 nm. For calibration a solution of 4-methylumbelliferone in MeCN was prepared and measured in triethanolamine buffer (using 0–2.5 nmol 4-methylumbelliferone). The calibration curve is shown in the Supporting Information.

(23) **Protein Expression**

Genes, coding for DERA (de °C) from *P. aerophilum*, *T. maritima* and *C. psychrerythraea* were ordered as synthetic genes from GenScript, followed by cloning into the pET-21a(+)-vector. For *S. halifaxensis*, the corresponding gene could be isolated from genomic DNA, whereas de °C genes from *E. coli* and *R. erythropolis* were isolated previously.[24] The proteins were expressed in *E. coli* BL21(DE3) strain, using TB medium. Expression was started by applying 0.1 mM IPTG, and cells were harvested after 16 h (24 h for psychrophilic enzymes) incubation at 25 °C (18 °C); 1 g cells were resuspended in potassium phosphate (KP_i) buffer (5 mL, 20 mM, pH 7). The cells were disrupted via sonification, and after centrifugation (15 min at 12000 × g) the supernatant was taken for kinetic measurements. A SDS-gel of all used proteins is shown in the Supporting Information.

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