Stereoselective synthesis and sialidase inhibition properties of KDO-based glycosyloxathiins

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Dedicated to Professor Pierre Vogel on the occasion of his 70th anniversary

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Abstract

The stereoselective synthesis of KDO-based glyco-1,4-oxathiins is described. Relying on a totally diastereoselective inverse electron demand hetero Diels-Alder, α,α′-dioxothiones as electron-poor heterodienes, and glycals as electron-rich dienophiles, reacted to give, in high yield, the KDO-based glyco derivatives **11** and **12a-c**. Taking into account their structural features, biological tests have been run to evaluate the properties of **11** and **12a** as sialidase inhibitors. The synthetic and biological data reported confirmed the versatility of this powerful [4+2] cycloaddition and showed the KDO-based cycloadduct **11** as attractive scaffold for the development of new sialidase inhibitors.

Keywords: Hetero Diels-Alder, KDO, exo-glycal, α,α′-dioxothiones, sialidase inhibitors, selective synthesis

Introduction

Pericyclic reactions represent one of the most powerful tools in synthetic chemistry. These reactions have been widely employed to obtain regio- and stereoselectively complex molecules with high atom economy degree. Among these, the inverse electron demand [4+2] Diels-Alder reactions (iEDDA) gained a great deal of attention[1-4] proving to be of pivotal importance for the synthesis of complex bioactive molecules and natural products[5-6].

In 2008[7] iEDDA were suggested as possible metal-free click reactions. In the same year, Fox[8] *et al.* proposed iEDDA as bioorthogonal ligation reactions for the bioconjugation of proteins. As matter of fact, they can proceed fast and in high yield, in aqueous or cell lysate

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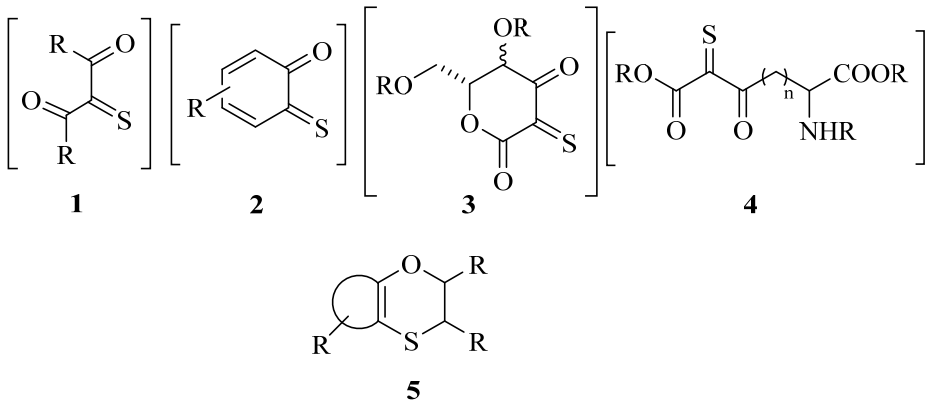
media and are compatible with many functional groups commonly occurring in biological substrates. More recently,[9-10] iEDDA have also been employed in the synthesis of radioimmunoconjugates and radiolabelled antibodies have been successfully prepared using norbornene-conjugated antibodies as dienophile and radiolabelled tetrazine as diene.

The huge number of reagents employed in iEDDA also includes heteroatom-containing dienes or dienophiles (inverse electron demand hetero-Diels-Alder, iHDA) to form the corresponding heterocyclic cycloadducts, important intermediates in the synthesis of natural and pharmacologically active products.[11-13]

From a mechanistic point of view, extensive kinetic and computational studies on iEDDA have been reported,[14-18] confirming the involvement of the HOMO orbital of the dienophile and the LUMO orbital of the diene.

In this context, in the last two decades, we successfully developed highly selective iHDA employing α,α′-dioxothiones as original electron-poor heterodienes (Scheme 1). These latter were aliphatic (**1**),[19] aromatic (**2**)[20] or saccharidic (**3**),[21-22] in addition we also prepared dioxothiones containing an aminoacid fragment (**4**).[23] α,α′-Dioxothiones are highly reactive species which can be generated in the presence of weak bases, under very mild conditions (room temperature or 40-60 °C) and trapped *in situ* with electron-rich dienophiles to undergo chemo-, regio- and steroselective iHDA to form the corresponding 5,6-dihydro-1,4-oxathiin derivatives **5**.



Scheme 1. Structure of α,α′-oxothiones **1**-**4** and of oxathiin **5**.

Relying on the stereochemical outcomes of these iHDA,[12,24] the proper selection of dienes and dienophiles allowed us to obtain an array of synthetically and biologically attracting molecules. In particular, focusing on the use of saccharidic dienophiles, we recently prepared diasteromerically pure glycosyl derivatives of relevant biological interest.[25-29] As matter of fact, α,α′-dioxothiones **1a** and **4a** were successfully employed in iHDA with exo-glycal **6**[30] and 1-galactal **7** to prepare respectively cycloadducts **8**[30] and **9**,[28] the first spiro sialyl derivative, the second a mimetic of the mucins Tn antigen. In addition, the micromolar water soluble matrix metalloproteinases inhibitors **10**[25] were formed by iHDA reaction of D(+) glucal with the thione **4b**, obtained from aspartic acid. (Scheme 2).

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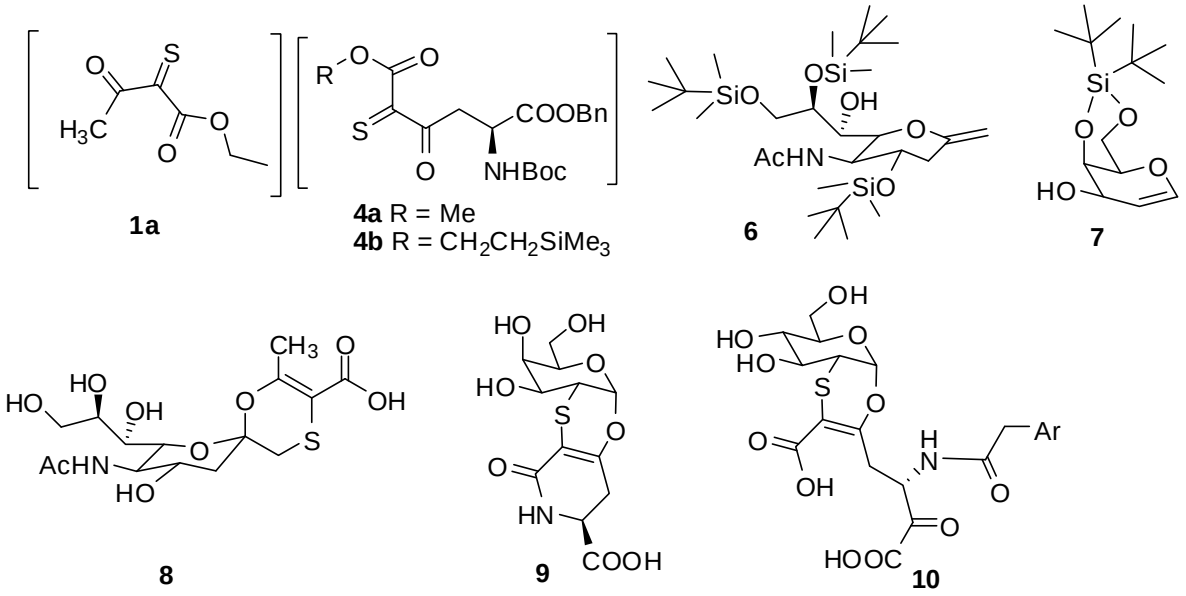
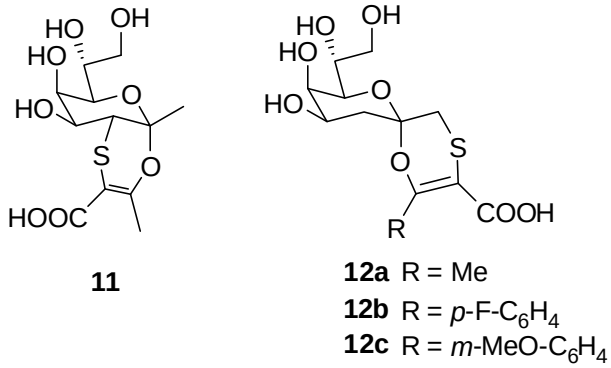


Scheme 2. Structure of electron-poor dienes **1a** and **4a-b**, electron-rich dienophiles **6** and **7** and of glycosyl derivatives **8**, **9** and **10**

We reported herein on the extension of this class of iHDA to KDO-related glycals as electron-rich dienophiles, to prepare the diasteromerically pure KDO-related glycosides **11** and **12a-c** (Scheme 3). The biological activity of **11** and **12a** as sialidase inhibitors was also investigated.



Scheme 3. Structures of the KDO-related glycosides **11** and **12a-c.**

Results and Discussion

Cycloadducts **12a-c** were prepared by iHDA reactions employing the KDO-based exo-glycal **13**[31] (Scheme 4) as electron-rich dienophile and α,α′-dioxothiones **1a-c**[30] as electron poor dienes. All the reactions were totally chemo- regio- and diasteroselective. The heterodienes **1a-c**

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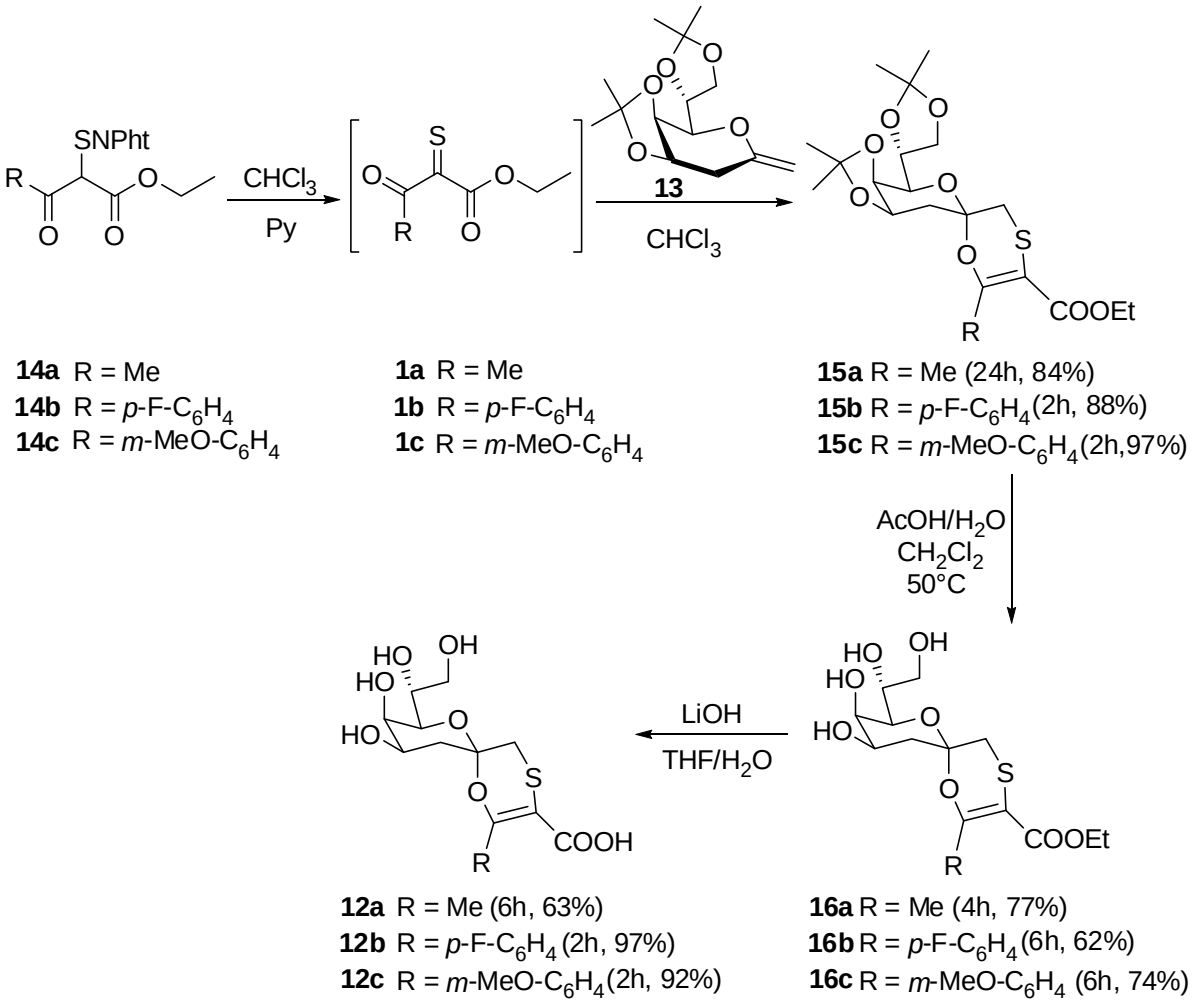
(Scheme 4), in turn, were obtained by the mild base treatment (pyridine, room temperature) of the parent sulfenyl derivatives **14a-c**, as previously reported.[30]



14a R = Me
14b R = *p*-F-C_6H_4
14c R = *m*-MeO-C_6H_4

1a R = Me
1b R = *p*-F-C_6H_4
1c R = *m*-MeO-C_6H_4

15a R = Me (24h, 84%)
15b R = *p*-F-C_6H_4 (2h, 88%)
15c R = *m*-MeO-C_6H_4 (2h,97%)

12a R = Me (6h, 63%)
12b R = *p*-F-C_6H_4 (2h, 97%)
12c R = *m*-MeO-C_6H_4 (2h, 92%)

16a R = Me (4h, 77%)
16b R = *p*-F-C_6H_4 (6h, 62%)
16c R = *m*-MeO-C_6H_4 (6h, 74%)

Scheme 4. Synthesis of the cycloadducts **12a-c.**

The highly reactive dienes **1a-c** were generated *in situ* in the presence of the dienophile **13** and afforded the corresponding cycloadducts **15a-c** in high yield (84-97%) (Scheme 4) and as single diasteroisomers. As expected, all cycloadducts (**15a-c**) were formed as the α-isomer, that is the isomer obtained from the preferred attack of the thiones **1a-c** to the bottom face of the dienophile **13**.[24,26] A matter of fact, all the iHDAs were totally chemo- regio- and stereoselective according with our previous data obtained and reported[26] employing **13** as electron-rich dienophile. Hydrolysis of the isopropylidene protecting groups of **15a-c** was accomplished in good yield (62-77%, **16a-c**) by treating **15a-c** with a 1.5/1 (v/v) mixture of acetic acid and water at 50°C. The final deprotection of the carboxylic residues of **16a-c** (Scheme 4) with lithium hydroxide (1M in H_2O) in THF as solvent, provided the desired glycosyl derivatives **12a-c**.

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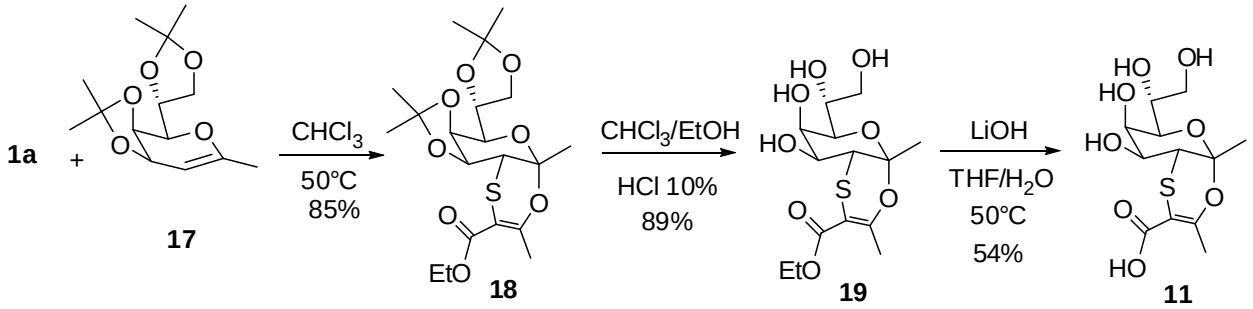
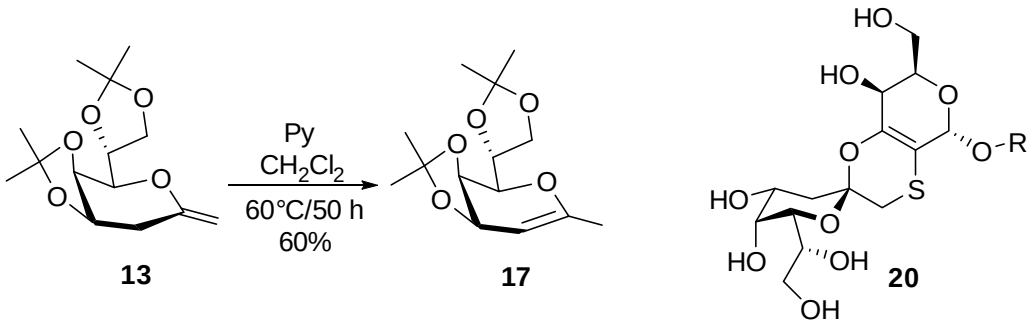
Analogously, the synthesis of the glycosyl derivative **11** was accomplished by an iHDA reaction between the α,α′-dioxothione **1a** and the glycal **17** (Scheme 5).



Scheme 5. Synthesis of cycloadduct **11**.

Glycal **17** (Scheme 6) was easily prepared by heating the exo-glycal **13** to 60°C for 50 h, in a 1.6/1 mixture of CH_2Cl_2/pyridine as solvent.



Scheme 6. Synthesis of glycal **17** and structure of the GM_3 lactone mimetic, **20**.

The iHDA of **1a** with **17** (Scheme 5) was performed in a 1/2 mixture of $CHCl_3$/pyridine at 50°C and the cycloadduct **18**, was formed as pure α isomer and in high yield (85%). The analysis of the ^{1}H NMR spectra of **18** allowed us to ascertain that also in this case, the electron-poor diene (**1a**) preferentially attacked the lower face of the dienophile (**17**) affording the thermodynamically more stable α-*O*-glycosyl derivative ($J_{3\text{-}4}$ 8.4 Hz). Furthermore, the value of the chemical shift of H-3 (2.78 ppm) confirmed the regioselectivite formation of the C_3-S linkage.[28]

Hydrolysis of the isopropylidene protecting groups of **18** with a solution of HCl (10% in EtOH), followed by the deprotection of the carboxylic residue of **19** with lithium hydroxide (1M in H_2O) in THF as solvent, provided the desired glycosyl derivative **11** (Scheme 5).

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Capitalizing on the biological issues recently reported[27] for **20** (see Scheme 6), a tricyclic thio-mimetic of the melanoma antigen GM_3 lactone ganglioside, characterized by the replacement of the native sialic acid moiety with a KDO-related residue, and taking into account the structural analogy of KDO-like fragment of compound **20** with that of derivatives **11** and **12a-c**, we investigated the potential activity of **11** and **12a** as inhibitors of influenza virus sialidase. The sialidase enzyme on influenza virus, recognizing sialic acid and its derivative, plays a major role in the virus life cycle by facilitating the release of virus progeny from the infected cells.[32]

To this end, we measured drug inhibition of the sialidase activity of A/Mississippi/3/2001 wild type H1N1 influenza virus[33] by the MUNANA based fluorescent assay[34] as previously described.[35] Both compounds **11** and **12a** did inhibit the enzyme activity of the H1N1 virus, but compared to zanamivir, the inhibition curves obtained for compounds **11** and **12a** showed unusual steep shifts in percent inhibition over a very narrow range. Because of this, a narrower range of dilutions was used to more accurately determine their IC_{50}s, (10, 100, 300, 600, 800, 1000, 3000, 10,000 for compound **11** and 10, 100, 300, 1000, 2000, 3000, 10,000 for compound **12a**).The IC_{50}s thus assessed were 760 μM for compound **11** and 1880 μM for **12a**, compared to 2.2 nM for zanamivir.

Conclusions

Herein we reported the extension of a powerful diasteroselective iHDA to the synthesis of KDO-based glycosyloxathiins **11** and **12a-c** (Scheme 3) efficiently prepared in few steps reacting two KDO-based enitols as electron-rich dienophiles. The data obtained confirmed that this peculiar class of iHDA is an efficient and versatile access to structurally heterogeneous diasteropure constructs.

Keeping in mind the recursively pandemic influenza A infections as well as the reported virus resistance to commonly used drugs like oseltamivir which make the discovery of new anti-influenza drugs compelling, the sialidase inhibition properties of **11** and **12a** were evaluated. The enzyme inhibition tests were carried out on the A/Mississippi/03/01 H1N1 virus sialidase, showing that both compounds, **11** and **12a**, inhibited the enzyme activity. In particular, compound **11** was about 2-fold more effective than **12a**. The striking steep inhibition curves obtained likely reflect a difference in the binding interactions of **11** and **12a** *vs.* the influenza NA active site with respect to zanamivir. In conclusion, though sensibly less effective than the golden standard zanamivir, the KDO-related glycosyloxathiin **11** inhibits sialidase and is characterized by an original and multifunctional scaffold helpful to develop a new generation of drugs. Structural modifications and further binding profile investigations are underway.

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Experimental Section

General. All solvents were of reagent grade quality and purchased commercially. All starting materials were purchased commercially and used without further purification. All NMR spectra were recorded on Varian instruments (200 or 300 MHz) and Mercury 400 instruments. The NMR spectra were referenced to solvent. Mass spectra were recorded on a LCQ-FLEET ion trap Thermo Fisher. ESI-MS analysis was performed both in positive or negative/ion mode. HRMS were performed on a LTQ-IT-Orbitrap with a spray voltage of 2.10 kV and a resolution of 100,000. Optical rotation measurements were carried out with a Jasco DIP-370 polarimeter.
Elementary analysis experiments of purified products were performed with an Elementary Analyzer 2400 Serie II Perkin-Elmer. Complete signal assignments from 1D and 2D NMR were based on COSY, HSQC correlations. The A/Mississippi/3/2001 wild type H1N1 influenza virus[34] was used to evaluate susceptibility in the enzyme inhibition assays. Zanamivir was provided by GlaxoSmithKline (Stevenage, UK)

Synthesis of compound 15a. To a solution of **13**[31] (0.168 g, 0.617 mmol) in dry pyridine (4.0 mL), **14a**[30] (0.208 mg, 0.68 mmol) was added. The mixture was warmed to 40°C and stirred for 6 h. After this time **14a** (0.208 mg, 0.68 mmol) was added and the mixture was stirred at 40°C for 18 h. The reaction mixture was cooled to rt, diluted with CH_2Cl_2 (50 mL) and washed with a saturated solution of NH_4Cl (2 × 10 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness to give a crude which was purified by flash column chromatography on silica gel (AcOEt:Petroleum Ether, 1:6) to give **15a** (0.222 g, 84%) as a yellow oil. $[\alpha]_{25}^{D}$ + 5.9 (*c* 0.44, CH_2Cl_2); ^{1}H NMR (400MHz, $CDCl_3$): δ 4.58 (dt, $J_{3\text{-}2a}$ =$J_{3\text{-}2b}$ 7.6 Hz, $J_{3\text{-}4}$ 3.2 Hz, 1H, H-3), 4.35 (dd, $J_{4\text{-}3}$ 7.6 Hz, $J_{4\text{-}5}$ 2.0 Hz, 1H, H-4), 4.29-4.24 (m, 1H, H-6), 4.19 (q, *J* 7.2 Hz, 2H, CH_2CH$_3$), 4.06-4.03 (A part of an ABX system, J_{AB} 9.2 Hz, J_{AX} 6.4 Hz, 1H, H-7a), 3.85-3.81 (B part of an ABX system, J_{BA} 8.8 Hz, J_{BX} 4.4 Hz, 1H, H-7b), 3.66 (dd, $J_{5\text{-}6}$ 7.6 Hz, $J_{5\text{-}4}$ 1.6 Hz, 1H, H-5), 3.07-3.04 (A part of an AB system, J_{AB} 12.8 Hz, H-1′a), 2.82-2.79 (B part of an AB system, J_{BA} 12.8 Hz, 1H, H-1′b), 2.45-2.40 (A part of an ABX system, J_{AB} 15.6 Hz, J_{AX} 3.2 Hz, 1H, H-2a), 2.29 (s, 3H, CH_3C=), 2.00-1.95 (B part of an ABX system, J_{BA} 15.6 Hz, J_{BX} 3.2 Hz, 1H, H-2b), 1.44 (s, 3H, (CH_3)$_2$C), 1.39 (s, 3H, (CH_3)$_2$C), 1.34 (s, 6H, (CH_3)$_2$C), 1.29 (t, *J* 6.8 Hz, 3H, CH$_2$CH_3); ^{13}C NMR (100MHz, $CDCl_3$): δ 164.9, 157.3, 109.3, 109.2, 97.7, 94.8, 73.3, 72.0, 70.5, 66.9, 60.8, 34.6, 34.0, 26.8, 26.2, 25.2, 25.0, 21.7, 14.3; ESI-MS: *m/z* 453.17 [M + Na]$^+$; Anal. Calcd for $C_{20}H_{30}O_8S$ (430.16): C, 55.80; H, 7.02%. Found: C, 55.82; H, 7.06%.
Synthesis of compound 16a. To a stirred solution of **15a** (0.102 g, 0.237 mmol) in glacial AcOH (3.5 mL), H_2O (1.5 mL) was slowly added. The reaction mixture was warmed to 50°C and stirred for 4 h. After this time, the solvent was co-evaporated with toluene under reduced pressure to give a crude which was purified by flash column chromatography on silica gel (AcOEt:MeOH, 10:1) to give **16a** (0.064 g, 77%) as a white solid. $[\alpha]_{25}^{D}$ + 94.6 (c 0.465, MeOH); mp: 134-136°C; ^{1}H NMR (400MHz, CD_3OD): δ 4.25-4.17 (m, 2H, CH_2CH_3), 4.12-4.06 (m, 2H, H-3, H-4), 3.92-3.87 (m, 1H, H-6), 3.77-3.73 (A part of an ABX system, J_{AB} 11.6 Hz, J_{AX} 4.0 Hz, 1H, H-7a), 3.58 (d, $J_{5\text{-}6}$ 8.8 Hz, $J_{5\text{-}4}$ 0.8 Hz, 1H, H-5), 3.46-3.42 (B part of an ABX

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system, J_{BA} 11.6 Hz, J_{BX} 6.4 Hz, 1H, H-7b), 2.93-2.90 (A part of an AB system, J_{AB} 13.2 Hz, 1H, H-1′a), 2.89-2.86 (B part of an AB system, J_{BA} 13.2 Hz, 1H, H-1′b), 2.33 (s, 3H, CH_3C=), 1.98-1.92 (m, 2H, H-1′a, H-1′b), 1.31 (t, J 7.2 Hz, 3H, CH_2CH_3); ^{13}C NMR (100MHz, CD_3OD): δ 166.7 (Cq), 157.6 (Cq), 99.4 (Cq), 96.2 (Cq), 74.9 (C-5), 70.2 (C-6), 67.6 (C-4), 67.3 C-3), 65.5 (C-7), 61.9 (CH_2CH_3), 37.1 (C-2), 33.7 (C-1′), 21.4 (CH_3C=), 14.5 (CH_2CH_3); ESI-MS: *m/z* 373.18 $[M + Na]^+$, 389.09 $[M + K]^+$; Anal. Calcd for $C_{14}H_{22}O_8S$ (350.10): C, 47.99; H, 6.33%. Found: C, 47.97; H, 6.29%.

Synthesis of compound 12a. To a stirred solution of **16a** (0.200 g, 0.571 mmol) in THF (14.0 mL), 3.42 mL of 1M solution of LiOH (3.42 mmol) in H_2O were added. The reaction mixture was warmed at 50°C for 6h then 1M solution of H_3PO_4 was added to reach pH 5. The solvent was co-evaporated with toluene under reduced pressure to give a crude which was suspended in dry MeOH and filtered through a PTFE membrane (pore size 0.20 uM). The filtrate was concentrated to dryness and purified by HPLC (column Zorbax RX-Silica, 9.4x250, 5 um, AcOEt:MeOH 90:10 to 50:50) to give **12a** (0.116 g, 63%) as an oil. $[\alpha]_{25}^{D}$ + 55.2 (c 0.30, MeOH); ^{1}H NMR (400MHz, CD_3OD): δ 4.10-4.04 (m, 2H, H-3, H-4), 3.89-3.84 (m, 1H, H-6), 3.76-3.72 (A part of an ABX system, J_{AB} 11.6 Hz, J_{AX} 3.6 Hz, 1H, H-7a), 3.55-3.53 (m, 1H, H-5), 3.44-3.40 (B part of an ABX system, J_{BA} 11.6 Hz, J_{BX} 6.8 Hz, 1H, H-7b), 2.90-2.87 (A part of an AB system, J_{AB} 13.2 Hz, 1H, H-1′a), 2.86-2.82 (B part of an AB system, J_{BA} 13.2 Hz, 1H, H-1′b), 2.3 (s, 3H, CH_3C=), 1.95-1.92 (m, 2H, H-2a, H-2b); ^{13}C NMR (50MHz, CD_3OD): δ 168.4, 157.3, 99.8, 96.0, 74.9, 70.2, 67.5, 67.3, 65.6, 37.2, 33.8, 21.4; HRMS: *m/z* calcd for $C_{12}H_{17}O_8S$ $[M - H]^-$ 321.06496, found 321.06476.

Synthesis of compound 17. To a stirred solution of **13** (0.500 g, 1.85 mmol) in CH_2Cl_2 (8.0 mL), dry pyridine (5.0 mL) was added. The mixture was warmed at 60°C for 50h then diluted with CH_2Cl_2 (80 mL) and washed with a saturated solution of NH_4Cl (2 × 10 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness to give a crude which was purified by flash column chromatography on silica gel (AcOEt:Petroleum Ether + NEt_3 0.1%, 1:8) to give **17** (0.300 g, 60%) as a yellow oil. $[\alpha]_{25}^{D}$ + 34.5 (*c* 0.44, CH_2Cl_2); ^{1}H NMR (400MHz, $CDCl_3$): δ 4.66-4.64 (m, 1H), 4.40-4.36 (m, 2H), 4.14-4.07 (m, 2H), 3.71 (dd, J 1.2 Hz, J 8.0 Hz, 1H), 1.73 (s, 3H), 1.43 (s, 3H), 1.42 (s, 3H), 1.38 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100MHz, $CDCl_3$): δ 152.7, 110.1, 109.4, 75.6, 74.1, 71.4, 69.6, 66.7, 28.0, 26.9, 26.8, 25.3, 19.6; ESI-MS: *m/z* 293.17 $[M + Na]^+$; Anal. Calcd for $C_{14}H_{22}O_5$ (270.14): C, 62.20; H, 8.20%. Found: C, 62.18; H, 8.24%.

Synthesis of compound 18. To a solution of **17** (0.300 g, 1.11 mmol) in $CHCl_3$ (2.5 mL) dry pyridine (5.5 mL) and **14a** (0.441 mg, 1.435 mmol) were added. The mixture was warmed at 50°C and stirred for 6 h. After this time **14a** (0.441 mg, 1.435 mmol) was added and the mixture was stirred at 50°C for 18 h. The reaction mixture was cooled at rt, diluted with CH_2Cl_2 (70 mL) and washed with a saturated solution of NH_4Cl (2 × 15 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness to give a crude which was purified by flash column chromatography on silica gel (AcOEt:Petroleum Ether + NEt_3 0.1%, 1:7) to give **18** (0.406 g, 85%) as a white solid. $[\alpha]_{25}^{D}$ + 96.8 (*c* 0.22, CH_2Cl_2); mp: 104-106°C; ^{1}H NMR (400MHz, C_6D_6): δ ^{1}H NMR (400MHz, C_6D_6): δ 4.48-4.43 (m, 1H, H-7), 4.14-4.11 (A part of an ABX

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system, J_{AB} 8.4 Hz, J_{AX} 5.2 Hz, 1H, H-8a), 4.11-4.08 (B part of an ABX system, J_{BA} 7.6 Hz, J_{BX} 2.4 Hz, 1H, H-8b), 4.063-3.93 (m, 4H, H-5, H-6, CH_2CH$_3$), 3.80 (dd, J_{4-3} 8.4 Hz, J_{4-5} 4.8 Hz, 1H, H-4), 2.78 (d, J_{3-4} 8.4 Hz, 1H, H-3), 2.33 (s, 3H, CH_3C=), 1.46 (s, 3H, H-1), 1.37 (s, 3H, (CH_3)$_2$C), 1.29 (s, 3H, (CH_3)$_2$C), 1.22 (s, 3H, (CH_3)$_2$C), 1.17 (s, 3H, (CH_3)$_2$C), 0.94 (t, J 7.2 Hz, 3H, CH$_2$CH_3); ^{13}C NMR (50MHz, C_6D_6): δ 164.5, 158.5, 108.8, 108.7, 98.6, 93.8, 73.9, 73.6, 72.0, 69.8, 66.7, 60.3, 43.7, 27.9, 26.4, 25.8, 24.9, 20.8, 13.6; ; ESI-MS: *m/z* 453.17 [M + Na]$^+$; Anal. Calcd for $C_{20}H_{30}O_8S$ (430.16): C, 55.80; H, 7.02%. Found: C, 55.88; H, 7.00%.

Synthesis of compound 19. To a solution of **18** (0.434 g, 1.00 mmol) in $CHCl_3$:EtOH / 1:2 (15.0 mL), 2.0 mL of HCl solution (10% in EtOH) were added. The reaction mixture was stirred overnight at rt then neutralized with NEt_3 and the solvent removed under reduced pressure. The crude was purified by flash chromatography on silica gel (AcOEt:MeOH 12:1) to give **19** (0.310 g, 89%) as a glassy solid. $[\alpha]_{25}^{D}$ + 146.0 (*c* 0.370, MeOH); ^{1}H NMR (400MHz, CD_3OD): δ 4.21-4.13 (m, 2H, CH_2CH$_3$), 4.05 (dd, J_{5-6} 2.8 Hz, J_{5-4} 0.8 Hz, 1H, H-5), 3.88-3.84 (m, 1H, H-7), 3.80-3.75 (m, 2H, H-6, H-8a), 3.58-3.53 (B part of an ABX system, J_{BA} 11.6 Hz, 6.4 Hz, 1H, H-8b), 3.43 (dd, J_{4-3} 10.4 Hz, J_{4-5} 3.2 Hz, 1H, H-4), 3.11 (d, J_{3-4} 10.4 Hz, 1H, H-3), 2.3 (s, 3H, CH_3C=), 1.59 (s, 3H, H-1), 1.27 (t, J 7.2 Hz, 3H, CH$_2$CH_3); ^{13}C NMR (100MHz, CD_3OD): δ 167.1, 160.3, 101.4, 94.4, 73.3 (C-6), 71.0 (C-7), 69.0 (C-5), 68.5 (C-4), 64.6 (C-8), 61.9 (*C*H$_2$CH$_3$), 44.3 (C-3), 26.7 (*C*H$_3$C=), 21.5 (C-1), 14.5 (CH$_2$*C*H$_3$); ESI-MS: *m/z* 373.18 [M + Na]$^+$, 389.09 [M + K]$^+$; Anal. Calcd for $C_{14}H_{22}O_8S$ (350.10): C, 47.99; H, 6.33%. Found: C, 48.02; H, 6.30%.

Synthesis of compound 11. To a stirred solution of **19** (0.300 g, 0.856 mmol) in THF (21.0 mL), 5.13 mL of 1M solution of LiOH (5.13 mmol) in H_2O were added. The reaction mixture was warmed at 50°C for 5 h then 1M solution of H_3PO_4 was added to reach pH 5. The solvent was co-evaporated with toluene under reduced pressure to give a crude which was suspended in AcOEt:MeOH 1:1 and filtered through a PTFE membrane (pore size 0.20 uM). The filtrate was concentrated to dryness and purified by HPLC (column Zorbax RX-Silica, 9.4x250, 5 um, AcOEt:MeOH 90:10 to 50:50) to give **11** (0.150 g, 54%) as a glassy solid. $[\alpha]_{25}^{D}$ + 90.7 (c 0.625, MeOH); ^{1}H NMR (400MHz, CD_3OD): δ 4.09-4.084 (m, 1H, H-5), 3.88-3.84 (m, 1H, H-7), 3.80-3.77 (m, 2H, H-6, H-8a), 3.58-3.54 (B part of an ABX system, J_{BA} 11.2 Hz, J_{BX} 6.0 Hz, 1H, H-8b), 3.49 (dd, J_{4-3} 10.8 Hz, J_{4-5} 3.2 Hz, 1H, H-4), 3.03 (d, J_{3-4} 10.8 Hz, 1H, H-3), 2.21 (s, 3H, CH_3C=), 1.56 (s, 3H, H-1); ^{13}C NMR (50MHz, CD_3OD): δ 173.6, 152.8, 100.3, 99.8, 73.1 (C-6), 71.1 (C-7), 69.0 (C-5), 68.3 (C-4), 64.6 (C-8), 44.5 (C-3), 26.8 (C-1), 20.6 (CH_3C=); ESI-MS: *m/z* 321.09 [M - H]$^-$; HRMS: *m/z* calcd for $C_{12}H_{17}O_8S$ [M - H]$^-$ 321.06496, found 321.06484.

Synthesis of compound 15b. To a solution of **13** (0.120 g, 0.444 mmol) in $CHCl_3$ (1.5 mL) dry pyridine (2.2 mL) and **14b**[30] (0.220 mg, 0.288 mmol) were added. The mixture was warmed at 50°C and stirred for 1 h. After this time, **14b** (0.220 mg, 0.288 mmol) was added and the mixture was stirred at 50°C for 1 h. The reaction mixture was cooled at rt, diluted with CH_2Cl_2 (50 mL) and washed with a saturated solution of NH_4Cl (2 × 10 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness to give a crude which was purified by flash column chromatography on silica gel (AcOEt:Petroleum Ether 1:6) to give **15b** (0.200 g, 88%) as a

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glassy solid. $[\alpha]_{25}^{D}$ -4.7 (c 0.65, CH_2Cl_2); ^{1}H NMR (400MHz, $CDCl_3$): δ 7.32-7.26 (m, 2H, *p*-F-C_6H_4), 7.05-7.00 (m, 2H, *p*-F-C_6H_4), 4.65-4.61 (m, 1H, H-3), 4.38 (dd, $J_{4\text{-}3}$ 8.0 Hz, $J_{4\text{-}5}$ 2.0 Hz, 1H, H-4), 4.33 (aq, J 6.4 Hz, 1H, H-6), 4.08-4.04 (A part pf an ABX system, J_{AB} 8.8 Hz, J_{AX} 6.0 Hz, 1H, H-7a), 4.02-3.95 (m, 3H, H-7b, CH_2CH$_3$), 3.83 (dd, $J_{5\text{-}6}$ 6.8 Hz, $J_{5\text{-}4}$ 2.0 Hz, 1H, H-5), 3.30-3.26 (A part of an AB system, J_{AB} 12.8 Hz, 1H, H-1′a), 2.97-2.93 (B part of an AB system, J_{BA} 13.2 Hz, 1H, H-2b), 2.44-2.39 (A part of an ABX system, J_{AB} 15.6 Hz, J_{AX} 3.2 Hz, 1H, H-1′a), 2.13-2.08 (B part of an ABX system, J_{BA} 16.0 Hz, J_{BX} 3.2 Hz, 1H, H-2b), 1.47 (s, 3H, $(CH_3)_2$C), 1.39 (s, 3H, $(CH_3)_2$C), 1.36 (s, 3H, $(CH_3)_2$C), 1.35 (s, 3H, $(CH_3)_2$C), 1.01 (t, J 7.2 Hz, 3H, CH_2CH_3); ^{13}C NMR (100MHz, $CDCl_3$): δ 164.9 (Cq), 162.99 (d, J 247.8 Hz, *p*-F-C_6H_4), 154.5 (Cq), 134.2 (Cq), 133.4 (d, J 3.8 Hz, *p*-F-C_6H_4), 130.5 (d, J 8.4 Hz, *p*-F-C_6H_4), 123.5, 114.8 (d, J 22.1 Hz, *p*-F-C_6H_4), 109.5 (Cq), 109.2 (Cq), 102.2 (Cq), 94.4 (Cq), 73.8 (C-6), 72.2 (C-5), 71.6 (C-4), 70.6 (C-3), 66.3 (C-7), 60.9 (CH_2CH_3), 35.1 (C-1′), 34.0 (C-2), 26.6 ($(CH_3)_2$C), 26.0 ($(CH_3)_2$C), 25.4 ($(CH_3)_2$C), 24.8 ($(CH_3)_2$C), 13.6 (CH_2CH_3); ESI-MS: *m/z* 533.08 $[M + Na]^+$; Anal. Calcd for $C_{25}H_{31}FO_8S$ (510.17): C, 58.81; H, 6.12%. Found: C, 58.85; H, 6.22%.

Synthesis of compound 16b. To a stirred solution of **15b** (0.214 g, 0.418 mmol) in CH_2Cl_2 (1.5 mL) glacial AcOH (6.0 mL) and H_2O (4.0 mL), were slowly added. The reaction mixture was warmed to 50°C and stirred for 6h. After this time, the solvent was co-evaporated with toluene under reduced pressure to give a crude which was purified by flash column chromatography on silica gel (AcOEt:MeOH, 20:1) to give **16b** (0.111 g, 62%) as a glassy solid. $[\alpha]_{25}^{D}$ +36.1 (c 1.7, CH_2Cl_2); ^{1}H NMR (400MHz, $CDCl_3$): δ 7.22-7.18 (m, 2H, *p*-F-C_6H_4), 6.96-6.92 (m, 2H, *p*-F-C_6H_4), 4.53 (bs, 1H), 4.09-4.02 (m, 2H, H-3, H-4 o H-5), 3.97-3.89 (m, 3H, H-6, CH_2CH$_3$), 3.72-3.63 (m, 2H, H-7a, H-5 o H-4), 3.53-3.49 (m, 1H, H-7b), 2.96-2.93 (A part of an AB system, J_{AB} 12.8 Hz, H-1′a), 2.83-2.80 (B part of an AB system, J_{BA} 12.8 Hz, H-1′b), 1.99-1.84 (m, 2H, H-2a, H-2b), 0.94 (t, J 7.2 Hz, 3H, CH_2CH_3); ^{13}C NMR (50MHz, C_6D_6): δ165.5 (Cq), 163.06 (d, J 248.1 Hz, Cq, *p*-F-C_6H_4), 153.1 (Cq), 132.2 (d, J 3.25 Hz, Cq, *p*-F-C_6H_4), 130.3 (d, J 8.35 Hz, CH, *p*-F-C_6H_4), 102.3 (Cq), 95.2 (Cq), 72.9 (C-5 o C-4), 69.4 (C-6), 66.6 (C-3), 66.2 (C-4 o C-5), 64.1 (C-7), 61.7 (CH_2CH_3), 36.4 (C-2), 33.6 (C-1′), 13.8 (CH_2CH_3); ESI-MS: *m/z* 453.08 $[M + Na]^+$; Anal. Calcd for $C_{19}H_{23}FO_8S$ (430.10): C, 53.02; H, 5.39%. Found: C, 53.08; H, 5.30%.

Synthesis of compound 12b. To a stirred solution of **16b** (0.074 g, 0.171 mmol) in THF (2.0 mL), 1.02 mL of 1M solution of LiOH (1.02 mmol) in H_2O were added. The reaction mixture was warmed at 60°C for 2h then 1M solution of H_3PO_4 was added to reach pH 5. The solvent was co-evaporated with toluene under reduced pressure to give a crude which was suspended in AcOEt (15.0 mL) and filtered through a PTFE membrane (pore size 0.20 uM). The filtrate was concentrated to dryness to give **12b** (0.066 g, 97%) as a glassy solid. $[\alpha]_{25}^{D}$ + 9.2 (c 1.1, MeOH); ^{1}H NMR (400MHz, CD_3OD): δ 7.39 (m, 2H, *p*-F-C_6H_4), 7.07-7.03 (m, 2H, *p*-F-C_6H_4), 4.07-4.03 (m, 2H, H-3, H-5), 3.92-3.88 (m, 1H, H-6), 3.80-3.77 (A part of an ABX system, J_{AB} 11.2 Hz, J_{AX} 3.2 Hz, 1H, H-7a), 3.73 (dd, J 8.8 Hz, J 0.8 Hz, 1H, H-4), 3.54-3.49 (B part of an ABX system, J_{BA} 11.6 Hz, J_{BX} 6.4 Hz, 1H, H-7b), 3.04-3.01 (A part of an AB system, J_{AB} 13.2 Hz, 1H, H-1′a), 2.99-2.96 (B part of an AB system, J_{BA} 13.0 Hz, 1H, H-1′b), 2.01-1.99 (m, 2H, H-

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2a, H-2b); ^{13}C NMR (100MHz, CD_3OD): δ 168.8, 164.4 (d, *J* 246.2 Hz, Cq, *p*-F-C_6H_4), 154.8, 134.7 (d, *J* 3.0 Hz, Cq, *p*-F-C_6H_4), 131.9 (d, *J* 8.4 Hz, CH, *p*-F-C_6H_4), 115.6 (d, *J* 22.0 Hz, CH, *p*-F-C_6H_4), 103.8, 96.6, 74.9, 70.3, 67.6, 67.4, 65.3, 37.1, 34.2; ESI-MS: *m/z* 401.1 $[M - H]^-$; Anal. Calcd for $C_{17}H_{19}FO_8S$ (402.07): C, 50.74; H, 4.76%. Found: C, 50.64; H, 4.83%.

Synthesis of compound 15c. To a solution of **13** (0.096 g, 0.355 mmol) in $CHCl_3$ (1.5 mL) dry pyridine (1.0 mL) and **14c**[30] (0.170 mg, 0.426 mmol) were added. The mixture was warmed at 50°C and stirred for 2h. The reaction mixture was cooled at rt, diluted with CH_2Cl_2 (40 mL) and washed with a saturated solution of NH_4Cl (2 × 10 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness to give a crude which was purified by flash column chromatography on silica gel (AcOEt:Petroleum Ether 1:6) to give **15c** (0.180 g, 97%) as a glassy solid. $[\alpha]_{25}^{D}$ -22.97 (c 0.48, CH_2Cl_2); ^{1}H NMR (400MHz, $CDCl_3$): δ 7.23-7.21 (m, 1H, *m*-OMe-C_6H_4), 6.91-6.85 (m, 3H, *m*-OMe-C_6H_4), 4.65-4.61 (m, 1H, H-3), 4.40-4.37 (dd, $J_{4\text{-}3}$ 5.2 Hz, $J_{4\text{-}5}$ 1.2 Hz, 1H, H-4), 4.34 (aq, *J* 4.8 Hz, 1H, H-6), 4.08-4.01 (m, 2H, H-7a, H-7b), 3.96 (q, *J* 7.2 Hz, 2H, C$H_2$$CH_3$), 3.83 (dd, $J_{5\text{-}6}$ 7.6 Hz, $J_{5\text{-}4}$ 2.0 Hz, 1H, H-5), 3.79 (s, 3H, OCH_3), 3.30-3.26 (A part of an AB system, J_{AB} 12.8 Hz, H-1′a), 2.97-2.94 (B part of an AB system, J_{BA} 12.8 Hz, 1H, H-1′b), 2.43-2.38 (A part of an ABX system, J_{AB} 16.0 Hz, J_{AX} 3.2 Hz, 1H, H-2a), 2.14-2.10 (B part of an ABX system, J_{BA} 15.8 Hz, J_{BX} 2.8 Hz, 1H, H-2b), 1.47 (s, 3H, $(CH_3)_2C$), 1.39 (s, 3H, $(CH_3)_2C$), 1.36 (s, 6H, $(CH_3)_2C$), 0.96 (t, J 7.2 Hz, 3H, CH_2CH_3); ^{13}C NMR (100MHz, $CDCl_3$): δ 165.26 (Cq), 159.1 (Cq), 155.0 (Cq), 138.6 (Cq), 134.2 (Cq), 128.8 (CH, *m*-OMe-C_6H_4), 123.5 (CH, *m*-OMe-C_6H_4), 121.14 (CH, *m*-OMe-C_6H_4), 115.0, 113.5, 109.6, 109.2, 102.1, 94.1, 73.7 (C-6), 72.2 (C-4), 71.6 (C-5), 70.6 (C-3), 66.4 (C-7), 60.9 (CH_2CH_3), 55.2 (OCH_3), 35.1 (C-1′), 34.1 (C-2), 26.8 ($(CH_3)_2C$), 26.1 ($(CH_3)_2C$), 25.4 ($(CH_3)_2C$), 24.9 ($(CH_3)_2C$), 13.6 (CH_2CH_3); ESI-MS: *m/z* 545.08 $[M + Na]^+$; Anal. Calcd for $C_{26}H_{34}O_9S$ (522.19): C, 59.75; H, 6.56%. Found: C, 59.77; H, 6.66%.

Synthesis of compound 16c. To a stirred solution of **15c** (0.180 g, 0.335 mmol) in CH_2Cl_2 (1.5 mL) glacial AcOH (5.0 mL) and H_2O (3.0 mL), were slowly added. The reaction mixture was warmed to 50°C and stirred for 6 h. After this time, the solvent was co-evaporated with toluene under reduced pressure to give a crude which was purified by flash column chromatography on silica gel (AcOEt:MeOH, 15:1) to give **16c** (0.104 g, 74%) as a glassy solid. $[\alpha]_{25}^{D}$ +30.2 (c 0.8, CH_2Cl_2); ^{1}H NMR (400MHz, $CDCl_3$): δ 7.23-7.19 (m, 1H, *m*-OMe-C_6H_4), 6.88-6.82 (m, 3H, *m*-OMe-C_6H_4), 4.15-4.11 (m, 2H, H-3, H-4 o H-5), 4.02-3.95 (m, 3H, H-6, CH_2CH_3), 3.78-3.72 (m, 3H, H-7a, H-4 o H-5) 3.72 (s, 3H, OCH_3), 3.59-3.55 (m, 1H, H-7b), 3.01-2.98 (A part of an AB systen, J_{AB} 13.2 Hz, 1H, H-1′a), 2.87-2.84 (B part of an AB system, J_{BA} 13.2 Hz, 1H, H-1′b), 2.04-1.89 (m, 2H, H-2a, H-2b), 0.98 (t, *J* 7.2 Hz, 3H, CH_2CH_3); ^{13}C NMR (50MHz, C_6D_6): δ 165.9 (Cq), 159.0 (Cq), 153.8 (Cq), 137.6 (Cq), 129.1 (CH, *m*-OMe-C_6H_4), 120.8 (CH, m-OMe-C_6H_4), 114.6 (CH, *m*-OMe-C_6H_4), 114.2 (CH, *m*-OMe-C_6H_4), 102.1 (Cq), 95.1 (Cq), 731. (C-4 o C5), 69.2 (C-6), 66.4 (C-3); 66.1 (C-5 o C-4), 64.3 (C-7), 61.4 (CH_2CH_3), 55.3 (OCH_3), 36.0 (C-1′), 33.3 (C-2), 13.6 (CH_2CH_3); ESI-MS: *m/z* 465.08 $[M + Na]^+$; Anal. Calcd for $C_{20}H_{26}O_9S$ (442.12): C, 54.29; H, 5.92%. Found: C, 54.32; H, 5.98%.

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Synthesis of compound 12c. To a stirred solution of **16c** (0.060 g, 0.142 mmol) in THF (2.0 mL), 0.852 mL of 1M solution of LiOH (0.852 mmol) in H_2O were added. The reaction mixture was warmed at 60°C for 2 h then 1M solution of H_3PO_4 was added to reach pH 5. The solvent was co-evaporated with toluene under reduced pressure to give a crude which was suspended in AcOEt:MeOH 8:1 (17.0 mL) and filtered through a PTFE membrane (pore size 0.20 uM). The filtrate was concentrated to dryness to give **12c** (0.054 g, 92%) as a glassy solid. $[\alpha]_{25}^{D}$ +5.0 (c 0.63, MeOH); ^{1}H NMR (400MHz, CD_3OD): δ 7.24-7.20 (m, 1H, *m*-OMe-C_6H_4), 6.96-6.78 (m, 3H, *m*-OMe-C_6H_4), 4.12-4.05 (m, 2H, H-3, H-5), 3.91-3.87 (m, 1H, H-6), 3.79-3.75 (m, 5H, H-4, H-7a, OCH_3), 3.58-3.53 (B part of an ABX system, J_{BA} 11.2 Hz, J_{BX} 5.6 Hz, 1H, H-7b), 3.03-3.00 (A part of an AB system, J_{AB} 12.8 Hz, 1H, H-1′a), 2.99-2.96 (B part of an AB system, J_{BA} 12.8 Hz, 1H, H-1′b), 2.01-1.99 (m, 2H, H2a, H2b); ^{13}C NMR (50MHz, CD_3OD): δ 169.7, 160.5, 154.1, 139.5, 129.8, 121.9, 115.3, 115.2, 104.4, 96.3, 74.7, 70.4, 67.6, 67.5, 65.2, 55.7, 37.2, 34.2; ESI-MS *m/z* 413.1 $[M - H]^-$; Anal. Calcd for $C_{18}H_{22}O_9S$ (414.09): C, 52.17; H, 5.35%. Found: C, 52.07; H, 5.42%.

Enzyme inhibition assays

The A/Mississippi/3/2001 wild type H1N1 influenza virus[34] was used to evaluate susceptibility in an enzyme inhibition assays. 25 μl of A/Mississippi/3/2001 wild type H1N1 influenza virus was mixed with 25 μl of inhibitor (Zanamivir or **11** or **12a**), and after preincubation for 30 min at room temperature 50 μl of MUNANA was added. After 60 min at 37°C the reaction was stopped with the addition of 200 mM Na_2CO_3. Fluorescence units were quantitated with a BMG FLUOstar with an excitation wavelength of 365 nm and an emission wavelength of 450 nm. Final concentrations in the assay were 50 mM sodium acetate pH 5.5, 5 mM $CaCl_2$ and 100 μM MUNANA. Serial 10-fold dilutions of Zanamivir were prepared in water. Dilutions of the compound **11** and **12a** ranged from 10 to 10000 μM.

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