Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

6-Substituted 1,2-benzoxathiine-2,2-dioxides are Isoform-Selective Inhibitors Towards Human Carbonic Anhydrases IX, XII and VA

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Supporting Information

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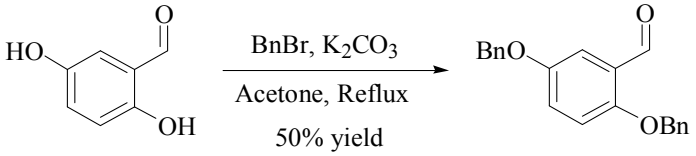
Experimental

1) Chemistry

Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, Alfa Aesar and TCI. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringes techniques to transfer solutions. Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR, DEPT-135, DEPT-90, HSQC, HMBC) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet; dd, double of doublet. The assignment of exchangeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck Silica gel 60 (230-400 mesh ASTM) as the stationary phase and ethyl acetate/*n*-hexane were used as eluents. Melting points (m.p.) were carried out in open capillary tubes and are uncorrected.

Synthesis of 2,5-bis-benzyloxy-benzaldehyde (1).



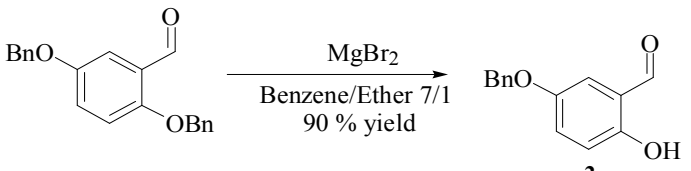
1

2,5-Dihydroxybenzaldehyde (0.15g, 1.0 eq) was dissolved in acetone (20 ml) followed by the addition of K_2CO_3 (1.0 eq) and benzyl bromide (1.0 eq) at r.t. The reaction mixture was stirred under reflux O.N., cooled down to r.t., quenched with a saturated $NaHCO_3$ aqueous solution (20 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with brine (3 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with 5% EtOAc in *n*-hexane to afford the title compound as a white solid.

2,5-Bis-benzyloxy-benzaldehyde **1**: 50 % yield; silica gel TLC R_f 0.27 (Ethyl acetate/*n*-hexane 5 % *v/v*); δ_H (400 MHz, DMSO-d_6) 5.12 (s, 2H), 5,25 (s, 2H), 7.43 (m, 14H), 10.4 (s, 1H); δ_C (100 MHz, DMSO-d_6) 70.6, 71.4, 112.4, 117.0, 119.6, 124.7, 128.5, 128.8, 128.9, 129.4, 129.5, 137.5, 137.8, 153.3, 156.2, 189.7.

Experimental data in agreement with reported data.[1]

Synthesis of 5-benzyloxy-2-hydroxy-benzaldehyde (2).



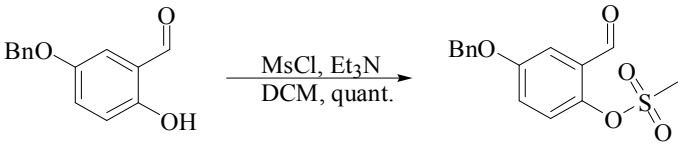
1 **2**

2,5-Bis-benzyloxy-benzaldehyde **1** (0.1 g 1.0 eq) was dissolved in 5 ml benzene/diethyl ether (7:1) and $MgBr_2$ (1.8 eq) was added at r.t. Then mixture was stirred under reflux for 4 hrs, cooled down to r.t. and quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml), extracted with ethyl acetate (3 x 15 ml), the combined organic layers were washed with brine (2x 20 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the title compound **2** as a white solid that was used as it is.

5-Benzyloxy-2-hydroxy-benzaldehyde **2**: 90 % yield; silica gel TLC R_f 0.36 (Ethyl acetate/*n*-hexane 10 % *v/v*); δ_H (400 MHz, DMSO-d_6) 5.10 (s, 2H), 6.99 (d, *J* 10.2, 1H), 7.26 (m, 2H), 7.41 (m, 5H), 10.30 (s, 1H), 10.33 (s, 1H); δ_C (100 MHz, DMSO-d_6) 70.7, 112.4, 119.6, 123.1, 126.0, 128.6, 128.8, 129.4, 138.0, 152.1, 156.4, 191.7.

Experimental data in agreement with reported data.[2]

Synthesis of methanesulfonic acid 4-benzyloxy-2-formyl-phenyl ester (3)



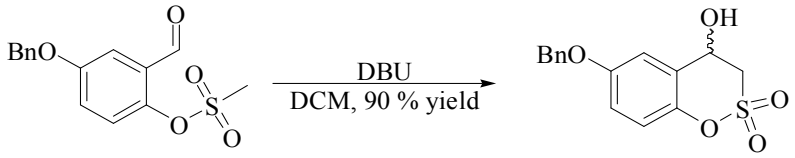
2 **3**

5-Benzyloxy-2-hydroxy-benzaldehyde **2** (0.04 g, 1.0 eq) and triethylamine (1.2 eq) were dissolved in dry DCM (2.0 ml) followed by addition of methanesulfonyl chloride (1,6 eq) at r.t. The reaction was stirred at r.t. under a nitrogen atmosphere until starting material was consumed (TLC monitoring) and then quenched with a 1.0 M hydrochloric acid aqueous solution (10 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the title compound **3** as a white solid that was used as it is.

4-Benzyloxy-2-formyl-phenyl ester **3** : quantitative; silica gel TLC R_f 0.47 (Ethyl acetate/*n*-hexane 40 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.58 (s, 3H), 5.24 (s, 2H), 7.50 (m, 8H), 10.22 (s, 1H); δ_C (100 MHz, DMSO-d_6) 32.5, 70.9, 113.8, 123.7, 126.3, 128.7, 129.1, 129.5, 130.97, 137.3, 144.4, 158.2, 189.4.

Experimental data in agreement with reported data.[3]

Synthesis of (±) 6-benzyloxy-2,2-dioxo-3,4-dihydro-2*H*-2λ⁶-benzo[e][1,2]oxathiin-4-ol (4)

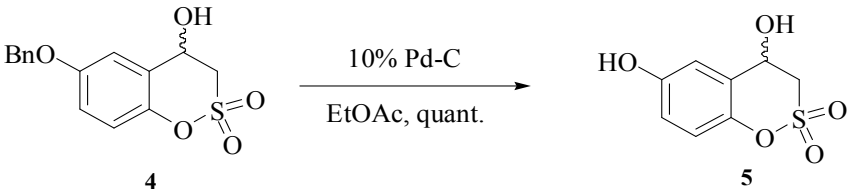


3 4

4-Benzyloxy-2-formyl-phenyl ester **3** (0.07 g, 1.0 eq) was dissolved in dry DCM (4.0 ml) and the solution was cooled down to 0°C and DBU (2.8 eq) was added drop-wise. The solution was stirred at r.t. for 4.5 hrs and then quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the title compound **4** as a white solid that was used as it is.

(±) 6-Benzyloxy-2,2-dioxo-3,4-dihydro-2*H*-2λ⁶-benzo[e][1,2]oxathiin-4-ol **4**: 90 % yield; silica gel TLC R_f 0.41 (Ethyl acetate/*n*-hexane 40 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.65 (dd, *J* 9.5, 14.2, 1H), 4.26 (dd, *J* 6.2, 14.2, 1H), 5.12 (m, 1H), 5.16 (s, 2H), 6.44 (d, *J* 7.04, 1H), 7.07 (dd, *J* 3.0, 9.0, 1H), 7.14 (d, *J* 9.0, 1H), 7.25 (d, *J* 3.0 Hz, 1H), 7.44 (m, 4H), 9.70 (s, 1H); δ_C (100 MHz, DMSO-d_6) 52.2, 65.7, 70.6, 115.0, 117.3, 120.1, 128.4, 128.6, 128.9, 129.4, 137.7, 143.5, 156.6. Experimental data in agreement with reported data.[3]

Synthesis of (±)2,2-dioxo-3,4-dihydro-2*H*-2λ⁶-benzo[e][1,2]oxathiine-4,6-diol (5)

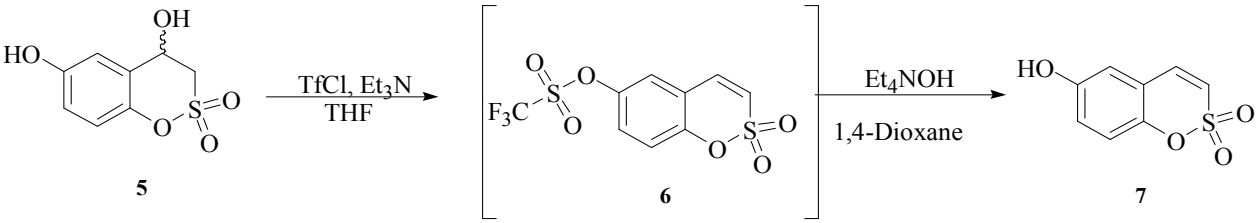


4 5

A solution of 6-benzyloxy-2,2-dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiin-4-ol **4** (0.1 g, 1.0 eq) in 20 ml of ethyl acetate was hydrogenated for 2.5hrs at 20 Psi using 10 % Pd/C (0.03g) as catalyst. The mixture was filtered through Celite®, the solvent was eliminated under *vacuo* to give the title compound **5** as a white solid that was used as it is.

(±)2,2-Dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiine-4,6-diol **5**: quantitative; silica gel TLC R_f 0.46 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.58 (dd, *J* 9.6, 14.0, 1H), 4.22 (dd, *J* 6.2, 14.0, 1H), 5.06 (m, 1H), 6.38 (d, *J* 7.3, 1H), 6.80 (dd, *J* 2.9, 8.8, 1H), 6.99 (d, *J* 5.2, 1H), 7.01 (s, 1H), 9.70 (s, 1H); δ_C (100 MHz, DMSO-d_6) 52.3, 65.6, 115.2, 117.4, 119.8, 128.3, 142.2, 155.8. Experimental data in agreement with reported data.[4]

Synthesis of 2,2-dioxo-2H-2λ^6-benzo[e][1,2]oxathiin-6-ol 7



5 **6** **7**

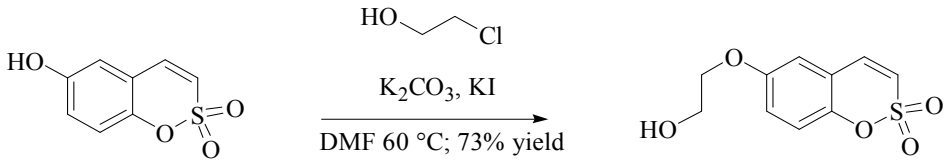
(±)2,2-Dioxo-3,4-dihydro-2*H*-2λ^6-benzo[e][1,2]oxathiine-4,6-diol **5** (0.1 g, 1.0 eq) was dissolved in dry THF (5ml) and the solution was cooled down to 0°C followed by drop-wise addition of triethylamine (1.2 eq) and trifluoromethanesulfonylchloride (1.6 eq) The reaction was stirred at r.t. under a nitrogen atmosphere until complete consumption of the starting material (TLC monitoring) and then quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give the intermediate trifluoro-methanesulfonic acid 2,2-dioxo-2H-2λ^6-benzo[e][1,2]oxathiin-6-yl ester **6** (0.22 g, 1.0 eq) which was dissolved in 1,4-dioxane (5.0 ml) and treated with a 20% aqueous solution of tetraethylammoniumhydoxhyde (1.0 g, 2eq). The solution was stirred at r.t. until

complete consumption of the starting material (TLC monitoring) and then quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with brine (2 x 15 ml), and dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with 50 % EtOAc in *n*-hexane to afford the title compound **7** as a white solid.

Trifluoro-methanesulfonic acid 2,2-dioxo-2H-2λ^6-benzo[e][1,2]oxathiin-6-yl ester **6**: silica gel TLC R_f 0.83 (Ethyl acetate/*n*-hexane 50 % *v/v*). The intermediate was not fully characterized as revealed to be instable.

2,2-Dioxo-2H-2λ^6-benzo[e][1,2]oxathiin-6-ol **7**: 79% yield; silica gel TLC R_f 0.41 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6): 6.97 (dd, *J* 3.0, 8.9, 1H), 7.07 (d, *J* 3.0, 1H), 7.23 (d, *J* 10.3, 1H), 7.46 (d, *J* 10.3, 1H), 7.64 (d, *J* 10.3, 1H), 9.98 (s, 1H); δ_C (100 MHz, DMSO-d_6) 116.0, 119.9, 120.3, 120.5, 123.5, 137.5, 144.3, 156.1. Experimental data in agreement with reported data.[5]

Synthesis of 2-(2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-6-yloxy)-ethanol (8)



7 **8**

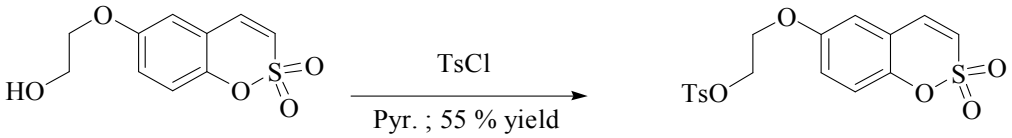
A solution of 2-chloroethanol (0.33g 2.0 eq) and KI (0.79 g 2.5eq) in dry DMF (10 ml) was stirred at 70 °C for 20 min. Then 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-6-ol **7** (0.4 g, 1.0 eq) and K_2CO_3 (1.9 g, 7.0 eq) were added and the reaction mixture was stirred at 60°C under a nitrogen atmosphere O.N.. The mixture was cooled down to 0°C, quenched with with a 1.0 M hydrochloric acid aqueous solution (15 ml) and extracted with ethyl acetate (3 x 15 ml). The

combined organic layers were washed with brine (2 x 15 ml), and dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with 50 % EtOAc in *n*-hexane to afford the title compound **8** as a white solid.

2-(2,2-Dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-6-yloxy)-ethanol **8**: 73% yield; silica gel TLC R_f 0.09 (Ethyl acetate/*n*-hexane 50 % *v/v*); δ_H (400 MHz, DMSO-d_6): 3.76 (q, *J* 6.2, 2H), 4.07 (t, *J* 6.2, 2H), 4.93 (t, *J* 6.2, 1H), 7.18 (dd, *J* 3.0, 9.0, 1H), 7.34 (d, *J* 3.0, 1H), 7.40 (d, *J* 9.0, 1H), 7.53 (d, *J* 10.3, 1H), 7.67 (d, *J* 10.3, 1H); δ_C (100 MHz, DMSO-d_6) 60.3, 71.2, 115.3, 119.8, 120.4, 120.5, 124.0, 137.4, 145.4, 157.1.

Experimental data in agreement with reported data.[6]

Synthesis of toluene-4-sulfonic acid 2-(2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-6-yloxy)-ethyl ester (9)

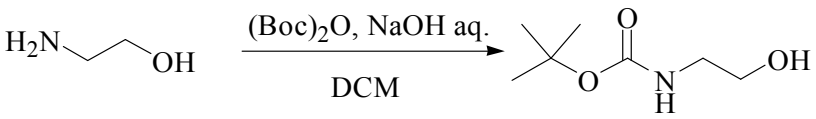


8 **9**

A solution of 2-(2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-6-yloxy)-ethanol **8** (0.03 g, 1.0 eq) was dissolved in dry pyridine (8.0 ml), and then cooled down to 0°C and tosyl chloride (0.47g, 2.0 eq) was added step-wise. The solution was stirred at r.t. until complete consumption of the starting material (TLC monitoring) and then cooled down to 0°C, quenched with with a 3.0 M hydrochloric acid aqueous solution (15 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with 60 % EtOAc in *n*-hexane to afford the title compound **9** as a white solid.

Toluene-4-sulfonic acid 2-(2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-6-yloxy)-ethyl ester **9**: 55% yield; silica gel TLC R_f 0.63 (Ethyl acetate/*n*-hexane 60 % *v/v*); δ_H (400 MHz, DMSO-d_6): 2.40 (s, 3H), 4.24 (m, 2H), 4.39 (m, 2H), 7.08 (dd, *J* 3.0, 9.0, 1H), 7.24 (d, *J* 3.0, 1H), 7.38 (d, *J* 9.0, 1H), 7.49 (d, *J* 8.1, 2H), 7.54 (d, *J* 10.3, 1H), 7.63 (d, *J* 10.3, 1H), 7.82 (d, *J* 8.3, 2H); δ_C (100 MHz, DMSO-d_6) 22.0, 66.9, 115.5, 119.6, 119.8, 120.4, 124.1, 128.6, 131.1, 133.1, 137.2, 143.5 145.7, 146.0, 156.0. Experimental data in agreement with reported data.[6]

Synthesis of (2-hydroxy-ethyl)-carbamic acid *tert*-butyl ester (10)



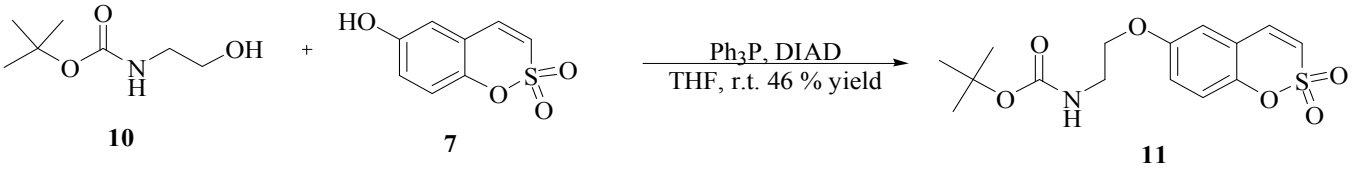
10

Ethanolamine (1.14 g, 1.0 eq) was treated at 0°C with a 1.0 M NaOH aqueous solution (2.0 ml) and then a solution of di-*tert*-butyl dicarbonate (1.1 eq) in DCM (5.0 ml) was added drop-wise at the same temperature. The mixture was vigorously stirres at r.t for 1.5 hrs and then quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with 60 % EtOAc in *n*-hexane to afford the title compound **10** as a white solid.

(2-Hydroxy-ethyl)-carbamic acid *tert*-butyl ester **10**: 59% yield; silica gel TLC R_f 0.30 (Ethyl acetate/*n*-hexane 60 % *v/v*); δ_H (400 MHz, DMSO-d_6): 1.41 (s, 9H), 3.00 (q, *J* 6.0, 2H), 3.39 (q, *J* 6.0, 2H), 4.60 (t, *J* 6.0, 1H), 7.70 (brt, *J* 6.0, 1H); δ_C (100 MHz, DMSO-d_6) 27.9, 43.7, 61.1, 78.5, 156.7.

Experimental data in agreement with reported data.[7]

Synthesis of [2-(2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-6-yloxy)-ethyl]-carbamic acid *tert*-butyl ester (11)



A solution of 2,2-dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-6-ol **7** (0.1 g, 1.0 eq), (2-hydroxy-ethyl)-carbamic acid *tert*-butyl ester **10** (1.2 eq) triphenyl phosphine (1.2 eq) in dry THF (12 ml) was stirred under N_2 N_2 atmosphere at r.t. for 10 min. Then the solution was cooled down to 0°C treated drop-wise with diisoprophylazadicarboxylate (1.2 eq) and stirred at r.t. for 5 hrs. The reaction was quenched with a 1.0 M hydrochloric acid aqueous solution (15 ml) extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with brine (2 x 15 ml), dried over Na_2SO_4, filtered and the solvent was eliminated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with 30 % EtOAc in *n*-hexane to afford the title compound **11** as a white solid.

[2-(2,2-Dioxo-2*H*-2λ^6-benzo[e][1,2]oxathiin-6-yloxy)-ethyl]-carbamic acid *tert*-butyl ester **11**: 46% yield; silica gel TLC R_f 0.18 (Ethyl acetate/*n*-hexane 30 % *v/v*); δ_H (400 MHz, DMSO-d_6): 1.41 (s, 9H), 3.32 (t, *J* 5.7, 2H), 4.04 (t, *J* 5.7, 2H), 7.06 (brt, *J* 5.7, 1H), 7.16 (dd, *J* 3.0 9.0, 1H), 7.34 (d, *J* 3.0, 1H), 7.40 (d, *J* 9.0, 1H), 7.53 (d, *J* 0.3, 1H), 7.66 (d, *J* 10.3, 1H); δ_C (100 MHz, DMSO-d_6) 29.1, 68.1, 78.7, 104.7, 113.8, 115.3, 119.7, 120.5, 124.0, 132.1, 137.4, 145.5, 156,8. Experimental data in agreement with reported data.[7]

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