Chemical genetics and cereal starch metabolism: structural basis of the non-covalent and covalent inhibition of barley β-amylase

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Supplemental Material

ENZYME ASSAYS

Residual BMY1 activity assay

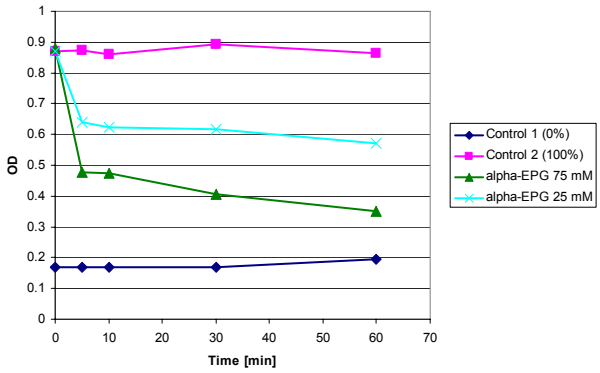


Fig. S1 Relative rates of barley β-amylase inactivation as a function of α-EPG concentration. The plot shows residual activity remaining as a function of time.

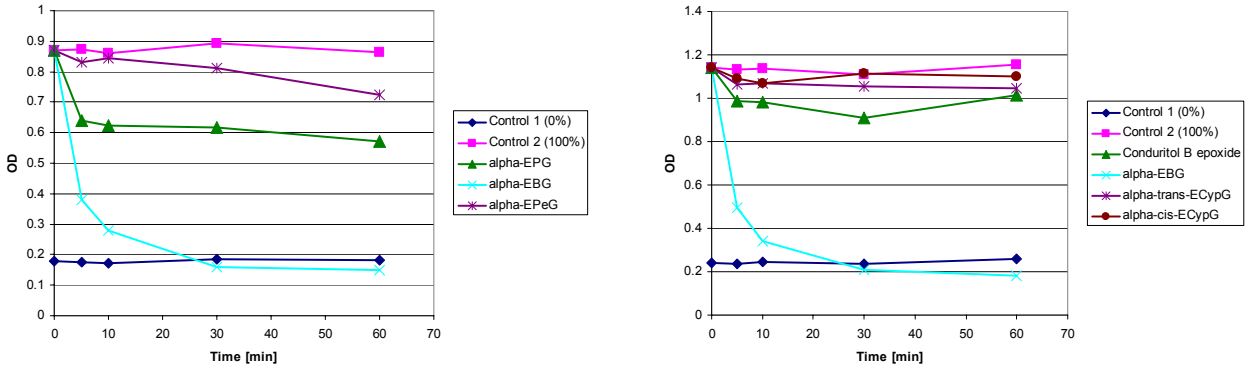


Fig. S2 Relative rates of barley β-amylase inactivation with various epoxyalkyl α-D-glucopyranosides (25 mM concentration). The plot shows residual activity remaining as a function of time.

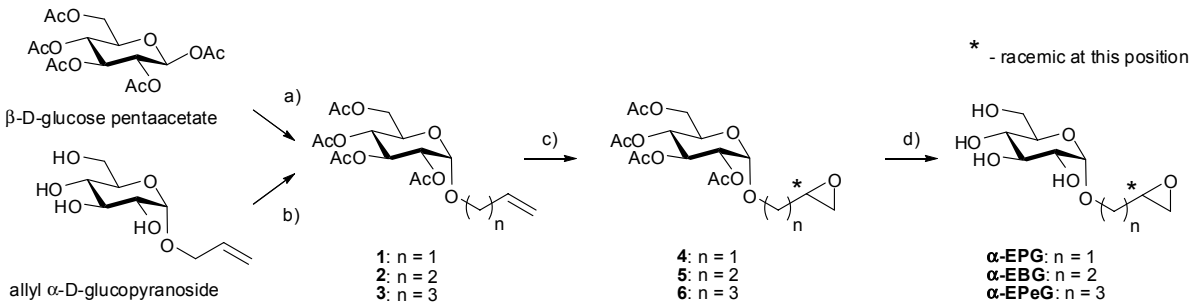
CHEMICAL SYNTHESIS AND ANALYSIS

Synthesis of epoxide based irreversible inhibitors (epoxyalkyl and epoxycycloalkyl α-D-glucopyranosides)

The epoxyalkyl α-D-glucopyranosides α-EPG, 3',4'-epoxybutyl α-D-glucopyranoside (α-EBG) and 4',5'-epoxypentyl α-D-glucopyranoside (α-EPeG) (Scheme S1) were synthesised following published procedures. α-EPG was prepared following Isoda and co-workers [1] who adopted procedures published earlier for preparation of 2',3'-epoxypropyl α-D-galactopyranoside[2] and 2',3'-epoxypropyl β-D-glucopyranoside.[3] As a starting material in our synthesis commercially available allyl α-D-glucopyranoside was used that was first peracetylated to give **1**. The olefin **1** was then oxidised to the corresponding epoxide **4** followed by a global deprotection and purification on silica gel to give α-EPG (Scheme S1).

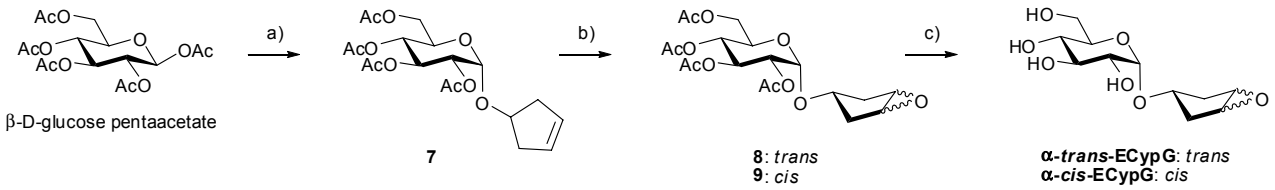
α-EBG and α-EPeG (Scheme S1) were prepared in a similar manner to α-EPG but starting from the corresponding alkenes **2** and **3**, respectively. The alkenes **2** and **3** were synthesised using $SnCl_4$-catalysed glycosylation essentially as described in the literature.[4,5] Synthesis of (1's,3'*R*,4'*S*)-3',4'-epoxycyclopentyl α-

D-glucopyranoside (α-*trans*-ECypG) and (1'r,3'*R*,4'*S*)-3',4'-epoxycyclopentyl α-D-glucopyranoside (α-*cis*-ECypG) followed the same route (Scheme S2), starting from cyclopenten-4-ol.



Scheme S1. Synthesis of epoxyalkyl α-D-glucopyranosides. **a**) $SnCl_4$, DCM abs., ROH, 2 days, 63 % (n = 2, ROH = 3-buten-1-ol), 56 % (n = 3, ROH = 4-penten-1-ol) **b**) Ac_2O, Py, DMAP, ~quant (n = 1) **c**) MCPBA (7 eq), $CHCl_3$, rt, 79 % (n = 1), 67 % (n = 2), 51 % (n = 3) **d**) i) MeONa, MeOH, 15 min, ii) dry ice, 81 % (n = 1), 85 % (n = 2), 95 % (n = 3)

In this case the 3-chloroperbenzoic acid (MCPBA) epoxidation of the cyclic olefin **7** afforded two separable *meso*-epoxides (1's,3'*R*,4'*S*)-3',4'-epoxycyclopentyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**8**) and (1'r,3'*R*,4'*S*)-3',4'-epoxycyclopentyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**9**) in a ~ 2.3 : 1 ratio. Both isomers were deprotected and purified to give the desired α-*trans*-ECypG and α-*cis*-ECypG, respectively (Scheme S2).



Scheme S2. Synthesis of epoxycyclopentyl α-D-glucopyranosides. **a**) $SnCl_4$, DCM abs., 3-cyclopenten-1-ol, 24 h, 51 % **b**) i) MCPBA (7 eq), $CHCl_3$, rt, ii) separation, 50 % (*trans*), 21 % (*cis*) **c**) i) MeONa, MeOH, 15 min, ii) dry ice, 86 % (*trans*), 98 % (*cis*)

Experimental Synthesis Procedures

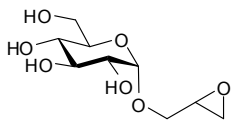
General methods. Allyl α-D-glucopyranoside was purchased from Carbosynth Ltd., UK and β-D-glucose pentaacetate from Sigma-Aldrich Co. All other reagents were commercially obtained from Acros, Aldrich, Alfa Aesar and Fluka and used without further purification. TLC were performed on pre-coated silica plates (Merck 60 F254, 0.25 mm) containing a fluorescence indicator. Compounds were visualised under UV (254 nm) and by heating after dipping in a solution of 5% H_2SO_4 in EtOH. Flash column chromatography was performed on silica gel (Biotage KP-SIL 60A, 40–63 μm). Standard column chromatography was performed on silica gel (Fluka 60, 63-200 μm). NMR spectra were recorded on a JEOL Lambda spectrometer at 400 MHz (^{1}H) or 100 MHz (^{13}C) or a Bruker Avance spectrometer at 600 MHz (^{1}H) or 150 MHz (^{13}C). ^{1}H NMR spectra recorded at 400 or 600 MHz were referenced to δ_H 7.26 for $CDCl_3$, δ_H 3.34 for CD_3OD, and ^{13}C NMR spectra recorded at 100 or 150 MHz were referenced to δ_C 77.0 for $CDCl_3$ and δ_C 49.05 for CD_3OD. Chemical

shifts of NMR signals recorded in D_2O are reported with respect to the methyl resonance of internal acetone at δ_H 2.22 ppm and δ_C 30.89 ppm. Assignments were made with the aid of COSY and HSQC experiments. Optical rotations were measured at ambient temperature on a Perkin-Elmer model 141 polarimeter using a sodium lamp. Low-resolution mass spectra, including MSn spectra (not reported), were obtained using a Thermo DecaXP^{plus} ion trap mass spectrometer equipped with electrospray ionisation (ESI-MS). High-resolution, accurate mass spectra were obtained using a Thermo LTQ Orbitrap mass spectrometer using positive electrospray ionisation. Matrix-assisted laser desorption/ionisation mass spectrometry was carried out using an UltraFlex™ MALDI-ToF/ToF incorporating the LIFT2™ module (Bruker Daltonics (UK) Ltd, Coventry, UK) and a 50 Hz 337 nm wavelength nitrogen laser. Samples were analysed in reflectron mode with an ion accelerating voltage of 25 kV using α-cyano-4-hydroxycinnamic acid matrix on pre-spotted anchor chip (PAC) plates (Bruker). Peptides were identified by searches of the SPtrEMBL database using an in-house version of the mass spectrometry search engine Mascot (Matrix Science, London, UK).

General procedure A: Epoxidation of terminal alkene. Appropriate alkenyl or cycloalkenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (2.6 mmol) was dissolved in $CHCl_3$ (10 ml) and the solution was cooled to 0°C with ice-bath. To this solution MCPBA (3.14 g, 18.2 mmol) dissolved in $CHCl_3$ (20 ml) was added dropwise. When the addition was finished, the ice-bath was removed and the reaction mixture was stirred overnight at room temperature. When conversion of the starting material was complete (followed by TLC) $CHCl_3$ (20 ml) was added to dissolve any precipitated material and the resulting solution was washed with saturated aq. solutions of sodium sulfite (Na_2SO_3, 10 ml), sodium bicarbonate $NaHCO_3$, 10 ml), sodium thiosulfate ($Na_2S_2O_3$, 10 ml), sodium bicarbonate (10 ml), and finally with water (10 ml). The organic layer was dried over $CaCl_2$, filtered and evaporated to give a crude mixture of products.

General procedure B: Deprotection. Appropriate epoxyalkyl or epoxycycloalkyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (1.08 mmol) was dried by azeotropic removal of water by addition of absolute benzene (10 ml) and evaporation at reduced pressure. To the residue abs. MeOH (5 ml) was added and the mixture was stirred vigorously at room temperature under nitrogen atmosphere. Then NaOMe in MeOH (1M, 276 µl) was added through a septum. The mixture was stirred vigorously at room temperature for exactly 15 min. The septum was removed and dry ice (~ 5g) was added to quench the reaction. The volatiles were evaporated and the residue was purified by standard column chromatography on silica gel (ethyl acetate / MeOH / H_2O 84:28:18).

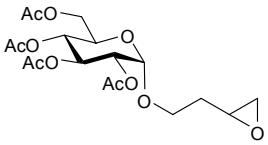
*(2'*R,S*)-2',3'-epoxypropyl α-D-glucopyranoside (α-EPG)*



(2'*R,S*)-2',3'-epoxypropyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside[1] (**4**) (714.6 mg, 1.77 mmol) was subjected to General procedure B to give pure α-EPG (336.1 mg, 81%) as a white amorphous solid. R_f = 0.39 (ethyl acetate / MeOH / H_2O 84:28:18); $[\alpha]^{20}_D$ +127.3 (*c* 1.0, H_2O), lit.[6] $[\alpha]_D$ +121.1 (*c* 0.51, H_2O); ^{1}H NMR (600 MHz; D_2O): spectrum is doubled δ = 4.99-4.94 (m, 1H, H1), 4.11-3.93 (m, 1H, H1'$_a$), 3.86-3.82 (m, 1H,

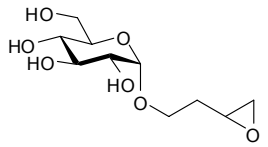
$H6_a$), 3.78-3.60 (m, 3H, H3, H4, $H6_b$), 3.59-3.34 (m, 4H, $H1'_b$, H2', H2, H5), 2.99-2.95 (m, 1H, $H3'_a$), 2.86-2.80 (m, 1H, $H3'_b$); ^{13}C NMR (150 MHz; D_2O): spectrum is doubled δ = 99.1, 99.0 (2 x d, 1C, C1 of epimers), 73.6 (d, 1C, C3), 72.5 (d, 1C, C4), 71.9 (d, 1C, C2), 70.1 (d, 1C, C5), 69.3, 68.6 (2 x t, 1C, C1' of epimers), 61.1 (t, 1C, C6), 52.4, 52.2 (2 x d, 1C, C2' of epimers), 45.7 (t, 1C, C3'); m/z (ESI$^+$) 495 ($[2M+Na]^+$, 100%), 259 ($[M+Na]^+$, 81%), 254 ($[M+NH_4]^+$, 84), 237 ($[M+H]^+$, 8); HR-MS calcd for $C_9H_{20}NO_7^+$ $[M+NH_4]^+$ 254.1234, found 254.1235. ^{13}C NMR spectrum was in good agreement with literature.[6]

*(3'*R,S*)-3',4'-epoxybutyl 2,3,4,6-tetra*-O-*acetyl-α-D-glucopyranoside (**5**)*



3'-Butenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside[4,5] (**2**, 1g, 2.49 mmol) was subjected to General procedure A. The resulting mixture was separated using flash column chromatography on silica gel (hexane / ethyl acetate, gradient from 12 to 100%) to give pure **5** (699.3 mg, 67 %) as a pale yellow oil. R_f = 0.43 (hexane / ethyl acetate 1:1); $[\alpha]^{25}_D$ +97.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$): spectrum is doubled δ = 5.48-5.44 (m, 1H, H2), 5.08-5.02 (m, 2H, H1, H4), 4.88-4.85 (m, 1H, H3), 4.24 (dd, 1H, $^2J_{6a,6b}$ = 12.3 Hz, $^3J_{5,6a}$ = 4.6 Hz, $H6_a$), 4.12-4.07 (m, 1H, $H6_b$), 4.03-3.99 (m, 1H, H5), 3.86-3.83 (m, 1H, $H1'_a$), 3.60-3.53 (m, 1H, $H1'_b$), 3.05-3.01 (m, 1H, H3'), 2.78 (bdd, 1H, $^2J_{4'a,4'b}$ = 9.9 Hz, $^3J_{3',4'a}$ = 5.0 Hz, $H4'_a$), 2.53-2.51 (m, 1H, $H4'_b$), 2.08, 2.05, 2.04, 2.01, 2.00 (5 x s, 12H, 4 x CH_3(CO)O); 1.98-1.92 (m, 1H, $H2'_a$), 1.75-1.66 (m, 1H, $H2'_b$); ^{13}C NMR 150 MHz; $CDCl_3$): spectrum is doubled δ = 171.0, 170.4, 169.9 (3 x s, 4C, 4 x CH_3(*C*O)O), 96.2, 96.0 (2 x d, 1C, C1 of epimers), 71.1 (d, 1C, C3), 70.4 (d, 1C, C2), 68.9, 68.8 (2 x d, 1C, C4 of epimers), 67.7 (d, 1C, C5), 65.9, 65.5 (2 x t, 1C, C1' of epimers), 62.2 (t, 1C, C6), 49.9, 49.8 (2 x d, 1C, C3' of epimers), 47.3 (t, 1C, C4'), 32.8, 32.5 (2 x t, 1C, C2' of epimers), 21.0, 20.9 (2 x q, 4C, 4 x *C*H_3(CO)O); m/z (ESI$^+$) 441 ($[M+Na]^+$, 100%), 436 ($[M+NH_4]^+$, 80), 331 (36); HR-MS calcd for $C_{18}H_{30}NO_{11}^+$ $[M+NH_4]^+$ 436.1813, found 436.1819.

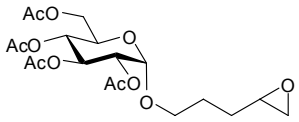
*(3'*R,S*)-3',4'-epoxybutyl α-D-glucopyranoside (α-EBG)*



(3'*R*,*S*)-3',4'-Epoxybutyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**5**, 589.1 mg, 1.408 mmol) was subjected to General procedure B to give pure α-EBG (298.5 mg, 85%) as a white amorphous solid. R_f = 0.45 ethyl acetate / MeOH / H_2O 84:28:18); $[\alpha]^{20}_D$ +120.2 (*c* 1.0, MeOH); ^{1}H NMR (600 MHz; D_2O): spectrum is doubled δ = 4.93 (bd, 1H, $^3J_{1,2}$ = 3.7 Hz, H1), 3.92-3.84 (m, 2H, $H6_a$, $H1'_a$), 3.76-3.62 (m, 4H, H3, H4, $H6_b$, $H1'_b$), 3.55 and 3.54 (2 overlapping dd, 1H, $^3J_{2,3}$ = 9.8 Hz, H2 of epimers), 3.42-3.38 (m, 1H, H5), 3.27-3.24 (m, 1H, H3'), 3.95 and 2.94 (2 overlapping dd, 1H, $^2J_{4'a,4'b}$ = 11.8 Hz, $^3J_{4'a,3'}$ = 4.3 Hz, $H4'_a$ of epimers), 2.73 and 2.72 (2 overlapping dd, 1H, $^3J_{4'b,3'}$ = 3.2 Hz, $H4'_b$ of epimers), 2.06-2.00 (m, 1H, $H2'_a$),

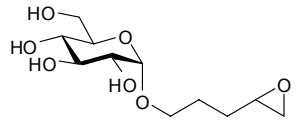
1.79-1.73 (m, 1H, H2'$_b$); ^{13}C NMR 150 MHz; D_2O): spectrum is doubled δ = 98.9, 98.7 (2 x d, 1C, C1 of epimers), 73.7 (d, 1C, C3), 72.5 (d, 1C, C4), 72.0, 71.9 (2 x d, 1C, C2 of epimers), 70.3, 70.2 (2 x d, 1C, C5), 65.9, 65.1 (2 x t, 1C, C1' of epimers), 61.2 (t, 1C, C6), 52.6, 52.3 (2 x d, 1C, C3' of epimers), 48.8, 48.2 (2 x t, 1C, C4' of epimers), 32.5, 32,3 (2 x t, 1C, C2'); m/z (ESI$^+$) 523 ([2M+Na]$^+$, 40%), 273 ([M+Na]$^+$, 100%), 268 ([M+NH_4]$^+$, 31); HR-MS calcd for $C_{10}H_{22}NO_7^+$ [M+NH_4]$^+$ 268.1391, found 268.1388.

*(4'*R, S*)-4',5'-epoxypentyl 2,3,4,6-tetra*-O-*acetyl-α-D-glucopyranoside (**6**)*



4'-Pentenyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside[4,5] (**3**, 430.0 mg, 1.03 mmol) was subjected to General procedure A. The resulting mixture was separated using flash column chromatography on silica gel (hexane / ethyl acetate, gradient from 8 to 60%) to give pure **6** (225.4 mg, 51 %) as a pale yellow oil. R_f = 0.41 (hexane / ethyl acetate 1:1); $[\alpha]^{20}_D$ +64.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$): spectrum is doubled δ = 5.46 and 5.44 (2 overlapping dd, 1H, $^3J_{2,3}$= 9.8 Hz, $^3J_{1,2}$ = 3.6 Hz,H2 of epimers), 5.05-5.02 (m, 2H, H1, H4), 4.86-4.83 (m, 1H, H3), 4.24 (dd, 1H, $^2J_{6a,6b}$ = 12.3 Hz, $^3J_{5,6a}$ = 4.6 Hz, H6$_a$), 4.08 (dd, 1H, $^3J_{5,6b}$ = 2.2 Hz, H6$_b$), 3.99 (ddd, 1H, $^3J_{4,5}$ = 10.2 Hz, H5), 3.77-3.72 (m, 1H, H1'$_a$), 3.49-3.44 (m, 1H, H1'$_b$), 2.95-2.91 (m, 1H, H4'), 2.76-2.75 (m, 1H, H5'$_a$), 2.48-2.46 (m, 1H, H5'$_b$), 2.08, 2.04, 2.02, 2.00 (4 x s, 12H, 4 x C*H*$_3$(CO)O); 1.81-1.73 (m, 2H, H2'), 1.72-1.65 (m, 1H, H3'$_a$), 1.58-1.51 (m, 1H, H3'$_b$); ^{13}C NMR (150 MHz; $CDCl_3$): spectrum is doubled δ = 171.0, 170.5, 169.9 (3 x s, 4C, 4 x CH_3(*C*O)O), 96.0 (d, 1C, C1), 71.2 (d, 1C, C3), 70.5 (d, 1C, C2), 68.9 (d, 1C, C4), 68.4 (t, 1C, C1'), 67.6 (d, 1C, C5), 62.3, 62.2 (2 x t, 1C, C6 of epimers), 52.2, 52.1 (2 x d, 1C, C4' of epimers), 47.3, 47.2 (2 x t, 1C, C5' of epimers), 29.4 (t, 1C, C3'), 26.2 (t, 1C, C2'), 21.0, 20.9 (2 x q, 4C, 4 x *C*H$_3$(CO)O); m/z (ESI$^+$) 455 ([M+Na]$^+$, 72%), 450 ([M+NH_4]$^+$, 100), 331 (23); HR-MS calcd for $C_{19}H_{32}NO_{11}^+$ [M+NH_4]$^+$ 450.1970, found 450.1970.

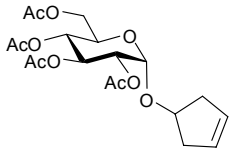
*(4'*R, S*)-4',5'-epoxypentyl α-D-glucopyranoside (α-EPeG)*



(4'*R, S*)-4',5'-epoxypentyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**6**, 179.6 mg, 0.415 mmol) was subjected to General procedure B to give pure α-EPeG (104.5 mg, 95%) as a white amorphous solid (limited solubility in H_2O). R_f = 0.48 (ethyl acetate / MeOH / H_2O 84:28:18); $[\alpha]^{20}_D$ +95.9 (*c* 1.0, MeOH); ^{1}H NMR (600 MHz; CD_3OD): spectrum is doubled δ = 4.81 (2 overlapping d, 1H, $^3J_{1,2}$ = 3.7 Hz, H1 of epimers), 3.85-3.81 (m, 2H, H6$_a$, H1'$_a$), 3.71-3.65 (m, 2H, H6$_b$, H3), 3.61-3.58 (m, 1H, H4), 3.55-3.51 (m, 1H, H1'$_b$), 3.42 (dd, 1H, $^3J_{2,3}$ = 9.7 Hz, H2), 3.33-3.25 (m, 1H, H5), 3.03-3.00 (m, 1H, H4'), 2.78 (2 overlapping dd, 1H, $^2J_{5'a,5'b}$ = 5.1 Hz, $^3J_{5'a,4'}$ = 4.3 Hz, H5'$_a$ of epimers), 2.54 (dd, 1H, $^3J_{5'b,4'}$ = 2.8 Hz, H5'$_b$), 1.87-1.78 (m, 2H, H2'), 1.78-1.71 (m, 1H, H3'$_a$), 1.64-1.56 (m, 1H, H3'$_b$); ^{13}C NMR (150 MHz; CD_3OD): spectrum is doubled δ = 100.2 (d, 1C, C1), 75.2 (d, 1C, C3), 73.8, 73.7 (2 x d, 1C, C4 of epimers), 73.6 (d, 1C, C2), 71.9 (d, 1C, C5), 68.7 (t, 1C, C1'), 62.8, 62.7 (2 x t, 1C, C6 of epimers), 53.4, 53.3 (2 x d, 1C, C4' of epimers), 47.8 (t,

1C, C5'), 30.5, 30.4 (2 x t, 1C, C3'), 27.2, 27.0 (2 x t, 1C, C2'); m/z (ESI$^+$) 287 ([M+Na]$^+$, 100%); HR-MS calcd for $C_{11}H_{24}NO_7^+$ [M+NH$_4$]$^+$ 282.1547, found 282.1547.

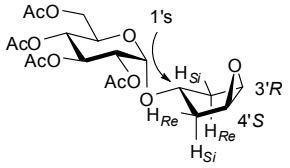
3'-Cyclopentenyl 2,3,4,6-tetra-O-*acetyl-α-D-glucopyranoside (**7**)*



To an oven-dried round bottom flask was added β-D-glucose pentacetate (1 g, 2.56 mmol) and abs. dichloromethane (50 ml). $SnCl_4$ in dichloromethane (1 M, 3.31 ml, 3.31 mmol) was added through a septum under nitrogen atmosphere. The mixture was stirred for 15 min at room temperature. 3-Cyclopenten-1-ol (278 mg, 3.31 mmol) was added and stirring was continued. The reaction course was monitored carefully by TLC and after 24 hrs when most of the undesired β-anomer (R_f = 0.24, hexane / ethyl acetate 3:1) disappeared, water (25 ml) was added to the mixture and stirring was continued for 15 min. The mixture was extracted with dichloromethane (4 x 50 ml). The combined extracts were neutralised with sat. aq. $NaHCO_3$ solution (50 ml), washed with water (50 ml), dried over Na_2SO_4, filtered and concentrated to give a crude mixture of products. The mixture was separated using flash column chromatography on silica gel (hexane / ethyl acetate, gradient from 6 to 100%) to give pure **7** (541.6 mg, 51 %) as a pale yellow oil. R_f = 0.31 (hexane / ethyl acetate 3:1); $[\alpha]^{20}_D$ +115.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$): δ = 5.69 (m, 2H, H3', H4'), 5.45 (dd, 1H, $^3J_{2,3} = {}^3J_{3,4}$ = 9.7 Hz, H3), 5.18 (d, 1H, $^3J_{1,2}$ = 3.7 Hz, H1), 5.05 (dd, 1H, $^3J_{4,5}$ = 9.7 Hz, H4), 4.81 (dd, 1H, H2), 4.46-4.42 (m, 1H, H1'), 4.25 (dd, 1H, $^2J_{6a,6b}$ = 12.3 Hz, $^3J_{5,6a}$ = 4.5 Hz, H6$_a$), 4.12-4.04 (m, 2H, H6$_b$, H5), 2.63-2.26 (m, 4H, H2', H5'), 2.09, 2.04, 2.02, 2.00 (4 x s, 12H, 4 x C*H*$_3$(CO)O); ^{13}C NMR (150 MHz; $CDCl_3$): δ = 170.7, 170.3, 170.2, 169.7 (4 x s, 4C, 4 x CH$_3$(*C*O)O), 128.4, 127.9 (2 x d, 2C,C3', C4'), 94.7 (d, 1C, C1), 78.0 (d, 1C, C1'), 70.9 (d, 1C, C2), 70.2 (d, 1C, C3), 68.9 (d, 1C, C4), 67.4 (d, 1C, C5), 62.0 (t, 1C, C6), 39.6, 39.0 (2 x t, 2C, C2', C5'), 20.7, 20.6 (2 q, 4C, 4 x *C*H$_3$(CO)O); m/z (ESI$^+$) 437 ([M+Na]$^+$, 100%), 432 ([M+NH$_4$]$^+$, 53), 331 (22); HR-MS calcd for $C_{19}H_{30}NO_{10}^+$ [M+ NH$_4$]$^+$ 432.1864, found 453.1861;

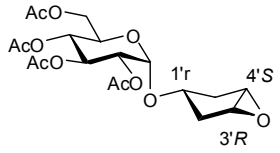
*(1's,3'*R*,4'*S*)-3',4'-epoxycyclopentyl 2,3,4,6-tetra*-O-*acetyl-α-D-glucopyranoside (**8**) and (1'r,3'*R*,4'*S*)-3',4'-epoxycyclopentyl 2,3,4,6-tetra*-O-*acetyl-α-D-glucopyranoside (**9**)*

3'-Cyclopentenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**7**; 1.078 g, 2.6 mmol) was subjected to General procedure A. The resulting mixture was separated using flash column chromatography on silica gel (hexane / ethyl acetate, gradient from 6 to 100%) to give pure **8** (554.2 mg, 50 %) as a pale yellow oil.



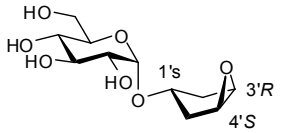
R_f = 0.43 (hexane / ethyl acetate 1:1); $[\alpha]^{23}_D$ +123.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$): δ = 5.43 (dd, 1H, $^3J_{2,3} = {}^3J_{3,4}$ = 10.0 Hz, H3), 5.05-5.01 (m, 2H, H1, H4), 4.81 (dd, 1H, $^3J_{1,2}$ = 3.8 Hz, H2), 4.25 (dd,

1H, $^{2}J_{6a,6b}$ = 12.3 Hz, $^{3}J_{5,6a}$ = 4.8 Hz, H6$_a$), 4.07 (dd, 1H, $^{3}J_{5,6b}$ = 2.1 Hz, H6$_b$), 4.01-3.97 (m, 2H, H1', H5), 3.51 (bd, 2H, $^{3}J_{3',4'}$ = 6.4 Hz, H3', H4'), 2.51-2.47 (m, 2H, H*Si*2', H*Re*5'), 2.10, 2.06, 2.03, 2.01 (4 x s, 12H, 4 x CH_3(CO)O), 1.81-1.76 (m, 1H, H*Re*2' or H*Si*5'), 1.63-1.59 (m, 1H, H*Re*2' or H*Si*5'); ^{13}C NMR (150 MHz; $CDCl_3$): δ = 170.6, 170.2, 170.0, 169.6 (4 x s, 4C, 4 x CH_3(*C*O)O), 95.4 (d, 1C, C1), 75.0 (d, 1C, C1'), 70.8 (d, 1C, C2), 70.0 (d, 1C, C3), 68.6 (d, 1C, C4), 67.5 (d, 1C, C5), 61.9 (t, 1C, C6), 55.4, 55.1 (2 x d, 2C, C3' and C4'), 34.4, 33.6 (2 x t, 2C, C2' and C5'), 20.7, 20.6 (2 q, 4C, 4 x *C*H_3(CO)O); m/z (ESI$^+$) 453 ([M+Na]$^+$, 100%), 448 ([M+NH$_4$]$^+$, 85), 331 (11); HR-MS calcd for $C_{19}H_{26}NaO_{11}^+$ [M+Na]$^+$ 453.1367, found 453.1367; and **9** (239.8 mg, 21 %) as a pale yellow oil.



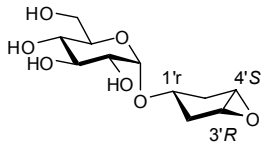
R_f = 0.23 (hexane / ethyl acetate 1:1); $[\alpha]^{23}_D$ +114.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$): δ = 5.45 (dd, 1H, $^{3}J_{2,3} = {}^{3}J_{3,4}$ = 9.7 Hz, H3), 5.15 (d, 1H, $^{3}J_{1,2}$ = 3.9 Hz, H1), 5.03 (dd, 1H, $^{3}J_{4,5}$ = 9.7 Hz, H4), 4.74 (dd, 1H, H2), 4.26-4.23 (m, 2H, H6$_a$, H1'), 4.17-4.14 (m, 1H, H5), 4.10 (dd, 1H, $^{2}J_{6a,6b}$ = 12.2 Hz, $^{3}J_{5,6b}$ = 2.3 Hz, H6$_b$), 3.51 (bs, 2H, H3', H4'), 2.35-2.32 and 2.12-1.95 (2 m, 4H, H$_{Si}$2', H$_{Re}$2', H$_{Si}$5', H$_{Re}$5'), 2.09, 2.08, 2.03, 2.00 (4 x s, 12H, 4 x CH_3(CO)O); ^{13}C NMR (150 MHz; $CDCl_3$): δ = 170.7, 170.5, 170.0, 169.8 (4 x s, 4C, 4 x CH_3(*C*O)O), 94.9 (d, 1C, C1), 77.8 (d, 1C, C1'), 71.1 (d, 1C, C2), 70.3 (d, 1C, C3), 68.7 (d, 1C, C4), 67.2 (d, 1C, C5), 62.0 (t, 1C, C6), 57.2, 56.9 (2 x d, 2C, C3' and C4'), 35.2, 34.8 (2 x t, 2C, C2' and C5'), 20.7 (q, 4C, 4 x *C*H_3(CO)O); m/z (ESI$^+$) 453 ([M+Na]$^+$, 56%), 448 ([M+NH$_4$]$^+$, 100), 331 (5); HR-MS calcd for $C_{19}H_{26}NaO_{11}^+$ [M+Na]$^+$ 453.1367, found 453.1363.

*(1's,3'*R*,4'*S*)-3',4'-epoxycyclopentyl α-D-glucopyranoside (α-*trans*-ECypG)*



(1's,3'*R*,4'*S*)-3',4'-epoxycyclopentyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**8**) (465.8 mg, 1.08 mmol) was subjected to General procedure B to give pure α-*trans*-ECypG (243.5 mg, 86%) as a white amorphous solid. R_f = 0.39 (ethyl acetate / MeOH / H_2O 84:28:18); $[\alpha]^{20}_D$ +125.8 (*c* 1.0, MeOH); ^{1}H NMR (600 MHz; D_2O): δ = 4.88 (d, 1H, $^{3}J_{1,2}$ = 3.9 Hz, H1), 3.99 (p, 1H, $^{3}J_{1',2'si} = {}^{3}J_{1',2'Re} = {}^{3}J_{1',5'si} = {}^{3}J_{1',5'Re}$ = 7.3 Hz, H1'), 3.83 (dd, 1H, $^{2}J_{6a,6b}$ = 12.3 Hz, $^{3}J_{5,6a}$ = 2.3 Hz, H6$_a$), 3.70 (dd, 1H, $^{3}J_{5,6b}$ = 5.3 Hz, H6$_b$), 3.71 (bs, 2H, H3', H4'), 3.66-3.63 (m, 2H, H3, H4), 3.50 (dd, 1H, $^{3}J_{2,3}$ = 9.9 Hz, H2), 3.40-3.36 (m, 1H, H5), 2.58-2.51 (m, 2H, H*Si*2', H*Re*5'), 1.87-1.83 (m, 1H, H*Re*2' or H*Si*5'), 1.80-1.76 (m, 1H, H*Re*2' or H*Si*5'); ^{13}C NMR (150 MHz; D_2O): δ = 98.6 (d, 1C, C1), 75.0 (d, 1C, C1'), 73.6 (d, 1C, C3), 72.7 (d, 1C, C4), 71.8 (d, 1C, C2), 70.2 (d, 1C, C5), 61.2 (t, 1C, C6), 58.2, 57.8 (2 x d, 2C, C3' and C4'), 34.5, 33.5 (2 x t, 2C, C2' and C5'); m/z (ESI$^+$) 285 ([M+Na]$^+$, 100%), 263 ([M+H]$^+$, 57); HR-MS calcd for $C_{11}H_{19}O_7^+$ [M+H]$^+$ 263.1125, found 263.1124.

*(1'r,3'*R*,4'*S*)-3',4'-epoxycyclopentyl α-D-glucopyranoside (α-*cis*-ECypG)*



(1'r,3'*R*,4'*S*)-3',4'-epoxycyclopentyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**9**, 209.8 mg, 0.487 mmol) was subjected to General procedure B to give pure α-cis-EcypG (125.5 mg, 98%) as a white amorphous solid. R_f = 0.27 (ethyl acetate / MeOH / H_2O 84:28:18); $[\alpha]^{20}_D$ +122.6 (*c* 1.0, MeOH); ^{1}H NMR (600 MHz; D_2O): δ = 4.89 (d, 1H, $^3J_{1,2}$ = 3.9 Hz, H1), 4.39-4.37 (m, 1H, H1'), 3.84 (dd, 1H, $^2J_{6a,6b}$ = 12.3 Hz, $^3J_{5,6a}$ = 2.3 Hz, $H6_a$), 3.74-3.71 (m, 3H, $H6_b$, H3', H4'), 3.69-3.63 (m, 2H, H3, H4), 3.45 (dd, 1H, $^3J_{2,3}$ = 9.9 Hz, H2), 3.38-3.34 (m, 1H, H5), 2.25-2.10 (m, 4H, H2', H5'); ^{13}C NMR (150 MHz; D_2O): δ = 97.9 (d, 1C, C1), 76.2 (d, 1C, C1'), 73.6 (d, 1C, C3), 72.7 (d, 1C, C4), 72.0 (d, 1C, C2), 70.4 (d, 1C, C5), 61.2 (t, 1C, C6), 60.3, 60.1 (2 x d, 2C, C3' and C4'), 35.9, 34.9 (2 x t, 2C, C2' and C5'); m/z (ESI$^+$) 285 ([M+Na]$^+$, 100%); HR-MS calcd for $C_{11}H_{18}NaO_7^+$ [M+Na]$^+$ 285.0945, found 285.0943.

MALDI-ToF mass spectrometry of trypsin/cyanogen bromide digests of BMY1-EBG adduct

In order to confirm the formation of a covalent bond and to assign the amino acid residue of BMY1 reacting with the irreversible inactivator α-EBG, a crystal of the enzyme was first treated with the inhibitor. The complex was subjected to proteolytic digestion with trypsin and analysed by MALDI-ToF mass spectrometry (Fig. S3). Specifically, a crystal of BMY1-α-EBG was dissolved in 10 µl water and a 1 µl portion was reductively alkylated and digested with porcine-modified sequencing grade trypsin (Promega). Acidified digests were spotted directly onto PAC target plates. MALDI ToF analysis showed a peak at m/z 2537.192 that corresponds well to the calculated monoisotopic mass for the expected modified peptide (Glu184 bound to α-EBG) fragment E_{165}FLDAGVIVDIEVGLGPAGE$_{184}$MR_{186} ([M+H]$^+$ = 2537.2697). An observed ion at m/z 2553.2647 (Fig. S3) is believed to be the same modified peptide fragment oxidised at Met185 (calculated monoisotopic mass for [M+H]$^+$ = 2553.2647). Consistent with the electron density maps, these data confirm the covalent modification of Glu184.

The trypsin digestion was relatively difficult to perform but the data were confirmed by an alternative chemical digestion approach based on cyanogen bromide cleavage. Specifically, 1 µl of the BMY1-α-EBG stock used for trypsin digestion was dissolved in 100 µl 0.1N HCl containing cyanogen bromide (5 mg, Sigma) and incubated at room temperature in the dark for 36 h. The sample was then freeze-dried and redissolved in water 3 times to remove the acid and cyanogen bromide. Finally the sample was redissolved in 10 µl of 0.1% trifluoroacetic acid and spotted directly onto PAC target plates for analysis by MALDI ToF mass spectrometry. The expected modified peptides (Glu184 bound to α-EBG; Met185 converted to homoserine lactone) K_{164}EFLDAGVIVDIEVGLGPAGE$_{184}$M$_{185}$ (calculated monoisotopic mass [M+H]$^+$ = 2461.2602) and K_{164}EFLDAGVIVDIEVGLGPAGE$_{184}$M$_{185}$ (Met185 converted to homoserine; [M+H]$^+$ = 2479.2708) were detected by MALDI-ToF to better than 50 ppm mass accuracy (Fig. S4). The modified peptide appearing at m/z 2461 was confirmed by coupled MS-MS to give the predicted sequence

$K_{164}EFLDAGVIVDIEVGLGPAGE_{184}M_{185}$ (Met185 converted to homoserine lactone) with modification at the C-terminal Glu184 residue (Fig. S5).

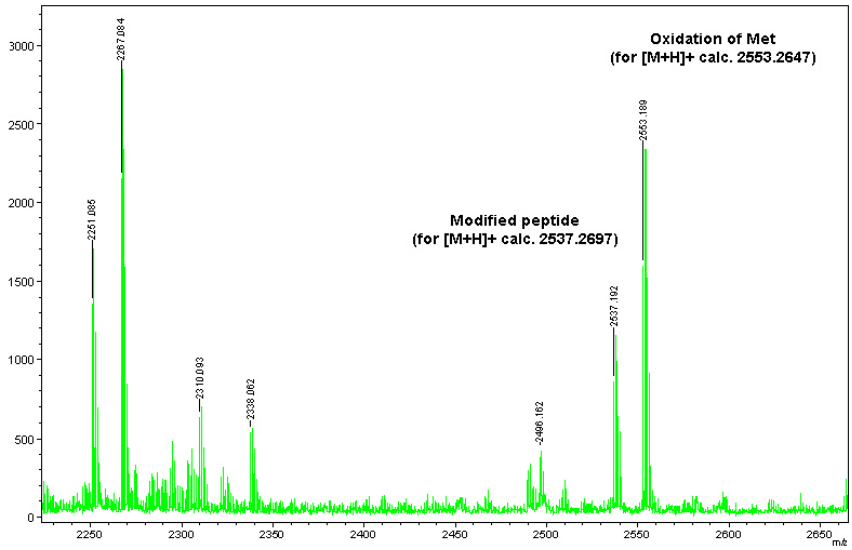


Fig. S3 MALDI-ToF spectrum of barley β-amylase-α-EBG trypsin digest

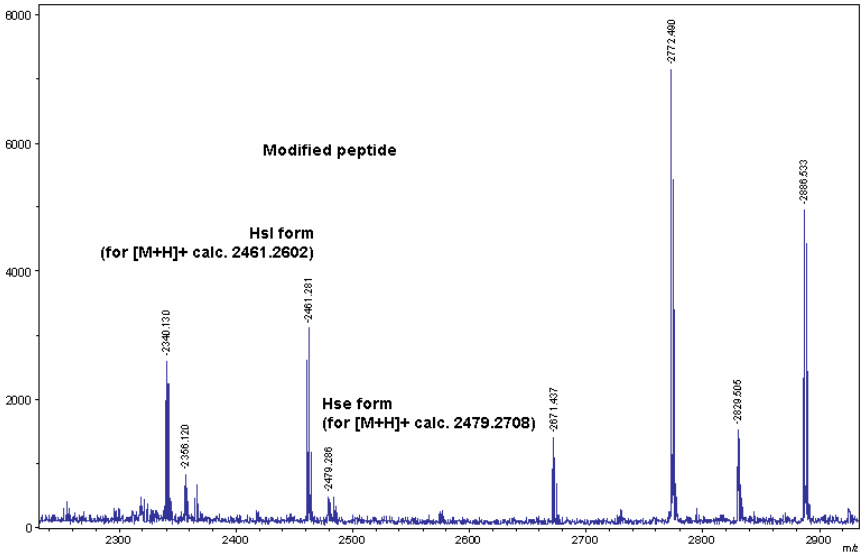


Fig. S4 MALDI-ToF spectrum of barley β-amylase-α-EBG cyanogen bromide digest (Hsl = homoserine lactone; Hse = homoserine)

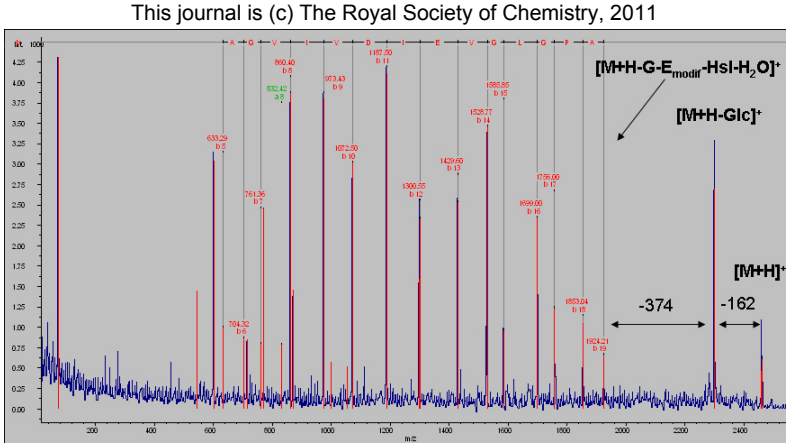


Fig. S5 Coupled MS-MS analysis of peptide fragment m/z 2461 (Hsl = homoserine lactone).

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