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Supporting information for article:

Novel mode of inhibition by D-tagatose-6-phosphate through a Heyns rearrangement in the active site of transaldolase B variants

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Table S1 Bacterial strains, plasmids and oligonucleotides which were used in this study. Bold sequences – mutagenised codons, underlined sequences - changed restriction site.

Strain designation	Relevant genotype/marker	Re./origin
BL21 (DE3) Star	<i>F</i> - <i>ompT hsdSB (rB-mB-) gal dcm</i>	Invitrogen, Karlsruhe,
pLysS	<i>rne131 (DE3) pLysS (Cam^R)</i>	Germany
LJ110 (DE3) talA ⁻	alias W3110, wild-type K-12, , talB ⁻ , prototroph, <i>DE3, talA-, talB-, pLysS</i>	(Schneider et al., 2008)
pLysS RARE2	<i>RARE2</i>	
Rosetta (DE3)	<i>F</i> - <i>ompT hsdSB(rB⁻ mB⁻) gal dcm (DE3)</i>	Novagen – Merck Millipore,
pLysS RARE2	<i>pLysS (Cam^R)</i>	Darmstadt, Germany
XL1Blue	<i>recA1, endA1, gyrA96, thi-1 hsdR17</i> (<i>rB⁻, mB⁺</i>), <i>supE44, relA1 lac</i> [<i>F'</i> , <i>proAB⁺, lacI^qZΔM15 Tn10 (Tet^R)</i>]	Stratagene, Amsterdam, Netherlands
Plasmids	Relevant markers	Re./origin
pET22b(+)	<i>PT7, Amp^R, lacI</i>	Novagen - Merck Millipore, Darmstadt, Germany
pJF119EH	<i>Ptac, Amp^R, lacI</i>	(Fürste et al., 1986)
pLysS RARE2	<i>lacI, Cam^R, p15a ori, tRNA genes: proL, leuW, metT, argW, thrT, glyT, tyrU, thrU</i>	Novagen - Merck Millipore, Darmstadt, Germany; (Novy et al., 2001)
pET22bfsaA	<i>fsaA</i> gene of <i>E. coli</i> K12 (strain MC4100) with N-term. 6xHis	(Sánchez-Moreno et al., 2012)
pET22bfsaA A129S	<i>fsaA</i> Ala129Ser gene of <i>E. coli</i> K12 (strain MC4100) with N-term. 6xHis	This study Variant was described previously in (Schürmann, 2001; Castillo et al., 2010)
pJF119frk	<i>frk</i> gene of <i>Zymomonas mobilis</i> in pJF119EH (<i>Kpn</i> I/ <i>Bam</i> HI)	N. Trachtmann, unpublished
pET28gatD	<i>gatD</i> gene of <i>E. coli</i> W3110 with N-term. 6xHis (<i>Nde</i> I/ <i>Eco</i> RI)	A. Samland, unpublished
pJF119talB	<i>talB</i> gene of <i>E. coli</i> W3110 amplified with N-term. 6xHis	(Schneider et al., 2008)
pJF119talB F178Y	<i>talB</i> Phe178Tyr gene with N-term. 6xHis	(Schneider et al., 2008)

pJF119*talB* E96Q *talB* Glu96Gln gene with N-term. 6xHis (Stellmacher et al., 2015)
pJF119*talB* E96Q *talB* Glu96Gln Phe178Tyr gene with N- (Stellmacher et al., 2015)
F178Y term. 6xHis

Oligonucleotides

FSAA A129S 5'-G GCA GGT GCG GAA TAT GTT TCG CCT TAC G-3'
forward

FSAA A129S 5'-C AAT ACG ATT AAC GTA AGG CGA AAC ATA TTC3'
reverse

Table S2 Half-life of the remaining specific activity. Calculation of the half-life is based on two or more independent measurements from which the exponential decrease of the specific activity was derived. Each measurement of the decrease consists of several assays of the remaining enzymatic activity at different points in time.

Protein	Excess of D-T6P compared to enzyme	Half-life of remaining specific activity	R ²
FSAA ^{A129S}	10-fold	22h 39 min	0.9368
FSAA	1-fold	25h 07 min	0.9793
	10-fold	14h 25 min	0.9623
TalB ^{E96Q F178Y}	1-fold	0h 27 min	0.9970
TalB ^{F178Y}	1-fold	3h 39 min	0.9650
	2-fold	2h 10 min	0.9914
	5-fold	1h 04 min	0.9932
	10-fold	0h 50 min	0.9959

Table S3 Overview of calculated and detected mass.

MS-Sample	Mass calculated [Da] (Gasteiger E. et al., 2005)	Mass detected [Da] main peak	Difference in mass of enzyme and inhibited enzyme [Da]
TalB ^{F178Y}	37297.4	37302.1	242.5
TalB ^{F178Y} + D-T6P	37541.2	37544.6	
TalB ^{E96Q F178Y}	37296.4	37301.6	242.3
TalB ^{E96Q F178Y} + D-T6P	37540.2	37543.9	
FSAA	23819.6	23823.2	242.1
FSAA + D-T6P	24061.3	24065.3	
FSAA ^{A129S}	23835.6	23839.8	241.4
FSAA ^{A129S} + D-T6P	24077.4	24081.2	

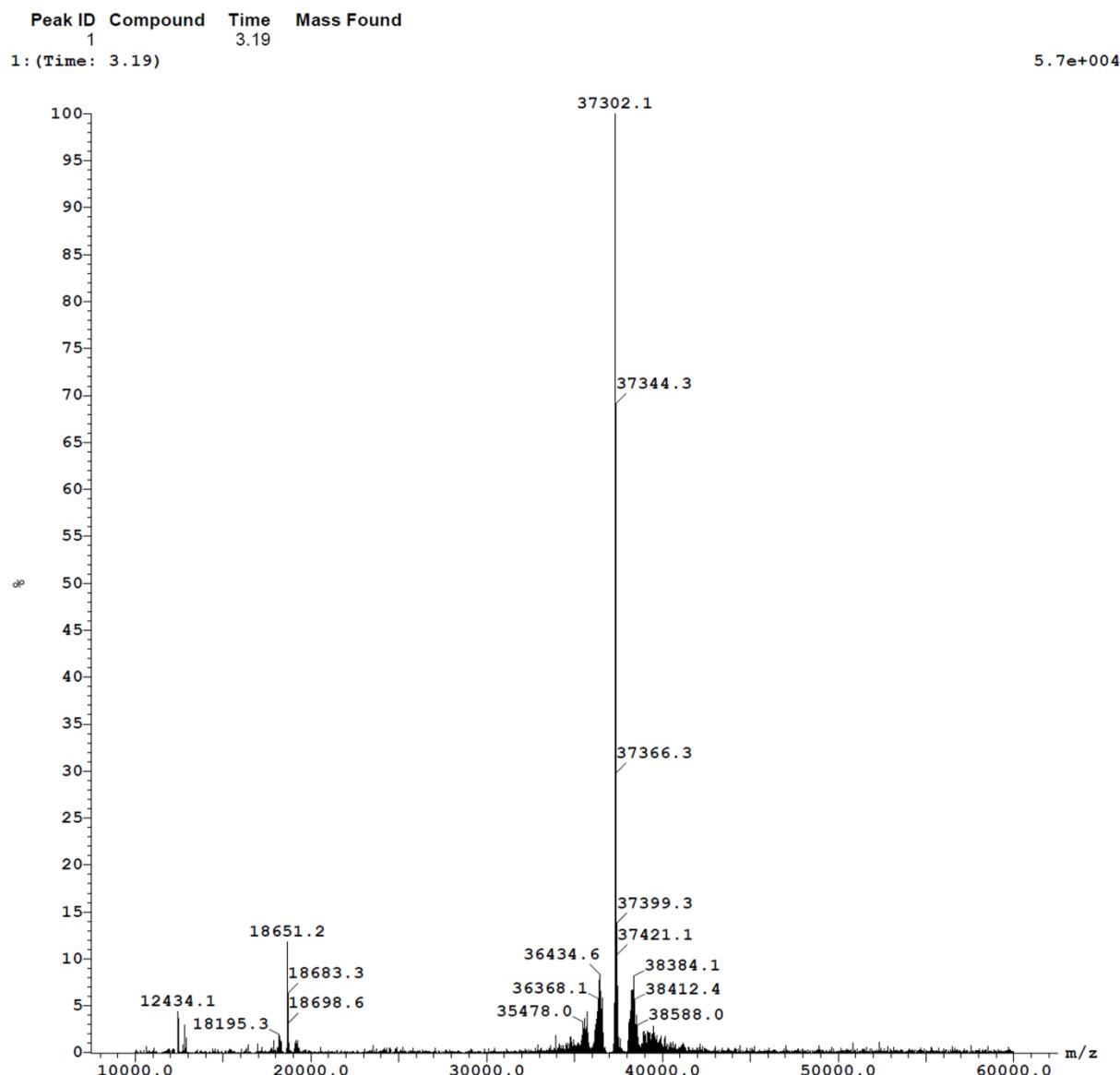


Figure S1 LC-ESI-MS of His₆-TalB^{F178Y}. Main peak displayed at 37302.1 Da.

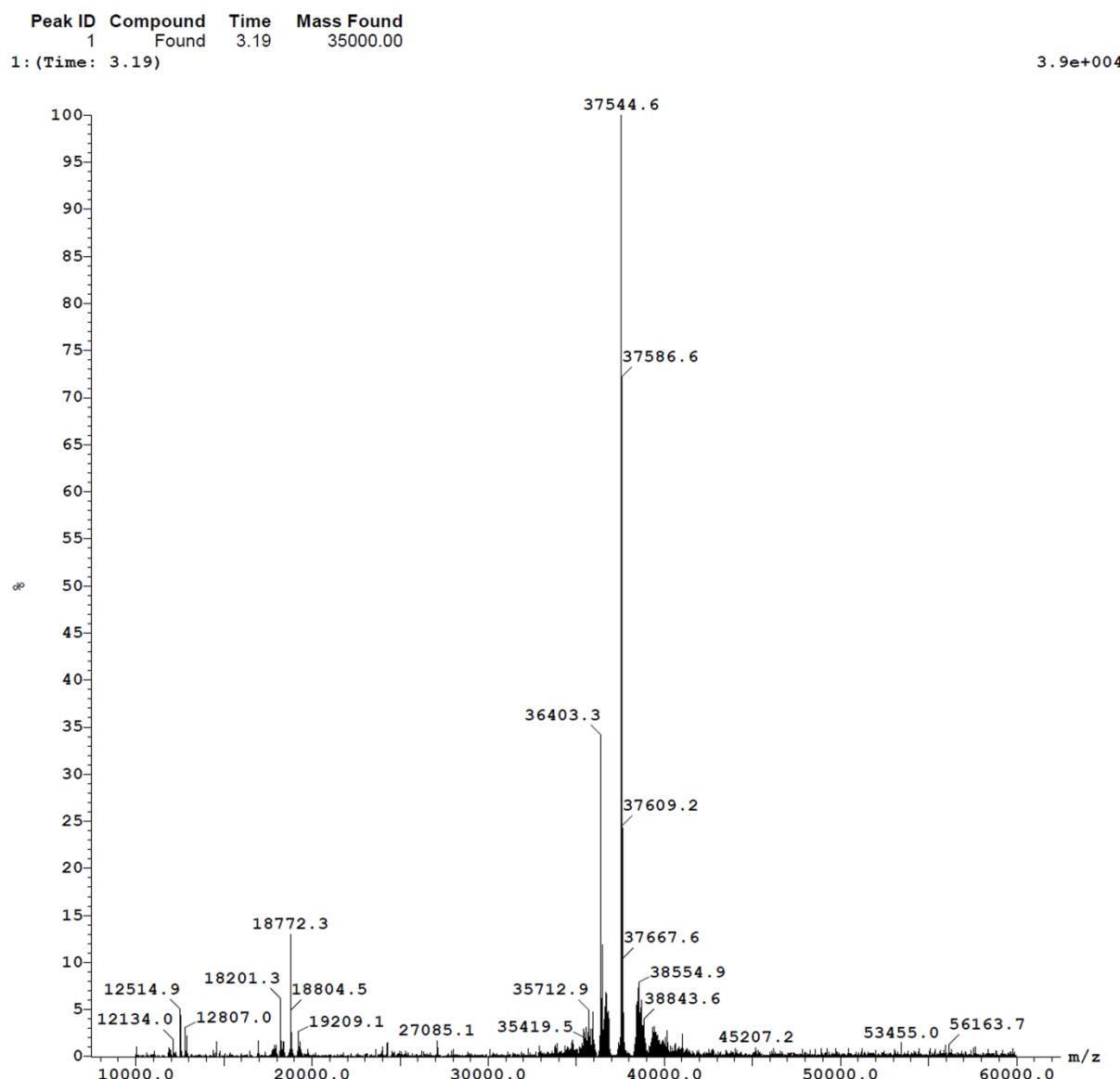


Figure S2 LC-ESI-MS of D-T6P bound to His₆-TalB^{F178Y}. Main peak displayed at 37544.6 Da.

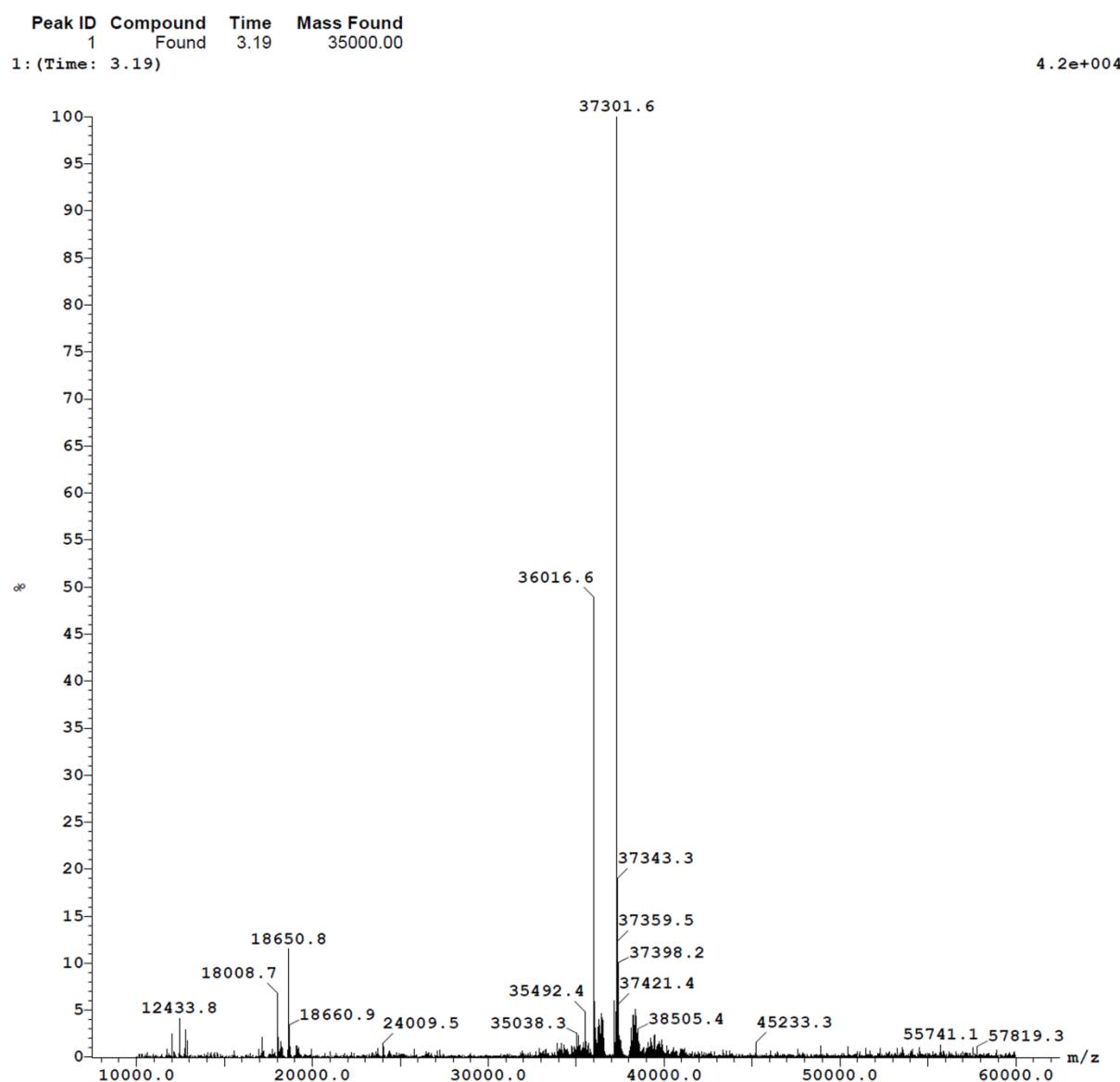


Figure S3 LC–ESI-MS of His₆-TalB^{E96Q F178Y}. Main peak displayed at 37301.6 Da.

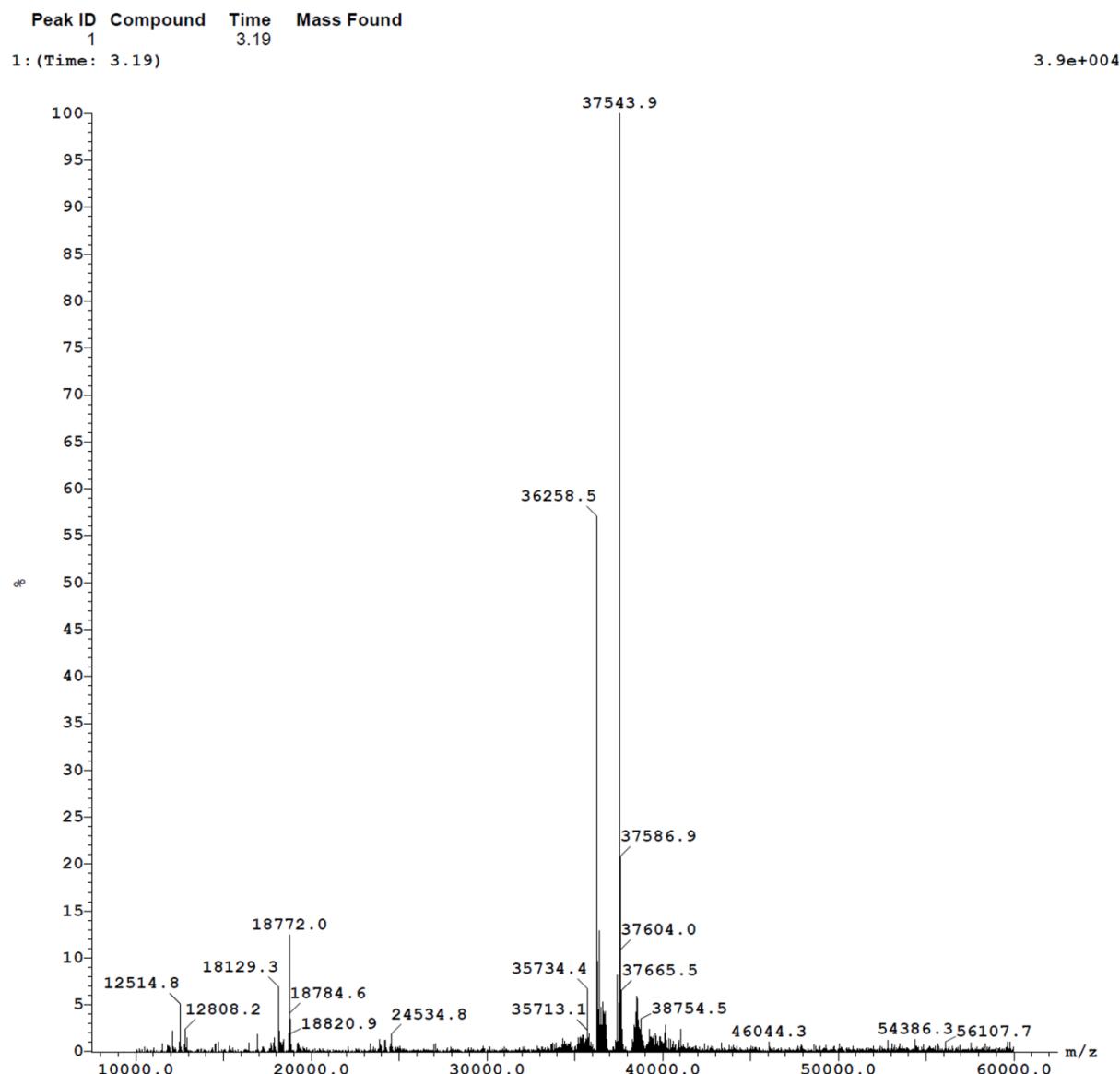


Figure S4 LC-ESI-MS of D-T6P bound to His₆-TalB^{E96Q F178Y}. Main peak displayed at 37543.9 Da.

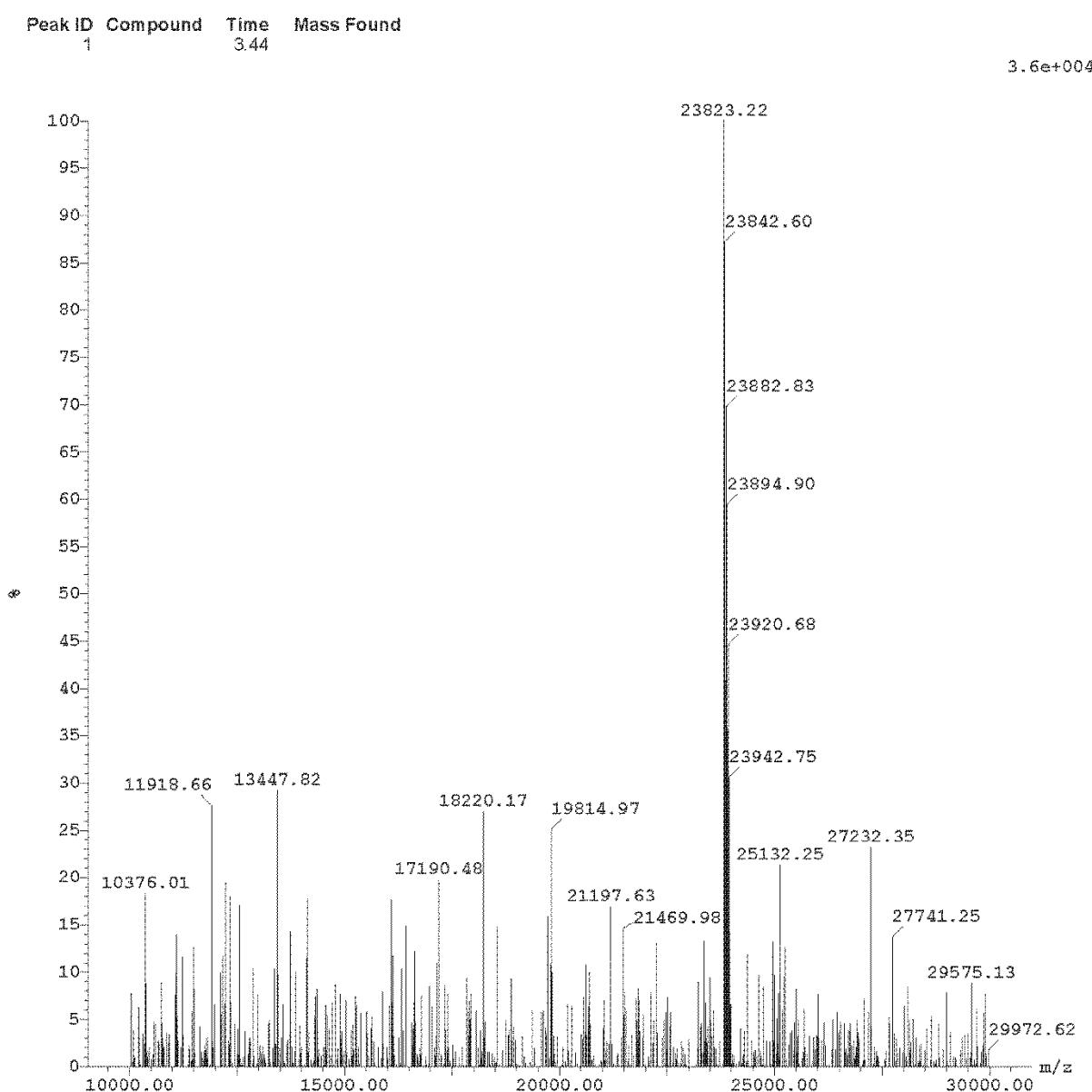


Figure S5 LC–ESI-MS of His₆-FSAA. Main peak displayed at 23823.2 Da.

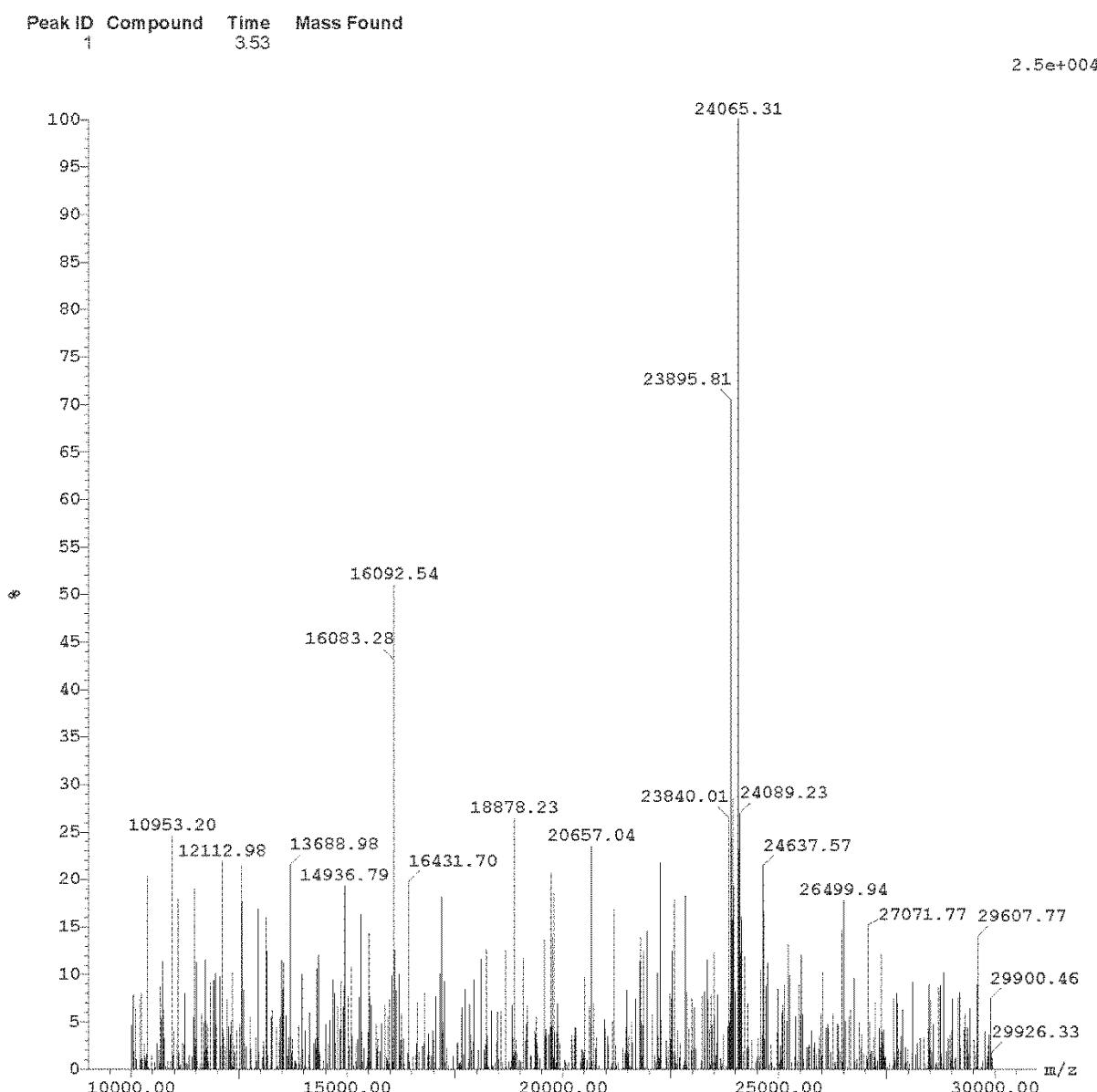


Figure S6 LC–ESI-MS of D-T6P bound to His₆-FSAA. Main peak displayed at 24065.3 Da.

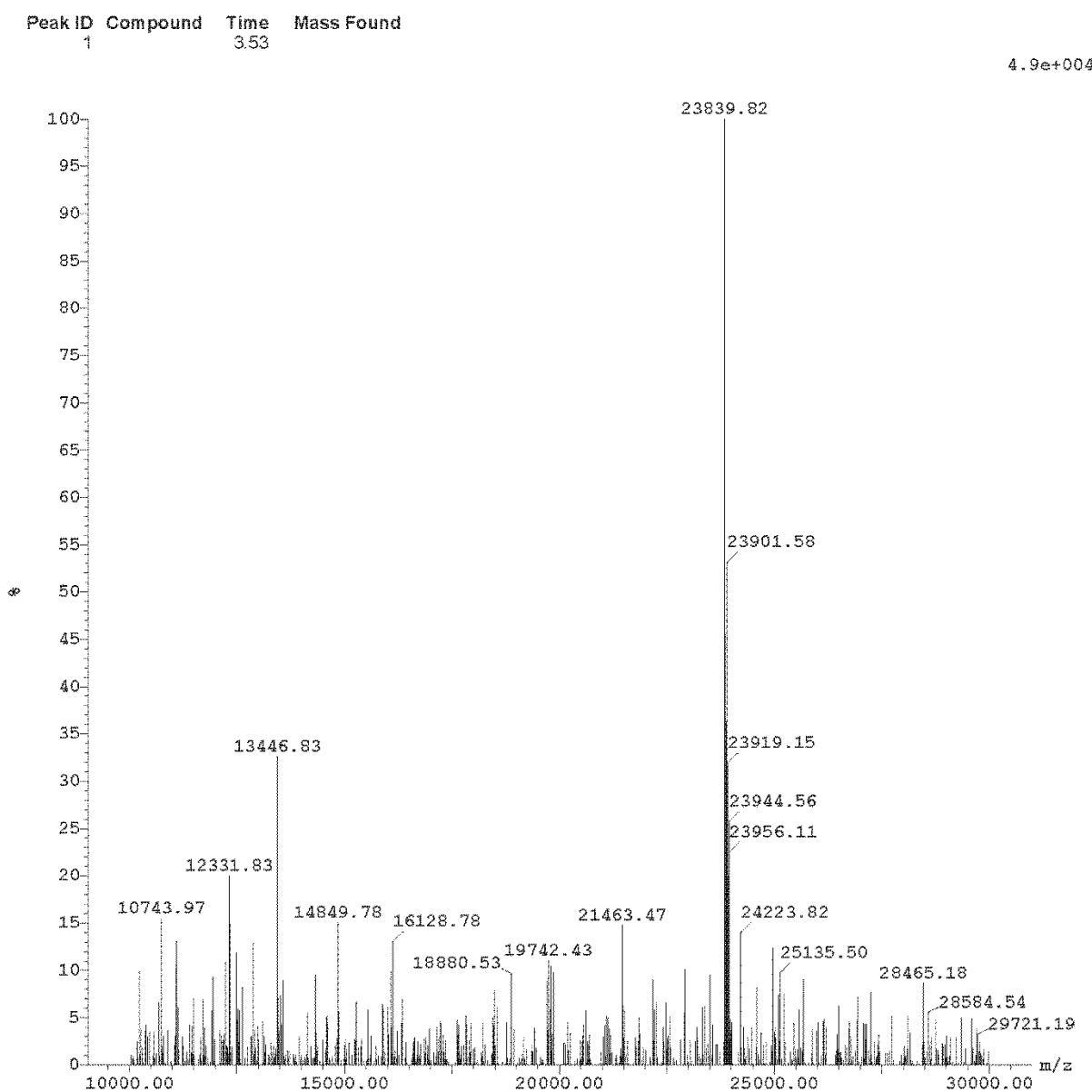


Figure S7 LC–ESI-MS of His₆-FSAA^{A129S}. Main peak displayed at 23839.8 Da.

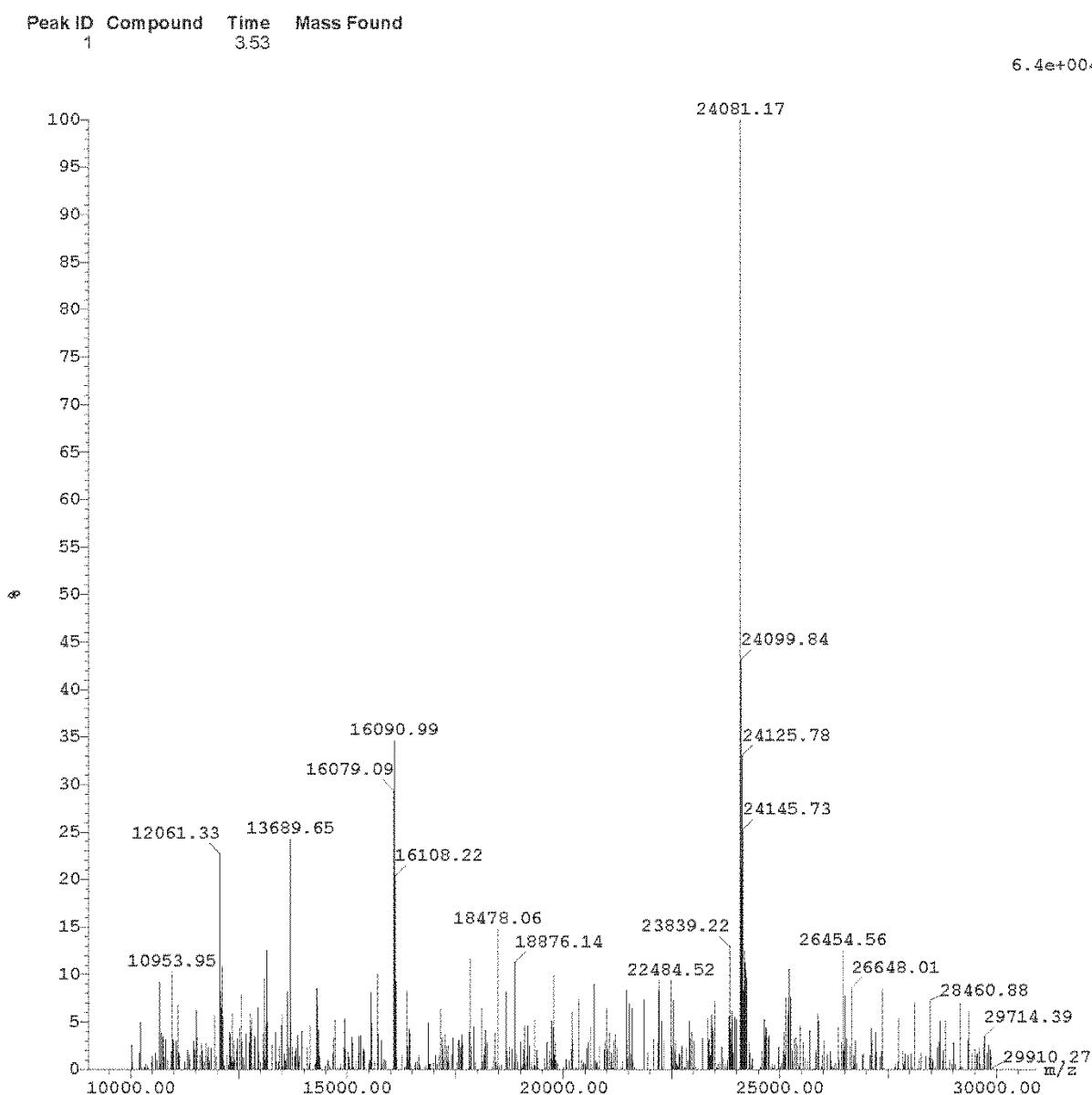


Figure S8 LC-ESI-MS of D-T6P bound to His₆-FSAA^{A129S}. Main peak displayed at 24081.2 Da.

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