

Mono-substitution of symmetric diesters: Selectivity of *Mycobacterium smegmatis* Acyltransferase variants

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Electronic supplementary information

Biocatalytic route for mono-substitution of divinyl adipate

¹H-NMR of structures of end points for the reactions between DVA and either 1-octanol or BVE catalyzed by MsAct wild type and variants: wt, L12A, T93A/F154A.

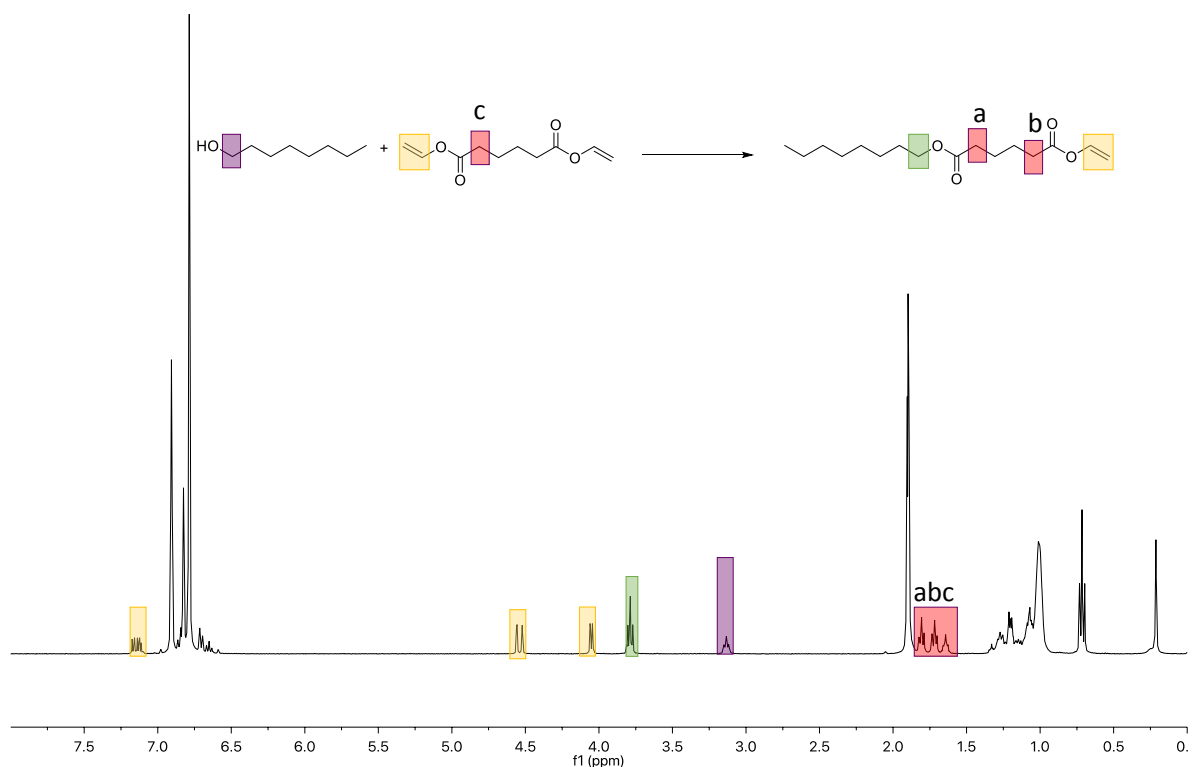


Figure S 1 ¹H-NMR of endpoint from the reaction between DVA and 1-octanol catalyzed by MsAct wt

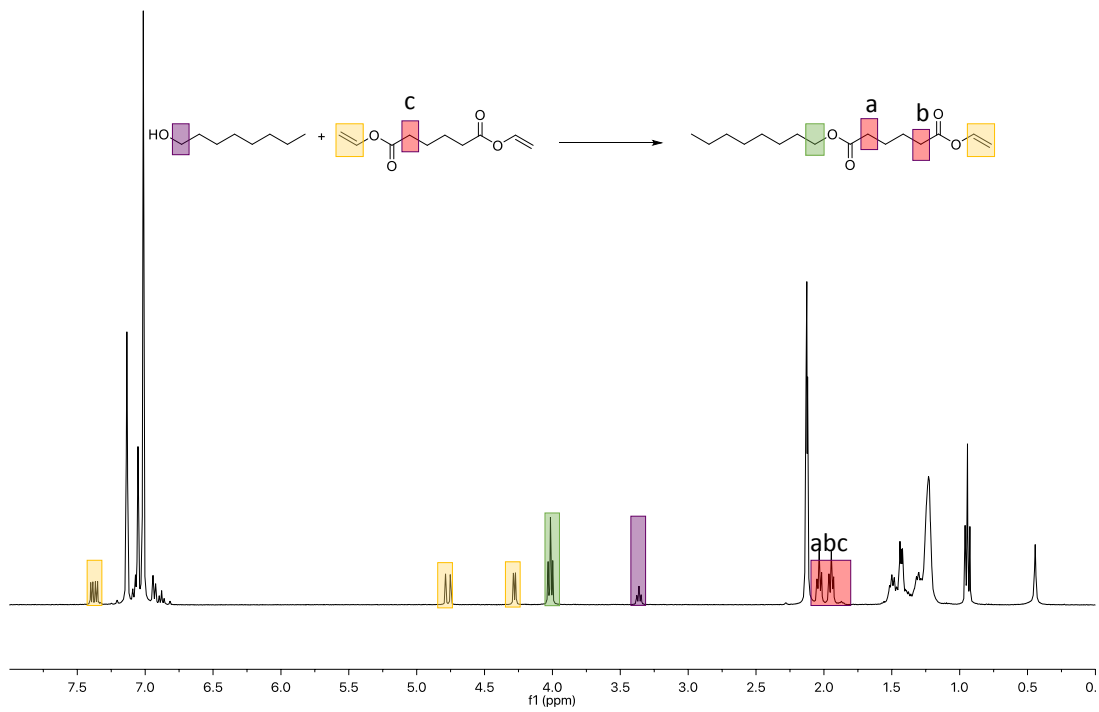


Figure S 2 $^1\text{H-NMR}$ of endpoint from the reaction between DVA and 1-octanol catalyzed by MsAcT L12A

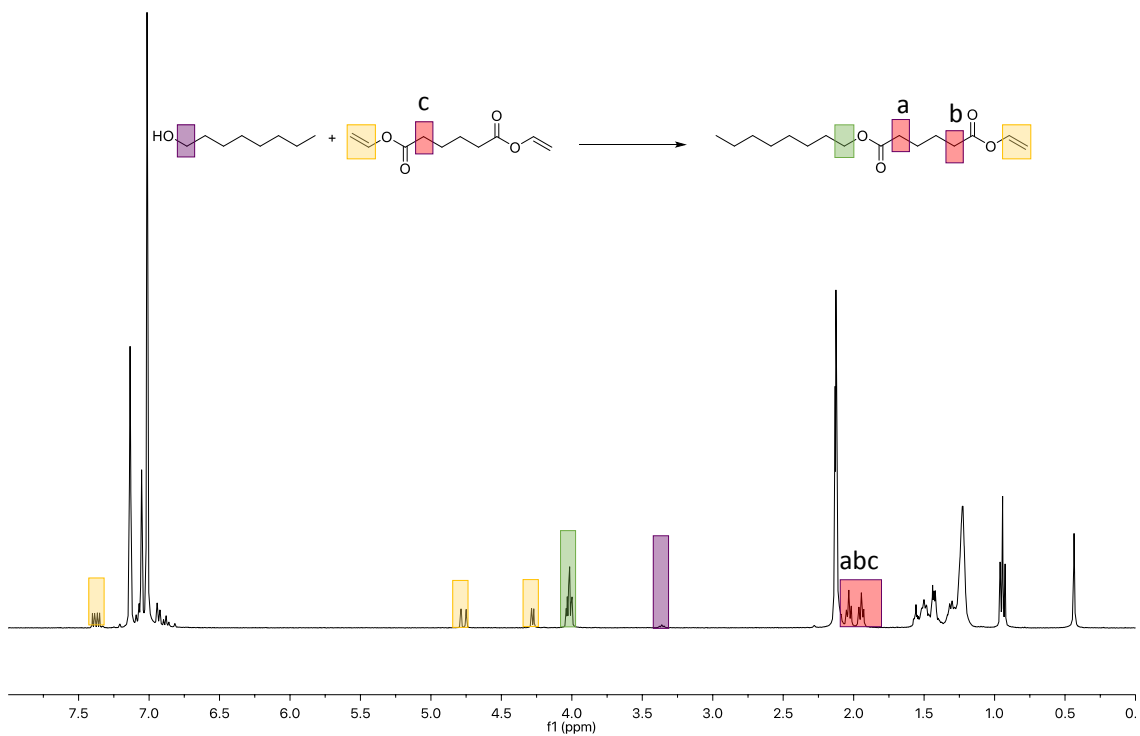


Figure S 3 $^1\text{H-NMR}$ of endpoint from the reaction between DVA and 1-octanol catalyzed by MsAcT T93A/F154A

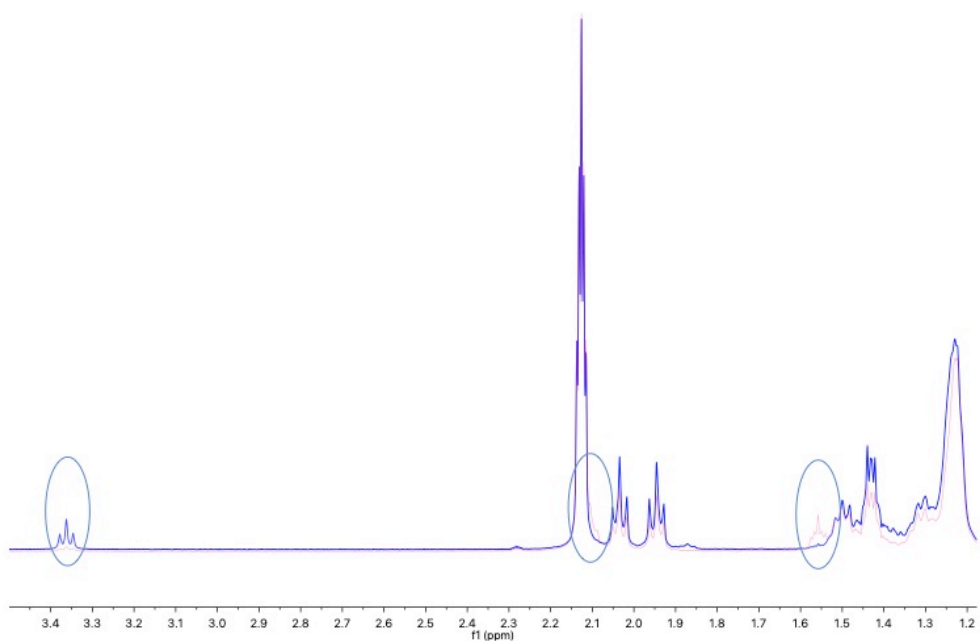


Figure S 4 Figure S2 and S3 superimposed. Spectrum from the reaction catalyzed by MsAcT L12A (Figure S2) shown in dark blue and spectrum from the reaction catalyzed by MsAcT T93A/F154A (Figure S3) shown in pink. The blue markings highlight difference in peaks corresponding to di-substituted DVA.

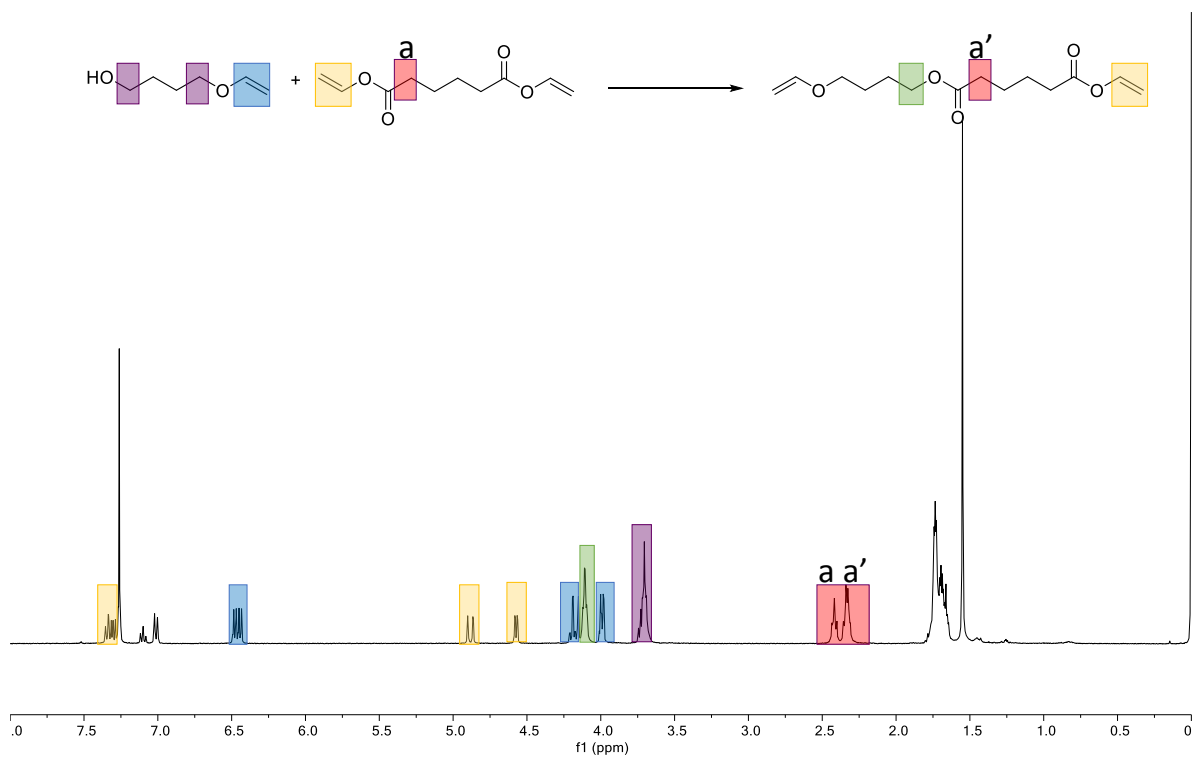


Figure S 5 $^1\text{H-NMR}$ of endpoint from the reaction between DVA and BVE catalyzed by MsAcT wt

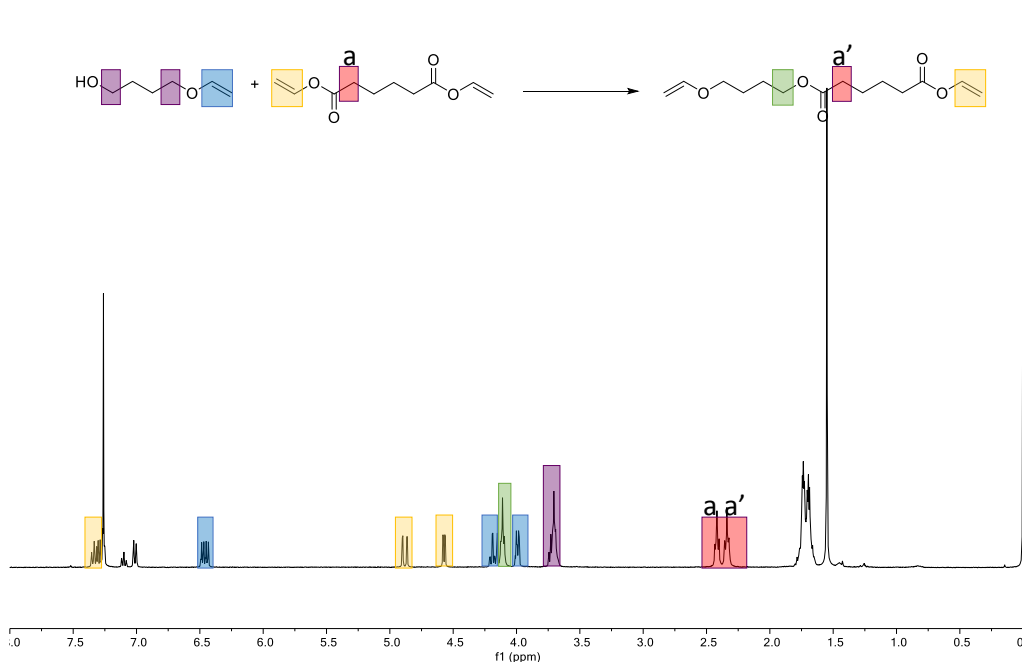


Figure S 6 ¹H-NMR of endpoint from the reaction between DVA and BVE catalyzed by MsAct L12A

The ratio between the acyl donor (DVA) and acyl acceptor (BVE) was 1:1.5 however the ratio between functional groups is 2:1.5 DVA:BVE, i.e. when full conversion of DVA and BVE is shown in GC spectra and the distribution between mono and di-substituted product is 50%. The integrals of the peaks corresponding to the vinyl groups on DVA will be 25% of their start value in spectra obtained from ¹H-NMR, which is observed from the reaction catalysed by MsAct T93A/F154A.

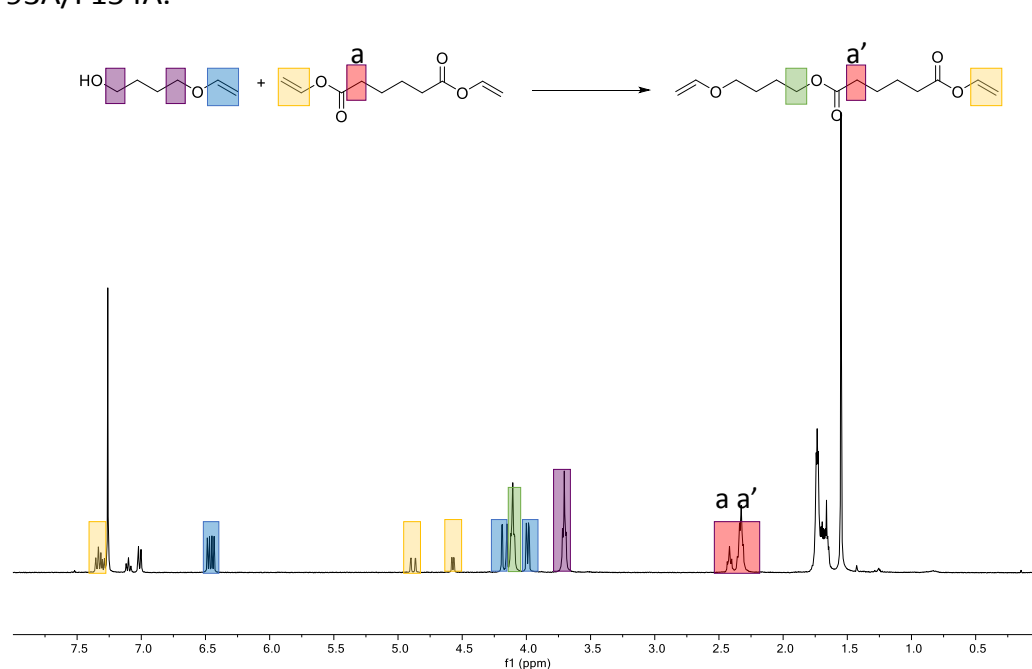


Figure S 7 ¹H-NMR of endpoint from the reaction between DVA and BVE catalyzed by MsAct T93A/F154A

Enzyme selectivity

Initial reaction velocities of MsAcT catalyzed transacylation of a mixture of methyl esters: succinate (C4), adipate (C6), suberate (C8) and sebacate (C10), Fig S8-S10. The conversions were calculated from product formation using relative response factors according to Scanlon et al. (1) The enzyme selectivity is the ratio of specificities (kcat/KM) towards the substrates and was determined from the initial reaction velocities by allowing the acyl donors to compete with each other.

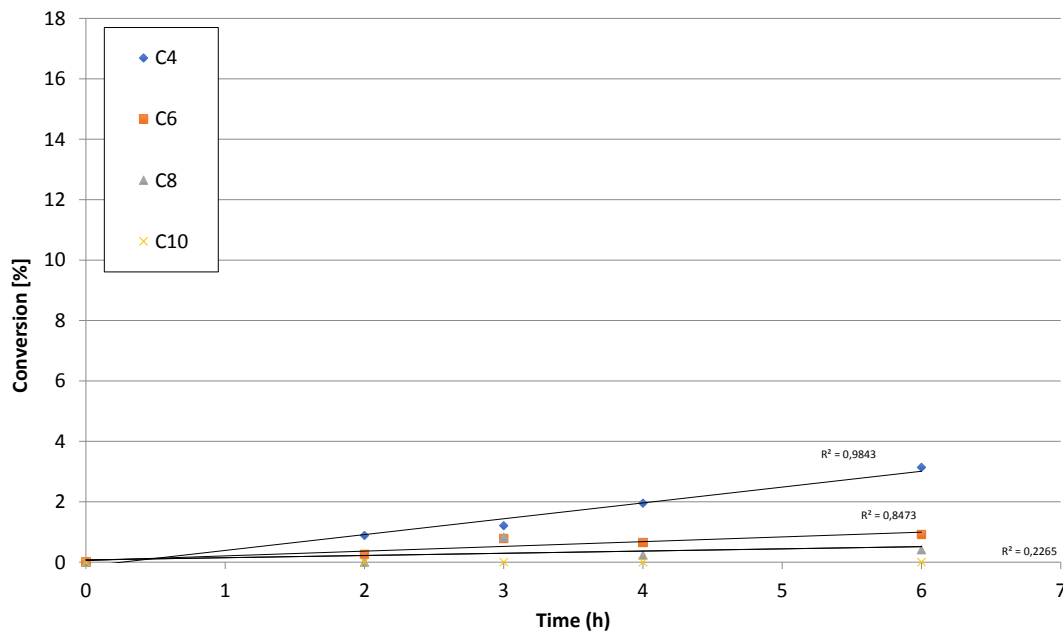


Figure S 8 Initial velocities of the transacylation reactions catalyzed by MsAcT wt.

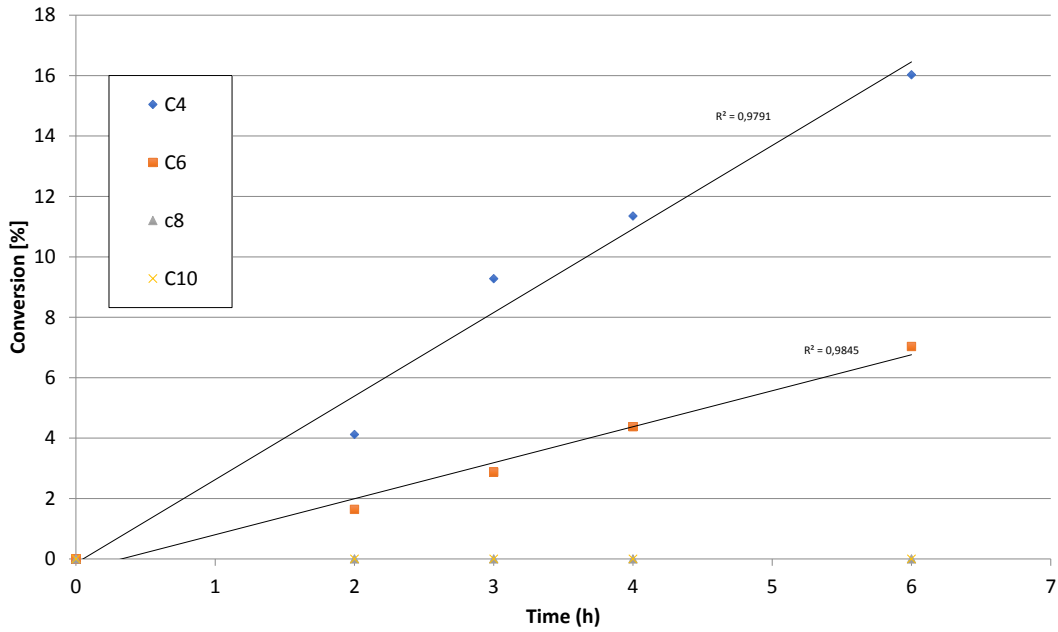


Figure S 9 Initial velocities of the transacylation reactions catalyzed by MsAcT L12A

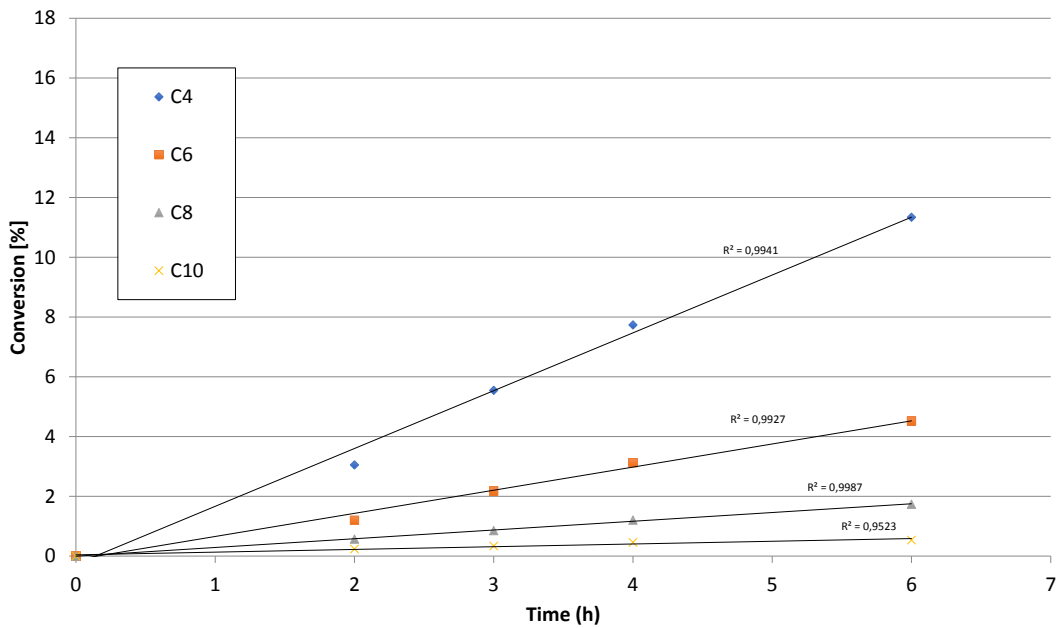


Figure S 10 Initial velocities of the transacylation reactions catalyzed by MsAcT T93A/F154A

1. Scanlon JT, Willis DE. Calculation of Flame Ionization Detector Relative Response Factors Using the Effective Carbon Number Concept. J Chromatogr Sci. 1985;23(8):333-40.