

Online Supplementary Information to:

**Bio-based route to the carbon-5 chemical glutaric acid and
bio-polyamide PA6.5 using metabolically engineered
*Corynebacterium glutamicum***

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Annotations and abbreviations of genes in central carbon metabolism and lysine biosynthesis (addendum to Figure 1).

aceA: isocitrate lyase; *aceB*: malic synthase; *aceEF*: subunits of pyruvate dehydrogenase; *acn*: aconitase; *ald*: aldolase; *asd*: aspartate semialdehyde dehydrogenase; *aspB*: aspartate aminotransferase; *dapA*: dihydrodipicolinate synthase; *dapB*: dihydrodipicolinate reductase; *dapC*: succinyl-amino-ketopimelate transaminase; *dapD*: tetrahydrodipicolinate succinylase; *dapE*: succinyl-diaminopimelate desuccinylase; *dapF*: diaminopimelate epimerase; *ddh*: diaminopimelate dehydrogenase; *eno*: enolase; *fbp*: fructose 1,6-bisphosphatase; *fum*: fumarase; *gapA*: glyceraldehyde 3-phosphate dehydrogenase; *gltA*: citrate synthase; *hom*^{V59A}: homoserine dehydrogenase with amino acid exchange valine to alanine at position 59; *icd*_{A1G}: isocitrate dehydrogenase with start codon exchange ATG to GTG; *lpd*: subunit of pyruvate/oxoglutarate dehydrogenase complex; *lysA*: diaminopimelate decarboxylase; *lysC*: aspartokinase; *malE*: malic enzyme; *mdh*: malic dehydrogenase; *odx*: oxaloacetate decarboxylase; *odhA*: subunit of oxoglutarate dehydrogenase complex; *pck*: phosphoenolpyruvate carboxykinase; *pfkA*: phosphofructokinase; *pgi*: phosphoglucoisomerase; *pgk*: phosphoglucokinase; *pgl*: 6-phosphogluconolactonase; *pgm*: phosphoglucomutase; *ppc*: phosphoenolpyruvate carboxylase; P_{sod}: promoter of the *sod* gene, encoding superoxide dismutase; *pyc*^{P458S}: pyruvate carboxylase with amino acid exchange proline to serine at position 458; *pyk*: pyruvate kinase; *sdhBAC*: succinate dehydrogenase complex; *sucB*: subunit of oxoglutarate dehydrogenase complex; *sucCD*: succinyl-CoA synthetase; *tal*: transaldolase; *tkt*: transketolase; *tri*: triose phosphate isomerase; *zwf*: glucose 6-phosphate dehydrogenase.

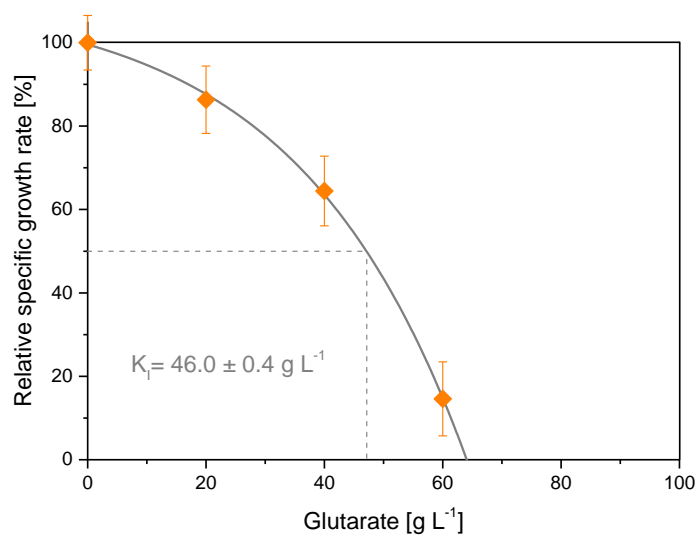


Fig. S1: The tolerance of *C. glutamicum* ATCC 13032 was obtained from cell growth measurement at different levels of glutarate. The data represent mean values and standard deviations from three biological replicates and are shown as relative values, normalized to the specific growth rate without added glutarate. The inhibitory constant K_i reflects the glutarate level at a 50% reduction in growth rate.

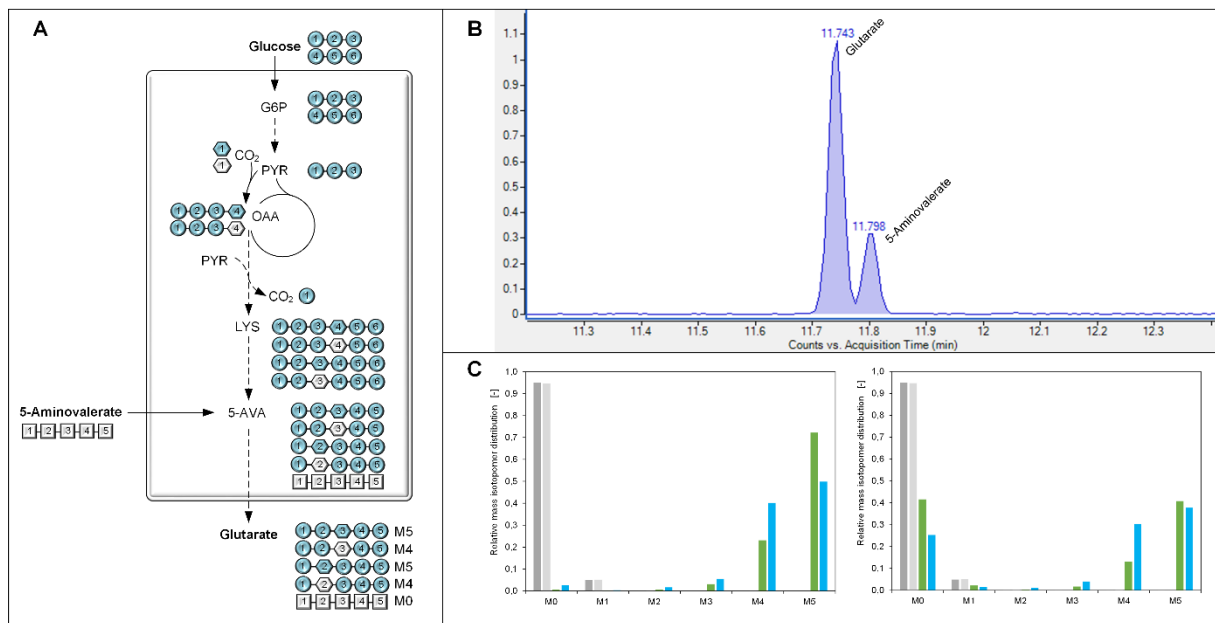


Fig. S2: Isotopic tracer study of *C. glutamicum* for elucidation of 5-aminovalerate/glutamate metabolism. Carbon transition during formation of glutamate from glucose and 5-aminovalerate. Non-labeled carbon atoms are given in white, labeled carbon atoms in turquoise (A). TIC spectrum from GC/MS analysis of culture supernatant from of *C. glutamicum* AVA-2, grown on naturally labeled glucose (B). Mass isotopomer distribution of glutamate, formed by *C. glutamicum* AVA-2 (green) and GTA-3 (turquoise), grown on U-¹³C glucose without (left) and with (right) addition of naturally labeled 5-aminovalerate (C). In addition, the mass isotopomer distribution of glutamate from a non-labeled standard (dark grey) and from glutamate formed by AVA-2 from naturally labeled glucose (light grey) are given. The data were corrected for natural isotopes.¹

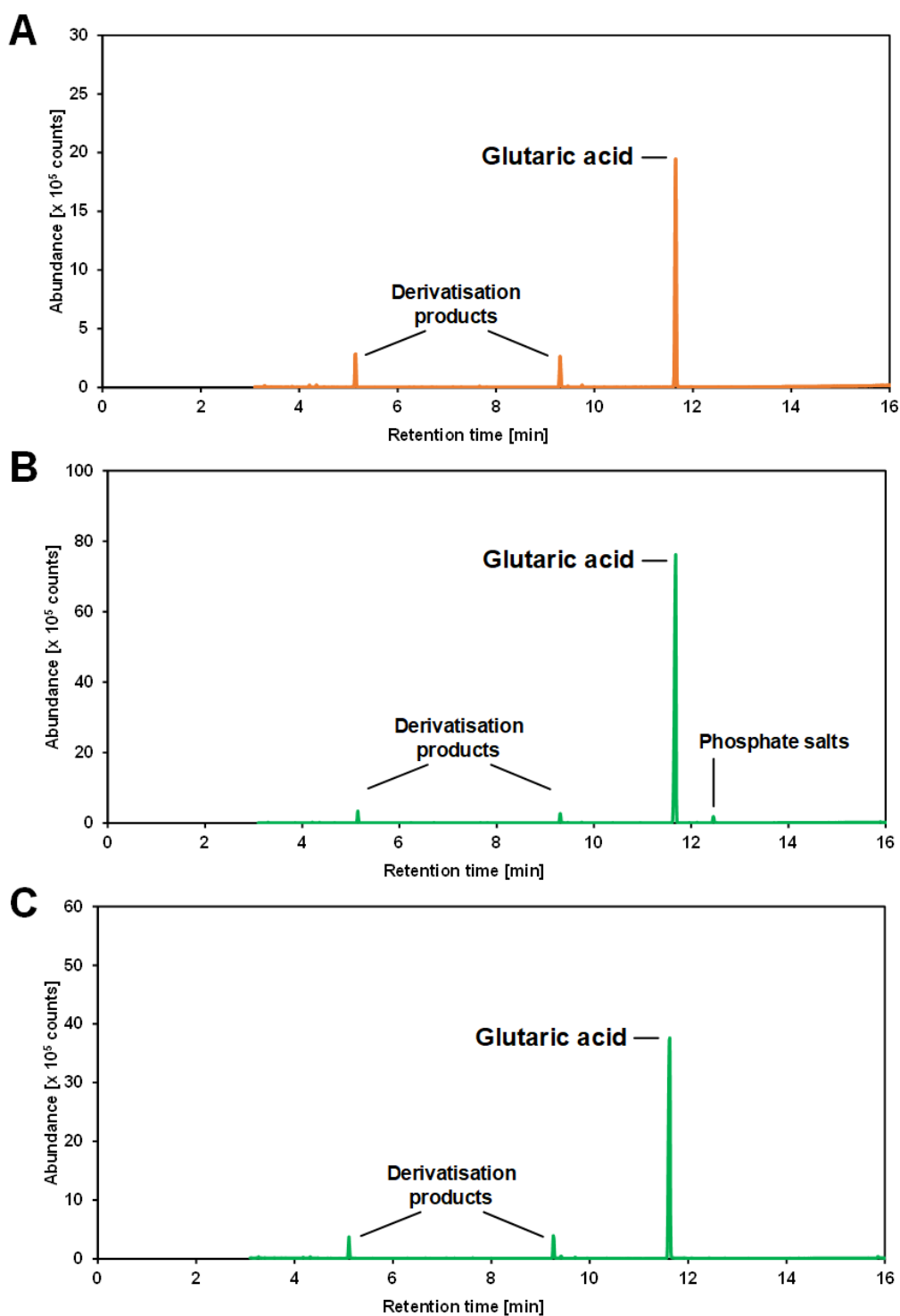


Fig. S3: Product analysis of glutaric acid using GC-MS. Total ion current (TIC) chromatograms of commercial glutaric acid of chemical origin (Sigma-Aldrich) (A), and bio-based glutaric acid after crystallization in acetone (B) and after crystallization in chloroform (C).

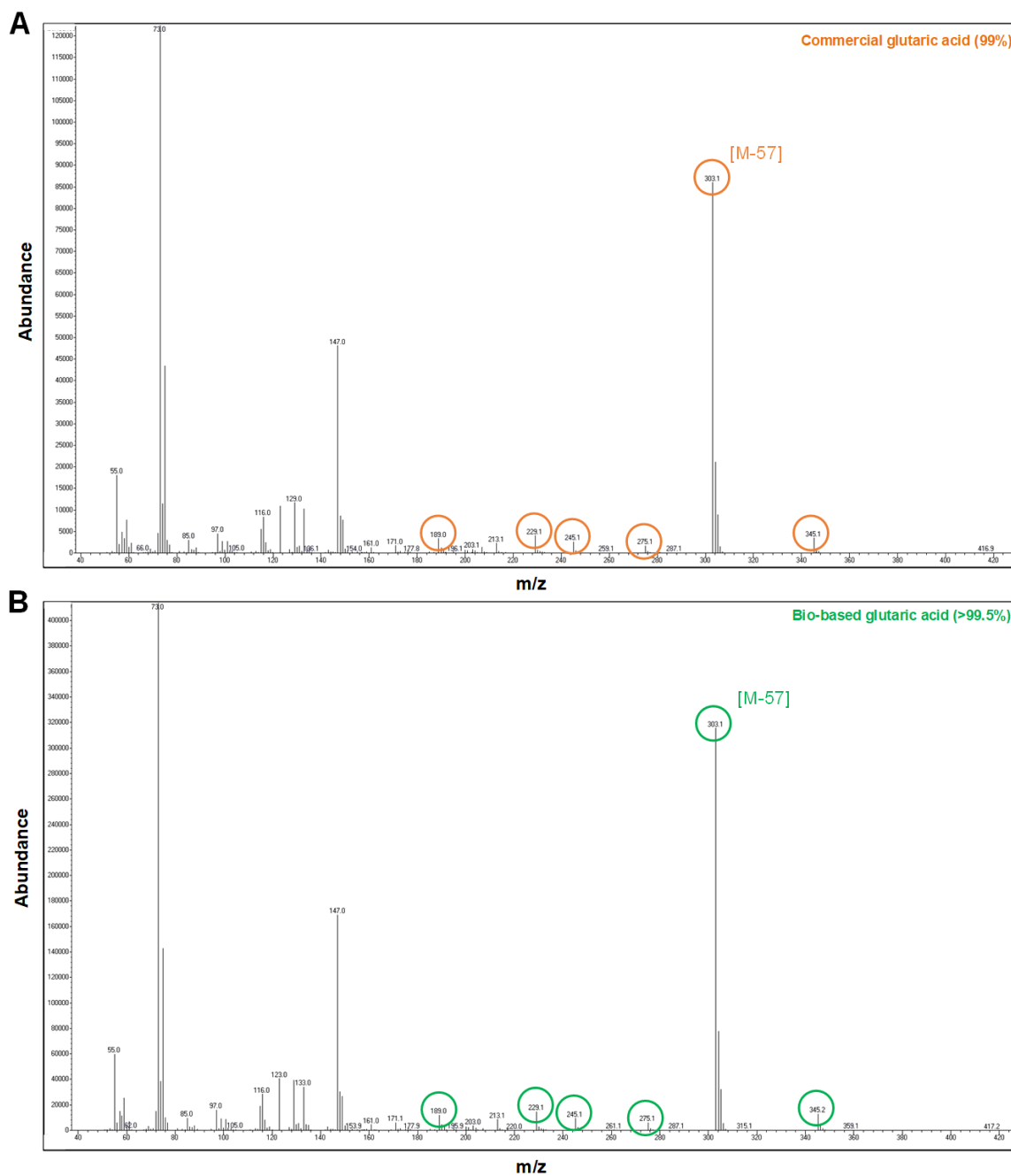


Fig. S3: Product analysis of glutaric acid using GC-MS. Mass spectra of commercial glutaric acid of chemical origin (A) and bio-based glutaric acid obtained in this work (B).

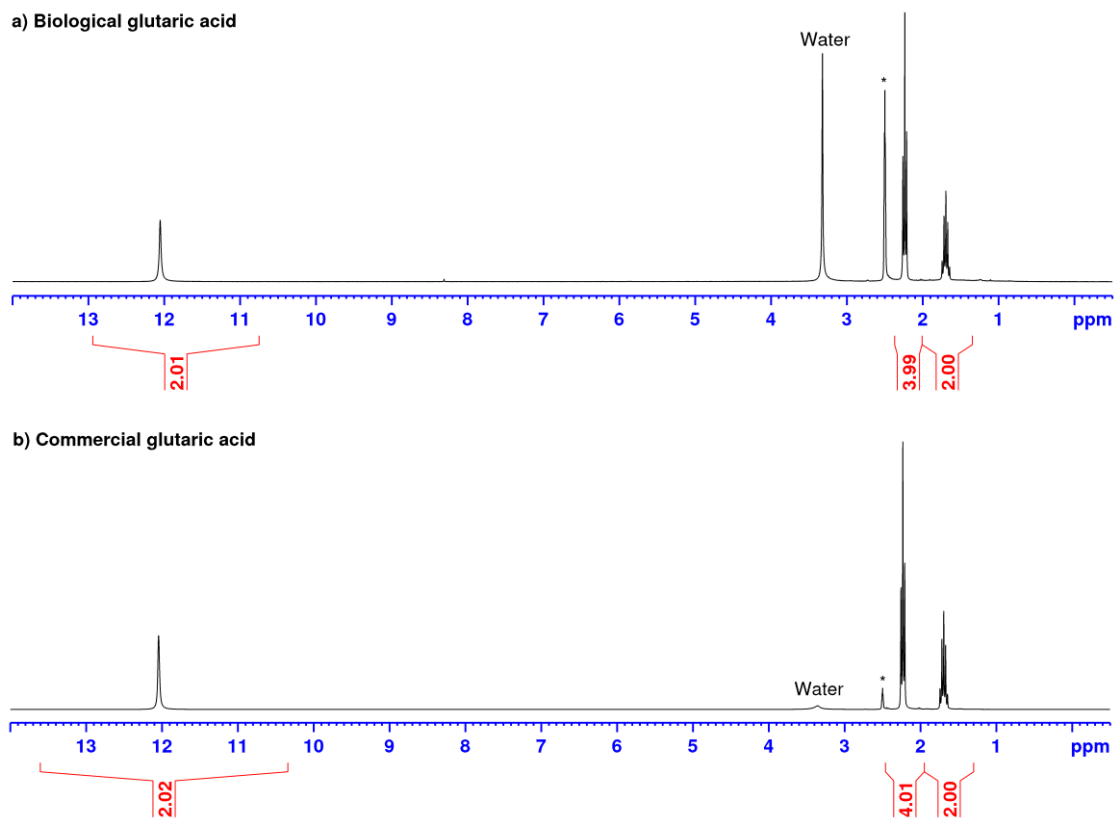


Fig. S5: ^1H NMR spectra (300 MHz, $\text{d}_6\text{-DMSO}^*$) of biological and chemical glutaric acid.

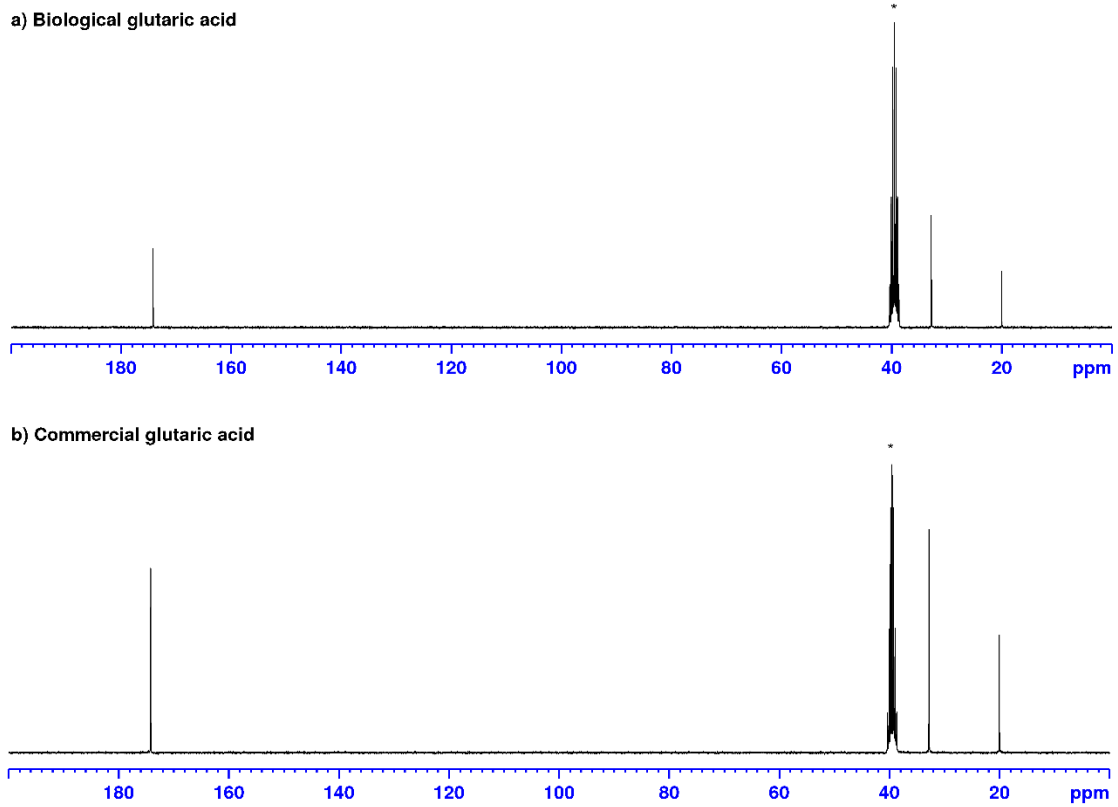


Fig. S6: ^{13}C NMR spectra (75 MHz, $\text{d}_6\text{-DMSO}^*$) of biological and chemical glutaric acid.

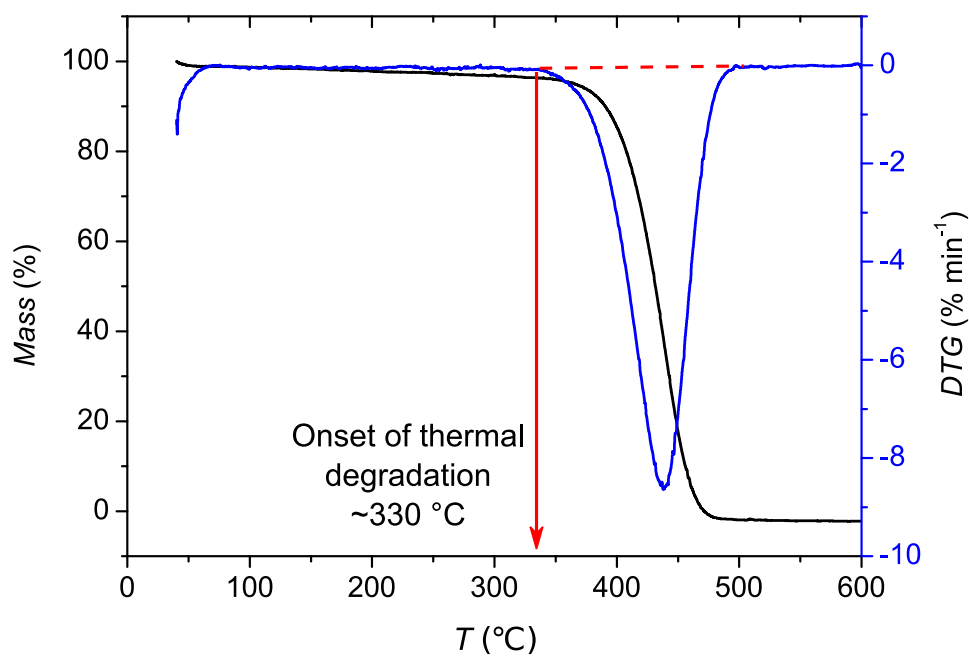
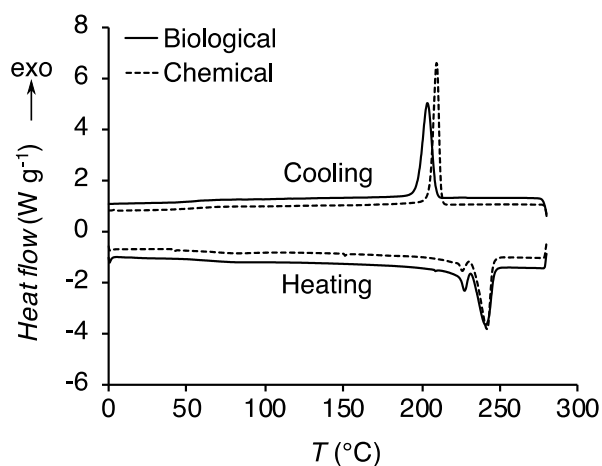


Fig. S7: Representative thermogravimetric analysis (TGA) thermogram of nylon-6,5 (here the melt polymer from commercial glutaric acid) performed under Ar atmosphere at a heating rate of 5 °C min⁻¹.

a) "Rope trick" polymers



b) Melt polymers

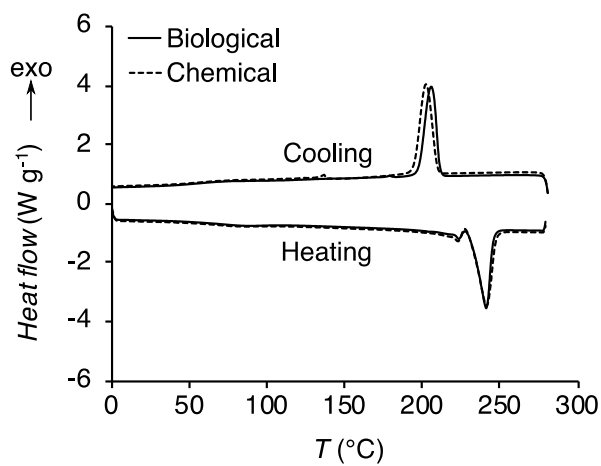


Fig. S8: Differential scanning calorimetry (DSC) of nylon-6,5 samples performed under Ar using heating and cooling rates of 20 °C min⁻¹. A prior heating above the melting temperature was performed to erase the thermal history. A small perturbation at the melting peak onset corresponded to melting and recrystallization of a minor crystalline phase² and the enthalpy of crystallization (rather than the enthalpy of fusion) was therefore preferred as an indicator of crystallinity due to its better peak definition.

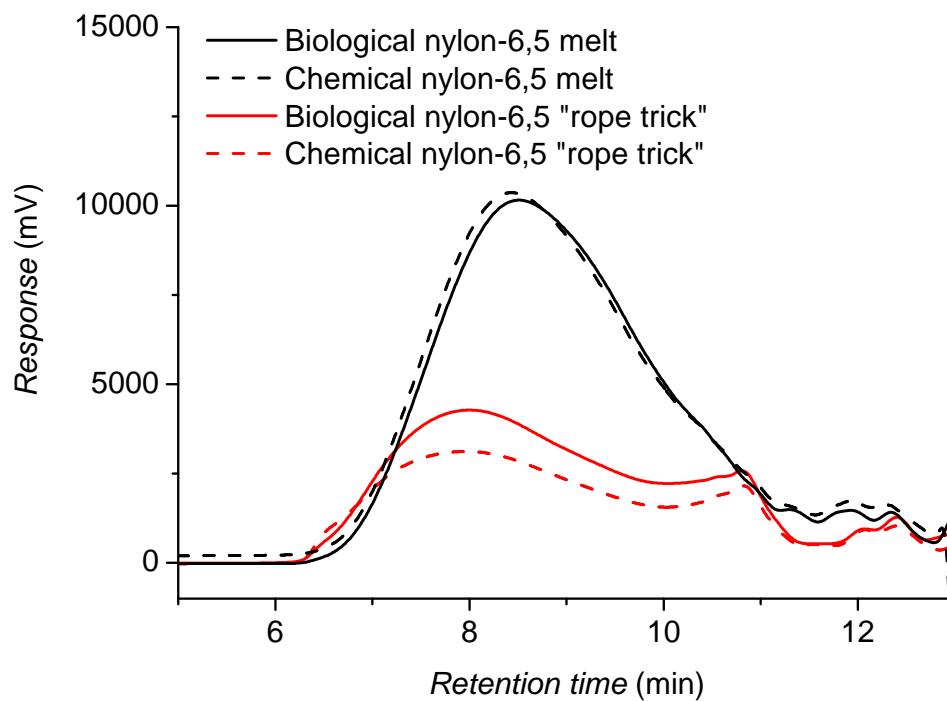


Fig. S9: Size exclusion chromatography (SEC) chromatograms of the four nylon-6,5 samples functionalized with trifluoroacetic anhydride and analyzed in tetrahydrofuran.

References

- W. A. van Winden, C. Wittmann, E. Heinzle and J. J. Heijnen, *Biotechnol Bioeng*, 2002, **80**, 477-479.
E. Navarro, L. Franco, J. A. Subirana and J. Puiggali, *Macromolecules*, 1995, **28**, 8742-8750.