Supplementary Information

Reaction behaviour of peptide based single thiol-thioesters exchange reaction substrate in the presence of externally added thiols

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Content

ESI-MS spectra of the TDP-LGLU reaction

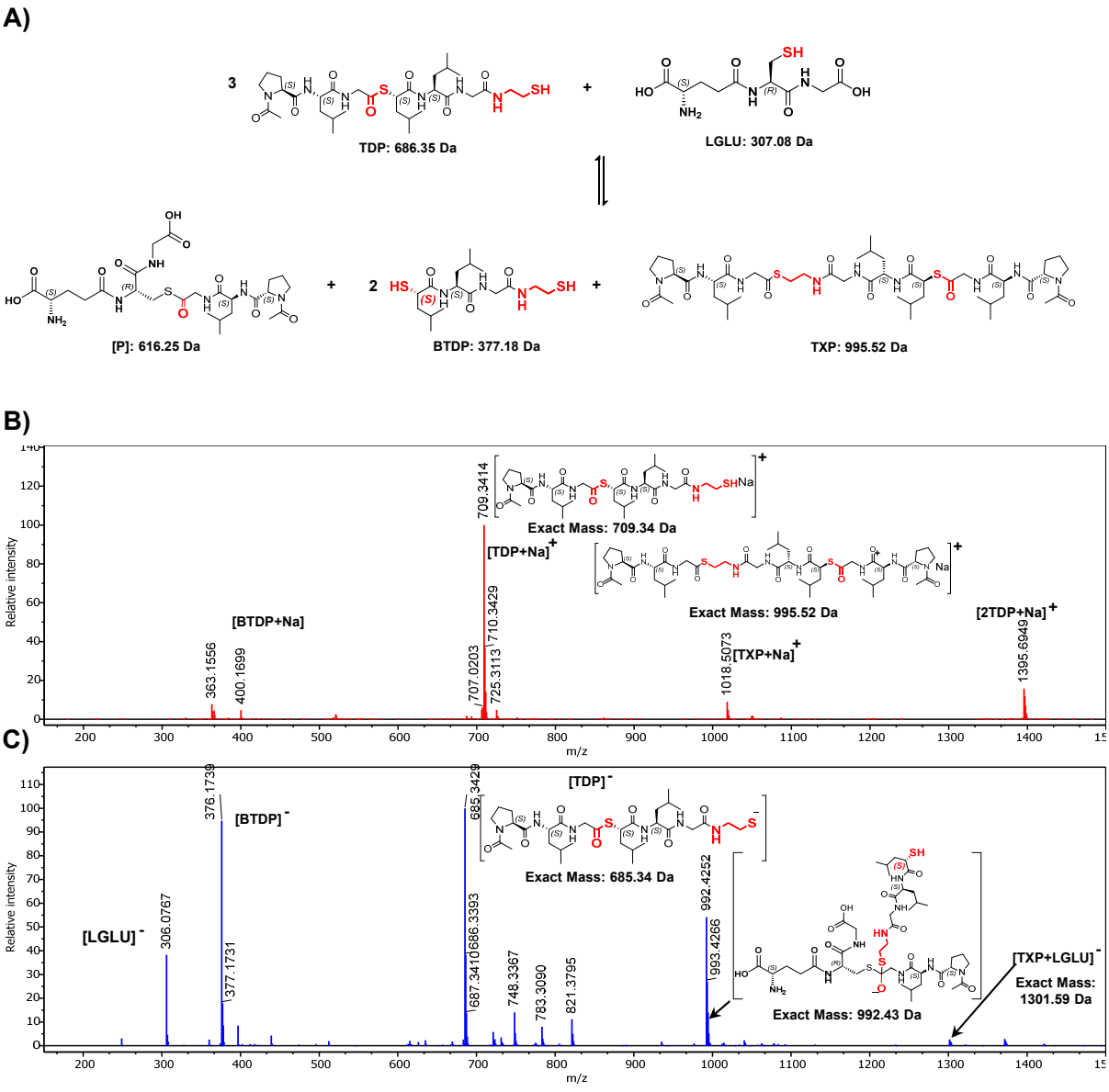

Figure S1. Overlay of ESI-MS spectra of TTE reactions of TDP with a commercial thiol, L-glutathione. (A) Reaction scheme, (B) ESI-MS spectrum in positive ion mode, (C) ESI-MS spectrum in negative ion mode. The signal at 992.4252 Da in the negative ion mode corresponds to the tetrahedral intermediate in the first step of the TTE reaction.

ESI-MS spectra of the TDP-MeTGC reaction

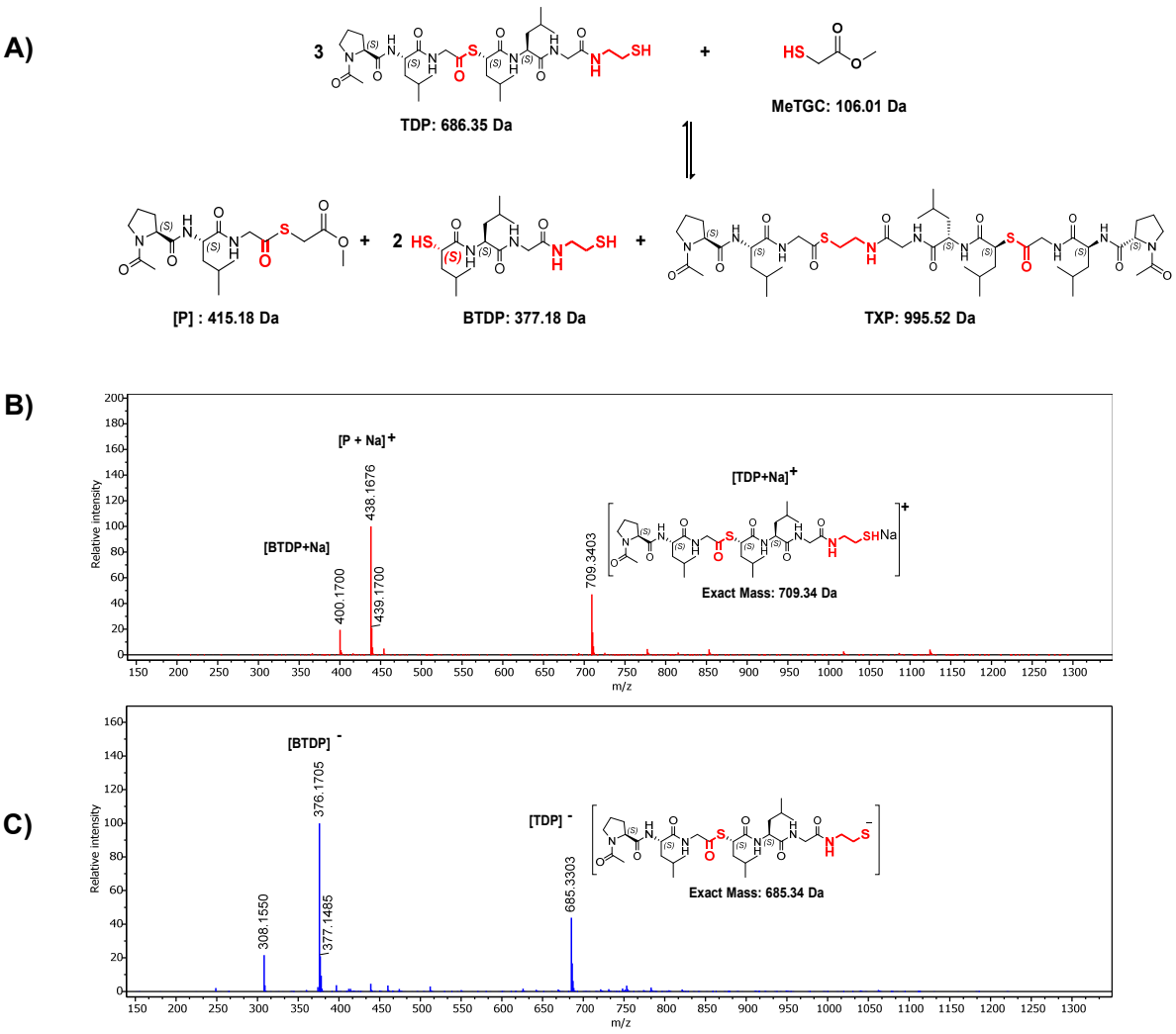

Figure S2. Overlay of ESI-MS spectra of TTE reactions of TDP with methylthioglycolate (MeTGC). (A) Reaction scheme, (B) ESI-MS spectrum in positive ion mode, (C) ESI-MS spectrum in negative ion mode.

ESI-MS spectra of the TDP-4MBA reaction

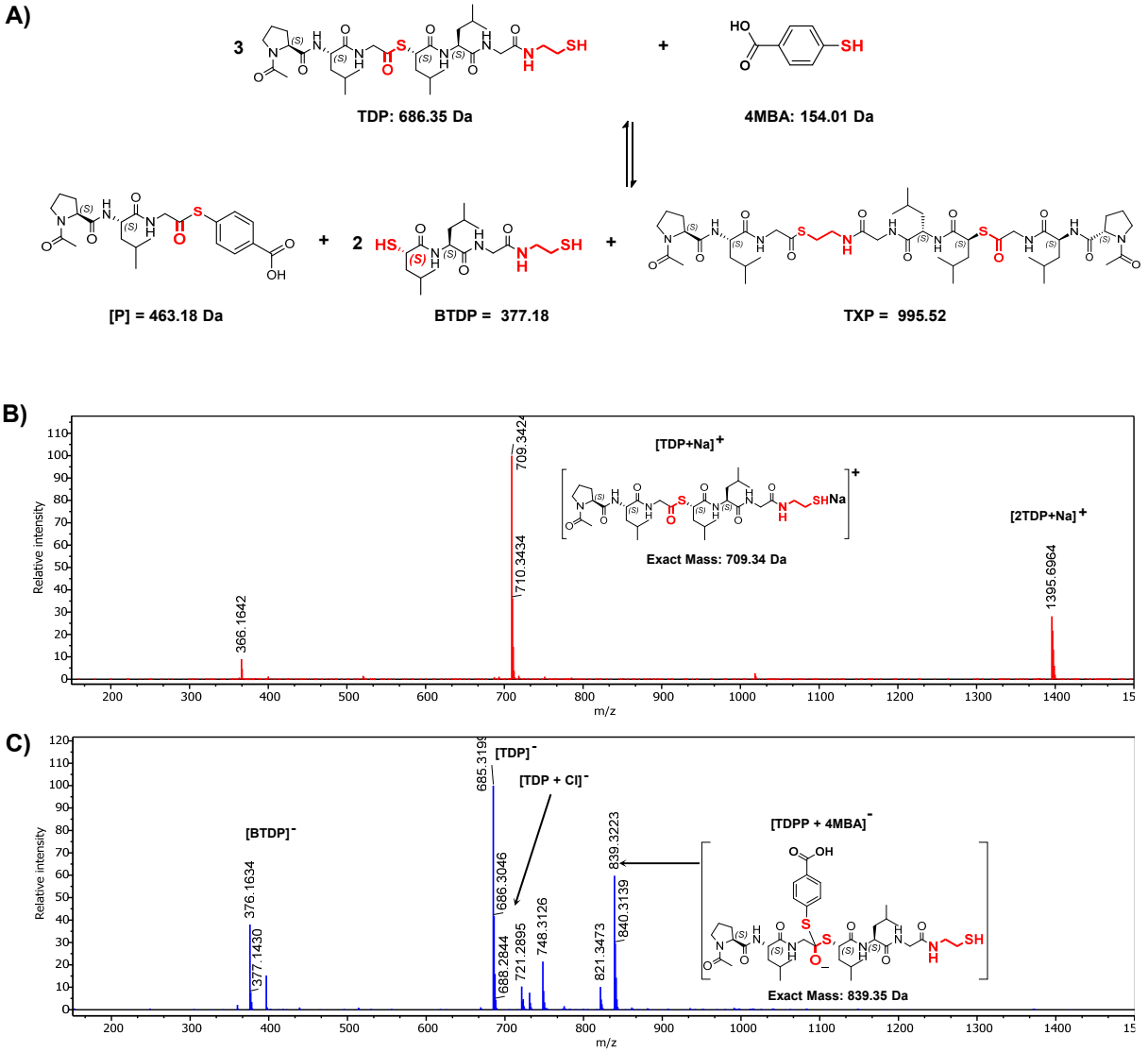

Figure S3. Overlay of ESI-MS spectra of TTE reactions of TDP with 4-mercaptobenzoic acid. (A) Reaction scheme, (B) ESI-MS spectrum in positive ion mode, (C) ESI-MS spectrum in negative ion mode. The signal at 992.4252 Da in the negative ion mode corresponds to the expected negatively charged tetrahedral intermediate in the first step of the TTE reaction.

ESI-MS spectra of the TDP-*p*NTP reaction

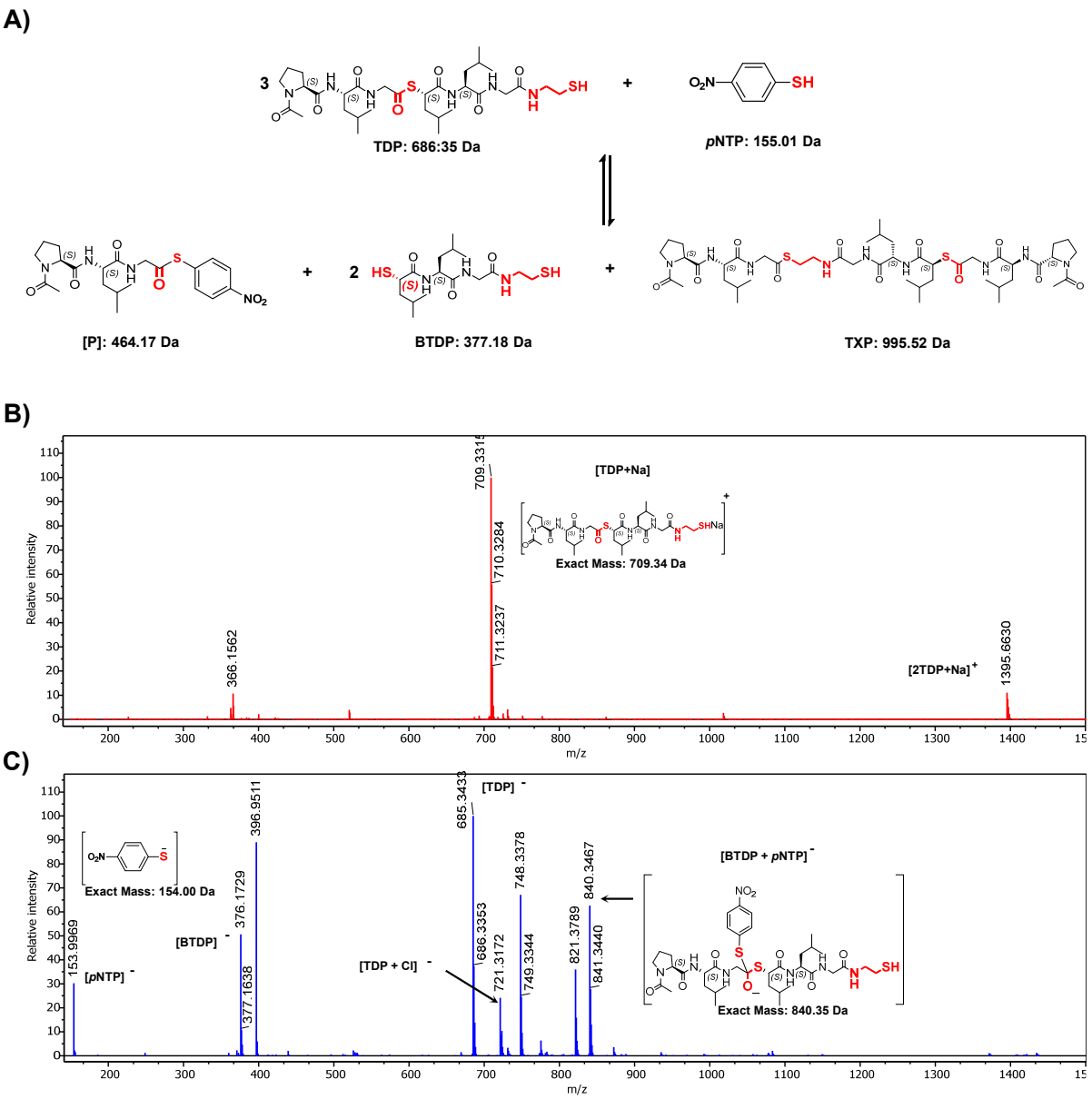

Figure S4. Overlay of ESI-MS spectra (red: positive ion mode, blue: negative ion mode) of TTE reactions of TDP with a commercial thiol, para-nitrothiophenol. (A) Reaction scheme, (B) ESI-MS spectrum in positive mode, (C) ESI-MS spectrum in negative mode. The signal at 840.3467 Da in the negative ion mode corresponds to the tetrahedral intermediate in the first step of the TTE reaction.

[1]H-NMR: Thiol-thioester reaction of TDP with MeTGC

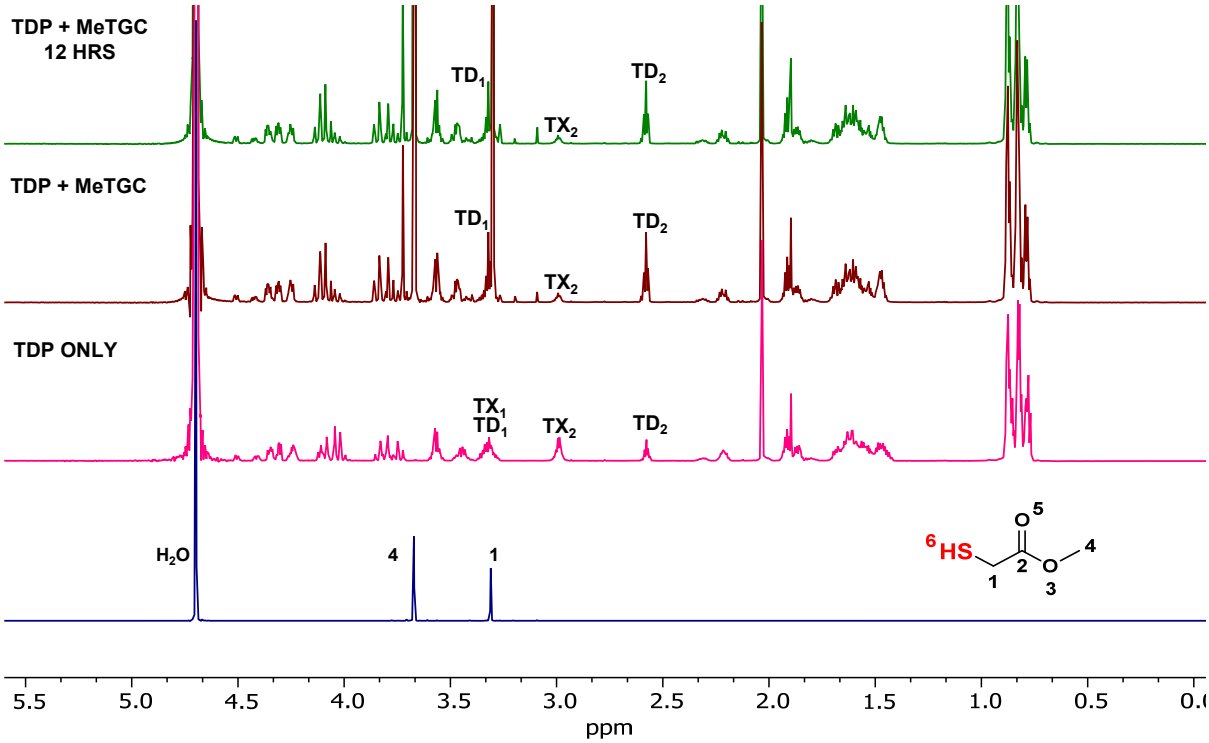

Figure S5. Overlay of [1]H-NMR spectra from bottom of MeTGC, TDP only (after 30 minutes), TDP-MeTGC (after 30 minutes) and TDP- MeTGC (after 12 hours) in methanol-d4

^{1}H-NMR: Solvent effect on thiol-thioester reaction of TDP

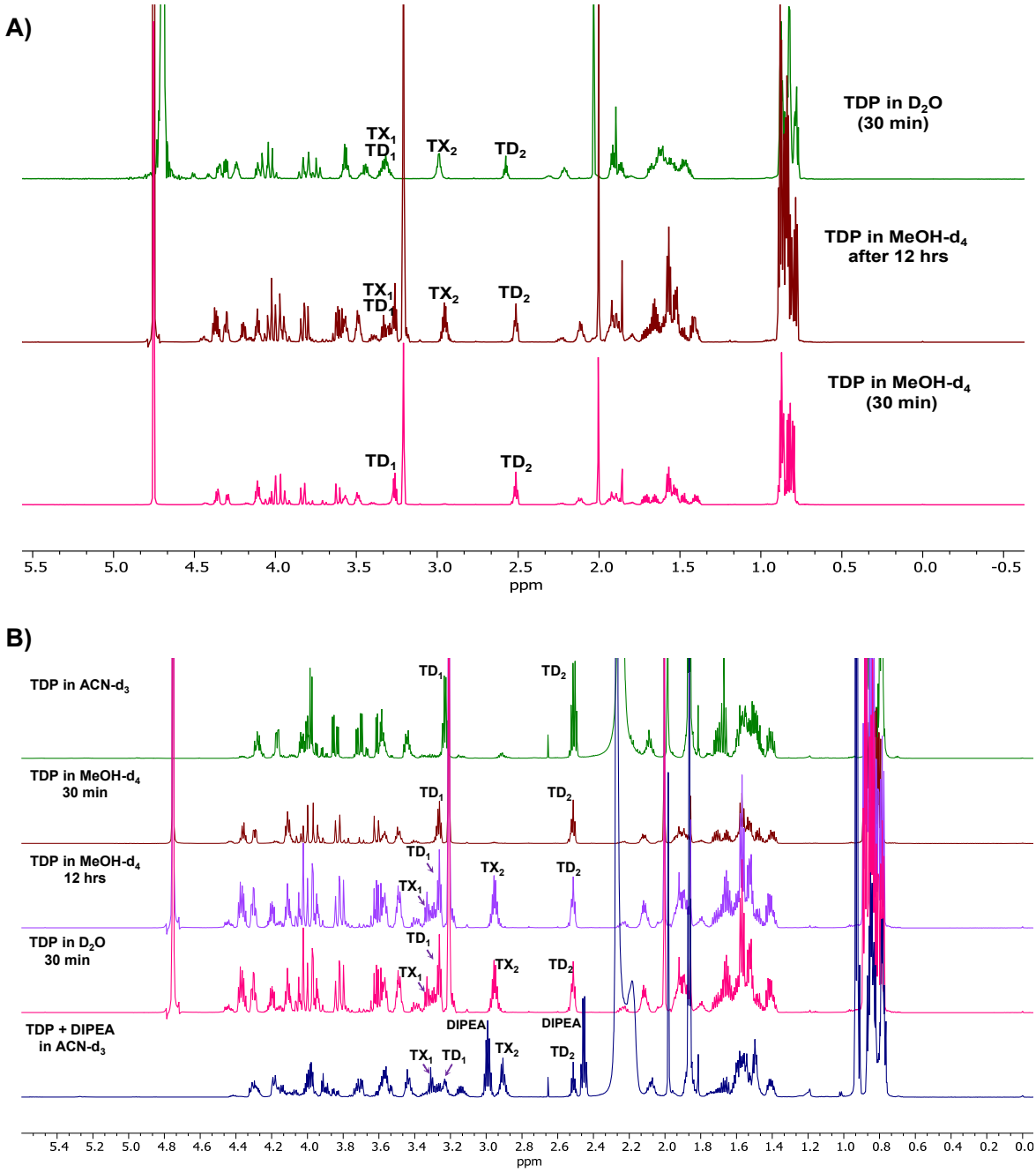

Figure S6. (A) Overlay of ^{1}H-NMR spectra from bottom of TDP in methanol-d4 (after 30 minutes), TDP in methanol-d4 (after 12 hours) and TDP in D2O (after 30 minutes) (B) Comparison of solvent effect on TTE of TDP. Polar protic solvents (D2O and MeOH-d4) aided TTE of TDP although TTE in D2O had faster kinetics. ACN-d3 which is polar aprotic however required addition of the organic base, diisopropylethylamine (DIPEA) to effect the TTE.

^{1}H-NMR: Thiol-thioester reaction of TDP with LGLU

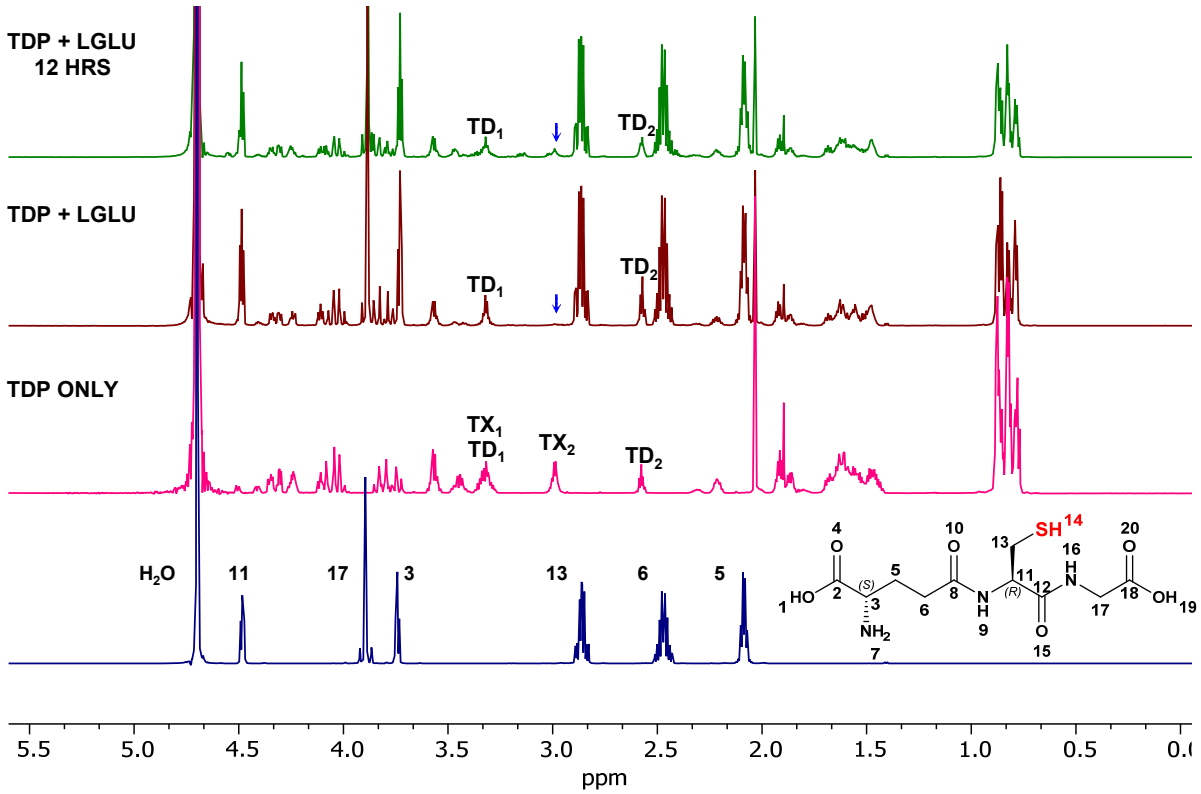

Figure S7. Overlay of ^{1}H-NMR spectra from bottom of LGLU, TDP only (after 30 minutes), TDP-LGLU (after 30 minutes) and TDP-LGLU (after 12 hours) in D_2O

^{1}H-NMR: Thiol-thioester reaction of TDP with 4MBA

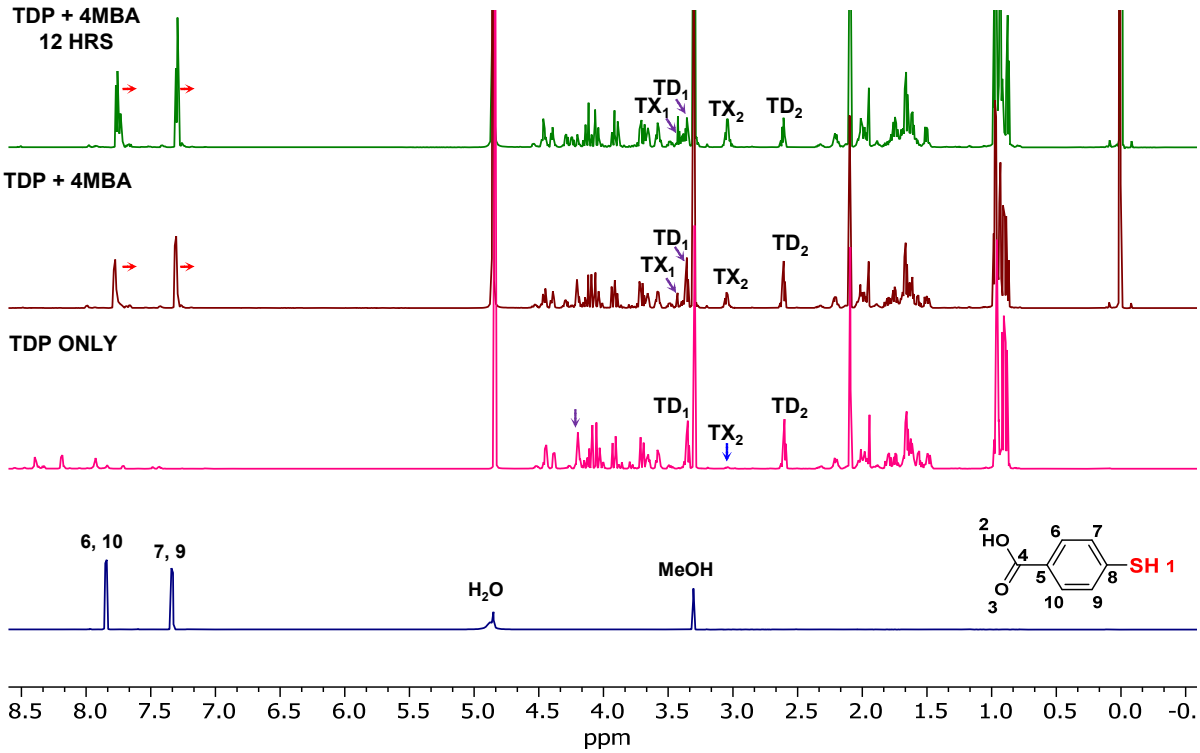

Figure S8. Overlay of NMR spectra from bottom of 4MBA, TDP only (after 30 minutes), TDP-4MBA (after 30 minutes) and TDP-4MBA (after 12 hours) in methanol-d4

^{1}H-NMR: Thiol-thioester reaction of TDP with *p*NTP

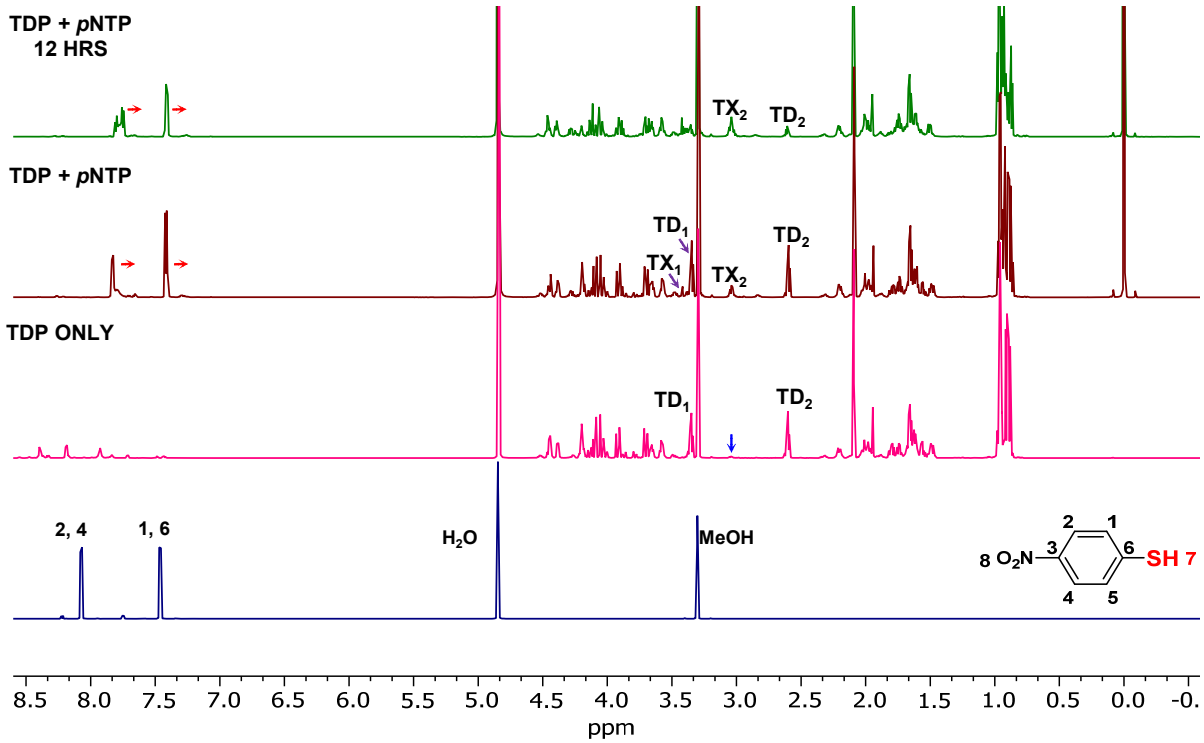

Figure S9. Overlay of ^{1}H-NMR spectra from bottom of *p*NTP, TDP only (after 30 minutes), TDP-*p*NTP (after 30 minutes) and TDP- *p*NTP (after 12 hours) in methanol-d_4

Comparison Multiplicity-edited HSQC spectrum of TDP in ACN-d_3 and D_2O

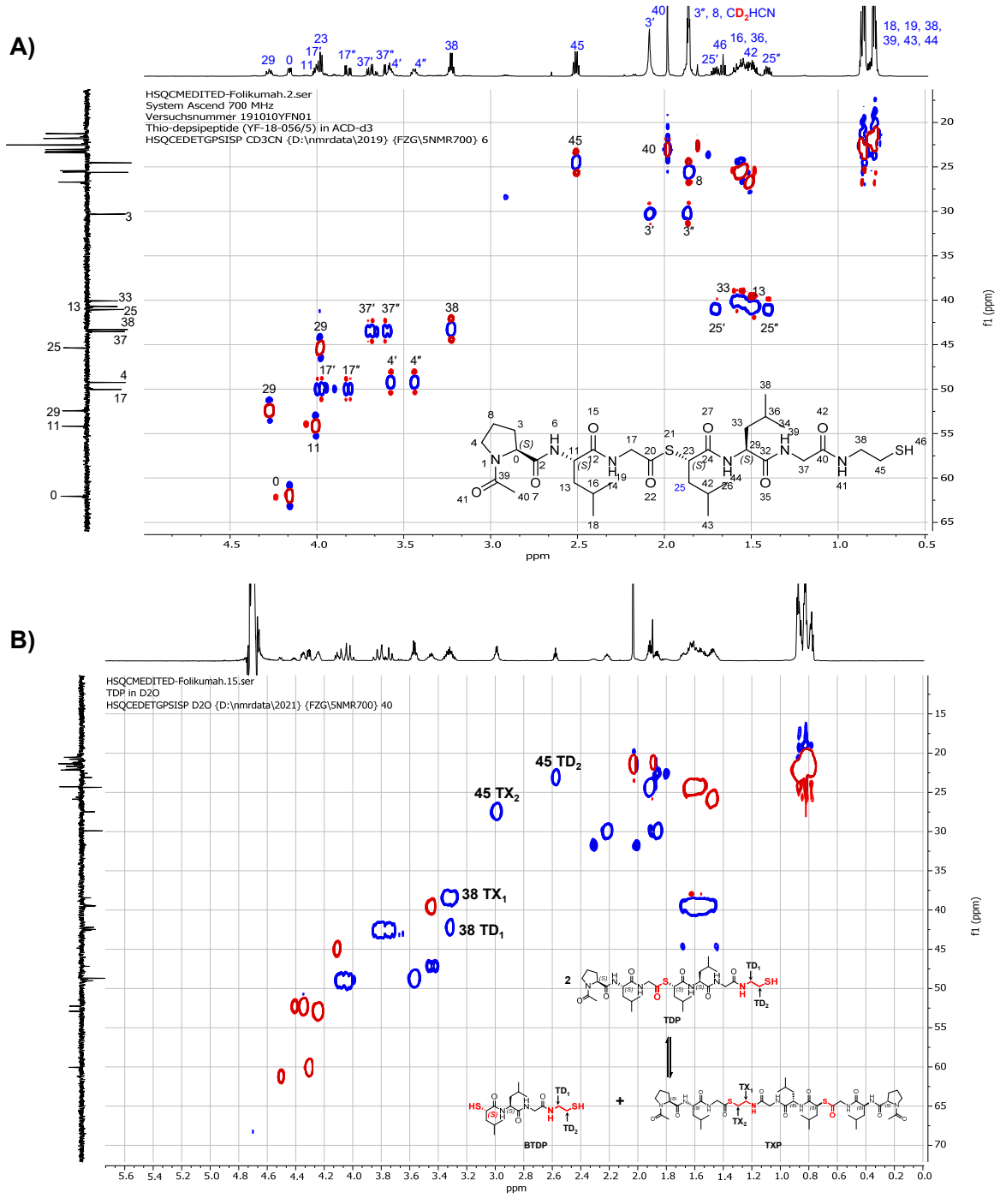

Figure S10. Multiplicity-edited HSQC spectrum of TDP (A) in ACN-d_3 with chemical structure and signal assignments. (B) in D_2O (30 minutes after preparing solution) showing additional resonances **TX_1** and **TX_2** belonging to TXP (insert)