

Supporting information

Set of small molecule polyurethane (PU) model substrates: Ecotoxicity evaluation and identification of PU degrading biocatalysts

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1. Chemical Methods

Solvents and Reagents

Solvents for dry-flash chromatography and MS analysis, as well as commercial materials and other solvents were purchased at the highest commercial quality from the providers Acros Organics, Alfa Aesar, Merck, Sigma Aldrich, and Thermo Fisher Scientific, in a purity of over 99% (HPLC-grade).

Chromatography

Thin-layer chromatography (TLC) was performed on precoated plates of silica gel F254 (Merck) with UV detection at 254 and 365 nm. Column chromatography was performed on silica gel Silica 10e18, 60 Å, ICN Biomedicals.

HR-MS

For high resolution mass spectrometry mass spectra were obtained on MS LTQ Orbitrap XL with heated ESI ionization (HESI).

NMR

¹H and ¹³C Nuclear Magnetic Resonance Spectra (NMR) were recorded on Varian/Agilent NMR 400 MHz (¹H at 400 MHz, ¹³C at 100 MHz). Chemical shifts (δ) are expressed in ppm and coupling constant (J) in Hz. TMS was used as an internal standard. The following abbreviation were used for signal multiplicities (s as singlet, t as triplet, q as quartet, dd as doublet of doublets, tt as triplet of triplets, m as multiplet).

Synthetic details

2-hydroxyethyl phenyl-carbamate (PU-1): Benzyloxy ethanol (2 eq) was added drop-wisely to the solution of phenyl isocyanate (1 eq) and ethyl-acetate (10 ml) and reflux overnight at 77°C. The next day, the reaction was stopped and extracted with ethyl-acetate and water. The organic layer was washed with sodium bicarbonate and Brine solution. The final product was purified by dry-flash chromatography using PE/EA=8:2, 7:3 eluents. **¹H NMR** (400 MHz, CDCl₃) δ 7.34-7.29 (m, ⁹H), 7.05 (t, ¹H), 6.76 (s, ¹H), 4.57 (s, ²H), 4.34 (t, ²H), 3.70 (t, ²H). **¹³C NMR** (400 MHz, CDCl₃) δ 153.32, 137.75, 129.01, 128.44, 127.79, 123.45, 73.20, 68.21, 64.24 and used further as a substrate in process of overnight hydrogenolysis in dichloromethane solution and a quantitative amount of Pd/C as a catalyst at room temperature. After overnight reaction the reaction mixture was filtered and pure by dry-flash chromatography (PE/EA=6:4) It was obtained 460 mg (77%) of the final product like a light-yellow oil. **¹H NMR** (400 MHz, CDCl³) δ 7.34-7.28 (m, 4H), 7.05 (t, 1H), 6.91 (s, ¹H), 4.29 (t, 2H), 3.85 (t, 2H), 2.26 (s, 1H). **¹³C NMR** (400 MHz, CDCl₃) δ 153.78, 137.56, 129.03, 123.67, 118.78, 66.83, 61.53.

2-ethoxyethyl phenylcarbamate (PU-2): Ethylene glycol-monoethyl ether (2 eq) was added drop-wisely to the solution of phenyl isocyanate (1 eq) and ethyl-acetate (6 ml) and reflux at 77 °C. The reaction was followed by TLC (PE/EA=7:3) and stopped after 3h. The extraction was done with ethyl-acetate and water. The organic layer was washed with sodium bicarbonate and Brine solution and the final product was purified by dry-flash chromatography using PE/EA=8:2, 7:3 eluents. The pure product was obtained like yellow oil, 487, 7 mg (60 %). **¹H NMR** (400 MHz, CDCl₃) δ 7.34-7.27 (m, 4H), 7.03 (t, 1H), 6.79 (s, 1H), 4.29 (t, 2H), 3.66 (t, 2H), 3.53 (q, 2H), 1.21 (t, 3H). **¹³C NMR** (400 MHz, CDCl₃) δ 153.33, 137.80, 128.99, 123.39, 118.60, 68.60, 66.61, 64.30, 15.07.

HR-MS(HESI+) m/z calculated for $C_{11}H_{15}NO_3$ [M+H]⁺: 210.11247, found: 210.11217, and [M+Na]⁺ : 232.09441, found: 232.09434.

Ethane-1,2-diyl bis(phenylcarbamate) (PU-3): Ethylene glycol (4.3 g, 3.9 ml, 0.07 mol) was dissolved in 30 ml of dried EtOAc and added drop wisely to the solution of phenyl isocyanate (17.5 ml, 0.14 mol) in EtOAc (20 ml). The reaction mixture was heated to reflux overnight. After cooling down the mixture, the product crystallized from the reaction mixture along with the impurities. After several recrystallizations from hot ethyl acetate (2 ml/1g) the pure product was obtained as white crystals 15 g (71 %). **¹H NMR** (400 MHz, DMSO-d6) δ 9.70 (s, 2H), 7.42 (t, J = 6.9 Hz, 4H), 7.24 (t, J = 7.7 Hz, 4H), 6.96 (t, J = 7.5 Hz, 2H), 4.30 (s, 4H). **¹³C NMR** (100 MHz, DMSO-d6) δ 153.8, 139.4, 129.2, 122.9, 118.6, 63.1. **HR-MS(HESI+)** m/z calculated for $C_{16}H_{16}N_2O_4$ [M+Na]⁺ : 323.10023, found: 323.10016.

2-((phenylcarbamoyl)oxy)ethyl hexanoate (PU-4): 2-hydroxyethyl phenylcarbamate was dissolved in dichloromethane (2 ml) and a solution of pyridine (1.5 eq) in dichloromethane (2 ml) was added to the carbamate, followed by the addition of a solution of hexanoyl chloride (1 eq) in dichloromethane (2 ml). The reaction was stirred at room temperature for 4h and followed by TLC (PE/EA=6:4). The extraction was done with ethyl-acetate and water, and the organic layer was washed with sodium bicarbonate and Brine solution. The pure product was obtained after dry-flash chromatography (PE/EA=7:3) like a yellow oil, 86%. **¹H NMR** (400 MHz, CDCl₃) δ 7.35-7.29 (m, 4H), 7.05 (t, 1H), 6.68 (s, 1H), 4.34-4.32 (dd, 4H), 2.32 (t, 2H), 1.62 (t, 2H), 1.28 (m, 4H), 0.86 (t, 3H). **¹³C NMR** (400 MHz, CDCl₃) δ 173.61, 152.98, 137.57, 129.04, 123.59, 118.64, 63.01, 62.16, 34.08, 31.23, 24.52, 22.25, 13.86. **HR-MS(HESI+)** m/z calculated for $C_{15}H_{21}NO_4$ [M+Na]⁺ : 302.13628, found: 302.13694.

Bis(2-((phenylcarbamoyl)oxy)ethyl) adipate (PU-5): 2-hydroxyethyl phenyl carbamate was dissolved in dichloromethane (2 ml) and a solution of pyridine (1.5 eq) in dichloromethane (2 ml) was added to the carbamate, followed by the addition of a solution of adipic-dichloride (1 eq) in dichloromethane (2 ml). The reaction was stirred at room temperature overnight and followed by TLC (PE/EA=6:4). The extraction was done with ethyl-acetate and water, and the organic layer was washed with sodium bicarbonate and Brine solution. The pure product was obtained after dry-flash chromatography (PE/EA=7:3, 6:4) like a colorless oil that crystallizes after fridge storage,

86%. **¹H NMR** (400 MHz, CD₃OD) δ 7.37-7.27 (m, 8H), 7.04 (t, 3H), 5.27 (s, 1H), 4.32 (q, 8H), 2.35 (t, 4H), 1.68 (m, 4H). **¹³C NMR** (400 MHz, CD₃OD) δ 173.27, 153.09, 137.76, 128.98, 123.50, 118.73, 62.77, 62.40, 33.73, 24.23. **HR-MS(HESI+)** *m/z* calculated for C₂₄H₂₇N₂O₈ [M+Na]⁺ : 495.17379, found: 495.17570.

Bis(2-hydroxyethyl) (4-methyl-1,3-phenylene)dicarbamate (PU-6): Toluene diisocyanate (1 eq) was added drop-wisely to the solution of ethylene glycol (25 eq) and ethyl-acetate (50 ml) under Ar atmosphere, and refluxed overnight at 77°C. The next day, the reaction was stopped, cooled down to room temperature, and extracted with ethyl-acetate and water. The organic layer was washed with sodium bicarbonate and Brine solution. The obtained yellow oil was dissolved in a minimal amount of hot ethyl-acetate and cooled in the fridge overnight to obtain white crystals. The process of recrystallization was repeated 2 times and it was obtained 78 % of pure white crystals. The reaction was followed by TLC=CHCl₃/MeOH=9:1. **¹H NMR** (400 MHz, CD₃OD) δ 7.54 (s, 1H), 7.14 (d, 1H), 7.08 (d, 1H), 4.36 (s, 1H), 4.16 (q, 4H), 3.74 (q, 4H), 3.57 (s, 1H), 3.32 (s, 1H), 3.28 (t, 2H), 2.17 (s, 3H). **¹³C NMR** (400 MHz, CD₃OD) δ 155.37, 154.54, 137.00, 136.06, 130.13, 115.52, 114.63, 66.20, 65.92, 62.88, 59.97, 15.82. **HR-MS(HESI+)** *m/z* calculated for C₁₃H₁₈N₂O₆ [M+Na]⁺ : 321.10571, found: 321.10525.

Bis(2-ethoxyethyl) (4-methyl-1,3-phenylene)dicarbamate (PU-7): Toluene diisocyanate (1 eq) was added drop-wisely to the solution of ethylene glycol monoethyl ether (25 eq) and ethyl-acetate (50 ml) under Ar atmosphere, and refluxed overnight at 77°C. The next day, the reaction was stopped, cooled down to room temperature, and extracted with ethyl-acetate and water. The organic layer was washed with sodium bicarbonate and Brine solution and purification was done by dry-flash chromatography (CHCl₃/MeOH=95:5, 9:1). The obtained yellow oil was repurified by dry-flash chromatography and it was obtained 50% of the product (90% of purity). **¹H NMR** (400 MHz, CD₃OD) δ 7.79 (s, 1H), 7.16 (s, 1H), 7.08 (d, 1H), 7.05 (d, 1H), 6.72 (s, 1H), 6.50 (s, 1H), 4.29 (m, 4H), 3.66 (m, 4H), 3.52 (m, 4H), 2.16 (s, 3H), 1.20 (m, 6H). **¹³C NMR** (400 MHz, CD₃OD) δ 153.45, 153.29, 136.56, 136.19, 130.73, 128.98, 128.17, 125.24, 68.63, 66.61, 64.45, 64.29, 20.74, 17.01, 15.07.

Bis(2-((phenylcarbamoyl)oxy)ethyl) (4-methyl-1,3-phenylene)dicarbamate (PU-8): Toluene diisocyanate (13.6 g, 11 ml, 0.1 mol) was dissolved in 25 ml of dried EtOAc and added drop wisely

into the solution of ethylene glycol (48 g, 43 ml, 1 mol) in 45 ml of EtOAc. Reaction mixture was heated to reflux overnight. Adukt was obtained by crystallization from hot ethyl-acetate. After several recrystallizations from ethyl acetate, pure product was obtained as white crystals 10 g, 35 % yield. **¹H NMR** (400 MHz, CD₃OD) δ 7.55 (s, 1H), 7.15 (d, J = 7.7 Hz, 1H), 7.07 (d, J = 8.3 Hz, 1H), 4.17 (dd, J = 10.2, 5.6 Hz, 4H), 3.75 (dd, J = 9.4, 4.5 Hz, 4H), 2.18 (s, 3H). **¹³C NMR** (100 MHz, CD₃OD) δ 155.4, 137.0, 136.1, 130.1, 115.5, 114.6, 66.2, 60.0, 15.8. Product from previous step (9.5 g, 0.03 mol) was dissolved in the mixture of solvents 120 ml EtOAc / DMF (1/1) and added drop wisely to the solution of phenyl isocyanate (10.8 ml, 0.1 mol) in ethyl acetate (60 ml) at room temperature, under the inert atmosphere. Reaction mixture was stirred overnight at 60 °C and then thoroughly washed with water (x7). Solvent was evaporated and the crude product was carefully purified by column chromatography (eluant: petroleum ether/EtOAc - 8/2). Final product (4.9 g, 30 %) was obtained in a form of white crystals. **¹H NMR** (400 MHz, DMSO-d6) δ 9.69 (s, 2H), 9.63 (s, 1H), 8.90 (s, 1H), 7.49 – 7.39 (m, 5H), 7.24 (t, J = 7.8 Hz, 4H), 7.12 (d, J = 7.9 Hz, 1H), 7.03 (d, J = 8.3 Hz, 1H), 6.96 (t, J = 7.4 Hz, 2H), 4.28 (s, 8H), 2.08 (s, 3H). **¹³C NMR** (100 MHz, DMSO-d6) δ 154.6, 153.8, 153.7, 139.4, 137.4, 136.7, 130.7, 129.2, 123.0, 118.7, 63.2, 63.1, 63.1, 63.0, 17.5. **HR-MS(HESI+)** *m/z* calculated for C₂₇H₂₈N₄O₈ [M+Na]⁺ : 559.17993, found: 559.18223.

NMR spectra

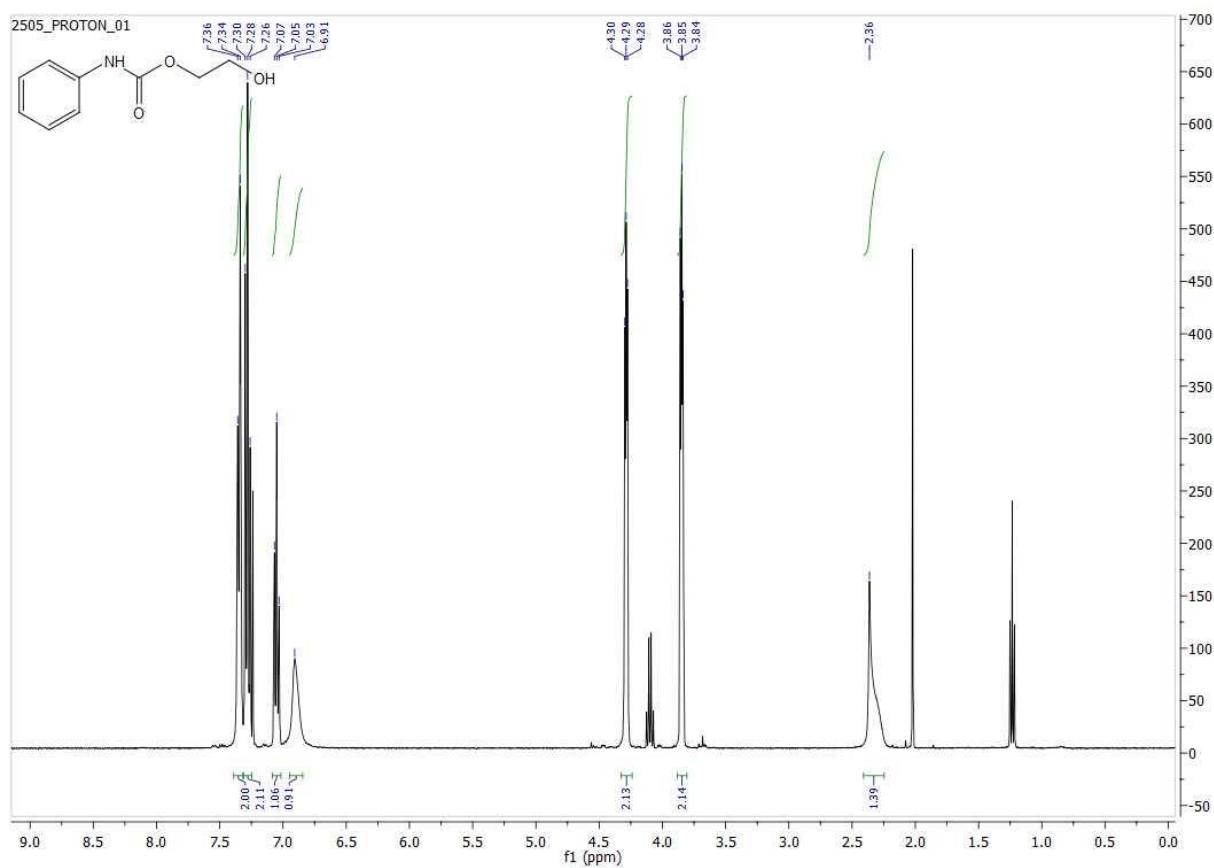


Figure S1.¹H-NMR spectrum of 2-hydroxyethyl phenyl-carbamate (**PU-1**)

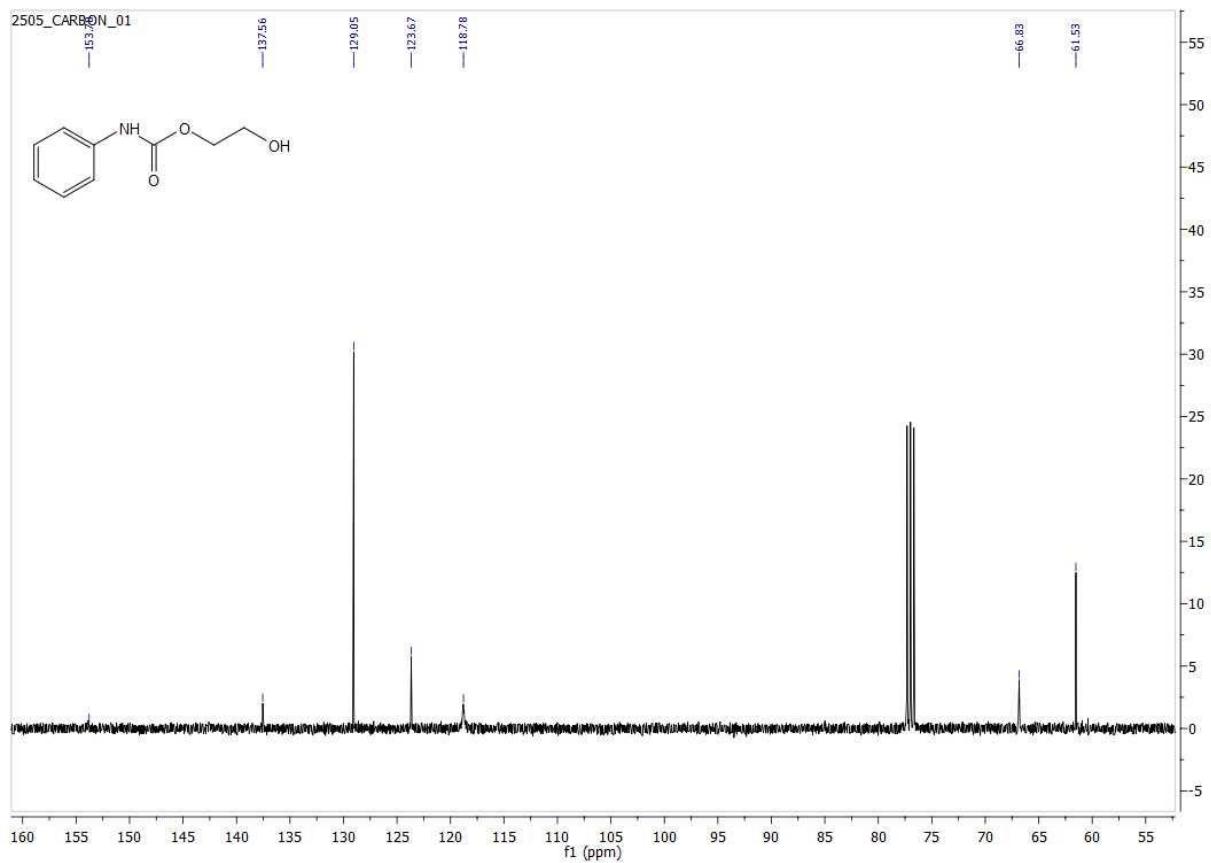


Figure S2.¹³C-NMR spectrum of *2-hydroxyethyl phenyl-carbamate (PU-1)*

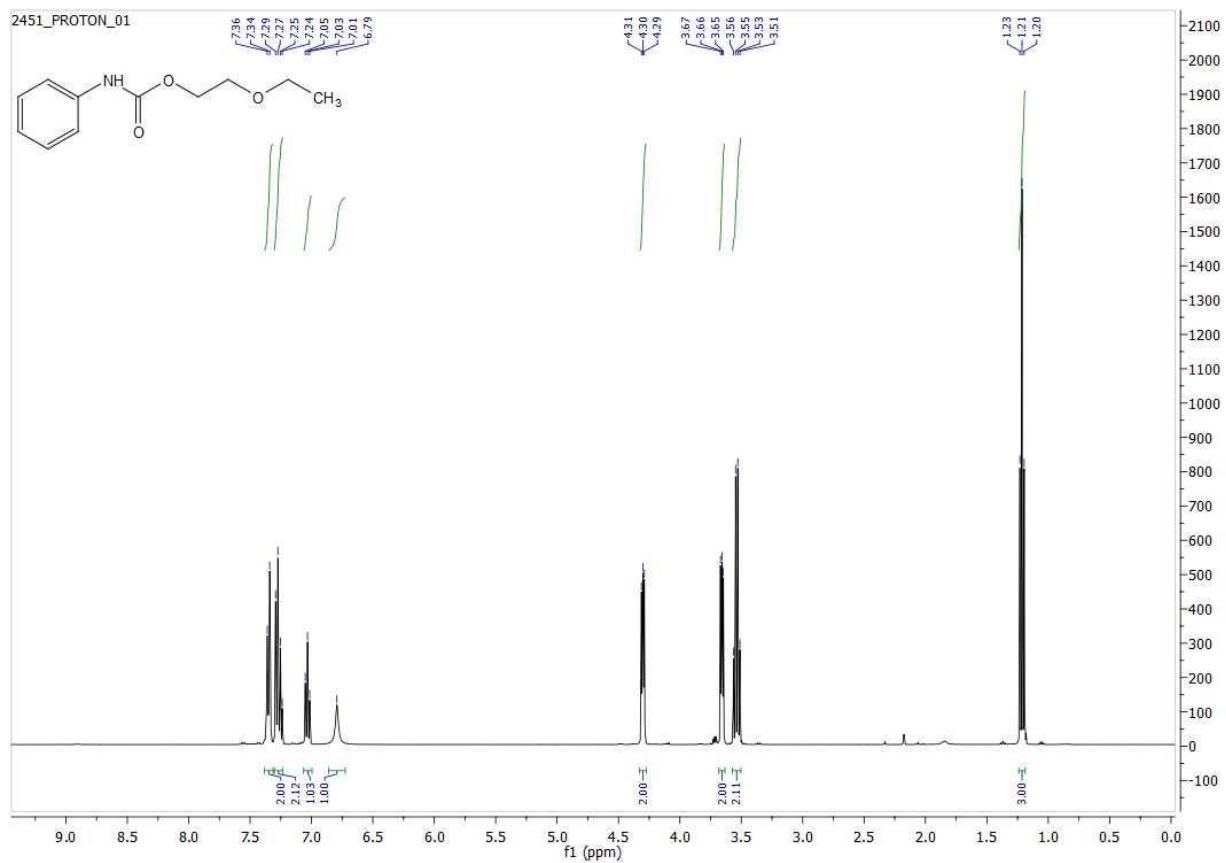


Figure S3.¹H-NMR spectrum of 2-ethoxyethyl phenylcarbamate (**PU-2**)

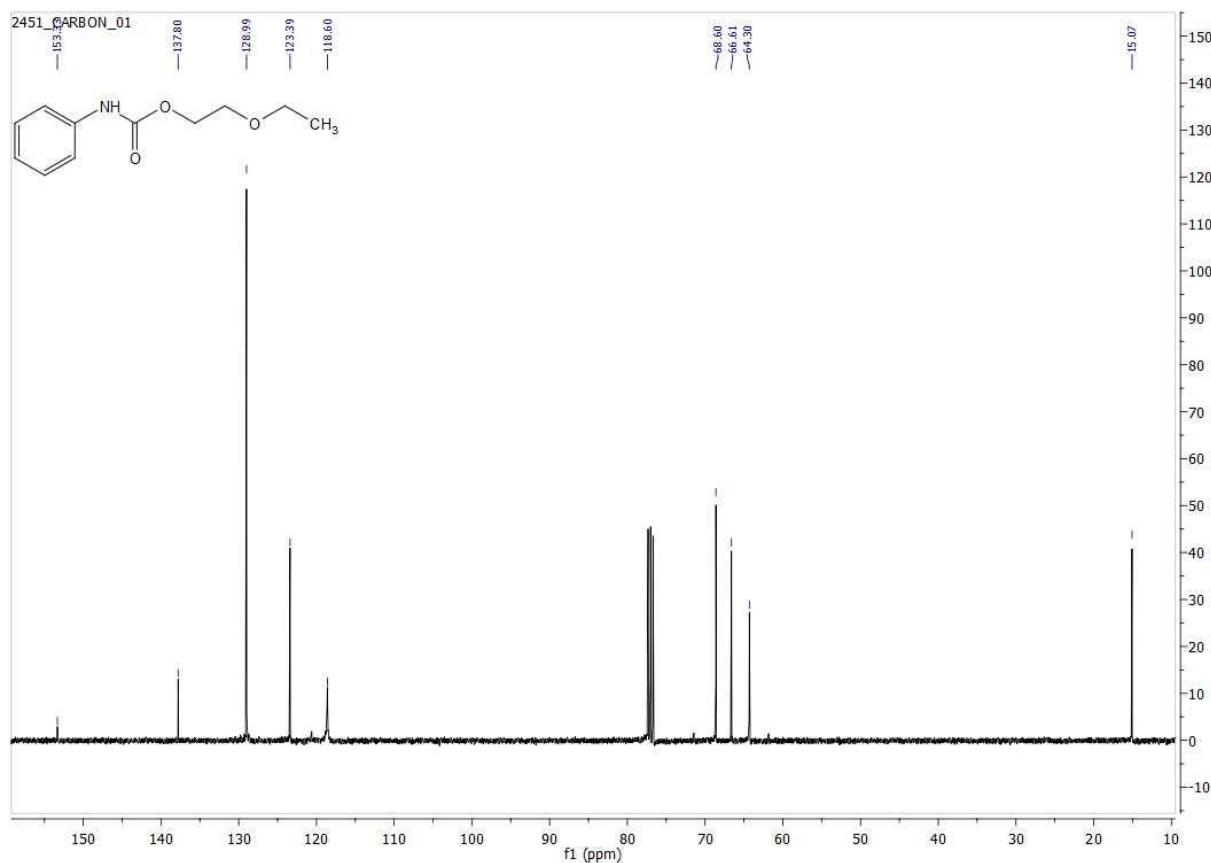


Figure S4.¹³C-NMR spectrum of 2-ethoxyethyl phenylcarbamate (**PU-2**)

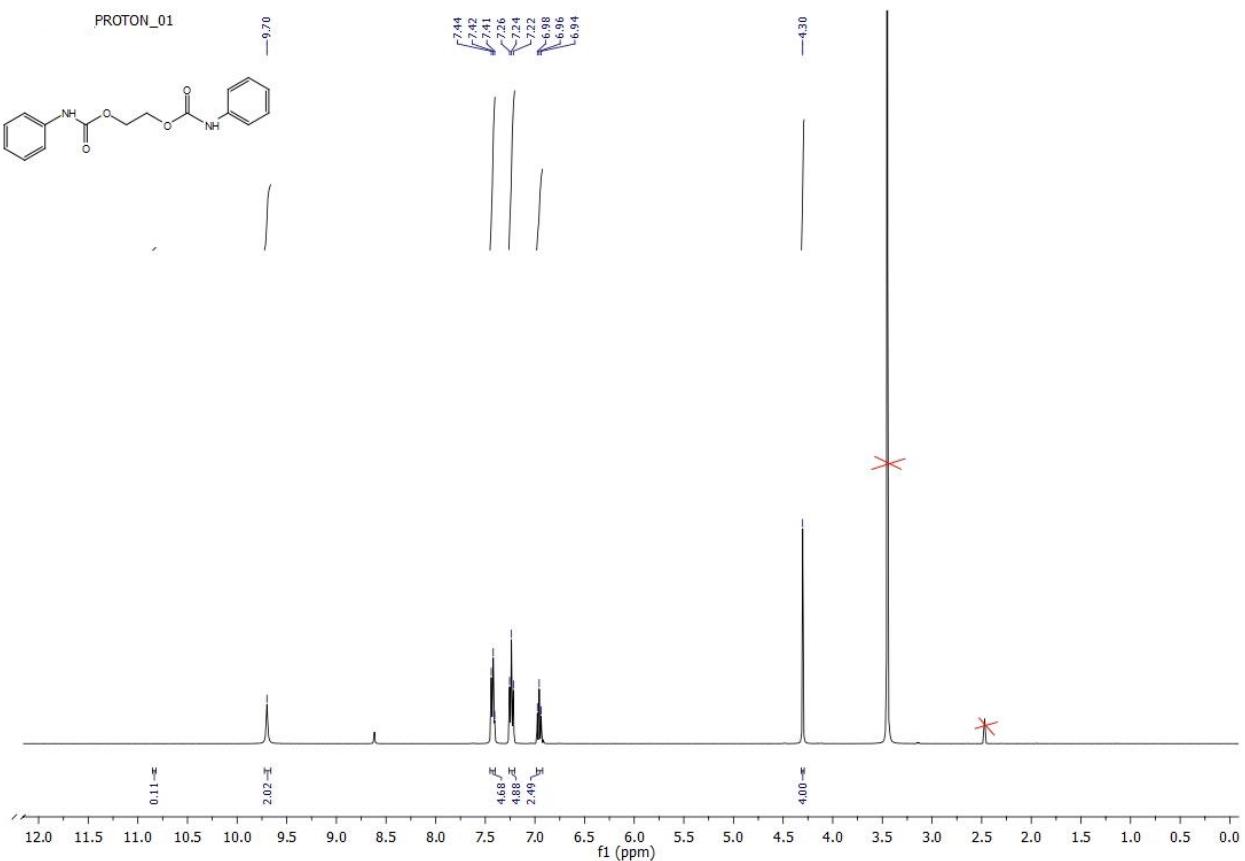


Figure S5.¹H-NMR spectrum of ethane-1,2-diyl bis(phenylcarbamate) (**PU-3**)

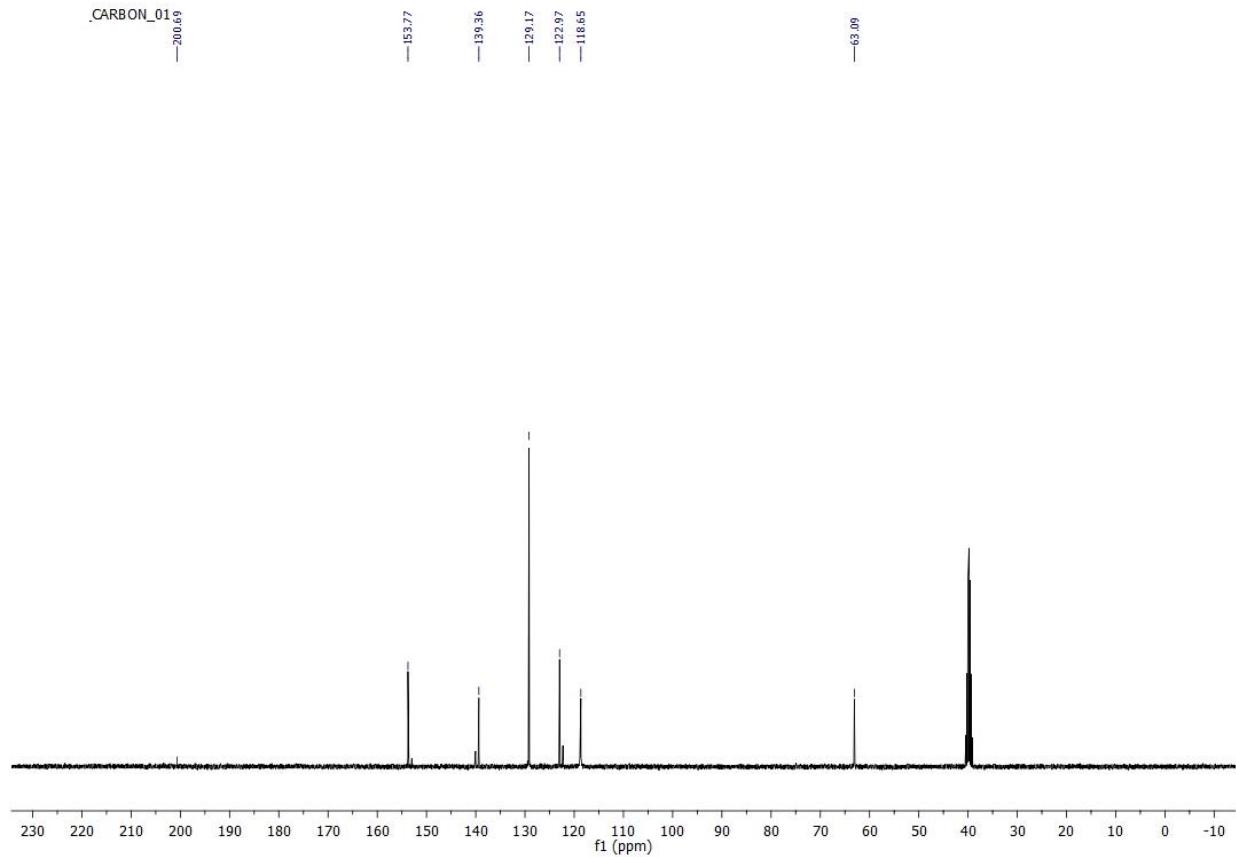


Figure S6.¹³C-NMR spectrum of *ethane-1,2-diyl bis(phenylcarbamate)* (**PU-3**)

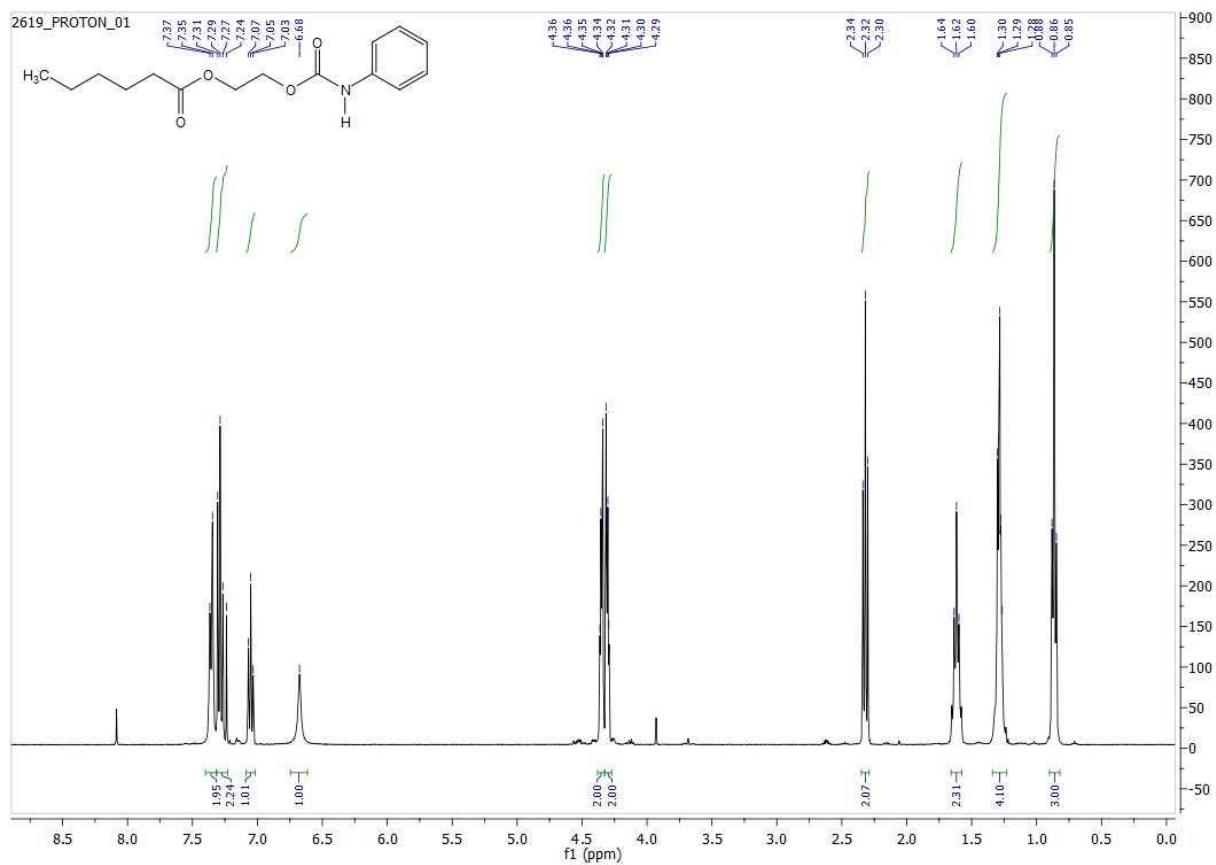


Figure S7. ^1H -NMR spectrum of 2-((phenylcarbamoyl)oxy)ethyl hexanoate (**PU-4**)

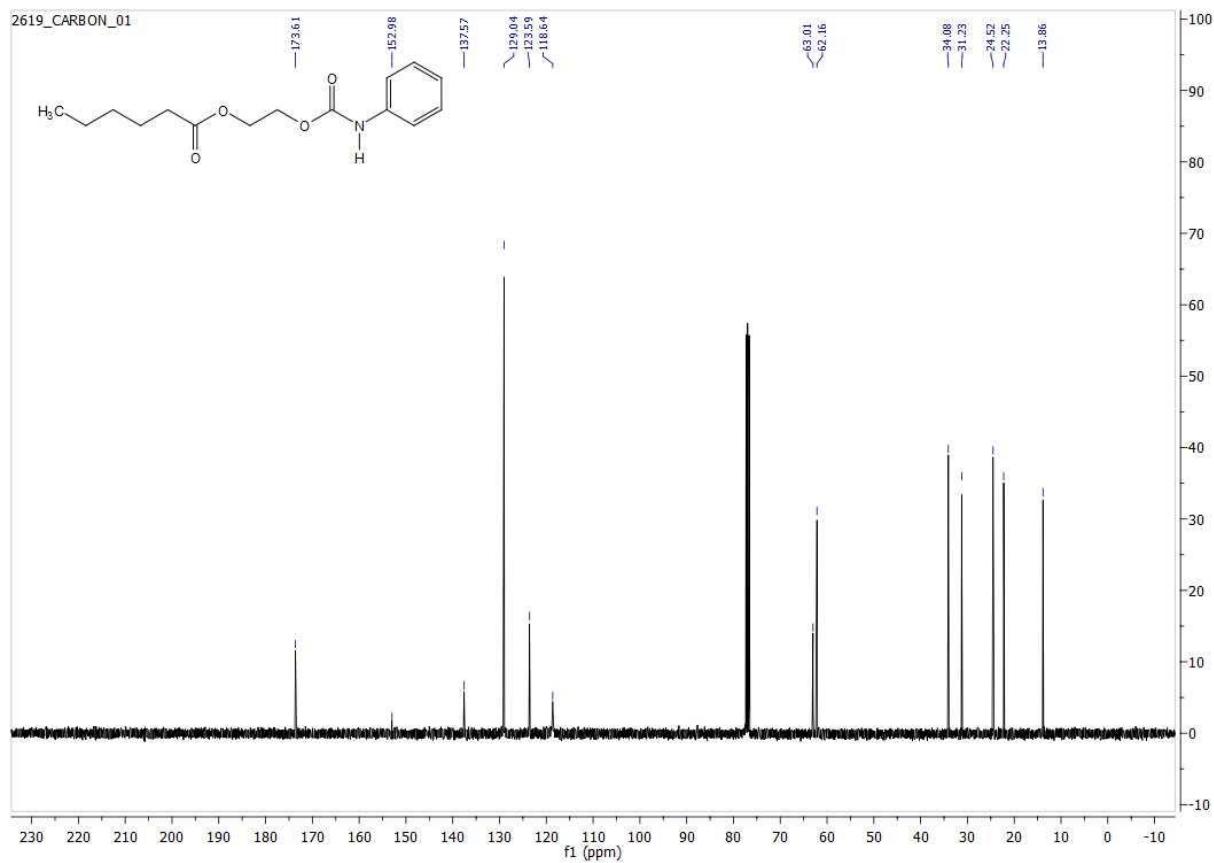


Figure S8. ^{13}C -NMR spectrum of 2-((phenylcarbamoyl)oxy)ethyl hexanoate (**PU-4**)

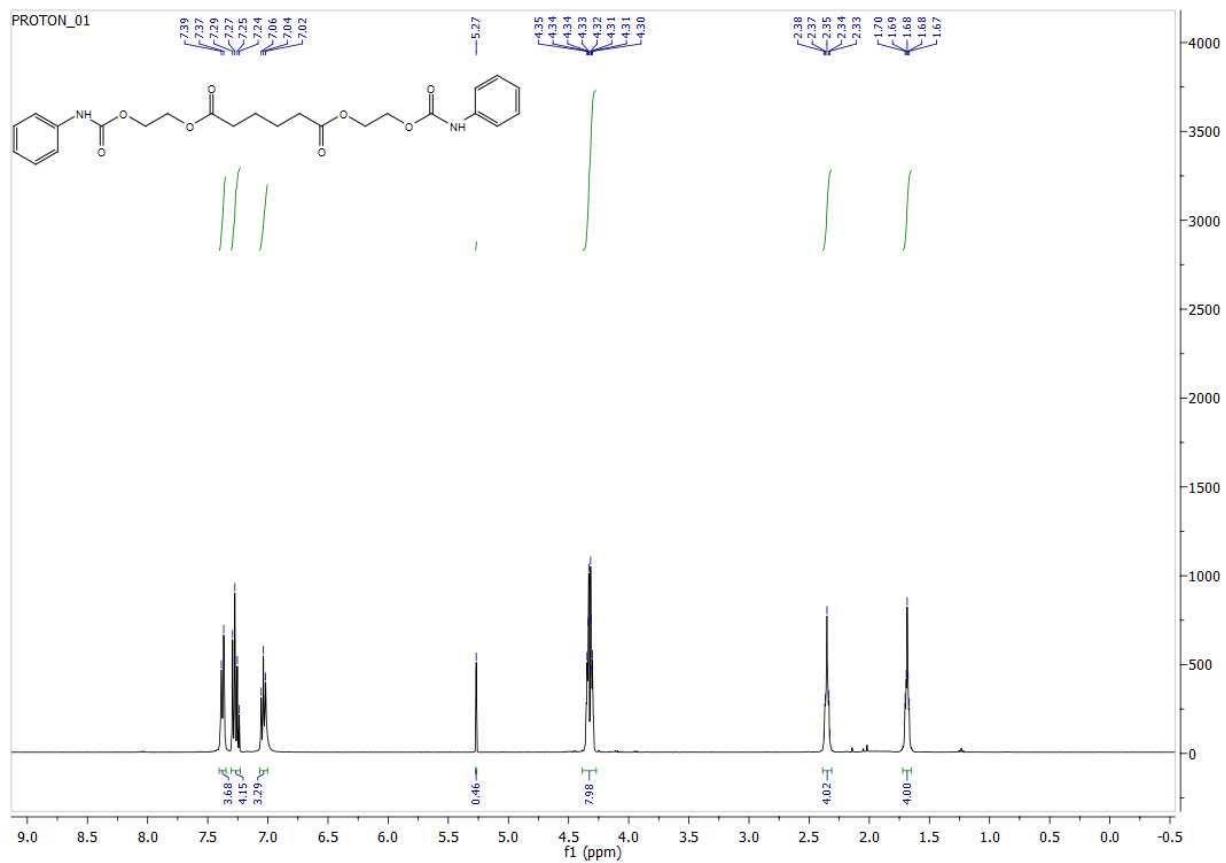


Figure S9.¹H-NMR spectrum of bis(2-((phenylcarbamoyl)oxy)ethyl) adipate (**PU-5**)

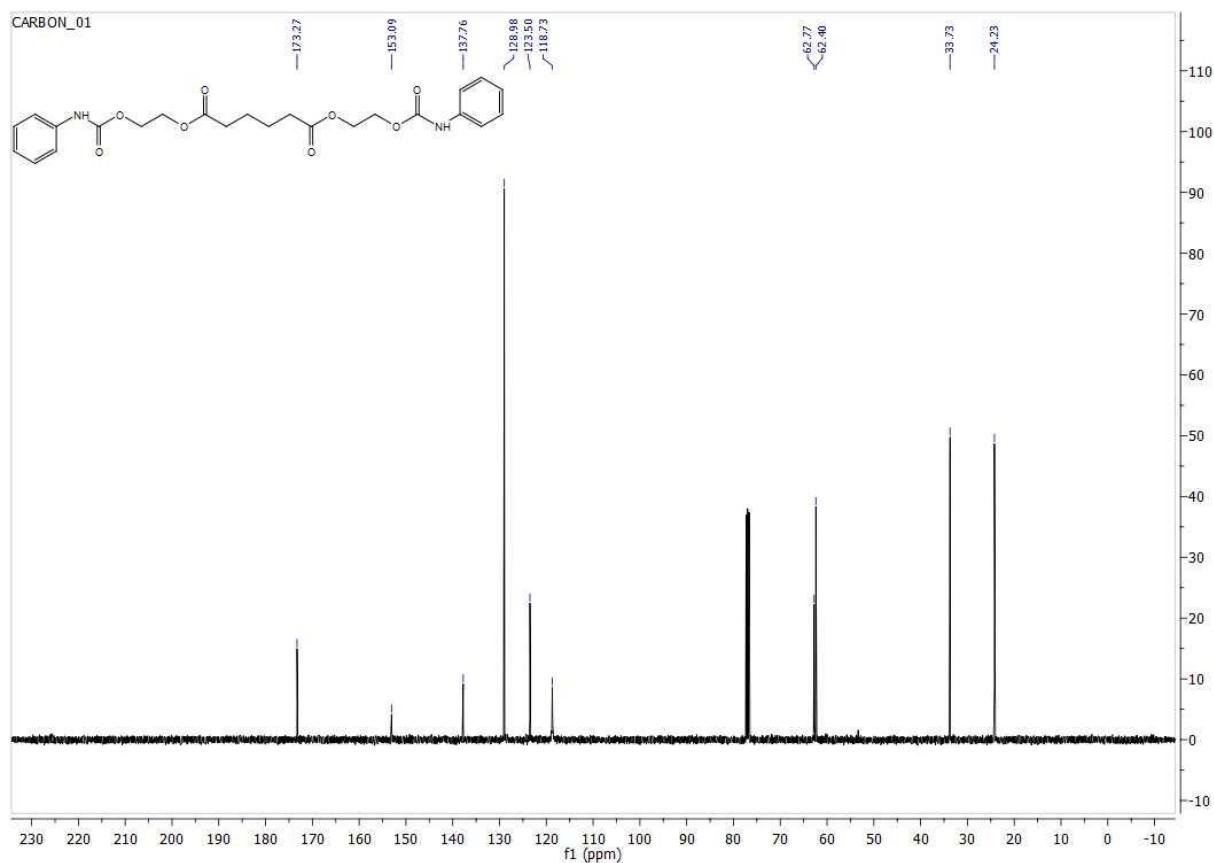


Figure S10.¹³C-NMR spectrum of *bis(2-((phenylcarbamoyl)oxy)ethyl) adipate (PU-5)*

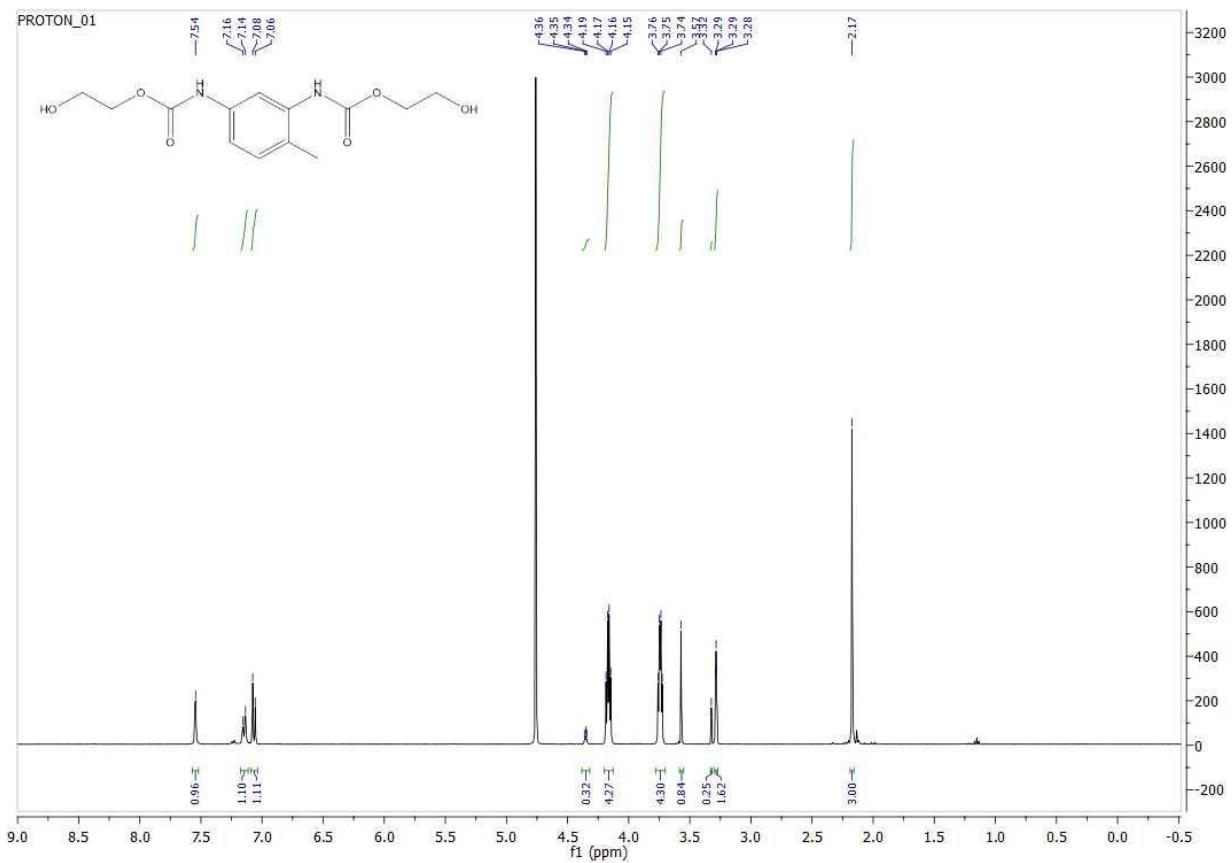


Figure S11.¹H-NMR spectrum of bis(2-hydroxyethyl) (4-methyl-1,3-phenylene)dicarbamate (PU-6)

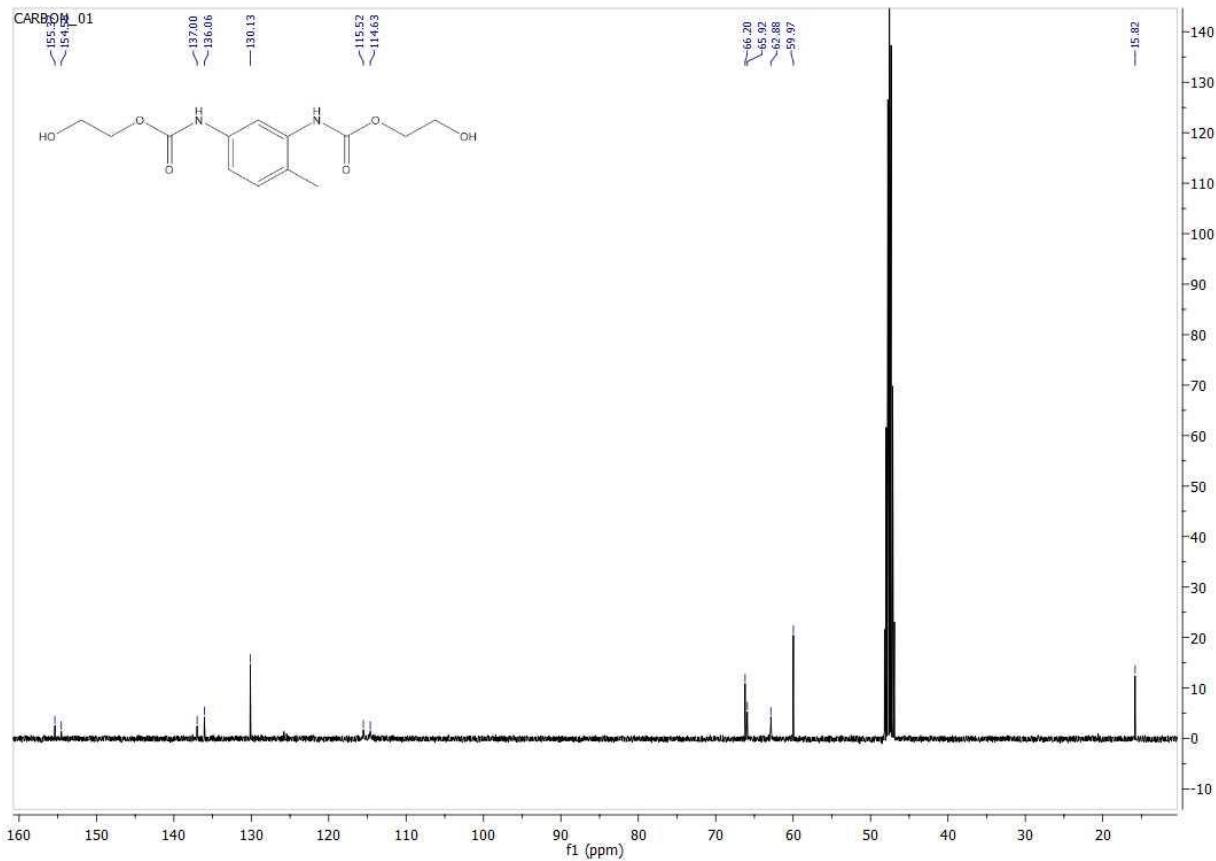


Figure S12.¹³C-NMR spectrum of *bis*(2-hydroxyethyl) (4-methyl-1,3-phenylene)dicarbamate (**PU-6**)

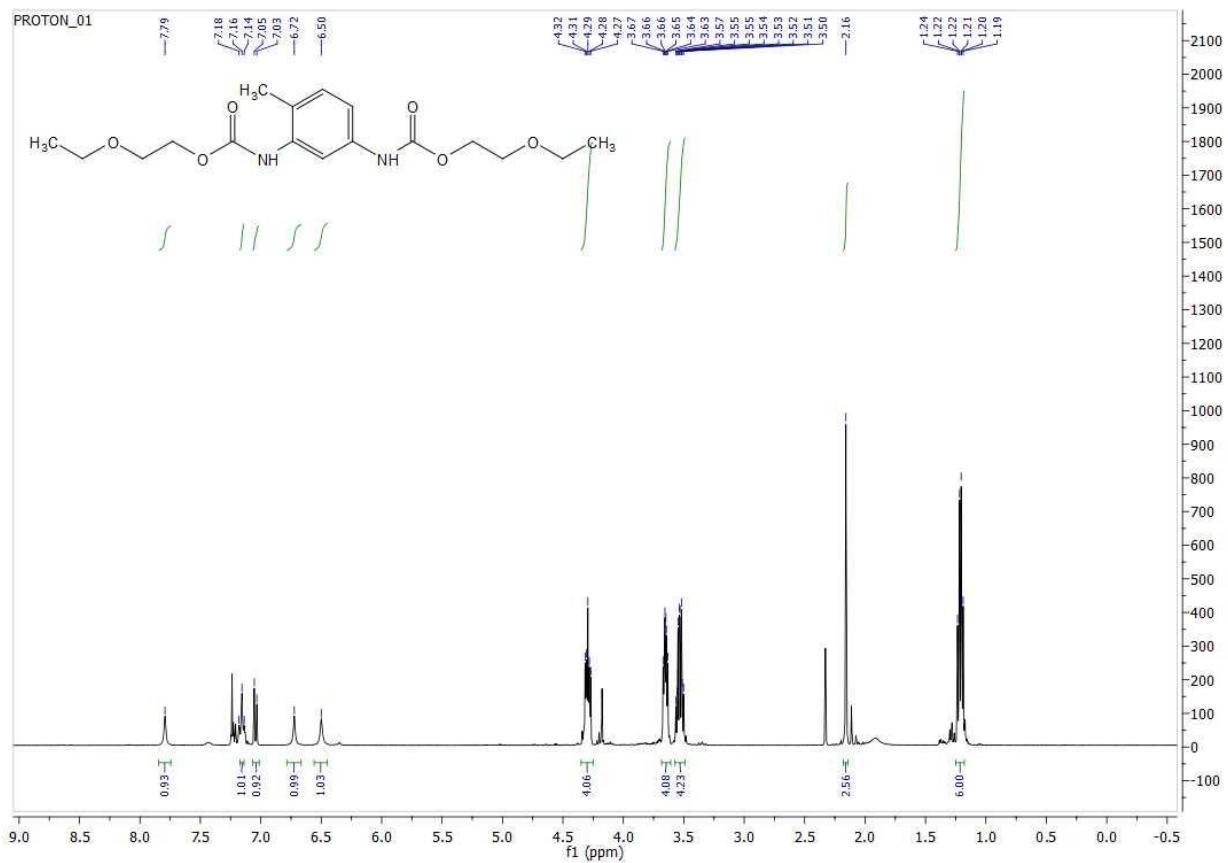


Figure S13.¹H-NMR spectrum of bis(2-ethoxyethyl) (4-methyl-1,3-phenylene)dicarbamate (PU-7)

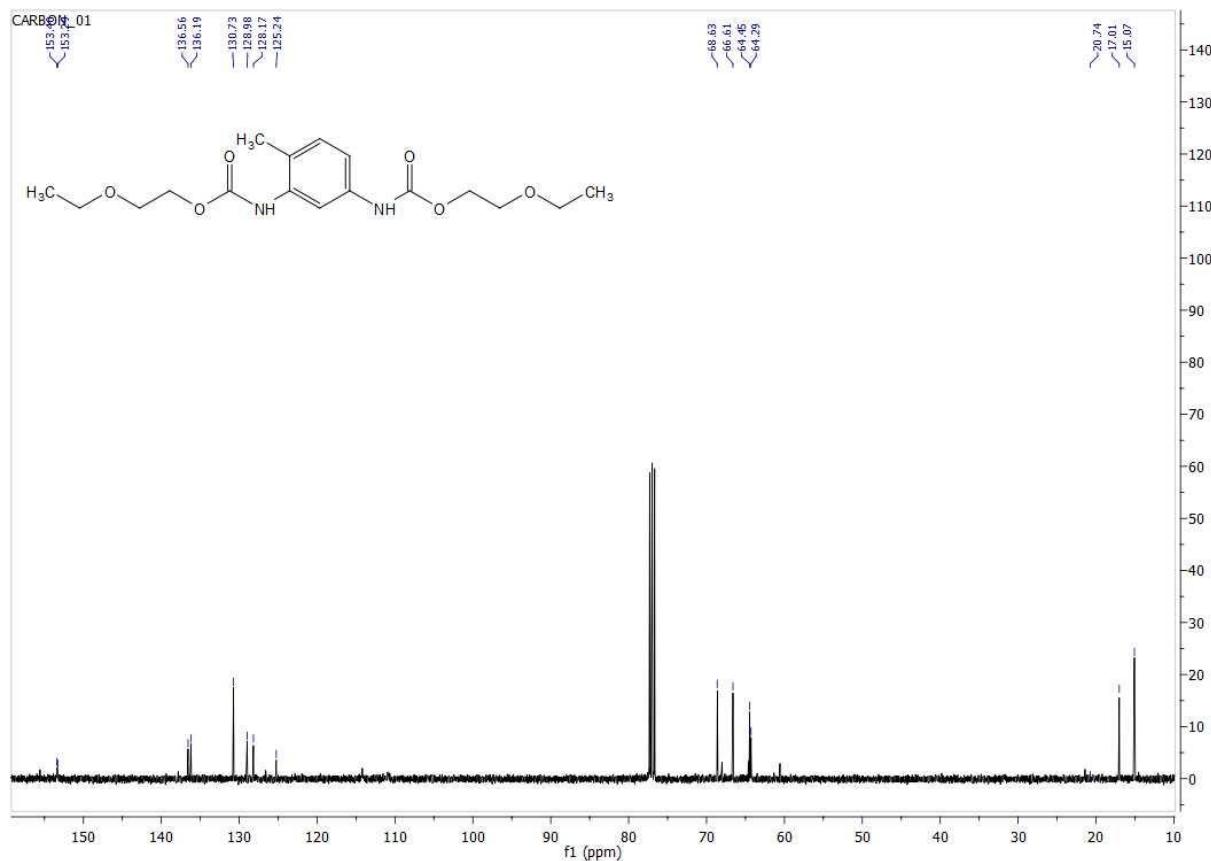


Figure S14.¹³C-NMR spectrum of *bis*(2-ethoxyethyl) (4-methyl-1,3-phenylene)dicarbamate (**PU-7**)

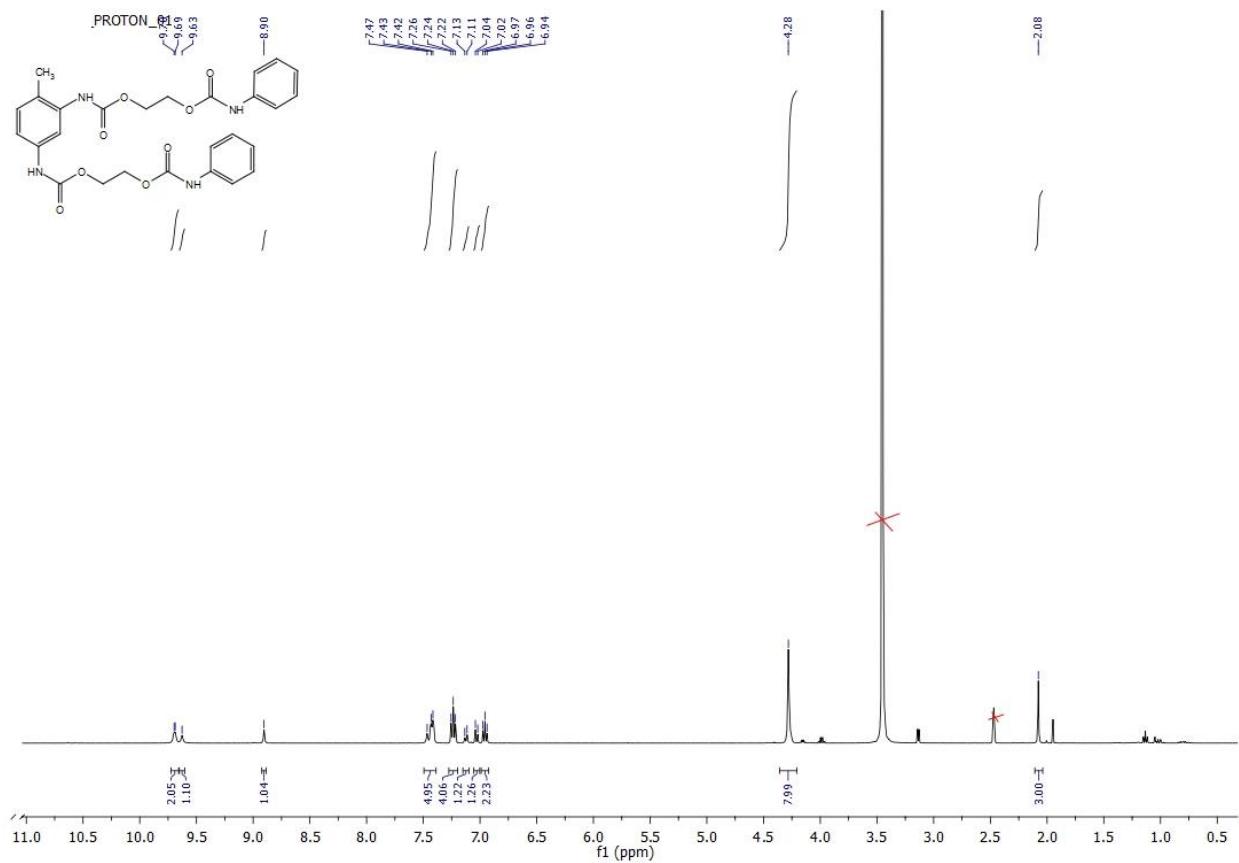


Figure S15. ¹H-NMR spectrum of bis(2-((phenylcarbamoyl)oxy)ethyl) (4-methyl-1,3-phenylene)dicarbamate (PU-8)

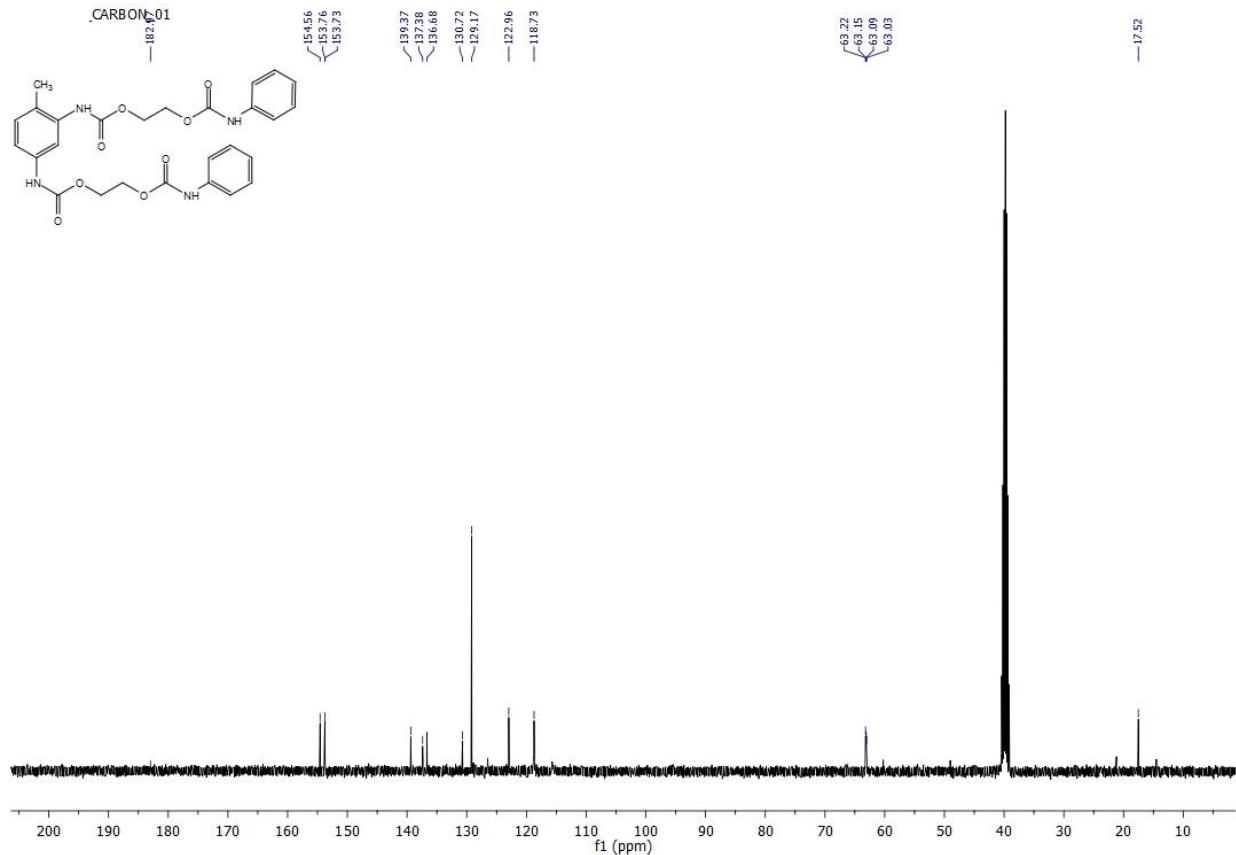


Figure S16. ^{13}C -NMR spectrum of bis(2-((phenylcarbamoyl)oxy)ethyl) (4-methyl-1,3-phenylene)dicarbamate (**PU-8**)

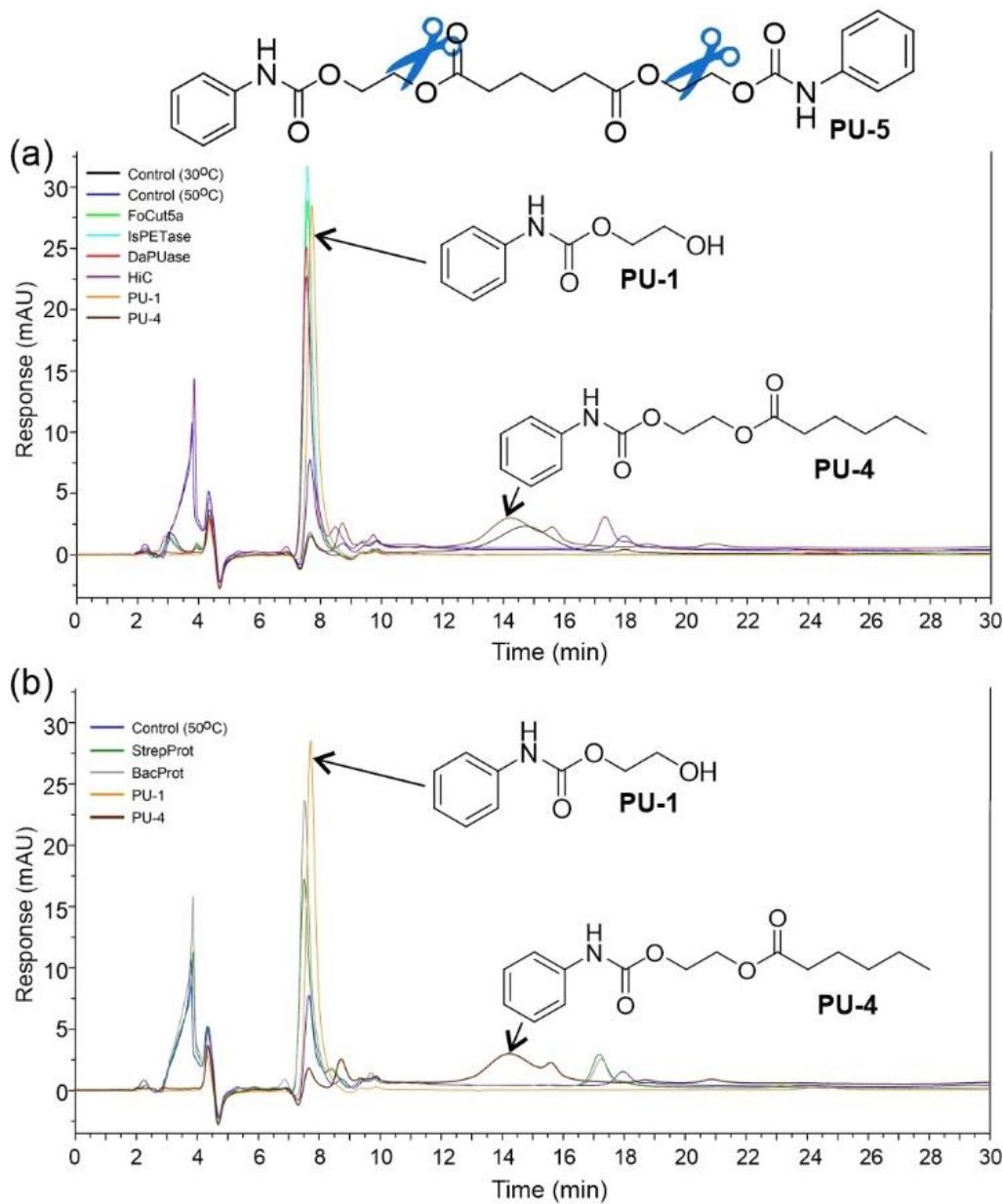


Figure S17. PU-5 degradation products (a) after incubation with a selection of esterases: FoCut5a (light green line), IsPETase (cyan line), DaPUase (red line), and HiC (purple line). Reactions without enzyme were performed at 30 °C (black line) and 50 °C (blue line); and two proteases: StrepProt (green line) and BacProt (grey line). Control

reactions without enzymes were performed at 50 °C (blue line). Possible degradation products PU-1 (orange line) and PU-4 (brown line) are also shown.

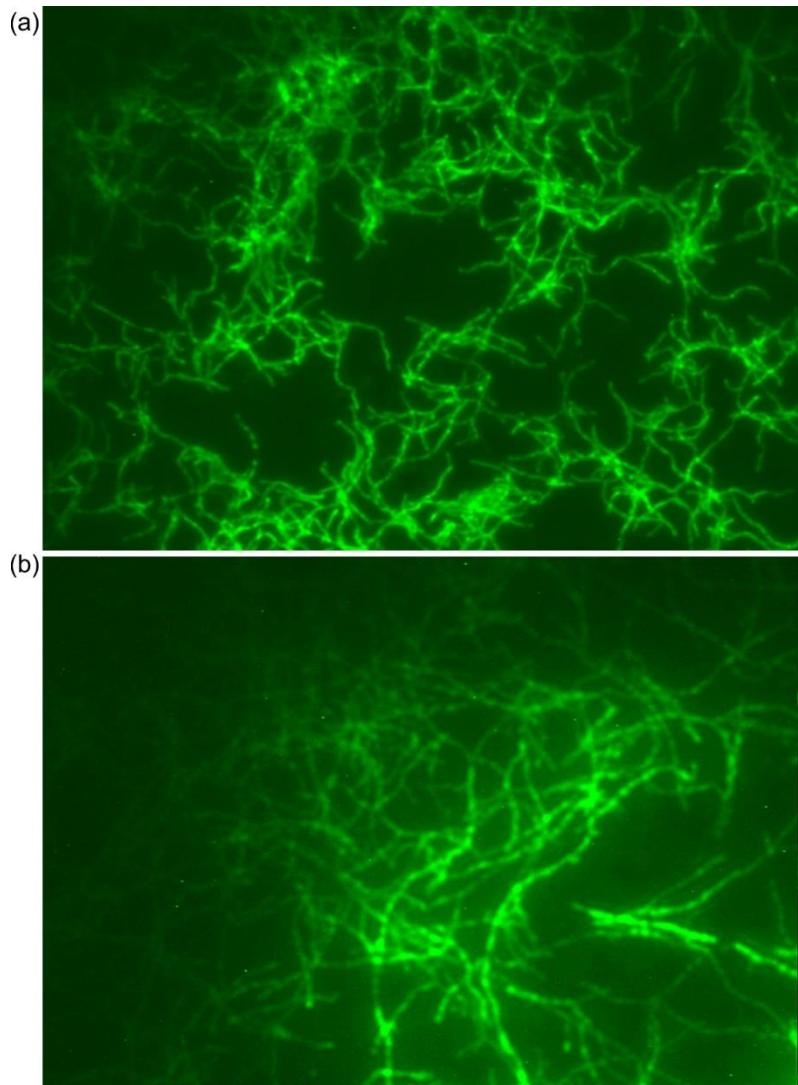


Figure S18. *Amycolatopsis mediterranei* ISP5501 cells stained with thiazole orange and visualized under fluorescent microscope (FITC channel). 60 × magnification (a) and 100 × magnification (b).

Table S1. Solubility of PU model compounds in selection of common organic solvents.

<i>Solvent compound</i>	EtOAc	DCM	MeOH	EtOH	DMF	DMSO
PU-1	***	***	***	***	***	***
PU-2	***	***	***	***	***	***
PU-3	***	***	***	***	***	***
PU-4	***	***	***	***	***	***
PU-5	***	***	***	***	***	***
PU-6	-	-	***	***	***	***
PU-7	***	***	***	***	***	***
PU-8	***	-	-	-	***	***

- not soluble

Solvents: EtOAc= ethylacetate; DCM=dichloromethane; MeOH= methanol EtOH= ethanol;
DMF= dimethylformamide; DMSO= dimethyl sulfoxide

Table S2. List of predicted PU-7 degradation products

<i>Compound</i>	Molecular mass	Molecular formula
PU-7	354.17910	C17H26N2O6 CCOCCOC(=O)NC1=CC=C(C)C(NC(=O)OCCOCC)=C1
PU7.13	106.02660	C3H6O4 OCCOC(O)=O
PU7.11 2,4-TDA	122.08440	C7H10N2 CC1=CC=C(N)C=C1N
PU7.12	134.0579	C5H10O4 CCOCCOC(O)=O
PU7.9	166.07420	C8H10N2O2 CC1=CC=C(N)C=C1NC(O)=O
PU7.10	166.07420	C8H10N2O2 CC1=CC=C(NC(O)=O)C=C1N
PU7.6	210.06410	C9H10N2O4 CC1=CC=C(NC(O)=O)C=C1NC(O)=O
PU7.7	210.10040	C10H14N2O3 CC1=CC=C(NC(=O)OCCO)C=C1N
PU7.8	210.10040	C10H14N2O3 CC1=CC=C(N)C=C1NC(=O)OCCO
PU7.14	238.1317	C12H18N2O3 CCOCCOC(=O)NC1=CC(N)=CC=C1C
PU7.15	238.1317	C12H18N2O3 CCOCCOC(=O)NC1=CC=CC(N)=C1
PU7.4	254.09030	C11H14N2O5 CC1=CC=C(NC(O)=O)C=C1NC(=O)OCCO
PU7.5	254.09030	C11H14N2O5 CC1=CC=C(NC(=O)OCCO)C=C1NC(O)=O
PU7.16	282.1216	C13H18N2O5 CCOCCOC(=O)NC1=CC=C(C)C(NC(O)=O)=C1
PU7.17	282.1216	C13H18N2O5 CCOCCOC(=O)NC1=CC(NC(O)=O)=CC=C1C
PU7.3	298.11650	C13H18N2O6 CC1=CC=C(NC(=O)OCCO)C=C1NC(=O)OCCO
PU7.1	326.14780	C15H22N2O6 CCOCCOC(=O)NC1=CC=C(C)C(NC(=O)OCCO)=C1
PU7.2	326.14780	CCOCCOC(=O)NC1=CC(NC(=O)OCCO)=CC=C1C

Table S3. Identification of 18 Impranil DLN degrading bacterial strains by 16S sequencing

Strain Identificati on	Sequence
MM46 <i>Streptomyces</i> sp.	TCCCGCATGGGAcGGGGTTAAAAGTTCCGGCGGtGAAGGATGAGCCCCCGGCCT ATCAGCTTGTGGGGTAATGCCCTACCAAGGCGACGGTAGCCGGCC TGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG GAGGCAGCACTGGGAATTATTGCACAATGGCGAAAGCCTGATGCAGCGACG CCGCGTGAGGGATGACGGCTTCGGGTTGTAACCTCTTCAGCAGGAAAGAA GCGAAAGTGACGGTACCTGCAGAAGAACGCCGGCTAACTACGTGCCAGCAG CCGCGTAATACGTAGGGCGAACCGTTGTCGGATTATTGGCGTAAAGAG CTCGTAGGCCGCTTGTACGTCGGATGTGAAAGCCCAGGCTTAACCCGGGT CTGCATTGATACGGCTAGCTAGAGTGTGGTAGGGAGATCGGAATTCTGG TGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGTGGCGAACGGCGA TCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGAGCGAACAGGA TTAGATAACCCTGGTAGTCCACGCCGTAAACGTTGGAACTAGGTGTTGGCGAC ATTCCACGTCGTGGTGCCGAGCTAACGCTTAAGTCCCCGCTGGGAGT ACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGCCGCACAAGCAGC GGAGCATGTGGCTTAATCGACGCAACCGCAAGAACCTTACCAAGGCTTGACA TATACCGAAAGCATCAGAGATGGTCCCCCTGTGGTCGGTATACAGGTGG TGCATGGCTGTCGTCACTCGTGTGAGATGTTGGGTTAAGTCCCACGCA GCGCAACCCCTGTTCTGTGTTGCCAGCATGCCCTCGGGGTATGGGACTCAC AGGAGACTGCCGGGTCAACTCGGAGGAAGGTGGGACGACGTCAAGTCATC ATGCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCGTACAATGAGCT GCGATGCCCGAGGCGGAGCGAATCTAAAAAcCCGGTCTCAGTCGGATTGG GGTCTGCAACTC

GGGACGGGgTtaAAAGTTCCGGCGGtGAAGGGAGACCCCCGGCCTTCAGTgTTGG
 TGGGgTAATGgCCTACCAAGgCGACGACGGTaCCGCCTGAGAGGGgCGACCGCCA
 cACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGgATTat
 TGCACAATGGCGAAAGCCTGATGCAGCGACGCCGTGAGGGATGACGGCC
 TTCGGGTTGTAAACCTCTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGC
 AGAAGAACGCCCCGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGGCG
 CAAGCGTTCCCGAATTATTGGCGTAAAGAGCTCGTAGGCGGCTGTCACG
 TCGGATGTGAAAGCCCCGGGCTTAACCCCGGTCTGCATTGATAACGGCTAG
 CTAGAGTGTGGTAGGGAGATCGAATTCTGGTAGCGGTGAAATGCCAG
 ATATCAGGAGGAACACCGGTGGCGAAGGGGATCTCTGGGCCATTACTGACGC
 TGAGGAGCGAAAGCGTGGGAGCGAACAGGATTAGATAACCTGGTAGTCCAC
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MM49

Streptomyces sp.

MM53

Streptomyces sp.

MM5

Streptomyces sp.

GATGACCCCGCGgCCTATCAGCTtGTTGGTGGGgTAATGgCCTACCAAGGCGAC
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GTCTGGCCTGCACACGTGCTACAATGGCCGGTACAATGA-CTGC-
ATGCCGCGA

MM61

Streptomyces sp.

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 GCCCAGCTAACCcCttTAfTTCCCCGCCCTGG

Streptomyces sp.
 BPN14

Streptomyces sp.
 BPS44

<p>JRD13 <i>Achromobacter sp.</i></p>	<p>TAATGGTCACCAAGGCACGACGGTAGCCGGCTGAGAGGGCGACCGGccA CCTGGGACTGAGACCAGGCCAGATTCTACGGAGGCAGCAGTGGGAATAT TGCACAATGGCGAAAGCCTGATGCAGCGACGCCGTGAGGGATGACGCC TTCGGTTGTAACCTTTCAAGCAGGAAAGAACGAAAGTACGGTACCTGC AGAAGAACGCCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGGCG CAAGCGTTGCCGAATTATTGGCGTAAAGAGCTGTAGGCCGTTGTCACG TCGATTGTGAAAGCCCAGGGCTAACCTCGGTCTGCAGTCGATACGGCTAG CTAGAGTGTGGTAGGGAGATCGGAATTCTGGTAGCGGTGAAATGCGCAG ATATCAGGAGGAACACCGGTGGCAAGGGGATCTCTGGCCATTACTGACGC TGAGGAGCGAAAGCGTGGGAGCGAACAGGATTAGATAACCTGGTAGTCCAC GCCGTAACCGTGGAACTAGGTGTTGCCGACATTCCACGTCGTCGGGCC AGCTAACGCATTAAGTCCCCGCTGGGAGTACGGCCGAAAGGCTAAAATC AAAGGAATTGACGGGGGCCGACAAGCAGCGAGCATGTGGCTAATTCA CGAACCGGAAGAACCTACCAAGGCTGACATACACCGAAAGCAGTCAGAG ATGGTGCCCCCTGTGGTCGGGTACAGGTGGCATGGCTGTCGTCAaCTCtT GTCG</p> <p>GCCTCCCTACGGTTAGGCTAACTACTTCTGGTAAAACCCACTCCATGGTGTG ACGGGCGGTGTGTACAAGGACCCGGAACGTATTACCGCGACATGCTGATCC GCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTGCAGACTGCGATCCGG ACTACGATCGGTTCTGGATTGGCTCCCCCTCGGGTTGGCACCCCTCTGT CCCGACCATTGTATGACGTGTGAAGCCCTACCCATAAGGCCATGAGGACTTG ACGTACATCCCCACCTTCCGGTTGTCACCGCAGTCTCATTAGAGTGCCCTT TCGTAGCAACTAATGACAAGGGTGCCTCGTGGGACTTAACCCAAACATC TCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTCCGGTCTTGC AGCACTTCAAATCTTCGAAATTCCAGACATGTCAAGGGTAGGTAAAGTTT TCGCGTTGCATCGAATTAAATCCACATCATCCACCGCTGCGGGTCCCCGTCA ATTCTTGTAGTTAATCTTGCCTCGACCGTACTCCCCAGCCGTCACCTCACCG GTTAGCTCGCTACTAAGGCCGAAGGCCCAACAGCTAGTTGACATCGTTA GGGCGTGGACTACCAGGGTATCTAACCTGTTGCTCCCCACGCTTGTGCAT GAGCGTCAGTGTATCCCAGGAGGCTGCCATCGGTGTTCCCTCCGCATA TCTACGCATTCACTGCTACACGCCGAATTCCACCTCCCTGACACACTCTAG CTCGGTAGTTAAAAATGCAGTTCAAAGTTAACGCTCTGGGATTTCACATCTTC TTTCCGAACGCCCTGCGCACGCTTACGCCAGTAATTCCGATTAACGCTGCA CCCTACGTATTACCGCGGCTGCTGGCACGTACTAGCCGTCCTATTCTGCAG GTACCGTCCGTTACGGGTATTAGCCATGACGTTCTTCCCTGCCAAAGTGC TTTACAACCGAAAGCCTCATCTCAACCCCGATGGCTGTATCAGGTTCC TGGACAAATTACCACTGCTGCTTCAGAAGGAAGGGGGGGCTTACCT GGGTGGCCTCCCAACCAACAAGGATCCTACCTGGGAATCTTACCCCCAA TAACAAACAGAATTGCTGCACAAATATGAGGGTTTCGACCCCTCCTTCC GAGTTGGGTTTATCTTGTATACCCCCACAAGG</p>
<p>JIA17 <i>Streptomyces sp.</i></p>	<p>TAATGGTCACCAAGGCACGACGGTAGCCGGCTGAGAGGGCGACCGGccA CCTGGGACTGAGACCAGGCCAGATTCTACGGAGGCAGCAGTGGGAATAT TGCACAATGGCGAAAGCCTGATGCAGCGACGCCGTGAGGGATGACGCC TTCGGTTGTAACCTTTCAAGCAGGAAAGAACGAAAGTACGGTACCTGC AGAAGAACGCCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGGCG CAAGCGTTGCCGAATTATTGGCGTAAAGAGCTGTAGGCCGTTGTCACG TCGATTGTGAAAGCCCAGGGCTAACCTCGGTCTGCAGTCGATACGGCTAG CTAGAGTGTGGTAGGGAGATCGGAATTCTGGTAGCGGTGAAATGCGCAG ATATCAGGAGGAACACCGGTGGCAAGGGGATCTCTGGCCATTACTGACGC TGAGGAGCGAAAGCGTGGGAGCGAACAGGATTAGATAACCTGGTAGTCCAC GCCGTAACCGTGGAACTAGGTGTTGCCGACATTCCACGTCGTCGGGCC AGCTAACGCATTAAGTCCCCGCTGGGAGTACGGCCGAAAGGCTAAAATC AAAGGAATTGACGGGGGCCGACAAGCAGCGAGCATGTGGCTAATTCA CGAACCGGAAGAACCTACCAAGGCTGACATACACCGAAAGCAGTCAGAG ATGGTGCCCCCTGTGGTCGGGTACAGGTGGCATGGCTGTCGTCAaCTCtT GTCG</p> <p>GCCTCCCTACGGTTAGGCTAACTACTTCTGGTAAAACCCACTCCATGGTGTG ACGGGCGGTGTGTACAAGGACCCGGAACGTATTACCGCGACATGCTGATCC GCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTGCAGACTGCGATCCGG ACTACGATCGGTTCTGGATTGGCTCCCCCTCGGGTTGGCACCCCTCTGT CCCGACCATTGTATGACGTGTGAAGCCCTACCCATAAGGCCATGAGGACTTG ACGTACATCCCCACCTTCCGGTTGTCACCGCAGTCTCATTAGAGTGCCCTT TCGTAGCAACTAATGACAAGGGTGCCTCGTGGGACTTAACCCAAACATC TCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTCCGGTCTTGC AGCACTTCAAATCTTCGAAATTCCAGACATGTCAAGGGTAGGTAAAGTTT TCGCGTTGCATCGAATTAAATCCACATCATCCACCGCTGCGGGTCCCCGTCA ATTCTTGTAGTTAATCTTGCCTCGACCGTACTCCCCAGCCGTCACCTCACCG GTTAGCTCGCTACTAAGGCCGAAGGCCCAACAGCTAGTTGACATCGTTA GGGCGTGGACTACCAGGGTATCTAACCTGTTGCTCCCCACGCTTGTGCAT GAGCGTCAGTGTATCCCAGGAGGCTGCCATCGGTGTTCCCTCCGCATA TCTACGCATTCACTGCTACACGCCGAATTCCACCTCCCTGACACACTCTAG CTCGGTAGTTAAAAATGCAGTTCAAAGTTAACGCTCTGGGATTTCACATCTTC TTTCCGAACGCCCTGCGCACGCTTACGCCAGTAATTCCGATTAACGCTGCA CCCTACGTATTACCGCGGCTGCTGGCACGTACTAGCCGTCCTATTCTGCAG GTACCGTCCGTTACGGGTATTAGCCATGACGTTCTTCCCTGCCAAAGTGC TTTACAACCGAAAGCCTCATCTCAACCCCGATGGCTGTATCAGGTTCC TGGACAAATTACCACTGCTGCTTCAGAAGGAAGGGGGGGCTTACCT GGGTGGCCTCCCAACCAACAAGGATCCTACCTGGGAATCTTACCCCCAA TAACAAACAGAATTGCTGCACAAATATGAGGGTTTCGACCCCTCCTTCC GAGTTGGGTTTATCTTGTATACCCCCACAAGG</p>

	AACTATTaCCGCAtACGT CCTGGGGAgAAAGCAGGGACCcTTcgGGCCTT GcGC
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	GTCCAGACTCCTACGGGAGGCAGCAGTGGgAATATTGGACAATGGCGAAA
ST11 <i>Pseudomonas</i> sp.	GCCTGATCCAGCCATGCCGGCtGTgtGAAGAAGGTCTCGGATTGTAAAGCACT
	TTAAGTTGGGAGGAAGGGCAGTAAGTTAACCTTGCTGCTTGACGTTACCA
	ACAGAATAAGCACCGGCTAACCTCGTGCCAGCAGCCGCGTAATACGAAGGG
	TGCAAGCGTTAACCGAATTACTGGCGTAAAGCGCGTAGGTGGTT CAGCA
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	GATATAGGAAGGAACtCCAGTG
	GTCCAGCAGCCGCGTAATACGTAGGGCGCAAGCGTTGTC CCGAATTATTGGG
	CGTAAAGAGCTCGTAGGC GGtGTGCGCTGGATGTGAAAGCCC GGCTTAA
	CCCCGGGTCTGCATTGATACGGG CAGACTAGAGTGTGGTAGGGGAGATCGGA
J1 <i>Streptomyces</i> sp.	ATT CCTGGTGTAGCGGTAAATGCGCAGATATCAGGAGGAACACCGGTGGCG
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	AAGGGGTGGCCACCGGCTCGGGTGTACCGACTTCGTACGTGACGGC
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	CAAGGGCCTGCTGGCAACACGGACAAGGGTCCGCTCGTCCGGACTTAAC
	CCAACATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTACACCGA
	CCACAAGGGGGCCCTGCTCCAGACGTTCCGGTATGTCAAGCCTGGTA
	AGTTCTCGCGTTGCGTCAATTAGCCACATGCTCCGCCGTTGTGCGGGCC
	CCCGTCAATT CCTTGAGTTAGCCTGCGGCCGTACTCCCCAGGGGGGCAC
VB659 <i>Streptomyces</i> sp.	TTAATGCGTTAGCTCGGCCACGGACAACGTGGAATGTTGCCACACCTAGTGC
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	TTTCGCTCCTCAGCGTCAGTATCGGCCAGAGATCCGCCTCGCCACCGGTGTT
	CCTCCTGATATCTGCGCATT CACCGCTACACCAGGAATTCCGATCTCCCTAC
	CGAACTCTAGCCTGCCGTATCGACTGCAGACCCGGGTTAAGCCCCGGCCT
	TCACAACCGACGTGACAAGCCGCTACGAGCTTTACGCCAATAATTCCGG
	ACAACGCTTGCGCCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGC
	GCTTCTTCTGCAGGTACCGTCACITTCGCTTCTCCCTGCTGAAAGAGGTTACA
	ACCCGAAGGCCGTATCCTCACCGCGCGTGCATCAGGCTTCCGCCATT
	GGGCAATATTCCCCACTGTCGCTCCCGTAGGAGTCTGGGCCGGTCTCAATCC
	CAGTGTGCCGGTGCCTTCAGGGCGGGTACCCGTCGTCGCCCTGGGAGCC
	ACTACCTCCCCACAAGCTGAAAGGCCCGGGTCCTCCTGAC

BV365

Streptomyces sp.

CCCTTCGGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGGCAATCTGCC
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TGGGA

RUJ1

Streptomyces sp.

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GCTAGTCTAACCC

CGACGGCTCCCTCCCACAAGGGGTTAACGCCACCGGCTCGGGTGTACCGACT
 TTCATGACGTGACGGGCAGGTGTACAAGGCCCGGAACGTATTACCGCAGC
 GTTGCTGATCTGCGATTACTAGCGACTCCGACTTCACGGGTCGAGTTGCAGAC
 CCCGATCCGAACTGAGACCAGCTTAAGGGATTGCTCCACCTCACGGTCTCG
 CAGCCCTCTGTACTGGCATTGTAGCATGTGTGAAGCCCTGGACATAAGGGC
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 ACGAGTCCCCACCATAACGTGCTGGCAACATAAGATAAGGGTTGCGCTCGTTG
 CGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAaGCCATGCACCAC
 CTGTATACCGACCACAAGGGGGCCACATCTCGCAGCTTCCGGTtATATGTC
 AAACCCAGGTAAaGGTCTTCGCGITGCATCGAAaTTAATCCACATGCTCCGCC
 GCTTGTGCGGGCCCCGTCAATTCTTGTAGTTAGCCTTGC GGCGTACTCCC
 CAGGCGGGCGCTTAATGCGTAGCTACGGCACGGATTCCGTGGAAGGAACCC
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 GCTACCCACGCTTCGTTCTCAGCGTCAGTTACTGCCAGAGACCCGCCCTCG
 CCACCGGTGTTCCCTCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCA
 GTCTCCCTGCAGTACTCAAGTCTGCCGTATGCCCTGCAAGCCAGCAGTTGAG
 CTGCTGGTTTCACAAACGACGCGACAAACCGCCTACGAACCTTACGCCCA
 GTAATTCCGGACAACGCTTGCACCCCTACGTATTACCGCGGCTGCTGGCACGTA
 GTTAGCCGGTGTCTCTGCAAGTACCGTCACTTGCCTGTTtCGTCCCTGCTGAA
 AGAGTTTACAACCCGAAaGGCCGTATCCCTCACGCCGCTCGCTGCATCAG
 GCTTTCGCCATTGTGCAATATTCCCCACTGgCTGCCCTCCGTAGGAGTCTGGG
 CCGTGTCTCAGTCCCAGTGTGGCCGGTACCCCTCTCAGGTCGGTACCCGCGT
 CGCCTGGTAGGCCATTACCCACCAACAAGCTGATAGGCCGGGGCCATCC
 TGCACCGATAAATCTTCCACCAACCCACCATGCGATAGGAGGTATATCCGGT
 ATTAGACCCAGTTCCCAGGCTTATCCCAGTGCAGGGCAGATACCCACGT
 GTTACTCACCCGTTGCCGCTCGTGTACCCGAAAGGCCTTACCGCTCGAC
 AAGCCTCGGGTGGATTAATGGCGAACGGGTGAGTAACACGTGGTAATCTGC
 CCTGCACTCTGGATAAGCCTGGAAACGGGTCTAATACCGGATATCACAAT
 CTCTCGCATGGGGGGTTGTGAAAGTTCTGGCGGTGCAAGGATGAACCCGCC
 CTATCAGCTTGTGGTAGTGGCTACCAAGGCGACGACGGTAGCCGG
 CCTGAGAGGGTGACCGGCCACACTGGACTGAGACACGCCAGACTCCTAC
 GGGAGGCAGCAGTGGGAATATTGCACAATGGCGCAAGCCTGATGCAGCGA
 CGCCCGTGAGGGATGACGGCTTCGGGTGAAACCTTTCGCCAGGGACG
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 AGCCCGGTAATACGTAGGGTGCAGCGTTGTCCGGATTATTGGCGTAAAG
 AGCTCGTAGGCGTTGTCCGTGCCGTGAAATCTCATGCTAACGTGGAG
 CGTGCAGGTGATAACGGGAGACTGAGTCGGTAGGGAGACTGGAATTCTG
 GTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCG
 GTCTCTGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGAGCGAACAGG
 ATTAGATAACCTGGTAGTCCACGCTGAAACGTTGGCGCTAGGTGTGGCGA
 CATCCACGTTGTCCGTGCCGTAGCTAACGCATTAACGCCCGCCTGGAGAGT
 ACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGCCCGACAAGCGGC
 GGAGCATGTGGATTAATTGATGCACCCGAAGAACCTTACGGGGCTTGGACT
 GCCCCAGAATCCTAAAATGGGGCTCCCTGGGTGGGTACGGGGTCGTT
 GTTGTCTT

MUG-A3

Rhodococcus sp.

ISP 5501

Amycolatopsis mediterranei

Table S4. Possible PU depolymerization associated enzyme families in *A. mediterranei* genomes

Genome	amidases	esterases	peptidases	ureases	other α/β hydrolases
ISP5501	87	161	387	9	146
GCF_000196835.1	88	161	385	9	147
GCF_000220945.1	88	161	387	9	146
GCF_000282715.1	88	162	388	9	146
GCF_000454025.1	88	164	387	9	145
GCF_000696405.1	82	162	375	9	142
GCF_000700945.1	88	163	387	9	145
GCF_001742805.1	88	162	387	9	146