Tetrazine Mediated Post-polymerization Modification

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S1. General Information

Potassium (Acros), Ethyl 2-diazidoacetate (Sigma Aldrich), 5-Amino-2-pyridinecarbonitrile (Sigma Aldrich), 2-Pyrimidinecarbonitrile (Acros Organics), 4-Cyanobenzoic acid (Sigma Aldrich) and 3,6-Diphenyl-1,2,4,5-tetrazine (Sigma Aldrich) were used as received. Allyl glycidyl ether (Sigma Aldrich) was distilled under vacuum. Naphthalene (Sigma Aldrich) was purified by recrystallization from hexane. Reactions, which required oxygen and moisture free conditions, were carried out using Schlenk techniques under a nitrogen atmosphere.

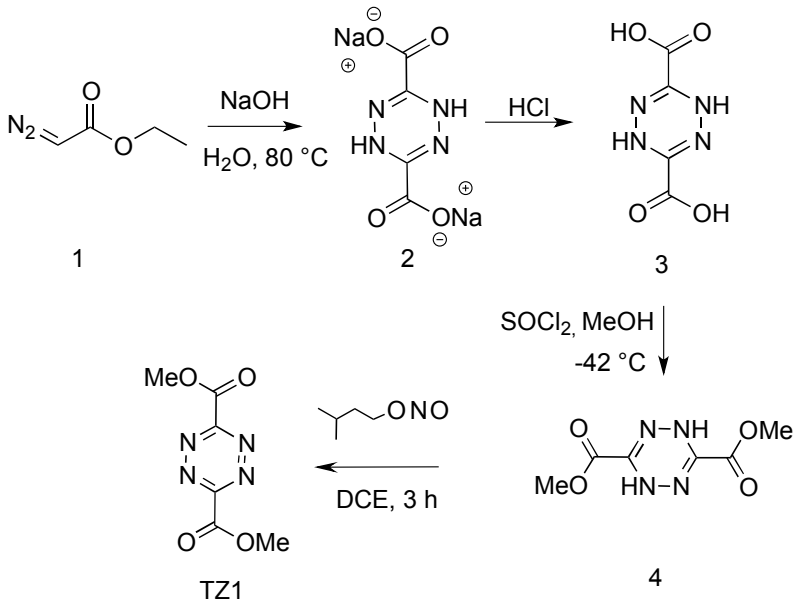
^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker AVA500 spectrometer (operating at 500 and 125 MHz respectively) at 298 K in deuterated solvents.

Residual protic solvent signal was used as a reference for H^1 NMR (($\underline{CHCl_3}$) δ_H = 7.26 ppm; CHD_2OD (δ_H = 3.30 ppm), and CHD_2SOCD_3 (δ_H = 2.50 ppm,) and $CDCl_3$ (δ_C = 77.0 ppm), $\underline{C}D_3OD$ (δ_C = 49.0 ppm), CD_3SOCD_3 (δ_C = 39.5 ppm) for C^{13} NMR.

Coupling constants were measured in Hertz (Hz). Chromatographic purifications were carried out on silica gel 60-120 mesh. Analytical thin layer chromatography was performed on silica gel F254 (Merck). Low Resolution Mass Spectra were obtained using a Hewlett Packard LCMS 110 ChemStation with a G1946B mass detector. Reverse phase analytical HPLC (RP HPLC) was performed using an Agilent 1100 Chemstation on a Kinetex 5uXB-C18 (50 × 4 × 60 mm), and compounds were detected using an UV detector. All solvents used were HPLC grade. Method A: eluent A: water and formic acid (0.1 %); eluent B: acetonitrile, formic acid (0.1%) (A = 95% 5 min, 95% to 5% over 10 min). Method B: eluent A: water and formic acid (0.1 %); eluent B: MeOH, formic acid (0.1%) (A = 95% 5 min, 95% to 5% over 10 min). Infrared absorption spectra were recorded on a Shimadzu Iraffinity-1 CE FTIR spectrometer. The number of repeating units in the polymers were determined by ^{1}H-NMR via integration of the initiator signal and integration of the allyl moiety and was used to calculate the molecular weight of the polymers. Polymers were analysed by gel permeation chromatography (GPC) using two PLgel MIXED-C columns (200 - 2,000,000 g mol^{-1}, 5 mm) using *N, N*-dimethylformamide (DMF) with 0.1M LiBr at 60 ºC at 1 mL min^{-1} as the eluent. The GPC was calibrated with PMMA as standards.

S2. Synthesis of Tetrazine

Synthesis of TZ1



Synthesis of 3

3 was synthesized following a literature procedure.[1] Ethyl 2-diazidoacetate 1 (20 mL, 190 mmol, 1 eq.) was added dropwise to a stirred solution of sodium hydroxide (35.0 g, 874 mmol, 4.6 eq.) in water (50 mL). The reaction mixture was heated to 80 °C and stirred for 1.5 hours. The reaction mixture was cooled to ambient temperature and diluted with ethanol (200 mL) then stirred for 30 minutes. The insoluble solid was removed by filtration and washed with ethanol (3 x 80 mL) then diethyl ether (3 x 80 mL) before drying under reduced pressure. Concentrated hydrochloric acid (38.2 mL, 37 % aq.) was added dropwise to a stirring solution in water (40 mL) at -10 °C. The insoluble solid was immediately removed by filtration and the precipitate resuspended in cold water (80 mL). The suspension was stirred at 0 °C for 30 minutes before the insoluble solid was removed by filtration. The precipitate was washed with cold water (3 x 20 mL) and dried under reduced pressure to yield **3** as a pale yellow solid (6.9 g, 46%). **^{1}H NMR** (500 MHz, DMSO) δ_H/ppm 8.85 (2H, s, RNH) **^{13}C NMR** (125 MHz, DMSO) δ_C/ppm 140.0 (2C, R*C*R), 160.4 (2C, RCO) **MS (ESI)**: m/z: 171.2 [M - H]$^-$

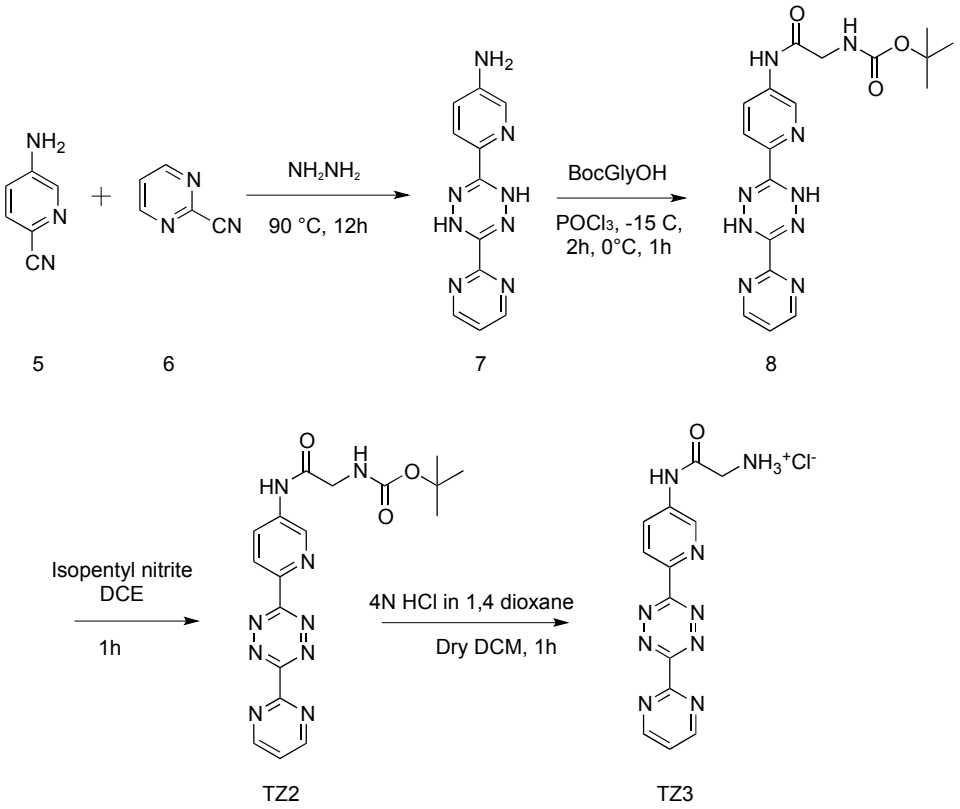
Synthesis of 4[1]

4 was synthesized following a literature procedure.[1] Thionyl Chloride (1.28 mL, 17.76 mmol, 3 eq) was added dropwise to a stirring solution of anhydrous methanol (7.5mL) at -30 °C under nitrogen. **3** (1 g, 5.88 mmol, 1 eq) in anhydrous methanol (7.5mL) was added to dropwise to the above solution at -30 °C under nitrogen. The reaction mixture was heated to 40°C and stirred for 2 hours before cooling to - 30 °C. The insoluble solid was removed by filtration and the filtrand was washed with cold MeOH (3 x 15mL) then cold diethyl ether (3 x 15mL) before drying under reduced pressure to yield crude product. The crude product was purified by flash chromatography eluting with a 0.5:95 mixture of MeOH:DCM to yield **4** as an orange solid (623.6 mg, 53%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H/ppm 7.45 (2H, s, RNH), 3.92 (6H, s, RCH_3) **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C/ppm 159.2 (2C, RCR), 138.2 (2C, RCO), 53.9 (2C, RCH_3) **MS (ESI)**: m/z (%): 222.8 $[M+Na]^+$

Synthesis of TZ1[2]

TZ1 was synthesized following a literature procedure with slight modification.[2] Isopentyl nitrile (1.14mL, 0.87 mmol, 3 eq) was added slowly to the solution of **4** (570 mg, 0.145 mmol, 1 eq) in 1, 2-Dicholoroethane (5mL). The reaction mixture was stirred for 3 hours before solvent was removed under reduced pressure to yield **TZ1** as a bright red solid in 57% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H/ppm 7.45 (2H, s, RNH), 3.92 (6H, s, RCH_3) **^{13}C NMR** (125 MHz, $CDCl_3$) δ_C/ppm 159.2(2C, R*C*R), 138.2(2C, RCO), 53.9 (2C, RCH_3)

Synthesis of TZ2 and TZ3



Synthesis of 7

7 was synthesised according to the literature procedure.[3] 5-amino-2-cyanopyridine (1.13 g, 9.52 mmol) and 2-cyanopyridine (1g, 9.52 mmol) were mixed with aqueous hydrazine (2.3 ml, 73.84 mmol) and heated for 12 h to 90ºC behind a blast shield. The mixture was allowed to cool to room temperature (rt), the orange precipitate was isolated by filtration, washed with cold water and dried under vacuum. The crude solid was dissolved in methanol, concentrated onto silica gel and was purified by chromatography on SiO2 (0% to 3% methanol in dichloromethane) as an orange solid (530 mg, 20%). ESI-MS (m/z): [M+H]+ 255.1.

Synthesis of 8

To a cold solution (-15 ºC) of N-(*tert*-butoxycarbonyl)glycine (530 mg, 2.28 mmol) and 7 (401 mg, 2.08 mmol) in pyridine (11 mL), phosphoryl chloride (218 µL,2.28 mmol) was added drop wise. The mixture was stirred for 2 hours at -15 ºC and then allowed to warm to room temperature and was stirred for an additional hour. The reaction mixture was quenched with water (1mL). The solvents were evaporated under reduced pressure. The crude solid was dissolved in methanol, concentrated onto silica gel and was purified by chromatography on

SiO_2 (0% to 3% methanol in dichloromethane) as an orange solid (588mg, 61%). **^{1}H-NMR** (500 MHz, d6-DMSO): δ 10.42 (s, 1H), 9.05 (s, 1H), 8.93 (d, 2H), 8.89 (s, 1H), 8.82 (m, 1H), 8.16-8.18 (dd, 1H), 7.94-7.96 (d, 1H), 7.62 (t, 1H), 7.13 (t, 1H), 3.79 (d, 2H), 1.41 (s, 9H) ppm; **^{13}C-NMR** (500 MHz, d6- DMSO): δ 169.14 (C), 157.66 (2 x CH), 155.98 (C), 155.91 (C), 145.64 (C), 145.55 (C), 141.40 (C), 138.95 (CH), 136.98 (C), 126.77 (CH), 122.08 (CH), 121.49 (CH), 78.14 (C), 43.82 (CH2), 27.34 (3 x CH3) ppm; **ESI-MS (m/z):** $[M+H]^+$ 410.3. Spectroscopic data matches literature.[3]

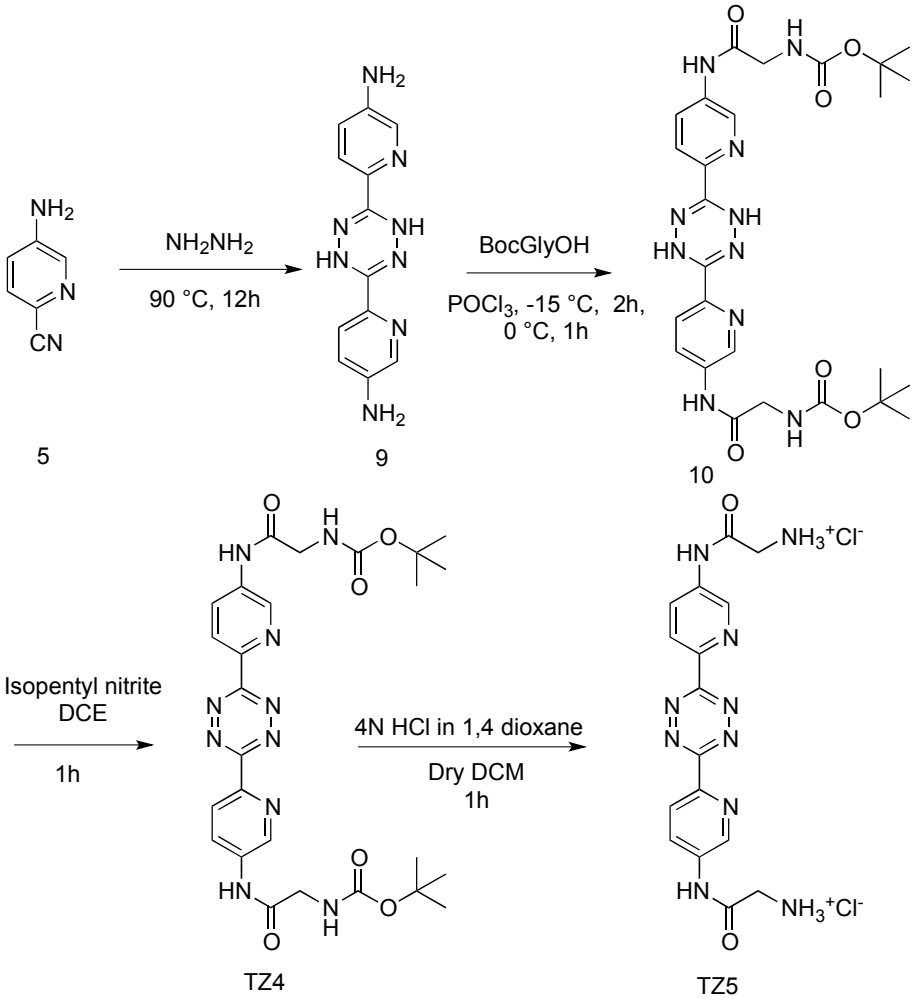
Synthesis of TZ2

To a stirred solution of **8** (600 mg, 1.46 mmol) in 1, 2-Dicholoroethane (35 ml) Isopentyl nitrite (0.39 ml, 2.9 mmol) was added dropwise at rt. The reaction was completed in 2 hour and the solvents were evaporated to afforded **TZ2** as a pink solid (525 mg, 88%). **1H-NMR** (500 MHz, d6-DMSO): δ 10.62 (s, 1H), 9.06 (d, J = 2.28, 1H), 8.94 (m, 1H), 8.65 (d, J = 8.68, 1H), 8.60 (d, J = 7.88, 1H), 8.43 (dd, J1 = 8.68, J2 = 2.36, 1H), 8.16 (dt, J1 = 7.76, J2 = 1.72, 1H), 7.73 (ddd, J1 = 7.76, J2 = 1.72, 1H), 7.18 (t, J = 6.0 Hz, 1H), 3.85 (d, J = 6.0 Hz, 1.42, s 9H) ppm; **13 C-NMR** (400 MHz, d6-DMSO): δ 169.5 (C), 163.0 (C), 162.7 (C), 156.0 (C), 150.6 (CH), 150.2 (C), 144.0 (C), 141.3 (CH), 138.2 (C), 137.8 (CH), 126.5 (CH), 126.3 (CH), 124.9 (CH), 124.2 (CH), 78.2 (CH2), 43.9 (C), 28.2 (CH3) ppm; ESI-MS (m/z): $[M+H]^+$ 409.6 Spectroscopic data matches literature.[3]

Synthesis of TZ3

To a stirred solution of **Tz2** (100 mg, 0.24 mmol) in dry dichloromethane (4 ml) a 4N HCl solution in dioxane (2 ml) was added and the reaction mixture was allowed to stir for 1 hour at rt., after which time complete consumption of the starting material was observed by LC-MS and TLC analysis. The solvent was removed and Et_2O was added and the precipitate recovered by filtration. The precipitate was dissolve in MeOH and the solvent was evaporated under reduce pressure to give compound Tz (85mg, 100%). The crude material was deemed pure enough for subsequent reactions. H-NMR (500 MHz, d6- DMSO): δ 11.79 (s, 1H), 9.13 (m, 3H), 8.62 (d, J = 4.4 Hz, 1H), 8.38-8.41 (m, br, 3H), 7.77 (t, J = 4.8 Hz, 1H), 3.88 (m, 2H) ppm; 13 C-NMR (500 MHz, d6-DMSO): δ 166.11 (C), 162.77 (C), 162.58 (C), 159.02 (C), 158.49 (2 x CH), 144.19 (C), 141.21 (CH), 137.90 (C), 126.61 (CH), 125.40 (CH), 122.99 (CH), 43.58 (CH2) ESI-MS (m/z): $[M+H]^+$ 309.2. Spectroscopic data matches literature.[3]

Synthesis of TZ5



Synthesis of 9

5-amino-2-cyanopyridine (5 g, 41.97 mmol) in aqueous hydrazine (10 ml, 321.99 mmol) was heated for 12 h to 90ºC behind a blast shield. The mixture was allowed to cool to room temperature (rt), the orange precipitate was isolated by filtration, washed with cold water and dried under vacuum. The crude solid was dissolved in methanol, concentrated onto silica gel and was purified by chromatography on SiO_2 (0% to 3% methanol in dichloromethane) as an orange solid (700 mg, 6%). ESI-MS (m/z): $[M+H]^+$ calcd for $C_{12}H_{13}N_8$ 269.12, found 269.

Synthesis of 10

To a cold solution (-15 ºC) of N-(*tert*-butoxycarbonyl)glycine (915 mg, 5.5 mmol) and 9 (700 mg, 2.61 mmol) in pyridine (16 mL), phosphoryl chloride (525 µL, 5.5 mmol) was added drop wise. The mixture was stirred for 2 hours at -15 ºC and then allowed to warm to room

temperature and was stirred for an additional hour. The reaction mixture was quenched with water (1mL). The solvents were evaporated under reduced pressure. The crude solid was dissolved in methanol, concentrated onto silica gel and was purified by chromatography on SiO_2 (0% to 3% methanol in dichloromethane) as an orange solid (200mg, 13%). ESI-MS (m/z): $[M+H]^+$ calcd for $C_{26}H_{35}N_{10}O_6$ 582.27, found 581.2.

Synthesis of TZ*4*

To a stirred solution of 10 (200 mg, 0.34 mmol) in 1, 2-Dicholoroethane (15 ml) Isopentyl nitrite (137 µl, 1.03 mmol) was added dropwise at rt. The reaction was completed in 1 hour and the solvents evaporated. Column chromatography on SiO2 (0% to 2% methanol in dichloromethane) afforded TZ4 as a pink solid (180 mg, 90%). ESI-MS (m/z): $[M+H]^+$ calcd for $C_{26}H_{32}N_{10}O_6$ 580.25, found 580.8.

Synthesis of TZ5

To a stirred solution of TZ4 (180 mg, 0.31 mmol) in dry dichloromethane (14 ml) a 4N HCl solution in dioxane (8 ml) was added and the reaction mixture was allowed to stir for 1 hour at rt., after which time complete consumption of the starting material was observed by LC-MS and TLC analysis. The solvent was removed and Et_2O was added and the precipitate was recovered by filtration. The precipitate was dissolve in MeOH and the solvent was evaporated under reduce pressure to give compound TZ5 (180 mg, 100%). The crude material was deemed pure enough for subsequent reactions. ESI-MS (m/z): $[M+H]^+$ 380.6.

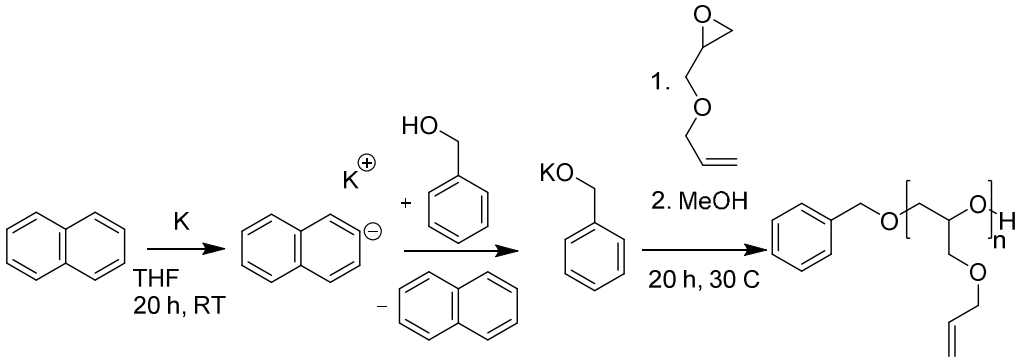
Synthesis of TZ7

TZ7 was prepared by the literature procedure.[4] 4-Cyanobenzoic acid (7.0 g, 48 mmol, 1.0 eq) and pyrimidin-2-carbonitrile (5 g, 48 mmol, 1.0 eq) were suspended in ethanol (20 mL), and hydrazine monohydrate (11.5 mL, 238 mmol, 5.0 eq) was added dropwise and the mixture was refluxed overnight. After cooling, the precipitate was removed by filtration and washed with small volumes of ethanol. To remove the bis(pyrimidin-2-yl)-1,2,4,5-dihydrotetrazine byproduct, the solid was stirred in acetone (30 mL) and filtered. This was repeated. The filtered solid was suspended in acetic acid (115 mL) and oxidised by the slow addition of isopentyl nitrite (4.5 mL, 33 mmol, 1.5 eq). After stirring overnight, diethyl ether (190 mL) was added to precipitate the product. After filtration, the crude product was purified by flash chromatography (1% acetic acid in hexane:DCM 3:7, followed by 1% acetic acid in DCM, followed by 10% methanol and 1% acetic acid in DCM) to yield 4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl) benzoic acid (1.8 g, 6.4 mmol, 13%) as a purple solid. 1H NMR (500

MHz, DMSO): δH 9.21 (2H, d, J = 4.9 Hz, CbHAr), 8.27-8.24 (2H, m, CgHAr), 8.19-8.16 (2H, m, ChHAr), and 7.84 (1H, d, J = 4.9 Hz, CaHAr). 13C NMR (125 MHz, CDCl3): δC 166.6 (C(O)O), 163.1 (CNN), 162.8 (CNN), 159.0 (qC), 158.4 (CHAr), 132.5 (qC), 130.2 (2 × CH), 129.8 (qC), 128.3 (2 × CH), and 122.3 (CH). MS (ESI) m/z:279.1 $[(M-H)]^-$.

S3. Synthesis of PAGE polymer

General procedure for synthesis of Poly (allyl glycidyle ether)



In a dry flask naphthalene (12.8 mg, 0.1 mmol) was dissolved in dry THF (2.5 mL). Potassium (4 mg, 0.1 mmol) was added and the suspension was stirred for 20 h at room temperature under nitrogen atmosphere.

Slowly the desired amount of initiator stock solution was added to the benzyl alcohol. The monomer allyl glycidyl ether was given to the solution under nitrogen atmosphere. The neat was stirred for 20 h under nitrogen atmosphere at 30 C. The reaction was quenched with methanol (0.1 ml).

^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.48 – 3.71 (m, 5H, -C<u>H</u>$_2$-C<u>H</u>(C<u>H</u>$_2$-O-CH$_2$-CH=CH$_2$)-O-); 4.00 (d, 2H, CH$_2$-O-C<u>H</u>$_2$-CH=CH$_2$); 4.56 (s, 2H, Ph-C<u>H</u>$_2$-O-); 5.18 and 5.29 (dd, 2H, CH$_2$-O-CH$_2$-CH=C<u>H</u>$_2$); 5.91 (m, 1H, CH$_2$-O-CH$_2$-C<u>H</u>=CH$_2$); 7.30 (m, 1H, <u>Ph</u>-CH$_2$-O); 7.34 (m, 4H, <u>Ph</u>-CH$_2$-O).

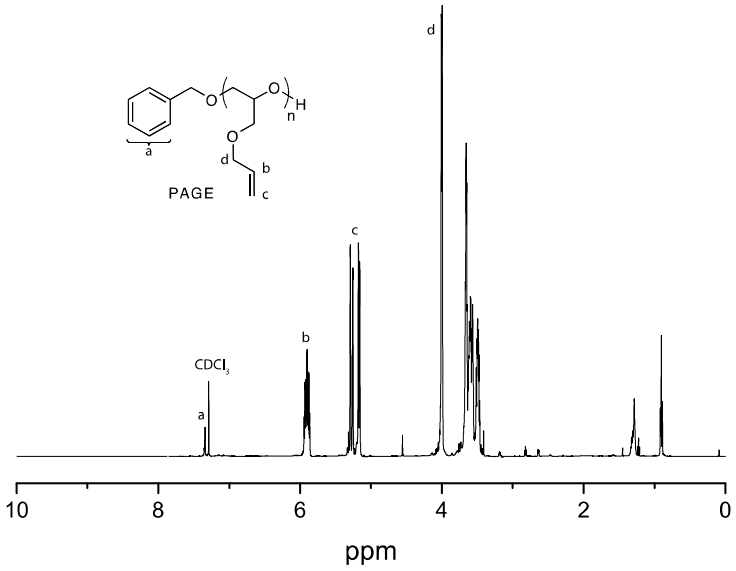


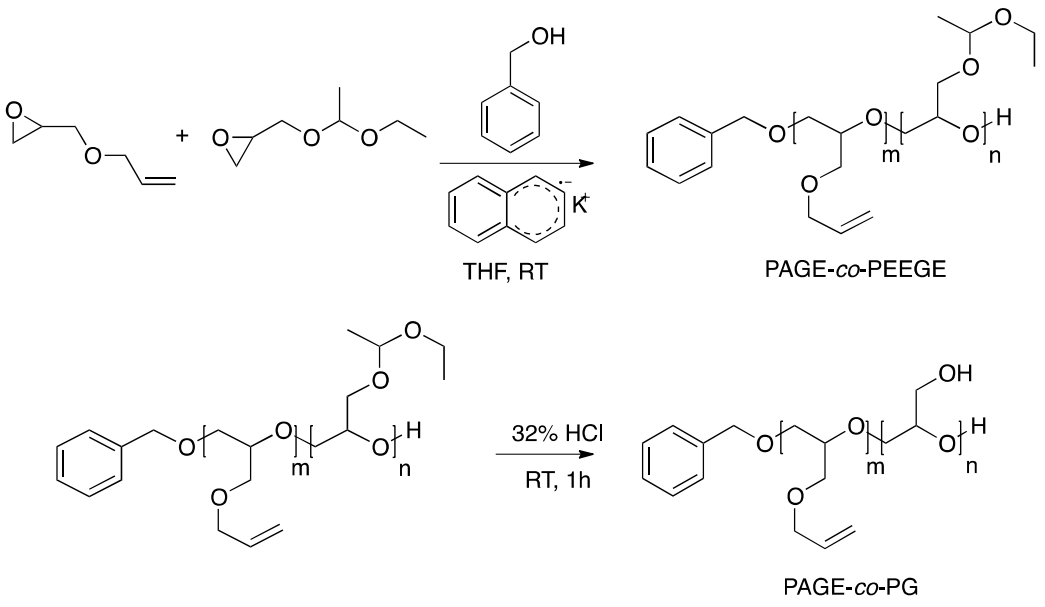
Figure S1. Proton NMR spectrum of PAGE2 in $CDCl_3$.

General procedure for synthesis of poly(allyl glycidyl ether-*co*-glycidol)

To synthesis the polymer, the round bottom flask were dried under vacuum then refilled with nitrogen for 5 times. Under a nitrogen atmosphere, 81.7 mg allyl glycidyl ether (0.716 mmol), 418.3 mg ethoxy ethyl glycidyl ether (EEGE) (2.863 mmol), 11.1 mg benzyl alcohol (0.103 mmol) were syringed in and stirred at 0 °C for 10 min. Freshly prepared potassium naphthalenide THF solution was carefully syringed in until a dark green colour persisted in solution, indicating the deprotonation of all alcohol. The reaction solution was then heated up to 30 °C and stirred overnight. The resulting polymer was washed with water and extracted with DCM, dried with sodium sulphate anhydride. After evaporating the solvent the polymer was obtained quantitatively as a dark brown liquid. (Mn = 13.7 kDa (NMR); PDI = 1.30 (GPC))

Synthesis of PAGE-*co*-PG

200 mg polymer was dissolved in 10mL THF and stirred for 5 min. 2.5 mL concentrated hydrochloric acid was slowly added (pH = 0.4) and stirred for 1 h. The solvent was evaporated and the resulting polymer was purified by dialysis (size 3500 Daltons). The polymer was yield quantitatively as a yellow liquid. (Mn = 9.96 kDa (NMR); PDI = 1.31 (GPC))



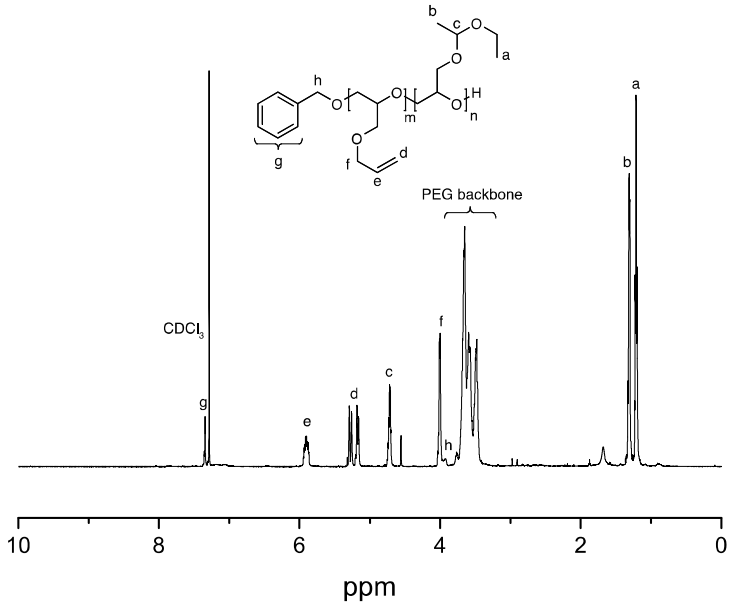


Figure S2. Proton NMR spectrum of PAGE-*co*-PEEGE in $CDCl_3$.

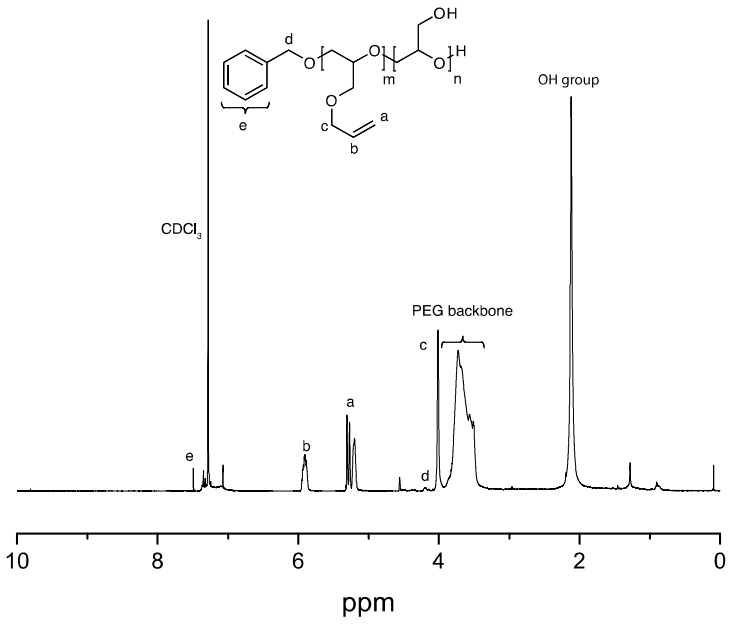


Figure S2. Proton NMR spectrum of PAGE-*co*-PG.

S4. Kinetic studies

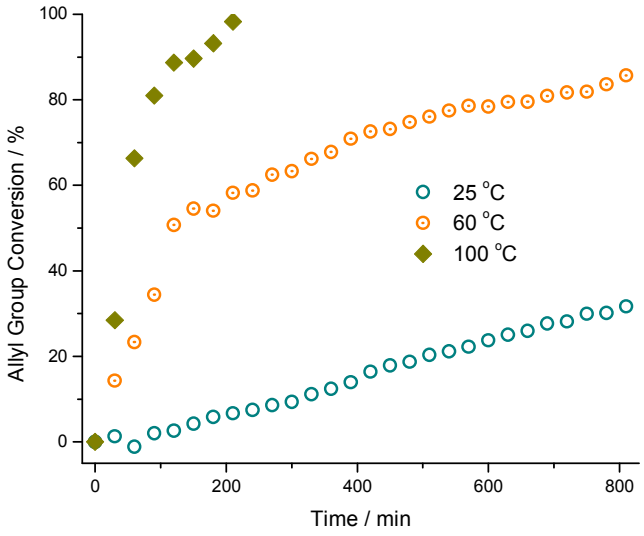


Figure S3. Reaction progress of TZ7 and AGE in DMSO at different temperatures: (○) 25°C; (⊙) 60°C; (◆) 100°C. Reaction condition: [AGE]/[TZ] = 1 : 4 using 14mM AGE.

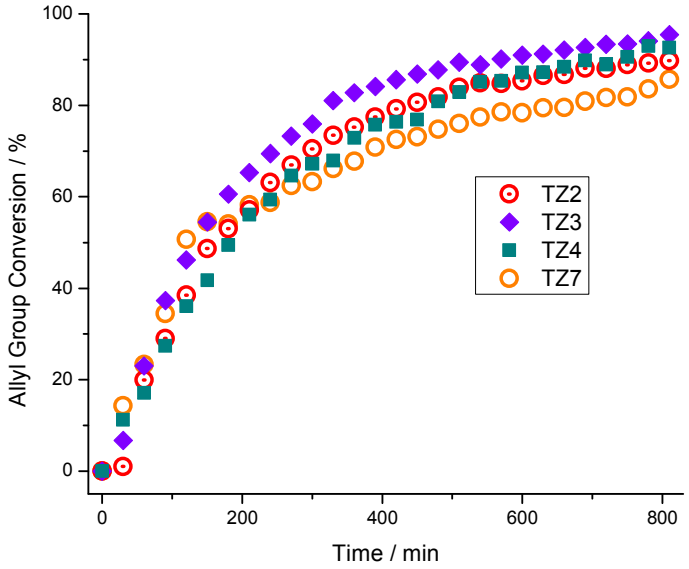


Figure S4. Reaction progress of tetrazine (⊙) TZ2; (◆) TZ3; (■) TZ4 and (○) TZ7 and AGE in [D6]DMSO at 60 °C. Reaction condition: [AGE]/[TZ] = 1 : 4 using 14mM AGE.

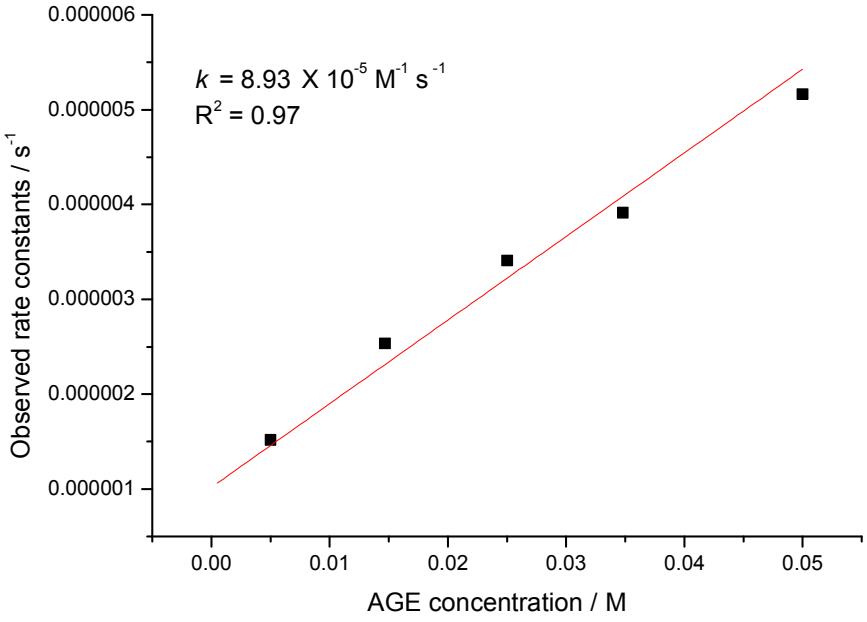


Figure S5. Observed rate constants k for $_{inv}$DA of AGE with TZ2.

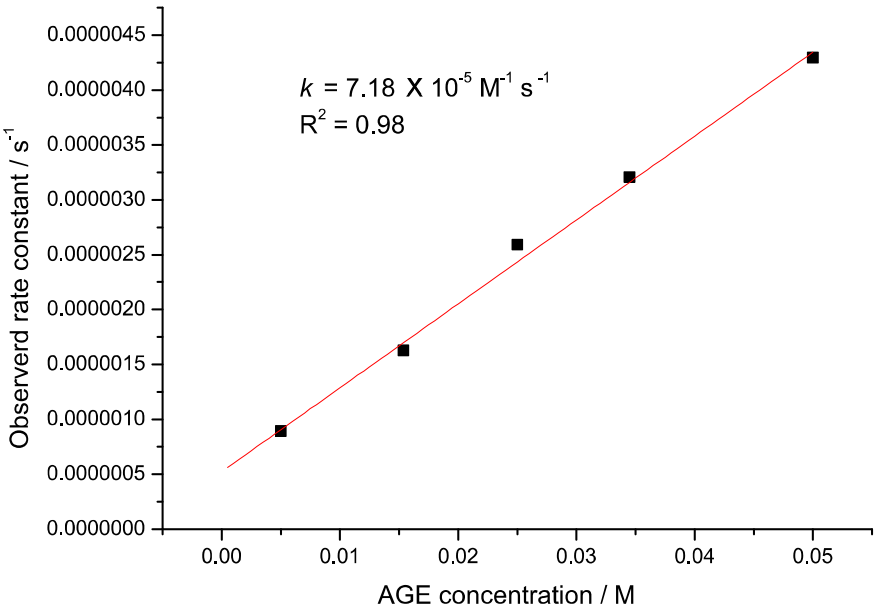


Figure S6. Observed rate constants k for $_{inv}$DA of AGE with TZ6.

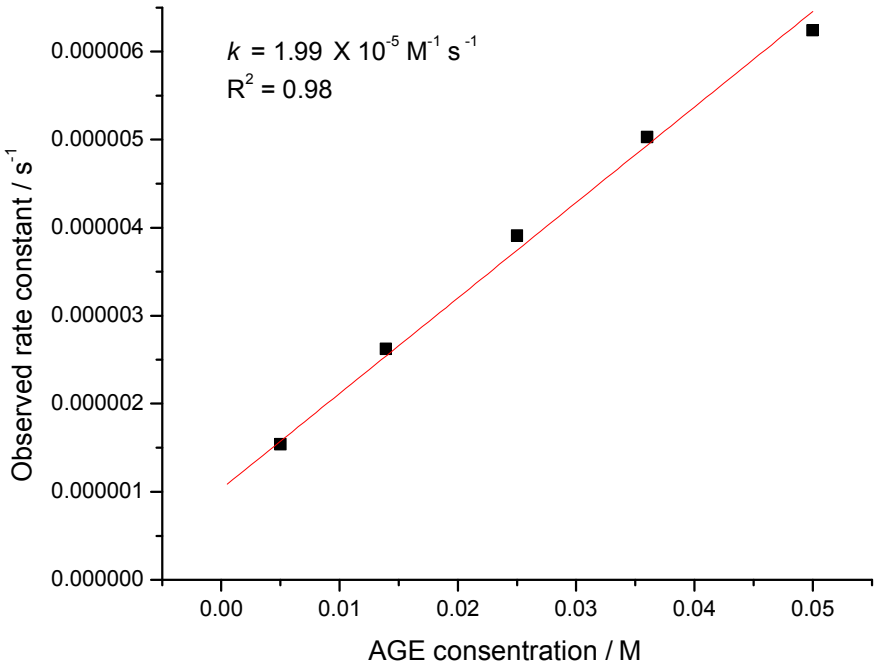


Figure S7. Observed rate constants k for $_{inv}$DA of AGE with TZ7.

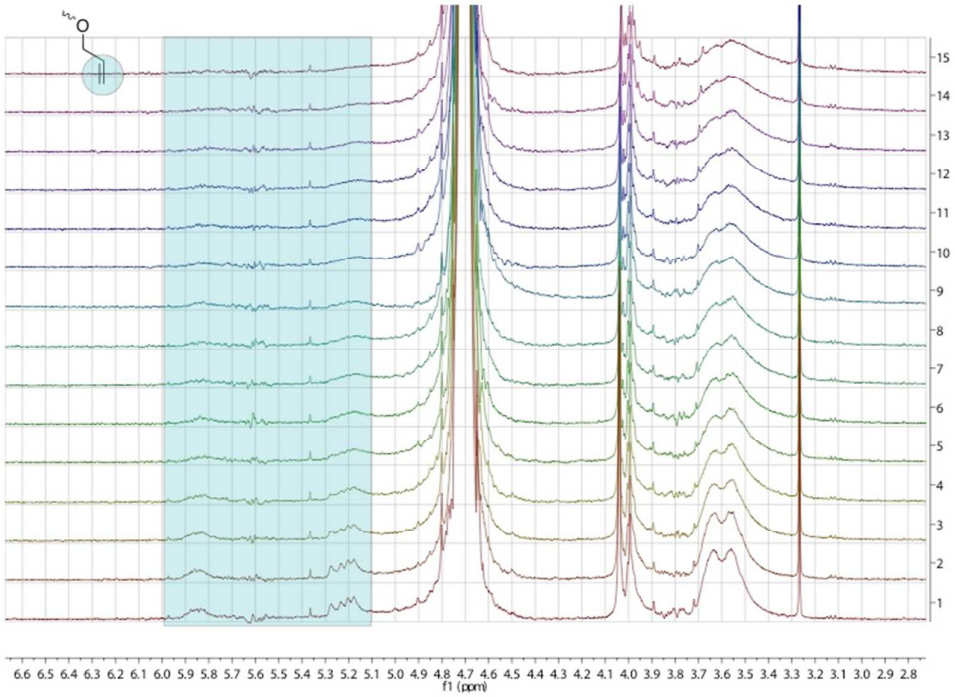


Figure S8. ^{1}H NMR analysis in D_2O at 25 ºC of PAGE-*co*-PG (alkene units 14 mM) and TZ3 (4 equiv.). Spectra were recorded at 30 min intervals from t = 0 (spectrum 1).

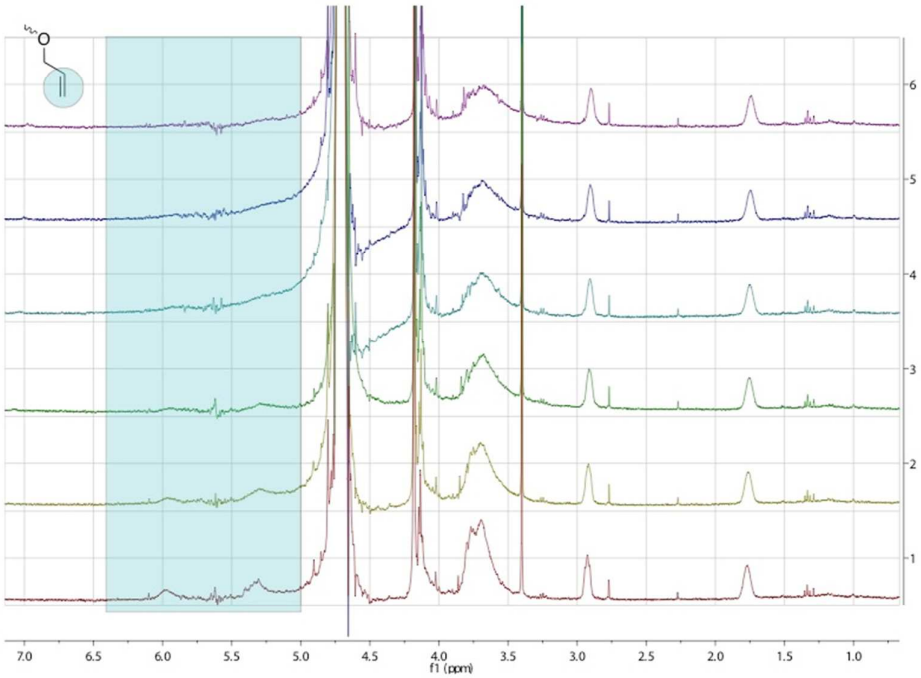


Figure S9. ^{1}H NMR analysis in D_2O at 37 ºC of PAGE-*co*-PG (alkene units 14 mM) and TZ3 (4 equiv.). Spectra were recorded at 30 min intervals from t = 0 (spectrum 1).

S5. References

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