Poly(vinyl alcohol)–*graft*-poly(ethylene glycol) resins and their use in solid-phase synthesis and supported TEMPO catalysis

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Materials and Instruments. Chemicals were used without further purification if not specially mentioned. Acetic anhydride (99% from Aldrich) was dried and distilled from sodium and stored tightly sealed. Pyridine was dried and distilled from sodium hydroxide. Dimethyl sulfoxide (DMSO) and benzene were dried and distilled from calcium hydride. Tetrahydrofuran (THF) was dried and distilled from sodium in the presence of benzophenone after the solution turned dark blue. Potassium naphthalene was prepared in dry THF from naphthalene and potassium at a concentration of 0.45 M (titrated with a standard hydrochloric acid solution using phenolphthalein as an indicator). Sodium hypochlorite (10-13% available chlorine, purchased from Aldrich) was diluted in a 0.4 M buffer with $KHCO_3$ at pH = 9.1 (0.5 g $KHCO_3$ in 10 mL 0.4 M bleach solution). N-(Boc)Aminoethanol was prepared from aminoethanol (Fluka) using $(Boc)_2O$ (Aldrich) in THF.[1] (2*R*)-2-[*N*-(Boc)Amino]-3-methyl-butane-1,3-diol was prepared as reported.[2] Treatment of pyrrolidin-3-ol with benzyl chloroformate (Cbz-Cl) and Na_2CO_3 in a biphasic mixture of ether-H_2O gave the known *N*-Cbz pyrrolidin-3-ol in 98% yield.[3] Lithocholic acid methylester was prepared by reacting lithocholic acid (Sigma) with methanol.[4] Merrifield (2% DVB crosslinking, 100-200 mesh, 2.0 mmol/g loading) and Wang resins (1% DVB crosslinking, 100-200 mesh, 1.0 mmol/g loading) were purchased from Fluka, and Tenta Gel S OH resin (90 μm, 0.2-0.3 mmol/g loading) from Rapp Polymere GmbH.

Magic angle spinning (MAS) ^{1}H NMR experiments were performed on a Bruker Avance 600 NMR spectrometer (600 MHz) equipped with a 4-mm HR-MAS ^{1}H probe with spin rate of ~ 6K Hz. Around 5 mg of beads was transferred into a Nano NMR tube and 40 μL of DMF-d_7 was then added. The spectra were recorded at room temperature and with a presaturation at 3.65 ppm. ^{1}H and ^{13}C NMR spectra of the PVA-PEG resins

and small molecular weight products were recorded on a Bruker Avance 300 (or 400) spectrometer operating at 300 MHz (or 400 MHz) for ^{1}H and 75 MHz (or 100 MHz) for ^{13}C. The optical images of resins (swollen in water) were recorded under an Axioskop 2 Plus (Zeiss) optical microscope. Liquid chromatography/mass spectrometry (LC/MS) traces were obtained on a coupled GILSON LC-ThermoFinnigan MSQ instrument equipped with a Prevall Allteck C18 (5 micron, 50 × 4.6 mm) column. The column condition: 20-80%B, A = H_2O/1%TFA, B = ACN/0.1%TFA, Flow = 0.5 mL/min, injection vol: 10 μL, column: C-18 50 × 4.6mm, UV wavelength = 214 nm. MS conditions: scan 100-500, cone voltage 30 kV, temperature 400 ^{o}C, mode (polarity) positive. UV-visible data was obtained on a UV-visible spectrophotometer (Cary 300 Bio, Varian).

Calculation of theoretical loading and the number of ethylene oxide units. In principle, no net hydroxyl group loss occurred during the anionic polymerization. The theoretical loading of PVA-PEG resin may thus be calculated by multiplying the loading of the PVA beads (L_0) by a ratio of the bead weights before (W_0) and after (W_1) the anionic polymerization:

$$L_t = \frac{L_0 \times W_0}{W_1} \qquad (1)$$

The theoretical hydroxyl group loading L_t for the PVA-PEG was determined using titrated loading L_0 of hydroxyl group on the crosslinked PVA resin (15 mmol/g). The number of ethylene oxide units attached on the PVA beads (n) can be calculated according to the weight increase and the amount of reactive species in the anionic polymerization:

$$n = \frac{W_1 - W_0}{44(C_i \times V_i)} \qquad (2)$$

where C_i and V_i are the concentration and the volume of the potassium naphthalene (initiator) solution in THF, respectively, and 44 is the molecular weight of the ethylene oxide repeating unit.

Loading obtained by titration. The loading of hydroxyl groups on the polymer beads were determined by titrating the excess acetic acid formed upon the acetylation of the hydroxyl groups on the resin after the addition of acetic anhydride.[5] About 100 mg of beads (PVA **1** or PVA-PEG **2**) were placed into a 20 mL flask, heated with 0.5 mL of

acetic anhydride and 5.0 mL of pyridine, stirred for 12 h at 60 ^{o}C then treated with 1 mL of water to hydrolyze the excess acetic anhydride into acetic acid. The mixture was completely transferred into an Erlenmeyer flask for the titration in the presence of resin. An excess aqueous solution of 0.4481 N NaOH (25 mL) was added to react with the acetic acid, then the excess of NaOH was titrated with a 0.1918 N HCl aqueous solution using phenolphthalein as an indicator. A blank titration was performed in the same way to avoid systematic errors.

Swelling. A glass capillary was used to transfer solvent to the beads (roughly 3 mg), which were weighed on a poly(tetrafluoroethylene) slice to avoid excess solvent adhering onto the matrix. The beads absorbed solvent from the capillary until equilibrium was reached between the solvent in the capillary and in the swollen bead. This procedure was repeated several times over a period of 10 minutes to allow the full swelling of the beads. The beads saturated with solvent were then weighed using a microbalance, and the weight gain was converted into the volume of solvent retained per gram of polymer.

Synthesis of Z-Gln-Gly-NH_2 4. Swollen PVA-PEG_5 resin **2** (35 mg) in 1.5 mL of DMF was treated with *N*-(Fmoc)glycine (309 mg, ~500 mol%), DIC (168 μL, ~500 mol%) and DMAP (2.5 mg, ~10 mol%), stirred overnight at room temperature, filtered and successively rinsed with 15 mL of DMF, 25 mL of DCM, and 10 mL of diethyl ether. The loading of the Fmoc-glycine was determined to be 1.1 mmol/g by UV measurement at 301 nm of the dibenzofulvene liberated by treatment of the resin with piperidine in DMF.[7] The swollen acylated resin was stirred for 2 h with 3 mL of acetic anhydride/pyridine solution (40:60) to acetylate the residual alcohol groups. The resin was successively washed with 15 mL of DMF and 25 mL of DCM. Fmoc cleavage was carried out with 3 mL of piperidine/DMF solution (20:80) for 1 h. The deprotection was monitored by a positive Kaiser test. [6]

The deprotected resin was treated with *N*-(Cbz)-L-glutamine (145 mg, ~250 mol%), HOBt (70 mg, 250 mol%) and DIC (84 μL, 250 mol%) in DMF (1.5 mL). The mixture was shaken overnight at room temperature and the dipeptide resin **3** was filtered and washed successively with 15 mL of DMF, 50 mL of DCM, and 10 mL of diethyl ether. Complete coupling was indicated by a negative Kaiser test.

Dipeptide resin **3** was treated with a saturated methanol solution of ammonia at room temperature overnight. After filtration, the resin was washed with methanol, and the filtrate and washings were combined and evaporated to yield a white solid. The amido-peptide (Z-Gln-Gly-NH_2) **4** was isolated by acetone trituration in 98% yield (14 mg from 35 mg of resin with a loading of 1.1 mmol/g) and in 92% purity (LC-MS analysis). ^{1}H NMR (300 MHz, DMSO) δ (ppm): 8.12 (t, 1H, J = 5.4 Hz, NH Gly), 7.56 (d, 1H, J = 7.4 Hz, NH Gln), 7.36 (m, 7H, CH aromatic benzyl and NH_2), 7.10 (s, 1H, NH_2 Gln), 6.78 (s, 1H, NH_2 Gln), 5.02 (s, 2H, CH_2 benzyl), 3.96 (m,1H, CH Gln), 3.63 (d, 2H, J = 5.5 Hz, CH_2 Gly), 2.10 (m, 2H, CH_2 γ Gln), 1.87 (m, 1H, CH_2 β Gln), 1.74 (m, 1H, CH_2 β Gln); ^{13}C NMR (75 MHz, CD_3OD) δ(ppm): 162.1, 161.3, 160.4, 146.8, 129.1, 121.3, 120.8, 120.7, 63.5, 53.3, 41.7, 32.1, 28.4; Anal. Calcd for $C_{15}H_{20}N_4O_5$: C, 53.56; H, 5.99; N, 16.66. Found: C, 52.62; H, 6.09; N, 16.68; $[\alpha]^{20}_D$: -18^o (c 0.0025, 50% aq. EtOH) [7]; LCMS: t_R 3.19 min, m/z 337.0 $[M+H]^+$. HR-MS: m/z calcd. for $C_{15}H_{21}N_4O_5$ $[M+H]^+$: 337.15064; Found $[M+H]^+$: 337.15107.

Methanesulfonate PVA-PEG resin 5. To a suspension of PVA-PEG resin **2** (0.5 g, 6.2 mmol/g for PVA-PEG_5 and 1.55 g, 2.0 mmol/g for PVA-PEG_20, respectively) and Et_3N (3.3 mL, ~1000 mol%) in DCM at 0 ^{o}C, methanesulfonyl chloride (3.48 g, ~1000 mol%) was added dropwise. The ice bath was removed and the reaction mixture was stirred overnight at room temperature. The resin was filtered, washed with DCM, THF and diethyl ether three times each, respectively, and dried under vacuum, to yield a light yellow methanesulfonate resin **5**. FTIR: 1352, 1174 cm^{-1}.

Azide PVA-PEG resin 6. A suspension of methanesulfonate resin **5** (~3 mmol) and NaN_3 (2.0 g, ~1000 mol%) in DMSO (40 mL) was heated and stirred overnight at 70 ^{o}C, filtered and washed with DMF, DCM, THF and diethyl ether, for 3 times each, respectively, and dried under vacuum to yield yellow azide resin **6**. The FTIR spectrum showed a strong adsorption at 2106 cm^{-1} for N_3. Azide loadings of resin **6** were determined to be 2.42 and 1.25 mmol/g by nitrogen elemental analysis for the resins with PEG chains of 5 and 20 units, respectively.

Preparation of 4-propargyloxy-TEMPO 7. To a solution of 4-hydroxy-TEMPO (1 g, 5.8 mmol) and Bu_4NBr (93.5 mg, 5 mol%) in THF (20 mL), NaH (0.334 g, 50% dispersion in mineral oil, 120 mol%) was added portion-wise at room temperature under

inert N_2 atmosphere. After stirring for 1 h at room temperature, the mixture was treated dropwise with an 80 wt% solution of propargyl bromide in toluene (3.45 g, 400 mol%) and the reaction mixture was heated at reflux overnight, cooled, filtered and concentrated under vacuum. The residue was purified by flash chromatography[8] (silica gel, 100% - 90% hexane in EtOAc) to give propargyl ether **7** as an orange-red crystalline solid (1.03 g, 84%): mp = 63-64 ^{o}C; FTIR: 3232, 2111, 1085 cm^{-1}. Anal. Calcd for $C_{12}H_{20}NO_2$: C, 68.57; H, 9.52; N, 6.67. Found: C, 68.73; H, 9.73; N, 6.50. LC/MS: t_R 12.95 min, ESI, m/z 211.0 $[M+H]^+$.

Preparation of 4-propargyloxy-benzyl alcohol 8. To a solution of 4-hydroxyl-benzyl alcohol (2.5 g, 20 mmol) in acetonitrile (50 mL), K_2CO_3 (3.96 g, 200 mol%) was added at room temperature. After stirring for 1 h, the mixture was treated dropwise with an 80 wt% solution of propargyl bromide in toluene (3.27 g, 110 mol%) and the reaction mixture was heated to 50 ^{o}C for 48 h, cooled, filtered and concentrated under vacuum. The residue was purified by chromatography with an eluent of 100% to 30% hexane in AcOEt. Evaporation of the collected fractions gave propargyl ether **8** as a yellow liquid (3.12 g, 95.5%): ^{1}H NMR ($CDCl_3$) δ (ppm): 7.28 (d, 2H, Ph-H), 6.96 (d, 2H, Ph-H), 4.69 (d, 2H, $PhCH_2$), 4.57 (s, 2H, CCH_2O), 2.54 (t, 1H, OH), 2.39 (s, 1H, CH); ^{13}C NMR (CD_3OD) δ (ppm): 157.4, 134.53, 128.97, 115.34, 78.97, 76.04, 65.10, 56.24. Anal. Calcd for $C_{10}H_{10}O_2$: C, 74.07; H, 6.17. Found: C, 73.64; H, 6.03.

Preparation of TEMPO resin 9. Azido-PVA-PEG_5 resin **6** (200 mg, 2.42 mmol/g) was agitated with 4-propargyloxy-TEMPO **7** (510 mg, ~500 mol%), CuI (9.3 mg, 10 mol%), DIPEA (0.48 mL, 525 mol%) and Ph_3P (14 mg, 10 mol%) in DMF (3 mL) at room temperature for 24 h. The suspension was filtered and the resin was rinsed successively with pyridine (3 × 20 mL), DCM (3 × 20 mL), THF (3 × 20 mL) and diethyl ether (3 × 20 mL), and dried under vacuum to yield a brown TEMPO resin **9**. Nitrogen elemental analysis indicated a loading of 1.85 mmol/g for TEMPO resin **9** with PEG chains of 5 units. FTIR: 1467 cm^{-1} of nitroxyl moiety and 3120 cm^{-1} of =C-H stretching band.

Preparation of Wang linker resin 10. Azido-PVA-PEG_20 resin **6** (400 mg, 1.25 mmol/g) was agitated with 4-propargyloxy-benzyl alcohol **8** (405mg, ~500 mol%), CuI (9.6 mg, 10 mol%), DIPEA (0.5 mL, 525 mol%) and Ph_3P (14 mg, 10 mol%) in DMF (3 mL) at room temperature for 24 h. The suspension was filtered and the resin was rinsed

successively with pyridine (3 × 20 mL), DCM (3 × 20 mL), THF (3 × 20 mL) and diethyl ether (3 × 20 mL), and dried under vacuum to yield a brown Wang linker resin **10**. The Wang linker loadings were determined by nitrogen elemental analysis to be 1.08 mmol/g for resins **10** with PEG chains of 20 units. The loading of hydroxyl groups was also measured to be 1.08 mmol/g by acylation of the resin with Fmoc-glycine and detection of the fluorescence of the fulvelene adduct after Fmoc removal. [9] The FTIR spectrum of Wang linker resin **10**: 1614, 1598, 1514, 1465 cm^{-1} and on-bead HR/MAS ^1H NMR in DMF-d_7 δ (ppm): 8.43 (s, 1H), 7.50 (d, 2H, J = 6.5 Hz), 7.22 (d, 2H, J = 6.0 Hz), 5.38 (s, 2H), 5.17 (bs, 1H), 4.81 (s, 2H), 4.74 (s, 2H), 4.10 (s, 2H), 3.68-3.80 (m, PEG-H), 1.28-1.92 (m, PVA-H)

General procedure for alcohol oxidation. A stoppered plastic tube-shaped reactor fitted with a Teflon filter was charged with 1 mL of a solution of alcohol **11** in DCM (0.4 M) and milligrams of PVA-PEG_5 TEMPO resin **9** (1.85 mmol/g, 3 mol% for primary alcohol and 10 mol% for secondary alcohol). The solution at room temperature was treated with 1.25 mL of bleach (0.4 M) buffered with $KHCO_3$ at pH 9.1 containing KBr (0.5 M, 0.08 mL), shaken at room temperature for 30 to 240 minutes, and monitored by TLC. The solution was filtered and the phases were separated. The aqueous phase was washed with DCM (2 × 1 mL). The combined organic phase was dried over Na_2SO_4 and concentrated to give the aldehyde or ketone **12**.

Reuse of TEMPO-PVA-PEG resin 9. Benzyl alcohol was used in a recycle oxidation study of resin **9**. Into a stoppered plastic tube-shaped reactor fitted with a Teflon filter, 1 mL of benzyl alcohol solution in DCM (0.4 M) and 7 mg of PVA-PEG TEMPO resin (3 mol%, 1.85 mmol/g) were added together with 1.25 mL of bleach (0.4 M) buffered with $KHCO_3$ at pH 9.1 containing KBr (0.5 M, 0.08 mL). The reaction was shaken vigorously at room temperature and monitored by TLC every 5 min. After a complete oxidation cycle was observed by the disappearance of the benzyl alcohol spot on TLC (R_f 0.28, hexane/ethyl acetate 4:1) and the formation of a single darker benzaldehyde spot under UV lamp (R_f 0.53, hexane/ethyl acetate 4:1), the solution was filtered and the phases were separated. The resin was washed with DCM (2 × 1 mL) and used again in the next oxidation cycle without any further treatment. Fresh portions of alcohol, bleach and KBr were charged into the reactor with the recycled TEMPO resin. The reaction mixture was

shaken at room temperature until TLC indicated the complete oxidation and the resin was filtered, washed and retreated with fresh alcohol as described above.

Characterization of aldehydes and ketones 12. 12a) Benzaldehyde: quantitive conversion by TLC; yield: 98%; R_f 0.53 (hexane/ethyl acetate 4:1); ^{1}H NMR ($CDCl_3$) δ (ppm) 10.06 (s, 1H), 7.92 (d, 2H, *J* = 7.34 Hz), 7.66 (t, 1H, *J* = 7.80 Hz), 7.57 (t, 2H, *J* = 7.80 Hz). **12b**) N-Boc glycinal: quantitive conversion by TLC; yield: 87%; R_f 0.45 (ethyl acetate/methanol 9:1); ^{1}H NMR ($CDCl_3$) δ (ppm): 9.68 (s, 1H), 5.21 (s, 1H), 4.1 (d, 2H, *J* = 4.61), 1.48 (s, 9H). **12c**) (2*R*)-2-[*N*-(Boc)Amino]-3-methyl-3-hydroxy-butanal: quantitive conversion by TLC; yield: 90%; R_f 0.55 (hexane/ethyl acetate 1:2); $[\alpha]^{20}_D$ 103^o (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm): 9.82 (s, 1H), 5.46 (s, 1H), 4.28 (d, 1H, *J* = 5.83), 2.74 (s, 1H), 1.48 (s, 9H), 1.36 (s, 3H), 1.34 (s, 3H). **12d**) 1-N-Cbz-3-pyrrolidinone: quantitive conversion by TLC; yield: 86%; R_f 0.46 (hexane/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.34-7.39 (m, 5H), 5.19 (s, 2H), 3.87 (t, 2H, *J*=7.75 Hz), 3.83 (s, 2H), 2.62 (t, 2H, *J*=7.75 Hz); ^{13}C NMR (CD_3OD) δ (ppm): 210.8, 155.3, 136.7, 129.0(2 C), 128.7, 128.5(2 C), 67.7, 52.8, 43.1, 37.2. **12e**) 5-α-Cholestan-3-one: 96% conversion by ^{1}H NMR; isolated yield: 80% after column chromatography: gradient of 5-10% EtOAc in hexanes; R_f 0.56 (hexane/ethyl acetate 4:1); ^{1}H NMR ($CDCl_3$): 0.70 (s, 3H), 0.88 (d, 3H), 0.89 (d, 3H), 0.93 (d, 3H), 1.03(s, 3H), 1.04-2.4 (m, 31 H); ^{13}C NMR ($CDCl_3$) δ (ppm): 212.6, 56.7, 56.6, 54.2, 47.1, 45.2, 43.0, 40.3, 39.9, 39.0, 38.6, 36.6, 36.2, 36.1, 35.8, 32.1, 29.4, 28.5, 28.4, 24.6, 23.2, 23.0, 21.9, 19.0, 12.5, 11.8. **12f**) Methyl 3-oxo-5-β-cholan-24-oate: 99% conversion by ^{1}H NMR; Isolated yield: 87% after column chromatography: gradient of 0-5% ethyl acetate in hexane; R_f 0.38 (hexane/ethyl acetate 4:1); ^{1}H NMR ($CDCl_3$): 0.70 (s, 3H), 0.94 (d, 3H), 1.04(s, 3H), 1.11-2.7 (m, 28 H), 3.69 (s, 3H); ^{13}C NMR ($CDCl_3$): 213.8, 175.2, 56.8, 56.4, 51.9, 44.8, 43.2, 42.8, 41.1, 40.4, 37.6, 37.2, 35.9, 35.3, 35.8, 31.5, 31.4, 28.6, 27.0, 26.1, 24.6, 23.0, 21.6, 18.7, 12.5.

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