Isotactic polyethylenimines induce formation of L-amino acids in transamination

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Supporting Information.

1. General information.

All reactions were carried out under an atmosphere of argon in flame- or oven-dried glassware with magnetic stirring unless otherwise indicated. Solvents and organic reagents were purchased from Aldrich and used without further purification unless otherwise mentioned. Methyl tosylate was purchased from TCI America, and used after distillation. Merck pre-coated 0.25 mm silica plates containing a 254 nm fluorescence indicator were used for thin-layer chromatography. Flash chromatography was performed on 230–400 mesh silica (Silica Gel 60) from EM Science. Analytical HPLC was run on a Waters 600 liquid chromatography equipped with a pumping system, an autosampler (Waters 717 plus), and a diode-array UV–vis detector (Waters 2996). Sunfire C-18 reverse-phase analytical columns (particle size 5 μm, 4.6×150 mm) were used as solid phase. NMR spectra were obtained on a Bruker DPX 300 MHz spectrometer. Infrared spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR spectrometer. GPC measurements were performed in Knauer GPC system with Knauer K-2301 refractive index detector equipped with a Spark Holland "Basic Marathon" autosampler. Three columns from Polymer Laboratories were used in series: 1. PLgel with particle size = 5μm, pore type = Mixed D, and length/I.D.=300mm/7.5mm, 2. PLgel with particle size = 5μm, pore type = Mixed D, and length/I.D. = 300mm/7.5mm 3. PLgel with particle size = 5μm, pore type = 100Angstrom, and length/I.D. = 300mm/7.5mm. The system was calibrated against polystyrene standards. THF was used as the eluent.

2. Syntheses.

1. A typical procedure for synthesis of the polyoxazoline:

To freshly distilled 4(S)-benzyl-2-oxazoline[1] (1.42 g, 8.81 mmol), methyl tosylate (79 μL, 0.53 mmol, 0.06 equivalent) was added under argon at room temp (20-25 ^{o}C). The tube was sealed and the mixture was first stirred at room temperature for 3 h, and then at 70 ^{o}C for 20 h. A glassy solid was formed as the reaction progressed. After the polymerization was complete, 3 mL of dry 1-butanol was added to the glassy solid and the mixture was heated at 117 ^{o}C for 14 hours. It was then cooled to room temp and the polymer precipitated after the addition of 5 mL of diethyl ether as a white solid. The polymer was washed repeatedly with ether (5 mL x 5) and centrifuged. The residue was collected, and on drying at 70 ^{o}C under reduced pressure for 24 h, yielded 1.32 g (~81%) of the white solid polymer. The degree of polymerization based on the ^{1}H NMR integration was 13. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.1-7.3 (br, 1H, >NCH=O), 7.3-6.1

[1] Leonard, W. R.; Romine, J. L.; Myers A. I. *J. Org. Chem*, **1991**, *56*, 1961-1963.

(br sh, C_6H_5, overlaps with chloroform peak), 4.1-1.8 (br sh, 5H, $CH(CH_2)_2$), 0.97 (br, CH_3 of OBn); IR (thin film, NaCl) cm^{-1}: 3028 (Ar C-H), 2930 (aliph C-H), 1668 (C=O), 1496, 1416, 1184, 1080, and 1033.

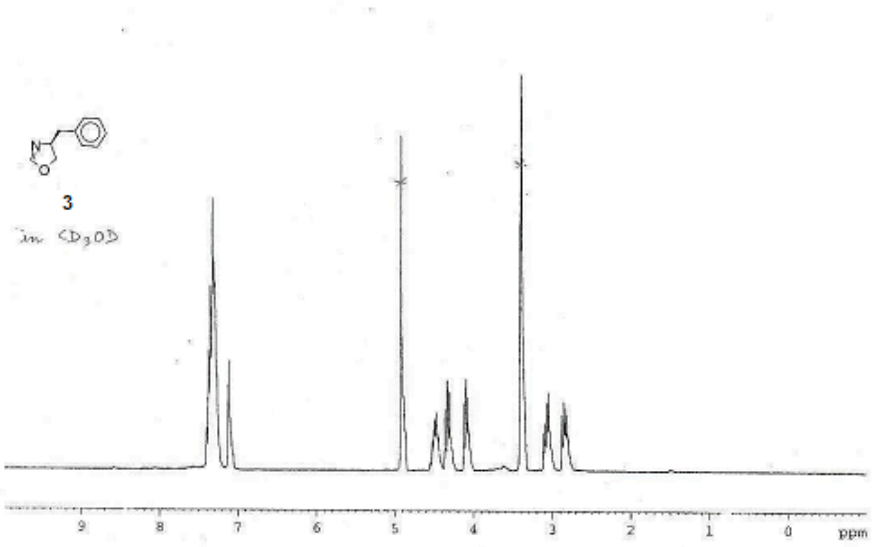
The spectroscopic properties of the three polyoxazolines are very similar. On the basis of the ^1H NMR integration, DP's calculated for the three polymers are ca. 13, 30 and 50. Spectral data for the polyoxazolines and the chiral PEI are in accordance with the literature.[2]

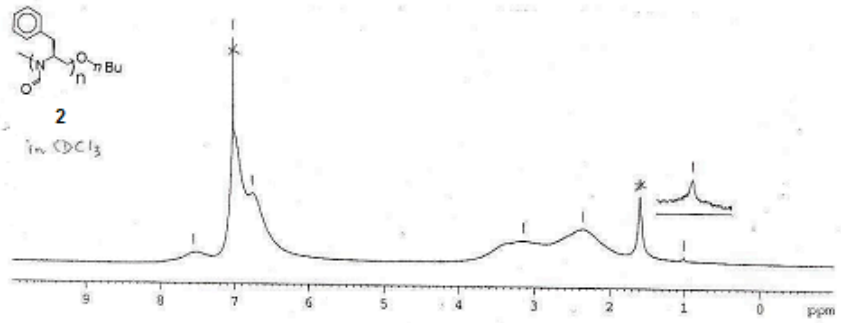
2. A typical deformylation condition:

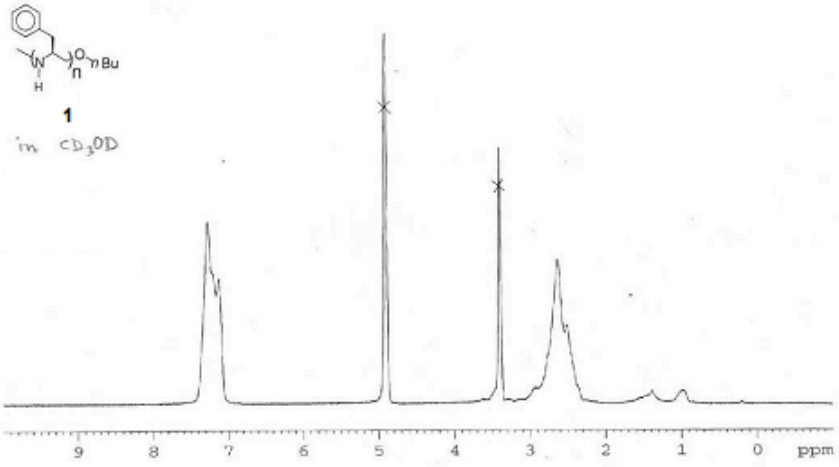
The formylated polymer (350 mg) was heated in 8 mL of 1-BuOH, until it completely dissolved. Then 700 mg of KOH (solid) was added and the mixture was refluxed for 16 h under argon. The mixture was cooled to room temp and the 1-BuOH was removed under reduced pressure. The product was dissolved in diethylether, washed with water (3 x 25 mL), dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was kept at 70 ^oC under reduced pressure for 2 days, after which 286 mg (95%) of the pale yellow gummy product was obtained. ^1H NMR (300 MHz, CD_3OD) δ 7.4-7.0 (br, sh, 5H, C_6H_5), 3.1-2.2 (br sh, 5H, $CH(CH_2)_2$), 1.6-1.2 (br sh, CH_2CH_2 of OBn), 1.02 (br, CH_3 of OBn); IR (thin film, NaCl) cm^{-1}: 3297 (N-H), 3035 (Ar C-H), 2923 (aliph C-H), 1452, and 1119.

[2] Saegusa, T.; Kobayash.S; Ishiguro, M. *Macromolecules* **1974**, *7*, 958- 959.

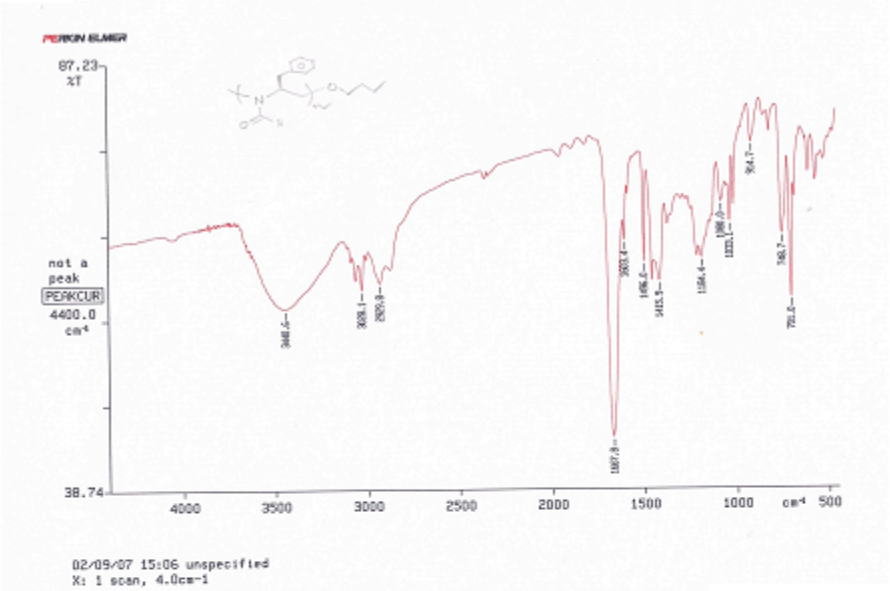
NMR Spectra of the 50mers.







IR spectra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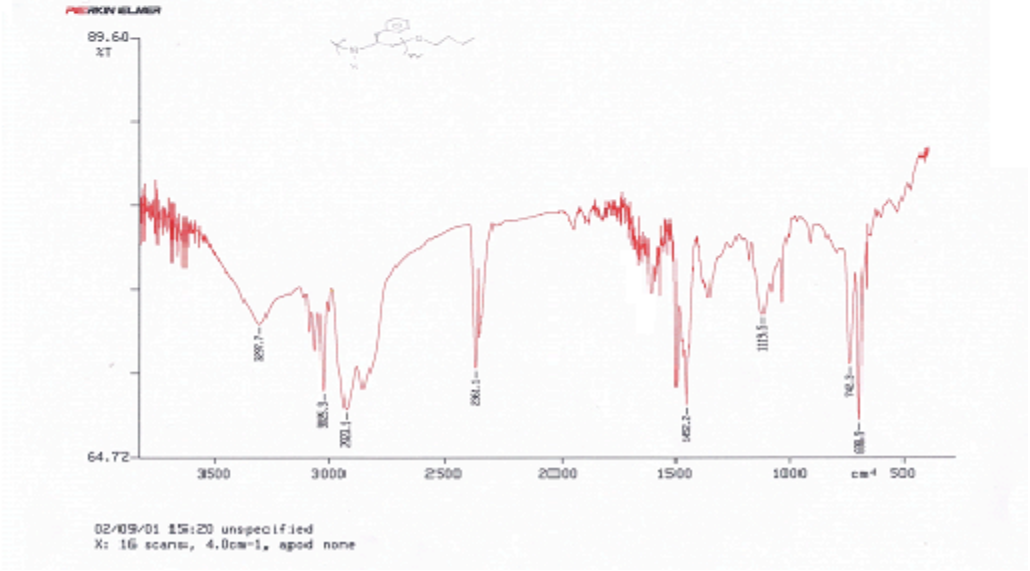
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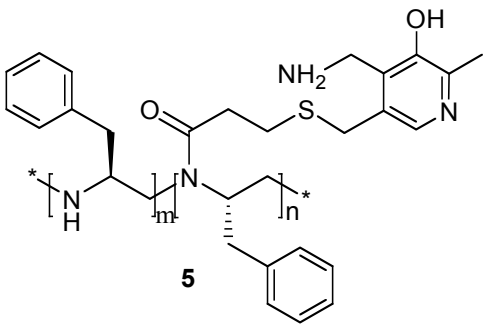
Covalently attached PEI-pyridoxamine system 5.

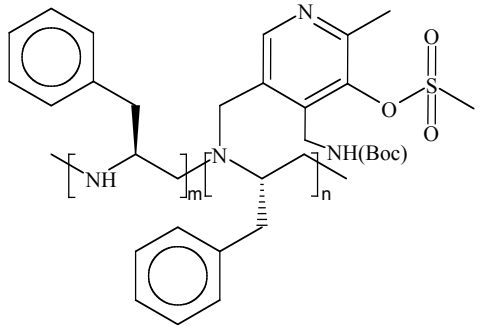


5

To a solution of 2,5-dioxoazolidinyl ester of 3,3'-dithiopropionic acid (197 mg, 0.49 mmol) in dichloromethane (15 mL) and sodium carbonate (100 mg), a solution of the PEI **1** (680 mg, 4.9 mmol) in dichloromethane (10 mL) was added at r.t. The reaction mixture was stirred at r.t. for 2 h. The solid sodium carbonate was removed by filtration, and after evaporation of the volatiles, the residue was dissolved in absolute ethanol (15 mL). Sodium borohydride (100 mg, 2.6 mmol, excess) was added to it, and the reaction mixture was stirred for 2 h at r.t. Then, 5-deoxomethyl-5-bromomethylpyridoxamine dihydrogen bromide (385 mg, 0.98 mmol) was added, and the mixture was stirred for another 1 h. The volatile materials were removed under reduced pressure, and the residue was dissolved in a mixture of dichloromethane-ether (1:1) (30 mL), washed, first with a solution of sodium carbonate (3 x 50 mL), and then with water (4 x 100 mL), and the organic layer was separated and concentrated. The polymer **5** was then precipitated via addition of a 1% solution of aqueous sodium carbonate, centrifuged and the residue was washed with water. The product was dried using a lyophilizer. Based on the ^{1}H integration, it was found that 9 % of the nitrogens of **5** carried the pyridoxamine units. ^{1}H NMR (300MHz, CD_3OD) δ 7.52 (br s, pyr ring H), 7.40-6.80 (br sh, Ph), 4.10 (br s), 3.70-3.50 (m), 3.10-2.00 (m br). UV-vis (MeOH; nm): 318.

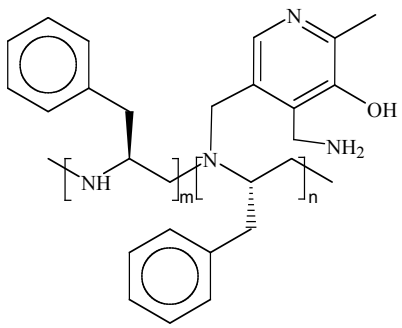
Covalently attached PEI-pyridoxamine system 6.

Step a.



To a mixture of 40 mg of 3-methanesulphoxy-4-(*t*-butoxycarbonylamino-methyl)-5-methanesulphoxy methyl-2-methylpyridin-3-yl ester[3] (**7**) in DMF-THF (1:1, 6 mL), a solution of the linear 50-mer PEI **1** (150 mg, 1.1 mmol) in 2 mL of THF, and anhydrous sodium carbonate was added at 0° C. The mixture was stirred at r.t. for 2 h. The product was extracted with diethylether, washed repeated (5 x 50 mL) with water, dried over anhydrous sodium sulphate and after removal of the solvent, 177 mg of the product was obtained. It was used in the following step without futher purification. Based on the ^{1}H integration, it was found that ~10% of the nitrogen atoms of the polymer were attached to the pyridoxamine units. ^{1}H NMR (300MHz, CD_3OD) δ 7.88 (s, pyr ring H), 7.52-6.88 (br sh, Ph), 4.22 (t, pyr-C**H**$_2$-PEI), 4.06 (s, pyrC**H**$_2$NHBOC), 2.96-2.09 (m br), 1.45 (s, N**H**BOC). UV-vis (MeOH; nm): 317.

Step b.

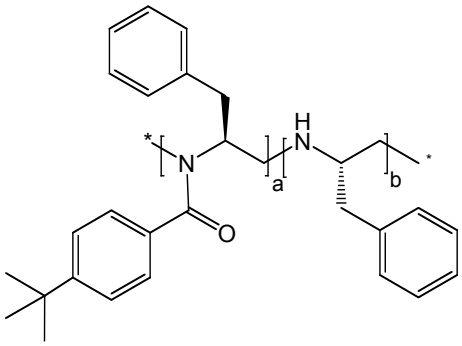


To a solution of 170 mg of the protected pyridoxamine-polymer in ethanol, 50 mg of sodium ethoxide was added and the reaction mixture was stirred at room temperature for 2 h. The solvent was then removed under reduced pressure, and the residue was extracted with dichloromethane-ether (1:1), washed with water and after removal of the solvent, redissolved in 5 mL of ether, and to it 1 mL of TFA added. The reaction mixture was stirred for 1 h, washed with a saturated solution of sodium carbonate (5 x 50 mL), and then with water (3 x 50 mL). The product **6** was precipitated via addition of a solution of aqueous sodium carbonate, centrifuged and the residue was washed with water. It was then dried using a lyophilizer. Based on the ^{1}H integration, it was found that ca. 8% of the nitrogen atoms of the polymer were attached to the pyridoxamine. Note that the pyridoxamine peak in the NMR spectrum was broad for this system. Based on the UV-vis spectrum, the percentage of the nitrogen atoms of the polymer attached to the pyridoxamine residues was about 9%, consistent to what was observed from the NMR spectrum.The ether layer was dried over anhydrous sodium sulphate, and after removal of the solvent, 144 mg of **6** was obtained. ^{1}H NMR (300MHz, CD_3OD) δ 8.00 (br sh,, pyr ring H), 7.70-6.70 (s br, Ph), 4.23 (s br, pyrC**H**$_2$NH$_2$), 4.08 (m br), 3.30-2.10 (br). UV-vis (nm) 316.

[3] Liu, L; Breslow, R. *J. Am. Chem. Soc.* **2003**, *125,* 12110-12111.

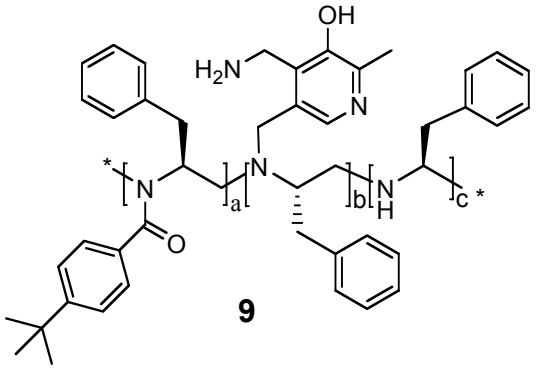
Covalently attached pyridoxamine-PEI system <u>10</u>.

Step a. Partial *N*-acylated PEI **8**.



To a solution of the linear PEI **1** (100 mg, 0.7 mmol) in dry dichloromethane (8 mL), 4-*t*-butylbenzoylchloride (28 μL, 0.14 mmol) and anhydrous sodium carbonate (40 mg) were added at 0^{o} C. The solution was allowed to stir under argon for 2 h. The organic layer was extracted with water, dried over anhydrous sodium sulphate and concentrated to afford 112 mg of **8.** The product was used in the next step without further purification. The NMR integration indicated that ca. 15% of the polymer nitrogens were acylated. ^{1}H NMR (300MHz, CD_3OD) δ 7.50-6.40 (br sh, Ph), 4.23 (s br, pyrC**H**$_2$NH$_2$), 3.20-2.10 (br), 1.30 (br m, *t*-Bu). IR (neat) 1664 cm^{-1}($\gamma_{C=O}$).

Step b. Attachment of the pyridoxamine unit via alkylation

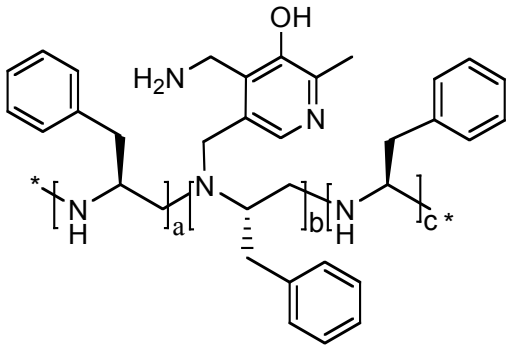


9

To the solution of partially benzoylated PEI **8** (55 mg) in dichloromethane-DMF (5:1) at rt, 37 mg of 5-bromopyridoxamine hydrochloride (0.095 mmol, ~25 mol% compared to **7**) in DMF (1 mL) and 40 mg of sodium carbonate (anhydrous) was added. The mixture was stirred for 8 h. The reaction mixture was extracted with ether, washed with water (200 mL x 4), and concentrated under reduced pressure. The product was then precipitated with addition of water, and the residue was separated and isolated after centrifugation, which on drying with a lyophilizer gave 59 mg of the product **9**. From the ^{1}H NMR spectrum, it was found that only ca. 8% of the polymer nitrogens were attached

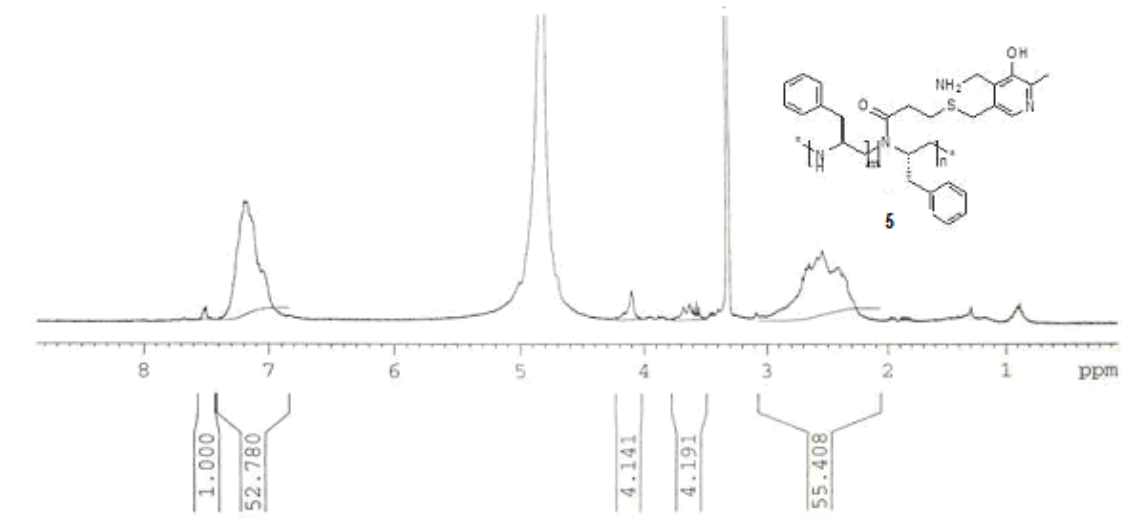
to pyridoxamine units. ^{1}H NMR (300MHz, CD_3OD) δ 8.20-7.70, (pyr ring H), 7.50-6.40 (br sh, Ph), 4.25 (s, pyrC**H**$_2$NH$_2$), 3.20-2.30 (br), 1.30 (br m, *t*-Bu); IR (neat) 1667cm^{-1} ($\gamma_{C=O}$), UV-vis (MeOH; nm) 318.

Step c. Deprotection of the *N*-acyl groups.



10

Potassium hydroxide (104 mg) was added to a solution of polymer **9** (40mg) in 10 mL of ethanol. The solution was refluxed for 14 h. The solvent was then removed under vacuum, and the residue was extracted with ether. The ether layer was washed with water (50 mL x 5), and then with a solution of sodium carbonate (50 x 4 mL), and concentrated under reduced pressure. It was then dissolved in 1 mL of methanol and precipitated with addition of water. The white precipitate was collected after centrifugation, and on drying using a lyophilizer yielded 28 mg of **10**. Based on the ^{1}H integration, it was found that ca. 8% of the polymer nitrogens were attached to the pyridoxamine, which was consistent with the UV-vis measurement. ^{1}H NMR (300MHz, CD_3OD) δ 8.20-7.70, (pyr ring H), 7.50-6.60 (br, Ph), 3.91 (s br, pyrC**H**$_2$NH$_2$), 3.20-2.30 (br). UV-vis (MeOH; nm) 316.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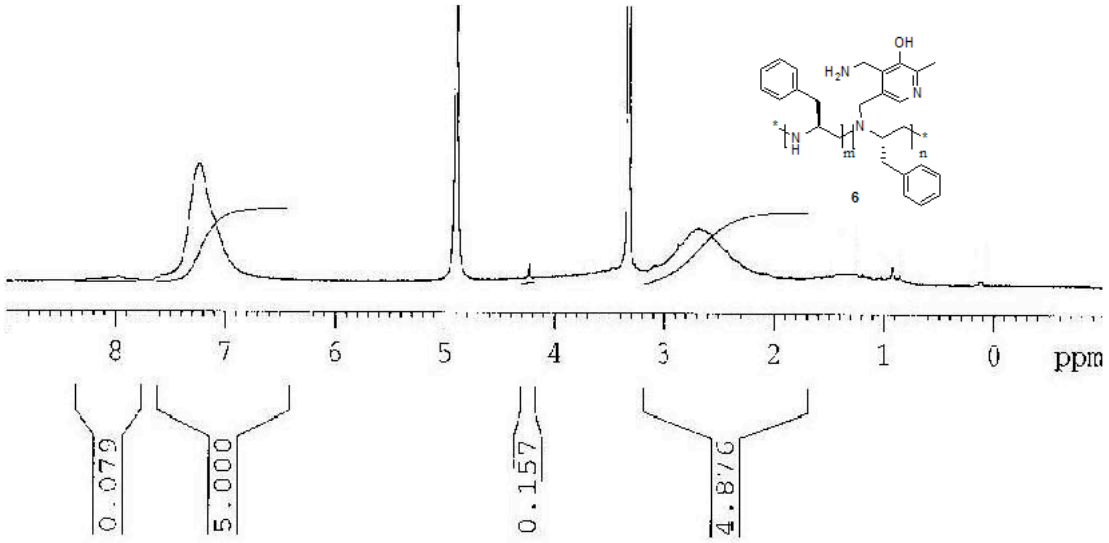
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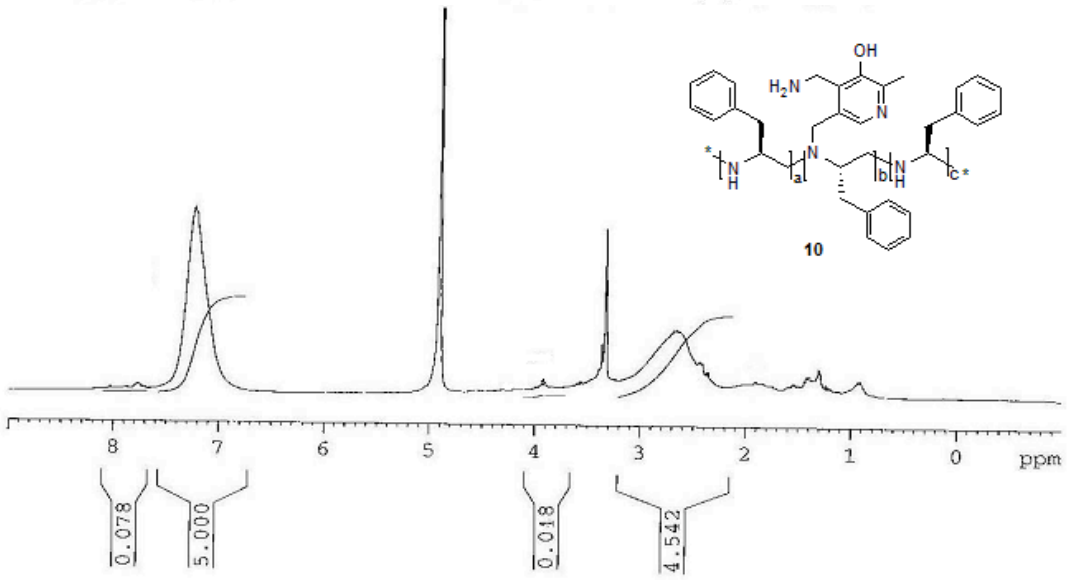
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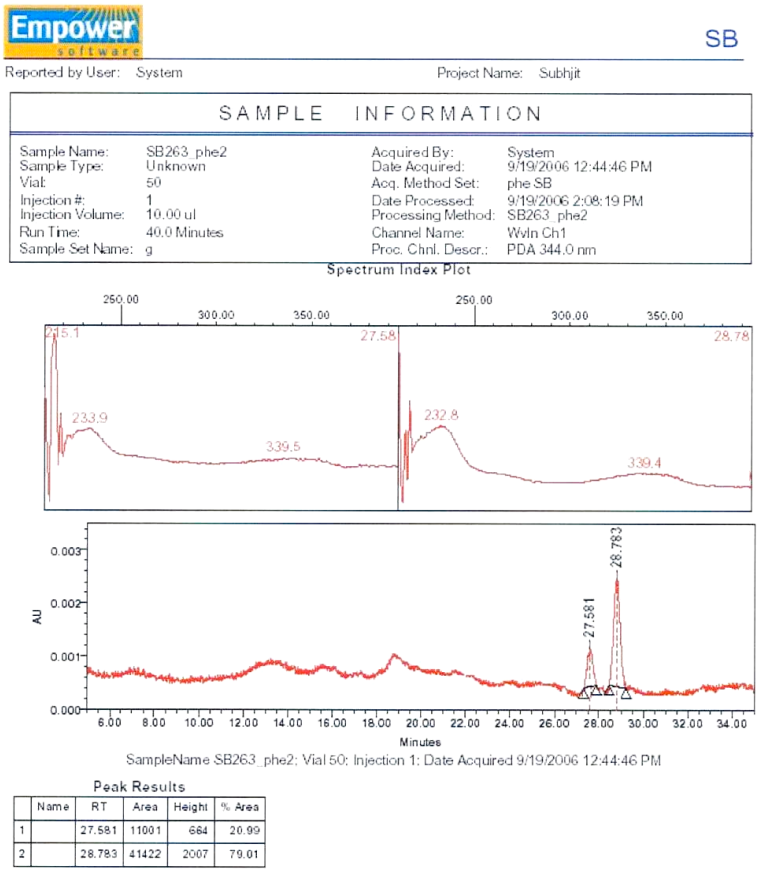
3. Transamination and analysis of the amino acid products with HPLC.

Methanolic solutions of pyridoxamine **4** (5.0×10^{-3} mol/L, 50 μL), PEI **1** (~100 g/L, 50 μL) was taken in a vial, and to it methanol (100 μL) was added. EDTA (1×10^{-2} mol/L, 25 μL, aq.), and ketoacid (5×10^{-2} mol/L, 50 μL, aq.) was then added at room temp, (20-25 ^{o}C) and with addition of water the total volume was made up to 500 μL. The pH of the solution was adjusted using 0.1 M solution of HCl or NaOH solution. The pH values reported here are as read with a glass-electrode calibrated against aqueous buffers. The reactions were run for 45 min to 24 h.

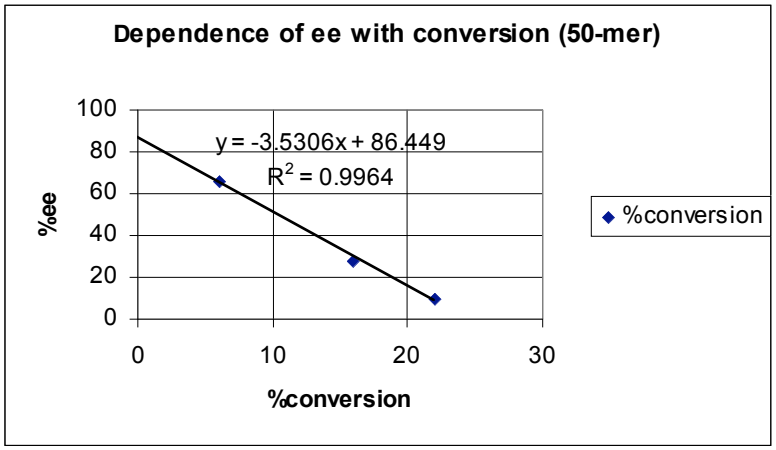
For the covalent systems **5**, **6** and **10**, the concentration of the polymer-pyridoxamine stock solution was ca. 5×10^{-2} mol/L. The pH was adjusted to ca. 5.5 with 0.1 M HCl. The reactions were run for 45 min to 24 h.

The ee values of the amino acid products were determined with HPLC. First, the amino acids were extracted with 1mL of water after evaporation of the solvents from the reaction mixture. Then the volume of the aqueous media containing the amino acids was reduced to 100 μL. 20 μL of a derivatizing solution containing 0.2 M *o*-phthalaldehyde and 0.2 M *N*-Boc-cysteine/*N*-acetyl-cysteine in methanol, 20 μL of an aqueous buffer solution of 1.0 M K_2HPO_4 (pH 8.0), and 20 μL of methanol were added to it. The resulting solutions of the diastreomeric 1-thioisoindoles were analyzed by HPLC, with UV-vis detection at 344 nm.[4] Samples were often diluted with aqueous methanol to obtain better resolution between the peaks of the enantiomers. At least, three independent runs were recorded to report the L/D ratio of the amino acids. Standard samples were run after the samples to ensure the right retention times of the productts. Standard deviations for the ee with the 13, 30 and 50-mers were 7.8, 3.1 and 4.6 respectively. The other standard deviation values are given in the text. A representative HPLC trace is provided here.

[4] Liu, L.; Rozenman, M.; Breslow, R. *Bioorg. Med. Chem.* **2002**, *10*, 3973-3979.



4. Dependence of ee with time with the 50-mer (in the case of the non-covalent system).



5. Comparative rates of transamination with the covalent PEI systems.

In a typical kinetics experiment, monitored by UV-vis spectrometer, the following materials were added to a 1 mL UV cell: (i) 100 μL of PEI-pyridoxamine solution (~10^{-3} M); (ii) 10 μL of EDTA (0.01 M , aq.) solution; (iii) 750 μL of H_2O-MeOH (35-40 % v/v.); (iv) 100 μL of phenyl pyruvic acid (0.05 M,aq.), and the pH was adjusted to 5.5 (uncorrected value, read directly with a glass electrode) with addition of 0.1 M HCl using a micropipette. The absorption at 315 nm was recorded. In another UV cell, a solution similar solution with a standard pyridoxamine solution (no PEI) that had the same absorption at 315 nm was prepared. The rate of transamination was monitored simultaneously using a Varian Cary IE UV-spectrophotometer by following the decrease of absorption at 315 nm, which corresponded to the disappearance of pyridoxamine for both of the solutions. Pseudo-first order of kinetics was assumed in the study, and the rates of transamination were provided by the computer regressions. The relative rates were calculated by comparing the rates of pyridoxamine-PEI systems with the pyridoxamine controls (no PEI). The values prodived are means of three trials. The agreement between different runs was within 15%.

Figure S1. A typical decrease in UV absorption at 315 nm during the course of transamination.